THE SHARK TRADE IN COSTA RICA: GENETICS, MERCURY CONTAMINATION AND HUMAN DIMENSIONS AND THE IMPLICATIONS FOR CONSERVATION

by

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DEDICATION

This is dedicated to my loving wife Jen, who always supported me during this endeavor, and our daughter Hannah.

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ABSTRACT

THE SHARK TRADE IN COSTA RICA: GENETICS, MERCURY CONTAMINATION AND HUMAN DIMENSIONS AND THE IMPLICATIONS FOR CONSERVATION

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In the past two decades shark populations have declined as a direct result of increased demand for shark products. As a result one quarter of shark species are now listed as "vulnerable", "endangered", or "critically endangered" by the International Union for Conservation of Nature (IUCN). For example, in Costa Rica, two threatened and commercially valuable species, the scalloped hammerhead (*Sphyrna lewini*) and silky shark (*Carcharhinus falciformis*), have experienced declines of ~90% and 80% respectively. However, within Costa Rica there is still a lack of: species-specific catch data for elasmobranchs in both artisanal and industrial fisheries, contamination levels of elasmobranch products, and information on the type of elasmobranch conservation measures that would potentially be supported by Costa Rican fishermen. Thus, it is impossible to monitor the impacts fisheries are having on specific elasmobranch populations, determine the potential health risk to elasmobranchs and consumers of their

products from contaminants, and determine the conservation measures that would prove to be most successful in protecting shark populations. Therefore, the objectives of this research were to attempt to fill some of these information gaps by determining: 1) artisanal fishermen's knowledge of sharks, their perceptions of local fisheries impacts on shark populations, and levels of potential public support for various conservation measures (i.e. MPAs); 2) species composition and abundance in artisanal and industrial fisheries using DNA barcoding; and 3) mercury (Hg) contamination levels in shark meat being sold for human consumption at markets. Using social surveys within two artisanal fishing communities we determined that support for new shark conservation measures was high (97%). However, support declined, to between ~60 to 6%, as proposed legislation potentially obstructed the fishermen's ability to continue their current use of their fishing grounds. This highlights the importance of fisheries managers to work with artisanal fishermen to develop regulations, which 86% of surveyed fishermen were willing to do, to develop effective legislation that has a greater chance of compliance by fishermen. Within these artisanal fisheries, we identified seven species of shark (*C. falciformis*, *C. porosus*, *C. limbatus Mustelus lunulatus*, *Nasolamia velox*, *Rhizoprionodon longurio*, *S. lewini*) and one ray (*Dasyatis longa*), with the scalloped hammerhead (*S. lewini*) accounting for ~75-80% of all sharks landed. Recorded total lengths for scalloped hammerheads in the artisanal fisheries sampled, based on observer data, suggests that each of the sharks sampled were either juveniles or neonates. The "endangered" conservation status of the scalloped hammerhead shark and its current susceptibility to artisanal fisheries methods highlights the needs for new shark

conservation measures in these fisheries. We also found that at least nine species of shark (*Alopias pelagicus*, *C. falciformis*, *C. obscurus*, *C. porosus*, *M. lunulatus*, *N. velox*, *R. longurio*, *S. lewini*, *S. zygaena*) and one ray (*D. longa*) were being sold in local markets, with the silky shark representing ~80% of samples tested. Therefore, silky sharks represent the most highly exploited pelagic shark in Costa Rica, which is of concern based of recent population declines and their listing as "vulnerable" in the Eastern Tropical Pacific by the IUCN. Within the markets total Hg concentrations in the shark products being sold were highest in *S. zygaena* (15.75 ± 2.11 ppm dry wt, 3.50 ± 0.47 ppm wet wt) and *C. limbatus* (11.89 ± 3.67 ppm dry wt, 2.50 ± 0.78 ppm wet wt). However, all shark species tested exceeded US Environmental Protection Agency (EPA) Hg limits of 0.3 ppm. Using previously established equations we were able to estimate THg concentrations (ppm dry wt.) for the livers of these sharks using the known muscle concentrations. *Sphyrna zygaena*, which is listed as "vulnerable" by the IUCN - had the highest estimated mean THg liver concentrations (4.67 ± 1.03 ppm dry weight). Thus, the consumption of shark products being sold in the Costa Rican markets poses a potentially serious health risk to consumers. The elevated Hg levels found in the muscle tissues and internal organs (liver) of this species also have the potential to negatively impact the health and conservation status of these species. It is apparent that new conservation measures are needed to protect elasmobranchs in Costa Rica, particularly the scalloped hammerhead in artisanal fisheries and the silky shark in pelagic fisheries. Despite the potential human health risk associated with the consumption of elasmobranch products, the level of contamination could prove to be a useful tool in reducing demand for

elasmobranch products, and thus aid in the conservation of threatened species. Regardless, monitoring of shark contamination, fisheries and market sales in Costa Rica is poor and further legislation will be needed to ensure the sustainability of their shark populations.

GENERAL INTRODUCTION

Sharks in decline

In the past several decades, shark populations have declined due to both directed shark fisheries and the bycatch of sharks (Abercrombie *et al*. 2005; Wallace *et al*. 2010; 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). These declines are a result of both increased global fishing pressure within all fisheries and higher demand for shark products (e.g. fins), that has lead to nearly 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). Sharks are generally not target species of pelagic fisheries, yet they still account for ~15% of the biomass for reported landings (Trujillo *et al*. 2012). Sharks are typically caught as bycatch, but do not survive due to either the retention of their bodies for their fins and meat (Whoriskey *et al*. 2011), or the high mortality rate associated with the stress of being caught (Frick *et al*. 2010). The increased fishing pressure currently experienced by sharks, whether from directed fisheries or indirect bycatch, has resulted in decreased catch rates for sharks around the globe.

Over 150 countries have participated in the trade, catch, sale, and transport, of shark products with reported landings of sharks peaking in 2003 (Hoelzel 2001; Cunningham-Day 2001; Lack 2006; Dulvy *et al*. 2014). Since that time, landings have

decreased by 20% and it is unclear if this is the result of better management or overfishing causing population declines (Dulvy *et al*. 2014). Of the products harvested from sharks, fins are of the highest value, being one of the most valuable fisheries products (Abercrombie *et al*. 2005; Topelko 2005). For example, in Hong Kong, the world's largest fin market, prices for shark fins can reach up to $700/kg for fins taken from highly valued species (Abercrombie *et al*. 2005; Topelko 2005). Because of the high prices for fins, the practice of shark-finning, removal and retention of shark fins and the discard of the carcass at sea, can be common practice (Topelko 2005). However, due to the implementation of finning bans in many countries there is a growing market for shark meat (Dent & Clarke 2015). Finning bans ultimately force fishermen to utilize the entire carcass of a shark helping to drive the market for shark meat and causing fishermen to view sharks as actual commercial species instead of just bycatch (Dent & Clarke 2015). There has actually been an increase in the trade value of shark meat in many key-trading countries, even as the quantity of shark meat being traded has risen substantially, which suggests demand for shark meat is increasing (Dent & Clarke 2015). Dogfish (*Squalus spp*.), mako (*Isurus spp*.), and school shark (*Galeorhinus galeus*) meat fetch the higher prices on the international shark meat market (Dent & Clarke 2015). While within the fin market, hammerhead (*Sphyrna spp*.), oceanic whitetip (*Carcharhinus longimanus*) and blue (*Prionace glauca*) sharks are the more highly valued species (Dent & Clarke 2015). The nations driving the markets (importers and exporters) for shark meat and fins are also quite different (Dent & Clarke 2015). Whereas many of the Asian countries (i.e. China, Hong Kong SAR, and Taiwan) drive the fin market, countries like Brazil - which

has increased shark meat imports eight fold since 2000 - and Mexico are responsible for the increased trade in shark meat (Dent & Clarke 2015).

The growing markets for shark products and increased global fishing pressure on all fish species has resulted in reduced catch rates (population declines) for many shark species. From 1986 to 2003, catch rates for hammerheads (89%), great whites (*Carcharodon carcharias*) (79%), tiger (*Galeocerdo cuvier*) (65%), thresher (*Alopias spp.*) (80%), blue (60%), and mako sharks (70%) all declined significantly in the Northwest Atlantic (Baum *et al*. 2003). In the Pacific Ocean catch rates of blue sharks in longline fisheries dropped by >50% from 1996 to 2009, while rates for oceanic whitetips declined by 90% (Clarke *et al*. 2013). Smooth hammerhead (*Sphyrna zygaena*), blue, mako, porbeagle (*Lamna nasus*), and common thresher shark (*Alopias vulpinus*) catch rates have all declined by 96 to 99.99% in the Mediterranean (Ferretti *et al*. 2008). While in the Gulf of Mexico, where shark used to make up 17% of the total catch of longline fisheries in the 1950's, sharks only accounted for 2% by the 1990's (Baum & Myers 2004). If current fishing pressure faced by shark populations is not reduced many may face the possibility of extinction, which could have major impacts for the ecosystems they inhabit (i.e. trophic cascades) (Myers *et al*. 2007).

Overfishing of sharks and rays through directed fisheries and as bycatch represent the largest threat to these species, but they are not alone. Habitat degradation poses a serious threat to coastal shark and ray populations. Commercial development of coastal areas, destruction of mangrove forests (potential nursing grounds), and pollution, which

can impact the health of the entire ecosystem, all pose serious threats to shark and ray populations (Dulvy *et al*. 2014).

One such pollutant is the heavy metal mercury (Hg), which is a naturally occurring element within the marine environment (Burger & Gochfield 2011; Maz-Courrau *et al*. 2012). However, Hg levels in the marine environment have been increasing due to anthropogenic sources including atmospheric releases from coal-fired power plants and coastal runoff from contaminated areas (Maz-Courrau *et al*. 2012). It is uncertain the exact impacts increased Hg contamination will have on shark and ray populations. However, in fish, Hg negatively impacted reproduction (Matta *et al*. 2001; Hammerschmidt *et al*. 2002; Drevnick & Sandheinrich 2003; Klaper *et al*. 2006; Crump & Trudeau 2009), liver function, metabolism (Adams *et al*. 2010), and behavior (Webber & Haines 2003). While also being shown to cause neurological damage (Basu *et al*. 2007, 2009; Scheuhammer *et al*. 2008), damage to gills and olfactory organs (Jagoe *et al*. 1996; Oliveira-Ribeiro *et al*. 1996, 2000) and even mortality (Wiener & Spry 1996). The longevity of sharks and rays and their generally high trophic level status results in them bioaccumulating substantial concentrations of Hg in their tissues (Lyle 1984; Adams 2004; Branco *et al*. 2004). This raises concerns over the potential impacts of Hg on the health of various shark and ray species and the implications this will have on current population declines. The growing market for shark and ray meat also poses a potential health risk to human populations, particularly to fetuses, infants, and children (Burger & Gochfield 2011, 2012; Lopez *et al*. 2013). The health concerns associated with shark and ray meat due to high levels of Hg could potentially be used as a deterrent to consumption

of these products. Possibly resulting in decreased fishing pressure due to lower demand for shark and ray meat and fins.

The persecution of shark and rays and climate change also pose a threat to the sustainability of their populations. Persecution of sharks and rays includes: population control measures (beach netting, directed hunts) to reduce the risk of an encounter between sharks and beachgoers, protect fishing gear from being damaged, and eliminating competition between sharks and rays and other commercially important fisheries (Dulvy *et al*. 2014). Currently, climate change is only known to threaten one species of shark or ray, however, sensitivity to climate change has been recorded in several other shark species (Chin *et al*. 2010).

Shark conservation

Despite current population declines there are currently no international or regional fisheries organizations with the specific goal of properly managing the exploitation of shark populations (Camhi *et al*. 2008). However, there are a variety of international organizations and regional fisheries management groups that attempt to regulate shark landings, directed or as bycatch, and provide these species with some level of protection. Of the shark and ray conservation measures currently in place, bans on shark "finning" are the most widespread (Dulvy *et al*. 2014). These prohibitions are implemented by a variety of states and fisheries management organizations and can enhance monitoring and compliance with other fisheries regulations, but they have not significantly reduced shark mortality or risk to threatened species (Clarke *et al*. 2013).

Regional Fisheries Management Organizations (RFMO's) are tasked with managing high seas, straddling and highly migratory fish stocks, with several of them taking steps to provide better protection for sharks in international water (Vincent *et al*. 2013). For instance, the International Commission for the Conservation of Atlantic Tuna (ICCAT) has adopted measures for protecting sharks landed in the fisheries they manage including: prohibiting the retention of silky sharks (*Carcharhinus falciformis*), bigeye thresher sharks (*Alopias superciliosus*), oceanic whitetip sharks, and all hammerhead sharks in their fisheries and trying to release them alive when possible (ICCAT). While the Inter-American Tropical Tuna Commission (IATTC), which is responsible for the eastern tropical pacific, has prohibited the landing of oceanic whitetip sharks and mobulid rays (IATTC Resolution C-11-10 & C-15-04).

The Migratory Sharks Memorandum of Understanding (MoU), a legally non-binding international instrument, adopted by the Parties of the Convention on Migratory Species (CMS) currently includes seven sharks under Annex I (Fowler 2012). The objectives of the MoU include: increasing understanding of migratory shark populations, ensuring sustainable shark fisheries, protecting critical habitat, increasing public awareness of threats to sharks, and enhancing cooperation regional and international cooperation (CMS 2010; Fowler 2012). Over 50 shark species have also been included in Annex I (Highly Migratory Species) of the 1982 Law of the Sea Convention, implemented on the high seas under the 1992 Fish Stocks Agreement (Dulvy *et al*. 2014). However, like the MoU, it is not a legally binding agreement. In 1999, the United Nations Food and Agriculture Organization (FAO) adopted the International Plan of

Action (IPOA) for Sharks, with the objective to ensure the conservation and management of sharks and their long-term sustainable use (FAO 2014). Through the IPOA-Sharks, states, which regularly catch sharks directly or indirectly, are instructed to implement a National Plan of Action (NPOA) for the conservation and management of shark stocks (FAO 2014). However, only a few nations have actually created a NPOA.

The Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES) regulates the trade of endangered or threatened species between countries (Vincent *et al*. 2013). Species are listed on one of three appendices that provide different levels of protection. Appendix I includes the most endangered species that have the highest risk of extinction. Listing on appendix I thus prohibits any international trade of these species. However, in certain cases (i.e. scientific research) trade of these species may be allowed if it is in the best interest of the species and the proper authorization is provided. Currently, the only shark species listed under CITES Appendix I are all members of the family *Pristidae* (sawfishes) (CITES 2013).

Appendix II includes species that are not necessarily currently threatened with extinction but may become threatened unless their trade is controlled (Vincent *et al*. 2013). It also includes "look-alike species", i.e. species whose specimens in trade look like those of species listed for conservation reasons (Vincent *et al*. 2013). International trade in these species is allowed with the proper export permits, but no import permits are required (Vincent *et al*. 2013). Current sharks and rays listed under Appendix II include: the whale shark (*Rhincodon typus*), great white shark, basking shark (*Cetorhinus maximus*), porbeagle shark, spiny dogfish (*Squalus acanthias*), oceanic whitetip shark,

scalloped hammerhead shark (*Sphyrna lewini*), and the mobulid rays (CITES 2013; Vincent *et al*. 2013). Under Article II paragraph 2 of the convention several other species of shark also have had the trade of their products regulated due to being "look-alike" species of the scalloped hammerhead shark (Vincent *et al*. 2013). These include the great hammerhead shark (*Sphyrna mokarran*), smooth hammerhead shark, sandbar shark (*Carcharhinus plumbeus*), and the dusky shark (*Carcharhinus obscurus*)(Vincent *et al*. 2013). However, even if a species is listed in an Appendix, Parties can submit reservations to the listing effectively making them a non-Party of CITES for that species (Vincent *et al*. 2013).

Appendix III includes species that already have their trade regulated by a member party, but they need the cooperation of other countries to prevent unsustainable or illegal exploitation. Appropriate permits or certificates are needed for international trade of these species listed in this appendix. There are no sharks or rays currently listed under Appendix III (CITES 2013).

Few nations have developed catch limits or restrictions on the directed fishing of sharks in their national waters. However, countries like the Republic of Congo, Ecuador, Egypt, Israel, and Palau (for foreign vessels), have all prohibited the directed fishing of all elasmobranchs (Camhi *et al*. 2008). Currently, no international or bilateral catch limits exist for elasmobranchs (Camhi *et al*. 2008). In many cases there is little interest in managing elasmobranchs 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; Spaet *et al*. 2012). Historically, 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). 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).

Sharks in Costa Rica

By the 1990's Costa Rica accounted for the majority of marine landings in the Central American region (Salas *et al*. 2011). Currently, Costa Rica is the sixth largest exporter of shark meat, and is the main supplier to the growing Mexican market (Dent & Clarke 2015). They are also an important exporter of shark fins and a key trading post for

the shark fishing fleet in Central America (Dent & Clarke 2015). However, there have been recent declines in domestic production of shark products with Costa Rica currently being ranked as the 28th largest producer in the world (Dent & Clarke 2015). Despite reductions in landings of marine organisms, concerns still remain on the impacts of fisheries on shark populations (Dapp *et al*. 2013).

In Costa Rica, overall catch rates for pelagic sharks declined by 60% from 1991 to 2000, while two commercially important and threatened species, the scalloped hammerhead and silky shark, have experienced catch rate declines of approximately 90% and 80% respectively (Arauz 2000; Arauz *et al*. 2004; Whoriskey *et al*. 2011). In 1991, sharks accounted for 27% of the total catch in Costa Rican fisheries, but dropped to 4.9% by 2003 (Arauz *et al*. 2004). Overall shark landings have also decreased by 72% in Costa Rica from 12,901 tonnes in 2000 to 3,635 tonnes in 2011 (Dent & Clarke 2015). 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 & Myers 2004). Fishermen have also reported observing reductions in the abundance of sharks in Costa Rica's coastal waters (Bystrom & Cardenes-Valenzuela *in prep*).

Directed fisheries for sharks are rare in Costa Rica, however some pelagic fisheries have been shown to shift their target species to sharks when their original target species are low in abundance (Swimmer *et al*. 2010). Despite not being the normal target species, sharks still account for ~15% of the biomass for reported landings in Costa Rica (Trujillo *et al*. 2012). This is because even when sharks are caught as bycatch they are either retained for their fins and meat (Whoriskey *et al*. 2011). Small shark fins can be

worth ~$10/kg, while larger shark fins can fetch ~$70/kg (Whoriskey *et al*. 2011; Dapp *et al*. 2013).

Of conservation concern in Costa Rica is documenting the catch rates of highly exploited species like silky, thresher, and hammerhead sharks. Silky sharks are the most commonly caught shark species in Costa Rican long-line fisheries (Dapp *et al*. 2013). However, due to reductions in catch rates from both target and non-target fisheries silky sharks have been listed as ‘‘near threatened’’ globally and ‘‘vulnerable’’ in the Eastern Tropical Pacific (Dulvy *et al*. 2008). 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 between 2004 and 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). There has also been a decline in catch rates and reduction in average size at capture, possible selecting for maturation at smaller sizes, for thresher sharks landed in Costa Rica (Whoriskey *et al*. 2011; Dapp *et al*. 2013). The scalloped hammerhead is known to aggregate in predictable locations making them easier to exploit and increases their vulnerability to fishing pressure (Abercrombie *et al*. 2005; Baum *et al*. 2007).

INCOPESCA (the Costa Rica Fisheries and Aquaculture Institute) is the governing body responsible for the implementation of all policies concerning marine fisheries management and aquaculture in Costa Rica (Cajiao-Jimenez 2003; Alpizar *et al*. 2006). However, there are ~50 organizations directly or indirectly involved in the fishing

industry in Costa Rica's central Pacific region alone (Araya 2006). In 1948, to combat potential overexploitation of marine resources, Costa Rica put in place fisheries laws to regulate and stimulate its fisheries (Salas *et al*. 2011; Trujillo *et al*. 2012). However, these laws were seen as unconstitutional by fishermen and challenged in court, causing long lapses in fisheries management in Costa Rica (Salas *et al*. 2011). Despite recent revisions to these laws in 2005 to combat complaints from fishermen, there are still issues limiting their application by INCOPESCA (Salas *et al*. 2011).

INCOPESCA has also been inefficient at regulating and enforcing catch rates of many species including sharks, due to small budgets and a lack of qualified personnel, resulting in non-compliance by many fishermen (Salas *et al*. 2011). In particular, sharks currently have no catch limits or size restrictions and there is a lack of species-specific catch data (i.e. shark are not recorded to species level in the fisheries) to help better manage these species (Camhi *et al*. 2008; Salas *et al*. 2011; Whoriskey *et al*. 2011; Trujillo *et al*. 2012). To try and combat some of the declines seen in shark populations due to shark finning, Costa Rica passed a law in 2005 that required all sharks landed at Costa Rican docks to have fins attached (Whoriskey *et al*. 2011). However, foreign flagged vessels sidestepped this law by landing their catches, including unattached shark fins, at private docks not regulated by the Costa Rican government (Salas *et al*. 2011). Therefore, in 2011 Costa Rica implemented Articles 211 and 212 of the Customs Law and Supreme Court Resolution 1109-2006, which required all foreign flagged vessels to land sharks at publicly operated docks to ensure their compliance with the finning ban (Arauz *personal communication*). However, shark-finning laws still lack enforcement by

the Costa Rican government (Whoriskey *et al*. 2011). Costa Rica has an abundance of laws, regulations, and decrees in order to try and protect the environment, but fishing legislation – especially as it concerns sharks - is fragmented and non-cohesive (Salas *et al*. 2011).

To protect sharks in Costa Rica, area or time closures of fisheries and shorter soak times for fishing gear have been suggested as means to protect the most vulnerable life stages of sharks (i.e. juveniles, times of migration)(Whoriskey *et al*. 2011; Dapp *et al*. 2013). Previous research has shown that Marine Protected Areas (MPA) can provide conservation benefits for shark populations by protecting these critical areas and life stages for various species (Knip *et al*. 2012). Highly migratory shark species also received benefits from MPAs, particularly those in coastal areas, by protecting juveniles and nursing grounds (Knip *et al*. 2012). The largest MPA in Costa Rica is the Cocos Island National Park and the Marine Seamount Management Area (Bessudo *et al*. 2011). The Cocos Island National Park was established in 1978 and is the second largest marine protected area (MPA) in the ETP and is a no-take zone for all marine species, protecting 1989 km^2 of ocean water surrounding the island (MarViva 2011). The Marine Seamount Management Area was established in 2011 as an extension of the Cocos Island National Park and partially protects an additional 9640 km^2 (MarViva 2011). Costa Rica also has several MPA's throughout its coastal waters, but few have restrictions on fishing practices (Arauz *et al*. 2004).

Conservation campaigns have also tried discouraging the consumption of any shark products owing to conservation concerns (Dent & Clarke 2015). While others

emphasize the levels of contaminants that can be found in shark tissue and the dangers that they then pose (Dent & Clarke 2015). However, within Costa Rica there is still a lack of: species-specific catch data for both artisanal and industrial fisheries, contamination levels of shark products, and what type of conservation measures would be potentially be supported by Costa Rican fishermen. Therefore, the objectives of this current research were to attempt to fill some of these information gaps by determining: 1) artisanal fishermen's knowledge of sharks, their perceptions of local fisheries impacts on shark populations, and levels of potential public support for various conservation measures (i.e. MPAs); 2) species composition and abundance in artisanal and industrial fisheries; and 3) and mercury (Hg) contamination levels in shark meat being sold for human consumption at markets.

EVALUATING SUPPORT FOR SHARK CONSERVATION AMONG ARTISANAL FISHING COMMUNITIES IN COSTA RICA

Introduction

Shark populations have declined globally due to increased exploitation rates caused by both a higher demand for their products and an increased level of bycatch (Abercrombie *et al*. 2005; Wallace *et al*. 2010; Dulvy *et al*. 2014). It is estimated that nearly 100 million elasmobranchs (sharks, skates and rays) are caught each year; however, true landings could be 3-4 times what is reported (Clarke *et al*. 2004; Abercrombie *et al*. 2005; Shivji *et al*. 2005; Worm *et al*. 2013; Dulvy *et al*. 2014). Due to current population declines one quarter of chondrichthyan species (includes elasmobranchs and chimeras) are listed as "vulnerable", "endangered", or "critically endangered" by the International Union for Conservation of Nature (IUCN) (Dulvy *et al*. 2014). Demand for shark fins, one of the overall highest valued seafood products, has been a main driver for increased shark landings globally (Dulvy *et al*. 2014). However, reported catch rates for sharks and trade in their fins peaked around 2003 and has steadily decreased by ~20% from 2000 to 2011 (Dulvy *et al*. 2014; Dent & Clarke 2015). It is unclear whether these declines are related to better management or reduced success from population declines (Dulvy *et al*. 2014). During this same time period the trade in shark meat increased by 4.5% annually despite its lower value, likely due to finning bans

requiring landing of whole sharks and growing markets for their meat in countries like Brazil (Dent & Clarke 2015).

Within global fisheries competition for resources exists between small-scale artisanal and advanced artisanal fisheries (Pauly *et al*. 2002). Small-scale artisanal fisheries are characterized by smaller vessels that stay close to shore compared to the advanced artisanal fishing fleet that target both pelagic and coastal areas and can travel great distances (Salas *et al*. 2007). However, in Latin America, competition is reduced due to the advanced fleet concentrating exclusively on pelagic fish species (Pauly 2006). Small-scale artisanal fisheries employ ~25 times as many fishers as advanced artisanal fleets and produce roughly the same amount of fish for human consumption (30 million tonnes) (Chuenpagdee & Pauly 2004; Pauly 2006). In Latin America, small-scale artisanal fisheries are an important source of food and employment for local communities, providing the main basis of revenue for ~2 million people (Salas *et al*. 2007). The overall importance of artisanal small-scale fisheries cannot be understated and if efforts are not first concentrated on understanding and properly managing these fisheries and the resources they utilize, long-term fisheries and fishing based cultures in these areas might not be sustainable (Pauly 2006).

Artisanal fisheries in Costa Rica account for 75-80% of the total annual catch in the country (Salas *et al*. 2011). Artisanal fisheries in Costa Rica include small-scale and mid-scale vessels that generally stay near the coast and use hand-lines, gillnets, and long-lines (i.e. bottom-lines, mid-water), and advanced artisanal fisheries that utilize long-lines in pelagic waters (Salas *et al*. 2011). There are approximately 3500 licensed fishing

vessels that make up the small-scale and mid-scale artisanal fishing fleet, accounting for 61% of the total annual catch (Salas *et al*. 2011). Despite the number of artisanal boats operating in Costa Rica, exports of marine products only represented 0.4% of the gross domestic product (GDP) in the 1990's. Regardless of the perceived low economic value attributed toward fisheries exports, Costa Rican fisheries still play an important role in creating employment for coastal areas and small-scale artisanal fisheries are still the main source of marine products for domestic consumption (Salas *et al*. 2011). Within Costa Rica there is also a domestic market for shark meat that is supplied by the artisanal fisheries (Dent & Clarke 2015). Fishermen from these artisanal fisheries have reported observing reductions in abundance of sharks in their coastal waters (Bystrom & Cardenes-Valenzuela *in prep*). For a majority of the shark landings in these fisheries there is a lack of information on species-specific catch rates resulting in limited regulations on either direct or indirect takes of these animals (Bonfil 2003; Camhi *et al*. 2008; Holmes *et al*. 2009; Spaet *et al*. 2012).

Artisanal fishing communities inherently gain knowledge on the species they exploit as well as how their fishery impacts those species, which is known as traditional ecological knowledge (TEK) (Neis *et al*. 1999; Drew 2005; Hartley & Robertson 2009). Traditional ecological knowledge is defined as "a cumulative body of knowledge, practice and belief evolving by adaptive processes and handed down through generations by cultural transmission, about the relationships of living beings with one another and with the environment" (Berkes *et al*. 2000). It is specific to individual locations and represents the information necessary for the people of that area to survive (Drew 2005).

Within the context of fisheries, TEK can aid researchers by providing information on the presence and distribution of particular species (Poizat & Baran 1997) including; key habitats to vulnerable life stages (i.e. nursing grounds) (Aswani & Hamilton 2004) as well as spawning sites (Johannes 1981) for certain species. The communities surveyed in this study commonly catch sharks as either target species or bycatch and from these experiences they develop TEK, as well as perceptions about, sharks and the impact their fishery has on them (Hickey & Johannes 2002; Thompson & Mintzes 2002; Foster & Vincent 2010; Salas *et al*. 2011). By understanding the general knowledge and perceptions these communities have about sharks and their fishery (current population trends within it, reasons for targeting sharks) we can try to determine the types of management practices that are best suited for shark conservation in these communities (Drew 2005; Foster & Vincent 2010). Previous research has shown that an individual's knowledge and perceptions of a species can guide their behaviors towards them, including whether they would support conservation of that species (Kraus 1995; Kellert 1995; Thompson & Mintzes 2002; O'Bryhim & Parsons 2015). Perceptions of the fishery and the species being exploited within the community are of particular importance because if there is not a perceived imminent threat to the sustainability of that resource then implementing protective measures and enforcing them can prove difficult (Alpizar 2006). Therefore, it is critical to work with the local fishing communities to develop effective fisheries management plans and to understand how this might impact their culture and fishery if researchers are to combat reductions seen in fish stocks (i.e. sharks) (Drew 2005).

To understand TEK from a target population, like fishermen, and their perceptions toward a species and its exploitation, social surveys (questionnaires) have previously been employed. Surveys allow researchers to obtain sociological data from large groups that can range over a wide area (i.e. fishermen scattered along a coastline). Researchers have previously used surveys to determine human impacts on wild species, and how people's perceptions can influence ecological management (White *et al*. 2005). Therefore, surveys make it possible to quantify how the perceptions of a group might be affecting the implementation of conservation measures (White *et al*. 2005; Anadon *et al*. 2009; Foster & Vincent 2010). Information that can be acquired includes: the types of gear being used by fishermen; the target species of a particular fishery; and the species of sharks being caught in these fisheries (Neis *et al*. 1999; Drew 2005; Moore *et al*. 2010). This can elucidate which species are most susceptible to different fishing methods, the time of year in which certain shark species are most frequently landed in their fishery, and whether they would support potential changes to their fishery to better protect sharks (Neis *et al*. 1999; Drew 2005; Jones *et al*. 2008).

By better understanding the fishing communities, policy-makers can work with them to develop legislation that is not only effective, but also supported by the fishermen (Drew 2005; Campbell *et al*. 2007). The behaviors of these communities are what will ultimately impact whether conservation measures are successful or not. Surveys provide the ability to quantify potential behaviors that may be exhibited by the fishermen toward differing fisheries regulations based on their knowledge and perceptions (White *et al*. 2005; Jones *et al*. 2008). For instances, O'Bryhim & Parsons (2015) determined that a

persons knowledge of sharks can predict their potential behaviors toward shark conservation. Surveys can also be used to determine what variables may be causing the varying knowledge levels and perceptions within a fishing community toward a particular species and fishery (Barney 2005; Kellert 2008). Figuring out what causes changes in both perceptions and potential behaviors can determine what needs to be done for major shifts in management policies to occur (Kellert 2008). Therefore, we used a social survey to determine the knowledge, perceptions, and potential behaviors Costa Rican fishing communities have toward sharks and possible legislation that would better protect them in their fishery. We also attempted to identify the variables that cause changes in perceptions and behaviors toward legislation protecting sharks in Costa Rica. Better understanding of these communities and involving them in the legislation process increases our chances of addressing their concerns. In addition, they will be more likely to follow the regulations thus making the difficult task of enforcement of regulations on the open ocean easier. Gaining support and understanding from the fishing community is crucial if conservation measures to protect sharks in Costa Rica are going to work (Barker & Schluessel 2005).

Methods

Fishing Communities

To determine the knowledge, perceptions and potential behaviors local fishermen in Costa Rica have about their fishery, sharks, and their support for conservation measures toward sharks on their fishing grounds, we distributed a structured survey

(n=72) to three fishing communities along Costa Rica's Pacific coast (Ojochal/Cortez, Coyote/Bejuco, and San Juanillo) from June 2013 to September 2014 (Figure 1).

The Ojochal fishing community consists of a single family and several transient fishermen that utilize a portion of the mouth of the Sierpe river estuary, adjacent to the Terraba-Sierpe national wetland. The local family consists of ~5 fishermen gillnetting from 2 to 3 boats. Another ~5 to 10 fishermen also utilize this area, but are not local. They are transported to this location by a seafood vendor from Puntarenas, where they utilize his 5 boats. All landings from these fishermen are immediately packed onto a truck with ice, which will travel to Puntarenas every few days to offload. The mangrove forests of the Terraba-Sierpe national wetland are officially off limits for fishing and the fishermen are supposed to only utilize the waters outside the river mouth.

Cortez is fishing community adjacent to Ojochal along the Sierpe river mouth. This was once a thriving fishing community but recent declines in the total catch have seen its population dwindle. We found few fishermen left in this community and surveyed those that we could. Due to the relatively small number of fishermen within the Ojochal and Cortez communities and their close proximity to one another surveys from these two communities were combined to represent the overall area. Between the Ojochal and Cortez communities 10 individuals completed surveys, with the majority coming from Ojochal.

Coyote and Bejuco are two adjacent fishing communities that utilize the same fishing grounds along the Nicoya peninsula (Figure 1). These two communities include up to 100 fishermen utilizing ~40 boats depending on time of the year and success of the

fishery. Both the Coyote and Bejuco fishing communities have fishermen associations, the Coyote Fishermen Association (ASPECOY) and the Bejuco Fishermen Association (BEJUCO) respectively. Within the fishing grounds utilized by these two communities exist two marine protected areas (MPAs) that are managed by Costa Rica's Environmental Ministry (MINAET); the Caletas-Arío refuge and Camaronal National Wildlife Refuge (MINAE 2005). These MPAs restrict certain fishing methods, but allow the use of bottom-lines within the MPAs (MINAE 2005). However, the use of gillnets, which are restricted in these areas, were witnessed being used when landings from bottom-lines were low. Between the Coyote and Bejuco communities 35 fishermen completed surveys, representing all the individuals that were available during sampling trips.

The San Juanillo fishing community can also be found along the Nicoya peninsula north of Coyote and Bejuco communities. It consists of ~30 fishermen who are members of the San Juanillo Fishermen Association (ASOPESJU). The fishing grounds of San Juanillo were recently declared a "Marine Area for Responsible Fisheries" (AMPR), which regulates fishing activity within an area, specifically banning the use of shrimp trawls (Alvarado *et al*. 2011; Fargier *et al*. 2014). This new protected area designation, AMPR, was created in 2008 by the Costa Rica's fisheries management organization, INCOPESCA, and requires members of the fishing community to shift toward sustainable fishing methods to protect fish populations and the community's livelihood (Alvarado *et al*. 2011). INCOPESCA requires local fishing associations to apply for AMPR status, which includes a code of ethics by which the fishermen will follow to

obtain a sustainable fishery (INCOPESCA 2010). This code, which can regulate fishing methods, allowable sizes of fish, and total landings, is supposed to be enforced by the fishermen and INCOPESCA (INCOPESCA 2010). The majority (27) of the members of the San Juanillo Fishermen Association were surveyed in this study.

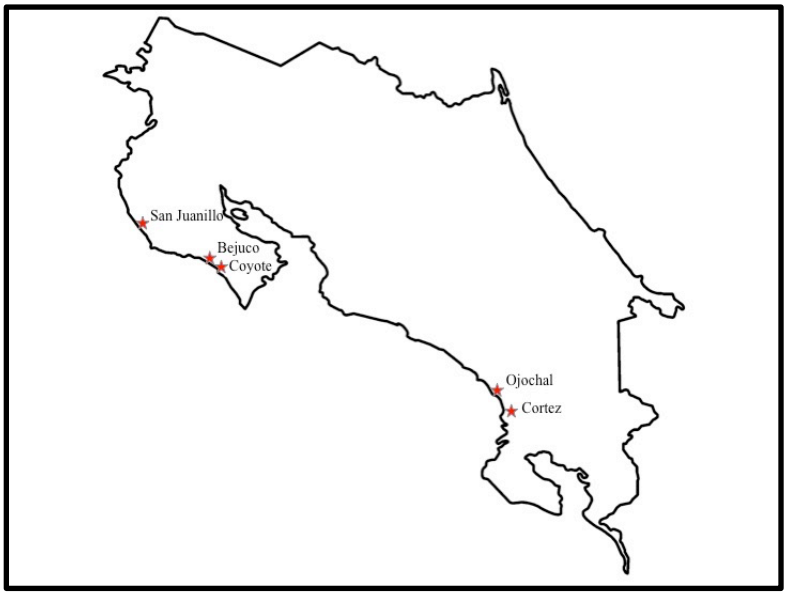


Figure 1. Map of Costa Rica indicating distribution locations of the social survey.

There were no incentives for taking the survey; it was completely voluntary. We used R statistical software (3.1.2) for all statistical analyses (R Development Core Team 2014). The survey instrument contained four sections separated *a priori* into the following categories: knowledge about sharks; perceptions of shark populations; potential behavior toward shark conservation; and participant demographics and fishing practices. Frequencies of responses for all questions were analyzed to determine variances between

the demographic make-up of the communities, perceptions of shark populations, and support for various conservation measures to protect sharks. Demographic and fishing pracice questions were treated as individual variables to analyze their impacts on potential behaviors, as well as perceptions and knowledge. For the categories knowledge about sharks, perceptions of shark populations, and potential behaviors towards shark conservation, we created indices using the respective questions from the survey instrument. We also analyzed frequencies of all responses within the demographic/fishing practices, knowledge, perceptions, and potential behavior categories.

The knowledge index consisted of 7 binary coded questions. Participants were also given the option of answering, "I don't know", which was scored the same as an incorrect answer. To create the knowledge index the responses on these questions were added together to give a total score out of 7. Participants' knowledge about sharks (knowledge index score) was based on the score they received with a higher score indicating more shark knowledge. However, the shark knowledge index proved to be internally unreliable (Chronbach's alpha[1]= -0.03) and, therefore, was not used in any further analyses.

The perception index (Chronbach's alpha = 0.56), based on 7 questions, was used to judge a participant's perceptions on the status of shark populations in Costa Rica, the impact of their fishery on shark populations, and whether they believed protective measures are needed to conserve them. Answers indicating a perception that some shark

[1] How well a set of items or variables measures a single uni-dimensional latent construct.

populations were not declining, and that protection was not needed, were scored with a -1, while fishermen who were not sure about a particular question and answered "I don't know" received a neutral score of 0. Fishermen that answered with a perception that some shark populations were declining and needed increased protection received a score of 1 or 2, depending on the question. Two possible positive scores were used for 2 of the 7 attitude questions showing varying levels of support for sharks. The score for each of the seven attitude questions were then added together for each surveyed fishermen to give them a total possible score from -7 to 9 with a higher score indicating the perceptions that shark populations are declining, due to negative impacts of their fishery, and greater protection is needed.

The potential behavior index (Chronbach's alpha = 0.72) was derived from 13 questions designed to measure a participant's potential behavior (i.e. support) toward legislation that would protect sharks in and around the areas that they currently fish. These included questions on the fishermen's potential willingness to support shark conservation legislation, the formation of marine protected areas (MPA's) near their fishing grounds, and whether they were willing to alter their fishing practices. The sums of the responses to these questions were used to create an index with a scale from -13 to 13 with higher scores representing the likelihood of more pro-shark conservation behavior (i.e. individuals more willing to support shark conservation in their areas).

Due to the knowledge index proving to be unreliable, it was not possible to test how demographic factors would impact knowledge about sharks, nor how knowledge about sharks may then impact perceptions and potential behaviors toward them. We

instead tested if the fishermen's perceptions of shark populations, as well as, varying demographic variables could directly impact their potential behavior toward shark conservation using a standard multivariate regression analysis. This approach was also used to test how various demographic variables (i.e. age, education, community) impacted perceptions of sharks and their fishery. We also used linear regressions to determine if individual variables could significantly predict potential behavior toward shark conservation or perceptions of shark populations. One-way analyses of variance (ANOVA) were used to compare demographic variability between communities, responses to individual questions and overall scores within the three indices between the three fishing communities. If responses between communities were significantly different we then used a Tukey's multiple comparison of means to determine which communities had significantly different answers.

Results

Demographics and Fishing Practices

Of the 72 fishers surveyed, all were male. The age of the fishermen ranged from 18 to 60 years, with a mean age of 37.6 years, for all three fishing communities combined. There was no significant difference between average ages between communities ($F=0.68$, $p=0.51$). The average education level was 6.4 years of schooling for all locations, with no significant difference between fishing communities ($F=1.29$, $p=0.28$). Fishermen from all surveyed communities had an average of 16 to 20 years of fishing experience, with bottom-lines being the most common fishing gear used for all communities (93%). However, significantly fewer fishermen in the Ojochal/Cortez

fishing community used bottom-lines compared to the other two ($F=13.23$, $p<0.001$). The Ojochal/Cortez fishing community also had more fishermen using hand-line fishing gear compared to the other communities ($F=4.07$, $p=0.02$). All fishermen responded that they fished within a 10km distance of shore, and averaged 31 to 40 hours of fishing effort per week. Fishermen of the San Juanillo community spent significantly less time fishing compared to the other communities ($F=6.70$, $p=0.002$). Overall, only 4% of fishermen indicated they directly targeted sharks, with no significant differences between communities ($F=1.78$, $p=0.18$). Of the shark species fishermen declared they encountered/caught, the smoothhound (*Mustelus spp*.) and scalloped hammerhead (*Sphyrna lewini*) were the two most commonly named by fishermen for each of the three fishing communities. However, fewer fishermen from Ojochal/Cortez indicated they had encountered/caught smoothhound compared to the other two communities ($F=4.34$, $p=0.02$). Fewer San Juanillo fishermen also reported catching sharpnose sharks (*Rhizoprionodon longurio*) than in the other fishing communities surveyed ($F= 8.63$, $p<0.001$). The majority of fishermen (86%) indicated they would retain captured sharks, with the majority selling their meat (85%) or keeping them for personal use (67%). Almost no fishermen surveyed (1%) kept sharks to remove and sell their fins. Average demographic and fisheries practices results can be found in Tables 1 and 2.

Table 1. Demographic information collected from artisanal fishermen from the San Juanillo, Coyote, Bejuco, Ojochal, and Cortez fishing communities in Costa Rica.

	Total (n = 72)	Ojochal/ Cortez (n = 10)	Coyote/ Bejuco (n = 35)	San Juanillo (n = 27)

Average age	37.6yrs	33.7yrs	37.5yrs	39.2yrs
Average years of education	6.4yrs	6.8yrs	5.9yrs	6.9yrs
Average years of fishing	16 to 20yrs	16 to 20yrs	16 to 20yrs	16 to 20yrs
Own their own boat	35%	40%	31%	37%

Table 2. Information on current fishing practices collected from artisanal fishermen from the San Juanillo, Coyote, Bejuco, Ojochal, and Cortez fishing communities of Costa Rica.

		Total (n = 72)	**Ojochal/Cortez (n = 10)**	**Coyote/ Bejuco (n = 35)**	**San Juanillo (n = 27)**
Fishing method					
	Hand-line	4%	20%	3%	0%
	Bottom trawl	1%	10%	3%	0%
	Gillnet	33%	50%	23%	41%
	Bottom-line	93%	60%	100%	96%
	Other	6%	40%	0%	7%
Distance from shore fished					
	0 – 10 km	100%	100%	100%	100%
Average hours spent fishing per week		31 – 40hrs	41 – 50hrs	31 – 40hrs	21 – 30hrs
Percentage of fisherman that regularly catch these shark species					
	Scalloped Hammerhead	86%	70%	89%	89%
	Silky	3%	10%	3%	0%
	Tiger	14%	20%	20%	4%
	Blue	1%	10%	0%	0%
	Sharpnose	25%	40%	40%	0%
	Blacktip	47%	50%	51%	41%
	Smooth-hound	88%	60%	91%	93%
	Bull	22%	40%	26%	11%
	Whitetip Reef	25%	10%	37%	15%
	Nurse	3%	10%	3%	0%
	Thresher	7%	10%	11%	0%

	Oceanic Whitetip	0%	0%	0%	0%
	Other	3%	0%	3%	4%
	Unsure of Species	3%	0%	0%	7%
What fishermen do with landed sharks					
	Keep the sharks	86%	90%	83%	85%
	Sell the meat	85%	90%	83%	85%
	Sell fins	1%	10%	0%	0%
	Personal use	67%	50%	63%	78%
Directly target sharks		4%	13%	0%	7%

Knowledge

The average number of correctly answered knowledge questions among the fishermen was 4.18 out of 7 questions in total. The average number of correct answers did not differ significantly between the three fishing communities ($F=2.07$, $p=0.14$). However, fishermen of the Ojochal/Cortez community answered correctly the question on shark reproduction significantly more often than the other two communities ($F = 4.07$, $p=0.02$) (Table 2). As noted above, the knowledge index proved to be unreliable and could not be used to represent fishermen's knowledge of sharks, to determine how this may impact their perceptions of shark populations or their potential behaviors toward shark conservation.

Table 3. Percentage (%) of respondents that answered the various knowledge question correctly or incorrectly.

	Total (n = 72)	**Ojochal/ Cortez (n = 10)**	**Coyote/ Bejuco (n =35)**	**San Juanillo (n =27)**
How many types/species of				

shark would you estimate there are in Costa Rican waters/					
	Correct	1%	0%	3%	0%
	Incorrect	99%	100%	97%	100%
Most Sharks will breed and reproduce within their first year of life like many other fish species?					
	Correct	25%	60%	20%	19%
	Incorrect	75%	40%	80%	81%
Sharks in general produce a small number (less than 20) of young per breeding season compared to other fish?					
	Correct	69%	70%	63%	78%
	Incorrect	31%	30%	37%	22%
Sharks are very susceptible to overfishing?					
	Correct	65%	60%	60%	74%
	Incorrect	35%	40%	40%	26%
Many of the shark species found in Costa Rica use its coastal waters for mating and raising their young?					
	Correct	97%	100%	94%	100%
	Incorrect	3%	0%	6%	0%
Sharks in general live for only a few years (less than 5) like many other fish species?					
	Correct	63%	70%	63%	59%
	Incorrect	37%	30%	37%	41%
Shark finning is banned in Costa Rica?					
	Correct	97%	100%	94%	100%
	Incorrect	3%	0%	6%	0%

Perceptions

Average score for surveyed fishermen's perceptions on the status of shark populations was 6.25 - on a scale from -7 to 9. Higher perception scores indicated fishermen with stronger beliefs that shark populations were in decline and that greater protection for sharks is currently needed. Perception index scores did not differ significantly between the three fishing communities ($F=0.28$, $p=0.76$). The majority of

fishermen from all three communities indicated they had encountered fewer sharks in their fisheries (83%) and believed that the numbers of sharks were decreasing (82%) in Costa Rican waters (Table 3). However, only 58% believed that too many sharks were currently being caught in the waters of Costa Rica. Of the fishermen surveyed, 89% believed it was important to protect sharks with 81% indicating that better laws were needed for their protection. Despite the vast majority of fishermen believing sharks needed to be protected, 57% of fishermen indicated that sharks were detrimental to their fishery. There was a significant difference between the Ojochal/Cortez and the Coyote/Bejuco communities on whether they believed sharks were helpful, or harmful, to their fishery ($F=3.267$, $p=0.044$) with, on average, more fishermen from Ojochal/Cortez indicating sharks were helpful to their fishery.

Table 4. The percentages (%) for respondent's perceptions toward sharks, shark conservation, and their fishery.

		Total (n = 72)	**Ojochal/ Cortez (n = 10)**	**Coyote/ Bejuco (n =35)**	**San Juanillo (n =27)**
Have you seen more, less, or the same number of sharks in your fishery in recent years?					
	Less	83%	80%	86%	82%
	Same	8%	0%	11%	7%
	More	3%	0%	3%	4%
	Don't Know	6%	20%	0%	7%
Do you think the number of sharks in Costa Rican waters is increasing, decreasing, or stable based on what you have observed in your fishery?					
	Decreased	82%	70%	83%	85%
	Stable	7%	0%	11%	4%
	Increased	5.5%	1%	6%	4%
	Don't Know	5.5%	2%	0%	7%

Do you think it is important to protect sharks?					
	Yes	89%	80%	89%	93%
	No	7%	0%	8%	7%
	Don't Know	4%	20%	3%	0%
Are laws to better protect sharks needed in Costa Rica?					
	Yes	81%	70%	83%	81%
	No	16%	10%	17%	19%
	Don't Know	3%	20%	0%	0%
Do you see sharks as being helpful or harmful to your fishery?					
	Helpful	35%	70%	20%	41%
	Harmful	57%	0%	3%	18%
	Don't Know	8%	30%	77%	41%
Do you think too many sharks are currently being caught in Costa Rica?					
	Yes	58%	60%	57%	59%
	No	22%	10%	23%	26%
	Don't Know	20%	30%	20%	15%
Do you think fishing for sharks should continue or that they should be protected?					
	Protect	86%	80%	97%	74%
	Fish	7%	0%	0%	19%
	Don't Know	7%	20%	3%	7

Potential Behaviors

On average, surveyed fishermen scored a 4.49, on a scale of -13 to 13, for their potential behaviors toward shark conservation in Costa Rica. Higher scores indicated potential behaviors more supportive of shark conservation. The potential behavior index scores were not significantly different between fishing communities ($F=0.57$, $p=0.57$). Almost all surveyed fishermen (97%) indicated they would be willing to potentially support shark conservation in Costa Rica (Table 4). However, support dropped to 67% if

the conservation measures resulted in any changes to their current fishing practices. The vast majority (86%) of fishermen indicated they would be more willing to potentially support shark conservation measures if fishing communities were included in the overall decision-making process. The same percentage (86%) also indicated they would be willing to work with researchers and public officials on shark conservation issues. When asked about the use of MPA's as a mechanism for shark conservation, 93% of fishermen indicated they would potentially support this. Though, as regulations for the MPA's became more intrusive on their fishing practices, potential support declined to a low of 6% when the proposed MPA was a year round, no take zone (Table 4). Overall, 82% of fishermen indicated the potential of attempting to stop landing specific shark species, with 71% potentially willing to stop landing all species of shark. When asked if they were possibly willing to use fishing gear that reduced the likelihood of catching or injuring sharks, 78% said they would if the new gear was provided to them with only 57% willing to if they had to purchase the gear on their own. There was no significant difference between each fishing community for all potential behavior questions except for whether they would be willing to stop catching all shark species. The Ojochal/Cortez community was significantly opposed to not catching any sharks, when compared to the other two communities. Results for all potential behavior questions can be found in Table 4.

Table 5. Percentage (%) of respondent's potential behaviors toward shark conservation.

	Total (N=72)	**Ojochal/ Cortez (N=10)**	**Coyote/ Bejuco (N=35)**	**San Juanillo (N=27)**
Would you be willing to				

support shark conservation in Costa Rica?					
	Yes	97%	100%	100%	93%
	No	3%	0%	0%	7%
	Don't Know	0%	0%	0%	0%
Would you still support shark conservation in Costa Rica if it resulted in you having to change some of your current fishing practices?					
	Yes	67%	40%	68.5%	74%
	No	28%	30%	28.5%	26%
	Don't Know	5%	30%	3%	0%
Would you support the formation of marine protected areas that would protect sharks?					
	Yes	93%	90%	97%	89%
	No	6%	10%	3%	11%
	Don't Know	0%	0%	0%	0%
Would you still support the formation of a marine protected area if it included portions of your current fishing grounds?					
	Yes	65%	50%	69%	67%
	No	34%	40%	31%	33%
	Don't Know	1%	10%	0%	0%
Would you still support the formation of a marine protected area if it restricted certain types of fishing gear?					
	Yes	39%	40%	37%	41%
	No	60%	50%	63%	59%
	Don't Know	1%	10%	0%	0%
Would you still support the formation of a marine protected area if it completely restricted all types of fishing in that area to only certain times of the year?					
	Yes	15%	20%	6%	26%
	No	84%	70%	94%	74%
	Don't Know	1%	10%	0%	0%
Would you still support the formation of a marine					

protected area if it restricted all types of fishing within the protected area during the entire year?					
	Yes	6%	10%	0%	11%
	No	93%	80%	100%	89%
	Don't Know	1%	10%	0%	0%
Would you be more likely to support legislation protecting sharks if fishermen were included in the decision making process?					
	Yes	86%	70%	86%	92%
	No	6%	0%	9%	4%
	Don't Know	8%	30%	5%	4%
If you had to purchase it, would you be willing to use a different type of fishing gear that is less likely to catch or injure sharks?					
	Yes	57%	40%	63%	56%
	No	35%	20%	34%	41%
	Don't Know	8%	40%	3%	3%
If you were provided it, would you be willing to use a different type of fishing gear that is less likely to catch or injure sharks?					
	Yes	78%	60%	83%	78%
	No	14%	0%	17%	15%
	Don't Know	8%	40%	0%	7%
Would you be willing to stop catching certain species of sharks?					
	Yes	82%	50%	89%	85%
	No	8%	10%	5.5%	11%
	Don't Know	10%	40%	5.5%	4%
Would you be willing to stop catching all species of sharks?					
	Yes	71%	10%	83%	78%
	No	19%	50%	11%	19%
	Don't Know	10%	40%	6%	3%
Would you be willing to work with researchers and public officials to help protect sharks?					

Yes	86%	80%	94%	78%
No	8%	0%	3%	19%
Don't Know	6%	20%	3%	3%

Indices model

The potential behavior of fishermen toward shark conservation could not be significantly predicted by the joint predictive power of their perceptions of sharks and their fishery, age, education, fishing experience, boat ownership, whether or not they used certain types of gear, distance from shore fished, hours fished per week, whether they targeted sharks, shark species landed, if they retained sharks and what they did with those sharks, nor which community they were from ($F=0.87$, $p=0.65$). However, a fisherman's perceptions of the status of shark populations could significantly predict his potential behavior when tested individually ($F=5.07$, $p=0.028$, $R^2=0.07$). With higher perception scores - that indicate they believed shark populations are declining and greater protection is needed for sharks - predicting potential behaviors more supportive of shark conservation. None of the demographic variables could significantly predict a fisherman's potential behavior toward shark conservation when tested individually. A fisherman's perception of sharks and their fishery could not be significantly predicted by the joint predictive power of age, education, fishing experience, boat ownership, whether or not they used certain types of gear, distance from shore fished, hours fished per week, whether they targeted sharks, shark species landed, if they retained sharks and what they did with those sharks, nor which community they were from ($F=1.03$, $p=0.47$). However, fishing experience ($F=4.05$, $p=0.05$, $R^2=0.05$), and whether or not they fished with

bottom-lines ($F=4.36$, $p=0.04$, $R^2=0.06$), could significantly predict perceptions of sharks and their fishery individually.

Discussion

Despite the geographical separation between the surveyed artisanal fishing communities, their demographic make-ups were quite similar. Overall the fishermen surveyed were of a similar age, education level, and had similar levels of fishing experience. Slight differences did exist between the species of sharks they caught, how long they spent fishing per week, and the percentage of fishermen within each community that used specific types of gear.

Fishermen surveyed from all three communities displayed an overall average knowledge about sharks and current shark legislation in Costa Rica. However, they lacked knowledge on the diversity of shark species in Costa Rica and some aspects of general shark biology. Many fishermen believed that sharks matured at a younger age - more similar to other teleost fish, which would allow them to potentially produce more offspring and, thus, help to replenish the population quicker. Believing that sharks can breed more frequently, allowing for greater population replacement, could potentially lead to the fishermen having the perception of their fishery having less of an impact on shark populations. Again, the knowledge index proved to be unreliable and further research would be necessary to better determine how knowledge of general shark biology could impact the perceptions of artisanal fishermen about the impact of the fishery on shark populations and their potential behavior toward shark conservation.

The vast majority of fishermen surveyed held the perception that shark populations in Costa Rica are declining and that protective measures are both needed and important for their conservation. However, despite the fact the majority indicated they have seen less sharks in recent years only about half of the fishermen indicated that their fisheries were catching too many sharks. These results could signify that nearly half of these fishermen do not view their fisheries as a significant contributor to the current shark population declines. Over half of the fishermen also perceived sharks as being harmful to their fishery in some way. Fishermen surveyed were not able to elaborate on how sharks would be harmful to their fishery, but perceived issues could include competition with fishermen for target species and damage to fishing gear caused by sharks. It is possible that artisanal fishermen do not perceive their particular fisheries as having a large enough impact on shark populations when compared to industrial fleets operating in Costa Rica. However, artisanal fishing communities employ substantially more fishermen than industrial fleets and are known to operate within shark nursing grounds (Pauley 2006; Clarke *et al*. 2011). Within Costa Rica, they have been found to catch substantial quantities of juvenile sharks like the endangered scalloped hammerhead (Chapter 3). The perception that their fisheries are not catching too many sharks could also be explained by their previously discussed misunderstanding of shark biology. If they believe shark populations have similar population growth patterns to other teleost fish they might not perceive the current exploitation rates of sharks by their fisheries as a problem. However, in this research no association was found between understanding that sharks mature later than other fish and the perception that shark population are declining and in need of

protection. Again, further research would be needed to determine if knowledge of shark biology could significantly impact fishermen's perceptions of shark populations.

Among the artisanal fishermen surveyed, increased fishing experience resulted in weaker perceptions that shark populations are in decline and that increased protection is needed. One would hypothesize that fishermen with more experience would be more likely to have witnessed declines in landings of sharks causing stronger perceptions that declines are occurring. However, this was not the case and could be the result of the older generation fishermen, with more experience, being less willing to admit that their fishery is having negative impacts on certain species. Alternatively, it is possible that there have been changes in the information being provided by schools and both governmental and non-governmental agencies. Thus, younger fishermen with less experience may have received different information that resulted in different perceptions about sharks. However, further research of these two groups would be needed to determine the cause of the differences in perception of shark populations.

The results from the multivariate regression analysis found that Costa Rican artisanal fishermen that perceived that shark populations were overfished and in decline, were more likely to potentially support new conservation measures to protect sharks in Costa Rica. Previous studies have found that attitudes, similar to perceptions, have the ability to guide, influence, direct, shape, or predict an individual's potential behavior towards a species and its conservation (Kraus 1995; Thompson & Mintzes 2002; Barney 2005). From this study it appears that we could predict that if fishermen do not perceive

an imminent threat to shark populations, and believe that some protective action needs to be taken, they will be less likely to support new conservation initiatives for sharks

Almost all fishermen surveyed indicated they would potentially support shark conservation in Costa Rica, including the potential use of Marine Protected Areas (MPAs). However, potential support for shark conservation and specifically the use of MPAs dropped dramatically as increasing restrictions to the fishermen's current fishing practices were introduced. This is despite that fact that two of the three surveyed locations (Coyote/Bejuco, San Juanillo) currently have some type of MPA within their fishing grounds. There was also no difference in support for various types of MPAs among the communities, despite protected areas currently existing in some of these locations. This is likely due to the fact that few restrictions currently exist within the protected areas of the surveyed communities and they are not strongly enforced. Therefore, it is almost as if these locations have no protected areas. In all communities surveyed, the more intrusive the proposed conservation measure was to their current fishing practices, the less potential support it garnered from fishermen, whether or not protected areas were currently present. This indicates that the fishermen surveyed would only potentially support conservation measures that do not substantively impact their current fishing practices. However, for conservation measures to be effective at protecting sharks, some changes to current fishing practices must occur. Therefore, a middle ground must be found between the fishermen and fisheries managers that will both garner support from the fishermen, but also provide an adequate amount of protection for shark populations. Based on the results from this survey, nearly half of the

fishermen were willing to potentially support restrictions on certain types of fishing gear within a proposed MPA. Restrictions on certain types of fishing gear could significantly reduce the number of sharks landed in these fisheries. Specifically banning the use of gillnets that catch fish indiscriminately, and generally do not allow for live release, could prove to be effective at reducing total shark landings. Currently, MPAs or "Marine Areas of Responsible Fisheries" exist at two of the three areas surveyed in this study. However, only the fishermen of the Coyote/Bejuco community, which are required to use only bottom-lines in the two MPAs within their fishing grounds, face any real restrictions on their current fishing practices. Despite the restrictions, it has been observed that if when using the required fishing method (bottom-lines) they do not meet necessary catch amount for subsistence, then many of the fishermen will start using gillnets (Arauz, personal communication, April 2013). Therefore, the challenge will be not only gaining support for new conservation measures but ensuring they are properly obeyed and enforced. The majority of fishermen surveyed also indicated they were willing to use different types of fishing gear that would be less detrimental to sharks, especially if the gear was freely provided. It should be noted that any fisheries managers attempting to regulate these fisheries must be careful not to implement regulations with too high a level of restrictions without the involvement and general consent from the communities. When increased restrictions on gear use and time limitations - how long and what time of year they could fish in an MPA - were introduced, they garnered almost no support. Agreement and support from local fishermen is of great importance if new conservation measures are to be successful. Enforcement of regulations within the marine environment

can prove to be difficult and expensive. By gaining support from these communities we can increase the chances that new conservation measures are followed with the possibility of these communities self- regulating, which reduces the need for expensive enforcement (Campbell *et al*. 2007). The vast majority of fishermen indicated they are potentially willing to stop catching sharks and are willing to work with researchers to come up with a way to accomplish that goal. Conservation measures must be developed that both limit the number of sharks being caught while not negatively impacting the economic value of these fisheries that these communities depend on to survive.

Conclusion

Developing effective legislation to protect the marine environment can be very difficult due to ineffective enforcement caused by the high price tag of attempting to patrol these areas. Methods of reducing enforcements costs and potentially increasing compliance among groups utilizing the protected resources (i.e. fish) is by including these groups in the decision making process and better understanding how they view their impact on the resource and their support for its conservation. Overall, support within the artisanal fishing communities in Costa Rica for new shark conservation measures was high. However, support declined as proposed legislation potentially obstructed the fishermen's ability to continue their current use of their fishing grounds. If effective legislation protecting sharks is to be created we must work with these fishing communities to develop management plans that would both protect sharks and cause minimal disruption to current fishing practices. Trying to enforce unsupported protective legislation on the approximately 3000 licensed artisanal fishers on Costa Rica's Pacific

coast would be ineffective. By working with the fishermen we increase the potential compliance of fishermen with the new legislation. Further educating the fishermen on the implications on the impacts of their fisheries and the potential benefits of new protective legislation is also important so they can make informed decisions about the direction of their fishery. Conducting sociological studies on artisanal fishing communities provides a valuable source of information on the current status of their fisheries and their willingness to work with conservationist to protect marine species. Implementation of social surveys, such as this one, can potentially increase the success of future conservation legislation and should be implemented in other areas (i.e. within Latin America) where small to mid-scale artisanal fisheries are important sources of revenue for a substantial portion of the population and are currently having substantially negative impacts on the marine environment.

FORENSIC SPECIES IDENTIFICATION OF ELASMOBRANCH PRODUCTS BEING SOLD IN COSTA RICAN MARKETS

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; Wallace *et al*. 2010; 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 nearly 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). 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. From 1986 to 2003, catch rates for hammerhead sharks (*Sphyrna spp.*) (89%), great whites sharks (*Carcharodon carcharias*) (79%), tiger sharks (*Galeocerdo cuvier*) (65%), thresher sharks (*Alopias spp.*) (80%), blue sharks (*Prionace glauca*) (60%), and mako sharks (*Isurus spp*.) (70%) all declined significantly in the Northwest Atlantic (Baum *et al*. 2003). Blue shark (>50%) and oceanic whitetip shark (*Carcharhinus longimanus*) (90%) catches rates in longline fisheries in the Pacific Ocean also declined from 1996 to 2009 (Clarke *et al*. 2013). While in the Mediterranean,

smooth hammerhead (*Sphyrna zygaena*), blue, mako, porbeagle (*Lamna nasus*), and common thresher shark (*Alopias vulpinus*) catch rates declined by 96 to 99.99% in the (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 & Myers 2004). However, by the 1990's they had dropped to only 2% of the total catch (Baum & 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 & 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, 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). Even though they are generally not the primary target species of pelagic fisheries, sharks account for 15% of reported landings (Trujillo *et al*. 2012). Sharks are typically caught as bycatch and 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). Of particular concern in these fisheries and others globally, is documenting the catch rates of highly exploited and endangered species like the scalloped hammerhead (*Sphyrna lewini*) and silky shark (*Carcharhinus falciformis*). Scalloped hammerheads are listed as globally 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 are the most commonly caught shark species in Costa Rican long-line fisheries (Dapp *et al*. 2013). However, due to reductions in catch rates from both target and non-target fisheries silky sharks have been listed as near threatened globally and vulnerable in the Eastern Tropical Pacific (Dulvy *et al*. 2008). 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).

In 1991, sharks accounted for 27% of the total catch in Costa Rican fisheries, but by 2003 they only made up only 4.9% of the total catch (Arauz *et al*. 2004). Overall shark landings have also decreased by 72% in Costa Rica from 12,901 tonnes in 2000 to 3,635 tonnes in 2011 (Dent & Clarke 2015). Catch rates for pelagic sharks in Costa Rica have declined by 60% from 1991-2000, while catch rates for two commercially important and threatened species, the scalloped hammerhead and silky shark, have experienced declines of approximately 90% and 80% respectively (Arauz 2000; Arauz *et al*. 2004; Whoriskey *et al*. 2011). On a larger scale, silky shark populations in the western and central Pacific are estimated to have declined by ~70% (Rice & Harley 2013). The declines in shark catch rates highlight the current threat to pelagic shark populations in Costa Rica from continued and increasing fishing pressure.

The Costa Rica Fisheries and Aquaculture Institute (INCOPESCA) is the

governing body responsible for the implementation of all policies concerning marine fisheries management and aquaculture in Costa Rica (Alpizar *et al*. 2006). To combat recent declines in shark populations due to shark finning, a law was passed in 2005 that required all sharks landed at Costa Rican docks to have fins attached (Whoriskey *et al*. 2011). However, this shark finning law lacked enforcement by the Costa Rican government (Whoriskey *et al*. 2011).

Costa Rica is also a member state of Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES), which regulates the trade of endangered or threatened species between countries (Vincent *et al*. 2013). Current sharks listed under Appendix II include: the whale shark (*Rhincodon typus*), great white shark, basking shark (*Cetorhinus maximus*), porbeagle shark, spiny dogfish (*Squalus acanthias*), oceanic whitetip shark, and the scalloped hammerhead shark (Vincent *et al*. 2013). Species listed under Appendix II require export permits in order to regulate their trade (Vincent *et al*. 2013). Article II paragraph 2 of the convention effectively leads to several other species of shark also having the trade of their products regulated due to being "look-alike" species of the scalloped hammerhead shark (Vincent *et al*. 2013). These include the great hammerhead shark (*Sphyrna mokarran*), smooth hammerhead shark, sandbar shark (*Carcharhinus plumbeus*), and the dusky shark (*Carcharhinus obscurus*)(Vincent *et al*. 2013). However, even if a species is listed in an Appendix, Parties can submit reservations to the listing effectively making them a non-Party of CITES for that species, and exempt from these trade restrictions (Vincent *et al*. 2013).

Regional Fisheries Management Organizations (RFMO's) are tasked with managing high seas, straddling and highly migratory fish stocks, with several of them taking steps to provide better protection for sharks in international water (Vincent *et al*. 2013). The International Commission for the Conservation of Atlantic Tunas (ICCAT) and the Inter American Tropical Tuna Commission (IATTC) are responsible for international waters extending out past Costa Rica's Atlantic and Pacific coasts respectively. The ICCAT has adopted several measures for protecting sharks landed in the fisheries they manage including: prohibiting the retention of silky sharks, bigeye thresher sharks (*Alopias superciliosus*), oceanic whitetip sharks, and all hammerhead sharks (*Sphyrna spp.*) in their fisheries and trying to release them alive when possible. The IATTC also prohibits the retention of oceanic whitetip sharks, but a similar mandate recently voted on to protect silky sharks did not pass due to lack of support - specifically from Costa Rica (IATTC Resolution C-11-10 & C-15-04; IATTC 2015; Project Aware 2015).

Sharks in Costa Rica also receive secondary protection from the Cocos Island National Park and the Marine Seamount Management Area (Bessudo *et al*. 2011). The Cocos Island National Park was established in 1978 and is the second largest marine protected area (MPA) in the Eastern Tropical Pacific (ETP) region and is a no-take zone for all marine species, thus protecting 1989 km^2 of ocean water surrounding the island (MarViva 2011). The Marine Seamount Management Area was established in 2011 as an extension of the Cocos Island National Park and partially protects an additional 9640 km^2 (MarViva 2011). The management area only prohibits industrial and semi-industrial

shrimp trawls, purse-seining for tuna, and petroleum exploration and exploitation, while still allowing long-lining and sports fishing (PRETOMA 2011). However, there are still major concerns over illegal fishing, shark finning, and an overall lack of enforcement for both the Cocos Island National Park and the Marine Seamount Management Area due to remoteness of these areas and the limited manpower to monitor them (Alpizar 2006; Baum *et al*. 2007). Costa Rica also has several MPAs throughout its coastal waters, but few have restrictions on fishing practices (Arauz *et al*. 2004). Even with this limited amount of protection, there are still no catch limits or size restrictions on sharks landed in Costa Rican waters (Whoriskey *et al*. 2011).

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, 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 & 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 and will then show 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 on the correct

taxonomic identifications of the reference sequences entered into the library (Dudgeon *et al*. 2012). Methods using several different protein-coding genes from the mitochondrial genome have been established for DNA barcoding of sharks with the cytochrome oxidase subunit I (COI) being the most commonly used (Ward *et al*. 2005; Ward *et al*. 2008; Naylor *et al*. 2012). However, the NADH dehydrogenase subunit 2 (NADH2) is another commonly used gene that is one of the fastest evolving of the 13 mitochondrial protein-coding genes, which can be better for distinguishing between closely related species compared to COI (Hoelzel *et al*. 2001; Broughton & Reneau 2006; Naylor *et al*. 2012). The NADH2 gene (~1044bp) is also longer than the COI gene (~650bp), allowing for more resolution when distinguishing between species (Moore *et al*. 2011). DNA barcoding methods can help reduce mis-identifications, with the identification of individual pieces of sharks (e.g. fins, meat), and help alleviate the issues of broadly categorized fisheries data (e.g. all species simply labeled shark) for fisheries managers (Tillett *et al*. 2012).

It was possible that current fishery catch data for sharks landed in Costa Rican pelagic fisheries were incorrect and that many shark products being sold in markets were being mislabeled. It is likely that species of conservation concern, such as the scalloped hammerhead, were being caught more often than was reported. Our objective, therefore, 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 composition 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).

Methods

Sample Collection

We collected 833 shark and ray 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 (Figure 2). 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 vertebrate), while ray meat is generally sold as fillets. For each sampling trip (day) a single sample of each of the available cuts of elasmobranch products was taken at each pescadaria. This was done to reduce the possibility of sampling the same individual shark more than once. In some instances tissue samples were collected from whole sharks that had yet to be processed into smaller cuts. Samples from the different cuts of shark or ray meat were taken using sterile 8mm biopsy punches. Shark and ray tissue were then stored in RNAlater and kept at -4◦ C.

Figure 2. Map of Costa Rica indicating locations of the San Jose and Heredia markets.

DNA Barcoding

We extracted total genomic DNA from the tissue samples using the Qiagen DNeasy Tissue Kit, following protocols recommended by the manufacturer. An approximately 1050bp of the NADH dehydrogenase subunit 2 (NADH2) gene were amplified for species identification using the ASNM and ILEM primer combination described in Naylor *et al*. (2012). Polymerase chain reaction (PCR) amplifications were conducted within a total volume of 25µl containing 10mM Tris pH 8.4, 50mM KCL, 0.2 mM each dNTP, 1.5mM $MgCl_2$, 0.4µM each primer, 1 U Amplitaq Gold Polymerase® (Life Technologies), and 4 µl of template DNA. The PCR thermal cycling profile was: 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 on 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. Cycle sequencing reactions were conducted 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.

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 the variation in species abundance and

composition among seasons, pescadarias (fish vendors) sampled, and the San Jose and Heredia markets we used an analysis of similarity (ANOSIM). In cases where species abundance and composition were 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.

Results

Species Identification

Of the 833 samples tested, 722 (401 full-length consensus sequences, 321 reverse sequences) resulted in positive species identifications. Overall, nine species of shark and one ray were represented in the samples (Table 5). Silky sharks accounted for the vast majority (77%) of samples tested followed by the longtail stingray (*Dasyatis longa*) (11.6%). Two species of hammerhead shark (scalloped and smooth) were also found in the markets making up 3.6% and 1.8% of the samples, respectively. The pelagic thresher (*Alopias pelagicus*) (2.9%) and blacktip (*Carcharhinus limbatus*) (1.8%) were the only other two shark species to account for more than 1% of the samples tested. However, the whitenose shark (*Nasolamia velox*), - which is considered uncommon to rare in the eastern tropical Pacific and is listed as data deficient by the IUCN - was also recorded (Ruiz *et al*. 2009).

Table 6. Percent (%) of each elasmobranch species being sold as bolillo (shark) or raya (ray) in local markets (San Jose and Heredia) in Costa Rica.

		Markets	
Species	**Total (n=722)**	**San Jose (n=516)**	**Heredia (n=206)**
Pelagic thresher shark (*Alopias pelagicus*)	2.9	0.2	9.7
Silky shark (*Carcharhinus falciformis*)	77	79.8	69.9
Dusky Shark (*Carcharhinus obscurus*)	0.4	0.6	-
Sicklefin smooth-hound (*Mustelus lunulatus*)	0.4	0.4	0.5
Blacktip shark (*Carcharhinus limbatus*)	1.7	0.2	5.3
Whitenose shark (Nasolamia velox)	0.3	-	1
Pacific sharpnose shark (*Rhizoprionodon longurio*)	0.1	0.2	-
Scalloped hammerhead shark (*Sphyrna lewini*)	3.6	4.8	0.5
Smooth hammerhead shark (*Sphyrna zygaena*)	1.8	1.7	1.9
Longtail stingray (*Dasyatis longa*)	11.8	12	11.2

Seasonal Variation

Shark species composition varied significantly among seasons (Table 6; Figure 3) ($R = 0.2246$, $p = 0.001$). However, based on the low R-value obtained by the ANOSIM analysis very little of the variation in species composition is explained by season. 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. Smooth hammerheads were not fund to be

present in the spring and pelagic threshers were not found in the winter. The remaining species account for very few of the samples and there omission from certain seasons may be due to their rarity in the markets.

Table 7. Percent (%) of shark species identified in each season.

Species	Fall (n=140)	Winter (n=111)	Spring (n=229)	Summer (n=156)
Pelagic thresher shark (*Alopias pelagicus*)	4	-	3	5
Silky shark (*Carcharhinus falciformis*)	81	91	89	88
Dusky Shark (*Carcharhinus obscurus*)	-	-	-	2
Sicklefin smooth-hound (*Mustelus lunulatus*)	1	-	1	-
Blacktip shark (*Carcharhinus limbatus*)	5	1	1	1
Whitenose shark (Nasolamia velox)	-	-	<1	1
Pacific sharpnose shark (*Rhizoprionodon longurio*)	-	-	<1	-
Scalloped hammerhead shark (*Sphyrna lewini*)	4	6	5	1
Smooth hammerhead shark (*Sphyrna zygaena*)	5	2	-	3

Variation among pescadarias and markets

Overall there was no difference in species composition between the two markets (R=0.1199, p=0.053, Table 5), but there was a difference among pescadarias (R=0.1141, p=0.002, Table 7). However, both variables explain a similar, and small, amount of the variation. There were some notable differences. For example, the pelagic thresher, which appears in relatively high abundance in the Heredia market, was never found in the San

Jose market. The blacktip shark was also in overall higher abundance at the Heredia, while the silky shark was more common at the San Jose market. More differences are apparent at the level of the pescadaria (Figure 4). It is clear that while some species (silky shark) are sold at every pescadaria, others are clearly associated with specific vendors. For example, the majority of scalloped hammerheads identified in the markets, which were only found at two of the fifteen pescadarias, were from the pescadaria "Caracol" (Figure 4). Similarly the smooth hammerhead was also identified in two pescadarias, with "Caracol" again accounting for the majority of these samples (Figure 4). Almost all identified pelagic thresher sharks were also almost exclusively from the pescadaria "Pulpo" (Figure 4). Blacktip sharks were most commonly observed in the pescadaria "Pulpo", where it accounted for 16% of samples tested while this species never accounted for over 5% in any other pescadaria. Species abundance was also most evenly distributed in the pescadarias "Caracol" and "Pulpo".

Table 8. Percent (%) of shark species identified at each pescadaria.

	Pescadarias in the San Jose Market										Pescadarias in the Heredia Market				
Species	**Bagre (n=49)**	**Caracol (n=79)**	**Corvina (n=48)**	**Despensia (n=40)**	**Dolfin (n=44)**	**Dorado (n=51)**	**Galapagos (n=41)**	**Malecon (n=17)**	**Marisco (n=42)**	**Rey (n=42)**	**Dos Mares (n=39)**	**Marina (n=40)**	**Nino (n=10)**	**Pulpo (n=51)**	**Unica (n=43)**
Pelagic thresher shark (*Alopias pelagicus*)	-	-	-	-	-	-	-	-	-	2	-	-	-	39	-
Silky shark (*Carcharhinus falciformis*)	100	54	98	100	100	100	93	100	100	98	82	100	90	45	93
Dusky Shark (*Carcharhinus obscurus*)	-	4	-	-	-	-	-	-	-	-	-	-	-	-	-
Sicklefin smooth-hound (*Mustelus lunulatus*)	-	-	-	-	-	-	5	-	-	-	3	-	-	-	-
Blacktip shark (*Carcharhinus limbatus*)	-	-	-	-	-	-	2	-	-	-	3	-	-	16	5
Whitenose shark (Nasolamia velox)	-	-	-	-	-	-	-	-	-	-	3	-	-	-	2
Pacific sharpnose shark (*Rhizoprionodon longurio*)	-	-	2	-	-	-	-	-	-	-	-	-	-	-	-
Scalloped hammerhead shark (*Sphyrna lewini*)	-	30	-	-	-	-	-	-	-	-	-	-	10	-	-
Smooth hammerhead shark (*Sphyrna zygaena*)	-	11	-	-	-	-	-	-	-	-	10	-	-	-	-

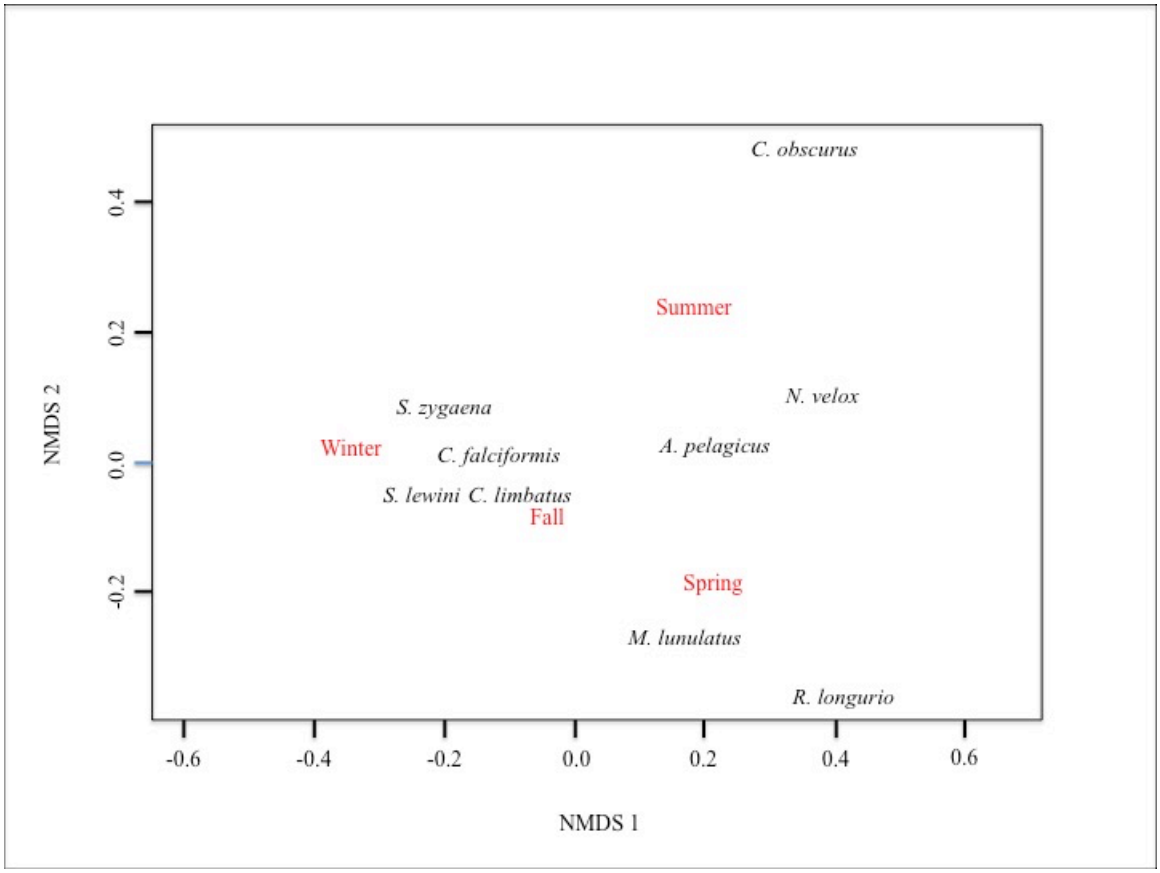


Figure 3. Non-metric multidimensional scale (nMDS) of shark species identified in the markets and their relationship to the sampling seasons. The closer a shark species is to a season the stronger relationship that species has with that season (i.e. more abundant in that that season than others).

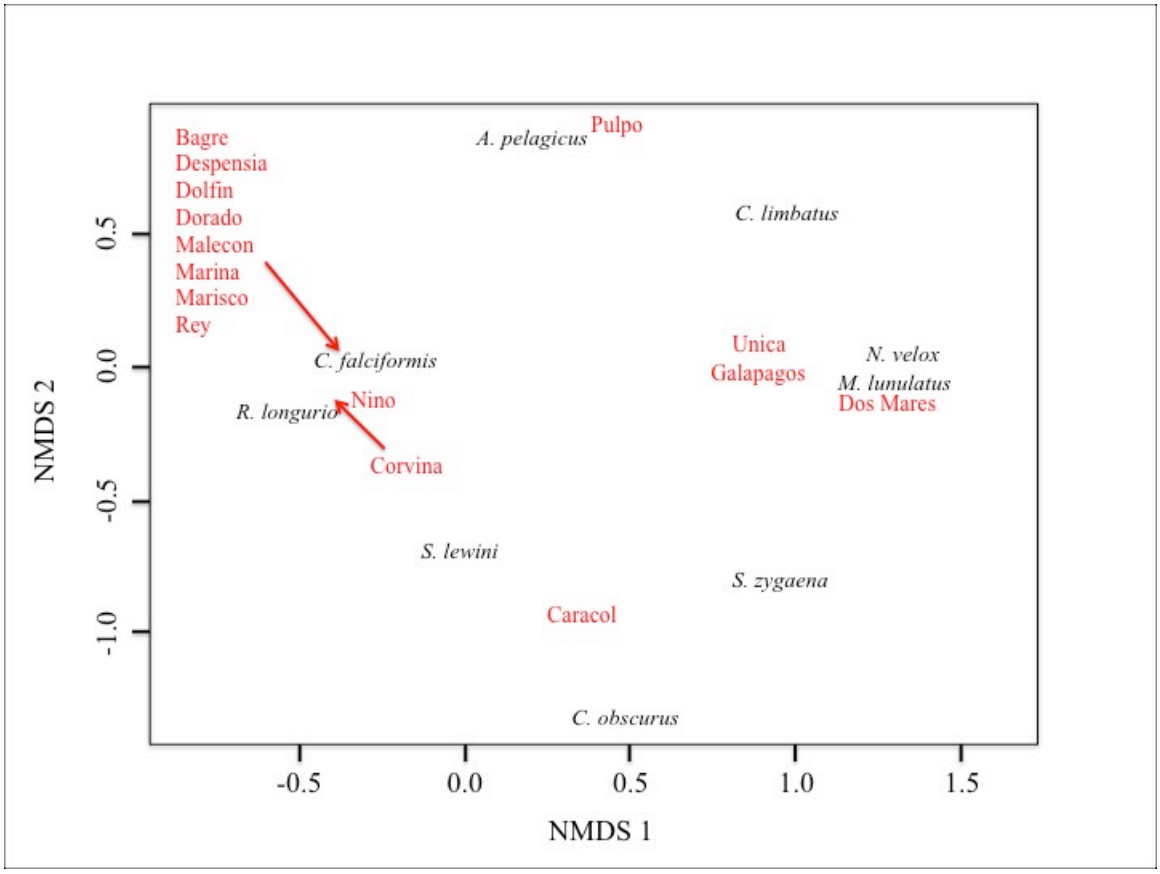


Figure 4. Non-metric multidimensional scale (nMDS) of shark species identified in the markets and their relationship to the various pescadarias (fish vendors). The closer a shark species is to a pescadaria the stronger relationship that species has with that pescadaria (i.e. more abundant in that pescadaria than others).

Discussion

Overall, 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.

Variation across seasons, markets and pescadarias

We found much more variation in shark species composition across markets and pescadarias than across seasons. Although these variables did not explain a large proportion of the variation there are some striking differences. As noted above, silky sharks dominated the market and in fact they made up 100% of the samples for seven pescadarias and ≥90% for five others. What is intriguing is that all seven of the pescadarias selling 100% silky shark and three out of the five selling ≥90% silky shark 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 have a darker coloring than 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. The large abundance of silky shark meat potentially supports the idea that most pescadaria owners and their customers prefer it and that the pescadarias of the larger San Jose market can better afford this species. 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.

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 the less desirable lower valued shark species that the pescadaria owners in San Jose don't want.

Species specific findings

Though we identified nine species of sharks, a vast majority (77.2%) 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 account for similar if not larger proportion of the overall shark landings now and 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 (Porras 1996). Ward & Myers (2005) estimated that the silky shark populations have declined in abundance and biomass by ~90%, resulting in them being listed as near threatened globally and vulnerable in the Eastern Tropical Pacific (Dulvy *et al*. 2008). The current status of silky shark populations along with the continued high fishing pressure they are experiencing makes Costa Rica's recent refusal to support the IATTC prohibition on the retention of silky sharks all the more concerning for the fate of this species in the eastern tropical Pacific (IATTC 2015; Project Aware 2015).

The longtail stingray accounted for the second highest proportion of elasmobranch species found in the markets and 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 elasmobranch 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 why longtail stingrays would be retained while other stingrays are discarded. However, through the fall of 2013 and into the winter, spring and summer of 2014 more pescadarias (fish vendors) began selling ray meat. More years of data would be needed to determine if this result is due to natural fluctuations in abundance or whether it indicates a new and 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.

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 & 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, the pelagic thresher shark only accounted for 2.9% of all elasmobranch species sampled, however, it did make up a relatively large portion (11%) 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. Though, even in the Heredia market, it was only sampled from two pescadarias and 95% of the samples came from a single vendor. The lack of pelagic thresher samples in the San Jose market may reflect changes in catch rates or in value. As noted above, the silky shark dominated the markets and seven of the fifteen

pescadarias sold exclusively silky shark meat. It is possible that silky shark has higher value and that most pescadarias are avoiding other species. 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.

Two hammerhead species, the scalloped and smooth hammerhead, were both found within the market samples. Although accounting for relatively low abundance of elasmobranch samples, the current conservation status, "endangered" and "vulnerable" respectively, and trade regulations, CITES Appendix II, makes any documentation of landings of these species important. As stated previously, Costa Rica does not record species-specific catch data for sharks so any information on possible exploitation rates of these species by pelagic fisheries is useful. Previous studies 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). The hammerhead sharks found in the markets were also not equally distributed among the pescadarias sampled. As mentioned above, one pescadaria, "Caracol", in the San Jose market accounted for the majority of hammerhead sampled identified. The reasoning behind why this particular pescadaria sold more hammerhead shark products 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.

Conservation implications

This study represents the first species identification of elasmobranch products in Costa Rican markets using DNA barcoding. Our findings further verified previous

observer data of pelagic fisheries that silky sharks are the most highly exploited elasmobranch species in Costa Rican waters. However, despite current conservation concern for this species, Costa Rica objected to new regulations at the 2015 annual meeting of the IATTC that would have protected silky sharks by requiring them to be released when landed as bycatch in pelagic fisheries. Based on our findings, continued fishing pressure of silky sharks at the current level could have severe consequences for the sustainability of this species populations into the near future. The endangered scalloped hammerhead was also found in the Costa Rican markets, but at levels far below that of silky sharks. However, due to their current conservation status, any landings of scalloped hammerheads 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 that could indicate further population declines and need for stronger protection.

When comparing species composition between the Costa Rican markets and data from observer programs on longline vessels in Costa Rican waters, we see that fewer elasmobranch species are actually sold in markets than are landed by the fishing vessels. This is likely due to low market value for many of the other elasmobranch species landed, resulting in the lower value species being discarded. Therefore, the DNA barcoding of markets 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 to provide a more accurate representation of all species of elasmobranch currently under fishing pressure in Costa Rica and if certain species are beginning to be targeted more. 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. Costa Rican pelagic fisheries and open-air markets utilize a variety of different elasmobranch species including ones that are currently overexploited and threatened with extinction. Therefore, the Costa Rican government needs to better monitor their pelagic fisheries and markets by collecting species-specific data and DNA barcoding can be an effective technique at achieving this goal.

FORENSIC SPECIES IDENTIFICATION OF ELASMOBRANCH BEING CAUGHT IN COSTA RICAN ARTISANAL FISHERIES

Introduction

Recent declines in shark populations are linked to intensified directed shark fisheries and larger numbers of bycaught sharks (Abercrombie *et al*. 2005; Wallace *et al*. 2010; Dulvy *et al*. 2014). Increased fishing pressure on sharks has ultimately led to a 7.5% global decrease in catch rates for all shark species from 1997 to 2010 (Worm *et al*. 2013). However, many species have experienced declines much greater than this. For example, catch rates for the smooth hammerhead (*Sphyrna zygaena*), blue (*Prionace glauca*), mako (*Isurus spp*.), porbeagle (*Lamna nasus*), and common thresher shark (*Alopias vulpinus*) all declined by >95% in the Mediterranean (Ferretti *et al*. 2008). While great whites (*Carcharodon carcharius*), tigers (*Galeocerdo cuvier*), blues, makos, hammerheads (*Sphyrna spp*.), and thresher sharks (*Alopias spp*.) experienced declines between 60 to 89% from 1986 to 2003 in the Northwest Atlantic (Baum *et al*. 2003). Oceanic whitetip sharks (*Carcharhinus longimanus*) have also experienced catch rate declines of 90% in pelagic long-line fisheries in the Pacific Ocean (Clarke *et al*. 2013). Current catch rate and population declines have resulted in one quarter of the over 500 shark species being listed as "vulnerable", "endangered", or "critically endangered" by the International Union for Conservation of Nature (IUCN) (Dulvy *et al*. 2014).

Within the global market for shark products, Costa Rica represents one of the largest producers in Central America (Dent & Clarke 2015). Currently, Costa Rica is the sixth largest exporter of shark meat in the world, and is the main supplier to the growing shark meat market in Mexico (Dent & Clarke 2015). Costa Rica is also an important exporter of shark fins, representing the eighth largest exporter in fin volume from 2000 to 2011, and is a key trading post for the shark fishing fleet in Central America (Dent & Clarke 2015). The growing market for shark products (fins and meat) has resulted in catch rates for pelagic sharks in Costa Rica to decline by 60% from 1991-2000, and overall landings for sharks declined by 72% from 2000 to 2011 (Arauz *et al*. 2004; Dent & Clarke 2015). However, a domestic market for shark meat in Costa Rica also exists and is supplied by artisanal fisheries that could be having significant impacts on shark populations (Dent & Clarke 2015).

In Latin America, small-scale to mid-scale artisanal fisheries are an important source of food and employment for local communities, providing the main basis of revenue for ~2 million people (Salas *et al*. 2007). In Costa Rica, these fisheries play a significant role in creating employment for coastal areas and are the main source of marine products for domestic consumption (Salas *et al*. 2011). In fact, approximately 60% of all marine organisms landed in Costa Rica come from these artisanal fisheries (Salas *et al*. 2011). Artisanal fisheries in Costa Rica can be broken up into three categories: small-scale, mid-scale, and advanced (Salas *et al*. 2011). Most Costa Rican artisanal fishing boats fall under the small-scale category with over 3,000 licensed vessels in the year 2000 (Salas *et al*. 2011). The mid-scale category follows with ~500 licensed

boats, but many more unlicensed vessels exist (Salas *et al*. 2011). Fishing licenses in Costa Rica theoretically give fishermen the right to exploit any marine resource they choose (Salas *et al*. 2011). The majority of these small- and mid-scale artisanal boats in Costa Rica can be found along the Pacific coast operating out of larger ports such as Puntarenas, Quepos, Playa del Coco, and Golfito (Sancho 2000; Li 2002). Artisanal small-scale and mid-scale fishermen generally stay near the coast and use hand-lines, gillnets, and shorter long-lines (i.e. bottom-lines, mid-water)(Salas *et al*. 2011). The less common artisanal advanced fisheries use large long-lines (surface and mid-water) targeting game fish like mahi-mahi, swordfish, tuna, and large pelagic sharks and contribute a majority of the sharks sold to overseas vendors and sold in large markets throughout Costa Rica (Salas *et al*. 2011). Based on forensic identification of shark products sold in markets in and near San Jose, silky sharks (*Carcharhinus falciformis*) make up a majority of the sharks landed in these fisheries, but endangered scalloped hammerheads (*Sphyrna lewini*) as well as pelagic threshers (*Alopias pelagicus*) were commonly found (Chapter 3).

To combat potential overexploitation of their marine resources, including sharks, in 1948 Costa Rica put in place fisheries laws to regulate and stimulate its fisheries (Salas *et al*. 2011; Trujillo *et al*. 2012). However these laws were seen as unconstitutional by fishermen and challenged in court, causing long lapses in fisheries management in Costa Rica (Salas *et al*. 2011). Despite recent revisions to these laws in 2005 to combat complaints from fishermen, there are still issues limiting their application by the governing body INCOPESCA (the Costa Rica Fisheries and Aquaculture Institute)(Salas

et al. 2011). INCOPESCA is responsible for the implementation of all policies concerning marine fisheries management and aquaculture in Costa Rica (Cajiao-Jimenez 2003; Alpizar *et al*. 2006). INCOPESCA has been inefficient at regulating and enforcing catch rates of many species including sharks, due to small budgets and a lack of qualified personnel, resulting in non-compliance by many fishermen (Salas *et al*. 2011). In particular, sharks currently have no catch limits or size restrictions and there is a lack of species-specific catch data to help better manage these species (Camhi *et al*. 2008; Whoriskey *et al*. 2011; Trujillo *et al*. 2012). In 2005, Costa Rica passed a law that required all sharks landed at their docks to have fins attached (Whoriskey *et al*. 2011). However, this shark finning law lacked enforcement by the Costa Rican government (Whoriskey *et al*. 2011). However, despite current legislation, artisanal fishermen have reported observing reductions in the abundance of sharks in Costa Rica's coastal waters (Bystrom & Cardenes-Valenzuela *in prep*). Overall, Costa Rica has also experienced decline in all fisheries landings since the mid-1980's, which can be attributed to the general overexploitation of marine resources (Salas *et al*. 2011).

In addition to INCOPESCA, marine resources in Costa Rica are also provided some protection through other avenues. For example, MINAET (Ministry of Environment, Energy and Telecommunications) is a Costa Rican governmental organization tasked with protecting marine species. In particular MINAET is responsible for marine protected areas (MPA's) in Costa Rica, the largest of which is Cocos Island National Park: a 1989 km^2 no take zone surrounding the island (Bessudo *et al*. 2011; MarViva 2011; Salas *et al*. 2011). Costa Rica also has several other MPA's protecting its

coastal waters, but few if any have restrictions on fishing practices or catch limits for sharks (Arauz *et al*. 2004). In addition to national organizations, Costa Rica is also a member state of the Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES), which regulates the trade of endangered or threatened species between countries (Vincent *et al*. 2013). Currently seven species of sharks are listed on Appendix II of this international agreement, putting regulatory conditions on the export of their products (Vincent *et al*. 2013). However, most of the small-scale and mid-scale artisanal fisheries in Costa Rica do not fall under CITES regulations because landed sharks are not exported due to inadequate preservation techniques for the meat (Dent & Clarke 2015). Costa Rica is also a member state of the International Commission for the Conservation of Atlantic Tunas (ICCAT) and the Inter American Tropical Tuna Commission (IATTC), which are responsible for international waters extending out past Costa Rica's Atlantic and Pacific coasts respectively. These groups are tasked with managing straddling and highly migratory fish stocks on the high seas (Vincent *et al*. 2013). However, their regulations do not extend to coastal fisheries and thus do not apply to most artisanal fishery operations.

A major barrier to establishing catch limits, and properly managing shark populations, is a lack of accurate species-specific extraction rates. Sharks landed in Costa Rica generally are not recorded to species level, or have had distinguishing characteristics removed, making accurate identification almost impossible (Camhi *et al*. 2008; Salas *et al*. 2011; Whoriskey *et al*. 2011; Trujillo *et al*. 2012). This problem is not unique to Costa Rica and to acquire species-specific catch data on sharks there is an increasing amount of

literature on the identification of sharks and their products (e.g. fins) using 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 & 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) of sequences of previously identified species and will then show the likelihood of that organism being a particular species (Hebert *et al*. 2003). Methods using several different protein-coding genes from the mitochondrial genome have been established for DNA barcoding of sharks, including the NADH dehydrogenase subunit 2 (NADH2) (Hoelzel *et al*. 2001; Ward *et al*. 2005; Broughton and Reneau 2006; Ward *et al*. 2008; Naylor *et al*. 2012). The NADH2 gene is ~1044bp long allowing for high resolution when distinguishing between species (Moore *et al*. 2011). DNA barcoding methods can help reduce mis-identifications, with the identification of individual pieces of sharks (e.g. fins, meat), and help alleviate the issues of broadly categorized fisheries data (e.g. all species simply labeled "shark") for fisheries managers (Tillett *et al*. 2012).

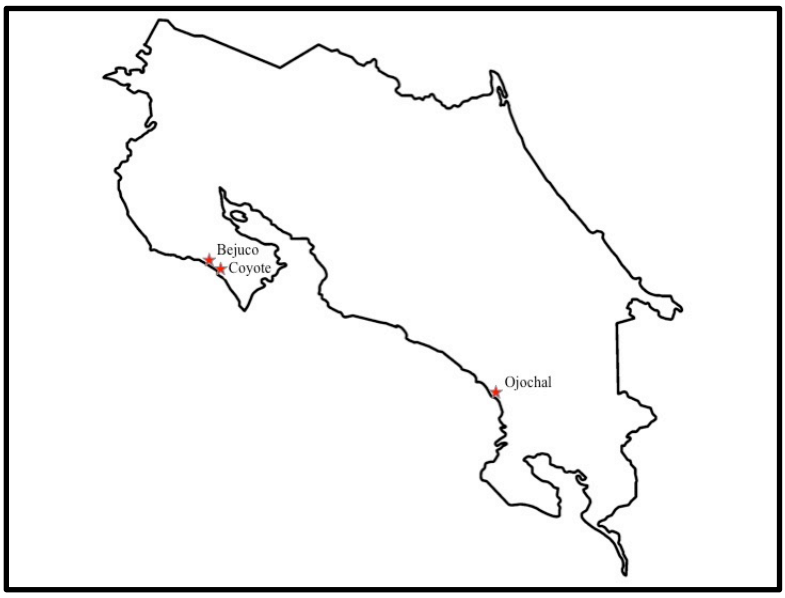
Our overall objective was to use DNA barcoding to conclusively identify the types and quantities of shark species being landed in several small to mid-scale artisanal fisheries along Costa Rica's Pacific coast. Of particular importance was determining the proportion of threatened species being landed in these fisheries. In addition, we also aimed to determine variation in the composition of species being caught 1) at different times of the year; 2) in different communities; and 3) with different fishing methods.

Finally, we wanted to determine if particular life stages of certain species were at a greater risk of overexploitation in these fisheries.

Methods

Sample Collection Artisanal Fisheries

We collected 416 shark tissue samples between April 2013 and September 2014, from artisanal small-scale to mid-scale fishermen in the Ojochal (n = 6) and Coyote/Bejuco (n = 8) fishing communities along the Pacific coast of Costa Rica (Figure 5). Both communities used gillnets and bottom-lines as their main fishing methods. Ojochal consisted of one individual using exclusively gillnets and 5 using bottom-lines. While in Coyote/Bejuco, 2 fishermen used both gillnets and bottom-lines while 3 used only gillnets and 3 more used only bottom-lines.



5. Map of Costa Rica indicating small-scale artisanal fishing communities sampled.

Prior to the onset of this research fisheries observer programs were already operating at both locations. The artisanal fishermen at these locations have agreed to allow researchers to examine their catch and record the fishing method used, species composition, abundance, sex, total lengths (TL), and overall landing weight by species. However, many sharks were landed without any identifying characteristics (i.e. no head) making observer species identification impossible. We would meet fishermen along the bank at established landing sites each morning and occasionally in the afternoon. For the Ojochal community this was done over an ~5 day period each month. While at the Coyote/Bejuco community we had an observer consistently present to collect data several times each month. As fishermen processed their catch (removal of heads and organs) they made all sharks available for morphological analysis and tissue collection. We collected tissue samples generally from muscle tissue around the gills (after heads had been removed) or from the apex of the upper lobe of the caudal fin. We used a sterile 8mm biopsy punch to collect samples from muscle tissue and sterile scissors for fins. When fishermen would bring in large amounts of sharks making it impossible to get length and sex data on all landed sharks, we would only collect tissue samples for these individuals. We were also not always able to collect information on the method of fishing used by the fishermen. We determined maturity level of particular shark species using their total length (TL) compared to recorded TL at maturity. Shark tissue samples were then stored in RNAlater and kept at -4◦ C.

Total genomic DNA was extracted from the tissue samples using the Qiagen DNeasy Tissue Kit, following protocols recommended by the manufacturer. An

approximately 1050bp of the NADH dehydrogenase subunit 2 (NADH2) gene were amplified for species identification using the ASNM and ILEM primer combination described in Naylor *et al*. (2012). Polymerase chain reaction (PCR) amplifications were conducted within a total volume of 25 µl containing 10mM Tris pH 8.4, 50mM KCL, 0.2 mM each dNTP, 1.5mM $MgCl_2$, 0.4µM each primer, 1 U Amplitaq Gold Polymerase® (Life Technologies), and 4 µl of template DNA, and 13.8ul of dH_2O. The PCR thermal cycling profile was: 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 on 1% TBE agarose gel containing Gel Red 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. Cycle sequencing reactions were conducted 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.

Data Analysis Artisanal Fisheries

Forward and reverse sequences were aligned using Sequencher v5.2.4 to generate a full-length consensus sequence for each sample. In some cases the forward sequencing reaction failed and we used only the reverse sequence (~700bp) for species identification.

This approach was previously shown to produce accurate identification for sharks and rays (Chapter 3). We aligned all consensus sequences using the MUSCLE tool and trimmed them to ~1000bp. 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. We used an analysis of similarity (ANOSIM) to determine the variation in species abundance and composition between months, artisanal fishing communities, and fishing methods.

Results

Species Identification

Of the 416 samples tested, 275 resulted in positive species identifications. Overall, 7 species of shark and 1 ray species were represented in the samples (Table 8). The scalloped hammerhead shark was the most frequently caught species in both the Coyote/Bejuco and Ojochal artisanal fishing communities accounting for 70.6% and 67.9% of all samples respectively (Table 8). The Pacific sharpnose shark (*Rhizoprionodon longurio*), was the second most abundant species in both communities, accounting for 15.1% and 12.8% of all samples respectively. Also commonly identified was the blacktip shark (*Carcharhinus limbatus*) (9.6%) in the Ojochal community and the sicklefin smooth-hound (*Mustelus lunulatus*) (6.7%) in the Coyote/Bejuco community. Other species identified in the study were the silky shark (*Carcharhinus falciformis*), smalltail shark (*Carcarhinus porosus*), and whitenose shark (*Nasolamia velox*). Only one

species of ray was recorded in either community: the longtail stingray (*Dasyatis longa*), and it was only represented in the Ojochal samples.

Table 9. Composition of species (percent of total elasmobranch samples) caught in two artisanal fisheries, Coyote/Bejuco and Ojochal, in Costa Rica.

Species	Artisanal Fisheries		Total (n=275)
	Coyote/Bejuco (n-119)	Ojochal (n=156)	
Carcharinus falciformis (Silky shark)	-	2.6	1.5
C. porosus (Smalltail shark)	-	0.6	0.4
C. limbatus (Blacktip shark)	4.2	9.6	7.3
Dasyatis longa (Longtail stingray)	-	5.1	2.9
Mustelus lunulatus (Sicklefin smooth-hound shark)	6.7	-	2.9
Nasolamia velox (Whitenose shark)	3.4	1.3	2.2
Rhizoprionodon longurio (Pacific sharpnose shark)	15.1	12.8	13.8
Sphryna lewini (Scalloped hammerhead shark)	70.6	67.9	69.1

Species Composition and Abundance

Species composition and abundance did not differ significantly among sampling periods (R=0.004, p=0.46). There was also no significant difference in species composition and abundance between the two fishing communities (R=-0.06, p=0.94), nor the fishing methods used (gillnet or bottomline) (R=0.09, p=0.12). Although, as can be

seen in Table 8, some species were only found at one or the other community. It also appears that within the Coyote/Bejuco community, gillnets catch proportionately more scalloped hammerheads than bottom-lines. The smalltail and blacktip sharks for both communities were caught exclusively by gillnets (Table 9). While longtail stingrays and sicklefin smooth-hounds were caught far more frequently by bottom-lines than gillnets.

Table 10. Comparison of the composition of species (percent of total elasmobranch samples) between two fishing methods (gillnet and bottom-line) from two artisanal fisheries, Coyote/Bejuco and Ojochal, in Costa Rica.

	Coyote/Bejuco		**Ojochal**		**Total**	
Species	**Gillnet (n = 61)**	**Bottom-line (n = 47)**	**Gillnet (n = 84)**	**Bottom-line (n = 21)**	**Gillnet (n = 145)**	**Bottom-line (n = 68)**
Carcharhinus porosus (Smalltail shark)	-	-	1.2	-	0.7	-
C. limbatus (Blacktip shark)	8.2	-	14.3	-	11.7	-
Dasyatis longa (Longtail stingray)	-	-	1.2	28.6	0.7	8.8
Mustelus lunulatus (Sicklefin smooth-hound)	-	17	-	-	-	11.8
Nasolamia velox (Whitenose shark)	-	6.4	1.2	4.8	0.7	5.9
Rhizoprionodon longurio (Pacific sharpnose)	3.3	34	13.1	4.8	9	25
Sphryna lewini (Scalloped hammerhead)	88.5	42.6	69.1	61.9	77.2	48.5

Size Ranges

For 191 out of the 416 sharks sampled, we also had observer data - including total length measurements (Table 10). For each of those species we reviewed size at sexual maturity and include those data in the table for comparison. Based on total length data, no sharks identified by observers as scalloped hammerheads, in either artisanal fishery, had reached sexual maturity. Individuals of the whitenose shark were below minimum size of sexual maturity for males, but it is currently unknown at what size females of this species mature. Some of the observer-dentified Pacific sharpnose sharks were large enough to be mature males, but not mature females. Based on size it appears that while only mature adult sicklefin smooth hounds were present in Coyote/Bejuco, and only juveniles were present in Ojochal.

Table 11. Total lengths (TL) of observer identified sharks landed in two artisanal fisheries and the species corresponding TL at which they reach maturity.

	Coyote/Bejuco		**Ojochal**		**Sexual Maturity**	
Species	**n**	**TL(cm)**	**n**	**TL(cm)**	**Female TL(cm)**	**Male TL(cm)**
Scalloped hammerhead (Sphyrna lewini)	62	46.5 – 77.2	99	10.9 – 83.4	~212*	140 – 165*
Whitenose shark (Nasolamia velox)	3	60.9 – 92	-	-	-	>106*
Pacific Sharpnose shark (Rhizoprionodon longurio)	12	50 – 88.7	8	48.4 – 66.2	~103*	58 – 69*
Sicklefin Smooth-hound (Mustelus lunulatus)	4	85 – 100.7	3	51.2 – 62.5	~97*	70 – 83*

*Compagno *et al*. 2005

Discussion

Overall, the small- and mid-scale artisanal fishing communities we examined are catching diverse array of shark species. Even though the communities are separated by ~100 miles of coastline, their catches are similar in both composition and abundance of species. This result suggests that other communities in the area are likely catching the same species of sharks as well. Statistically, the species of elasmobranchs being caught was not affected by whether fishermen used gillnets or bottom-lines. However, when looking at data it appears that scalloped hammerheads are caught more frequently by gillnets than bottom-lines. Blacktip sharks landed in both communities were also found to be caught exclusively by gillnets. Gillnets in general catch a wider variety of species and are particularly lethal due to the amount of area they can cover and the fact that they are far less discriminatory than other fishing methods, with fish easily become entangled in them. Therefore, it could be expected for this method to catch a greater variety and larger quantity of fish (i.e. sharks) than other more discriminatory methods. For example, bottom-lines in this study generally caught larger quantities of demersal elasmobranchs, like the longtail stingray and sicklefin smooth-hound, while gillnets caught species from the entire water column. Although scalloped hammerheads frequently prey on demersal organisms (i.e. crabs) making them susceptible to bottom-lines, they still spend a great deal of time in the upper layers of the water column making them very susceptible to gillnets. Based on conversations with the fishermen, sharks caught in bottom-lines often can be released alive while those in gillnets are already deceased when the nets are retrieved. Therefore, gillnets pose a greater potential threat to certain shark populations,

like the endangered scalloped hammerhead, because there is little to no chance for releasing caught individuals.

The most frequently caught species caught by both communities was the scalloped hammerhead: representing ~70% of all shark landings. It is evident, based on the total length data, that all of the scalloped hammerheads caught were either juveniles or neonates. This strongly suggests that the areas where they were caught are nursing grounds for the species. These results are concerning given that the scalloped hammerhead is listed as globally endangered by the IUCN with population declines between 50-90% depending on ocean basin (Baum *et al*. 2007). They are also listed on CITES Appendix II, which regulates international trade in their products (Vincent *et al*. 2013). The propensity of individuals of this species to aggregate in predictable locations, including the use of nursing grounds, has made them easier to exploit and increases their vulnerability to fishing pressure (Abercrombie *et al*. 2005; Baum *et al*. 2007). Small-scale to mid-scale artisanal fisheries account for ~60% of landings of all marine organisms in Costa Rica (Salas *et al*. 2011). In previous studies of pelagic fisheries in Costa Rica, scalloped hammerheads made up only ~1-3% of total landings (Whoriskey *et al*. 2011; Dapp *et al*. 2013; Chapter 3). Therefore, it appears that the small to mid-scale artisanal fisheries are a greater threat to the scalloped hammerhead population. If our data are indicative of what happens regularly and at other communities, then artisanal fisheries along the Pacific coast could have severe consequences for scalloped hammerhead populations in this region.

The remainder of the shark species landed by these artisanal fishing communities is mainly of the family Carcharhinidae. Many of the species in this family have similar physical characteristics and it can be hard to distinguish among them in the field. This can lead to mis-identification of species. For example, in this study, sharks that were identified by observers as Pacific sharpnose sharks were actually whitenose sharks. These two species have similar body morphology with long snouts, similar size ranges, and grey-brown coloring. However, the key distinguishing characteristic between the two, a black spot outlined in white on the snout, is removed when the sharks are processed (Compagno *et al*. 2005). This would make these two species almost indistinguishable at first glance. Using genetic methods we also identified two species (silky and smalltail sharks) previously unrecorded by the current observer programs. These two species represented a very small portion of the total landings but their occurrence in these fisheries could still be of importance. The use of genetic techniques for identification are clearly important for acquiring accurate data on the composition and abundance of shark species being caught in fisheries.

The species composition found in these artisanal fisheries was very different from that in the San Jose and Heredia markets (Chapter 3). All of the species landed by artisanal fishermen were also found in the markets, but in very different frequencies. For example, in the markets, the most frequently identified species was the silky shark, which accounted for >75% of all samples. In fact, most of the vendors sold silky shark exclusively (Chapter 3). However, in the current study, silky sharks comprised < 3% of the total catch. The opposite is true for scalloped hammerheads that dominated the

artisanal landings but only accounted for 3.6% of the market samples. Similarly, Pacific sharpnose sharks were the second most common shark identified in this study but was only identified as one of the 732 market samples. We were not surprised that the same species are represented in both studies given that they represent the shark species found off the Pacific coast of Costa Rica. However, the completely different abundances of each species emphasizes that the small to mid-scale artisanal fishing vessels are utilizing very different areas. Importantly, the differences between this study and the market (Chapter 3) study strongly suggest that artisanal fisheries in Costa Rica do not play a key role in supplying shark products to the markets in major cities like San Jose. In fact, from our observations during this research, sharks landed in these fisheries generally remain in the local community: either sold to local restaurants or consumed by the fishermen themselves.

Management implications

Immature scalloped hammerhead accounting for the majority of sharks being landed in these fisheries indicates that the Pacific coast of Costa Rica contains many important nursing grounds for this species. This highlights the necessity for Costa Rica to develop effective fisheries management legislation to protect this species. If large portions of the juvenile scalloped hammerhead population continue to be exploited at current levels it could threaten the ability of this species to replenish the adult breeding population. Marine protected areas have been shown to provide conservation benefits for shark populations (Knip *et al*. 2012). However, MPA's will only be effective if they are properly enforced and are implemented in areas known to be important to the species of

concern. We showed that fishermen in small to mid-scale artisanal fishing communities were willing to support shark conservation in the form of MPAs (Chapter 2). However, MPAs that would infringe on their current fishing practices by creating no-take zones, time closures, or fishing gear restrictions gained almost no support (Chapter 2). Our results also found that there is no specific time of year during which MPAs with time closures would allow for greater protection for scalloped hammerheads, and similar compositions and quantities of shark were landed for all gear types used in these communities. It would appear that fishermen in these communities were in support of shark conservation, in theory, but were unwilling to allow it to impact their current fishing practices (Chapter 2). Without some support from these communities enforcement of any conservation measure would be almost impossible. However, the fishermen of these communities indicated they are willing to stop catching sharks; they just want to know how to do so without harming their livelihoods. Therefore, it will be up to fisheries managers to work with these fishermen to develop legislation that will gain the necessary community support for it to actually be followed, but also provide the necessary protection for these vulnerable species.

RELATIONSHIPS OF MERCURY CONCENTRATIONS ACROSS TISSUE TYPES AND REGIONS FOR TWO SHARK SPECIES

Introduction

Mercury (Hg) pollution poses a serious potential threat to the health of organisms within the marine environment. In fish, Hg accumulates principally from dietary sources (Trudel & Rasmussen 2001) and has been found to negatively impact reproduction (Matta *et al*. 2001; Hammerschmidt *et al*. 2002; Drevnick & Sandheinrich 2003; Klaper *et al*. 2006; Crump & Trudeau 2009), liver function and metabolism (Adams *et al*. 2010), and behavior (Webber & Haines 2003). Mercury has also been shown to cause neurological damage (Basu *et al*. 2007, 2009; Scheuhammer *et al*. 2008), damage to gills and olfactory organs (Jagoe *et al*. 1996; Oliveira Ribeiro *et al*. 1996, 2000) and even mortality (Wiener & Spry 1996).

A great deal of attention has been paid to Methylmercury (MeHg) contamination in high trophic level species such as tuna, swordfish and sharks. Particular emphasis has been placed on the threat that consumption of such organisms pose to humans (Rice *et al*. 2000; Escobar-Sánchez *et al*. 2010), including significant neurodevelopmental effects on early developmental stages (Burger & Gochfeld 2011; Lopez *et al*. 2013). Because they are long lived and often occupy high trophic positions, many shark species also accumulate substantial concentrations of Hg (Lyle 1984; Adams 2004; Branco *et al*. 2004). To better understand the human health implications of Hg, it is critical to examine

the potential exposure levels that result from the consumption of shark meat and fins (Swain *et al*. 2007).

Mercury concentrations can vary between individuals within a shark species due to differences in length (age) (Walker 1976; Hornug *et al*. 1993; Hueter *et al*. 1995; Lacerda *et al*. 2000; Endo *et al*. 2008), sex (Pethybridge *et al*. 2010), season (Escobar-Sánchez *et al*. 2010), and habitat type and location (Hornug *et al*. 1993; Hueter *et al*. 1995; McMeans *et al*. 2007). Different species of sharks often exhibit different Hg concentrations (Hueter *et al*. 1995; Storelli *et al*. 2002; McMeans *et al*. 2007; Endo *et al*. 2008) likely due to variations in prey type and habitat, in addition to the previously mentioned variables. Mercury concentrations also vary among tissue types within an individual shark (Branco *et al*. 2007; Endo *et al*. 2008; Gutiérrez-Mejía *et al*. 2009; Escobar-Sánchez *et al*. 2010; Pethybridge *et al*. 2010; Nam *et al*. 2011; Delshad *et al*. 2012; Hurtado-Banda *et al*. 2012; Lopez *et al*. 2013). The highest concentrations of total mercury (THg) have been found in muscle tissue, followed by internal organs (e.g. kidney, liver), fins, and skin (Branco *et al*. 2007; Endo *et al*. 2008; Escobar-Sánchez *et al*. 2010; Pethybridge *et al*. 2010; Nam *et al*. 2011; Delshad *et al*. 2012; Hurtado-Banda *et al*. 2012).

While the Hg levels in shark tissue may negatively affect human health through consumption, there is also concern about potential implications that Hg will have on shark health (Swain *et al*. 2007). However, there are insufficient data on Hg contamination in shark organs and how those levels relate to health to adequately assess the direct impacts elevated concentrations may have on sharks and their populations.

Methods to obtain key internal organs from sharks typically involve lethal sampling techniques. When possible, transition from lethal to non-lethal sampling techniques is especially important given that 25% of all chondrichthyan fishes (sharks, skates, rays, and chimeras) are currently threatened with extinction (Dulvy *et al*. 2014). Non-invasive techniques that would utilize a subset of tissues to estimate Hg concentrations throughout a shark's body (i.e. organs) would provide the ability to expand our understanding of how Hg is distributed throughout shark tissues, and remove the need for lethal sampling. However, no previous studies have looked at the relationship of Hg concentrations among a wide array of tissue types and organs.

The objectives of this study were thus to: 1) examine total mercury (THg) concentrations in tissues from two species of shark, with varying life histories, i.e., bonnethead shark (*Sphyrna tiburo*) and silky shark (*Carcharhinus falciformis*); 2) examine the relationships of THg concentrations across a wide range of tissues including eight muscle regions (dorsal axial, interdorsal, caudal peduncle white muscle, caudal peduncle red muscle, mid-flank, mid-abdominal, cranial, pre-pectoral), fin cores and fin trailing margins from four different fins (first dorsal, left and right pectoral, caudal), and five organs (liver, heart, kidney, spleen, epigonal); and 3) determine if measurements of THg via less invasive fin clips (fin trailing margins) or muscle biopsy punches can be used to effectively estimate THg concentrations in other tissue types. Previous studies on amphibians (Todd *et al*. 2012) and birds (Eagles-Smith *et al*. 2008) have shown that THg concentrations in one tissue can be used to accurately predict relationships among other tissues.

Methods

Study Species

The bonnethead shark (*Sphyrna tiburo*) is a smaller shark species, only reaching a maximum total length (TL) of 150cm (Castro 2011). In waters around the southeastern U.S. female *S. tiburo* mature between 6 to 7 years of age (~81.9cm), while males mature between 3 to 9 years (~61.9cm) (Frazier *et al*. 2014). Based on the TL for the *S. tiburo* sampled for this study, male and female, juvenile and adult sharks were sampled (38.6-120.1 cm TL). The individuals collected in this study came from the southeastern U.S. where these sharks are known to inhabit estuarine and shallow coastal waters (Compagno *et al*. 2005). These sharks are relatively common in these waters with females generally inhabiting estuaries from late spring to early autumn while males generally remain in shallow coastal waters (Ulrich *et al*. 2007). In the western North Atlantic Ocean females of this shark have been found to live up to 17.9 yrs with males reaching 16 yrs (Frazier *et al*. 2014). The estuarine waters of the southeastern U.S. coast likely represent important feeding grounds for *S. tiburo* (Driggers *et al*. 2014). They are specialized to feed on hard-shelled prey and have been widely documented throughout their range to feed primarily on crustaceans, particularly blue crabs (Cortés *et al*. 1996; Lessa & Almeida 1998; Wilga & Motta 2000; Bethea *et al*. 2007; Gurshin 2007), which comprises their almost exclusive food source in these estuaries (~99% of their diet from individuals collected from April to August; Ulrich *et al*. 2007).

The silky shark (*Carcharhinus falciformis*) is a semi-pelagic species that frequents both coastal and open waters in tropical oceans around the world (Bonfil 2008). It is most commonly found on the edge of continental and insular shelves, particularly

around seamounts, at depths averaging 200m (Compagno *et al.* 2005; Bonfil 2008). *Carcharhinus falciformis*, like most species of shark, is relatively long-lived (22+ yrs), with a maximum observed TL of ~330cm (Bonfil 1990; Compagno *et al*. 2005; Bonfil 2008). The age and size of maturity for this species varies between ocean basins, but in general males reach maturity after 5-10 yrs (>180cm) and females 6 to 10 years (>180cm) (Branstetter 1987; Bonfil *et al*. 1993; Hoyos 2003; Oshitani *et al*. 2003). Based on their TL, individuals of *C. falciformis* sampled in this study encompassed juvenile males and females and adult male sharks (86.6-220.0 cm TL). *Carcharhinus falciformis* feeds primarily on fish such as the sea catfish (family Ariidae), mullets (family Mugilidae), mackerel (family Scombridae), yellowfin tuna (*Thunnus albacares*), albacore tuna (*T. alalunga*), porcupine fish (family Diodontidae), and other fish species, and is also known to prey on a variety of cephalopods (Bonfil 1990; Compagno *et al*. 2005).

Sample Collection

We collected *S. tiburo* (n=42) and *C. falciformis* (n=5) in 2012 and 2013 from estuarine and coastal areas of the southeastern United States (St. Catherine's Island, GA and the Indian River Lagoon, FL and adjacent nearshore waters). We used gill nets, haul seines and hook and line gear to capture sharks from this study area. We placed captured sharks on ice directly after removal from water, processed them and then stored samples in a -20◦C freezer. In addition, eight *C. falciformis* were collected during market surveys in Jeddah, Saudi Arabia (Spaet & Berumen 2015). Precaudal length (PCL), fork length (FL), total length (TL), total weight, and sex were recorded for each specimen. We used a clean stainless-steel knife or scalpel to dissect muscle tissue samples from eight specific

regions (dorsal axial, interdorsal, caudal peduncle white muscle, caudal peduncle red muscle, mid-flank, mid-abdominal, cranial, pre-pectoral) along the body (Figure 6). Caudal peduncle red muscle was only sampled for *S. tiburo*. Care was taken during dissection to ensure that muscle samples did not come into contact with shark epidermal or dermal layers, or surrounding surfaces (Adams *et al*. 2003). Also dissected for THg analyses were a sample of the liver, the posterior region of the kidney, spleen, epigonal organ, and the entire heart. We also removed the entire first dorsal, caudal, and both pectoral fins using a sterile knife. Four of the five *C. falciformis* from the southeastern U.S., and 28 of the 42 *S. tiburo* only had dorsal axial muscle and liver samples taken and were only included in regression analyses comparing these two sample types. The remaining *C. falciformis* individual from the southeastern U.S. had each of its muscle regions and organs sampled and Hg concentrations for these tissues were used in the appropriate analyses, but no fin samples were available. Tissue and fin samples were immediately placed in new sterile polyethylene containers and frozen at −20° C or −80° C (which was dependent on the storage location before transfer to the University of Georgia's Savannah River Ecology Laboratory).

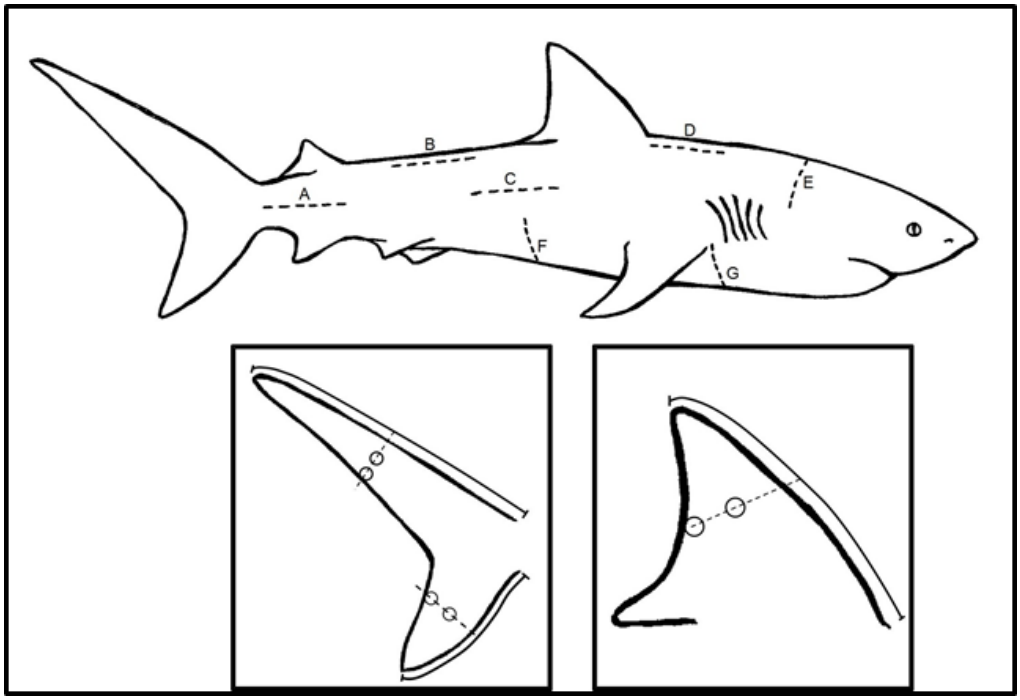


Figure 6. Muscle regions sampled from sharks. Two types of caudal peduncle muscle were sampled from the same location where one muscle overlays the other. A) caudal peduncle white and red muscle, B) interdorsal, C) mid-flank, D) dorsal axial, E) cranial, F) mid-abdominal, G) pre-pectoral.

Sample Processing

We allowed samples for individual sharks to thaw at room temperature before taking sub-samples. We used 8 mm disposable biopsy punches to take two samples from each of the previously collected fins (fin trailing margin and inner core) (Figure 6). We determined the fin inner core sample location by measuring the halfway point on the leading edge of the respective fin and then measuring to the center point between the leading and trailing edge. We then took fin trailing margin samples from the trailing edge of the fin on the same bisecting line as the fin inner core sample. We treated the upper

and lower lobes of the caudal fin as two separate fins for sub-sampling, resulting in a fin trailing margin and inner core sample for both the upper and lower lobe. However, lower lobe caudal fin samples were only obtained for the species *S. tiburo* (N = 14). To sub-sample the muscle regions and organs we used sterile forceps and scissors to remove ~1 g samples. We then placed all sub-samples into individual 1 oz clean polyethylene plastic sample bags.

Chemical Analysis

We lyophilized all tissue samples (muscles, organs, and fins) and then homogenized muscle and organ samples by crushing them by hand or with mortar and pestle while they remained inside the polyethylene plastic sample bags. This approach reduced cross contamination and we made sure samples did not puncture the polyethylene plastic sample bags. Fin tissue samples were either analyzed whole or they were cut in half using sterile scissors (when duplicates of that fin needed to be run). We analyzed THg content by thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry (DMA 80; Milestone, Monroe, CT, USA), according to U.S. Environmental Protection Agency (EPA) method 7473 (U.S. E.P.A. 2007). Concentrations of THg for all samples were analyzed on a dry weight basis to standardize comparisons and avoid the confounding factor of variable moisture loss among samples.

For quality-assurance measures we included the analysis of two certified reference materials (dogfish liver DOLT-4, and lobster hepatopancreas TORT-2; National Research Council of Canada, Ottawa, ON) and system and method blanks. We

initially ran three system and method blanks followed by the reference materials to verify system calibration. Then for every 10-15 samples we ran a single duplicate of the last sample run, followed by a system and method blank, the two reference materials, and finally by two more system and method blanks. Our recoveries averaged 101.85% ± 7.10% (n = 149) and 99.60% ± 5.96% (n = 67) for the certified reference materials and calibration checks respectively. We found the absolute relative percentage difference (RPD) for duplicates in all tissues combined averaged to be 11.12% ± 13.34%.

Statistical Analyses

We used R statistical software (3.1.2) for all statistical analyses (R Development Core Team 2014). We tested for normality using the Shapiro-Wilk test (Shapiro & Wilk 1965) and homogeneity of variance using a Bartlett test (Bartlett 1937) for all tissue groups (muscles, organs, fin inner cores, fin trailing margins). To better meet the assumptions of standard parametric statistical tests and to normalize residuals we natural-log transformed all THg data. After transformation, each of the tissue groups met at least one of the assumptions of ANOVA, but generally not both assumptions. However, ANOVA is fairly robust to violations of a single assumption, especially if sample sizes are fairly large, as in this study.

We therefore used ANOVA to determine if THg concentrations were significantly different from each other within different sample location groupings (e.g. dorsal axial, interdorsal, cranial, pre-pectoral, mid-abdominal, mid-flank, caudal peduncle white, and caudal peduncle red muscle), or tissue grouping (muscle, organ, fin inner core, fin trailing margin). If samples within a tissue grouping were significantly different we then used a

Tukey's multiple comparison of means to determine which sample types had significantly different THg concentrations. Using the results from the non-significant ANOVAs some tissue groups were reduced to a single representative variable (e.g. dorsal axial muscle representing all muscle samples) for further analyses. The ANOVAs for the tissue groups only included samples from sharks that had data for each of the respective tissues types (*S. tiburo* n=14, *C. falciformis* n=8), excluding samples from the lower lobe of the caudal fin and caudal red muscle for *C. falciformis*.

We used linear regressions to create predictive relationships between tissue types for each species. The goal was to use non-invasive fin trailing margins (fin clips) and muscle samples to predict Hg concentrations in tissues that are typically collected via lethal sampling. In the first set of regressions, we used the fin trailing margin as the independent variable and performed separate regressions for the following dependent variables: muscle (dorsal axial); fin inner core (first dorsal, inner core of the upper caudal lobe (IUCL); liver; kidney; spleen; heart; and epigonal organ. In the second set of regressions, we used muscle (dorsal axial) as the independent variable, and ran separate regressions for the following dependent variables: fin inner core (first dorsal), IUCL, liver, kidney, spleen, heart, and the epigonal organ. From this we developed equations for predicting THg concentrations in tissues based on the concentration found in either the fin trailing margin or muscle tissue. When significant relationships between two variables were found in both species, we compared confidence intervals of the regression lines to assess whether the relationships were the same for both species.

Results

Comparison of THg concentrations between and within tissue groups

Overall muscle tissue for both species tended to have the highest concentrations of THg (Table 11). However, THg levels in the organs were only slightly lower and overlapped some of those in muscle tissue. The five organs sampled did not have significantly different THg concentrations (F= 1.947, p= 0.107). Fin trailing margins, interestingly, had higher THg concentrations than the fin inner cores from the first dorsal, left and right pectoral, and lower lobe of the caudal fin. The fin inner core of the upper caudal lobe (IUCL) had much higher concentrations of THg compared to all other fin samples, likely due to the increased muscle tissue in that region of the caudal fin. The IUCL THg concentrations more closely resembled those seen in muscle tissue and organs, while the other fin samples had far lower THg concentrations.

Table 12. Total mercury (THg) concentrations in muscle regions, organs, and fins of *S. tiburo* and *C. falciformis* with standard deviations.

		S. tiburo		*C. falciformis*	
Muscle		n	Mean THg (ppm dry wt)	n	Mean THg (ppm dry wt)
	Dorsal Axial	42	2.37 ± 1.52	13	3.09 ± 2.38
	Interdorsal	14	2.78 ± 1.85	8	2.55 ± 1.61
	Cranial	14	2.95 ± 1.94	8	2.52 ± 1.56
	Mid-abdominal	14	2.42 ± 1.74	8	2.14 ± 1.24
	Mid-flank	14	2.97 ± 2.18	7	2.30 ± 1.65
	Pre-pectoral	14	3.10 ± 2.12	8	2.61 ± 1.46
	Caudal Peduncle (White)	14	3.01 ± 2.02	8	2.67 ± 1.46
	Caudal Peduncle (Red)	14	2.53 ± 1.58	0	NA
Organs					
	Liver	42	1.82 ± 2.94	13	2.10 ± 3.64
	Kidney	14	2.15 ± 2.41	8	0.66 ± 0.44
	Spleen	14	1.43 ± 2.07	8	0.77 ± 0.84
	Heart	14	1.62 ± 1.02	7	1.06 ± 0.52
	Epigonal	14	0.86 ± 0.90	8	1.43 ± 2.23
Fin Inner Cores					
	First Dorsal	18	0.04 ± 0.03	8	0.02 ± 0.01
	Left Pectoral	18	0.04 ± 0.03	8	0.02 ± 0.01
	Right Pectoral	18	0.04 ± 0.02	8	0.02 ± 0.01
	Upper Caudal Lobe (IUCL)	18	0.68 ± 0.50	8	0.98 ± 0.82
	Lower Cuadal Lobe	14	0.06 ± 0.08	0	NA

Fin trailing margin					
	First Dorsal	17	0.07 ± 0.06	8	0.05 ± 0.03
	Left Pectoral	18	0.07 ± 0.05	8	0.03 ± 0.01
	Right Pectoral	18	0.07 ± 0.05	8	0.03 ± 0.01
	Upper Caudal Lobe	18	0.10 ± 0.09	8	0.04 ± 0.02
	Lower Cuadal Lobe	14	0.08 ± 0.07	0	NA

Total Hg concentrations between the eight muscle regions sampled were not significantly different from one another for either species of shark (*S. tiburo*: F = 0.185, p = 0.988; *C. falciformis*: F = 0.199, p = 0.976). Thus, we used THg concentrations of the dorsal axial muscle (most commonly used tissue in previous studies) to represent all muscle tissue in our subsequent linear regression models. Trailing fin margin THg concentrations for each of the sampled fins were not significantly different for either species (*S. tiburo*: F = 0.282, p = 0.406; *C. falciformis*: F = 2.509, p = 0.0792). Since the first dorsal fin represents a commonly sampled region in sharks with seemingly easier access during live sampling, we used it as a representative "fin" sample for our regression analyses.

Fin inner core samples for each species (excluding the lower caudal lobe for *C. falciformis*, which was not sampled) were initially found to have significantly different THg concentrations (*S. tiburo*: F = 49.25, p < 0.001; *C. falciformis*: F = 66.86, p < 0.001) (Table 11). However, the fin IUCL accounted for all the variation between these tissues, being significantly different from each of the other fin inner core samples in both species (Tukey's p-values < 0.001 for each fin inner core compared to the IUCL). When the IUCL is excluded from the analysis, all the other fin inner core samples do not have significantly different THg concentrations (*S. tiburo*: F = 0.001, p = 1.000; *C. falciformis*:

F = 0.793, p = 0.465). Consequently, we used the fin inner core THg concentrations of the first dorsal fin to represent fin inner cores from the pectoral and lower caudal lobe (for *S. tiburo*), while the IUCL remained its own variable for the linear regression models.

Relationships between tissues

Of particular interest to this study was determining if less invasive sampling methods (e.g. fin clips or muscle biopsy punches) could be used to estimate THg concentrations in a variety of shark tissues. The THg concentrations in the fin trailing margin (first dorsal fin) were significantly related to THg concentrations in all other tissue types for *S. tiburo* (muscle, fin inner core, IUCL, liver, kidney, spleen, heart, epigonal) (Table 12; Figure 7). Within *C. falciformis* fin trailing margin tissue was only significantly related only to heart tissue, but explained a low percentage of the variation (Table 12). There were also significant relationships between THg concentrations in muscle tissue (dorsal axial muscle) and all of the other tissue types for *S. tiburo* and for all but the epigonal organ for *C. falciformis* (Table 12; Figure 8). For the relationship between muscle tissue and liver THg concentrations we were able to increase our sample size from 22 total sharks between the two species (individuals with samples taken from all tissue regions) to 55 sharks (33 sharks with only muscle and liver sampled). With the increased sample size there was a significant relationship between the two tissues for both species independently, and the regression lines for the two species were not significantly different from one another, with overlapping confidence intervals (CI) (Table 12; Figure 8a). However, for the other tissue samples, even when the relationship

was significant within both species, the regression lines were different between species (Figure 8b-d).

Table 13. Regression results showing the relationships of the fin trailing margin of the first dorsal fin (from the center of the fin) and dorsal axial muscle tissue to the other sampled tissue types for *S. tiburo* and *C. falciformis*.

			n	F	p	R^2	log y = log x + b
Fin trailing margin							
	Muscle	*S. tiburo*	16	42.18	<0.001*	0.75	y = 0.87x + 3.42
		C. falciformis	8	1.43	0.280	0.19	y = 0.45x + 2.12
	Fin Inner Core	*S. tiburo*	17	135.20	<0.001*	0.90	y = 0.88x – 0.93
		C. falciformis	8	0.45	0.530	0.07	y = 0.22x – 3.28
	IUCL	*S. tiburo*	17	24.77	0.001*	0.62	y = 0.71x + 1.49
		C. falciformis	8	2.13	0.200	0.26	y = 0.71x + 1.86
	Liver	*S. tiburo*	16	32.40	<0.001*	0.70	y = 1.55x + 4.87
		C. falciformis	8	0.74	0.420	0.11	y = 0.59x + 1.74
	Kidney	*S. tiburo*	13	45.87	<0.001*	0.81	y = 1.50x + 4.76
		C. falciformis	7	0.17	0.690	0.03	y = 0.22x + 0.08
	Spleen	*S. tiburo*	13	33.24	<0.001*	0.75	y = 1.52x + 4.27
		C. falciformis	8	1.07	0.340	0.15	y = 0.75x + 1.52
	Heart	*S. tiburo*	13	44.42	<0.001*	0.80	y = 0.75x + 2.66
		C. falciformis	8	7.04	0.05*	0.59	y = 0.74x + 2.30
	Epigonal	*S. tiburo*	13	60.76	<0.001*	0.85	y = 1.27x + 3.31
		C. falciformis	8	0.00	0.960	0.00	y = 0.04x – 0.67
Muscle							
	Fin Inner Core	*S. tiburo*	17	21.82	<0.001*	0.59	y = 0.70x – 4.13
		C. falciformis	8	16.17	0.007*	0.73	y = 0.69x – 4.44
	IUCL	*S. tiburo*	17	38.78	<0.001*	0.72	y = 0.75x – 1.24
		C. falciformis	8	12.06	0.013*	0.67	y = 1.10x – 1.13
	Liver	*S. tiburo*	42	99.08	<0.001*	0.71	y = 1.65x – 1.31
		C. falciformis	13	50.54	<0.001*	0.82	y = 1.65x – 1.55
		Species combined	55	144.90	<0.001*	0.73	y = 1.63x – 1.35
	Kidney	*S. tiburo*	14	191.00	<0.001*	0.94	y = 1.63x – 1.21
		C. falciformis	9	13.52	0.008*	0.66	y = 1.03x – 1.34
	Spleen	*S. tiburo*	14	96.69	<0.001*	0.89	y = 1.69x – 1.83
		C. falciformis	9	16.73	0.005*	0.71	y = 1.54x – 1.83
	Heart	*S. tiburo*	14	298.80	<0.001*	0.96	y = 0.86x – 0.39
		C. falciformis	8	7.40	0.035*	0.55	y = 0.80x – 0.52
	Epigonal	*S. tiburo*	14	107.20	<0.001*	0.90	y = 1.37x – 1.77
		C. falciformis	9	0.53	0.489	0.07	y = 0.58x – 0.96

*Indicates significant relationship between tissue types.

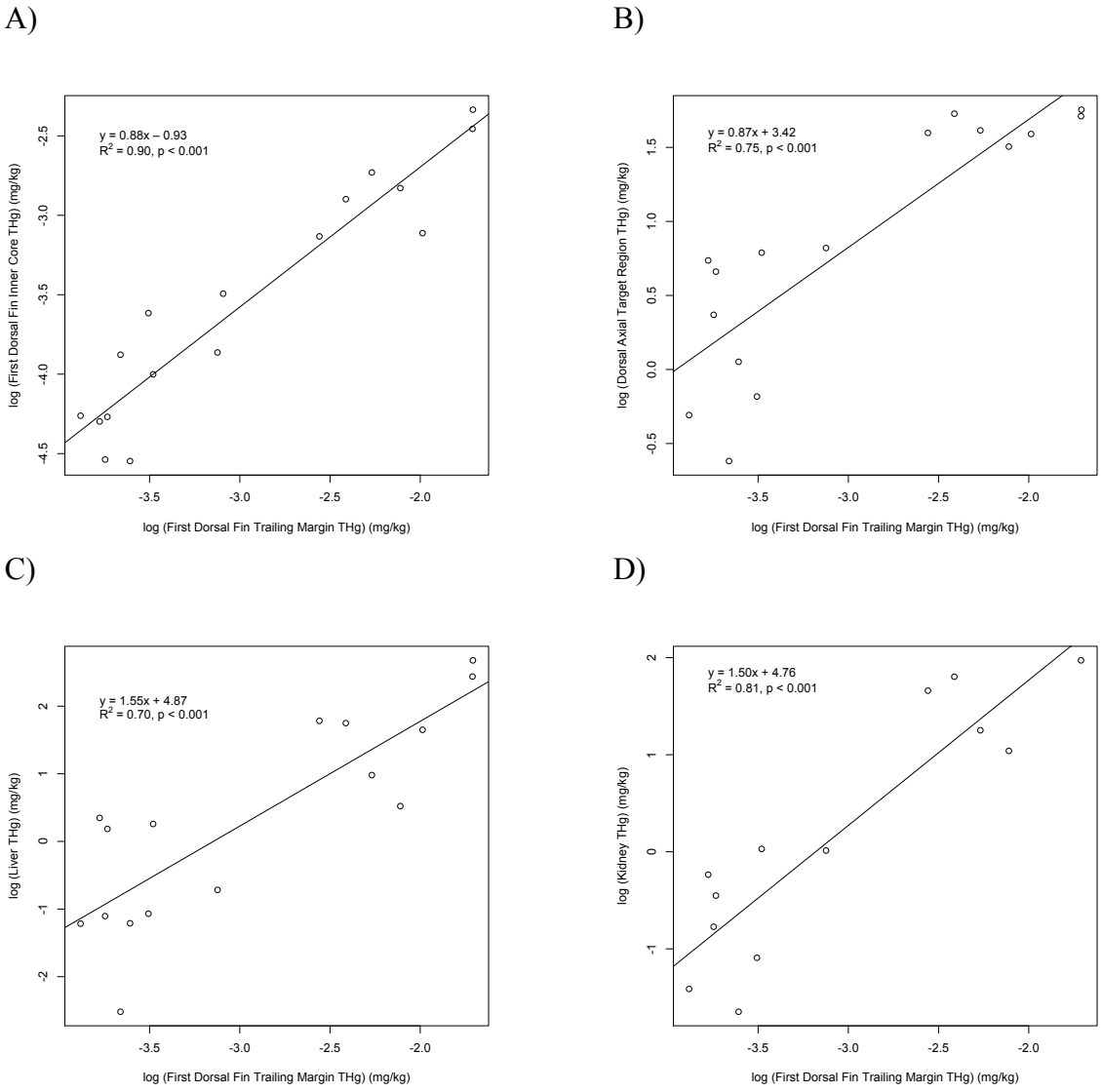


Figure 7. Linear relationships of THg concentrations in first dorsal fin trailing margin and A) first dorsal fin inner core, B) dorsal axial muscle, C) liver, and D) kidney of *S. tiburo*.

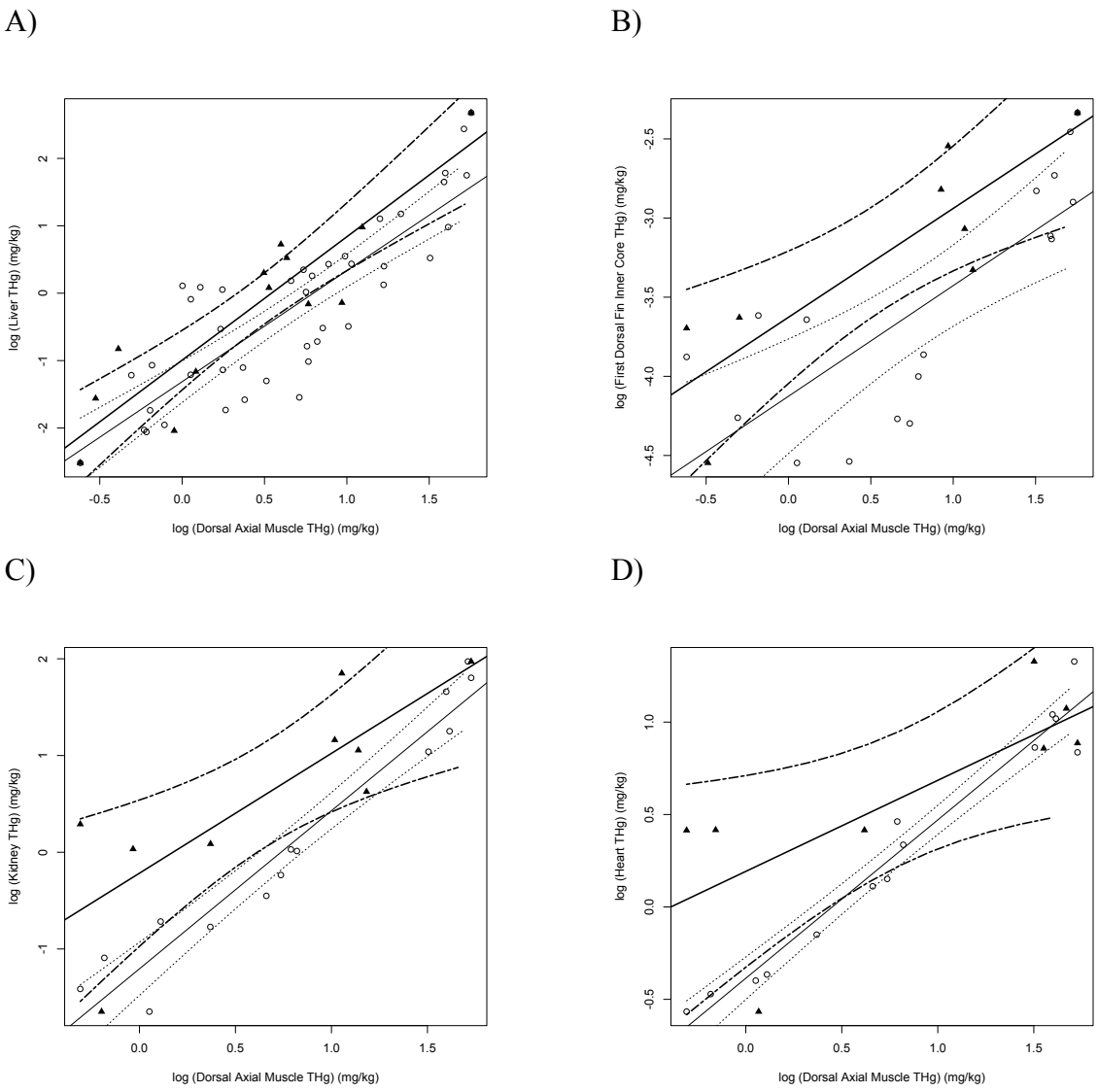


Figure 8. Linear relationships of THg concentrations in dorsal axial muscle and A) liver, B) first dorsal fin inner core, C) kidney, D) heart for *S. tiburo*(○) and *C. falciformis*(▲), with 95% confidence intervals (bold regression and confidence interval lines = *C. falciformis*).

Discussion

For both species analyzed, THg concentrations were highest in muscle tissue, followed by the organs, which all had similar THg concentrations, and lowest in the fins (inner core of the upper lobe of the caudal fin, trailing fin margins, fin inner cores respectively), which is in general agreement with previous studies (Branco *et al*. 2007; Coelho *et al*. 2010; Pethybridge *et al*. 2010). Total Hg concentrations in each of the muscle regions examined were not significantly different from one another in either *S. tiburo* or *C. falciformis*. This suggests that sampling muscle from any of the eight locations would be representative of each of the other muscle sample locations. This could be important for monitoring how consumption of various types of shark muscles may impact human Hg exposure as samples from the whole shark is not needed (Adams & McMichael 1999; Storelli *et al*. 2002; Nam *et al*. 2011). Of particular interest was that red muscle from the caudal peduncle in *S. tiburo* showed similar THg concentrations as the other seven white muscle samples. Cizdziel *et al*. (2002) found that red muscle had significantly higher concentrations of Hg than white muscle. Given that red muscle uses aerobic respiration (Tyus 2012), and blood is a vector for the distribution of Hg to various tissues (Eagles-Smith *et al*. 2008) we expected to also find significantly higher THg concentrations in red muscle. At this point we have no explanation for this discrepancy.

Analogous to our findings for muscle tissue, neither species showed a significant difference in THg concentrations among fin inner core samples (excluding the upper caudal lobe), which provides useful information on the exposure of humans to Hg through the consumption of shark fins (i.e. shark fin soup). A recent study showed that MeHg accounts for 62 ± 22% of THg in dried shark fins (Nalluri *et al*. 2014). Nalluri *et*

al. (2014) also found that while shark fin soup does represent a potentially important source of MeHg in humans, other seafood products (i.e. muscle tissue of top predators) contain much higher concentrations of THg and would result in higher exposure. The most commonly recorded, and likely consumed, fin positions in the Hong Kong fin market (worlds' largest fin market) are the first dorsal, lower caudal lobe and pectoral fins (Clarke *et al*. 2004). Based on our results, consumption of any of these would provide similar levels of THg exposure. However, the IUCL consistently had higher concentrations of THg compared to the other fin inner core samples in both species analyzed, likely due to the increased amounts of muscle found in this inner regions of the caudal fin compared to the other fins sampled. Thus, consumption of this would result in increased mercury exposure compared to the other fins and lower caudal lobe. The trailing fin margins had proportionately higher concentrations of THg than fin inner core samples for both species. The fin inner cores for the first dorsal fin, pectoral fins, and lower lobe of the caudal fin are composed primarily of ceratotrichia (i.e. cartilaginous fibers) that give structure to the fins, while the respective fin trailing margins are composed almost entirely of skin. The variation in tissue composition could explain the variations seen in THg concentrations between these two regions of the fin, with skin containing higher concentrations of THg. Total Hg exposure could be reduced by removing the skin when preparing fins for consumption (a common practice), although only by a marginal amount. Therefore, the THg concentrations for fin inner core samples from this study, which still had the skin attached, could be slight overestimates of potential exposure to people consuming dishes like shark fin soup. With or without skin,

our results for the shark species and life stages tested, support Nalluri *et al.*'s (2014) finding that exposure to Hg from consumption of fins alone would be substantially less than from consumption of other shark products (i.e. muscle, liver).

One objective of this study was to determine if THg concentrations in certain tissue samples could accurately predict THg concentrations in other tissues. Particularly we wanted to determine if less invasive samples (dorsal axial muscle biopsy punches, first dorsal fin trailing margin fin clips) could be used to estimate THg concentrations in other shark tissues (e.g. fin inner core, liver, kidney, spleen, heart, epigonal). If so, the ability to predict THg concentrations in these tissues could then be used to estimate general impacts of Hg on population health without causing undue harm or death to the sampled individual. Of the samples collected, fin clips from the dorsal fin trailing margins and dorsal axial muscle samples from biopsy punches represent relatively less invasive sample types. Based on linear regressions, the use of THg concentrations from the dorsal axial muscle tissue outperformed the dorsal fin trailing margin for estimating THg in the IUCL, liver, kidney, spleen, heart, and epigonal for *S. tiburo*. For *C. falciformis*, dorsal axial muscle THg concentrations were better at estimating THg concentrations in the fin inner core, IUCL, liver, kidney, and spleen, compared to the dorsal fin trailing margin. The THg concentrations for the dorsal fin trailing margin yielded slightly more accurate predictions of THg concentrations in fin inner cores (first dorsal, pectoral, lower and upper caudal lobes) within *S. tiburo*. However, the dorsal fin trailing margin THg concentrations of *C. falciformis* could not significantly predict THg concentrations in fin inner cores. Overall, for *C. falciformis*, the fin trailing margins THg

concentrations did not explain a lot of the variation in other tissue samples. Total Hg concentrations from dorsal fin trialing margins were also able to significantly predict THg concentrations in dorsal axial muscle for *S. tiburo*. To determine THg concentrations throughout a shark's body for either of these species we recommend collecting muscle biopsy samples: this allows for not only the most accurate values and estimations for a majority of the tissue types, but also causes minimal harm to the animal or its products. In cases where the THg concentrations in the fin inner cores are of the most interest, fin trailing margin samples could be used, as seen in *S. tiburo*. This may be useful in instances where researchers are interested in THg concentrations in shark fins at fish markets, where only fins are available, and vendors will not allow them to damage the product (fin) with biopsy punches.

Overall, relationships between either dorsal fin trailing margins or dorsal axial muscle and the other tissue types were stronger for *S. tiburo* compared to *C. falciformis*, which could be attributed to the increased sample size for *S. tiburo*. Dorsal axial muscle THg concentrations were able to significantly predict THg concentrations in liver tissue for both species and the confidence intervals for the corresponding regression lines overlapped. This supports the possibility that the relationship between dorsal axial muscle and liver THg concentrations may not only be consistent within each species, but also across species. If this relationship holds across several shark species, muscle tissue could be used as an estimator for liver THg concentrations, which could be used as an indicator for overall shark health. Additional sampling for both of these species and the inclusion

of new shark species would help to verify if this relationship and the others reported here remain constant, not only within a species, but between them as well.

The potential impacts of Hg on the health of already declining shark populations are not well understood, but in other fish species high Hg concentrations can negatively impact both reproduction and general survival (Wiener & Spry 1996). Using muscle biopsy punches to test for Hg could provide both baseline estimates for Hg contamination in shark populations and information to researchers about Hg contamination within the sharks' ecosystem. The ability to estimate Hg concentrations throughout a shark's body from less invasive samples would also allow for greater monitoring of the Hg contamination in sharks, a larger variety of their products, and their environment. Mercury concentrations, even within the same species, can vary greatly depending on a shark's geographic location and this sampling method would provide a mechanism to compare variations in contamination and uptake of Hg between locations. This is of importance for both understanding the health of the different shark populations and for humans that are consuming products of sharks from different places.

MERCURY CONTAMINATION IN ELASMOBRANCH PRODUCTS IN COSTA RICA

Introduction

Mercury (Hg) is a naturally occurring element within the marine environment resulting from a variety of geological processes including erosion and volcanic emissions (Burger & Gochfield 2011; Maz-Courrau *et al*. 2012). However, in recent history Hg levels in the marine environment have been increasing due to anthropogenic sources including atmospheric releases from coal-fired power plants and coastal runoff from contaminated areas (Maz-Courrau *et al*. 2012). Increased Hg concentrations within the marine environment pose a threat to both the health of marine organisms and the human population that consumes these organisms (Burger & Gochfield 2011; Maz-Courrau *et al*. 2012).

Aquatic organisms can accumulate Hg through biotic and abiotic processes (Rand *et al*. 1995). Routes of exposure for Hg to marine species include: ingestion (primary source), inhalation, and dermal absorption (Solis *et al*. 2000; Moreno *et al*. 2005). The bioaccumulation of Hg in larger predatory fishes and its biomagnification through the marine food web has been well established (Rand *et al*. 1995; Gomes *et al*. 2004; Escobar-Sánchez *et al*. 2010). Sharks, which are long-lived and occupy high trophic levels, can bioaccumulate substantial concentrations of Hg in their tissues (Lyle 1984; Adams 2004; Branco *et al*. 2004). This raises concerns that Hg may be impacting the

health of various shark species and possibly reducing the commercial, recreational, and cultural value of sharks and the marine ecosystems they inhabit (Swain *et al*. 2007).

Few studies have looked at the possible health implications of acute and chronic exposure to Hg in sharks and rays. In teleost fish, Hg has been found to negatively impact reproduction (Matta *et al*. 2001; Hammerschmidt *et al*. 2002; Drevnick and Sandheinrich 2003; Klaper *et al*. 2006; Crump and Trudeau 2009), liver function, metabolism (Adams *et al*. 2010), and behavior (Webber & Haines 2003). Mercury has also been shown to cause neurological damage (Basu *et al*. 2007, 2009; Scheuhammer *et al*. 2008), damage to gills and olfactory organs (Jagoe *et al*. 1996; Oliveira-Ribeiro *et al*. 1996, 2000) and even mortality (Wiener & Spry 1996). Many shark species exhibit slow growth rates and low fecundities making them particularly vulnerable to the potential negative impacts caused by Hg contamination (Lyle 1984; Adams 2004; Branco *et al*. 2004). Many shark populations are experiencing population declines due to increased demand for their products, with an estimated one-quarter of all chondrichthyan fishes (sharks, skates, rays, and chimeras) being currently threatened with extinction (Dulvy *et al*. 2014). However, it is unclear whether increased levels of contaminants, like Hg, could further impair shark populations' abilities to rebound from these declines.

Human exposure to Hg primarily results from the consumption of fish products and thus a great deal of attention has been paid to Hg levels in high trophic level species such as tuna and swordfish (Rice *et al*. 2000; Escobar-Sanchez *et al*. 2010). The increasing global demand for shark fins and meat is directly negatively impacting shark populations and causing declines, but it may also be negatively impacting the human

population via exposure to potentially high levels of Hg in consumed sharks (Adams & McMichael 1999; Dulvy *et al*. 2008, 2014). Sharks represent 0.5-1% of total landings reported to the United Nations (UN) Food and Agriculture Organization (FAO), with an estimated 1,412,000 tons being caught for their meat or fins in 2010 (Worm *et al*. 2013). Given the amount of shark meat and fins being consumed, Hg from shark consumption could pose a serious health risks to humans, especially to fetuses, infants, and children (Burger & Gochfield 2011, 2012; Lopez *et al*. 2013). Mercury has been found to cause significant neurodevelopmental effects on these early developmental stages (Burger & Gochfeld 2011; Lopez *et al*. 2013). Therefore, it is critical to better understand the potential implications of shark consumption on human health (Swain *et al*. 2007).

Costa Rica is known to have a substantial domestic market for shark meat that is primarily sourced by its artisanal fishing fleet (Dent & Clarke 2015). However, the total amount of shark being sold is unknown (Dent & Clarke 2015). The objectives for this study included determining: 1) THg concentrations in sharks, rays, and reference fish species inhabiting Costa Rican waters, 2) the potential THg exposure to consumers of shark, ray, and fish meat from two open air markets in San Jose, Costa Rica, 3) whether these concentrations exceed recommended levels for human consumption, and 4) estimate THg concentrations in livers for the shark species *Carcharhinus falciformis* and *Sphyrna spp*. as a possible indicator for overall shark health within Costa Rica. Shark and ray samples had previously been identified down to species level using DNA barcoding allowing us to determine if difference in THg concentrations existed between species.

Methods

Sample Collection and Identification

We collected 170 shark, ray, and fish muscle tissue samples from Costa Rican central markets in San Jose (10 pescadarias) and Heredia (5 pescadarias) over a 5-day period in September 2014 (Figure 9). Shark meat being sold at pescadarias from these locations is generally sold as either a fillet or chuleta (a cross section of the shark including a single vertebrate), while ray meat is generally sold as fillets. On each visit, we only obtained one sample of each type of cut from each pescadaria to reduce to possibility of the same individual shark or ray being sampled twice. As a comparison to shark and ray meat, we also sampled a variety of fish fillets (i.e. corvina, vela) from pescadarias. Within Costa Rica markets corvina is generally represented by either a species of croaker or drum. While species in the family Lutjanidae (snappers) make up fish products labeled as pargo. Fish sold under the monikers "corvina" and "pargo" are in lower trophic levels compared to the sharks and ray sampled in this study. Therefore, lower concentrations of THg would be expected. Fish labeled as "dorado" in Costa Rican markets is generally Mahi-Mahi (dolphin fish), a large highly valued fish, that has directed long-line fisheries established for its capture. Vela on the other hand most commonly refers to various billfish species (i.e. sailfish, swordfish). Species representing "dorado" and "vela" are large predatory fish (secondary consumers) that generally feed on smaller fish allowing them to bioaccumulate larger quantities of Hg. Thus they are more similar to the shark species sampled in this study than the other reference fish. Each day, after the visit to the pescadaria, we took subsamples from each piece of meat using sterile 8mm biopsy punches and stored them in sterile 1.5 mL eppendorf tubes at -4◦ C.

Shark and ray samples included in this study had previously been identified down to species using DNA barcoding techniques outlined from Chapter 3.

Figure 9. Map of Costa Rica indicating location of the two markets (San Jose and Heredia) where muscle samples were collected for mercury (Hg) analysis.

Chemical Analysis

Before analysis, we recorded wet weights for each tissue sample then lyophilized and reweighed them for their dry weights. We analyzed whole samples (half for duplicates) for THg content by thermal decomposition, catalytic conversion, amalgamation, and atomic absorption spectrophotometry (DMA 80; Milestone, Monroe, CT, USA), according to U.S. Environmental Protection Agency (EPA) method 7473 (U.S. E.P.A. 2007). Concentrations of THg for all samples were analyzed on a dry weight basis to standardize comparisons and avoid the confounding factor of variable moisture

loss among samples. However, wet weight concentrations were calculated using known water loss values, based on wet and dry weights, for each lyophilized sample. For quality-assurance measures we included the analysis of two certified reference materials (marine sediment PACS-2, and lobster hepatopancreas TORT-3; National Research Council of Canada, Ottawa, ON) and system and method blanks. We initially ran three system and method blanks followed by the reference materials to verify system calibration. Then for every 10-15 samples we ran a single duplicate of the last sample run, followed by a system and method blank, the two reference materials, and finally by two more system and method blanks. Our recoveries averaged 96.01% ± 9.60% (n = 36) and 102.93% ± 8% (n = 15) for the certified reference materials and calibration checks respectively. We found the absolute relative percentage difference (RPD) for duplicates in all tissues combined averaged to be 7.75% ± 6.27%.

Statistical Analyses

We used R statistical software (3.1.2) for all statistical analyses (R Development Core Team 2014). To better meet the assumptions of standard parametric statistical tests and to normalize residuals we natural-log transformed all THg data. We used an analysis of variance (ANOVA) to determine if THg concentrations between different species of shark, ray, and fish were significantly different from one another. If THg concentrations between the species were significantly different we then used a Tukey's multiple comparison of means to determine which were significantly different from each other. Using linear regression equations described in Chapter 5 (*Sphyrna spp*.: y=1.65x -1.31, *Carcharhinus spp*.: y=1.63x -1.35) we determined THg concentrations in livers for

Carcharhinid and Sphyrnid shark samples based on the THg concentrations from their muscle tissue.

Results

Muscle THg Concentrations

Sphyrna zygaena had the highest THg concentrations followed by *Carcharhinus limbatus* and *Sphyrna lewini*. *Carcharhinus falciformis* muscle tissue, which represented the majority of the samples collected, had a mean THg concentration that exceeded levels found in all but one of the reference teleost fish groups sampled (Table 13). It should be noted that THg concentrations for the shark species *Mustelus lunulatus* and the fish groups of dorado, and vela, were represented by only one sample. Therefore, THg concentrations within these species could not be compared to other species and they do not represent a clear picture of exposure to THg from consumption of these species. *Dasyatis longa*, the only stingray species represented in the samples, had a mean THg concentration below all but one shark species (*Alopias pelagicus*) and was also lower than three of the four fish reference groups. Reference fish labeled as "corvina" had the lowest mean THg concentrations of all species analyzed. There was a significant difference in THg concentrations between species (excluding groups with only one sample) sampled ($F=23.07$, $p<0.001$). Using the Tukey's multiple comparison we found that the fish labeled as corvina had significantly lower THg concentrations than all other species sampled (Table 14). *S. zygaena* was found to have muscle THg concentrations significantly higher than all other species, excluding *C. limbatus*. *A. pelagicus* had significantly lower THg concentrations than *S. zygaena* and *C. limbatus*, but similar concentrations to all other species sampled (excluding corvina). Again THg

concentrations in *C. falciformis* were significantly lower than those in *C. limbatus* and *S. zygaena*, but significantly higher than those in *D. longa*. *C. limbatus* was found to have significantly higher THg concentrations than all other species, excluding *S. zygaena* and vela. *C. falciformis*, *S. lewini*, *A. pelagicus*, and the fish labeled as vela all had THg concentrations that were not significantly different from one another.

The mean THg concentrations for all shark and ray species sampled exceeded the US EPA guideline of 0.3ppm wet weight in seafood products (US EPA 2001). Only the mean THg concentration for corvina and the singe sample of pargo fell below this standard. However, only two shark species (*C. limbatus*, *S. zygaena*) and the single sample of *M. lunulatus* exceeded the more lenient 1ppm wet weight standard for seafood products of the US Food and Drug Administrations (FDA) (US FDA 2007).

Estimated Liver THg Concentrations

Using linear regression equations described in Chapter 5 we determined dry weight THg concentrations in livers for *C. falciformis*, *C. limbatus*, *S. lewini*, and *S. zygaena*, from their muscle sample THg concentrations. The two linear regression equations used were developed using samples from *C. falciformis* and *S. tiburo*. The equation developed from *C. falciformis* samples was used to estimate THg concentrations in *C. falciformis* and *C. limbatus*. These species have somewhat similar life history characteristics allowing the previously developed *C. falciformis* equation to provide rough estimates of liver THg concentration for the other two species. The *S. tiburo* equation from Chapter 5 study was used to estimate liver THg concentrations in *S. lewini* and *S. zygaena*, species within the same genus and with similar life histories. Based on

these equations estimated liver THg concentrations were highest in *S. zygaena*, followed by *C. limabtus*, *S. lewini*, and *C. falciformis* (Table 13).

Table 14. Total Hg concentrations (wet and dry weight) for muscle tissue of sharks, ray, and reference fish and estimated THg concentrations (dry weight) for livers of several shark species.

Species	n	Mean Muscle THg (ppm dry wt)	Mean Muscle THg (ppm wet wt)	Est. Mean Liver THg (ppm dry wt)
Alopias pelagicus	5	1.87 ± 0.64	0.36 ± 0.12	-
Carcharhinus falciformis	115	3.75 ± 4.39	0.76 ± 0.84	0.40 ± 0.87
Carcharhinus limbatus	6	11.89 ± 3.67	2.50 ± 0.78	1.75 ± 0.78
Sphyrna lewini	5	3.85 ± 3.41	0.81 ± 0.83	0.60 ± 0.79
Sphyrna zygaena	7	15.75 ± 2.11	3.50 ± 0.47	4.67 ± 1.03
Mustelus lunulatus	1	6.25	1.22	-
Dasyatis longa	16	1.99 ± 1.66	0.39 ± 0.33	-
Corvina	8	0.32 ± 0.26	0.06 ± 0.05	-
Vela	5	3.47 ± 0.64	0.79 ± 0.16	-
Dorado	1	2.8	0.52	-
Pargo	1	0.55	0.1	-

Table 15. Results of the Tukey's multiple comparison test comparing THg concentrations between species.

Species	*C. falciformis*	*C. limbatus*	*S. lewini*	*S. zygaena*	*D. longa*	Corvina	Vela
A. pelagicus	0.894	0.001*	0.974	<0.001*	0.998	<0.001*	0.678
C. falciformis	-	<0.001	1.000	<0.001*	0.016*	<0.001*	0.963
C. limbatus	-	-	0.039*	0.986	<0.001*	<0.001*	0.245
S. lewini	-	-	-	0.002*	0.578	<0.001*	0.996
S. zygaena	-	-	-	-	<0.001*	<0.001*	0.022*
D. longa	-	-	-	-	-	<0.001*	0.116
Corvina	-	-	-	-	-	-	<0.001*

*Indicates significant result.

Discussion

Overall, sharks found farther off the coast (i.e. silky shark) of Costa Rica had similar THg concentrations compared to other regions, but the patterns are species specific. However, sharks known to frequent more coastal waters (i.e. smooth hammerhead, blacktip shark) had THg concentrations higher than what has been described elsewhere. Higher THg concentrations in coastal shark species from Costa Rica could be due to deforestation, agricultural practices, topsoil erosion, and excessive use of fertilizers and agrochemicals, resulting in the increased runoff of chemicals like Hg into coastal habitats and causing increased exposure rates (Guzman & Jimenez 1992; Bastidas & Garcia 1999). The THg concentrations discovered in Costa Rican shark products are of concern for shark and human health. Below we discuss some of the species-specific data and address the potential impact on both shark and human populations.

Carcharhinus falciformis samples from this study had mean muscle THg concentrations similar to individuals from the same species along the Baja peninsula (3.40 ± 1.42 ppm dry wt) (Maz-Courrau *et al*. 2012). This species is known to be highly migratory and it is likely that individuals can easily traverse between these two adjacent locations. In chapter 5 we found that individuals of *C. falciformis* from the Atlantic coast of Florida (4.18 ± 3.33 ppm dry wt) and the Red Sea (2.41 ± 1.40 ppm dry wt) also to have comparable THg concentrations in their muscle tissue. This would indicate that individuals of this species are experiencing similar exposure rates to Hg, which is likely in such a highly migratory species like *C. falciformis*.

Sphyrna zygaena, which had the highest THg concentrations in this study, were recorded having considerably lower THg concentrations off the Baja peninsula (0.98 ±

0.92 ppm dry wt) (Maz-Courrau *et al*. 2012) and the Pacific coast of Mexico (0.025 to 7.62 ppm dry wt/ 0.005 to 1.93 ppm wet wt) (Escobar-Sanchez *et al*. 2010). However, this could be due to the fact that mercury concentrations can vary between individuals within a shark species due to differences in length (age) (Walker 1976; Hornug *et al*. 1993; Hueter *et al*. 1995; Lacerda *et al*. 2000; Endo *et al*. 2008), sex (Pethybridge *et al*. 2010), season (Escobar-Sánchez *et al*. 2010), and habitat type (Hornug *et al*. 1993; Hueter *et al*. 1995; McMeans *et al*. 2007) and prey types utilized could explain the variations seen in *S. zygaena* between these locations. The small sample size of S. zygaena in this study could also account for the difference seen between locations. More samples would be need to determine if this species continues to have elevated THg concentrations compared to other locations in the Eastern Pacific. However, Storelli *et al*. (2002) did report similar muscle THg concentrations (18.29 ± 0.03 ppm dry wt) in *S. zygaena* to this study in the Mediterranean Sea off the coast of Italy.

Carcharhinus limbatus in this study had the second highest mean THg concentration. This is considerably higher than THg concentrations (0.77 ± 0.71 ppm wet wt) found by Adams and McMichael (1999) in the same species off the Atlantic coast of Florida. *C. limbatus* is a generally coastal species utilizing bays and estuaries to feed, give birth, and use as nursing grounds for their young (Burgess & Branstetter 2009). Therefore, individuals of this species in Costa Rica are likely experiencing increased exposure rates compared to Florida, due to higher levels of contaminated runoff in Costa Rica as previously mentioned.

The mean THg concentrations for two shark species (*C. limbatus*, *S. zygaena*) and the single sample of *M. lunulatus* all exceeded the US Food and Drug Administrations (FDA) legal limit of 1 ppm wet wt for seafood products (US FDA 2007). However, the US Environmental Protection Agency (US EPA) has established its own guidelines tissue residue guidelines for Hg contamination in seafood, with a limit of 0.3 ppm wet wt (US EPA, 2001). This was exceeded by all shark and ray species sampled, with only the THg concentrations for corvina and pargo falling below it. Therefore, both *C. falciformis* and *D. longa,* which account for >70% of all shark meat and 100% of ray meat being sold in the markets sampled exceeded recommended contamination levels (Chapter 5). Based on our results patrons of these markets are likely to purchase and consume shark meat that is over twice the US EPA limit and ray meat that just exceeds it. However, to fully understand the health risk posed THg in the markets we need to gain a clearer picture of how much shark and ray meat is being consumed by individuals purchasing these products.

Shark meat, based on observations from sampling trips, appeared to sell at a much higher rate compared to "corvina" and other seafood products. This is likely due to the comparatively inexpensive nature of shark meat in these markets. Within Costa Rican markets sharks and rays are not generally labeled down to species. Therefore, attempting to avoid specific species with higher THg concentrations would be nearly impossible. Based on this it would be in the best interest of consumers to either avoid shark and ray products entirely or at least limit consumption of these products as much as possible. This is especially true for young children and pregnant women. Our data suggests that

customers are safest consuming fish labeled as “corvina” and possibly “pargo” (more testing needed). The other reference fish species (dorado and vela), pose a similar threat for Hg contamination as the sharks and rays sampled in these markets. However, due to the cheaper price of shark and ray meat dissuading customers from purchasing these products could prove difficult. Since shark and ray products potentially pose a serious health risk to the Costa Rican population, the Costa Rican government may also need to take steps to better regulate the products being sold in these markets. This could include posting cautionary signage about the potential health risks associated with Hg exposure from specific seafood products, to beginning to test and regulate products for Hg levels above what is safe for human consumption.

There is also concern over the possible impacts Hg may be having on the health of various shark species and populations, particularly for those species already with conservation concerns (Swain *et al*. 2007). Currently it is unclear how Hg may impact shark physiology and whether exposure to Hg could be impacting population declines, but it has been shown to have several deleterious effects on teleost fish including mortality. This study did not attempt to determine the potential physiological impacts of Hg contamination on sharks, but rather begin the process of monitoring Hg concentrations found in sharks and their products in Costa Rica. Monitoring liver Hg concentrations in shark are of particular importance because it is believed that sharks, like mammals, may be able to protect against Hg toxicity through the interaction of Hg and selenium (Se) in their livers (Cardellicchio *et al*. 2002; Endo *et* al. 2005; Branco *et al*. 2007). Previous studies (Branco *et al*. 2007; Nam *et al*. 2011) have found shark livers to

contain disproportionately more inorganic Hg than organic Hg when compared to their muscle tissue. Organic Hg (i.e. Methylmercury), the toxic form of Hg, is thought to bind to Se in the liver, which converts it into its inorganic form (Nam *et al*. 2011). This then allows it to be more easily excreted from the body (Nam *et al*. 2011). However, for Se to continue to help with the detoxification of Hg there must be at least a 1:1 molar ratio of the two elements. Once the 1:1 molar ratio of Hg and Se is exceeded continually higher levels of Hg could accumulate in their livers, and other tissues, without the ability for detoxification (Das *et al*. 2000; Storelli & Marcotrigian 2002; Endo *et al*. 2002, 2005, 2006). Using regression equations (*Sphyrna spp*.: y=1.65x - 1.31, *Carcharhinus spp*.: y=1.63x - 1.35) to predict liver THg concentrations from muscle concentrations we found *S. zygaena* and *C. limbatus* to have the highest potential liver concentrations. In fact they were three times higher than liver concentrations for *C. falciformis* and *S. lewini*. *Sphyrna zygaena* also had estimated mean liver THg concentrations higher than those previously described in sharks from the same genus in Chapter 5. The exceptionally high THg concentrations found in the livers of *S. zygaena* in this study could indicate they have exceeded this 1:1 molar ratio of Hg/Se and no longer have the ability to detoxify and eliminate this contaminant. This would have potential health implications for both the sharks and for consumers of their products who may experience increased exposure levels.

The mean liver THg concentration for *C. falciformis* in Costa Rica was more than four times less than the combined mean THg concentrations from the Red Sea and Atlantic coast of Florida (2.10 ± 3.64 ppm dry wt.)(Chapter 5). *Sphyrna lewini*, a species

of conservation concern, also had lower estimated liver THg concentrations compared to individuals of *S. tiburo* (1.82 ± 2.94 ppm dry wt.), sampled from the coast of South Carolina (Chapter 5). This could be due to higher levels of industrial runoff contaminating coastal waters in South Carolina where *S. tiburo* inhabits compared to the pelagic water where the *S. lewini* from this study were likely caught. Further research is still needed into the possible negative impacts of Hg contamination in shark livers and muscle to determine the potential implications for the sharks in Costa Rica and elsewhere. Since Se may mitigate toxicity of Hg, it has also been suggested to look further into the molar ratios of Hg/Se in edible muscle and livers, as this may be an important criterion for assessing the true health risk posed by Hg to consumers (Nam et al. 2011).

Conclusion

Overall, all shark and ray muscle THg concentrations exceeded recommended levels established by the US EPA. Two of the four (dorada, vela) reference fish groups also exceeded these limits, while corvina and pargo were found to have safe concentrations of THg. Previous research found *C. falciformis* to account for ~70% of all shark meat being sold in markets in Costa Rica. This species was found to have a mean THg concentration over twice the EPA recommended level and should be cause for concern for consumers of shark products in these markets. With shark meat being comparably inexpensive compared to other fish in these markets they appear to be consumed in relatively larger amounts and it will likely be up to the Costa Rican government to better regulate these products and provide a safe food source for their population. This study also found one species of shark sampled to have relatively high

concentrations of THg in their livers compared to other species, which could indicate a shutdown of the livers detoxifying properties for mercury. This could pose a threat for both the Costa Rican populations of this species and consumers of their products as Hg levels in these species could grow exponentially. It is our recommendation based on these findings that continued testing of shark, ray, and fish products in Costa Rican markets is needed to better determine the safety of these products and that the government should strongly consider at least providing consumers with cautionary information on the consumption of shark and ray products.

CONCLUSIONS

Currently, no catch limits or size restrictions exist in Costa Rica for elasmobranchs, which can be partially attributed to a lack of species-specific catch data for both artisanal and industrial fisheries (Camhi *et al*. 2008; Salas *et al*. 2011; Whoriskey *et al*. 2011; Trujillo *et al*. 2012). The fisheries management organization, INCOPESCA, which is responsible for collecting this data has proven to be ineffective at regulating and enforcing catch rates for many species, including elasmobranchs, due to small budgets and a lack of qualified personnel, which has resulted in non-compliance by many fishermen (Salas *et al*. 2011). The development of effective shark conservation measures in Costa Rica will also benefit from information on contamination levels of shark products, and whether potential support currently exist for shark conservation within Costa Rican artisanal fishing communities. Therefore, in this study we attempted to fill in these gaps by determining: 1) species composition and abundance in artisanal and industrial fisheries; 2) artisanal fishermen's knowledge of sharks, their perceptions of local fisheries impacts on shark populations, and levels of potential public support for various conservation measures (i.e. MPAs); and 3) and mercury (Hg) contamination levels in shark meat being sold for human consumption at markets.

Species abundance and composition in markets

Large open-air markets within the central valley of Costa Rica sell seafood products predominantly landed by pelagic fisheries operating off the Pacific coast. The characteristics of these pelagic fisheries vary greatly from the small to mid-scale artisanal fisheries, which are scattered along the coastline. Specifically, pelagic fisheries generally consist of larger vessels; many foreign flagged, operating well offshore, while small to mid-scale artisanal fisheries operate small vessels in close proximity to the coastline. As a result of the differences between these two fisheries, the elasmobranch species composition and abundance varies greatly between them. Within the open-air markets in Costa Rica's central valley, silky sharks dominate the elasmobranch products being sold. The silky shark is listed by the IUCN as "threatened" globally and "vulnerable" in the Eastern Tropical Pacific yet it is still one of the, if not the most, exploited shark species in pelagic fisheries. Within Costa Rica it is by far the most important elasmobranch species for pelagic fisheries and the domestic shark meat market. However, despite its high exploitation rates no protection exists for this species inside of Costa Rican waters. Recently, Costa Rica declined to support a proposal at the Inter-American Tropical Tuna Commission (IATTC) that would have required the release of silky sharks landed within the pelagic fisheries that supply these markets. A common issue with any attempts to regulate large-scale pelagic fisheries in Costa Rica can be attributed to fishing rights purchased by foreign investors/ fishing fleets. China and Taiwan, which operate vessels within Costa Rican waters, both have vested interests in a continued landing of silky sharks to supply fin markets that contain high percentages of this species (Clarke *et al*. 2006, Dent & Clarke 2015). It is also likely these foreign vessels sell the remaining silky

shark carcasses to fish vendors operating domestically in areas like San Jose (Dent & Clarke 2015).

When comparing species composition data from the markets and observer programs on long-line vessels in Costa Rican waters, we see that fewer elasmobranch species are actually sold in markets than are landed by the fishing vessels (Whoriskey *et al*. 2011; Dapp *et al*. 2013). This is likely due to low market values or catch rates for many of the elasmobranch species being landed, resulting in these species being discarded. Therefore, DNA barcoding of markets does not identify all the elasmobranch species facing fishing pressure in Costa Rica. However, species that are sold in these markets are likely the facing higher levels of fishing pressure, and are therefore at the greatest risk of overexploitation. As a result of market sampling not accounting for all species being landed in pelagic fisheries, the inability of observer programs to monitor many different vessels at a given time, and the misidentification by observers of already processed shark species with similar morphological characteristics. We recommend the use of DNA barcoding at landing docks to provide a more accurate representation of all species of elasmobranch currently facing fishing pressure in Costa Rica. This will help determine current catch rates and possible populations declines for all elasmobranchs being landed and help potential growing markets for new elasmobranch species. This will require significant support from the Costa Rican government, as gaining access to pelagic vessels, particularly foreign flagged ones, can be difficult and dangerous. Costa Rican pelagic fisheries and open-air markets utilize a variety of different elasmobranch species including ones that are currently overexploited and threatened with extinction. Therefore,

the Costa Rican government needs to better monitor their pelagic fisheries and markets by collecting species-specific data. Here we have shown DNA barcoding to be an effective technique for achieving this goal.

Based on observations during this research the possibility of a growing market for stingray products also exists. Previously, observers on longline vessels noted stingrays as being discarded back into the ocean (Dapp *et al*. 2013). However, recently it appears more vendors in the Costa Rican markets are beginning to sell stingray meat. Stingrays can be more vulnerable to increased fishing pressure than other elasmobranch species, which has resulted in rays representing 5 of the 7 most threatened chondrichthyan families (Dulvy *et al*. 2014). Therefore, strict monitoring for species preference of fishers and vendors of stingray products within the markets should be considered to determine species that could be undergoing the greatest fishing pressure and potential overexploitation. The continued use of DNA barcoding would be recommended for monitoring of stingray products in these markets.

Hg contamination in shark products

Based on the Costa Rican government's stance on the IATTC proposal to protect silky sharks, their relationships with foreign governments, and the lack of current legislation protecting elasmobranchs it is unlikely they would support new legislation for their conservation in pelagic fisheries. Therefore, other methods may be necessary to help reduce the exploitation of elasmobranch populations. This includes attempting to reduce the domestic demand for shark products. Elasmobranch products obtained from markets around San Jose were tested for mercury (Hg) contamination. The mean total mercury

(THg) concentrations for all elasmobranch species tested exceeded US EPA recommended limits for human consumption. However, consumers at these markets are not provided information on the potential health risks associated with elasmobranch products. Since elasmobranch products are also far less expensive than other fish in these markets, this increases their risk. Therefore, a program aimed at educating the public that frequents these markets on the dangers of Hg, and the potential dosages they may be receiving by consuming elasmobranch products, could reduce overall demand for elasmobranch products. Such a program could be similar to the "Seafood Watch" program run by the Monterrey Bay Aquarium, which provides recommendation for safe and sustainable seafood products. Currently, the Costa Rican non-governmental organization "PRETOMA" is running a "Yo no como Tiburon" (I don't eat shark) campaign and this Hg data could help in convincing more people not to eat sharks, and thus reduce demand and exploitation of threatened stocks.

As previously stated, foreign nations have a vested interest in the pelagic fisheries in Costa Rica. Shark fins also still represent an important export to many of these countries. Currently, anti-finning laws prohibit vessels in Costa Rican waters from removing fins and discarding the bodies at sea but sharks can still have their fins removed once onshore. Therefore, reducing demand for elasmobranch products in Costa Rica markets will not necessarily reduce fishing pressure on pelagic elasmobranch populations or their retention in these fisheries. However, with current global campaigns aimed at stopping the shark fin industry, simply reducing demand for other products domestically could be very helpful in the near future. Again, a campaign highlighting the potential

health risks associated with Hg in elasmobranch products could be useful in reducing demand for these products. However, the goal should be to educate the public with accurate information and not employ scare tactics that could backfire on any potential conservation campaigns.

Species abundance and composition in artisanal fisheries

Elasmobranch species composition and abundance differed significantly between small to mid-scale artisanal fisheries and the open-air markets in Costa Rica. Our results support the hypothesis that small to mid-scale artisanal fisheries are not responsible for supplying the markets in the central valley with elasmobranch products. Therefore, separate species –specific monitoring of artisanal fisheries is necessary. The variation in species composition and abundance between the artisanal fisheries and markets (pelagic fisheries) also highlights the need for the development of differing fisheries management plans for the conservation of elasmobranch between these two distinct fisheries.

Immature scalloped hammerheads represented the most abundant species in the small to mid-scale artisanal fisheries sampled. The scalloped hammerhead is currently listed as "endangered" by the IUCN and the Pacific coast of Costa Rica is a known nursing area for this species. With over 3000 small to mid-scale artisanal fishing vessels operating on this coastline the potential for significant negative impacts on scalloped hammerhead populations in the Eastern Tropical Pacific is high. Currently, Costa Rican fishing licenses do not limit the quantity or species of fish that can be caught by fishermen. This allows for overexploitation of fish populations that could potentially lead to population collapses of various species and negatively impact the continued viability

of these fisheries. However, effectively managing artisanal fisheries with ~ 3000 licensed, and countless unlicensed vessels, can prove to be very difficult due to the costs associated with enforcement. Therefore, working with artisanal fishing communities to educate them on the benefits of new legislation that could ensure the sustainability of their fishery and to create new legislation supported by these communities is of the utmost importance.

Potential support for elasmobranch conservation

It is important to understand that in small to mid-scale artisanal fishing communities in Costa Rica that any reduction in landings of target species directly impacts that fishermen's ability to feed their family. If any new regulations protecting elasmobranchs are to gain support from local fishing communities, which could reduce the need for continuous enforcement, they must have as little impact as possible on current catch rates of target species. This is evident in the results from this research where almost all fishermen surveyed supported potential new shark conservation legislation. However, as prospective legislation (i.e. creation of marine protected areas) increasingly restricted current fishing practices, support nearly dropped to zero. Some change to the fishery will be necessary if the protection of elasmobranchs is to be successful, and most fishermen indicated they are willing to work with researchers to figure out a solution.

Based on our findings the majority of artisanal fishermen are aware that shark populations are declining, which has resulted in stronger beliefs in the need for protection. However, they do not view these declines as a direct result of their fisheries. This could be caused by the current lack of understanding by the fishermen about how

sharks are biologically different from other more fecund fish species in their ability to replenish their populations. For instance, despite witnessing declines in the population approximately half of the fishermen surveyed didn't think they were catching too many sharks. Therefore, a first step in the process of working with fishermen to develop new legislation to protect elasmobranchs in Costa Rica is to develop an understanding of the importance of these species to the environment (many see them as harmful to the fishery), how they differ from their target species, and the impacts their fishery is having on elasmobranch populations. This would hopefully further increase support - even for legislation that would be slightly invasive to the fishery. The most efficient mechanism for distributing this information would be to meet with local fisheries organizations in the various artisanal fishing communities. Due to there being so many artisanal fishermen in Costa Rica, identifying particularly vulnerable areas to distribute the above mentioned information to, would prove to be most effective.

Legislation that might garner the most support from fishermen, and provide adequate protection for elasmobranchs, would be the creation of coastal MPAs and restrictions on fishing methods. Support for MPAs varied greatly depending on the restrictions they enforced on the current fishing grounds. The creation of MPAs adjacent to current fishing grounds may avoid confrontation with fishermen, while potentially still protecting important habitat. It is uncertain what level of restriction would be supported for these fishery adjacent areas (i.e. no take, gear restrictions), but no take zones would obviously be most effective. The ability of MPAs to help increase population sizes of local species should also be emphasized to the fishermen.

Possibly the least invasive legislation that could be implemented on these communities to protect elasmobranchs would be gear restrictions. Approximately half of the fishermen indicated they would support gear restrictions on their fishing grounds. Based on our results, the composition and relative abundances of shark species caught by fishermen using gillnets was the same as by those using long-lines. However, there were far fewer fishermen using gillnets, meaning more sharks were caught per fishermen using this method and scalloped hammerheads appeared to be more susceptible to gillnets as well. Gillnetting also removes the possibility of releasing sharks alive. We recommend banning the use of gillnets in coastal areas to significantly reduce the number of elasmobranchs per fishermen being landed. However, it should be noted that as production in the fisheries decline due to various reasons, many fishermen would potentially revert back to gillnetting despite the ban. For example, within the Coyote/Bejuco community a restriction on gillnets was implemented by the local fisheries organization but as the fisheries yields declined, fishermen became desperate and started using gillnets again. Despite the possibility of fishermen reverting back to gillnets when fishing is less productive, removing them for any portion of the year would be advantageous to the conservation of elasmobranch populations.

Between the two communities surveyed, bottom long-lines were the most commonly used fishing method. This method allows for the possibility of sharks to be released alive if handled properly. The majority of fishermen indicated they would be willing to use new fishing gear if it was provided to them. “Circle hooks” have been shown to be effective at allowing increased survival rates of hooked elasmobranchs

(Swimmer *et al*. 2011). Therefore, by providing fishermen with circle hooks and training for the safe removal of these hooks from elasmobranchs could significantly reduce elasmobranch bycatch, particularly of the endangered scalloped hammerhead, in these fisheries. In conversations with the fishermen they indicated that the sharks have no real value to their fishery and they would be willing to stop catching them if possible. Therefore, circle hooks provide a potential solution to move toward that goal.

Based on the history of marine conservation in Costa Rica getting the government to create new legislation to protect marine species, including elasmobranchs, could be difficult. Reducing demand for elasmobranch products from pelagic fisheries is a plausible method at trying to reduce the number of elasmobranchs being landed. Creating new legislation protecting elasmobranchs in artisanal fisheries in Costa Rican has the benefit of not having to account for foreign interests. Researchers could also work with local fisheries organizations to develop new self-implemented regulations that do not require the involvement of the federal government. Current issues related the shark trade (fisheries and markets) in Costa Rica exists. However, here we have also presented several possible solutions to these issues that have potential to help change shark conservation in this country. It is not too late for effective management to mitigate current shark population declines as well as properly regulate for potential contamination of sharks and the humans with toxic chemicals.

APPENDIX

Social Survey Spanish Version

1) ¿Qué edad tiene Usted? _____

2) ¿Cuántos años de estudio ha completado? _____

3) ¿Aproximadamente hace cuanto tiempo está pescando con licencia comercial?
5 años o menos 6 a 10 años 11 - 15 años 16 - 20 años 21 - 25 años
26 - 30 años más de 30 años

4) ¿Especifique el cargo que tiene Usted en el bote pesquero en el que trabaja actualmente:

---

5) ¿Qué tipos de artes de pesca se utiliza? (Marque todo lo que corresponden)
palangre de superficie redes línea de mano red de arrastre trasmallo
palangre demersal/ línea de fondo Otros:_____

6) ¿Cuáles son las especies objetivo de su barco?
_____

7) ¿A qué distancia de la costa suelen viajar a pescar?
0-10 kilómetros 11-20 kilómetros 21-30 kilómetros 31-40 kilómetros
Más de 41 kilómetros No estoy seguro

8) ¿Cuántas horas pesca normalmente a la semana?
A menos de 10hrs 10-20hrs 21-30hrs 31-40hrs 41-50hrs Más de 50 horas

9) ¿Suele atrapar alguna de las siguientes especies de tiburones? (Encierra en un círculo todas las que corresponden)
Tiburón martillo Sedoso Tigre Azul Cazón
Perro (punta blanca oceánica) Punta Negra Mamón Toro

Punta Blanca del arrecife Zorra

10) ¿En general, se utiliza los tiburones que se capturan en la pesca?
No Sí

Si es así, ¿por qué? (Marque todo lo que corresponda)
Para vender su carne Para vender / exportar sus aletas
Para consumo personal Otros

11) ¿En cuales de los meses mencionados a continuación suelen atrapar la mayor cantidad de tiburones (Marque todo lo que corresponda)
Enero Febrero Marzo Abril Mayo Junio Julio
Agosto Septiembre Octubre Noviembre Diciembre
No estoy seguro Igual cantidad durante todo el año

12) ¿Usted dirige su pesquería directamente hacia los tiburones?
No Sí

13) ¿Cuántos tipos / especies de tiburones cree usted que hay en las aguas de Costa Rica?
Menos de 5 6-10 11-20 21-40 41-60 Más de 60

14) Cree Usted, que la mayoría de los tiburones se aparean y reproducen dentro de su primer año de vida al igual que muchas otras especies de peces?
No Sí

15) ¿Cree Usted, que los tiburones, en general, producen una pequeña cantidad de juveniles (menos de 20) por temporada de cría en comparación con otros peces?
No Sí

16) ¿Cree Usted, que los tiburones son muy vulnerables a la sobrepesca?
No Sí

17) Muchas de las especies de tiburones que se encuentran en Costa Rica utilizan sus aguas costeras para aparearse y criar a sus pequeños?
Verdadero Falso

18) Tiburones en general viven sólo unos pocos años (menos de 5) al igual que muchas otras especies de peces.
Verdadero Falso

19) El aleteo de tiburón está prohibida en Costa Rica.

Verdadero Falso

20) ¿Usted ha visto más, menos o el mismo número de tiburones en la pesca en los últimos años?
Igual Más Menos

21) ¿Cree que el número de tiburones en aguas de Costa Rica ha crecido, disminuido, o se encuentra estable basado en lo que ha observado durante su pesca?
Estable Aumento Disminución

22) ¿Usted cree que es importante proteger a los tiburones?
No Sí

23) ¿Se necesitan mejores leyes para proteger a los tiburones en Costa Rica?
No Sí

24) ¿Ve a los tiburones útil o perjudicial para su pesquería?
No Sé útil Nocivo

25) ¿Usted cree que demasiados tiburones están siendo capturados en CR?
No Sí

26) ¿Usted cree que la pesca de tiburones debe continuar o que se deben proteger?
Debe ser protegidos Deben pescarlos

27) ¿Estaría dispuesto a apoyar la conservación de tiburones en Costa Rica?
No Sí

28) Si usted contestó si a la pregunta anterior:

¿Todavía apoyaría la conservación de tiburones en Costa Rica si resultara en que usted tenga que cambiar algunas de sus prácticas de pesca actuales?
No Sí

29) ¿Apoyaría la creación de áreas marinas protegidas que protejan a los tiburones?
No Sí

30) Si su respuesta es "no" a la pregunta # 29, por favor explique por qué no apoyaría la formación de áreas marinas protegidas que protejan a los tiburones.

_____
_____
_____
_____

31) Si su respuesta es "si" en la pregunta # 29, seguiría apoyando la formación de un área marina protegida si esta incluyera partes de los caladeros de pesca actuales?

No Sí

32) Si su respuesta es "si" en la pregunta # 29, seguiría apoyando la formación de un área marina protegida si en esta se prohibiera el uso de ciertos tipos de artes de pesca?

No Sí

33) Si su respuesta es "si" en la pregunta # 29, seguiría apoyando la formación de un área marina protegida si en esta prohibieran todos los tipos de pesca en esa zona durante ciertas épocas del año?

No Sí

34) Si su respuesta es "si" en la pregunta # 29, seguiría apoyando la formación de un área marina protegida si en esta se prohibieran todos los tipo de pesca dentro de la zona protegida durante todo el año?

No Sí

35) ¿Sería más probable que apoyen la legislación que protege los tiburones si los pescadores se incluyeron en el proceso de la toma de decisiones?

No Sí

36) Si tuvieras que comprar la, ¿estaría usted dispuesto a utilizar otro tipo de artes de pesca que tiene menos posibilidades de atrapar o herir a los tiburones?

No Sí

37) Si se le proporciona, ¿estaría usted dispuesto a utilizar otro tipo de artes de pesca que tiene menos posibilidades de atrapar o herir a los tiburones?

No Sí

38) ¿Estaría dispuesto a parar la captura de determinadas especies de tiburones?

No Sí

39) ¿Estaría dispuesto a parar la captura de todas las especies de tiburones?

No Sí

40) ¿Estaría dispuesto a trabajar con investigadores y funcionarios públicos para ayudar

a proteger a los tiburones?
No Sí

Social Survey English Version

1) How old are you? _____

2) How many years of schooling have you completed?_____

3) Approximately how long have you been fishing commercially?
5 years or less 6 to10 years 11to15 years 16 to 20 years
21 to 25 years 26 to 30 years more than 30 years

4) What is your job on the fishing vessel on which you currently work?
_____

5) What types of fishing gear do you use? (Mark all that apply)
Pelagic Longline Mid-water trawl Hand-lines Bottom Trawl Gillnet
Demersal Longline/ Bottom Line Others
_____

6) What are the target species of your fishery?
_____

7) How far from shore do you generally travel to fish?
0-10km 11-20km 21-30km 31-40km More than 41km Not sure

8) How many hours do you spend on the water fishing in an average week?
Less than 10hrs 10-20hrs 21-30hrs 31-40hrs 41-50hrs More than 50hrs

9) Do you regularly catch any of the following shark species in your fishery? Circle all that apply.
Scalloped Hammerhead Silky Tiger Blue Sharpnose
Oceanic Whitetip Blacktip Smooth Hound Bull Whitetip Reef

10) Do you usually keep the sharks that you catch in your fishery?
Yes No

If yes, why? (Mark all that apply)

To sell their meat To sell/export their fins

For personal consumption Other

11) Which of the months listed below do you generally catch the most sharks (Mark all that apply)?

January February March April May June
July August September October November December
Equal amount throughout the year Not Sure

12) Do you directly target sharks in your fishery?

Yes No

13) How many types/species of sharks would you estimate there are in Costa Rican waters?

Less than 5 6-10 11-20 21-40 41-60 More than 60

14) Most sharks will breed and reproduce within their first year of life like many other fish species?

True False

15) Sharks in general produce a small number (less than 20) of young per breeding season compared to other fish?

True False

16) Sharks are very susceptible to overfishing.

True False

17) Many of the shark species found in Costa Rica use its coastal waters for mating and raising their young.

True False

18) Sharks in general live for only a few years (less than 5) like many other fish species.

True False

19) Shark finning is banned in Costa Rica.

True False

20) Have you seen more, less, or the same number of sharks in your fishery in recent years?

More Less Same

21) Do you think the number of sharks in Costa Rican waters is increasing, decreasing, or stable based on what you have observed in your fishery?

Increased Decreased Stable

22) Do you think it is important to protect sharks?
Yes No

23) Are laws to better protect sharks needed in Costa Rica?
Yes No

24) Do you see sharks as being helpful or harmful to your fishery?
Helpful Harmful I Don't Know

25) Do you think too many sharks are currently being caught in CR?
Yes No

26) Do you think fishing for sharks should continue or that they should be protected?
Fish for them Protect them

27) Would you be willing to support shark conservation in Costa Rica?
Yes No

28) If you answered yes on the previous question:

Would you still support shark conservation in Costa Rica if it resulted in you having to change some of your current fishing practices?
Yes No

29) Would you support the formation of marine protected areas that would protect sharks?
Yes No

30) If you answered "no" on question #29 please explain why you would not support the formation of marine protected areas that would protect sharks.

_____
_____
_____
_____

31) If you answered "yes" on question #29, would you still support the formation of a marine protected area if it included portions of your current fishing grounds?
Yes No

32) If you answered "yes" on question #29, would you still support the formation of a marine protected area if it restricted certain types of fishing gear?
Yes No

33) If you answered “yes” on question #29, would you still support the formation of a marine protected area if it completely restricted all types of fishing in that area to only certain times of the year?

Yes No

34) If you answered “yes” on question #29 would you still support the formation of a marine protected area if it restricted all types of fishing within the protected area during the entire year?

Yes No

35) Would you be more likely to support legislation protecting sharks if fishermen were included in the decision making process?

Yes No

36) If you had to purchase it, would you be willing to use a different type of fishing gear that is less likely to catch or injure sharks?

Yes No

37) If you were provided it, would you be willing to use a different type of fishing gear that is less likely to catch or injure sharks?

Yes No

38) Would you be willing to stop catching certain species of sharks?

Yes No

39) Would you be willing to stop catching all species of sharks?

Yes No

40) Would you be willing to work with researchers and public officials to help protect sharks?

Yes No

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