Using Genetic and Chemical Techniques to Aid Elasmobranch Conservation

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Declaration

I declare that I have written and produced all the contents of this PhD thesis unless otherwise stated.

Signed: 

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Abstract

Elasmobranchs encompass some of the most threatened species on our planet, with their biggest threat being overfishing, either when they are targeted as a group themselves (i.e., for fins and meat) or when they are incidentally caught as bycatch. Elasmobranch products are sold and consumed all over the world, with threatened and CITES-listed species dominating the trade. Conservation and management of elasmobranch populations requires a multidisciplinary approach and to address some of these conservation issues, this thesis uses genetic and chemical techniques to aid elasmobranch conservation. In Chapter 2 a total of 85 studies were included in a systematic review, which found that 11.3% of samples were mislabelled and 10.1% labelled using umbrella terms. Species listed as threatened made up 48.7% of mislabelled elasmobranchs and 53.7% of species labelled using umbrella terms. In Chapter 3, I developed a paper-based Lab-on-a-Chip (LOC) for the identification of three threatened and CITES-listed sharks (bigeye thresher, pelagic thresher and shortfin mako shark) that incorporated DNA amplification and visualisation using Loop Mediated Isothermal Amplification (LAMP). I was able to successfully identify the three sharks, where when target species where present there was a simple colour change from pink to yellow. In Chapter 4, a total of 176 studies were included in a systematic review of pollutants in elasmobranchs. The highest concentrations of pollutants were found in sharks occupying top trophic levels (Carcharhiniformes and Lamniformes). A human health risk assessment carried out in both Chapter 4 and 5 identified that humans consuming shark as little as once a week are exposed to more mercury than is recommended by the US EPA. This not only poses a risk to local fishing

communities and international consumers of shark-based products but also those subject to the widespread mislabelling of elasmobranch products. Overall, this thesis has helped to address a significant gap in our understanding of mislabelling and pollutant levels in elasmobranchs. Additionally, the LOC for identifying CITES-listed sharks has promising implications for shark conservation efforts as it has the potential to enhance the monitoring of trade in protected and threatened shark species.

Key words: *Elasmobranchs; Conservation; Genetics; Pollutants; Lab-on-a-Chip (LOC); Threatened*

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Chapter 1

1 General Introduction

1.1 Background

The planet is currently undergoing its sixth mass extinction where we are seeing the alarming loss of species at an exponential rate. This rapid loss in biodiversity has mainly been caused by human activities such as deforestation, intense farming and agricultural activities, climate change, introduction of non-native and invasive species, climate change, overexploitation of natural resources, pollution, and habitat fragmentation (Derraik, 2002; Sanderson et al., 2002; Islam and Tanaka, 2004; McKee et al., 2004; Cardinale et al., 2012; Dulvy et al., 2014, 2021; Patel and Haldar, 2022).

Marine ecosystems are particularly vulnerable to human activities, with climate change, pollution and overfishing being the main driver causing rapid declines of marine populations. The overexploitation of fisheries has led to the depletion of numerous fish stocks, disrupting the balance of marine ecosystems. Overfishing can be when species are targeted and incidentally caught (e.g. bycatch), further contributing to the decline in fish, sea turtle, marine mammal (cetacean), and elasmobranch (sharks, rays, and skates) populations. This loss of biodiversity in the ocean not only threatens marine life but also negatively impacts human livelihoods, as millions of people depend on fisheries for food as well as economic security.

1.2 Evolution and Biology of Elasmobranchs

Chondrichthyans are a diverse group of cartilaginous fish that include sharks, rays, skates, and chimaeras. They have existed on our planet for over 420 million years, making them one of the oldest and most ecologically diverse vertebrae lineages on earth (Ebert et al., 2013; Dulvy et al., 2014). Elasmobranchs, comprising of sharks (Selachimorpha), rays and skates (Batoidea), are a particularly diverse ecological group that can be found in every ocean across the world, from shallow coastal waters to deep seas (Lucifora et al., 2011; Ebert et al., 2013). There are over 1,200 known species of elasmobranchs made up of approximately 500 species of sharks and 700 rays and skates (Ebert et al., 2013). Sharks, particularly those occupying positions at the top of the food chain in tropical climates have been shown to exert top-down control of prey species, including mesopredators such as smaller sharks, rays and skates (Myers et al., 2007; Ferretti et al., 2008; Baum and Worm, 2009; Prugh et al., 2009; Barría et al., 2017). Many sharks exhibit traits like that of large bodied cetaceans where they have reduced number of offspring, long gestation periods and late maturity (Reynolds et al., 2005; Field et al., 2010; Simpfendorfer et al., 2011). It's a combination of these traits, as well as their high trophic level and migratory behaviour and their relatively low economic value that make them more susceptible to anthropogenic threats such as overfishing and bycatch, pollution exposure, habitat loss and degradation and climate change (Tiktak et al., 2020; Pacoureau et al., 2021).

1.3 Conservation Status of Elasmobranchs

Despite their evolutionary success, many species of elasmobranchs are now facing significant threats from human related activities. It is estimated that over one third (37.5%) of chondrichthyan (sharks, rays, skates and chimaeras) are currently

threatened with extinction, where 21% of rays and skates, and 17% of sharks are classified as threatened according to the IUCN Red List. The IUCN Red List threatened categories encompass Critically Endangered (CR), Endangered (EN) and Vulnerable (VU). The true number of threatened elasmobranchs is likely to be higher as many elasmobranchs are listed as Data Deficient (DD) (*n* = 438) or their population has not yet been assessed by the IUCN, making their true status unknown (Dulvy et al., 2014, 2021; Gray and Kennelly, 2018; Tiktak et al., 2020; Niedermüller et al., 2021; Pacoureau et al., 2021; Cardeñosa et al., 2022; IUCN, 2023). Dulvy et al., (2021) estimated that the current rate of extinction for Chondrichthyes's is potentially 24 extinctions a year. Some species of elasmobranchs, such as oceanic white tip (*Carcharhinus longimanus*), thresher sharks (*Alopias* spp.), smooth, great, and scalloped hammerheads (*Sphyrna* spp.) and shortfin mako sharks (*Isurus oxyrinchus*) have experienced population declines of over 90% in the last few decades and are now at risk of extinction (Baum et al., 2003; Dulvy et al., 2008; Ferretti et al., 2008; Clarke et al., 2013; Cortés et al., 2015; Pacoureau et al., 2021).

Currently 154 elasmobranch species are listed in either Appendix I or II of The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (CITES, 2023a). CITES is "an international agreement between governments. Its aim is to ensure that the international trade in specimens of wild animals and plants does not threaten the survival of species"(CITES, 2023b). CITES works by enforcing certain restrictions for specimens that are internationally traded. Species that are covered by CITES are listed under three different Appendices: Appendix I, II or III. Appendix I is for species that are threatened with extinction and trade in these species is highly restricted and can only be done in exceptional circumstances, only a

handful of elasmobranchs are listed in this Appendix. Appendix II encompasses species that are not immediately at risk of extinction, but where trade regulation is essential to prevent utilization incompatible with their survival. Appendix III includes species that are protected in at least one country, and where other CITES Parties are asked to assist in controlling the trade (CITES, 2023b). An additional 54 requiem sharks (Carcharhinidae), six hammerhead sharks (Sphyrnidae), seven freshwater stingrays (*Potamotrygon* spp.) and 37 guitarfishes (Rhinobatidae) were added to CITES Appendix I or II following the 19th Conference of the Parties (CoP19) of CITES held in 2022 (CITES, 2023a).

The decline of these populations could have devastating effects on the health and productivity of marine ecosystems across the world. A loss of our large predatory sharks has serious consequences to the health of our oceans (Myers et al., 2007; Pacoureau et al., 2021). They are crucial for the top-down control of food webs as they regulate the natural mortality in a range of their prey, contributing to changes in the abundance, distribution, and behaviour of small elasmobranchs (mesopredators), marine mammals, and sea turtles which only have a few other natural predators (Myers et al., 2007; Ferretti et al., 2010; Hammerschlag et al., 2018). Their disappearance will also significantly impact the global fishing industry, causing a potential collapse of one of the most important sources of food and income for many countries.

1.4 Overexploitation

The main threat to elasmobranchs is overfishing, whether it's when they are targeted specifically as a group or when they are incidentally caught, in bycatch. It is estimated that between 63 and 273 million sharks are killed a year because of

overfishing, with this number likely to be higher due to the underreporting of species (i.e., Illegal Unregulated and Unreported (IUU) fishing, illegal trade, and mislabelling) (Worm et al., 2013). One of the main challenges in managing overfishing of elasmobranchs is the difficulty in monitoring these activities as many fisheries operate beyond jurisdiction (high seas), such as IUU fishing, and sharks cross many jurisdictions (Dulvy et al., 2017). Elasmobranchs are often considered bycatch, and not as a targeted group themselves which makes it difficult to monitor which species are caught and sold, as well as receiving support from government and other institutional bodies.

1.5 Trade in Elasmobranchs and their Related Products

Elasmobranchs are traded and consumed all over the world, the WWF recently reporting that the trade in elasmobranch fins and meat generated over $4.1 billion USD globally between 2012 and 2019 (Niedermüller et al., 2021). Elasmobranch products include dried fins often for shark fin soup, meat, traditional Chinese medicine (e.g. gill plates), dietary supplements (e.g., liver oil and cartilage supplements), cosmetic and beauty products (e.g., lipstick, mascara, anti-ageing creams), vaccines, and pet food (Wong et al., 2009; Caballero et al., 2012; Zeng et al., 2016; Steinke et al., 2017; Almerón-Souza et al., 2018; Cardeñosa et al., 2018; Cardeñosa, 2019; Hobbs et al., 2019; Ferretti et al., 2020; Tiktak et al., 2020; Niedermüller et al., 2021; Prasetyo et al., 2023).

The most “valuable” and frequently traded fins belong to those species that are threatened as well as protected under Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), such as scalloped, great, and smooth hammerhead sharks (*Sphyrna* spp.), thresher sharks (*Alopias* spp.),

shortfin mako shark (*Isurus oxyrinchus*), oceanic whitetip shark (*Carcharhinus longimanus*) and other Carcharhinid species (Abercrombie et al., 2005; Cardeñosa et al., 2018, 2022).

While the shark fin trade has often been thought as being the primary threat to shark populations, there has been growing concern for the elasmobranch meat trade. Between 2012 and 2019, the meat trade generated over $2.6 billion USD, which was more than the fin trade during that period (~$1.5 billion USD) (Niedermüller et al., 2021). The elasmobranch meat trade has expanded in the last few decades with regulations in place that require elasmobranchs to be landed with fins and heads intact ("antifinning") as well as the increase in demand in elasmobranch meat (Dent and Clarke, 2015). The growing meat trade might be contributing to the increased frequency of mislabelling, leading to instances where elasmobranchs may be sold as other species (e.g. teleost fish), or where threatened species are sold as non-threatened species. Mislabelling raises significant concerns as it not only hinders elasmobranch conservation efforts, but also poses a risk to humans that may be consuming elasmobranch products without their knowledge. Mislabelling also contributes to overfishing of already vulnerable species as we are uncertain of true numbers caught and sold, which can increase their likelihood of extinction. It also deceives consumers who may not know they are purchasing elasmobranch meat and prevents them from making informed decisions about their food choices (Luque and Donlan, 2019).

Identifying elasmobranchs and elasmobranch-related-products is one of the main challenges in monitoring their trade. Physical features such as fin shapes, teeth structure, colouration, body markings, size and length are often used to identify

elasmobranchs. When elasmobranchs are landed at fisheries, they might lack important identifying features such as heads, fins, or wings (rays and skates). Carcasses may also lose colour and degrade due to sun exposure, for example when caught at open sea and left for days or when landed at a market and left in direct sunlight. Additionally, some species share many physical characteristics that make them difficult to tell apart even for experts, for example sharks belonging to the genus *Carcharhinus* and *Mobula* (Corrigan et al., 2017; Pazmiño et al., 2019; Zhang et al., 2021). In some cases, products such as a fish fillet may be sold to consumers with ambiguous labelling, or where a general term is used such as “shark”, and in both cases consumers are unaware of what species is being sold. This lack of species-specific identification poses a significant challenge as understanding which species are caught and traded is crucial for effective conservation and management of elasmobranch populations. However, identifying elasmobranchs down to species level often requires specialised expertise in either morphological identification, or genetic identification techniques such as DNA barcoding. This expertise is essential for accurate species identification among the ambiguity that arises from the sale of processed products (e.g., dried fins) as well mislabelling.

Genetic identification techniques are some of the most powerful tools in elasmobranch conservation as it allows for accurate identification, down to species level, even for heavily degraded and processed samples such as dried fins, shark fin soup, pet food etc (Fields et al., 2015; Cardeñosa et al., 2017; French and Wainwright, 2022). DNA Barcoding is the most widely used genetic technique for the identification of elasmobranchs in the trade (Abercrombie et al., 2005; Ward et al., 2005; Pinhal et al., 2012). It can assist conservation policies by increasing capacity for local

biodiversity assessments that help prioritise conservation programs and evaluating the success of already implemented conservation efforts. Identifying sharks using genetic tools is the most reliable technique though it comes with some drawbacks. Techniques such as these require specialist facilities and equipment, as well as trained experts in the field to carry out the analysis. It's also more time consuming as it can take a few days (or longer) to process samples, especially if it's done by external researchers or international research institutes. Most species are found in the tropics of developing countries where resources are often limited for thorough biodiversity assessments and monitoring programs (Krishna Krishnamurthy and Francis, 2012). In some cases, input from international experts and institutions is needed to carry out genetic analysis, but our aim is to divert away from this approach and collaborate directly with local stakeholders and communities. There is a real need for rapid, field-based genetic identification techniques that can be used by non-scientifically trained personnel at the market source and in the country of origin.

1.6 Pollutant Exposure

Pollutants enter the marine environment through a variety of sources, mainly human activities, such as industrial discharge, agricultural and urban runoff, vehicle emissions, waste incineration, sewage, as well as natural processes such as volcanic activity and forest fires (Wang et al., 2004; El-Shahawi et al., 2010; Morrison and Murphy, 2010; Megson et al., 2013; O'Sullivan and Megson, 2013; Briffa et al., 2020; WHO, 2020). When pollutants are introduced into the ocean, they can have long lasting effects and cause irreversible damage to marine ecosystems. Persistent organic pollutants (POPs), heavy metals, crude oil and marine debris (e.g. marine litter or microplastics) are the most common marine pollutants found globally (United

Nations Environment Program, 2017; Briffa et al., 2020; Patel and Haldar, 2022). Pollutants encompass a wide range of substances, including heavy metals, such as mercury (Hg), lead (Pb) and cadmium (Cd), as well as various persistent organic pollutants (POPs) such as industrial compounds and chemicals like pesticides (e.g. DDT), flame retardants, and dioxins and furans (Patel and Haldar, 2022).

Sharks are exposed to pollutants in various ways, though the main pathway is through their diet. They may also be exposed through their gills (limited research on this). Mercury transfers up the food chain through the prey sharks feed on, which varies significantly per species from small to large fish species, crustaceans, cephalopods, marine mammals, sea birds, turtles, and other elasmobranchs (Teffer et al., 2014; Estupiñán-Montaño et al., 2017; Gonzalez-Pestana et al., 2017). Many pollutants bioaccumulate and biomagnify up the food chain, eventually concentrating in apex predators such as sharks and marine mammals (cetaceans) (Arnot and Gobas, 2004; Bezerra et al., 2019; Cagnazzi et al., 2019; Tiktak et al., 2020). Therefore, apex predators such as sharks may have high concentrations of heavy metals (e.g. methylmercury, cadmium, arsenic, and lead) and POPs in their tissue due to their high trophic level (Tiktak et al., 2020). In many cases, these concentrations are above the legal limit set by health regulators (e.g. WHO, EPA etc) (Domingo and Bocio, 2007; Vračko et al., 2007; WHO, 2010; U.S. Environmental Protection Agency, 2011).

Inadvertently consuming elasmobranchs and their related products could have significant health implications. The hidden costs of consuming elasmobranch meat products are that not only are they crucial for marine ecosystem functioning, but many elasmobranchs (especially large-bodied sharks) have high concentrations

of pollutants, such as mercury in their tissue (Tiktak et al., 2020). Humans are exposed to pollutants through their diet (Linares et al., 2010) which means consuming apex predators puts them at greater risk of overexposure to pollutants and bioaccumulation in their tissue. Humans that consume elasmobranch products without their knowledge, i.e. in the case of mislabelling, are exposed to high concentrations of these pollutants often without their knowledge. Humans that knowingly consume shark may not know the species of shark and are therefore also at risk of exposure especially if they are consuming pelagic shark species with high concentrations of mercury.

Pollutants can have detrimental effects on human health, where even low concentrations of toxic metals can have a devastating impact on the global population (Linares et al., 2010; Nordberg et al., 2022). Some of the health impacts include cell damage, cellular function loss, neurotoxicity, impaired reproductive success, birth defects, lowered fertility, endocrine disruption, immunosuppression, increased risk of cancer and in some cases can even lead to death (Vračko et al., 2007; Zheng et al., 2007; Kim et al., 2013; Sharma et al., 2014; Briffa et al., 2020). Pregnant women and young children are at increased risk to the health risks associated with exposure to these contaminants (Patandin et al., 1999; Bruce-Vanderpuije et al., 2019; EFSA et al., 2019)

The effects pollutants have on elasmobranchs remains relatively unknown (Bezerra et al., 2019; Merly et al., 2019; Tiktak et al., 2020). Elasmobranchs exhibit k-life strategies, characterised by producing very few offspring, maturing late, and slow growth (García et al., 2008; Dulvy et al., 2021). This makes them particularly susceptible to toxic pollutants which bioaccumulate over time. Nevertheless, there is

limited information regarding the effects of pollutants on elasmobranchs despite their threat status. Pollutant studies are often done in isolation, or only on a few species or pollutants at a time. Therefore, it's important to incorporate all these components and understand biases within the literature by identifying gaps in previous literature. This enables us to focus research efforts in key and understudied areas.

1.7 Conservation Efforts and Challenges

The media has historically presented sharks in a negative way, using emotive language, "one liner" or attention grabbing headlines, and terms expressing negative personification frames (Neff and Hueter, 2013; McCagh et al., 2015). The actual number of humans attacked by sharks each year remains relatively low, and the number killed even lower (Simpfendorfer et al., 2011; Crossley et al., 2014; Friedrich et al., 2014). Despite these statistics, elasmobranchs have a reputation that often paints them in a negative light, leading to the public's negative perception and a challenge to elasmobranch conservation (Friedrich et al., 2014; McCagh et al., 2015; Neff, 2015). Jaws is one of the most well-known horror films to date but has now become the discussion of many conservationists (McCagh et al., 2015). Jaws had a significant impact on how the public viewed sharks, and Steven Spielberg has since dedicated himself to shark conservation after the impact Jaws had on real-world sharks with an increase in trophy hunting, shark culls and reduced support for shark conservation and policy implementations (Neff and Hueter, 2013; McCagh et al., 2015; Neff, 2015). The media went into a frenzy after the death of Steve Irwin caused by a stingray barb. Though his death was considered a freak accident, it still caused a

shift in people's perception of stingrays, leading to acts of retribution where stingrays were killed (Guardian, 2006; Lunney and Moon, 2008).

Conservation efforts for elasmobranchs often face challenges from public perception as many people struggle to see the immediate importance of "saving" elasmobranchs and negative media attention may stop them from wanting to help them at all. If conservation messages are angled from a "human health" perspective, gathering support may be easier as a direct connection can be made. This can be done when assessing the potential human health risks arising from pollutant exposure through the consumption of elasmobranch and their related products. If consumers are given the choice of consuming elasmobranch products, they may opt against it. This, however, is made difficult in the case of mislabelling, where the true identity of a seafood product is unknown. This highlights the importance of focussing research efforts on pollution exposure, and identification of mislabelled elasmobranchs, as this will enhance our understanding of the health risks associated with their consumption (Tiktak et al., 2020), as well as facilitating informed consumer decisions.

1.8 Ecuador

Ecuador, a relatively small country situated in South American, is recognised as a global hotspot for many marine species including cetaceans, sea turtles, sea birds and elasmobranchs. Its unique geographical location allows for this ecologically complex and diverse marine ecosystem (Bustamante et al., 2000; López-Angarita et al., 2021). The mainland coastline of Ecuador stretches for over 2,200 km, offering a diverse range of marine environments, from estuaries within mangroves to deep offshore waters. This high marine biodiversity supports productive fisheries as well as

providing a large range of important habitats that serve as nurseries, breeding grounds, and migratory routes for numerous important elasmobranch species such as scalloped (*S. lewini*) and smooth hammerhead (*S. zygaena*) sharks and tiger sharks (Galeocerdo cuvier) (López-Angarita et al., 2021).

Unfortunately, many elasmobranchs in Ecuador have experienced significant population declines driven by the demand for shark fins and meat in international markets, as well as locally. Elasmobranchs are traditionally sold and consumed in fish markets along the coast of Ecuador (Dominguez and Cobeña, 2019; Hearn et al., 2022). Ecuador is one of the world's leading countries in shark conservation, with the implementation of its second National Shark Action Plan (Gobierno del Ecuador, 2020). In 2007, the Ecuadorian government prohibited shark finning and directed shark fisheries in the country to reduce the illegal trade but allowed for the sale of incidentally whole caught sharks (fins and body), except in the Galápagos Marine Reserve where sharks are fully protected (Executive Decree 486 of 2007, cited in Hearn et al., 2022). Despite the legislation, over one million shark landings were reported in Ecuador between 2008 and 2012 with CITES-listed shark species: bigeye thresher (*Alopias superciliosus*), pelagic thresher (*A. pelagicus*) and shortfin mako (*Isurus oxyrinchus*) sharks accounting for 61% of the total landings during that period (Martínez-Ortiz et al., 2015).

1.9 Aims and Objectives

Efforts to conserve shark populations requires a multidisciplinary approach that addresses threats from overexploitation, habitat loss and degradation, and climate change. However, it is not possible to tackle all the threats above, and therefore in this thesis I aim to tackle two, namely overexploitation and illegal trade of sharks, and

pollutant exposure. The main aim of this thesis is to use genetic and chemical techniques to aid elasmobranch conservation (Figure 1 1). Ecuador provides a perfect location for conducting this research as despite its rich elasmobranch diversity, there is limited information about the status of these species in this region. Ecuador also plays a key role in the contributing to the global elasmobranch trade. Given Ecuador's long-term commitment to elasmobranch conservation, my research has the potential to make a meaningful contribution to these conservation efforts by answering a wide range of questions (Figure 1 1).

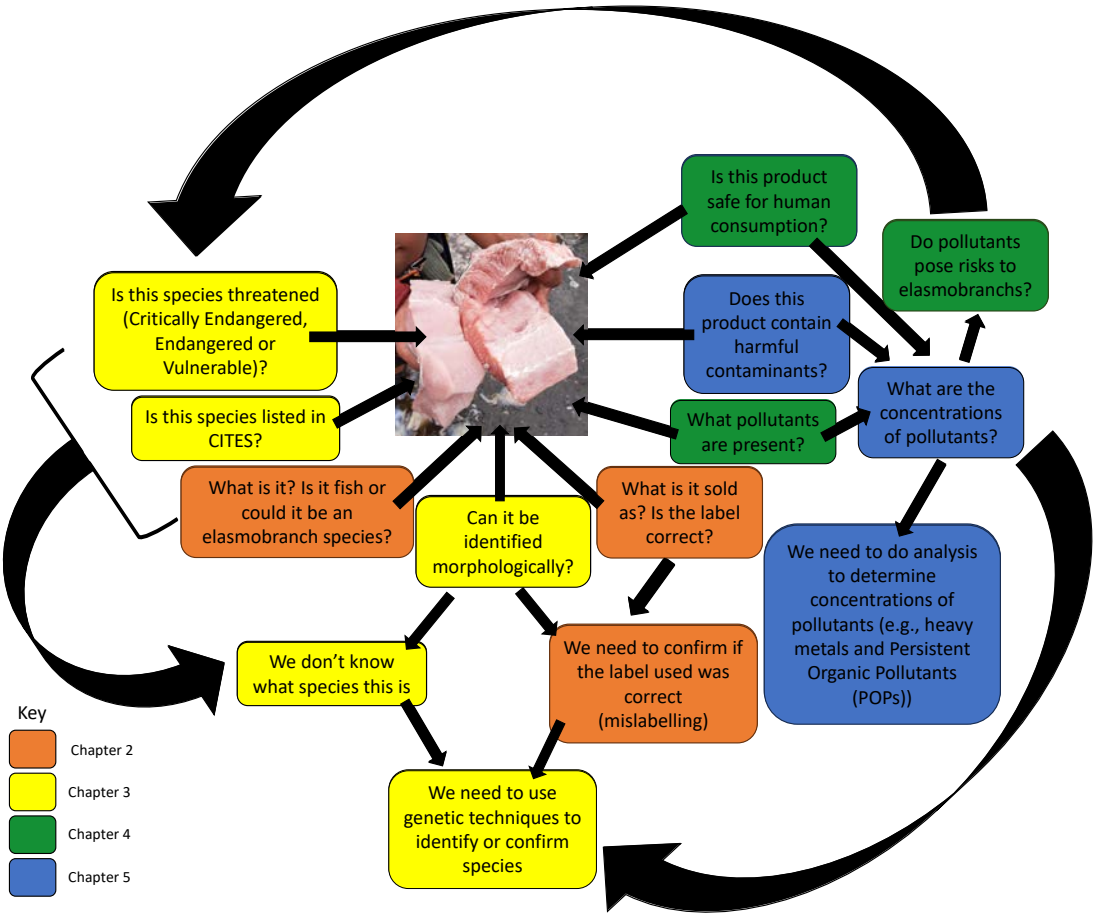

Figure 1-1 Flow chart of the four key elements of my PhD thesis: Chapter 2 - Review on identification and mislabelling of elasmobranchs, Chapter 3 – Lab-on-a-Chip (LOC) for the identification of three threatened and CITES-listed sharks, Chapter 4 – Systematic review of pollutants in elasmobranchs, and

Chapter 5 – Trace metals and heavy metals in the tissue of five commercially important shark species in Ecuador. Note that some chapters have elements that overlap.

This thesis will address the following objectives:

Chapter 2: Explore methods for identifying elasmobranchs and elasmobranch-related-products in the international trade and provide a comprehensive and up-to-date synthesis of the existing evidence on the mislabelling of elasmobranchs across the world.

Chapter 3: Develop a rapid, field-based identification tool in the form of a Lab-on-a-Chip (LOC) for genetic analysis of three CITES-listed sharks: bigeye thresher (*Alopias. superciliosus*), pelagic thresher (*A. pelagicus*) and shortfin mako shark (*I. oxyrinchus*) belonging to the order Lamniformes.

Chapter 4: Assess and evaluate the concentrations of pollutants in elasmobranchs (sharks, rays, and skates) across the world by systematically gathering and analysing the available scientific literature.

Chapter 5: Determine trace element and heavy metal concentrations in five commercially important shark species, bigeye thresher (*A. superciliosus*), pelagic thresher (*A. pelagicus*), silky shark (*Carcharhinus falciformis*), blue shark (*Prionace glauca*) and smooth hammerhead shark (*Sphyrna zygaena*) landed at artisanal fish markets in Ecuador, and to evaluate the potential risks to human health and ecological impacts associated with the consumption of these species.

Chapter 6: Conclude the main findings and discuss conservation implications of chapters two to five and provide future recommendations for the conservation of elasmobranchs.

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Chapter 2

2 Identifying patterns of mislabelling in elasmobranchs (sharks, rays, and skates): A global review

Chapter Overview

This chapter is a systematic review on mislabelling worldwide in elasmobranchs (sharks, rays, and skates) following the 2020 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

Author Contributions

As lead author I designed the project, collected the data, performed the statistical analysis, wrote the manuscript. I supervised a MSc student along with Professor Richard Preziosi, who provided support with data collection to add an additional level of rigour on the data collected from the literature. This chapter will be streamlined for submission to Conservation Biology early 2024.

Abstract

Elasmobranchs (sharks, rays, and skates) currently comprise one of the most vulnerable taxa on the planet, with many species facing rapid population declines largely driven by overexploitation and bycatch. The trade in elasmobranchs and elasmobranch-related-products has prompted their capture at unsustainable rates, as well as increasing rates of species mislabelling and substitution, allowing for the sale of endangered and CITES-listed species. Here we present the first systematic review aimed at exploring methods of identifying elasmobranchs and elasmobranch-related-products in the trade, as well as identifying patterns of mislabelling worldwide. Eighty-three studies were included in this review, covering 306 elasmobranch species, 45 families and 12 orders. Thirty-five percent (*n* = 109) of elasmobranchs caught were classified as threatened by the IUCN Red List and 9.48% (*n* = 29) were listed in CITES Appendix I, II or III at the time of study. Around 44.7% (*n* = 38) of studies focussed on mislabelling, revealing that 11.3% (*n* = 824) of samples were mislabelled and 10.1% (*n* = 744) were labelled using umbrella terms such as "Cação" and "Flake". Blue sharks (*Prionace glauca*) were the most frequently mislabelled species, representing 12.5% (*n* = 103) of all cases of mislabelling. Additionally, blue sharks were also the most common species sold under umbrella terms, accounting for 13.7% (*n* =.102) of these cases. Species listed as threatened made up 48.7% (*n* = 38) of mislabelled elasmobranchs and 53.7% (*n* = 29) of species labelled using umbrella terms. Thirteen percent (*n* = 10) of mislabelled and 9.26% (*n* = 5) of umbrella labelled elasmobranch species originated from CITES-listed species. Mislabelling and the use of umbrella terms was widespread, with many studies being carried out in Europe (*n* = 14) and South America (*n* = 14) but some of the most

important trading hubs for elasmobranchs had limited (Hong Kong, China, Indonesia, and Singapore) to no studies (Vietnam), highlighting the urgent need for research in these understudied regions. DNA barcoding using the mitochondrial (mtDNA) cytochrome oxidase 1 (CO1) region was the most widely used genetic identification technique (81.2%), though studies highlighted the need for faster, cheaper, and field based identification techniques. The results of this review underline the negative effect of mislabelling on monitoring trade for conservation efforts, especially for species facing rapid population declines. The prevalence of threatened and CITES-listed species in the trade, especially when mislabelled, indicates the heightened threat to these already vulnerable populations, emphasising the need for stronger enforcement measures and additional conservation efforts to protect these species from extinction.

Keywords: *Elasmobranchs; Mislabelling; CITES; Identification; Trade; Conservation*

2.1 Introduction

Elasmobranchs (sharks, rays and skates) have been identified as one of the most threatened vertebrates on the planet, with one third of all chondrichthyans (sharks, rays, skates and chimeras) currently threatened with extinction (Dulvy et al., 2008, 2014, 2021; Ferrette et al., 2019a; Bernardo et al., 2020; Pacoureau et al., 2021). The trade in elasmobranchs and their related products has been the biggest driver of their mortalities worldwide (Dulvy et al., 2021). Elasmobranchs are especially susceptible to threats from overexploitation as many of them exhibit similar traits to that of large-bodied mammals with slow growth, delayed maturity, long gestation periods and low fecundity (Abercrombie et al., 2005a; Clarke et al., 2006b; García et al., 2008; Almerón-Souza et al., 2018). Their populations require long periods of recovery following overfishing, which may span decades (Macbeth et al., 2018; Ferrette et al., 2019a).

Elasmobranchs and elasmobranch-related products are consumed by humans all over the world, with only a small number of species currently protected under national and international laws that make them illegal to fish and trade (Abercrombie et al., 2005a; Cardeñosa et al., 2018, 2022; Ferretti et al., 2020; Van Houtan et al., 2020; Dulvy et al., 2021). The combined value of the shark fin trade (~1.5 billion USD) and meat trade (~2.6 billion USD) generates over four billion USD annually (Niedermüller et al., 2021). This makes the elasmobranch trade one of the most valuable seafood commodities globally. Other elasmobranch-derived products include vitamin and beauty supplements, gill rakers, cosmetics, souvenirs, traditional medicine, and pet food (Wainwright et al., 2018; Cardeñosa, 2019; Niedermüller et al., 2021). Recently there has been growing concern for mislabelling and species

substitution of elasmobranchs that occurs globally, even in non-coastal countries and cities. Many elasmobranchs have been found with high concentrations of mercury (Hg) as well as other pollutants (e.g., dichloro-diphenyl-trichloroethane(DDT)) which puts consumers at greater risk to serious health concerns from overexposure to pollutants, especially when they may be unaware that they are consuming elasmobranchs when they are being mislabelled (Bezerra et al., 2019; Tiktak et al., 2020; EPA, 2022).

Identifying elasmobranchs or elasmobranch-related products is one of the main challenges in the trade. Various methods are used for identification, including morphological identification, which can be limited when dealing with heavily processed products. Morphological identification is typically carried out on whole specimens and experts trained in visual identification (Clarke et al., 2006a; Holmes et al., 2009). DNA barcoding is often used to identify sharks when they cannot be identified based on their morphological features. It may also be used as a tool to confirm visual identification, for example in the case of heavily processed shark fins (Ward et al., 2005; Clarke et al., 2006a, 2006b; Cardeñosa et al., 2017; Filonzi et al., 2021). Studies often use fish primers or species-specific primers to perform polymerase chain reaction (PCR)-based amplification of DNA from mitochondrial genes such as cytochrome b (CytB), cytochrome oxidase subunit 1 (CO1), Internal Transcriber Space 2 (ITS2), 16S ribosomal RNA, and NADH dehydrogenase subunit 2 (NADH2). The DNA requires downstream processes such as visualisation of bands through gel electrophoresis and/or DNA sequencing (Shivji et al., 2002; Fields et al., 2015; Feitosa et al., 2018; Delpiani et al., 2020). DNA barcoding uses a short, standardised sequence of genomic DNA from a particular region against a ‘barcode’

sequence within a database of reference samples to distinguish between species (Marchetti et al., 2020; Filonzi et al., 2023). Because the primers used to amplify and sequence DNA fragments are universal, no prior knowledge of the species present in a sample is required to genetically identify species within samples. As a result, this technique is applicable to large fish markets where the point of origin of a wide range of products may be unknown (Hobbs et al., 2019; Cardeñosa et al., 2021), though also useful in seizures from IUU fishing vessels or at customs.

DNA barcoding has been recognised as being most effective in the identification of elasmobranch species in fresh and lightly processed products, due to the large section of sequences available for PCR amplification (around 650bp is the standard) (Marchetti et al., 2020). When elasmobranch products have been heavily processed such as dried fins, shark fin soup, cartilage, pet food and vitamin supplements, liver oil capsules and beauty products (i.e., lipstick and mascara) the DNA often becomes degraded, making it difficult to attain large amplicons for PCR (Marchetti et al., 2020). For heavily degraded samples, mini-barcode assays can be used as they produce shorter fragments of DNA from the mtDNA CO1 region for amplification (~100-200bp) which is more effective than full DNA barcoding. Mini-DNA barcoding allows for the identification of heavily processed and degraded samples down to species level (Fields et al., 2015; Cardeñosa, 2019; Giovos et al., 2020; Zahn et al., 2020; Zhang et al., 2021).

The aim of this systematic review was to explore methods for identifying elasmobranchs and elasmobranch-related products in the international trade and provide a comprehensive and up-to-date synthesis of the existing evidence on the mislabelling of elasmobranchs globally. This will be achieved by;

1) Identifying trends, geographical distribution, and knowledge gaps within existing literature on the elasmobranch trade;

2) Assessing the species composition and conservation status of elasmobranchs within existing literature;

3) Evaluating the prevalence and extent of mislabelling of elasmobranch products in global seafood markets across different geographic regions;

4) Identifying gaps in knowledge and research needs related to the mislabelling of elasmobranchs and provide recommendations for future research and policy actions to address this issue; and finally;

5) Evaluating and discussing the morpholical and genetic methods for the identification of elasmobranchs and elasmobranch-related-products in the international trade.

2.2 Methods

2.2.1 Study Selection

A comprehensive search was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for 2020 (Page et al., 2021), to identify relevant research articles related to the identification and mislabelling of elasmobranchs. Studies were considered for inclusion based on the following inclusion criteria: the study reported on the genetic identification or mislabelling of elasmobranchs within the scope of the seafood trade, published between 1970 (the earliest start date on Scopus) and 2023 (cut off October), was original research, published within a peer-reviewed journal and written in English. 'Gray Literature' were not included in this review as they often do not undergo the

same peer-review process and may not be available online. Studies that focussed on the development of a method (e.g., species-specific markers, PCR multiplex) and shark depredation were not included in this review.

The following search terms were used separately or in combination for the identification of papers (Figure 2-1) within the search engines Scopus and Web of Science: “mislabel*”, “identif*”, “species substitution”, “elasmobranch*”, “shark*”, “ray*”, “skate*”, “batoid*”. The following text “AND NOT” combined with “X-ray”, “gamma”, “attack”, “Rayleigh”, “cosmic”, “receptor”, “ray tracing”, “skater*” and “skateboard*”. Search terms were kept broad to obtain as many studies as possible. Google Scholar was used to cross-reference papers, and any suggested papers that were relevant were also included.

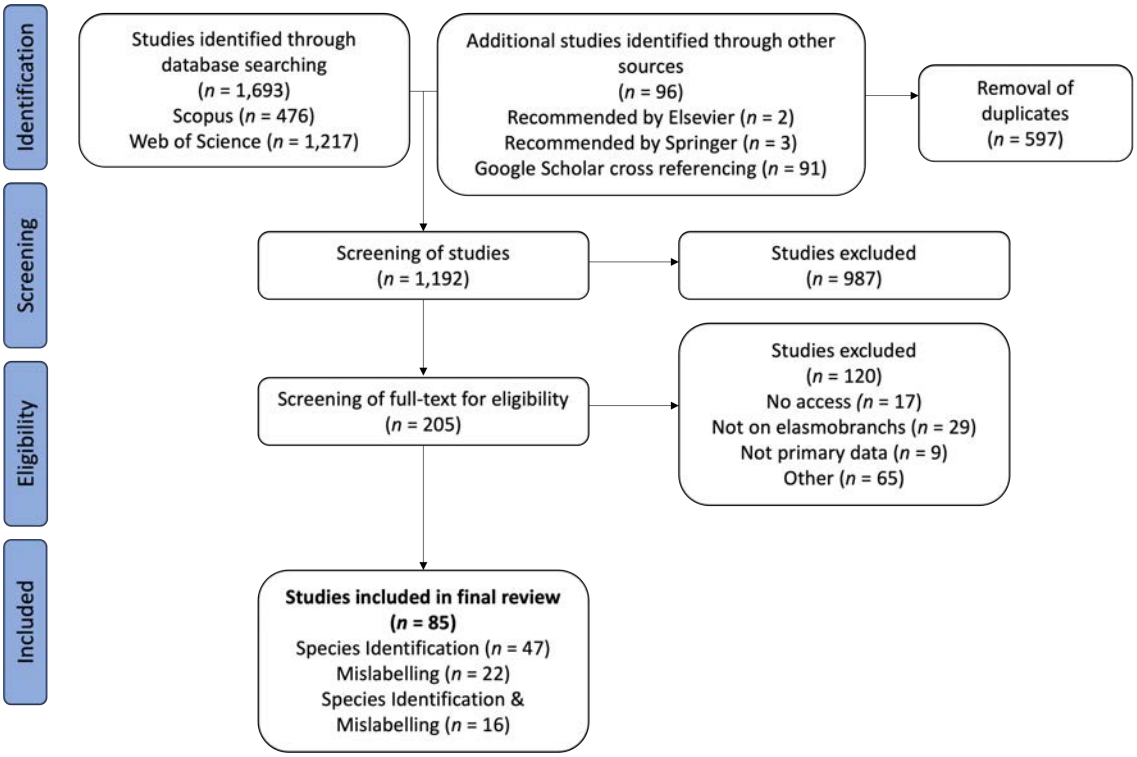

Figure 2-1 PRISMA flow diagram used for the inclusion and exclusion of studies at each stage of the review’s screening process. A total of 85 studies were included in the final review.

2.2.2 Data Collection

Relevant information was extracted from each eligible study and recorded, including: the title of the study, author(s), year of publication, and journal. Taxa-specific data were collected to include common and scientific names, family, order, superorder, subclass, class, number of samples collected per species and most common species within the study (number and percentages where applicable), in addition to their International Union for Conservation of Nature (IUCN) Red List status (based on the time of publication as well as their status in 2023) and their inclusion in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) Appendices I, II or III (CITES, 2022, 2023; IUCN, 2023). The country and region of the study were recorded, the surrounding ocean(s), the place and number of samples attained, the number of species identified, type of sample (e.g. fin, muscle tissue, dried/fresh/frozen), identification method and genetic marker used (where available). Studies were split into two categories: species identification, and mislabelling. Species identification studies were defined as studies that focussed on identifying elasmobranchs using morphological (visual) or genetic techniques, and mislabelling studies reported on the mislabelling of elasmobranchs. Studies could belong to one or both categories, depending on the scope.

In the literature mislabelling cand be defined in a number of different ways, in this review we have defined mislabelling under two specific terms: mislabelling and umbrella labelling. Mislabelling is defined as any seafood product that has false or incorrect labelling which may include species substitution, for example when a threatened species is mislabelled as a non-threatened species (Ryburn et al., 2022). Umbrella labelling is defined as when various species or organisms are grouped under

a general term without specifying each individual species (Giagkazoglou et al., 2022). An umbrella term might be the use of “cacao” which is deliberately broad to incorporate elasmobranchs. Several studies grouped mislabelling and the use of umbrella terms together for certain species without distinguishing between the two categories. As it was not possible to separate these instances, they were excluded from the analysis to ensure clarity and accuracy in reporting.

Additional data was collected that included: the number of samples mislabelled, price of product (where applicable), taxa information (species, family, order, subclass, class, etc), IUCN status and CITES listing. IUCN status of non-threatened species were categorised as DD = Data Deficient, LC = Least Concern, LR/CD = Lower Risk/Conservation Dependent, LR/LC = Lower Risk/Least Concern, LR/NT = Lower Risk/Near Threatened, NE = Not Evaluated, NT = Near Threatened, Species were classified as threatened if they were categorised within the following IUCN categories: VU = Vulnerable, EN = Endangered and CR = Critically Endangered (IUCN, 2023).

2.2.3 Mislabelling Terminology

In total, 140 different commercial names were used across the 38 mislabelling studies. To standardise reporting the names were grouped into broader commercial categories for example, “tuna” was grouped with “átun” (tuna in Spansih), “átun rojo” and “albacore” and categorised as “tuna species”. If common or latin names were present, these were classified as “shark species” or “ray species” where applicable, if no species names were present, the umbrella term “shark” or “ray” was used. By recategorizing the terms, it reduced the number of categories for mislabelling terms

drastically, from 140 down to 26. Latin names were written as common species names for simplicity. Any samples which did not fall into any of the above categories were classified as “other”. For full breakdown of terms see Section 2.6. Supplementary Information (Table S2 -2).

2.2.4 Data Analysis

All data analysis and visualisation were carried out R (3.6.1 (2019-07-05)) (R Core Team., 2019). Data for longitude and latitude of each country were taken from Google’s data set: “countries.csv” (https://developers.google.com/public-data/docs/canonical/countries_csv). Maps were made in R using “Leaflet” (Cheng et al., 2023) and the map base layer “esri.worldmap”. A chi-square goodness of fit test was used to examine whether the frequency of studies published in each country was evenly distributed.

2.3 Results and Discussion

2.3.1 Research Trends

A systematic review was performed on the identification of elasmobranchs with a focus on mislabelling worldwide. A total of 85 relevant studies were identified for this review that were published between 1970 and 2023. Seventy-six percent (*n* = 65) of these were solely focussed on elasmobranchs, and 24.7% (*n* = 21) included other organisms (i.e., teleost’s, crustaceans, and chimeras). For the purpose of this study only elasmobranchs were discussed. Forty-five percent (*n* = 38) of these studies discussed elements of mislabelling of elasmobranchs within the seafood trade, while 55.3% (*n* = 47) focussed only species identification, 3.6% (*n* = 3) (Figure 2-2). Nineteen

percent (*n* = 16) of studies focussed on both species identification and mislabelling (Figure 2-2).

There has been an increase in studies over the last five years, with a spike in the number of studies published between 2019 and 2021 (Figure 2-2). The search for this review concluded in October 2023, thus the real figure of 2023 (*n* = 7) could be higher (Figure 2-2). A possible explanation for the increase in studies could be due to the advancement in genetic techniques, as they have become cheaper, faster, and more accurate (Hellberg and Morrissey, 2011; Dudgeon et al., 2012; Durmaz et al., 2015). There has been an increase in studies focussing on mislabelling in elasmobranchs in the last six years (Figure 2-2) though it's difficult to infer whether this is due to an increase in mislabelling or other factors, such as the above suggested advancement of genetic techniques, or increased interest in elasmobranchs due to their growing population concerns etc.

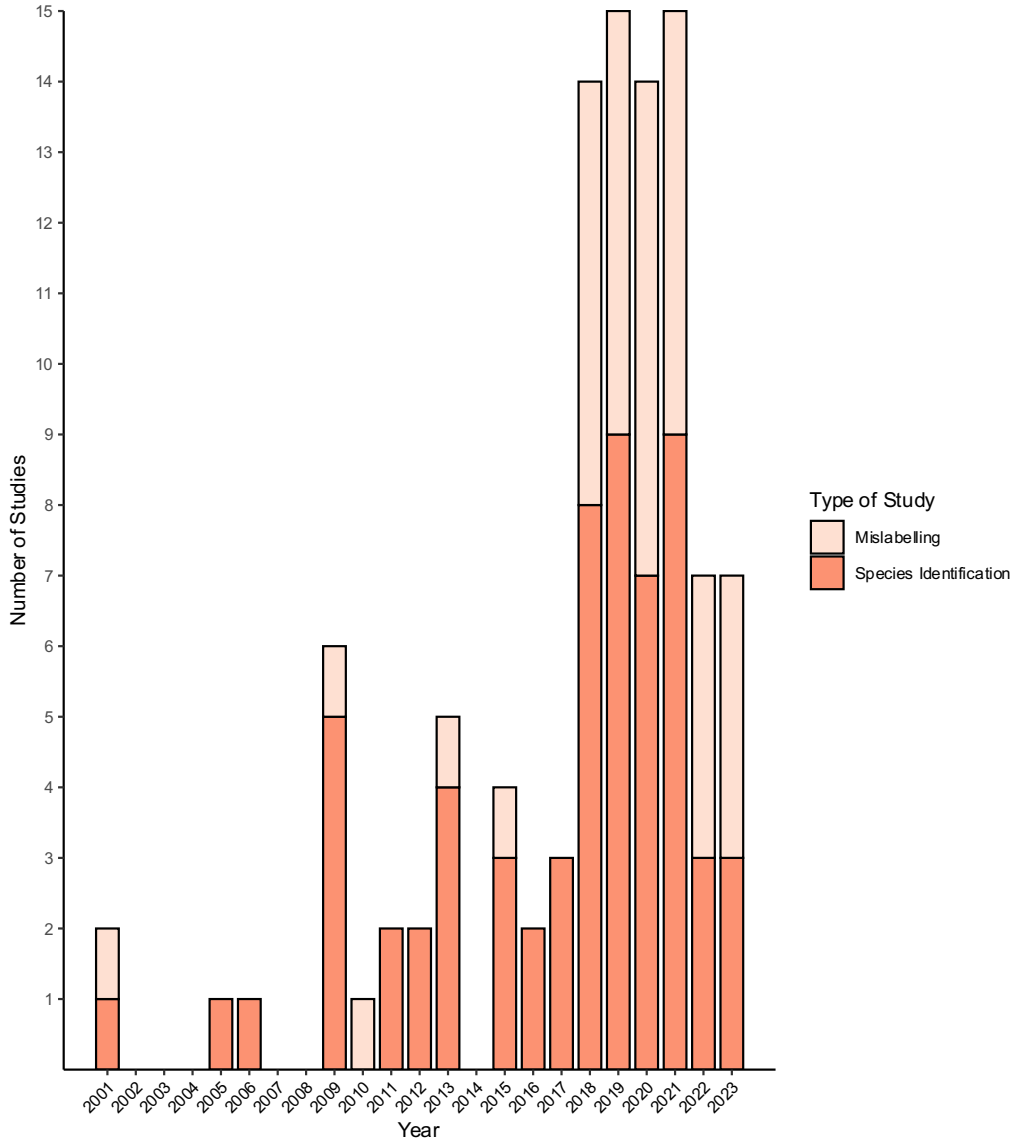

Figure 2-2 Total number (*n* = 85) of publications between 2001 and 2023 by topic (bycatch, elasmobranch landings, mislabelling and species identification). No relevant studies were found before 2001.

2.3.2 Global Distribution of Studies

Samples originated from 50 countries, where studies were mainly carried out in Europe (*n* = 25), Asia (*n* = 21), or South America (*n* =21). Most studies were conducted in Brazil (*n* = 16), followed by Mexico (*n* = 6) and the USA (*n* = 5) (Figure 2-3). Mislabelling of elasmobranchs was evaluated in 19 countries, though 14 of those countries only included one to two studies. Brazil (*n* = 9), Italy (*n* = 4) and Greece

(*n* = 3) were the countries with the greatest number of studies on mislabelling (Figure 2-3).

East and Southeast Asia remain the main hub for shark fins, with Hong Kong SAR, Taiwan province and Singapore being amongst the highest importers of elasmobranch-related products (e.g., shark fins) (Shea et al., 2022). Shark fin soup is still widely consumed in East and Southeast Asia despite the decrease in demand, as well as the use of traditional Chinese medicine and vitamin supplement, usually coming from cartilage and shark liver oil (Oceana, 2010). Hong Kong imported nearly 10,000,000 kg of shark fins in 2008, where most originated from Spain, Singapore and Taiwan (Oceana, 2010). Europe is a significant player in the global shark fin trade providing 45% of shark fins to the trade hubs in Asia, but also 22% of the global shark meat supply (Niedermüller et al., 2021; Shea et al., 2022). Between 2003 and 2020, Spain supplied Hong Kong SAR, Singapore and Taiwan with an annual average of 2,877.52 metric tonnes of shark fins (Shea et al., 2022).

While many studies focussing on elasmobranchs were carried out in Europe, it's important to point out that a limited number of studies were carried out in individual European countries. Despite the implementation of various measures to regulate shark fishing and trade, challenges persist in effectively monitoring and controlling these activities within the EU.

Industrial and artisanal fleets across the world supply the Asian markets for shark fins (including fins from batoid species), while the meat from these captured sharks is traded to different countries, for example meeting the growing demand in markets in Brazil (Dent and Clarke, 2015). The elasmobranch meat trade has expanded significantly with regulations in place that require carcasses to be landed

with fins attached (“antifinning”) as well as the increase in demand in elasmobranch meat (Dent and Clarke, 2015). The elasmobranch meat trade generated 2.6 billion USD between 2012 and 2019, exceeding the total income of 1.5 billion USD from shark fins, which shows this increased demand for elasmobranch meat. Considering the value of the global elasmobranch fin and meat trade, there remains a pressing need for increased research efforts.

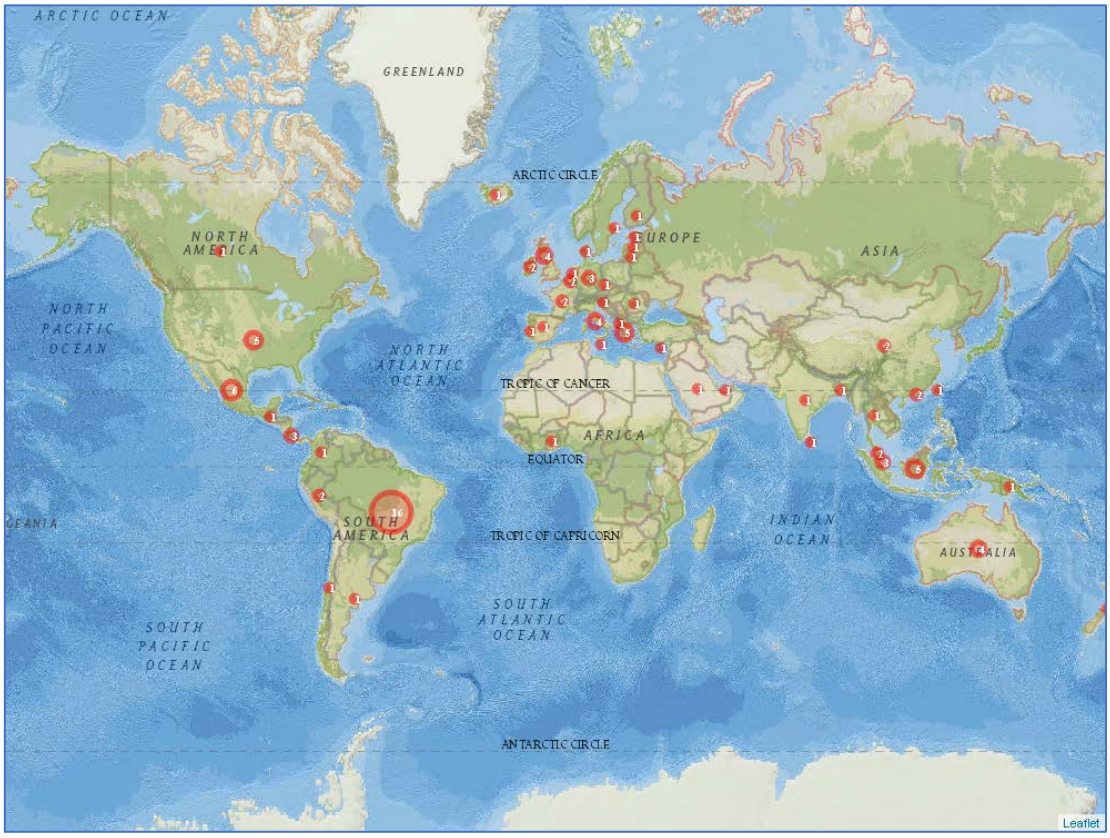

Figure 2-3 Geographical distribution of studies published between 2001 and 2023 (cut off October) on elasmobranchs in the trade (bycatch, elasmobranch landings, species identification and mislabelling). The numbers represent the number of studies carried out in each country. The number of studies were not even across area of study ($\chi^2 = 137.35$, df = 49, $p < 0.0001$) where more studies were published in Brazil than anywhere else in the world. Species Composition of Global Elasmobranch Trade and Conservation Status

2.3.3 Species Identification

A total of 146,794 individual elasmobranchs were sampled, where 19,813 biological samples were taken for further genetic analysis (i.e., tissue, cartilage etc) and 126,981 were identified morphologically (Figure 2-4). Most studies targeted elasmobranchs specifically, aiming to identify elasmobranchs from a range of samples across different points of the supply chain, i.e., fishing vessels, artisanal and commercial fish markets, restaurants, and supermarkets (Table 2-1). Most studies were carried out at fish markets (*n* = 20), food markets and street vendors (*n* = 12), supermarkets and grocery stores (*n* = 11) and artisanal fish markets (*n* = 11) (Table 2-1).

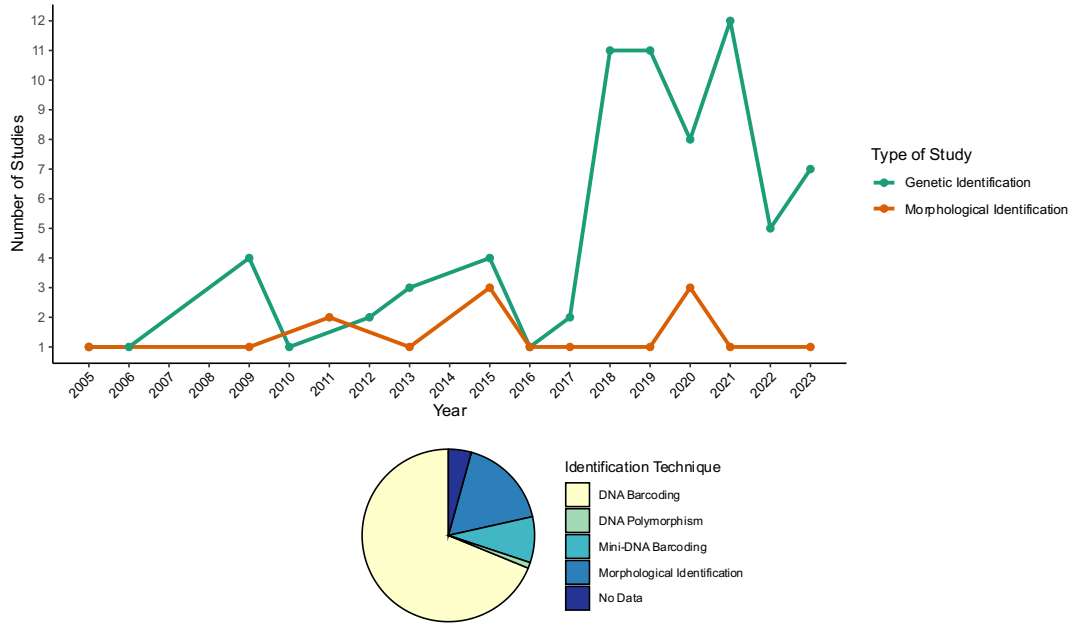

Figure 2-4 This figure shows (a) the identification techniques used over time, specifically showing genetic and morphological identification, and (b) provides a more detailed breakdown of the studies conducted. One study identified elasmobranchs using protein fingerprinting in 2001, but these findings have not been included in the figure.

Biological samples were derived from a range of products, with the most frequent sample type being muscle tissue (9.72%, *n* = 14,274) and dried fin (0.64%, *n* = 933). Many elasmobranchs were morphologically identified (86.5%, *n* = 126,981) across 16 studies (Figure 2-4), and 13.5% (*n* = 19,813) were identified using genetic techniques across 73 studies. Morphological identification studies were often carried out on boats where elasmobranchs were for example caught as bycatch, as well as when they were landed whole at ports or artisanal markets.

Table 2-1 Market sectors for each of the studies conducted on mislabelling and species identification of elasmobranchs. The total number of market sectors was 28. Some studies were conducted in more than one market sector.

Market Sector	Count of Studies
Food markets and street vendors	12
Artisanal fish market	11
Supermarkets and grocery stores	11
Restaurants and takeaway	10
Fish mongers and vendors	9
Landing sites and docks	7
Seizures	6
Fish nets	6
Department stores and retail outlets	4
Auction	3
Pharmacies	3
Fishing vessel	3
Wholesalers	2
Commercial fish market	2
Catering facilities	2
Fin trading hub	2
Online	2
Commercial shark fisheries	1
Fish processing industries	1
Customs	1
Fin auction	1
Fin drying facilities	1
Dry seafood market	1
Food service establishment	1

School	1
University	1
Seafood company	1

2.3.3.1 Molecular Species Identification

A total of 81.2% of studies (*n* = 69) used DNA barcoding (including mini-DNA barcoding) to identify samples (Figure 2.4), with the majority using the CO1 gene (81.2%, *n* = 56). The CO1 region sometimes struggles to accurately discriminate between some shark species due to introgression, hybridisation or low genetic variation between species (i.e. Squalidae and Triakidae, and sharks belonging to the genus *Carcharhinus*) (Corrigan et al., 2017; Pazmiño et al., 2019; Zhang et al., 2021). Carcharhinid sharks for example, are very difficult to tell apart visually, but also genetically (Sebastian et al.,2008; Smith et al., 2009; Fields et al., 2017; Cardeñosa, 2019; Hobbs et al., 2019; Hacohen-Domené et al., 2020). Several studies (*n* = 18) used additional genetic markers, used min-DNA barcoding or used other markers entirely for more accurate species identification, or when samples were heavily degraded (Shivji et al., 2005; Rodrigues-Filho et al., 2009; Caballero et al., 2012; Zeng et al., 2016; Vella et al., 2017; Fields et al., 2017; Feitosa et al., 2018; Pazartzi et al., 2019; Cardeñosa, 2019; Ferrette et al., 2019b; Marchetti et al., 2020; Pardo and Jiménez, 2020; Alvarenga et al., 2021a; Giovos et al., 2021; Domingues et al., 2021; French and Wainwright, 2022; Giagkazoglou et al., 2022).

Some studies used ribosomal RNA (rRNA) markers (*n* = 12), such as 16S (average length 544 bp) or the Internal Transcriber Spacer 2 (ITS2) which have been used in global-scale applications. ITS2 has a high degree of sequence conservation within shark species and can amplify the target species sequence regardless of its

origin (Shivji et al., 2002; Abercrombie et al., 2005b; Nachtigall et al., 2017; Fields et al., 2018). This is particularly valuable when examining samples from trade hubs in Asia, where products on sale can originate from various sources.

The associated costs of downstream analysis, such as sequencing (e.g., Sanger) may be excessive in large-scale applications or in situations where resources are limited (Canfield and Bowen, 2021). A popular alternative method of shark identification in the field involves the use of a multiplex PCR mini-barcode assay that can identify processed shark products. This method significantly reduces to cost per sample to just $1 (USD) by using a multiplex assay that allows for rapid species identification (Cardeñosa et al., 2017), which is a fraction of the cost of DNA barcoding (Canfield and Bowen, 2021).This method still needs to be carried out by trained personnel and the use of specialistic equipment such as thermocyclers and sequencers (Tiktak et al., 2024). Low-cost field-based techniques remain limited in conservation applications, posing an ongoing challenge in addressing the trade in elasmobranch and their related products. Despite the increase in genetic studies carried out on elasmobranchs, information is still limited, for example, with only a handful of nuclear genomes being sequenced (*n* = 9) (Hara et al., 2018; Marra et al., 2019; Pearce et al., 2021).

DNA barcoding is the most common tool for the identification of elasmobranchs and challenges the identification issues posed by the absence of visual features (Ward et al., 2005; Holmes et al., 2009; Kuguru et al., 2018). Genetic techniques such as these offer a promising opportunity for improving the accuracy of elasmobranch trade data (e.g., catch and landing records, as well as mislabelled samples. This data could potentially help to reduce IUU fishing activities whilst

providing informed data on species composition, as well as reducing the illegal trade of threatened and/or prohibited species. Enhanced seafood traceability, inspections (i.e., at customs and fishery landing sites), field DNA identification methods are needed to manage the influx of fins and meat from threatened CITES-listed elasmobranchs (Cardeñosa et al., 2018).

2.3.4 Species Composition of Global Elasmobranch Trade and Conservation Status

Across the 85 studies, a total of 306 different elasmobranch species were found belonging to 45 families and 12 orders. A total of 53.3% (*n* = 163) species were sharks, Selachimorpha, and 46.7% (*n* = 143) were rays and skates, Batoidea, belonging to 45 Families from 12 Orders (Figure 2 5). Carcharhinidae was the most represented family, making up 52.9% (*n* = 77,597) of all elasmobranch families found, followed by Rhinobatidae (11.5%, *n* = 16,809), Lamnidae (6.69%, n = 9,817), Triakidae (5.98%, *n* = 8,773), Trygpnorhinidae (5.10%, n = 7,480), and Sphyrnidae (4.85%, *n* = 7,113) (Figure 2 5). The most common elasmobranch species found were silky sharks (*Carcharhinus falciformis*) (33.1%, *n* = 48,521), blue sharks (*Prionace glauca*) (12.5%, *n* = 18375), shovelnose guitarfish (*Pseudobatos productos*) (11.2%, *n* = 16,470) and shortfin mako sharks (*Isurus oxyrinchus*) (6.55%, *n* = 9,599) (Figure 2 5).

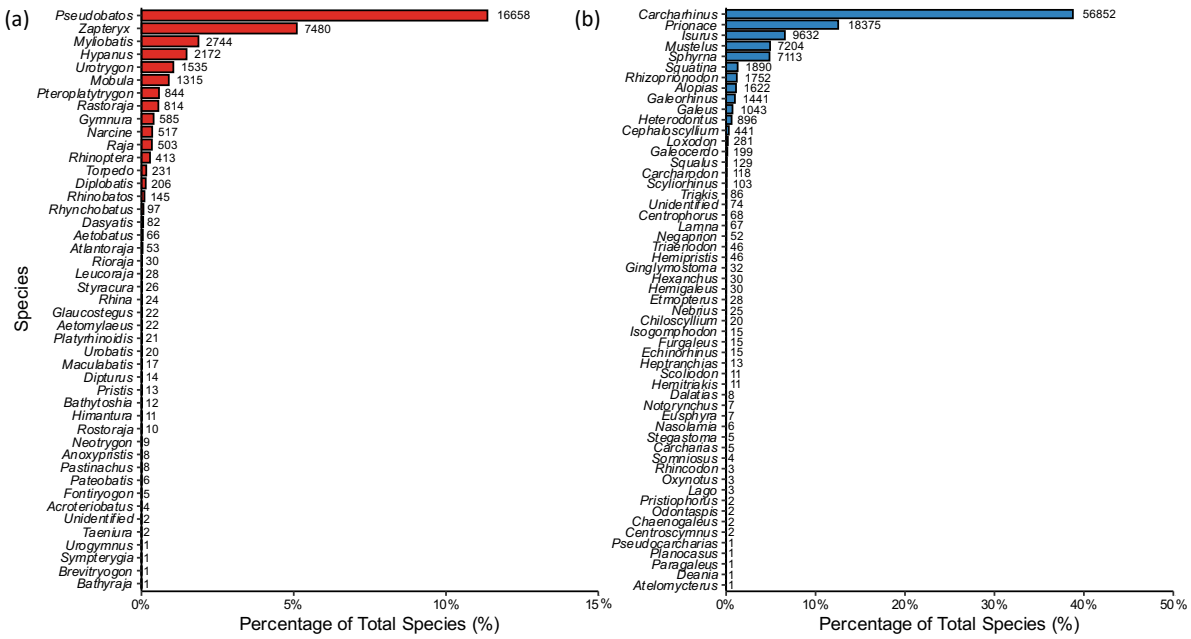

Figure 2-5 The percentage of total species (%) for different genera across the two elasmobranch superorders: (a) Batoidea (rays and skates) and (b) Selachimorpha (sharks), calculated based on data from the 85 studies. Each bar's height indicates the total count (*n*) of each genus within the two superorders. Some species were excluded from the dataset due to missing sample numbers in the studies, despite their presence in the records. The excluded species were *Lago* sp./*Mustelus mosis*, *Isistius brasiliensis*, *Lamna* spp., *Bathyraja brachyurops* and *Himantura* sp.

Thirty-five (*n* = 126) of elasmobranch species were listed as threatened (CR = 18, EN = 34 and VU = 74), 19.6% (*n* = 60) were NT, and 13.4% (*n* = 41) LC. Thirty-five percent (*n* = 109) of species were NE and 15.4% (*n* = 47) were data deficient (DD) showing the limited amount of information we still have about elasmobranchs and their threat status. Additionally, 29 species were listed in CITES which represented 9.48% of the species found in this study.

Our study confirms the existing literature that suggests predominantly pelagic species supply the international elasmobranch trade (Dulvy et al., 2014; Tolotti et al., 2015; Cardeñosa et al., 2018, 2022). The most common species found in this study, silky sharks are not only listed as VU by the IUCN red list, but they have also been

listed in Appendix II of CITES since 04/10/2017. Blue sharks are currently listed as NT globally, but in November 2023 (25/11/2023) they were included in Appendix II of CITES, and their status in the Mediterranean, where they are targeted frequently, is CR. Blue sharks are one of the most targeted shark species globally, and they are often found in elasmobranch catches due to their high distributional overlap with fishing hotspots (Queiroz et al., 2019; Dulvy et al., 2021). Shortfin mako sharks are listed as EN by the IUCN red list. Many longline fisheries overlap with hotspots for commercial valuable and international protected species of sharks (Lucifora et al., 2011; Queiroz et al., 2019; Dulvy et al., 2021). There is limited to no protection for pelagic sharks from fishing efforts in the high seas as these fisheries often operate in areas beyond national jurisdiction (Queiroz et al., 2019). Where there is protection in place (i.e., marine protected areas (MPA)), due to the highly mobile and migratory nature of many pelagic sharks, these areas may not offer enough refuge and leaves them vulnerable to fishing efforts (Heupel et al., 2015; MacKeracher et al., 2019).

The IUCN status and CITES listing were recorded for each species at the time of study and in 2023. This was performed to compare their current status to their previous status as Dulvy et al., (2021) indicated that 32.6% (*n* = 391) chondrichthyans are currently threatened with extinction, with the main cause being overfishing. A total of 52.6% (*n* = 161) of the species found in this review would now be threatened according to their current IUCN Red List Status, and 33.9% (*n* = 104) would now be listed in one of the CITES Appendices. If the current trajectory of the elasmobranch trade continues, it could lead to the depletion of these species. Existing regulations for the protection of elasmobranchs is limited to those that are the most endangered

and threatened, which allows for the continued fishing of other “non-threatened” elasmobranchs to near depletion (Shea et al 2022).

Treaties such as CITES work well when implemented correctly but relies on other national and international fisheries management measures (Vincent et al., 2014), which if not managed effectively may lead illegal trade of threatened wildlife. There are also challenges with enforcing CITES, especially as many elasmobranch products traded are highly processed and degraded (i.e., fins) and are extremely difficult to identify, thus requiring additional resources such as genetic techniques which are costly, required trained personnel and specialist facilities, and in some cases, the involvement of international experts if samples are sent to laboratories in other countries (Helmy et al., 2016; Zhu et al., 2020). CITES focusses on regulating the international trade and may not directly address the other pressing threats to elasmobranch populations such as overfishing, bycatch and habitat destruction. Conservation of elasmobranchs thus requires a multidisciplinary approach and relevant protection in place across every stage of the seafood supply chain.

Another concerning factor was the occurrence of species in this review initially categorised as DD or NE (50.9%) by the IUCN Red List during the study period. This highlights the significant gap in our understanding of elasmobranch populations and their true status, potentially being threatened by the time the assessment is carried out (IUCN, 2023). By 2023, the proportion of species that were categorised as DD or NE had reduced from 50.9% (*n* = 156) to 2.61% (*n* = 8), indicating the increased research efforts dedicated to our understanding of elasmobranch populations.

2.3.5 Summary of Mislabelling Findings in Elasmobranch Products

Our review resulted on the inclusion of 38 studies reporting on mislabelling in elasmobranchs. Thirty-three different products made up of 7,295 individual samples were tested for mislabelling, where 11.3% (*n* = 824) were mislabelled and 10.1% (*n* = 744) were labelled using umbrella terms (Figure 2-6).

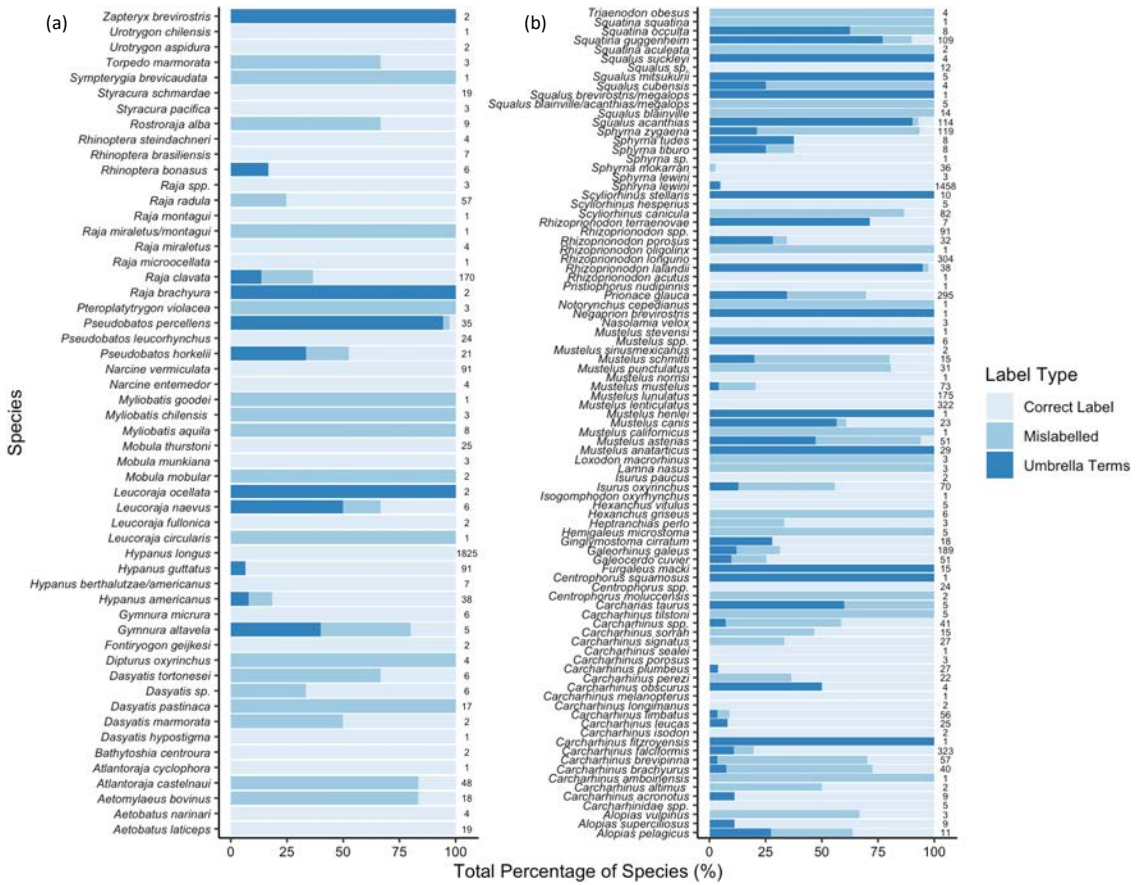

Figure 2-6 Level of mislabelling per species. This figure shows the total percentage of species identified in studies with correct labels, mislabelled or labelled using umbrella terms for the two elasmobranch orders (a) Batoidea (rays and skates) and (b) Selachimorpha (sharks). Each bar represents the percentage of species within each order that are classified as correctly labelled, mislabelled, or labelled using umbrella terms. The *n* represents the total number of species identified

Samples tested for mislabelling included dried and fresh fins (7.33%, *n* = 535), muscle tissue (25.6%, *n* = 1864), amongst others. The most mislabelled samples were muscle tissue (including fillets) (8.21%, *n* =599), or fresh and dry fins (1.89%, *n* = 138). Other mislabelled samples included processed elasmobranch products such as cosmetics (<0.1%, *n* = 3), pet food (0.946%, *n* = 69), capsules (<0.1%, *n* = 1), whole body (<0.1%, *n* = 1) and processed shark meat (0.178%, *n* = 13). Most samples that were labelled using umbrella terms were tissue samples (7.09%, *n* = 517) and processed meat (1.15%, *n* = 84).

2.3.5.1 Mislabelling

Mislabelling rates ranged from 3.70% to 100%, which resulted in an average percentage of 44.3% ± 28.1%. The mislabelled elasmobranchs were composed of nine orders, 20 families, and 75 species across 23 different studies. The most common families found amongst mislabelled samples were Carcharhinidae, Squatinidae and Sphyrnidae. The most mislabelled species were blue sharks (12.5%, *n* = 103), followed by smooth hammerhead shark (*Sphyrna zygaena*) (10.4%, *n* = 86) and small-spotted catshark (*Scyliorhinus canicula*) (8.62%, *n* = 71) (Figure 2.6). Elasmobranchs were mislabelled as fish speices 70 times (8.50%).

Many shark species were mislabelled as other incorrect shark species (31.3%, *n* = 272) across 16 studies across the globe. Blue sharks were mislabelled as other shark species 73 times, followed by small sotted catshark (*n* = 38), spinner shark (*Carcharhinus brevipinna*) (*n* = 37) and silky shark (*n* = 21). These sharks were mislabelled as *Mustelus* species, tope shark (*Galeorhinus galeus*), sandbar shark *(C. plumbeus*), pelagic thresher shark, nurse shark (*Ginglymostoma cirratum*), “hammerhead” shark and others (Marín et al., 2018; Pazartzi et al., 2019; Pardo and

Jiménez, 2020; Agyeman et al., 2021; Cruz et al., 2021). Ninety-six samples were labelled as sandbar shark or brown shark in ten different studies. This type of mislabelling is also frequently known as “species substitution” as often less valuable species are substituted for more valuable ones, or threatened species are substituted by non-threatened species.

In total 48.7% (*n* = 38) of elasmobranch species in mislabelling studies were listed as threatened according to the IUCN red list, where 11.5% were CR (*n* = 9), 15.4% were EN (*n* = 12), and 21.8% were VU (*n* = 17). Thirteen percent (*n* = 10) of mislabelled species were listed in CITES Appendix II, this figure jumps to 39.7% (*n* = 32) in 2023 with the addition of species to CITES over the last two decades (CITES, 2022, 2023). The prevalence of threatened and CITES-listed species amongst the mislabelled samples raises significant concerns. The high level of mislabelling observed across the studies might be in part attributed to the lack of regulations and legislation on elasmobranch fishing and conservation. Stronger enforcement of CITES-listed species is required in exporting and importing nations, as well as more protection for elasmobranchs within nations (i.e., implementation of elasmobranch action plans).

Determining whether mislabelling takes place intentionally or accidently is challenging, as many studies do not address this aspect. Investigating the underlying reasons for why seafood fraud takes place may fall outside the scope of the studies reviewed. Gathering this type of data requires social research, which can be challenging due to the need for specific permits and ethical approval. Individuals engaging in illegal practices may also be reluctant to disclose accurate information, deliberately concealing products or withholding the truth. As a result, mislabelling or

providing false information may be a way to conceal non-compliant or unlawful behavior. Additionally, time constraints, and protection of fishing practices, especially in artisanal fisheries, can further limit data collection. Only a handful of studies (Barbuto et al., 2010; Garcia-Vazquez et al., 2011; Hobbs et al., 2019; Alvarenga et al., 2021a; Zhang et al., 2021) provided pricing information of products which could provide insights into the motivation behind mislabelling (Donlan and Luque, 2019).

2.3.5.2 Umbrella Labelling

Umbrella terms were used for 56 elsmobranch species, made up of 18 families and eight orders across 17 different studies. The most common families found sold using umbrella terms were Carcharinidae, Sphyrnidae and Squatinidae. Umbrella terms were most frequently used for blue sharks (13.7%, *n* = 102), scalloped hammerhead sharks (*Sphyrna lewini*) (9.14%, *n* = 68) and silky sharks (7.73%, *n* = 58) (Figure 2.6).

The umbrella term “Cação” was the most common umbrella term (55.7%, *n* = 418), used throughout studies carried out in Brazil. It’s considered an umbrella term as the species are not defined (Bornatowski et al., 2015; Bernardo et al., 2020). Cação was mostly used for the sale of shark meat (48.5%, *n* = 364), where 19 species were sold under the name Cação, with the most common species sold under this label being blue sharks (*n* = 88), angular angel sharks (*n* = 84) and scalloped hammerhead sharks (*n* = 64). Cação was used for labelling ray products in 7.20% (*n* = 54) of the samples, where chola guitarfish (*n* = 33), Brazilian guitarfish and longnose stingray (*n* = 6) were found most frequently.

In the UK, the terms “dogfish”, “flake”, “huss”, “rigg”, “rock eel” and “rock salmon” are used to describe several species of sharks in the genus *Galeorhinus*,

Mustelus, *Scyliorhinus*, and two specific species of shark blackmouth catshark (*Galeus melastomus*) and spiny dogfish (*Squalus acanthias*) (Hobbs et al., 2019). Sixty-six percent (*n* = 77) of the samples collected in the study conducted by Hobbs et al., (2019) in the UK were spiny dogfish which are listed as VU by the IUCN red list (IUCN, 2023). These terms are considered umbrella terms as they include several species grouped under a single nomenclature. Not only are these labels misleading for consumers, but they may also hinder conservation efforts as there no way of recording which actual species are sold. These terms are also misleading as they closely resemble fish names. If consumers aren't aware that these names refer to shark, they might mistakenly assume it is a type of fish.

Over half of (53.7%, *n* = 29) threatened elasmobranch species were sold using umbrella terms, with 9.26% (*n* = 5) species listed in either CITES Appendix II or III. Using umbrella terms that include both sharks and rays has real implications for their conservation as threatened and/or prohibited species can easily be concealed under these labels making it difficult to enforce and regulate the trade of threatened elasmobranchs.

Although mislabelling is prevalent in the elasmobranch trade, there is a lack of consistency in describing the types of mislabelling that take place. Terms are often used interchangeably despite describing different practices. The varying national and international policies concerning seafood labelling and traceability further complicates efforts to define and track species mislabelling. Characterisation of mislabelling is often done at the study-level which hinders efforts to monitor mislabelling trends. Studies should aim to standardise mislabelling terminology to get a better understanding of the broader mislabelling trends for elasmobranchs.

provide insights into the motivation behind mislabelling (Donlan and Luque, 2019).

2.3.5.3 Conservation Implications of Mislabelling

Blue sharks are the most targeted species for the global trade of sharks as well as in fisheries (Agyeman et al., 2021). Once processed, blue and silky sharks have white meat (muscle tissue) which may resemble fish species such as cod, marlin, and even tuna species as indicated in this review, or other Carcharhinid sharks (Pardo et al., 2018; Agyeman et al., 2021; Alvarenga et al., 2021a; French and Wainwright, 2022), and thus allows them to be traded under these false labels and using generic labels such as “ocean fish” and “white fish” (French and Wainwright, 2022).

Demands for fins may drive the sale of shark meat as many countries have adopted legislation that require the fins, body, and head of sharks to be attached when landed (Oceana, 2010, 2022; Passantino, 2014; Ferretti et al., 2020; Niedermüller et al., 2021). Cardeñosa et al., (2018) found that some of the most traded species in the fin trade were two CITES listed hammerheads, silky sharks, blue sharks, and black tip shark species complex (*Carcharhinus limbatus*, *C. tilstoni*, *C. leidon* and *C. amblyrhynchoides*). Fins belonging to the threatened hammerheads (great, smooth and scalloped) are some of the most valuable and sought after fins in the global fin trade due to their large size (Abercrombie et al., 2005a; Cardeñosa et al., 2018), and may explain why they are amongst the most mislabelled species.

Studies reporting on mislabelling in elasmobranchs often had differing sampling designs, frequently reporting brief sampling methodologies. Many studies focusssed specifcally on detecting mislabelling which can overestimate true mislabelling rates. Sampling was typicaly limited in both scope and duration, often

carrying out “convenience sampling” which may be due to the high costs, time requirements and need for specialised facilities and expertise assocatied with genetic identification techniques (Frézal and Leblois, 2008;Farrokhi and Mahmoudi-Hamidabad, 2012; Luque and Donlan, 2019). Whilst this type of sampling has its benefits, for example being cost effective and speeding up data collection, it introduces difficult-to-measure biases (Luque and Donlan, 2019). As a result, reported mislabelling rates only present a snapshot and may not be rrepresentative of broader trends. Additionally, there may be sampling bias favoring countries with greater resources and genetic exepertise.

As elasmobranchs are facing increasing global concern, the limited availability of papers reporting on the issue of mislabelling could be concerning. Nevertheless, this study did not include ‘Gray Literature’ such as fishing data, export and import data, studies carried out by NGOs, governmental institutions, and other research bodies which may provide additional scope if scrutinised at the same standards as peer review. The studies included in this review were limited by the search terms used. As a result, mislabelling studies outside these parameters may have been missed. Therefore, one of the main limitations of this review is the scope of the search terms employed. A recommendation for future studies is to broaden the search terms and databases used, incorporating a wider range of synonyms and related terms. This approach would help encompass a more comprehensive range of mislabelling studies, addressing the limitations of the current review.

2.3.5.4 The Hidden Costs of Mislabelling

Mislabelling may expose consumers to products containing high concentrations of pollutants without their knowledge potentially putting them at risk

of serious health concerns (Taylor et al., 2014; Sandoval-Herrera et al., 2016; Marchetti et al., 2020; Tiktak et al., 2020). Health concerns may include supressed reproductive development effects, immunosuppression, neurological effects, endocrine disruption and oxidative stress (Chien et al., 2002; Sundeland, 2007; Mohammed Abdul et al., 2015; Nadal et al., 2016). Large-bodied sharks, often pelagic, tend to have higher body mass as well as higher trophic positions which makes them more susceptible to bioaccumulation of toxic pollutants (Lee et al., 2015; Sandoval-Herrera et al., 2016; Tiktak et al., 2020). Most elasmobranch species found mislabelled in this review belonged to species of higher trophic levels, such as the blue shark, smooth hammerhead, and silky sharks. Carcharhiniformes have been found with an average of 1.43 mg kg^{-1} (wet weight) of mercury in their muscle tissue which greatly exceeds the recommended weekly intake as set by the Environmental Protection Agency (EPA) (EPA, 2020; Tiktak et al., 2020). It is critical that elasmobranch products are accurately tracked and correctly labelled due to the human risk associated with the consumption of elasmobranch and elasmobranch related products.

Mislabelling was found all across Europe indicating a serious concern as many elasmobranchs in the Mediterranean Sea and Atlantic Ocean are facing serious population declines, with commonly traded and mislabelled species such as spiny dogfish, silky sharks, smooth hammerhead sharks, shortfin mako shark listed as either Vulnerable or Endangered by the IUCN Red List (IUCN, 2023). In 2011 and 2014 the EU implemented stricter regulations (legislation n1169/2011 and 1379/2013 and) for seafood labelling which require essential information such as commercial and scientific names and that products have clear, comprehensible and legible labels

(FAO, 2011, 2014; Hobbs et al., 2019). These regulations have been put in place to ensure that consumers are appropriately informed about the food they are eating and can make informed choices regarding their health, as well as economic, environmental, social and ethical considerations (FAO, 2011). Although these measures have been put in place, our findings support the current literature that broad terms, such as umbrella terms, are used for seafood product descriptions in the EU, creating ambiguity around products sold, especially in the case of elasmobranchs (FAO, 2014).

Consumers frequently receive deceptive, incomplete, confusing, or misleading information about the seafood they buy and consume. This widespread problem of seafood substitution, mislabelling and lack of transparency is prevalent globally (Luque and Donlan, 2019). Mislabelling can lead to the misidentification of threatened species, for example if threatened species are sold as non-threatened species, it can undermine conservation efforts by misrepresenting the true number of threatened species caught or sold. There is no way of monitoring which species are being caught or sold and may therefore lead to the overexploitation of threatened species as true numbers are concealed. This leads to inaccurate data for conservation planning/priorities and can lead to mismanagement of elasmobranch populations, as it may give an inaccurate indication of population health. Mislabelled species might allow bypassing laws that are aimed at conserving species, particularly with certain elasmobranch species that share similar morphological and genetic characteristics. Hopefully with the addition of Carcharhinid sharks, and hammerhead sharks to CITES, it may protect these species from being mislabelled, i.e., under umbrella terms, in the future.

Seafood mislabelling is a global concern, and solving this crisis requires a collaborative and joint global approach. This means improved transparency throughout the supply chain, stricter regulations, further national and international protection of threatened elasmobranch species, advanced technological and genetic tracing methods, and international collaboration to not only protect human health but also ensure the safeguarding of elasmobranch populations.

2.4 Conclusion and Future Recommendations

This review identified 84 relevant studies published between 1970 and 2023 focussed on four different aspects of the elasmobranch trade, namely species identification, mislabelling, bycatch, and elasmobranch landings. Studies were widespread, though mainly carried out in Europe, Asia, or South America leaving areas such as Africa unstudied despite being fishing and elasmobranch hotspots (Lucifora et al., 2011; Queiroz et al., 2019).

Forty-two percent of elasmobranch species were classified as threatened by the IUCN Red List, and 10.6% of species were listed in one of the CITES Appendices. Although batoids were reported on across a wide range of studies, there is still a larger focus on sharks. Our study supports the literature that many threatened, and CITES-listed species make up the international elasmobranch trade, with Carcharhinidae being the most represented family, making up 52.9% of the total numbers. As this review covers the global elasmobranch trade, we can start to understand the severity of the situation with such a significant percentage of threatened species present in the elasmobranch trade.

Thirty-eight studies were identified that covered aspects of mislabelling. This review has uncovered a wide array of elasmobranch species being sold, and

mislabelled, among many which were threatened and CITES-listed. The most frequently mislabelled species were blue sharks, smooth hammerhead shark and small-spotted catshark. The species sold most frequently under umbrella terms, were blue sharks, scalloped hammerhead sharks and silky sharks. Most of these are listed in CITES Appendix II, and catogorised as either EN, VU or CR by the IUCN Red List. Given the global reach of seafood markets and supply chains, this review offers an initial insight into our understanding of mislabelling and the use of umbrella terms in elasmobranchs globally.

We have found significant gaps in our understanding of mislabelling of elasmobranchs within the seafood trade, especially concerning where mislabelling is taking place and what is being mislabelled (i.e., species, products etc.). Monitoring the trade of elasmobranch and elasmobranch-related products is difficult as not only are there challenges in identifying species, but many species are incorrectly labelled with the use of umbrella labels across the world. Labels frequently used included umbrella terms such as 'Cação' for a wide range of threatened and non-threatened elasmobranch species, or incorrect labels such as fish species or other elasmobranchs. Mislabelling poses a significant threat to elasmobranch populations as one third of all chondrichthyans are currently threatened with extinction. The high level of mislabelling observed across the studies highlights the need for further research being carried out, as well as the necessity for better regulations aimed at seafood traceability and sustainability. As mislabelling was found across the world, tackling this issue requires a global approach. Additional conservation measures and protection are needed within countries but also internationally.

It is important to note that this review is based on peer-reviewed literature and therefore only represents a snapshot of mislabelling in elasmobranchs. Carrying out studies such as these are not only time consuming, but they are also resource intensive and expensive, especially as they rely on specialist facilities (i.e., laboratories for genetic analysis) and trained experts to carry out the research. Therefore, studies may be biased towards countries where most research has been carried out not where the most mislabelling has taken place.

With elasmobranchs facing increasing global concern, the limited availability of papers reporting on the issue of mislabelling as well as species identification is worrying. Our understanding of the global elasmobranch trade and the true extent of mislabelling remains limited. Although there is a positive movement in the conservation and management of elasmobranchs, stronger enforcement measures are required as well as the development of faster, cheaper, and more portable DNA identification techniques (Tiktak et al., 2024). This will help international treaties such as CITES or other regulatory bodies monitor the trade in elasmobranch products, such as meat that are not easy to identify down to species, and tackle emerging tactics used by illegal actors to disguise fins and meat from threatened and CITES-listed species by changing their morphology or mislabelling them (Cardeñosa et al., 2017).

Future studies should aim to focus their research on areas that have received less attention as well as important fisheries and trading hubs for elasmobranchs. This is crucial as in combination with other threats from pollutant exposure, overfishing, habitat loss and climate change, we may see the loss of threatened species entirely. The loss of elasmobranchs from our seas will not only have devastating impacts on marine ecosystems but also the livelihoods of many people that depend on fishing

and/or tourism as a source of income and sustenance. Despite this concern, there has been a positive movement in the management and conservation of elasmobranchs, with the addition of more species to CITES and many countries adopting national shark action plans. More countries are promoting eco-tourism where tourists can swim, dive or snorkel with elasmobranchs all over the world. This growing interest in elasmobranch conservation will help encourage the involvement of government bodies, increasing the funding and capacity building opportunities aimed at protecting threatened elasmobranch species.

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Supplementary Information

Table S 2-1 Studies included in the final review (*n* = 85) including the paper number, author (year), paper type, type of identification technique, country, and continent.

Author(s) and Year	Paper Type	Type of Identification Technique	Country or Place	Continent
Smith and Benson, 2001	Species Identification and Mislabelling	Other	New Zealand	Asia
Shivji et al., 20005	Species Identification	Genetic and Morphological	USA	North America
Clarke et al., 2006	Species Identification	Genetic	Hong Kong	Asia
Silva Rodrigues-Filho et al.,	Species Identification and Mislabelling	Genetic	Bazil	South America
Hernandez et al., 2009	Species Identification	Genetic	Chile	South America
Holmes et al., 2009	Species Identification	Genetic	Australia	Asia
Smith et al., 2009	Species Identification	Morphological	Mexico	Central America
Rodrigues et al., 2009	Species Identification	Genetic	Brazil	South America

Author(s) and Year	Paper Type	Type of Identification Technique	Country or Place	Continent
Barbuto et al., 2010	Mislabelling	Genetic	Mexico	South America
Belcher and Jennings, 2011	Species Identification	Morphological	USA	North America
Cartamil et al., 2011	Species Identification	Morphological	Mexico	Central America
Caballero et al., 2012	Species Identification	Genetic	Colombia	South America
Wallace et al., 2018	Species Identification	Genetic	Canada and USA	North America
Griffiths et al., 2013	Species Identification and Mislabelling	Genetic	Ireland and UK	Europe
Liu et al., 2013	Species Identification	Genetic	Taiwan	Asia
Domingues et al., 2013	Species Identification	Genetic	Brazil	South America
Ramirez-Amaro, 2013	Species Identification	Morphological	Mexico	Central America
Bernard-Capelle et al., 2015	Mislabelling	Genetic	France	Europe

Author(s) and Year	Paper Type	Type of Identification Technique	Country or Place	Continent
Spaet and Berumen, 2015	Species Identification	Genetic and Morphological	Saudi Arabia	Asia
Jabado et al., 2015	Species Identification	Genetic and Morphological	United Arab Emirates	Asia
Sembiring et al., 2015	Species Identification	Genetic and Morphological	Indonesia	Asia
Zeng et al., 2016	Species Identification	Genetic	China	Asia
Clarke et al., 2016	Species Identification	Morphological	Costa Rica	Central America
Vella et al., 2017	Species Identification	Genetic	Malta	Europe
Piovano et al., 2017	Species Identification	Morphological	Fiji	
O'Bryhim et al., 2017	Species Identification	Genetic	Costa Rica	Central America
Almeron-Souza et al., 2019	Mislabelling	Genetic	Brazil	South America
Marin et al., 2018	Species Identification and Mislabelling	Genetic	Peru	South America

Author(s) and Year	Paper Type	Type of Identification Technique	Country or Place	Continent
Pardo et al., 2018	Mislabelling	Genetic	23 European countries (Portugal, Spain, Finland, Baltic States (Latvia, Lithuania and Estonia), France, Sweden, Italy, Belgium, Netherlands, Denmark, Greece, Cyprus, United Kingdom, Ireland, Germany, Slovenia, Czech Republic and Romania), Iceland)	Europe
Bunholi et al., 2018	Species Identification and Mislabelling	Genetic	Brazil	South America
Sarmiento-Camacho et al., 2018	Species Identification and Mislabelling	Genetic	Mexico	Central America
Christiansen et al., 2018	Mislabelling	Genetic	Belgium	Europe
Appleyard et al., 2018	Species Identification	Genetic	Papua New Guinea	South America
Feitosa et al., 2018	Species Identification	Genetic	Brazil	South America

Author(s) and Year	Paper Type	Type of Identification Technique	Country or Place	Continent
Wainwright et al., 2018	Species Identification	Genetic	Singapore	Asia
Fields et al., 2018	Species Identification	Genetic	Hong Kong	Asia
Md-Zain et al., 2018	Species Identification	Genetic	Malaysia	Asia
Cardeñosa, 2019	Mislabelling	Genetic	USA	North America
Hellberg et al., 2019	Species Identification and Mislabelling	Genetic	USA	North America
Caelgri et al., 2019	Mislabelling	Genetic	Brazil	South America
Ferrito et al., 2019	Species Identification and Mislabelling	Genetic	Italy	Europe
Pazartzi et al., 2019	Mislabelling	Genetic	Greece	Europe
Ferrette et al., 2019b	Species Identification	Genetic	Brazil	South America

Author(s) and Year	Paper Type	Type of Identification Technique	Country or Place	Continent
Haque et al., 2019	Species Identification	Genetic	Bangladesh	Asia
Fernando et al., 2019	Species Identification	Genetic	Sri Lanka	Asia
Manojkumar et al., 2019	Species Identification	Morphological	India	Asia
Ferette et al., 2019b	Species Identification	Genetic	Brazil	South America
Muttagin et al., 2019	Species Identification	Genetic	Indonesia	Asia
Hobbs et al., 2019	Species Identification and Mislabelling	Genetic	United Kingdom	Europe
Marchetti et al., 2020	Mislabelling	Genetic	Italy	Europe
Pardo and Jimenez, 2020	Mislabelling	Genetic	Spain	Europe
Bernardo et al., 2020	Species Identification and Mislabelling	Genetic	Brazil	South America

Author(s) and Year	Paper Type	Type of Identification Technique	Country or Place	Continent
Giovos et al., 2020	Mislabelling	Genetic	Greece	Europe
Widowati et al., 2020	Species Identification and Mislabelling	Genetic	Indonesia	Asia
Delpiani et al., 2020	Mislabelling	Genetic	Argentina	South America
Abdullah et al., 2020	Species Identification and Mislabelling	Genetic	Indonesia	Asia
Rodrigues et al., 2020	Species Identification	Genetic	Brazil	South America
Clavareau et al., 2020	Species Identification	Morphological	Atlantic and Indian Ocean	Africa
Hacohen-Domene et al., 2020	Species Identification	Morphological	Guatemala	Central America
Bakiu et al., 2020	Species Identification	Morphological	Albania	Europe
Alvarenga et al., 2021	Mislabelling	Genetic	Brazil	South America

Author(s) and Year	Paper Type	Type of Identification Technique	Country or Place	Continent
Zhang et al., 2021	Species Identification	Genetic	China	Asia
Cruz et al., 2021	Species Identification and Mislabelling	Genetic	Brazil	South America
Agyeman et al., 2021	Mislabelling	Genetic	Ghana and Spain	Africa and Europe
Filonzi et al., 2021	Mislabelling	Genetic	Italy	Europe
Giovos et al., 2021	Species Identification and Mislabelling	Genetic	Greece	Europe
Munguia-Vega et al., 2021	Mislabelling	Genetic	Mexico	Central America
Martins et al., 2021	Species Identification	Genetic	Brazil	South America
da Cruz et al., 2021	Species Identification	Genetic	Brazil	South America
Castillo et al., 2021	Species Identification	Morphological	Guatemala	Central America

Author(s) and Year	Paper Type	Type of Identification Technique	Country or Place	Continent
O'Bryhim et al., 2021	Species Identification	Genetic	Costa Rica	Central America
Villate-Moreno et al., 2021	Species Identification	Genetic	Germany	Europe
Choo et al., 2021	Species Identification	Genetic	Singapore	Asia
French and Wainwright, 2022	Mislabelling	Genetic	Singapore	Asia
Dufflocq et al., 2022	Mislabelling	Genetic	Brazil	South America
Giagkazoglou et al., 2022	Species Identification and Mislabelling	Genetic	Greece	Europe
Manguia et al., 2022	Species Identification and Mislabelling	Genetic	Mexico	Central America
Seah et al., 2022	Species Identification	Genetic	Malaysia	Asia
Sharrad et al., 2023	Mislabelling	Genetic	Australia	Asia

Author(s) and Year	Paper Type	Type of Identification Technique	Country or Place	Continent
Cundy et al., 2023	Mislabelling	Genetic	Australia	Asia
Niedermeier et al., 2023	Mislabelling	Genetic	Germany	Europe
Khali et al., 2023	Mislabelling	Genetic	Australia	Asia
Klangnurak et al., 2023	Species Identification	Genetic	Thailand	Asia
Alfaro-Cordova et al., 2023	Species Identification	Genetic and Morphological	Peru	South America
Prasetyo et al., 2023	Species Identification	Genetic	Indonesia	Asia

Table S 2-2 Definitions of terms summarised in the review.

Term	Country	Definition	Author(s) (Year)
Raie	Greece	Ray	(Giovos et al., 2020; Giagkazoglou et al., 2022)
Salchi	Greece	Common name for Batoids	(Giovos et al., 2020; Giagkazoglou et al., 2022)

Vatos	Greece	*Raja* species	(Giovos et al., 2020; Giagkazoglou et al., 2022)
Rinovatos	Greece	Guitarfish	(Giovos et al., 2020; Giagkazoglou et al., 2022)
Rina	Greece	Angel shark	(Giovos et al., 2020; Giagkazoglou et al., 2022)
Galeos	Greece	*Mustelus* spp.	(Giovos et al., 2020; Giagkazoglou et al., 2022)
Palombo	Italy, Spain and South America	*M. asterias* and *M. mustelus*	(Barbuto et al., 2010; Pazartzi et al., 2019; Marchetti et al., 2020; Dufflocq et al., 2022)
Shark species	Various	When shark species were listed (this includes full genus and species)	This study
Fish species	Various	When fish species were listed (this includes full genus and species)	This study
Fish	Various	When the label stated "fish"	Various
Shark	Various	When the label stated "shark"	Various

Ray	Various	When the label stated "ray"	Various
Guitarfish	Various	When the label stated "guitarfish"	Various
Tuna species	Various	Tuna was the most common fish species, and often used as a label, therefore tuna species were their own group	This study and others
Cação	Brazil	Elasmobranch	Various
Flake	Australia and United Kingdom	Shark fillets	(Cundy et al., 2023; Khalil et al., 2023; Sharrad et al., 2023)
Other	Various	Any other term used for elasmobranchs	Various
Dogfish, huss, rigg, rock eel, rock salmon	United Kingdom	Used to describe species of sharks in the genus *Galeorhinus*, *Mustelus*, *Scyliorhinus*, and two specific species of shark blackmouth catshark (*Galeus melastomus*) and spiny dogfish (*Squalus acanthias*)	(Hobbs et al., 2019)

Table S 2-3 Different identification techniques used for identifying elasmobranchs in this review.

Technique	Count of Studies
DNA Barcoding	64
Morphological Identification	16
Mini-DNA Barcoding	8
DNA Polymorphism	1
Isoelectric Focusing (Protein Fingerprints)	1
No Data	4

Note: Some studies may have used a combination of one or more techniques to identify species.

Chapter 3

3 Genetic identification of three CITES-listed sharks (bigeye thresher, pelagic thresher and shortfin mako shark) using a paper-based Lab-on-a-Chip (LOC)

Chapter Overview

This chapter presents the development of a paper-based Lab-on-a-Chip (LOC) to identify three CITES-listed sharks, bigeye thresher (*Alopias superciliosus*), pelagic thresher (*A. pelagicus*) and shortfin mako shark (*Isurus oxyrinchus*) in the field. The LOC gives a simple colour change when one of the target species is present. The LOC has the potential to act as a rapid in-field screening test.

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Author Contributions

As lead author I designed the project, collected the samples and data, carried out the method development in the lab as well as in the field, wrote, and submitted the manuscript. I supervised two final year A-level, and two BSc students that provided support with carrying out preliminary laboratory analyses. Other co-authors provided support with conceptualisation, applying for funding, data interpretation and revising the manuscript.

3.1. Abstract

Threatened shark species are caught in large numbers by artisanal and commercial fisheries and traded globally. Monitoring both which shark species are caught and sold in fisheries, and the export of CITES restricted products, are essential in reducing illegal fishing. Current methods for species identification rely on visual examination by experts or DNA barcoding techniques requiring specialist laboratory facilities. The need for specialist equipment and/or input from experts means many markets are currently not monitored. We have developed a paper-based Lab-on-a-Chip (LOC) to facilitate identification of three threatened and CITES-listed sharks, bigeye thresher (*Alopias superciliosus*), pelagic thresher (*A. pelagicus*) and shortfin mako shark (*Isurus oxyrinchus*) at market source. DNA was successfully extracted from shark meat and fin samples and combined with DNA amplification and visualisation using Loop Mediated Isothermal Amplification (LAMP) on the LOC resulting in the successful identification of the target species of sharks, with a working positive and negative control. The LOC provided a simple "yes" or "no" result via a colour change from pink to yellow when one of the target species was present. The LOC serves as proof-of-concept (PoC) for field-based species identification as it does not require specialist facilities. It can be used by non-scientifically trained personnel, especially in areas where there are suspected high frequencies of mislabelling or for the identification of dried shark fins in seizures. We anticipate that the development of the LOC has the potential to greatly facilitate the monitoring of the trade in shark and shark-related products.

Keywords: *Lab-on-a-Chip (LOC); CITES; Identification; Sharks; Portable; LAMP*

3.2. Introduction

Elasmobranchs (sharks, rays and skates) represent one of the most vulnerable taxa on the planet, where over one third of all elasmobranchs are threatened with extinction (Dulvy et al, 2021; IUCN, 2020). One of the primary drivers of decline across the group is overfishing. Some species have experienced population declines of over 90%, and without effective protection many species may go extinct (Dulvy et al., 2021; Pacoureau et al., 2021). Elasmobranchs often occupy tertiary positions in food chains as meso and apex predators, playing a crucial role in ecosystem functions (Abercrombie et al., 2005; Ferretti et al., 2010; Matich and Heithaus, 2014). Many elasmobranch species exhibit similar traits to that of large mammals with long gestation periods, slow maturity, and low fecundity, which makes them especially vulnerable to overexploitation (Dulvy et al., 2014; Sims, 2015).

Shark meat is traded and consumed globally, and there is growing concern for the widespread practice of species mislabelling and substitution, even in non-coastal regions where sharks may not be typically considered a primary food source (Almerón-Souza et al., 2018). Mislabelling and species substitution occurs when sharks are sold as other elasmobranch species or teleost fish, and consumers may thus be unaware that they are consuming shark products (Hobbs et al., 2019; Pazartzi et al., 2019). Mislabelling can pose a threat to the safety of consumers as they may be exposed to allergens, zoonotic diseases, and high concentrations of pollutants, without their knowledge (Spink and Moyer, 2011; Tiktak et al., 2020). Countries where mislabelling and species substitution has occurred include the UK, Brazil, Greece, Indonesia, Singapore, Taiwan, Peru, Italy, Spain, and the USA, amongst others

(Bornatowski et al., 2014; Almerón-Souza et al., 2018; Hellberg et al., 2019; Hobbs et al., 2019; Pazartzi et al., 2019; Bernardo et al., 2020).

Ecuador is home to a diverse array of shark species, but populations have experienced declines driven by the demand for shark fins and meat in international markets. They have also been traditionally sold and consumed in fish markets along the coast of Ecuador (Dominguez and Cobeña, 2019). In 2007, the Ecuadorian government prohibited shark finning and directed shark fisheries in the country to reduce the illegal trade but allowed for the sale of incidentally whole caught sharks (fins and body), except in the Galápagos Marine Reserve where sharks are fully protected (Executive Decree 486 of 2007, cited in Hearn et al., 2022). Additional efforts to protect sharks included prohibiting the fishing of hammerhead (Sphyrnidae) and oceanic whitetip sharks (*Carcharhinus longimanus*) (Ministerial Agreement MCEIP-SRP-2020-0084-A, cited in Hearn et al., 2002). Despite these efforts, over one million shark landings were reported in Ecuador between 2008 and 2012 with the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) listed shark species: bigeye thresher (*Alopias superciliosus*), pelagic thresher (*A. pelagicus*) and shortfin mako (*Isurus oxyrinchus*) sharks accounting for 61% of the total landings during that period (Martínez-Ortiz et al., 2015).

Current monitoring is primarily undertaken using DNA barcoding as a technique to identify sharks when they cannot be identified based on their morphological features. It may also be used to confirm visual identification, for example when shark fins have been processed and dried (Ward et al., 2005; Clarke et al., 2006; Cardeñosa et al., 2017). Many studies use species-specific primers to perform polymerase chain

reaction (PCR)-based amplification of DNA from mitochondrial genes such as cytochrome b, cytochrome oxidase I (CO1), ITS2 and NADH2, and then visualised through gel electrophoresis and/or DNA sequencing (Shivji et al., 2002; Feitosa et al., 2008; Fields et al., 2015). More recently, Loop Mediated Isothermal Amplification (LAMP) has been demonstrated to amplify short DNA fragments from 12 CITES-listed shark species (But et al., 2020; Lin et al., 2021), not including shortfin mako shark. Although these genetic techniques have become increasingly popular over the past few decades, they remain time consuming and expensive, especially as they rely heavily on access to costly laboratory facilities, trained personnel, specialised field equipment, and even international export if they are sent to labs in other countries (Helmy et al., 2016; Zhu et al., 2020). One example of shark identification in the field involves the use of a multiplex PCR mini-barcode assay that can identify processed shark products. The cost of each sample is reduced to $1 by using a multiplex assay that can identify species rapidly (Cardeñosa et al., 2017). Nevertheless, this method still requires the use of trained personnel and specialistic equipment such as thermocyclers and sequencers. Developments in genetic techniques have allowed scientists to sequence DNA in the field, i.e., using a hand-held sequencing device such as the minION (Nanopore, UK) (Johri et al., 2019; Oxford Nanopore, 2023), nevertheless it costs approximately $60 - $80 USD per sample to run and requires considerable expertise (Zhang et al., 2020). Thus, these techniques may not be suitable for implementing in countries where control authorities have limited or no access to these technologies or technical expertise.

Miniaturised laboratory techniques are becoming increasing popular as they are often rapid, cost-effective, and portable (e.g., can be carried out in the field). One

example of these techniques is Lab-on-a-Chip (LOC) technology. The LOC incorporates several laboratory processes on a small device that is usually only a few square centimetres in size (Azizipour et al., 2020). LOCs are typically made of glass or polydimethylsiloxane (PDMS), though recent development in the field has incorporated the use of paper-based microfluidic chips which further decreases the cost of application and can be as low as $0.01 per sample (Esfandyarpour et al., 2017; McNeill et al., 2021). LOCs have primarily been used for clinical diagnostics and biomedical research, for example glucose monitoring for diabetes, covid-19 detection, and HIV (Azizipour et al., 2020). Significant development in this field over the past 15 years has allowed for point-of-care (PoC) diagnostics, though there has been limited use of LOC technology in conservation (Wimbles et al., 2021).

We have developed a simple, on-site identification tool in the form of a LOC which can be easily deployed to monitor the trade of three CITES-listed sharks: bigeye thresher, pelagic thresher and shortfin mako belonging to the order Lamniformes. This study specifically aimed to 1) develop a field-based cell lysis and DNA extraction method that would be suitable for shark muscle and wet fin tissue samples; 2) design species-specific LAMP primers for the three CITES-listed sharks for visual identification; 3) combine the two previous steps, along with positive and negative controls, into an integrated paper-based LOC device; and 4) evaluate the applicability of the LOC device through proof-of-concept field testing and end-user workshops.

3.2. Methods

3.2.1. Sample Collection

For the initial development of the LOC, a total of 31 tissue samples were collected from 26 different species of sharks, rays and fish, and confirmed by Sanger Sequencing of the CO1 gene (see section 2.2. for further details). Eleven were collected from fishing ports and markets across three regions in Ecuador between June and July 2018 (Supplementary Information (SI); Table S 3-1). The sampling sites included Mercado de Mariscos Santa Rosa of Salinas in the province of Santa Elena (coordinates: 2° 13' 0" South, 80° 58' 0" West), Playita Mía in Manta, province of Manabí (coordinates: 0° 57' 10" South, 80° 48' 45" West) and Puerto Pesquero Artesanal de Esmeraldas situated in the province of Esmeraldas (coordinates: 0.9682° North, 79.6517° West) [CITES Permit: No. 18EC000020/VS]. One fin clip of whale shark (*Rhincodon typus*) was collected from the Galapágos Islands, Ecuador [CITES Permit: No. 18EC000020/VS], 17 tissue samples were also received from the USA [UK CITES No. GB040, and U.S.A. CITES No. US044], and opportunistic fin clips were taken from two species of shark from Sea Life Paris Aquarium. An additional 12 samples were collected from Ecuador between June and July 2018 (bigeye thresher shark, pelagic thresher shark, shortfin mako shark, and blue shark; n = 3 per species) for evaluation of the prototype LOC. These sharks were identified visually and were confirmed by LAMP. No live specimens were involved in the sample collection. All samples were stored in nucleic acid preservation (NAP) buffer, except for aquarium tissue samples which were stored in 95% ethanol. All samples were kept at room

temperature for short-term storage (2 months) and then at -20 °C for long-term storage.

3.2.2. Confirmation of Species Identification

DNA was extracted from ~25 mg of tissue from the 26 different species of elasmobranchs and teleost fish (*n* = 31; Table S 3-1) using a Bioline ISOLATE II Genomic DNA Kit (Bioline, UK) according to the manufacturer's instructions. In the final step of the kit-based extraction, samples were eluted with Nuclease-Free Water (Merck, Germany) in place of elution buffer to ensure compatibility with LAMP. DNA from these 26 species of elasmobranchs and teleost fish were used for validation of species identity for testing species-specific primers in LAMP (section 2.4).

Species identifications were confirmed via PCR and Sanger Sequencing. Fish primers FishF1: 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1: 5' TAGACTTCTGGGTGGCCAAAGAATCA-3', were used to amplify ~655 bp of DNA from the CO1 region of the mitochondrial genome (Ward et al., 2005). PCR was performed in a total volume of 20 µL, which included, 10 µL of 2x MyTaq^{TM} Red Mix (Bioline, UK), 0.4 µL of both forward and reverse primers (20 µM), 1 µL of DNA (5 ng/µL), and 8.2 µL of Nuclease-Free Water (Merck, Germany). All PCR reactions were run with a negative control (no template DNA) and positive control (scalloped hammerhead). Gel electrophoresis prior to sequencing demonstrated a product size of approximately 650 bp in length. ExoSAP-IT™ Express (Thermo Fisher Scientific, UK) was used to treat PCR products prior to sequencing. Briefly 2 µL of ExoSAP-IT Express reagent was added to 5 µL of PCR product followed by incubation at 37°C for 4 minutes and another incubation period at 80°C for 1 minute.

Approximately 20 ng of DNA for both forward and reverse sequences were sent for Sanger Sequencing at the Medical Research Council Protein Phosphorylation and Ubiquitylation Unit (MRC PPU) (Dundee, Scotland) (Table S 3-1). Resulting sequences were confirmed by eye, trimmed for quality (~50 bp from 5' and 3' ends) and any residual primer sequences were removed. Forward and reverse sequences were then aligned in BioEdit v7.05 (Windows 95/98/NT) and a Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov) was used to confirm species by comparing the sequences against all taxa in GenBank.

3.2.3. LOC: Loop Mediated Isothermal Amplification (LAMP)

3.2.3.1. Primer Design

PrimerExplorer V5 (http://primerexplorer.jp/lampv5e/index.html) was used to develop species-specific primers for three CITES-listed species of sharks: bigeye thresher, pelagic thresher and shortfin mako shark. Primers for bigeye thresher shark (KC204935) were developed from the ITS2 region. Primers for pelagic thresher (KF020876) and shortfin mako shark (MH760159) were developed based on the non-coding mitochondrial D-Loop region of the mitochondrial genome (Ardura et al., 2013) (Table 3-1; Table S 3-3). ITS2 and D-LOOP genes were chosen for primer development because of their high mutation rates and variation between species. ITS2 has been previously used to develop species-specific primers in sharks (Shivji et al., 2002), and therefore was chosen for the bigeye thresher shark. For the full primer development process see SI 3.

Primers were generated using the default settings in PrimerExplorer and for each primer sequence a BLAST search against all taxa in GenBank was performed.

Loop primers were developed for each species by putting the LAMP primers back into PrimerExplorer and automatically generating loop primer sets. Loop primer sequences were also run against all taxa in GenBank using BLAST. LAMP primers consist of a forward primer (F3), forward inner primer which is made up of F1c and F2 (FIP), a reverse primer (B3), reverse inner primer make up of B1c and F2 (BIP) and optional loop forward (LF) and/or reverse primers (LR).

3.2.3.2. Amplification Reaction

LAMP was performed in a total volume of 10 µL containing 3 µL of Nuclease-Free Water (Merck, Germany), 5 µL of WarmStart® Colorimetric LAMP Master Mix (NEB, UK), 1µL of primers FIP (0.8 µM), BIP (0.8 µM), F3 (0.2 µM), B3 (0.2 µM), LF (0.4 µM) and specific primers for the three sharks (bigeye thresher, pelagic thresher and shortfin mako shark; Table 3-1) or Lambda (λ) LF and/or LR (0.4 µM), and 1 µL of DNA (approximately 5 ng/µL). Negative controls, containing no template DNA, were also prepared. LAMP primers for the positive control using λ DNA were obtained from Merck (Germany) and λ DNA was purchased from New England Biolabs (NEB, UK) (250 ng λ DNA, stored in 10mM Tris HCl pH 7.5, 10mM NaCl and 1mM EDTA). Five nanograms per microliter of λ DNA was used for the positive control LAMP reaction (Supplementary Information).

Table 3-1. LAMP and loop primers for bigeye thresher shark (*Alopias superciliosus*), pelagic thresher shark (*A. pelagicus*) and shortfin mako shark (*Isurus oxyrinchus*).

Species	Primer Sequences (5'-3')
Bigeye thresher shark	F3: TCCGGATGGTAGCCGTGG
	B3: GGAAGGAGCCTCAACTCCAG
	FIP: GGACCAAACCAGTCACTGCGTTCAGGTGCAGGCGTTACC
	BIP: GCTGGTGGTGTGTTCGCTTTGGGCGTCAGCGCAGCCAA
	LF: GCTCCGCTTCACCTCCTAC
	LR: TGGCATTTCGGACGTGAGT
Pelagic thresher shark	F3: ATTTGTGGCACTGCACTC
	B3: CTCGGTGTCCCAGATCAG
	FIP: GGTACATTCATTCTTGACGCGATTACTAATCCCCATTAATTGACCAG
	BIP: CTCCCTTTTATGCCATTTTCGTCCAGTAATTGCTTCATCCCCG
	LR: TTGATCGTCTCAAGATTTCTTGTCC
Shortfin mako shark	F3: CCCCATTACTGTACTAATCACT
	B3: GGGATTAATCGAGTACAGCG
	FIP: GAGGGTGGAAGGAGTAATATGATGATTTCATTACACTCTATTCTTAGTCC
	BIP: ATCTCTGTATATCTTATGCGGGCTCACAAATAGAGCAATTTTTTCCT
	LR: GGTAAGAACATCACATCCCGC

The mixture was incubated at 65 °C for 1 hour and then visualised on a 1.5% agarose gel, following electrophoresis, using a Biorad Gel Doc EQ system w/ Universal Hood II (UV transilluminator) (Bio-Rad Laboratories, USA). Successful amplification was indicated by a colour change (pink to yellow) and presence of bands on the gel.

3.2.3.3. Primer Specificity

To ensure amplification of the three target species of sharks, bigeye thresher, pelagic thresher, and shortfin mako shark, the species-specific primers (Table 3-1) were initially tested against DNA from target species (in triplicate) to confirm successful amplification. The specificity of the primers was then determined by LAMP amplification of 26 different species of elasmobranchs and fish previously confirmed by Sanger Sequencing (Section 3.2 and Table S 3-1). A DNA concentration of

approximately 5 ng/µL was used for all subsequent reactions. All reactions were performed in triplicate for each primer set and amplification was further confirmed by gel electrophoresis.

3.2.4. LOC and LAMP Optimisation

3.2.4.1. LOC Design & Fabrication

The LOC was designed to integrate DNA extraction, amplification and visualisation using LAMP. Method development and optimisation, including portable lysis and extraction for the LOC can be found in SI 2. The LOC incorporated three CITES-listed sharks namely bigeye thresher, pelagic thresher shark, and shortfin mako shark, as well as a negative and positive control using λ primers and DNA (DNA only for positive control). LOCs were created in Microsoft Word, and a wax design was printed onto Whatman Grade 1 filter paper (Fisher Scientific, UK) using a Xerox ColorQube 8580 Printer (Xerox, USA) to produce the design in Figure 3-1b. The printed template was then incubated at 130 °C for 3 minutes allowing the wax to melt. One Whatman© GC/F glass microfiber filters (6.4 mm in size; grade 1.2 µm) (Merck, Germany) was placed in-between the second (green) and third (orange) panel on the chip (panel 2 and 3) (Figure 3-1b & c), and five were placed on a separate plastic mould (Figure 3-1e, panel 6).

3.2.4.2. LOC Standard Operating Procedure (SOP)

For lysis, approximately 25 mg of tissue from each of the above species (one species per chip) were added to 500 µL of 5 M GuHCl and agitated using a pipette tip ("cutting"; mechanical lysis) (Figure 3-1a). The DNA solution was then left at room temperature for 15 minutes. Whilst the sample was lysing, a mastermix was made for

each of the sharks, the negative and positive λ control (enough for two reactions) which contained 2 µL of species-specific primer, 10 µL of WarmStart® Colorimetric LAMP Master Mix (NEB, UK), and eight microliters of Nuclease-Free Water (Merck, Germany) for the negative control (additional details on controls can be found in SI 2 and 5). Each solution was gently pipetted up and down to mix all the components and left on ice until used on the LOC.

Thirty microlitres of the lysed DNA solution was loaded onto the orange (3) panel (Figure 3-1c). Then 30 µL of 70% ethanol was loaded onto the chip (panel 3 again), and once that was absorbed another 30 µL was added. When the ethanol had dried completely (after ~30 seconds), the black waste panel (1) was discarded, and the orange (3) and green (2) (in this order) were placed over the purple (4) and blue (5) panel (Figure 3-1d). Whilst the ethanol was drying, the 3 µL of λ DNA was added to the positive control mastermix and mixed by pipetting gently up and down.

The LOC was then re-folded and placed on top of the plastic mould that is stuck onto an adhesive Polyester PCR Sealing Film (Starlab, UK) containing the five filters. One hundred microliters of Nuclease-Free Water (Merck, Germany) was gently loaded onto the pink panel (3) to elute the DNA which travelled along the channels on the cross shaped purple panel (4) and into the four separate DNA amplification chambers on the blue tab (5) that sits on top of the plastic mould (Figure 3-1e, panel 6). Once the water had been fully absorbed by all four chambers, all paper panels were removed. Next, the LAMP mastermixes were loaded onto the corresponding chambers (6 µL of either bigeye thresher, pelagic thresher, shortfin mako shark chambers, seven microliters for the λ positive control and 10 µL for the negative control).

The plastic LOC was sealed together by folding the adhesive Polyester PCR Sealing Film (Starlab, UK). The LOC was then then placed onto a portable Miniature Incubator (TC-MIW) with a Temperature Controller (TC-1-100-1) (BioScience Tools, USA) for 30 minutes at 65 °C. Each of the chambers on the plastic mould contained the LAMP mix which turned from pink to yellow if the target species was present, a positive control was also included on the LOC to ensure that the set-up was working. A working LOC was considered if the positive control changed from pink to yellow, and the negative control stayed pink (see Figure S 3-10 for decision making flowchart). Colour changes of the three sharks was dependent on what species were used for the initial lysis step.

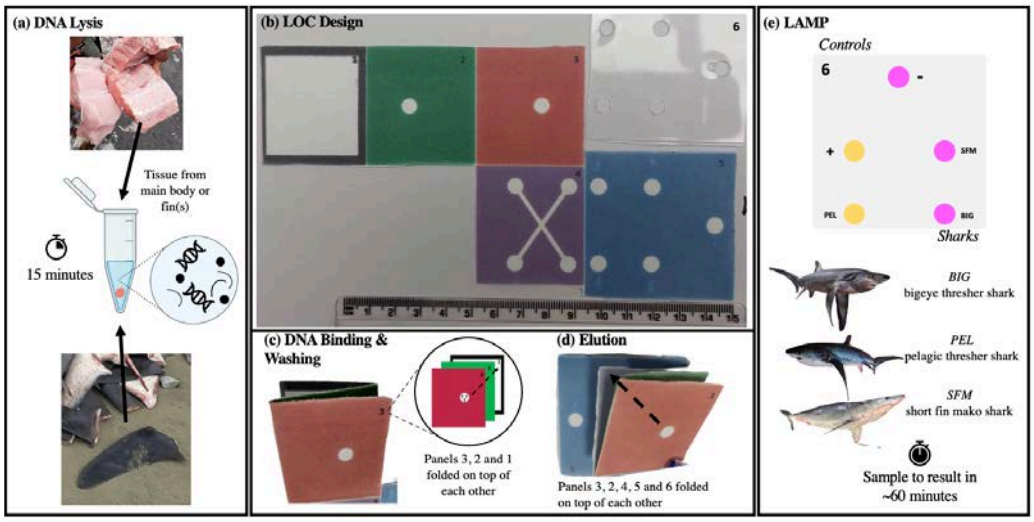

Figure 3-1. a) Overview of the procedure for cell lysis from a small piece of meat or wet fin sample taken from markets; b) Photograph showing the paper LOC design with five different coloured areas (panels) that was folded in a origami-style manner to enable different steps of the genetic analysis to be performed; c) Schematic showing the folding of the LOC for DNA binding and washing steps; d) Schematic showing the alternative folding of the LOC for DNA elution; e) Location of the five LAMP chambers for species-specific amplification, as well as positive and negative controls.

LOCs were tested in the lab on tissue belonging to each of the target species (bigeye thresher, pelagic thresher and shortfin mako shark) as well as one non-target species (blue shark, *Prionace glauca*). Blue shark was chosen as the non-target species as it is commonly found at the fish markets in Ecuador and is an important commercial species globally (Clarke et al., 2006; Martínez-Ortiz et al., 2015). The LOCs were tested ≥ 3 times per species, pictures were taken before and after to show the colour change from pink to yellow. Positive and negative controls were run on a thermocycler conjunctively to every two LOC's using the same mastermix with λ DNA (positive control only), H_20 and λ primers.

3.2.4.3. Testing of Real-World Samples and Workshop

A small-scale preliminary test was carried out in Ecuador in 2022 on six LOCs namely two bigeye threshers, two pelagic threshers, and two shortfin mako sharks. These samples were collected opportunistically from markets in Santa Rosa and Manta, Ecuador.

A workshop about the LOC devices was conducted in Manta, Ecuador with 31 attendees from various departments across the Viceministerio de Acuacultura y Pesca. Participants were asked to complete a survey to gauge their experience both before and after the event. These findings were used to further optimise the LOC.

3.3. Results and Discussion

3.3.1. Lab-on-a-Chip (LOC): Lysis and Extraction

A LOC was designed to integrate field-based DNA extraction, amplification and visualisation using LAMP into single cost-effective system which could be employed by non-specialists to monitor the trade in sharks and shark products (see SI 2 for methods and SI 4 for lysis results).

3.3.1.1. Optimisation and Evaluation of Cell Lysis Techniques

Although molecular techniques are advancing rapidly and we are now able to amplify and sequence DNA in the field (e.g., MinION), there has been limited research on portable extraction techniques, including cell lysis (Kim et al., 2009). We have applied a lysis method that incorporates easy steps that can be carried out in the field by combining the use of a chaotropic salt (5 M GuHCl) with mechanical disruption in a single test tube (Figure S 3-1 and Figure S 3-2). This removed the need for common laboratory equipment (e.g., vortex, incubators, and centrifuges) and numerous steps

involving different chemicals (e.g., Proteinase K, lysis buffer 1 and 2). Despite the simpler, portable nature of the lysis method, it was still effective on complex samples, such as shark fin, which are made of cartilage with very little muscle tissue and a lot of collagen fibres making them rigid and tough (Wakeman and Corwin, 2014).

3.3.1.2. Optimisation and Evaluation of Field-based DNA Extraction

We aimed to develop a portable extraction method that could produce high yields of DNA whilst also being simple, cost-effective, and rapid. The lysis method above using 5 M GuHCl was used to determine the capture efficiency of the GF/C filters on the LOC. The GF/C filter used per reaction had an average capture efficiency of 83.6% and the total amount that could be bound was 260 ng of DNA which is more than adequate for downstream application as LAMP only requires as little as six copies of DNA for successful amplification (Figure 3-2) (Notomi et al., 2000). The GF/C filters provide a straightforward and cost-effective method of capturing DNA on the LOC and offer versatility as the user can cut them into whatever shape or size necessary for DNA capture. (Minamoto et al., 2016).

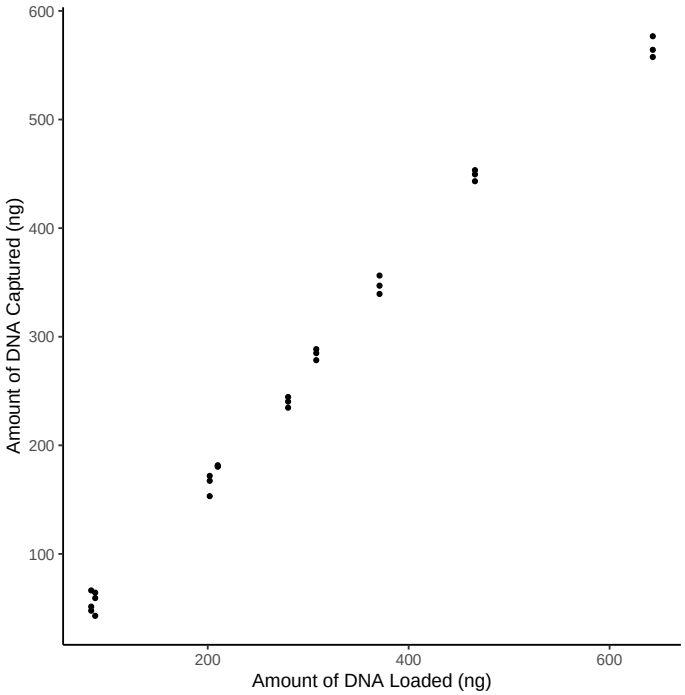

Figure 3-2. DNA capture efficiency of Whatman© glass microfiber filters (GF/C). The DNA solution was made up to 25 µL with 5M GuHCl in concentrations ranging from 8 to 64 ng/µL. The maximum retention of the GF/C filters were recorded.

Once bound, it was important to then wash the DNA to remove any impurities which may inhibit the subsequent LAMP reaction, and therefore a purity (A_{260}/A_{280} nm ratio) ranging between 1.7 and 2.0 (Promega UK, 2023) was required. The volume of ethanol loaded onto the LOC depended on tissue type, impurity levels and protein concentration. The optimal volume of ethanol required to remove most impurities from both fin and muscle tissue samples was 60 µL (Figure 3-3). At 70 µL the GF/C filter became oversaturated, and the LOC could not maintain its structural integrity. One hundred microliters of Nuclease-Free water were then needed to physically transfer the eluted DNA into the amplification chambers (Figure 3-1d & e).

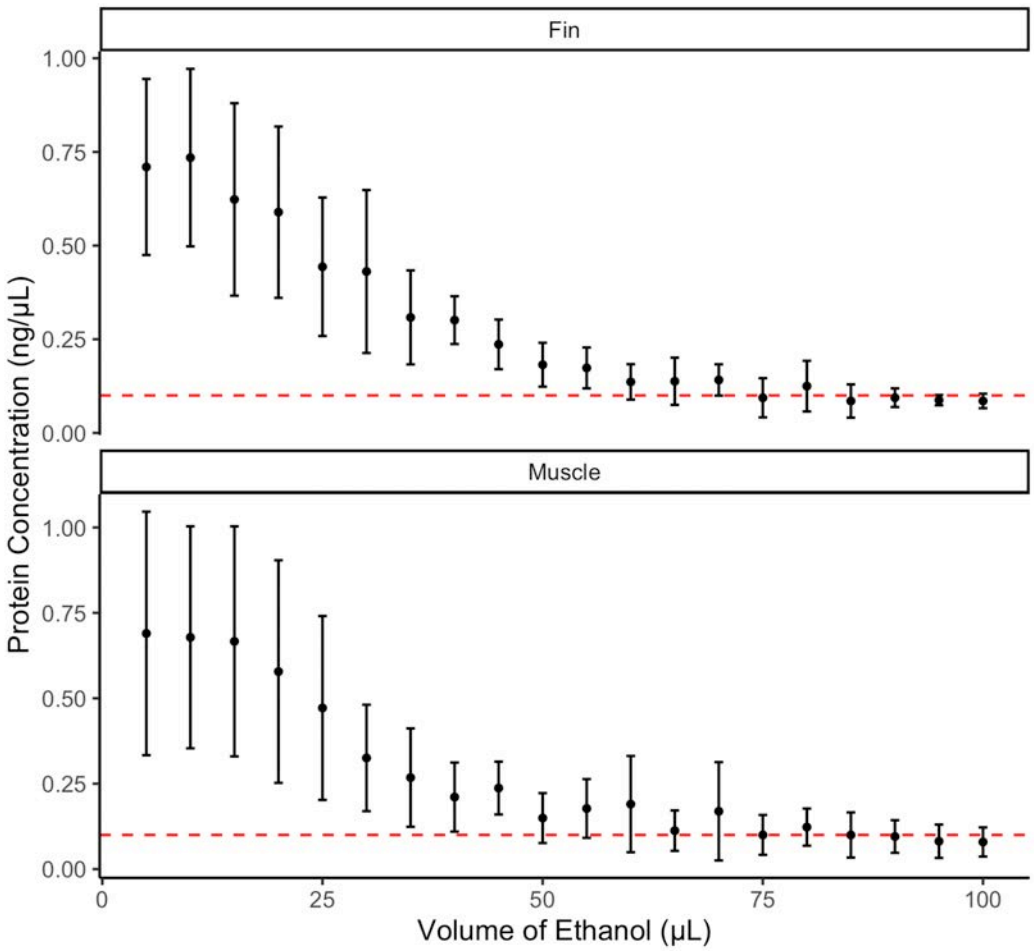

Figure 3-3. DNA from fin and muscle tissue samples was purified using 70% ethanol in 5 µL increments. The protein concentrations of the resulting samples were compared. The red dashed line indicates the plateau in protein concentration at 0.1 ng/µL when 60 µL of ethanol is loaded onto the LOC.

3.3.2. LAMP

3.3.2.1. Primer Specificity

The specificity of the LAMP primers was tested against the target species, as well as 26 non-target species as listed in Table S 3-1. LAMP primers for all three sharks were specific, only amplifying DNA from the target species (see Figures S3 8 & S3 9 for colour change and gel electrophoresis results). The primers proved to be specific even when testing against closely related species such as the common thresher shark (*A. vulpinus*), great white shark (*Carcharodon carcharias*) and salmon shark (*Lamna*

ditropis) which are in the same Order as the three target sharks, and the common thresher shark in the same genus as bigeye and pelagic thresher shark (*Alopias*) (Shimada, 2005; Vélez-Zuazo and Agnarsson, 2011).

Species identification is predominantly carried out using DNA barcodes that amplify specific regions of DNA (e.g CO1, Cytochrome B, NADH2) or limited nuclear genes (e.g., ITS2, 12S and 16S) between species. The LAMP primers developed here are designed to amplify only one species. The three sharks used in this study are very closely related to each other and to other sharks within their order (Lamniformes), and even sharks belonging to other orders (Shimada, 2005; Vélez-Zuazo and Agnarsson, 2011). Therefore, developing completely species-specific LAMP primers for additional species of sharks may prove challenging as DNA is highly conserved and mutation occurs at very slow rates in sharks (Martin et al., 1992).

3.3.2.2. Integrated LOC

3.3.2.2.1. LOC Standard Operating Procedure (SOP)

The optimised lysis, extraction, and amplification (using LAMP) were combined on a LOC which were then tested against the three target species of sharks (bigeye thresher, pelagic thresher, and shortfin mako shark) and one non-target species of shark (blue shark) in the lab ($n \geq 3$) (Figure S3 8 and S3 9). Results indicated successful amplification of each target species, namely pelagic thresher, bigeye thresher and shortfin mako shark. The controls that were incorporated on the chip also worked successfully, where there was amplification of the positive control and no amplification of the negative control. Example of the LOC results are shown in Figure 3-4 (full details can be found in Figure S 3 8). There were no instances of cross-

contamination for the non-target blue shark (*n* = 4). For bigeye thresher and shortfin mako shark, one false negative was observed for each (Figure S3 9).

For our method development we incorporated the use of a portable miniaturised incubator (~$1,500) for reliable results but for future applications of the LOC in the field any heating or incubating device (e.g., heating plate or hot water bath) could be used that can supply a constant temperature of 65°C for 30 to 60 mins. This operating system is much simpler than that required for PCR, which relies on complex temperature control for stages of denaturation, annealing and extension of DNA/RNA sequences. Although there are currently many methods of identifying shark products, for example visually using fin and meat guides (Hernández et al., 2018; Flores-Rivera et al., 2023) 3D fins with TRAFFIC (Bürgener et al., 2021), mini-DNA and DNA barcoding (Clarke et al., 2006; Cardeñosa et al., 2017, 2018), many of these techniques are costly, rely on trained personnel for visual identification of whole caught sharks or fins, laboratory facilities and experts to carry out genetic analysis or visual identification, or are time-consuming.

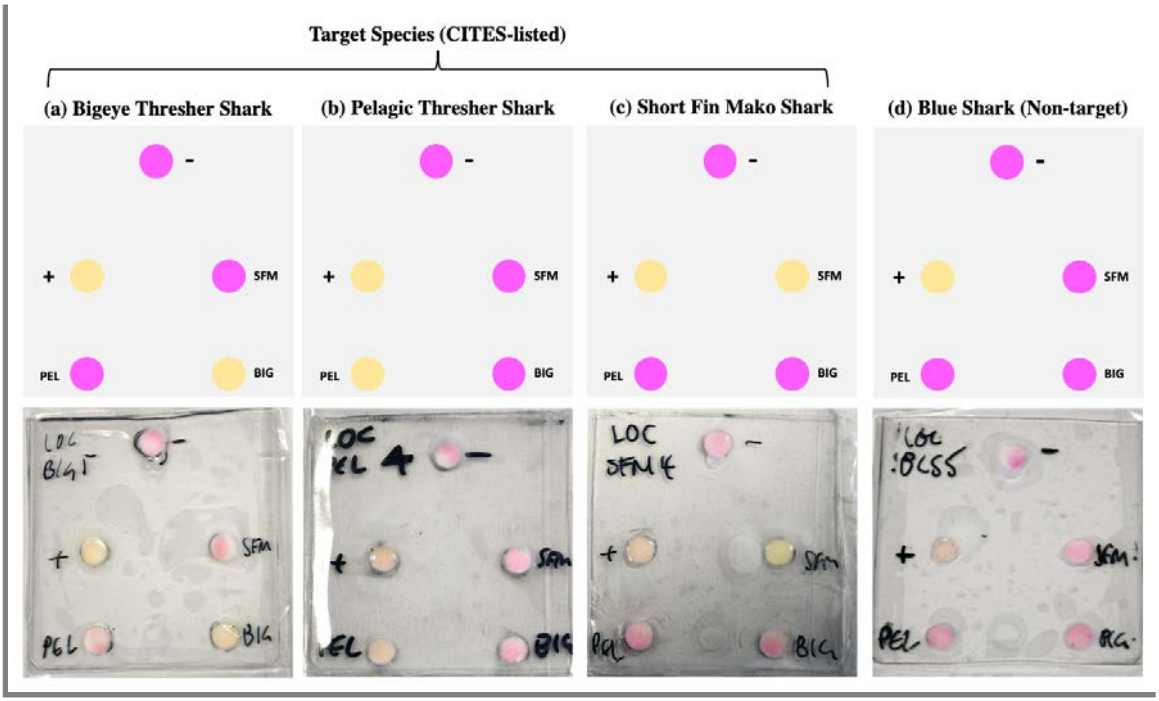

Figure 3-4. Schematic (*top panel*) and photographic (*bottom panel*) examples of LOC results showing amplification of target species (a) bigeye thresher shark (*Alopias superciliosus*), (b) pelagic thresher shark (*A. pelagicus*), and (c) shortfin mako shark (*Isurus oxyrinchus*), and no amplification of non-target species (d) blue shark (*Prionace glauca*).

3.3.2.2.2. Testing of Real-World Samples and Workshop

LOCs were initially tested in the field to identify shark species from six fresh muscle tissues of sharks landed at commercial fish markets in Ecuador. The LOCs were tested without the use of a laboratory or any laboratory equipment in a hotel room. Of the six LOCs, five worked successfully (one bigeye thresher shark and four shortfin mako sharks) and one failed due to a false negative. This demonstrates proof-of-concept in the field but would require an increased number of tests to be carried out for validation. The key learnings from this field-study were how to store the LAMP mix during long-haul flights (>14 hrs) and in the field to ensure no CO_2 entered the vials, importance of the distance of the negative control chamber from the sample

chamber, and drying time needed for the removal of contaminants step on the LOC (using 70% ethanol).

The LOC works as a screening test and therefore the cost per sample is less compared to PCR, £4.73 vs. £10.5 prior to Sanger Sequencing which will further increase the cost (Fields et al., 2017) (for further breakdown of cost see Figure S 3-11). The LOC is also rapid (less than an hour from extraction to visualisation), field-based and can be carried out by non-scientifically trained personnel. It is important to note that non-scientifically-personnel require some initial training prior to using of the LOC.

As the LOC works as a screening test, further laboratory analysis would be required to determine species identity to an accredited standard, for example relating to the handling of illegal products (e.g., dried fins from CITES-listed sharks). The LOC can reduce the number of samples that would further require downstream laboratory analysis and greatly reduces the cost as only a few samples need to be sequenced rather than all unknown samples. This is especially true in the case of high volumes of unidentified shark fins or unlabelled meat which can be expensive, approximately $10 per sample. In 2019, one of the largest seizures was recorded to date in the Galápagos Marine Reserve, where an illegal shipping vessel contained over 7,600 sharks (Bonaccorso et al., 2021). The sample cost can be reduced to $0.94 and can be done in <4 hours using Cardeñosa et al., (2018) multiplex real-time PCR assay, but this still requires a Real-Time PCR machine which may not be present in every lab and can be costly.

We carried out a workshop in Manta, Ecuador where ministerial officials (*n* = 31) were shown some genetic techniques and a demonstration on the use of the LOC. Of

the 31 ministerial officials who attended the LOC workshop, 18 had experience of carrying out species identification, with 22% having experience in visual identification, 22% in use of the genetic techniques and 38% with both. Over 80% of the participants had either worked with or confiscated shark products as part of their roles. Following the workshop, the participants knowledge of LOC technology increased from average of 1.88 (on a scale of 1 = nothing, 2 = very little, 3 = more or less, 4 = very good and, 5 = excellent) to 3.80. All participants (100%) responded positively that the LOC would be useful, highlighting benefits for identification of CITES-listed species in processed products where visual identification would not be possible and to verify exports at border control. Constructive feedback revolved around inclusion of additional CITES-listed species but not just restricted to sharks, e.g., mobula rays (Mobulidae), and further development on the SOP for the LOC.

3.4. Conclusion

We present the first completely field-based technique in the form of a paper-based LOC that can used to identify threatened and CITES-listed species of sharks. Previous LOCs incorporate one or two stages of DNA extraction, amplification, and visualisation; we provide all three. LOC technology is still an up-and-coming field. Despite the 15 years of research, most of their applications have been on clinical diagnostics and biomedical research; there has been limited to no research on the use of LOC devices for conservation. There is still a great deal of uncertainty surrounding the techniques used, and therefore our LOC for identifying sharks is a proof-of-concept and can provide a screening of shark species detected but further validation is required. Whilst this work was carried out in Ecuador, the LOC can be applied to any market globally and further development could see the inclusion of

other CITES-listed elasmobranchs or taxa entirely. The LOC provides us with the ability to identify sharks in the field without the use of expensive laboratory equipment and can distinguish between sharks, rays and fish, and identify the three CITES-listed sharks: bigeye thresher, pelagic thresher and shortfin mako shark.

Despite recent genetic advances in identifying sharks, there is still an urgent need to involve local stakeholders in the conservation of sharks. Attendees of the workshop reported that the LOC would be useful, in identifying processed products or at border control. Genetic tools should be available to non-specialists and people with limited access to expensive or specialised equipment, especially in countries where sharks are targeted the most, and where there are fewer regulations on the sale of shark and shark-related products.

3.5. References

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Supplementary Information

Table S 3-1 Elasmobranch and teleost fish species collected in Ecuador, USA and in captivity (Aquarium France as well as the percentage (%) match from Sanger Sequencing for both Forward (F) and Reverse (R) sequences for each species.

Common name	Species	Location	Tissue type	F	R
Clearnose skate	*Raja eglanteria*	USA	Muscle	90.4%	89.7%
Sandbar shark	*Carcharhinus plumbeus*	USA	Muscle	99.1%	98.5%
Salmon shark	*Lamna ditropis*	USA	Muscle	NA	97.9%
Shortfin mako (1)	*Isurus oxyrinchus*	USA	Muscle	99.4%	99.3%
Blue shark	*Prionace glauca*	USA	Muscle	91.3%	83.6%
Pelagic stingray	*Pteroplatyrygon violacea*	USA	Muscle	96.7%	97.1%
Common thresher	*Alopias vulpinus*	USA	Muscle	99.7%	98.6%
Great white shark	*Carcharodon carcharias*	USA	Muscle	98.9%	NA
Leopard shark	*Triakis semifasciata*	USA	Muscle	98.9%	99.5%
Bigeye thresher (1)	*Alopias superciliosus*	USA	Muscle	99.7%	88.1%
Kitefin shark	*Dalatis licha*	USA	Muscle	89.9%	89.4%
Black tip shark	*Carcharhinus limbatus*	USA	Muscle	90.4%	NA
Great hammerhead shark	*Sphyrna mokarran*	USA	Muscle	99.0%	99.4%
Scalloped hammerhead (1)	*Sphyrna lewini*	USA	Muscle	98.5%	99.1%
Sand tiger shark	*Carcharias taurus*	USA	Fin	99.4%	99.3%
Smooth dogfish	*Mustelus canis*	USA	Fin	99.5%	99.7%
Cownose ray	*Rhinopetera bonasus*	USA	Fin	99.6%	99.4%
Scalloped hammerhead (2)	*Sphyrna lewini*	Playita Mia	Muscle	99.8%	100%
Whale shark	*Rhincodon typus*	Galapagos	Muscle	98.0%	97.9%
Bigeye thresher (2)	*Alopias superciliosus*	Santa Rosa	Muscle	100%	98.8%
Pelagic thresher	*Alopias pelagicus*	Santa Rosa	Muscle	99.7%	99.7%
Oceanic whitetip	*Carcharhinus longimanus*	Playita Mia	Muscle	99.7%	97.3%

Shortfin mako (2)	*Isurus oxyrinchus*	Esmeraldas	Muscle	99.0%	99.3%
Long tail stingray	*Hypanus longus*	Santa Rosa	Muscle	98.9%	99.1%
Sicklefin smoothhound	*Mustelus lunulatus*	Santa Rosa	Muscle	99.6%	99.4%
Skipjack tuna (1)	*Katsuwonus pelamis*	Playita Mia	Muscle	99.7%	99.1%
Yellowfin tuna	*Thunnus albacares*	Playita Mia	Muscle	97.4%	98.8%
Skipjack tuna (2)	*Katsuwonus pelamis*	Playita Mia	Muscle	99.4%	98.9%
Tiger shark	*Galeocerdo cuvier*	Santa Rosa	Muscle	99.8%	98.1%
Black tip reef shark (captive)	*Carcharhinus melanopterus*	France	Fin	95.8%	98.0%
Zebra shark (captive)	*Stegastoma fasciatum*	France	Fin	98.2%	97.9%

(1) and **(2)** indicate where there are multiple samples for the same species.

NA = where sequences were inconclusive/sequencing failed. This was most likely because of highly degraded DNA.

Lab-on-a-Chip (LOC): Lysis and Extraction

A LOC was designed to integrate the field-based DNA extraction and, amplification and visualisation using LAMP.

Comparison of Lysis Methods

For optimisation experiments, a variety of cell lysis methods were evaluated using ~25 mg of either fin or muscle tissue belonging to scalloped hammerhead (*Sphyrna lewini*). Methods included thermal lysis, chemical lysis, chemical lysis with the addition of surfactants and mechanical lysis. Methods were compared to find a field-based lysis method that gave comparable results to conventional DNA extraction kit, with consideration given to speed, amount of DNA yielded, quality of DNA, cost of analysis and ease of use. DNA from the scalloped hammerhead was used as the model species for all the testing of LOC processes in sections 2.3.1. – 2.3.3 (please refer to main manuscript) as good quality tissue was readily available.

For the lysis of tissue using a DNA extraction kit, tissue samples were added to 180 µL of Lysis Buffer GL and 25 µL Proteinase K solution following the pre-lysis steps of the Bioline ISOLATE II Genomic DNA Kit (Bioline, UK) protocol. Samples were then incubated at 56°C for 3 hours until completely digested Samples were vortexed briefly and added to 200 µL Lysis Buffer G3, and incubated at 70°C for 10 min. For thermal lysis, tissue samples were added to 500 µL Nuclease-Free Water (Merck, Germany) and incubated at 100 °C for 15 minutes.

For chemical lysis, tissue samples were added to 500 µL guanidine hydrochloride (GuHCl) (Thermo Fisher Scientific, UK) of varying concentrations ranging from 3M to 8M (Montgomery and Sise, 1990; Tian et al., 2000; Shaw, Joyce, et al., 2009; Shaw, Thain, et al., 2009; Kashkary et al., 2012; Mosley et al., 2016). Samples were left at room temperature for 5, 10 and 15 minutes.

Mechanical lysis was carried out in 5M GuHCl with 2% SDS, different mechanical lysis techniques were used including no disruption (control), cutting, pipetting, rolling, shearing, and vortexing (lab equipment) (Table S 3-2). Vortexing was carried out using a vortex mixer found in the laboratory. It was used as to compare DNA concentrations yielded from laboratory equipment versus "field-based" mechanical lysis methods.

Table S 3-2 Mechanical lysis methods used to physically break down cell membranes.

Method	**Description**
Control	The samples were not disturbed.
Mashing	A pipette tip was used to break apart fin and tissue samples for 15 seconds.
Pipetting	Samples were pipetted up and down ten times using a 1000 µL pipette set at 500 µL.

Rolling	Samples in Eppendorf tubes were rolled across a tube rack for 15 seconds.
Shearing	Samples were sheared up and down 15 times with a needle syringe.
Vortexing	Samples were agitated for 15 seconds using a vortex (laboratory equipment).

The total DNA concentration (ng/µL) and purity (260:280 nm ratio) of the lysed DNA samples were analysed using a Thermo Scientific™ NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, UK).

Optimisation of Field-based DNA Extraction Protocol

Scalloped hammerhead DNA was lysed in 5 M GuHCl and used to determine the retention (capture efficiency) of the Whatman© glass microfiber filters (GF/C) (Merck, Germany) Figure 3-2). DNA concentrations (ng/µL) for all subsequent reactions were quantified using Thermo Scientific™ NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, UK).

The lysed DNA was made up to 25 µL with 5M GuHCl in concentrations of DNA ranging from 8 to 64 ng/µL and loaded onto the Whatman© GF/C glass microfiber filters (Merck, Germany) to determine their binding efficiency. The maximum retention of the GF/C filters were recorded by collecting the DNA solution in a 0.2 mL PCR tube and recorded in the DNA concentration (ng/µL) for each eluate.

Next the bound DNA samples underwent a wash step to remove any contaminants. A 70% ethanol solution was loaded onto the LOC in 5 µL increments until a total of 100 µL had passed through the GF/C filter. Eluted fractions were collected on the other side of the LOC in 0.2 mL PCR tubes, and the protein and DNA concentrations (ng/µL) as well as purity were recorded. DNA was then eluted using 100 µL of Nuclease-Free Water (Merck, Germany).

Statistical Analysis

Statistical analysis and data visualisation were carried out in R 3.6.1 (R Core Team., 2019). Data was tested for normality with all variables found to be not normally distributed; non-parametric statistical methods were used. Wilcoxon rank-sum tests were used to compare differences in DNA concentrations between fin and muscle tissue. Kruskal-Wallis rank-sum tests and pairwise multiple comparison test ('Kruskalmc') using the "pgirmess" package (Giraudoux, 2018) were used to assess differences in DNA concentrations across different lysis methods, including chemical and mechanical lysis.

Positive and Negative Controls

A positive control was incorporated onto the LOC (Figure 3-1) using λ primers (Merck, Germany) and readily available λ DNA (NEB, UK). The design of the LOC physically separated the negative control from the other chambers (including target species primers and positive control) by less than 1.33 cm to ensure that it was DNA free. A concentration of 5 ng/µL DNA was used on the LOC chip. The positive and negative controls (including primers and DNA) were added to the LOC after elution of DNA. A mastermix was made for the positive and negative containing 10 µL of LAMP mix and 2 µL of λ primers. For the negative control an additional 8 µL of Nuclease-Free Water (Merck, Germany) was added, and for the positive control, 2 µL of 5 ng/µL λ DNA (NEB, UK) was added. No H_2O was added to the positive control as this was added during elution of DNA on the LOC. Both mastermixes were homogenised by pipetting up and down gently until the solution was fully mixed. Seven µL of the positive control mastermix was pre-loaded onto the positive control chamber, and 10 µL of negative control mastermix in the negative control chamber on a separate

plastic chip (mould with five chambers containing each a Whatman© glass microfiber GF/C filter (Merck, Germany).

Primer Development

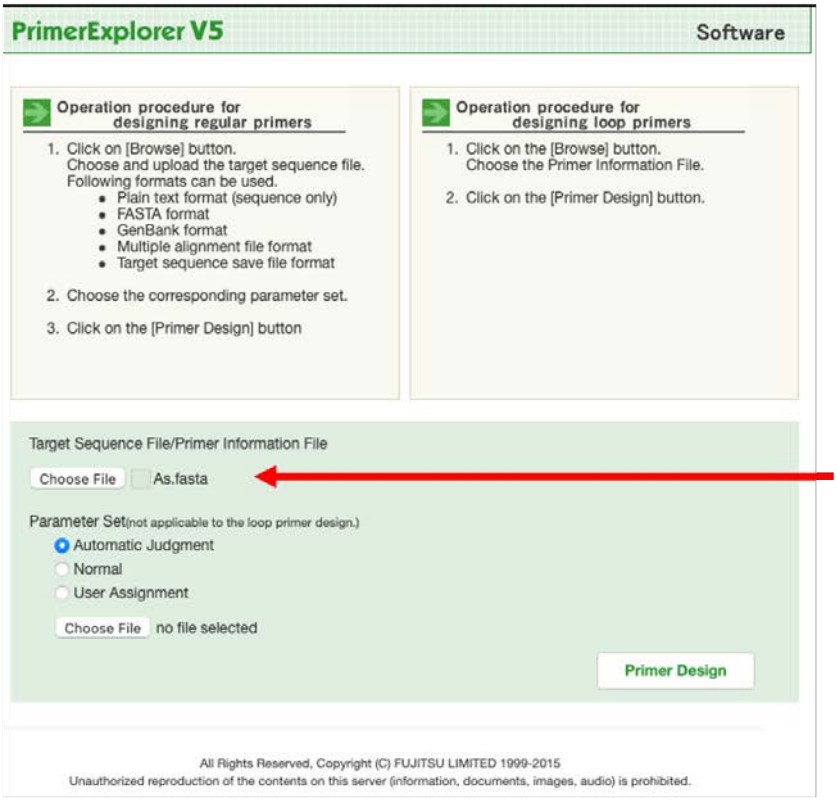
Sequences for LAMP Primer Development

Table S 3-3 Sequences used for LAMP primer development.

Common Name	Species	Type of Sequence	Gene	Genbank Accession No.	Length (bp)
Biegeye thresher shark	Alopias superciliosus	Nuclear ribosomal RNA (rRNA)	Internal Transcriber Spacer 2 Locus (ITS2) (located between 5.8S and 28S)	KC204935	740
Pelagic thresher shark	Alopias pelagicus	Mitochondrial DNA (mtDNA)	D-LOOP (1563 – 16692 bp)	KF020876	1059
Shortfin mako shark	Isurus oxyrinchus	Mitochondrial DNA (mtDNA)	D-LOOP	MH760159	791

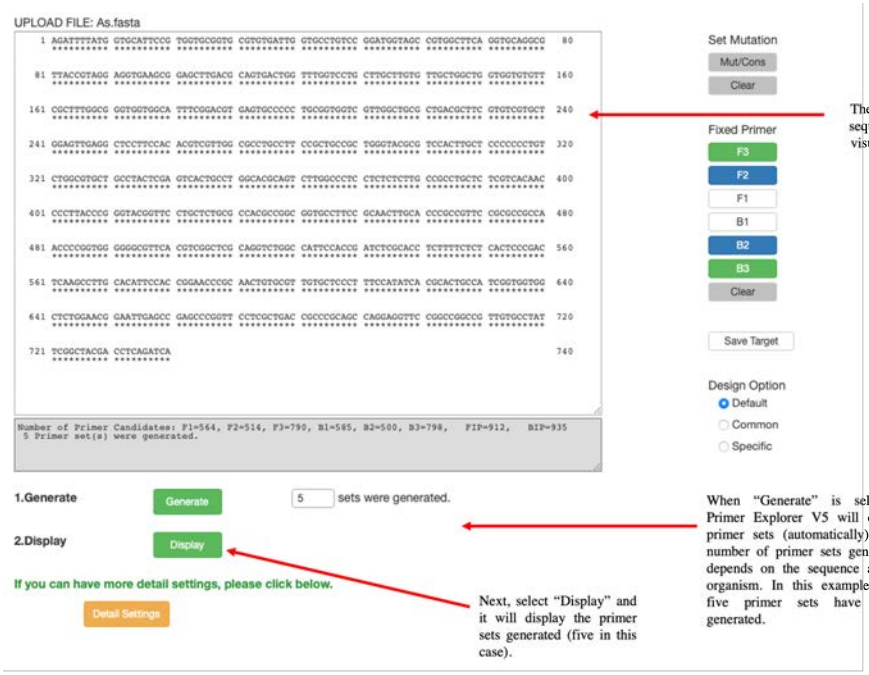
Step-by-step LAMP Primer Development

Using the sequences above, primers were developed in Primer Explorer V5 (http://primerexplorer.jp/lampv5e/index.html) for the three CITES-listed sharks: bigeye thresher, pelagic thresher and shortfin mako shark. Outlined here is a full overview of how the primers were developed (using bigeye thresher shark as an example).



Input DNA sequence here (as FASTA file)

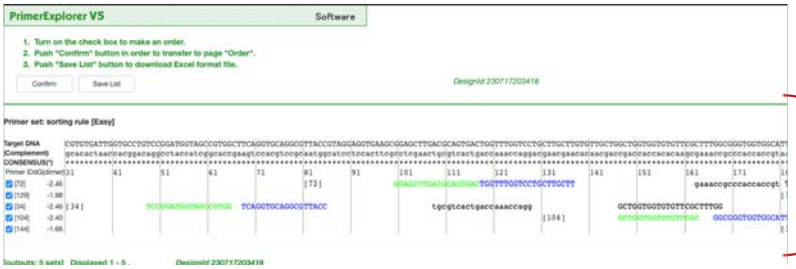
e.g., Bigeye thresher shark (*Alopias supercilious*) (KC204935)



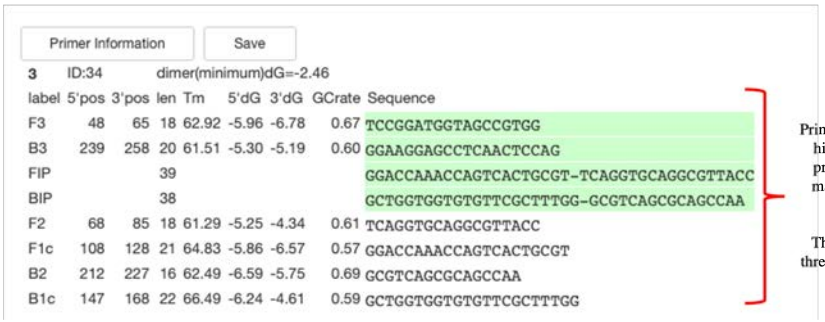
The DNA/RNA sequence can be visualized here.

When "Generate" is selected, Primer Explorer V5 will output primer sets (automatically). The number of primer sets generated depends on the sequence and/or organism. In this example only five primer sets have been generated.

Next, select "Display" and it will display the primer sets generated (five in this case).



Primer sets are generated and then selected to "confirm"



Primer Information | Save

3 ID:34 dimer(minimum)dG=-2.46

label	5'pos	3'pos	len	Tm	5'dG	3'dG	GCrate	Sequence
F3	48	65	18	62.92	-5.96	-6.78	0.67	TCCGGATGGTAGCCGTGG
B3	239	258	20	61.51	-5.30	-5.19	0.60	GGAAGGAGCCTCAACTCCAG
FIP			39					GGACCAAACCAGTCACTGCGT-TCAGGTGCAGGCGTTACC
BIP			38					GCTGGTGGTGTGTTCGCTTTGG-GCGTCAGCGCAGCCAA
F2	68	85	18	61.29	-5.25	-4.34	0.61	TCAGGTGCAGGCGTTACC
F1c	108	128	21	64.83	-5.86	-6.57	0.57	GGACCAAACCAGTCACTGCGT
B2	212	227	16	62.49	-6.59	-5.75	0.69	GCGTCAGCGCAGCCAA
B1c	147	168	22	66.49	-6.24	-4.61	0.59	GCTGGTGGTGTGTTCGCTTTGG

Primer sets are displayed. Primers highlighted in "green" are the primers used. FIP and BIP are made up of F1/B1c and F2/B2 below.

This was the output for bigeye thresher shark and the primers we selected for the LOC.

The next step is to BLAST each of the primer sets generated against "all taxa" in BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) with the aim to produce a high match with your target species, and lower match with non-target species. In our case, we were aiming to have a lower match with other elasmobranchs and teleost fish. Then once a suitable primer group are found, they are put back into Primer Explorer V5 to create loop primers.

Lab-on-a-Chip (LOC): Optimisation and Evaluation of Cell Lysis Techniques

Optimisation and Evaluation of Cell Lysis Techniques

Nine lysis methods were tested to establish the most suitable method for field-testing that were cost effective and efficient. When evaluating chemical lysis methods, significant differences were observed between GuHCl concentrations used and total DNA released for fin (Kruskal-Wallis: $\chi^2 = 14.344$, df = 5, $p < 0.02$; 78.7±38.3 ng/µL, Figure S 3-1) and muscle tissue (Kruskal-Wallis: $\chi^2 = 16.013$, df = 5, $p < 0.01$; 39.3 ± 28.0 ng/µL, Figure S 3-1). Five molar GuHCl was chosen as the optimum concentration as it yielded the highest concentrations of DNA from muscle and fin tissue (90.6 ± 8.22 ng/µL and 113.4 ± 18.8 ng/µL, respectively). Using the same concentration of GuHCl for both samples also increased the simplicity for end users as the lysis method is consistent and therefore not dependant on what type of sample is used (i.e., whether it is fin or muscle tissue).

Significant differences in total DNA concentration were observed between the mechanical lysis methods for fin (Kruskal – Wallis: χ^2 = 16.112, df =8, $p < 0.05$, Figure S 3-2) and muscle tissue (Kruskal – Wallis: χ^2 =18.5, df = 8, $p < 0.02$, Figure S 3-2). Pipetting yielded higher concentrations of DNA in fin tissue (374 ± 19.7 ng/µL) and cutting (386 ± 102 ng/µL) in muscle tissue, though the multiple comparison test revealed no significant differences (Figure S 3-2). Mechanical disruption by mashing was able to successfully yield high concentrations from both fin and muscle tissue.

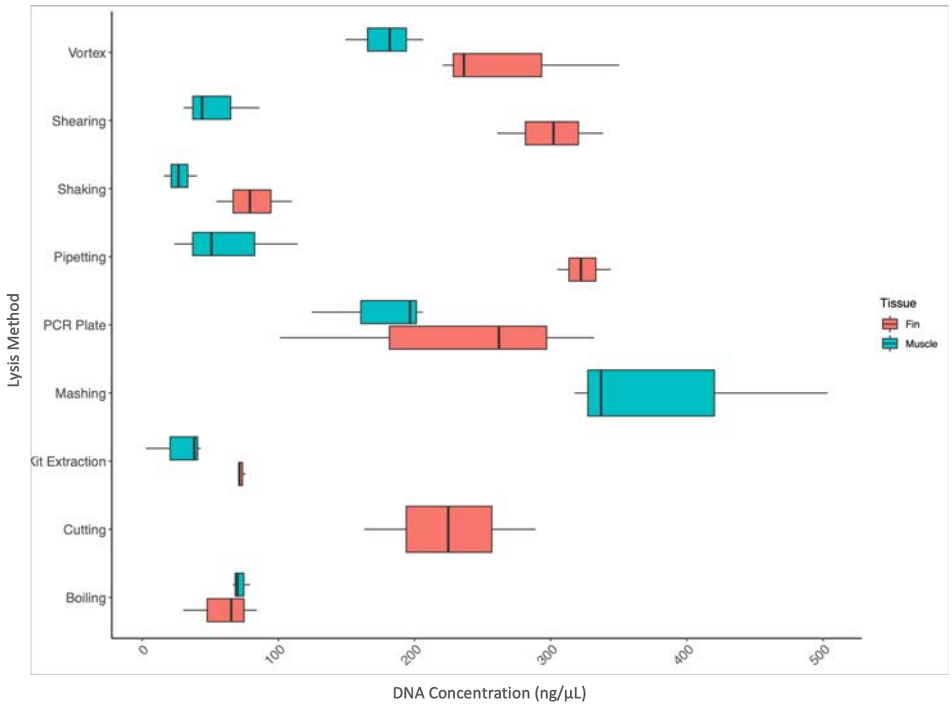


Figure S 3-1 Comparison of different mechanical lysis methods used to yield DNA from fin and muscle tissue of scalloped hammerhead sharks (*Sphyrna lewini*).

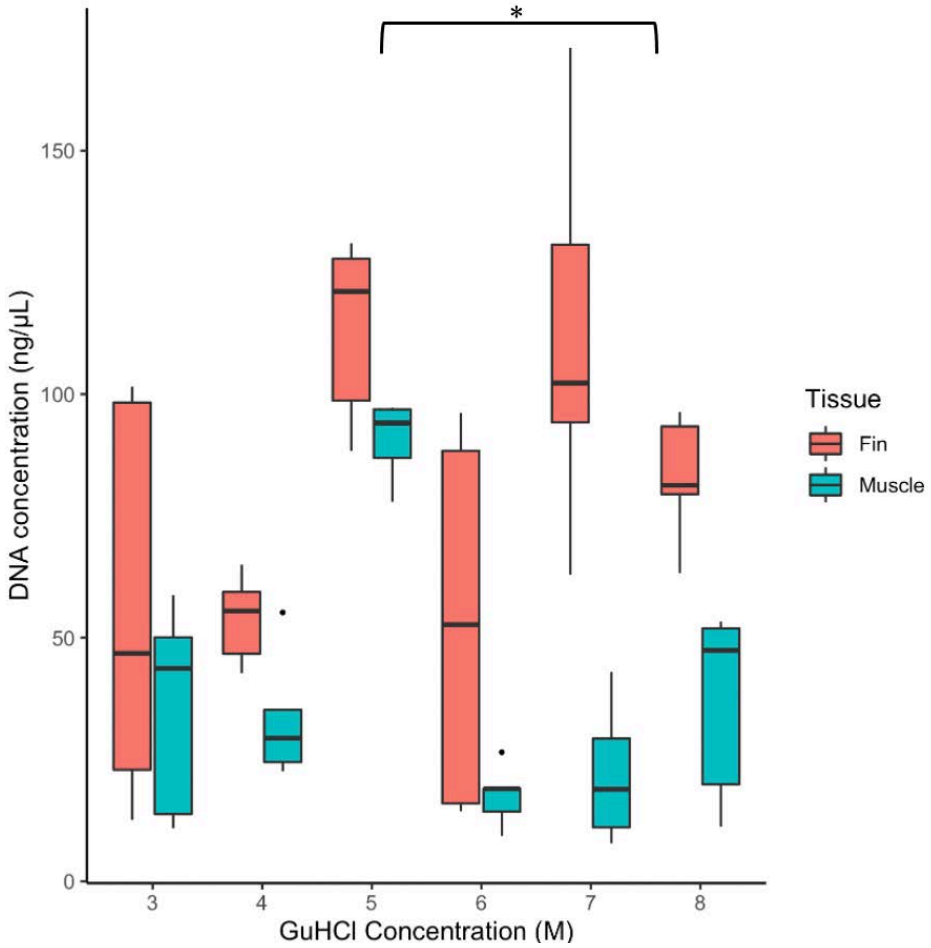


Figure S 3-2 Comparison of different concentrations of GuHCl (ranging from 3 M to 8 M) used to obtain DNA from 25 mg of fin and muscle tissue of scalloped hammerhead sharks (*Sphyrna lewini)*.

Positive and Negative Control

A positive and negative control were incorporated onto the LOC using λ DNA (positive control only) and λ primers (Nagamine et al., 2002). For the positive control, 5 ng/µL of λ DNA was required to produce a consistent reaction, any lower and false negative were observed. For the negative control, the distance from the reaction wells was important (move earlier information on the distance down to here). Any detectable failure in the controls meant that the LOC was discarded immediately. See S3-8 & S3-9 for LOC's.

All three primer sets (bigeye thresher (Figure S 3-3), pelagic thresher (Figure S 3-4; Figure S 3-5) and shortfin mako (Figure S 3-6; Figure S 3-7) shark) proved to be specific when testing against the 26 (Table S 3-1) different species of elasmobranchs and fish. Repeat assays were carried out where contamination was present and revealed these were caused by human error.

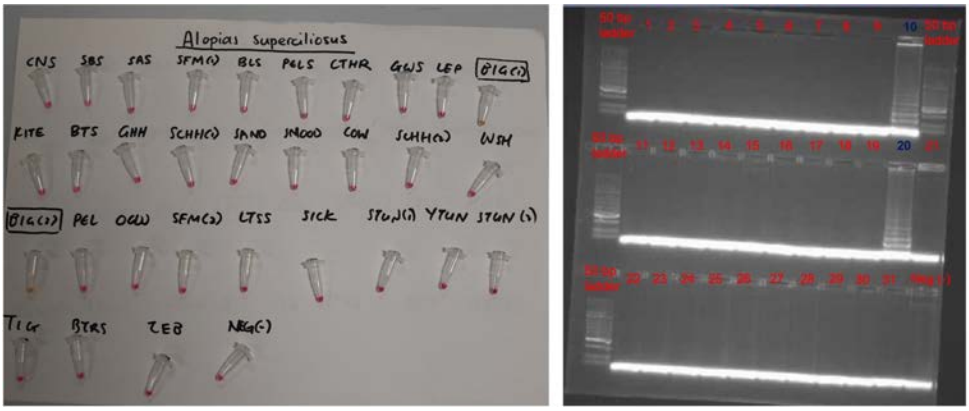


Figure S 3-3 Primer specificity for target species bigeye thresher shark against 26 different species of elasmobranchs and fish, (a) Showing LAMP colour change with target and non-target species in order from top right to bottom left and (b) gel image showing LAMP amplification of same species (numbered 1 – 31); red = non-target species, dark blue = target species: (1) CNS = clearnose skate (*Raja eglanteria*), (2) SBS = sandbar shark (*Carcharhinus plumbeus*), (3) SAS = salmon shark (*Lamna ditropis*), (4) SFM (1) = shortfin mako shark (*Isurus oxyrinchus*), (5) BLS = blue shark (*Prionace glauca*), (6) PELS = pelagic stingray (*Pteroplatyrygon violacea*), (7) CTHR = common thresher shark (*Alopias vulpinas*), (8) GWS = great white shark (*Carcharodon carcharias*), (9) LEP = leopard shark (*Triakis semifasciata*), (10) BIG (1) bigeye thresher shark (*A. superciliosus*), (11) KITE = kitefin shark (*Dalatis longa*), (12) BTS = black tip shark (*Carcharhinus limbatus*), (13) GHH = great hammerhead (*Sphyrna mokarran*), (14) SCHH (1) = scalloped hammerhead (*S. lewini*), (15) SAND = sand tiger shark (*Carcharias taurus*), (16) SMOOD = smooth dogfish (*Mustelus canis*), (17) COW = cownose ray (*Rhinoptera bonasus*), (18) SCHH (2), (19) WSH = whale shark (*Rhincodon typus*), (20) BIG (2), (21) PEL = pelagic thresher shark (*A. pelagicus*), (22) OCW = oceanic whitetip (*Carcharhinus longimanus*), (23) SFM (2), (24) LTSS = long tail stingray (*Dasyatis longa*), (25) SICK = sicklefin smoothhound (*M. lunulatus*), (26) STUN (1) skipjack tuna (*Katsuwonus pelamis*), (27) YTUN = yellowfin tuna (*Thunnus albacores*), (28) STUN (2), (29) TIG = tiger

shark (*Galeocerdo cuvier*), (30) BTRS = black tip reef shark (*C. melanopterus*), (31) ZEB = zebra shark (*Stegastoma fasciatum*) and Neg (-) = negative control.

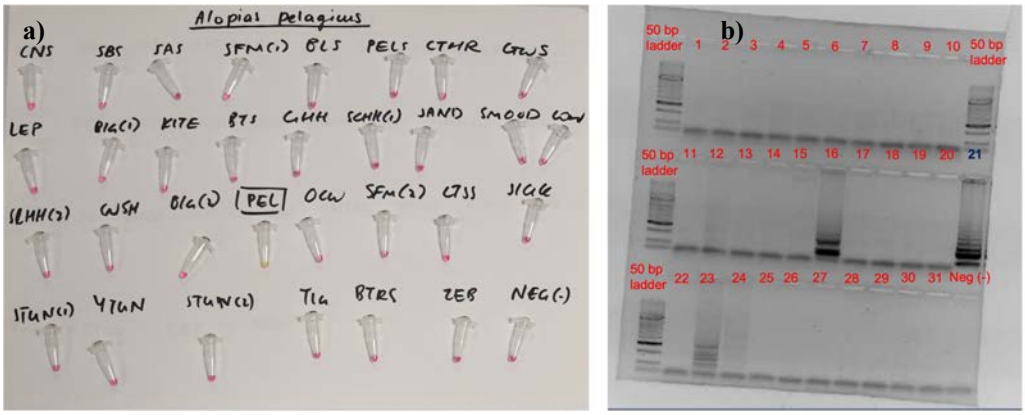


Figure S 3-4 Primer specificity for target species pelagic thresher shark against 26 different species of elasmobranchs and fish, (a) showing LAMP colour change with target and non-target species in order from top right to bottom left and (b) gel image showing LAMP amplification of same species (numbered 1 – 31); red = non-target species, dark blue = target species: (1) CNS = clearnose skate (*Raja eglanteria*), (2) SBS = sandbar shark (*Carcharhinus plumbeus*), (3) SAS = salmon shark (*Lamna ditropis*), (4) SFM (1) = shortfin mako shark (*Isurus oxyrinchus*), (5) BLS = blue shark (*Prionace glauca*), (6) PELS = pelagic stingray (*Pteroplatyrygon violacea*), (7) CTHR = common thresher shark (*Alopias vulpinas*), (8) GWS = great white shark (*Carcharodon carcharias*), (9) LEP = leopard shark (*Triakis semifasciata*), (10) BIG (1) bigeye thresher shark (*A. superciliosus*), (11) KITE = kitefin shark (*Dalatis longa*), (12) BTS = black tip shark (*Carcharhinus limbatus*), (13) GHH = great hammerhead (*Sphyrna mokarran*), (14) SCHH (1) = scalloped hammerhead (*S. lewini*), (15) SAND = sand tiger shark (*Carcharias taurus*), (16) SMOOD = smooth dogfish (*Mustelus canis*), (17) COW = cownose ray (*Rhinoptera bonasus*), (18) SCHH (2), (19) WSH = whale shark (*Rhincodon typus*), (20) BIG (2), (21) PEL = pelagic thresher shark (*A. pelagicus*), (22) OCW = oceanic whitetip (*Carcharhinus longimanus*), (23) SFM (2), (24) LTSS = long tail stingray (*Dasyatis longa*), (25) SICK = sicklefin smoothhound (*M. lunulatus*), (26) STUN (1) skipjack tuna (*Katsuwonus pelamis*), (27) YTUN = yellowfin tuna (*Thunnus albacores*), (28) STUN (2), (29) TIG = tiger shark (*Galeocerdo cuvier*), (30) BTRS = black tip reef shark (*C. melanopterus*), (31) ZEB = zebra shark (*Stegastoma fasciatum*) and Neg (-) = negative control.

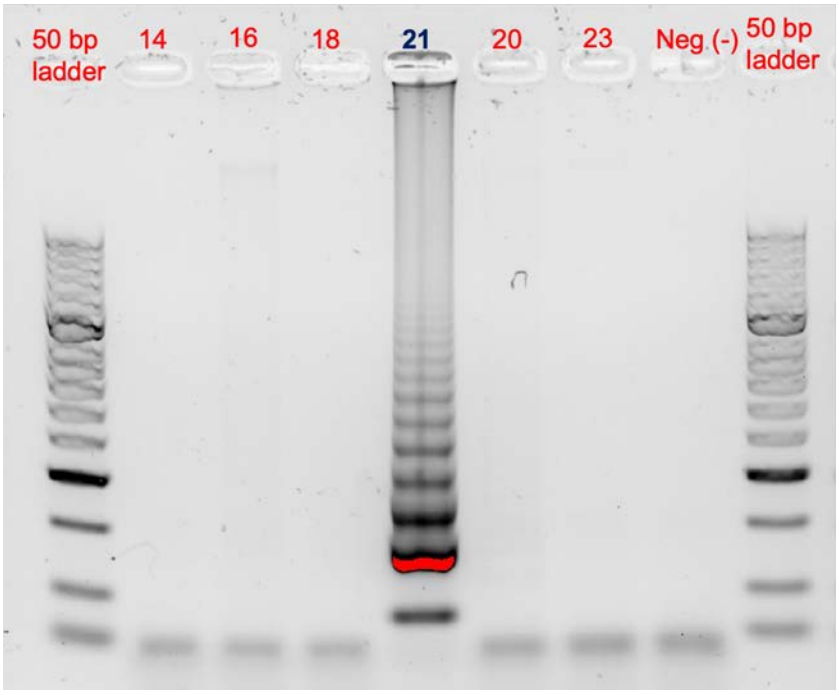


Figure S 3-5 Repeat assay for pelagic thresher shark against five non-target species (as there was contamination in gels): 14 = scalloped hammerhead (*Sphyrna lewini*), 16 = smooth dogfish (*Mustelus canis*), 18 = scalloped hammerhead, 21 = pelagic thresher (*Alopias pelagicus*), 20 = bigeye thresher (*A. superciliosus*), 23 = shortfin mako shark (*Isurus oxyrinchus*) and Neg (-) = negative control, where red = non-target species and dark blue = target species.

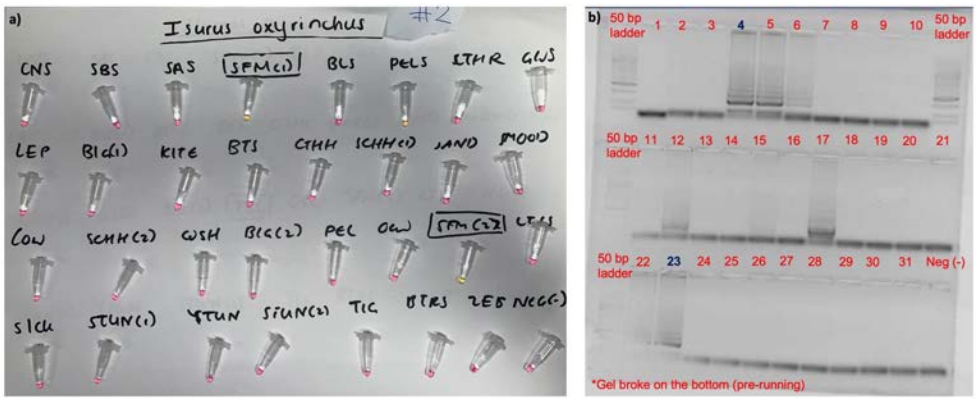


Figure S 3-6 Primer specificity for target species shortfin mako shark against 26 different species of elasmobranchs and fish, (a) showing LAMP colour change with target and non-target species in order from top right to bottom left and (b) gel image showing LAMP amplification of same species (numbered 1 – 31); red = non-target species, dark blue = target species: (1) CNS = clearnose skate (*Raja eglanteria*), (2) SBS = sandbar shark (*Carcharhinus plumbeus*), (3) SAS = salmon shark (*Lamna ditropis*),

(4) SFM (1) = shortfin mako shark (*Isurus oxyrinchus*), (5) BLS = blue shark (*Prionace glauca*), (6) PELS = pelagic stingray (*Pteroplatyrygon violacea*), (7) CTHR = common thresher shark (*Alopias vulpinas*), (8) GWS = great white shark (*Carcharodon carcharias*), (9) LEP = leopard shark (*Triakis semifasciata*), (10) BIG (1) bigeye thresher shark (*A. superciliosus*), (11) KITE = kitefin shark (*Dalatis longa*), (12) BTS = black tip shark (*Carcharhinus limbatus*), (13) GHH = great hammerhead (*Sphyrna mokarran*), (14) SCHH (1) = scalloped hammerhead (*S. lewini*), (15) SAND = sand tiger shark (*Carcharias taurus*), (16) SMOOD = smooth dogfish (*Mustelus canis*), (17) COW = cownose ray (*Rhinoptera bonasus*), (18) SCHH (2), (19) WSH = whale shark (*Rhincodon typus*), (20) BIG (2), (21) PEL = pelagic thresher shark (*A. pelagicus*), (22) OCW = oceanic whitetip (*Carcharhinus longimanus*), (23) SFM (2), (24) LTSS = long tail stingray (*Dasyatis longa*), (25) SICK = sicklefin smoothhound (*M. lunulatus*), (26) STUN (1) skipjack tuna (*Katsuwonus pelamis*), (27) YTUN = yellowfin tuna (*Thunnus albacores*), (28) STUN (2), (29) TIG = tiger shark (*Galeocerdo cuvier*), (30) BTRS = black tip reef shark (*C. melanopterus*), (31) ZEB = zebra shark (*Stegastoma fasciatum*) and Neg (-) = negative control.

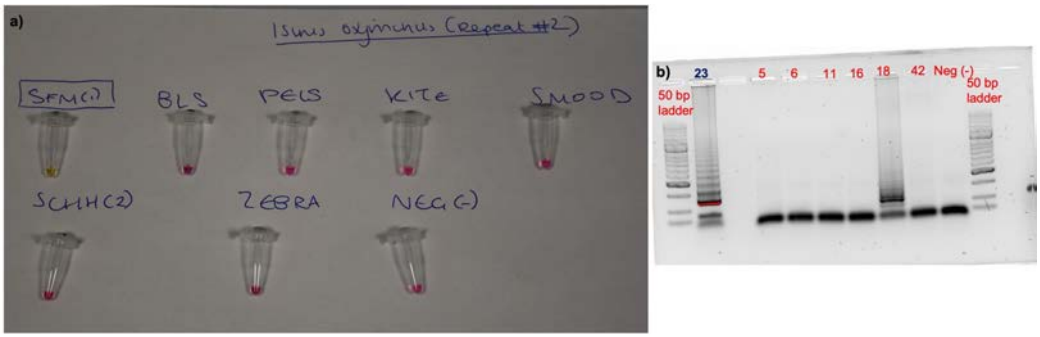


Figure S 3-7 Repeat assay for shortfin mako shark against seven non-target species (as there was contamination in colour changes and gels): LAMP showing colour change (a) and (b) gel for shortfin mako shark against seven non-target species in order: (23) SFM = shortfin mako shark (*Isurus oxyrinchus*), (5) BLS = blue shark (Prionace glauca), (6) PELS = pelagic stingray (*Pteroplatyrygon violacea*), (11) KITE = kitefin shark (*Dalatis licha*), (16) SMOOD = smooth dogfish (*Mustelus canis*), (18) SCHH = scalloped hammerhead (*Sphyrna lewini*), (42) ZEBRA = zebra shark (*Stegastoma fasciatum*) and Neg (-) = negative control, where red = non-target species and dark blue = target species.

Integrated LOC

LOCs were tested against the three target species of sharks (bigeye and pelagic thresher, and shortfin mako shark) and one non-target species of shark (blue shark (Prionace glauca)) in the lab ($n \geq 3$) (Figure S 3-8). Please note that although these pictures look grey, they were photographed on a white piece of A4 paper as background.

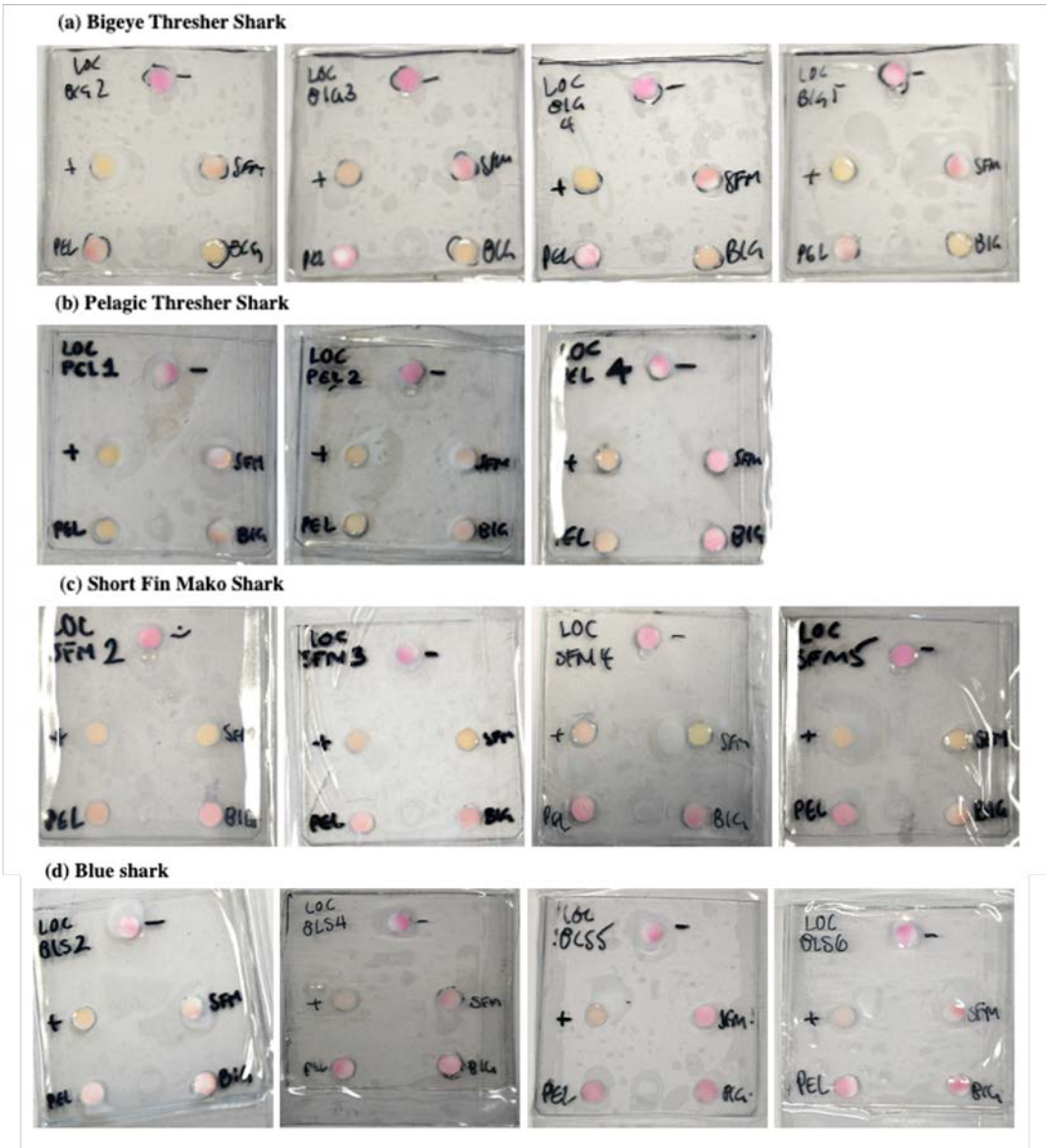


Figure S 3-8 Successful LOCs for target CITES-species where yellow indicates amplification and pink indicates no amplification.

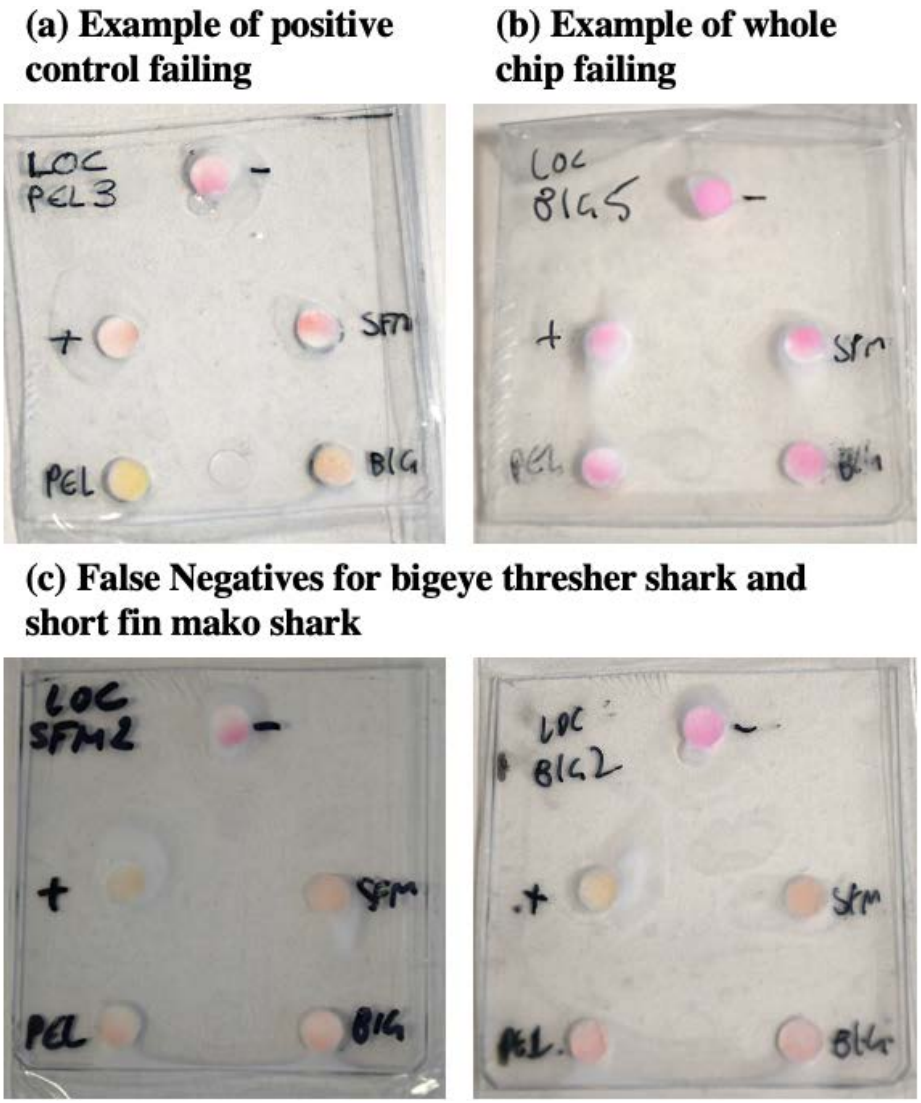


Figure S 3-9 Examples of LOCs that did not work, (a) where either the positive control did not change, (b) where the whole chip failed or (c) the target species did not change colour, but the positive control did (False Negative) for bigeye thresher and shortfin mako shark (SFM 2, left panel) and bigeye thresher (BIG 2, right panel).

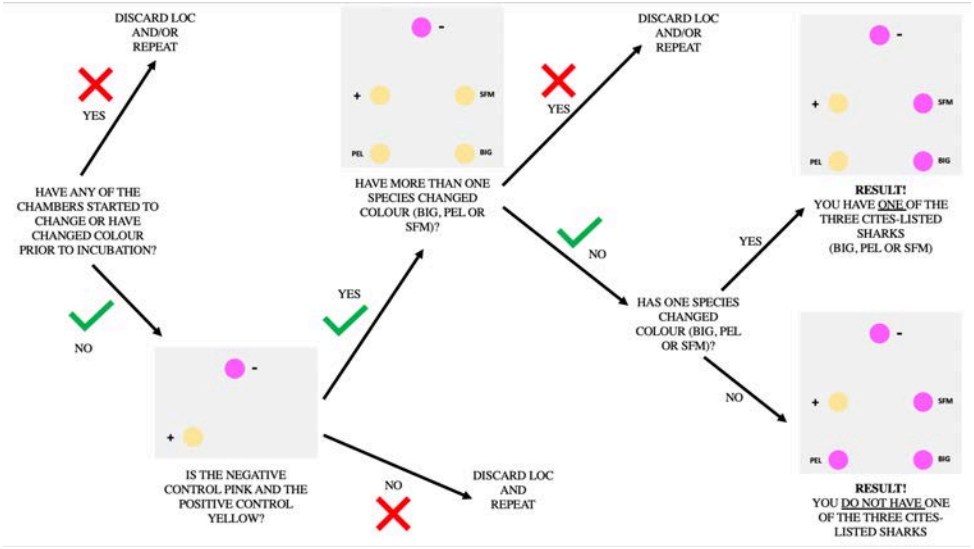


Figure S 3-10 Decision making flowchart for the LOC Standard Operating Procedure (SOP) where BIG = bigeye thresher shark, PEL = pelagic thresher shark and SFM = shortfin mako shark. The (+) and (-) indicate positive and negative control. The positive should always turn yellow and negative stay pink.

Cost comparison with conventional methods

Cost comparisons were based on prices in the UK in 2023 and therefore these are just an estimation for comparison as costs and availability of reagents in different places and countries with differ and be hard to quantify. Costs of the DNA Barcoding method were based on Field et al., (2017). The cost to carry out one LOC is £4.73 per sample (Figure S 3-11a) and for DNA Barcoding is £10.5 (Figure S 3-11b). The cost breakdown for the DNA barcoding is prior to any upstream processing/analysis such as Sangar Sequencing which would increase the cost further.

(a)

ITEM	UNIT	NET COST (£)
WHATMAN© GF/C FILTERS	6	£0.231
WARMSTART© COLORIMTRIC LAMP MIX	0.025 mL	£2.920
LAMBDA DNA (500 µg/mL)	0.001 mL	£0.001
INK	1	£0.043
FILTER PAPER	1	£0.407
PRIMERS	0.005 mL	£0.376
GuHCL	0.5 mL	£0.391
NUCLEASE FREE H20	0.11 mL	£0.002
ETHANOL (70%)	0.06	£0.006
ADHESIVE PCR FILM	1	£0.350

(b)

ITEM	SEQUENCING	UNIT	COMPANY	CAT NO.	NET COST (£)	ONE UNIT (£)
DNEASY COMMERCIAL KIT	PRE-SEQUENCING	250	QIAGEN	69506	£947.00	£3.788
PRIMERS (FISHF2_t1, VF2_t1 & SHARK COI-MINIR)	PRE-SEQUENCING	5000	MERCK	NA	£51.94	£0.031
Hot StarTaq Master Mix Kit	PRE-SEQUENCING	2500	QIAGEN	203446	£1,221.00	£0.488
AGAROSE	PRE-SEQUENCING	1.6	MERCK	A9539-500G	£888.00	£2.842
1 X TBE BUFFER	PRE-SEQUENCING	25000	MERCK	1061772500	£113.00	£0.362
DNA LADDER	PRE-SEQUENCING	100	MERCK	D3812-1VL	£147.00	£2.940
ETHIDIUM BROMIDE	PRE-SEQUENCING	37500	MERCK	1116080030	£59.50	£0.003
LOADING BUFFER	PRE-SEQUENCING	30000	MERCK	G2526-5ML	£27.50	£0.001

Figure S 3-11 Cost breakdown of (a) one Lab-on-a-Chip (LOC) and one sample for DNA barcoding based on UK prices in 2023.

Chapter 4

4. Are concentrations of pollutants in sharks, rays, and skates (Elasmobranchii) a cause for concern? A systematic review

Chapter Overview

This chapter is a systematic review with meta-analysis on pollutants in sharks, rays, and skates (Elasmbobranchii) following the 2009 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.

This chapter is based in part on the original research of (Tiktak et al., 2020), which was published in Marine Pollution Bulletin (DOI: 10.1016/j.marpolbul.2020.111701).

Author Contributions

As lead author I designed the project, collected the data, performed the analysis, wrote, and submitted the manuscript. I supervised a MSc student who provided support with data collection to add an additional level of rigour on the data collected. Other co-authors provided support with data interpretation and revising the manuscript.

4.1. Abstract

This review represents a comprehensive analysis on pollutants in elasmobranchs including meta-analysis on the most studied pollutants: mercury, cadmium, PCBs, and DDTs, in muscle and liver tissue. Elasmobranchs are particularly vulnerable to pollutant exposure which may pose a risk to the organism as well as humans that consume elasmobranch products. The highest concentrations of pollutants were found in sharks occupying top trophic levels (Carcharhiniformes and Lamniformes). A human health risk assessment identified that children and adults consuming shark once a week are exposed to over three times more mercury than is recommended by the US EPA. This poses a risk to local fishing communities and international consumers of shark-based products, as well as those subject to the widespread mislabelling of elasmobranch products. Wider screening studies are recommended to determine the risk to elasmobranchs from emerging pollutants and more robust studies are recommended to assess the risks to human health.

Keywords: *Elasmobranch; Pollution; Mercury; Cadmium; PCB; DDT.*

4.2. Introduction

Human activities are the main driver behind the rapid loss of the world's biodiversity (Derraik, 2002; Sanderson et al., 2002; McKee et al., 2004). Factors such as pollution, climate change, overexploitation and habitat loss now affect most marine ecosystems on the planet, with human activities causing irreversible damage (Derraik, 2002; Islam and Tanaka, 2004; Dulvy et al., 2014; EEA, 2018). In recent years there has been growing concern for the increasing prevalence of pollutants in the marine environment, their effect on marine organisms, and subsequent effects on humans (Tanabe et al., 1983; Blocksom et al., 2010; Corsolini et al., 2014; Jepson et al., 2016). Persistent organic pollutants (POPs), heavy metals, crude oil and marine debris (e.g. marine litter or microplastics) represent the most common marine pollutants globally (United Nations Environment Program, 2017). Some of these substances are used intentionally as disease and pest control, as well as in manufacturing and industrial processes. These substances can also be produced unintentionally as by-products through industrial processes such as waste incineration, vehicle emissions, and cigarette smoke, as well natural processes such as volcanic activity and forest fires (El-Shahawi et al., 2010; Megson et al., 2013; WHO, 2020). Pollutants can enter the aquatic environment through atmospheric deposition, erosion, urban discharge, combustion, and industrial charges (Wang et al., 2004; Morrison and Murphy, 2010; Megson et al., 2013).

Many pollutants bioaccumulate and biomagnify, and thus, apex predators usually have exposure to disproportionately high concentrations of pollutants compared to environmental levels. Pollutants in teleost fish, molluscs and marine mammals have been well-studied (Tanabe et al., 1983; Streit, 1998; Blocksom et al.,

2010; Sharma et al., 2014; Jepson et al., 2016; Barone et al., 2018; Desforges et al., 2018), and have been shown to cause adverse health effects including suppressed reproductive development effects, immunosuppression, endocrine disruption, and oxidative stress (Letcher et al., 2010). Less attention has been paid to pollutants in elasmobranchs compared to other vertebrate groups, which is especially concerning considering the high trophic position of elasmobranchs and their continued population decline (Dulvy et al., 2014).

Elasmobranchs belong to the class Chondrichthyans, which are cartilaginous fish that make up one of the oldest and most ecologically diverse vertebrate lineages, arising over 420 million years ago. They occupy the top tiers of aquatic food chains and are present in every ocean. Many elasmobranchs play a crucial role in the top-down control of coastal and oceanic ecosystem structure and function (Ebert et al., 2013; Dulvy et al., 2014). It is estimated that 30% of all Chondrichthyan species are currently threatened with extinction, where 21% of rays and skates, and 17% of sharks are classified as threatened (encompassing IUCN Red List categories 'critically endangered', 'endangered' and 'vulnerable'). In reality, this number is likely to be higher due to the large proportion (n = 438) of species that are listed as 'data deficient' and have not (yet) been assessed (Dulvy et al., 2008, 2014; Gray and Kennelly, 2018; IUCN, 2020). Elasmobranchs exhibit biological and ecological traits similar to those of large-bodied mammals; maturing late, reproducing slowly, having small numbers of offspring (García et al., 2008; Dulvy et al., 2014). The combination of these traits and their high trophic level puts elasmobranchs at relatively higher risk from exposure to pollutants.

All humans are exposed to pollutants throughout their lifetime, with diet being the most significant exposure pathway for many pollutants that bioaccumulate (e.g. lipophilic compounds such as PCBs) (Johansen et al., 2004; Fleming et al., 2006; Sharma et al., 2014). Twenty seven percent (1.9 billion people) of the world's population lives within 100 km of the coast (Fleming et al., 2006; Kumma et al., 2016). Although variable globally, many of these coastal countries and communities depend on fishing as a source of income, and seafood can make up the majority of their diet (Johansen et al., 2004; Fleming et al., 2006; Zheng et al., 2007; Brunner et al., 2009; Sharma et al., 2014; Bruce-Vanderpuije et al., 2019). Exposure to pollutants such as PCBs, mercury and dioxins, have been linked to cancer, liver and kidney damage, immunosuppression, reproductive defects, and endocrine disruption (Vračko et al., 2007; Zheng et al., 2007; Kim et al., 2013; Knutsen et al., 2019). Pregnant women and young children are especially vulnerable to the health risks associated with exposure to these contaminants (Patandin et al., 1999; Bruce-Vanderpuije et al., 2019).

Although elasmobranchs may not typically be considered as a primary food source in many non-coastal regions, products deriving from sharks, rays and skates are consumed, and used worldwide (Staffen et al., 2017; Almerón-Souza et al., 2018; Bernardo et al., 2020). Examples of consumption include shark fin soup, the use of traditional Chinese medicine (e.g. gill plates) and the intake of dietary supplements (e.g. liver oil and cartilage supplements). In addition, compounds deriving from elasmobranchs have been found in cosmetic products (Wong et al., 2009; Liu et al., 2013; Dulvy et al., 2014; Fields et al., 2015; Zeng et al., 2016; Cardeñosa et al., 2017; Steinke et al., 2017; Almerón-Souza et al., 2018; Ferretti et al., 2020). Shark meat is also often unintentionally consumed when it is mislabelled (e.g. as other types of

elasmobranchs or teleost fish), which means that consumers are unaware that they are consuming shark products (Hobbs et al., 2019; Pazartzi et al., 2019). Shark may be traded under names such as 'white fish', 'corvina', 'toyo', or 'cação', and can end up being consumed in countries where eating sharks is not culturally popular (Bornatowski et al., 2014; Almerón-Souza et al., 2018; Bernardo et al., 2020), as shown in the recent study that found threatened shark species (e.g. spiny dogfish) being sold at fish and chip shops in the UK (Hobbs et al., 2019). This is especially concerning due to the high concentrations of pollutants found in sharks (Holmes et al., 2009; Barbuto et al., 2010; Filonzi et al., 2010; Gilbert, Baduel, et al., 2015; Gilbert, Reichelt-Brushett, et al., 2015; Alves et al., 2016).

Despite the ecological and economical importance of elasmobranchs, the impact contaminants have on their health is poorly understood, as are the risks to humans through consumption of shark meat. No previous reviews have been carried out for pollutants in all elasmobranchs, the most recent review was performed on rays and skates only (Batoids) (Bezerra et al., 2019). The aim of this manuscript is to address this current knowledge gap by providing a thorough review of pollutant concentrations in all elasmobranchs (but with a specific focus on sharks). Specifically this review aims to 1) identify publication trends for elasmobranch pollution studies, 2) examine the variation in pollution concentrations between taxa, and determine elasmobranch groups most at risk from exposure to marine pollution, 4) relate concentrations of pollutants to toxic thresholds and discuss potential risks of consuming shark meat from a human health perspective, and 5) identify current knowledge gaps and discuss future recommendations.

4.3. Methods

4.3.1. Study Selection

The present systematic review follows the 2009 PRISMA guidelines (Moher et al., 2009) to identify research articles on marine pollution in elasmobranchs (flowchart, Supplementary Material 1). Eligibility for inclusion in this review was assessed independently by two reviewers (GPT and DB). Studies were incorporated based on the following inclusion criteria: the study reported on pollutant concentrations in elasmobranchs (though the study did not have to focus primarily on elasmobranchs to be considered for inclusion), the study was published between January 1999 and November 2019, the study was published in a peer-reviewed journal, and the study reported original research. Studies were considered from any country or region and on any contaminant type, as long they were published in English. Information on other taxa (non-elasmobranchs) were not included in this study. 'Grey literature' was not considered in this study as these papers often do not undergo the same peer-review process and are often not available online.

The following search terms were used to identify papers on two separate search engines (Web of Science and Scopus): *"shark*", "ray*", "sawfish*", "skate*", "elasmobranch*", "contaminant*", "contamination", "heavy metal*", "persistent organic pollutant*", "microplastic*", "organochloride", "tissue*", "fin*", "ingest*", "bioaccumulation", "bioaccumulate*"*. The following text "AND not *x-ray"* had to be specified as an exclusion criterion due to the high volume of papers identified in the initial search that were not relevant. Google Scholar was excluded, as it returned a large number of non-relevant papers (over 1000). A number of papers were found

based on the studies identified through the above search; for example, three additional papers were added based on the systematic review published on trace metals and POPs in rays and skates (Batoids) (Bezerra et al., 2019).

4.3.2. Data Collection

For every eligible study, general information was collected including author(s), year published, journal, pollutant (e.g. POPs, trace elements, plastic and radionuclides), taxa (species, family, order and superorder), common name, total number of elasmobranchs, area of study, ocean, risk to organism and/or humans and whether the primary focus was on elasmobranchs. The trophic level for all species identified from the scientific literature was sourced from FishBase (Froese and Pauly, 2019). The tissue type analysed was also recorded, specifically whether this concerned liver, fin, kidney, gills, reproductive organs, gastrointestinal system, or other. Reproductive organs included: egg, embryo, gonads, yolk, ovaries and ova; digestive system included: stomach, stomach content, digestive system, intestine and intestinal tract. The current IUCN Red List status (IUCN, 2020) of each species was recorded. Species were also grouped into their superorder Selachimorpha or Batoidea. IUCN status 2020 was categorised as followed; DD = Data Deficient, LC = Least Concern, NT = Near Threatened, VU = Vulnerable, EN =Endangered and CR = Critically Endangered. Pollutants were grouped into five categories: POPs, plastic, trace elements, radionuclides and other (see Supplementary Material 1 and 2).

4.3.3. Meta-analysis

A meta-analysis was carried out on total mercury (THg), cadmium (Cd), ΣPCB and ΣDDT concentrations in the muscle and liver tissue of elasmobranchs and were

recorded on a wet weight basis (dry and lipid weight in Supplementary Material 1 and 2). Muscle and liver tissue were recorded as these were the most reported tissue types, as well as being the most significant in terms of human exposure (through consumption). Data was converted to ng g^{-1} when necessary. Mean values were calculated when more than one individual was reported for one species. Where ranges were reported, a simple average of the upper and lower bounds of the range was calculated. Genders were grouped together, as were different age classes, so the meta-analysis could be focused on evaluating trends in the concentration of pollutants in different elasmobranch groups. Mean concentrations were reported to three significant figures.

4.3.4. Statistical Analysis

Statistical analysis and data visualisation were carried out in R 3.6.1 (R Core Team, 2019). Data was tested for normality with all variables found to be not normally distributed; non-parametric statistical methods were used. A chi-square goodness of fit test was used to examine whether the frequency of studies published across oceans and seas was evenly distributed. Wilcoxon rank-sum tests were used to compare differences in pollutant concentrations between muscle and liver tissue and, also between Selachimorpha and Batoidea. Kruskal-Wallis rank-sum tests and pairwise multiple comparison test ('Kruskalmc') using the "pgirmess" package (Giraudoux, 2018) were used to assess differences in pollutant concentrations across orders for both tissue types.

4.4. Publication Trends

4.4.1. General Information

This review examined a total of 176 studies on pollutants in elasmobranchs that were published between January 1999 and November 2019. Sixty-five percent of these studies were solely focussed on elasmobranchs (*n* = 115) and 35% included other organisms (e.g. fish and marine mammals) (*n* = 61). The most-studied tissue types included muscle (68%), liver (47%) and organs within the gastrointestinal tract (17%). Other tissue types included fin (14%), reproductive system (14%), gills (9%), kidney (7%), unknown (1%) and other (10%).

4.4.2. Overview of pollutants studied

A total of 111 papers focussed on trace elements, 59 on POPs, 12 on plastic, 7 on radionuclides, 3 on cholinesterases (ChEs) and lipid peroxidation (LP), 1 on endocrine-disrupting chemicals, and 1 on synthetic musk fragrances. Sixty three percent (*n* = 111) of all studies were focussed on trace elements, with 84% (*n* = 93) of these papers examining mercury (Hg) and 41% (*n* = 45) examining cadmium (Cd). Studies on POPs made up 32% (*n* = 57) of the total number of studies, where PCBs (74% of these studies; *n* = 42) and DDTs (55% of these studies; *n* = 31) were the most studied POPs. Other POPs included polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like PCBs (DL-PCBs), non-dioxin-like PCBs (NDL-PCBs), organochlorine pesticides (e.g. DDT and its metabolites, dieldrin, endrin and chlordane), hexachlorocyclohexane (HCH) and hexachlorobenzene (HBH), chlorobenzene, per- and polyfluoroalkyl substances (PFAS), polycyclic aromatic hydrocarbons (PAHs), and

halogenated flame retardants (HFR). Ninety-two studies (52%) discussed pollutant exposure risk in elasmobranchs, and 96 (55%) discussed the risks to humans. Forty-five studies (26%) discussed both the risks to elasmobranchs and humans, while 33 (19%) studies did not discuss risks to either elasmobranchs or humans.

There was a spike in the number of studies focussing on pollutants in elasmobranchs from 2013 to 2017, especially regarding trace elements and POPs (Figure 4-1). This could be due to the recent advances in cheaper, faster, and more accurate analysis techniques as well as an increase in interest from human health and environmental perspectives (Cole et al., 2011; Wright et al., 2013; Boucher and Friot, 2017). Plastics, such as microplastics and single-use-plastic, have become a recent important environmental concern and focus for researchers, this is evident from the increase in studies from 2016 onwards (Figure 4-1) (Wright et al., 2013; Ivar Do Sul and Costa, 2014; Gall and Thompson, 2015; Miranda and de Carvalho-Souza, 2016; Alomar and Deudero, 2017; Fossi et al., 2017; Pegado et al., 2018; Smith, 2018). The media and documentaries, such as Blue Planet II (presented by the BBC), have shifted consumers' views, as well as aided in the adoption of new laws on microplastics and single-use-plastic (Barboza and Gimenez, 2015; Xanthos and Walker, 2017; Henderson and Green, 2020). The recent advances in analysis techniques have allowed for a wider scope of studies focussing on emerging pollutants (Nikolaou et al., 2009); in spite of this, most of the studies targeted trace elements, PCBs, and DDTs, which may be because there are more standardised methods to analyse these pollutants in elasmobranchs rather than microplastics, and some of the more emerging and toxic POPs that require lower detection limits (e.g. dioxins, PFAS and halogenated flame retardants).

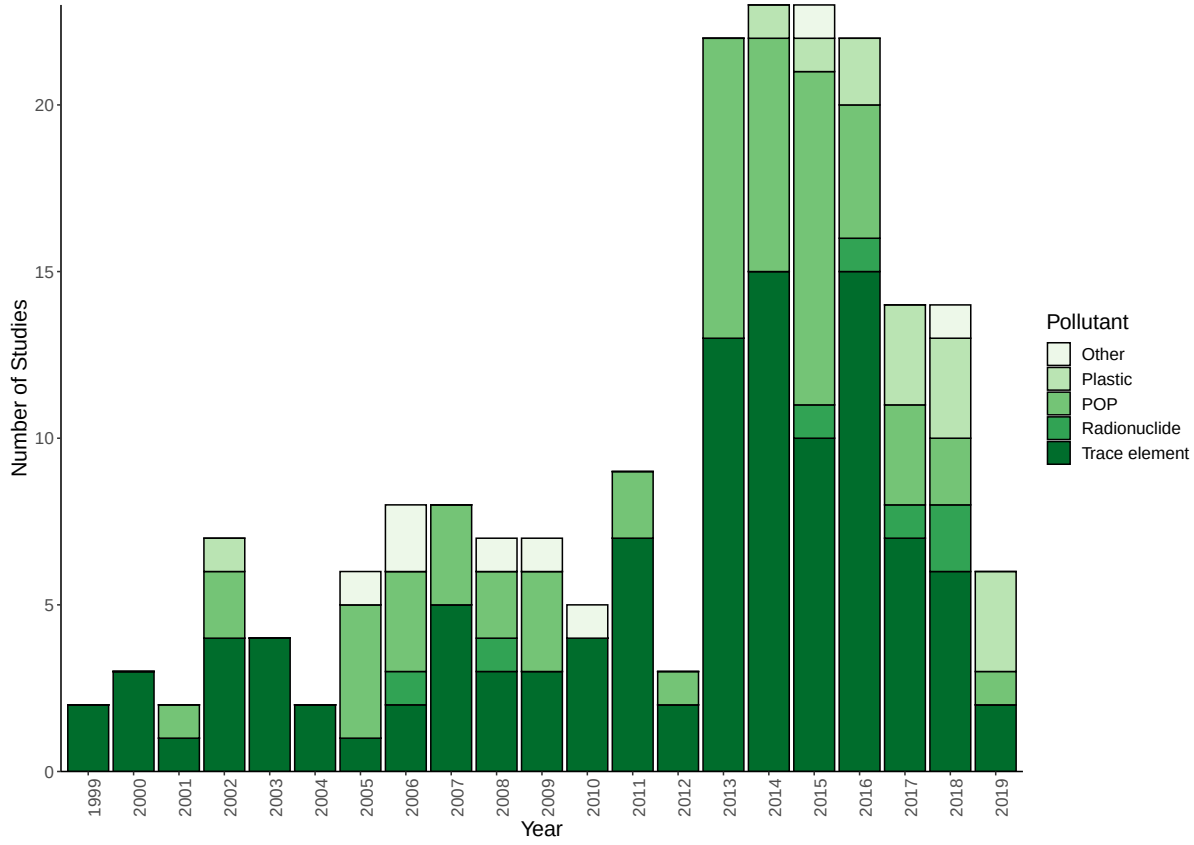


Figure 4-1 Total number of studies carried out on different pollutant types (Plastic, POP, Radionuclide and Trace Element, Other). Total number of studies (n=176); some studies reported on more than one type of pollutant (January 1999 – November 2019).

Most studies were published on trace elements (*n* = 62) and POPs (*n* = 31) in Carcharhiniformes, followed by trace elements in Rajiformes (*n* = 20), Squaliformes (*n* = 20) and Lamniformes (*n* = 19). Forty-nine species of rays and skates (Batoidea), and 47 sharks (Selachimorpha) were reported on three times or less. Fifty-five species, 13 families and four orders were recorded for superorder Batoidea. The most represented Batoid species were thornback skates (*Raja clavata*) (8 studies), brown skates (*Raja miraletus*) (5 studies) and starry skates (*Raja asterias*) (5 studies). A total of 80 species, 20 families and six orders were recorded for superorder Selachimorpha. The most reported on shark species were blue sharks (*Prionace glauca*) (28 studies), short fin mako sharks (*Isurus oxyrinchus*) (22 studies) and small spotted catsharks

(*Scyliorhinus canicula*) (19 studies). Hence, there appears to be a publication bias towards common and globally occurring species of sharks that are frequently caught in longline fisheries.

4.4.3. IUCN status

Species reported were categorised into groups based on their IUCN Red List status (IUCN, 2020), and their superorder (Selachimorpha or Batoidea). Three species of sharks were classed as CR, 7 as EN, 17 as VU, 18 as LC, 14 as DD, and 5 species were unknown. For rays and skates, one species was classed as CR, 6 as EN, 9 as VU, 7 as NT, 17 as LC, 13 as DD and 7 species were unknown. No studies reported on species of sawfish from the order Pristiformes despite their endangered and critically endangered IUCN status (IUCN, 2020). Most studies focussed on species that were listed as vulnerable or least concern.

4.4.4. Geographical distribution

There was relatively good global coverage of studies focussing on elasmobranchs, but the majority were carried out in the North Atlantic Ocean (63 studies), North Pacific Ocean (42 studies) and Mediterranean Sea (36 studies). Lesser studied areas included the South Pacific Ocean (14 studies), Indian Ocean (15 studies) and South Atlantic Ocean (21 studies) (Figure 4-2). Areas in the Southern Hemisphere such as the South Pacific (including Eastern Tropical Pacific), South Atlantic and Indian Ocean (including the Red Sea and Persian Gulf) received proportionately less attention despite being global hotspots for elasmobranch occurrence (Lucifora et al., 2011; Dulvy et al., 2014; Gray and Kennelly, 2018; Derrick et al., 2020). With a bias towards certain regions, we may not understand the full extent to which

elasmobranchs are exposed to pollutants. This is especially concerning as large-scale commercial fisheries often overlap with these hotspots putting humans at risk from exposure to high concentrations of pollutants if they consume products from these areas (Lucifora et al., 2011; Ferretti et al., 2020). It is crucial that future studies focus on regions that have received less attention in order to accurately identify the global threats to marine organisms as well as the humans that consume these products.

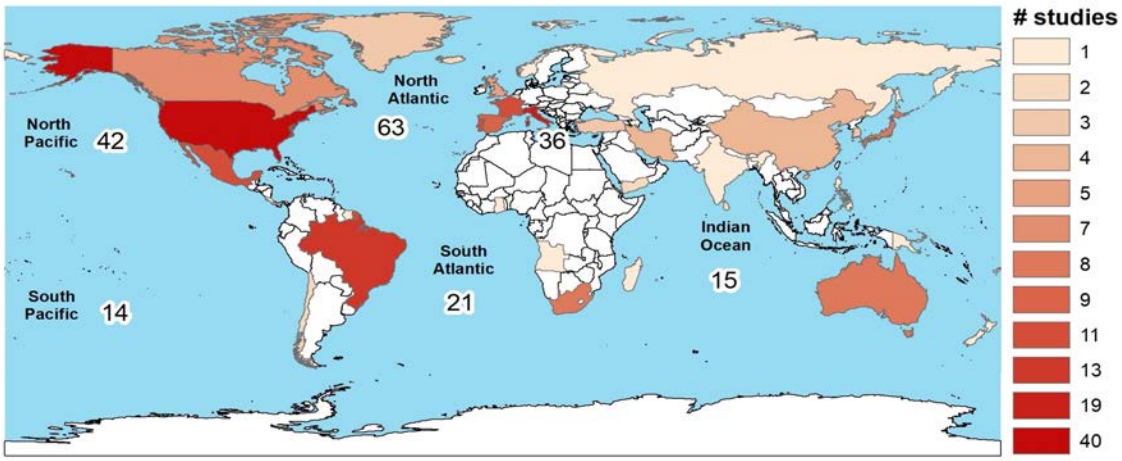


Figure 4-2 Geographical distribution from studies published on pollutants in elasmobranchs. The numbers represent the number of studies performed for each ocean (North Pacific, South Pacific, North Atlantic, South Atlantic and Indian Ocean), with the coloured shading showing how many studies were conducted by each country. The number of studies were not even across area of study (χ^2 = 56.885, df = 5, $p < 0.001$) where more studies were published in the North Atlantic Ocean than other locations.

4.4.5. Concentrations of pollutants in elasmobranchs

A meta-analysis was carried out on the concentrations of THg, Cd, ΣPCBs and ΣDDTs in the muscle and liver tissue of different elasmobranch orders, as these were the most represented pollutants in the literature. A total of 108 from the initial 176 studies were included in the meta-analysis (74 studies on mercury, 35 on cadmium, 41 on ΣPCBs and 28 ΣDDTs). Within the literature there was variation in how

concentrations were reported as either dry weight, lipid weight or wet weight was used. To allow comparisons between pollutants and enable a human health risk assessment, only wet weight (*n* = 75) is discussed within the body of this review, however all dry weight and lipid weight data is presented in Supplementary Material 1 and 2 via Science Direct.

4.4.5.1. Total mercury (THg)

Mercury concentrations were significantly higher in muscle (1430 ± 2330 ng g^{-1}) than in liver tissue (522 ± 971 ng g^{-1}) (Wilcoxon: W = 966, p < 0.001). Pairwise comparisons indicated that THg concentrations of muscle tissue in Carcharhiniformes (1520 ± 1900 ng g^{-1}, *n* = 826) and Lamniformes (2580 ± 4790 ng g^{-1}, *n* = 195) were significantly higher than concentrations in liver tissue (839 ± 1438 ng g^{-1}, *n* = 84 and 85.5 ± 53.6 ng g^{-1}, *n* = 108) (Figure 4-3). THg did not differ between orders in liver tissue (Kruskal – Wallis: χ^2 = 9.79, df = 5, p = 0.081), but did in muscle tissue (Kruskal – Wallis: χ^2 = 25.965, p < 0.01). A multiple comparison test on muscle tissue indicated that concentrations of mercury were higher in Carcharhiniformes (1520 ± 1900 ng g^{-1}, *n* = 1739), Lamniformes (2580 ± 4790 ng g^{-1}, *n* = 508) and Squaliformes (1610 ± 1040 ng g^{-1}, *n* = 415) than in Myliobatiformes (383 ± 350 ng g^{-1}, *n* = 195) (Figure 4-3). Mercury concentrations in liver tissue ranged between 4 ng g^{-1} in giant manta rays (*Mobula birostris*) (*n* = 6) caught along the coast of Takoradi, Ghana (Essumang, 2009) and 20,800 ng g^{-1} in short fin mako sharks from Southern California, North Pacific (Lyons et al., 2015). Mercury concentrations in muscle tissue ranged between 4 ng g^{-1} in giant manta rays (*n* = 6) from Takoradi, Ghana (Essumang, 2009) and 4620 ng g^{-1} in smooth tooth black tip sharks (*Carcharhinus leiodon*) (*n* = 7) from the Arabian Gulf (Moore et al., 2015).

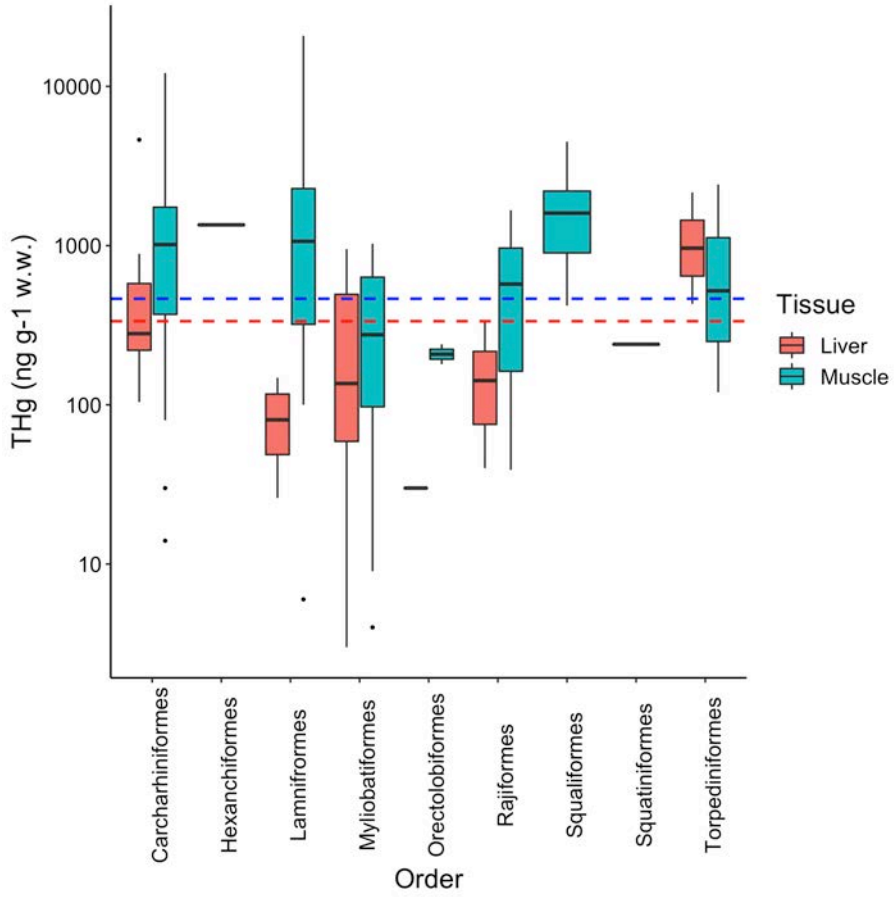


Figure 4-3 Total mercury (THg) concentrations in the muscle and liver tissue of different elasmobranch groups reported globally. Values are reported in ng g^{-1} on wet weight (w.w.) basis. The tolerable concentration of THg in one serving of fish (113 g) for adults indicated with a blue dashed line, and one serving of 28 g in children (two-years-old) with a red dashed line. The upper limit was set at 464 µg kg^{-1} (ng g^{-1}) per week for adults and the lower limit at 335 µg kg^{-1} (ng g^{-1}) per week in children (EPA, 2020).

4.4.5.2. Cadmium (Cd)

Cd concentrations were significantly higher in liver tissue (7050 ± 21200 ng g^{-1}) than in muscle tissue (160 ± 397 ng g^{-1}) (Wilcoxon: W = 917, p<0.001). Pairwise comparisons indicated that Carcharhiniformes (7730 ± 15100 ng g^{-1}) and Rajiformes (16300 ± 34200 ng g^{-1}) had significantly higher concentrations of Cd in muscle than liver tissue (451 ± 813 ng g^{-1} and 115 ± 181 ng g^{-1}) (Figure 4-3). Cd concentrations did

not differ between orders in muscle tissue (Kruskal – Wallis: χ^2 = 6.802, df = 6, p = 0.339) but did in liver tissue (Kruskal – Wallis: χ^2 =12.51, df = 5, p < 0.05). A multiple comparison test indicated that Carcharhiniformes (7730 ± 15100 ng g^{-1}, *n* = 84) had significantly higher concentrations of Cd in their liver than Torpediniformes (45 ± 19 ng g^{-1}, *n* = 155) (Figure 4-4). The lowest concentrations of Cd in muscle tissue of 10 ng g^{-1} were observed in sandy (*Leucoraja circularis*) (*n* = 20) and shagreen skates (*Leucoraja fullonica*) (*n* = 24) from Bay of Biscay and the Celtic Sea (Nicolaus et al., 2017), blue sharks (*n* = 20) from southwest waters of Portugal, North East Atlantic (Alves et al., 2016), and whitespotted bamboo sharks (*Chiloscyllium plagiosum*) (n=26) from the southern waters of Hong Kong (Cornish et al., 2007). The highest Cd concentrations in muscle tissue of 2000 ng g^{-1} were observed in small tail sharks (*Carcharhinus porosus*) (*n* = 12) from Atlantic waters surrounding Trinidad and Tobago (Mohammed and Mohammed, 2017). Cd in the liver ranged between 17 ng g^{-1} in giant manta rays (*n* = 6) from Takoradi, Ghana (Essumang, 2009) and 87,200 ng g^{-1} lesser guitarfish (*Acroteriobatus annulatus*) (*n* = 19) from False Bay and Saldanha Bay, South Africa (Morris et al., 2016).

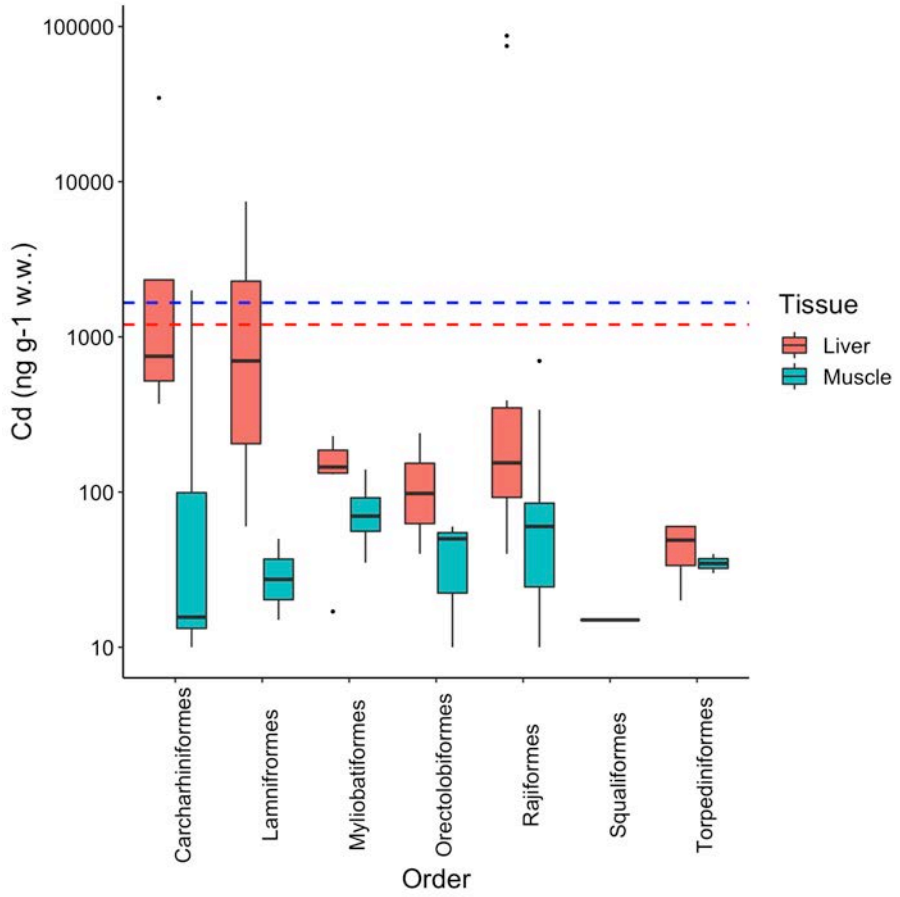


Figure 4-4 Cadmium (Cd) concentrations in the muscle and liver tissue of different elasmobranch groups reported globally. Values are reported in ng g^{-1} on a wet weight (w.w.) basis. The maximum concentration of Cd in one serving (113 g) of fish for adults is indicated with a blue dashed line, and one serving of 28 g in children (two-years-old) with a red dashed line. The upper limit was set at 1660 µg kg^{-1} (ng g^{-1}) per week for adults and the lower limit at 1200 µg kg^{-1} (ng g^{-1}) per week in children (FAO and WHO, 2013; EFSA, 2011).

4.4.5.3. Polychlorinated biphenyls (PCBs)

ΣPCB concentrations were significantly greater in liver tissue (6380 ± 9720 ng g^{-1}) than in muscle tissue (14 ± 14 ng g^{-1}) (Wilcoxon: W = 125, $p < 0.001$), though a pairwise comparison did not indicate any significant differences within orders. No significant difference was observed in ΣPCB concentrations between each order in muscle (Kruskal – Wallis: $\chi^2 = 5.42$, df = 3, p = 0.143) and liver tissue (Kruskal – Wallis:

χ^2 = 6.959, df = 3, p = 0.073) (Figure 4-5). Concentrations of ΣPCBs in muscle tissue ranged from 1 ng g^{-1} in barndoor skates (*Dipturus laevis*) (*n* = 13) from Cape Cod, Massachusetts, USA (Lyons and Adams, 2017) to 44.5 ng g^{-1} in Greenland sharks (*n* = 3) from North East Greenland waters (Corsolini et al., 2014). ΣPCBs in liver tissue ranged from 35.6 ng g^{-1} in Greenland sharks (*n* = 43) from the Kongsfjorden area, Svalbard, Norway (Molde et al., 2013) to 30,000 ng g^{-1} in one short fin mako shark from Huntington Beach, California, USA (Lyons et al., 2015). Although the total PCB concentration of elasmobranch orders are reported here, these values should be taken tentatively. Due to the different approaches of each study (i.e. taking a subset of PCBs or excluding DL-PCBs), it makes comparing PCB concentrations across orders and the two tissues types challenging. This is an inherent issue when comparing PCB data sets as researches use different analytical techniques and report "total PCBs" in different ways (Megson et al., 2019). Therefore, these values should only be used as a conservative guideline to indicate the potential health risks to elasmobranchs as well as humans consuming products deriving from elasmobranchs.

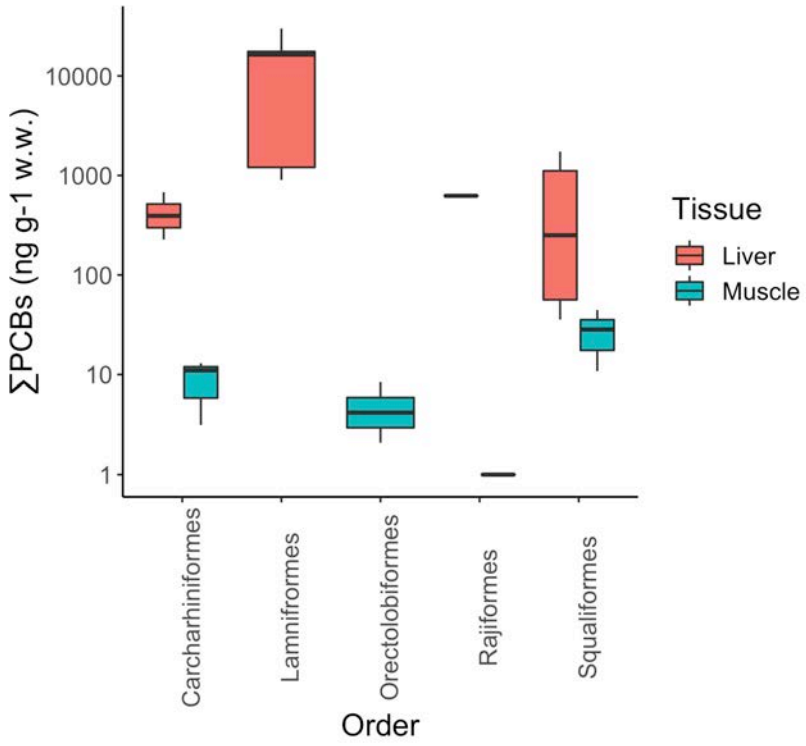


Figure 4-5 ΣPCB concentrations in the muscle and liver tissue of different elasmobranch groups reported globally. Values are reported in ng g^{-1} on a wet weight (w.w.) basis. No tolerable limit was considered against this data due to inconsistencies in reporting PCB concentrations in the literature.

4.4.5.4. Dichlorodiphenyltrichloroethane (DDT)

ΣDDT concentrations were significantly greater in liver tissue (19500 ± 37100 ng g^{-1}) than in muscle tissue (10 ± 14 ng g^{-1}) (W = 145, $p < 0.001$). A pairwise comparison of muscle and liver tissue did not indicate any significant differences within each order (Figure 4-6). No significant differences were observed between orders for muscle (Kruskal – Wallis: $\chi^2 = 3.99$, df = 4, p = 0.408) and liver tissue (Kruskal – Wallis: $\chi^2 = 4.08$, df = 3, p = 0.253) (Figure 4-6). Concentrations in muscle tissue ranged from 0.28 ng g^{-1} in barndoor skates (*n* = 1) collected in offshore waters adjacent to Cape Cod, Massachusetts, USA (Lyons and Adams, 2017) to 49.3 ng g^{-1} in gulper sharks (*Centrophorus granulosus*) (*n* = 25) from the Mediterranean Sea (Storelli and Marcotrigiano, 2001). Concentrations in the liver ranged from 0.537 ng

g^{-1} in Greenland sharks (*n* = 3) (Corsolini et al., 2014) from North East Greenland to 103,000 ng g^{-1} in great white sharks (*n* = 30) from North Pacific waters surrounding California, USA (Lyons et al., 2013).

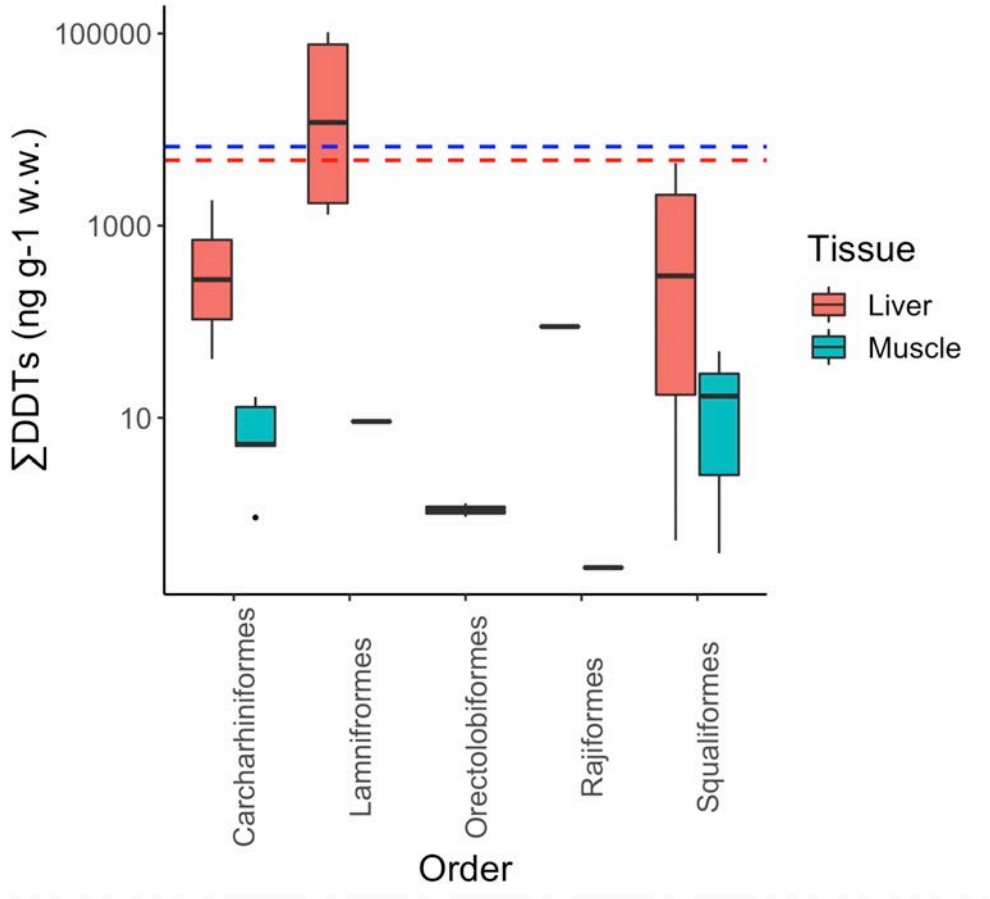


Figure 4-6 ΣDDT concentrations in the muscle and liver tissue of different elasmobranch groups reported globally. Values are reported in ng g^{-1} on a wet weight (w.w.) basis. The maximum concentration of DDT in one serving (113 g) of fish for adults is indicated with a blue dashed line, and one serving of 28 g in children (two-years-old) with a red dashed line. The upper limit was set at 6.64 mg kg^{-1} (6640 ng g^{-1}) per week for adults and the lower limit at 4.79 mg kg^{-1} (4790 ng g^{-1}) per week in children (WHO, 1961; WHO and FAO, 2000).

4.4.6. Risk to elasmobranchs

Elasmobranchs are exposed to high concentrations of pollutants throughout their lifetime. Sharks had higher concentrations of pollutants than rays and skates

(Table 4-1), with the exception of Cd in bluntnose guitarfish (*Acroteriobatus blochii*) and lesser spotted guitarfish (*Acroteriobatus annulatus*) belonging to the order Rajiformes. Species belonging to the orders Carcharhiniformes and Lamniformes had the highest concentration of all four pollutants (Figure 4-3 to Figure 4-6). The variation observed between groups can be explained by the diversity of elasmobranchs, as well as their different habitats, size, age, trophic position, life strategies and diet (Pethybridge et al., 2010; Olin et al., 2014; Beaudry et al., 2015; Sandoval-Herrera et al., 2016; Matulik et al., 2017; McKinney et al., 2017; Morris et al., 2016). Many shark species are migratory predators that feed continuously and as pollutants can vary across geographic regions, species may be exposed to pollutants in different ways (Teffer et al., 2014). Trophic level data revealed that there was a significant positive correlation between THg concentration and trophic level in muscle tissue for sharks, rays and skates. A positive trend was observed between trophic level and concentration of PCBs and DDTs in both tissue types, and THg in liver tissue, however, these trends were not statistically significant which could be as a result of the limited data available. Interestingly Cd concentrations seemed to decrease as trophic level increased; this anomaly seemed to be primarily driven by high concentrations observed in three elasmobranch species (bluntnose and lesser spotted guitarfish, and megamouth shark).

Previous studies have found that sharks, rays and skates accumulate organic (e.g. PCBs, DDTs and organochlorines) and inorganic (e.g. trace elements) pollutants (Olin et al., 2014; Beaudry et al., 2015; Gilbert et al., 2015; Weijs et al., 2015; Cagnazzi et al., 2019). Elasmobranchs occupying high trophic positions also tend to be long-lived and large-sized, mature late, and have relatively few offspring, which allows for

the bioaccumulation of pollutants (Fisk et al., 2002; Cagnazzi et al., 2019; Matulik et al., 2017; McKinney et al., 2016). As well as bioaccumulation, trophic level analysis revealed strong evidence of biomagnification of organic and inorganic pollutants through the food chain. The lowest concentrations of pollutants were observed in rays and skates, especially THg and Cd in giant manta rays. Giant manta rays are secondary consumers that predominantly feed on zooplankton (e.g. krill, shrimp and crabs), which means they may not accumulate pollutants at the same rate as some of the other rays and skates that feed on larger prey (Essumang, 2009; Bezerra et al. 2019; Burgess et al., 2016). Further discussion on pollutant accumulation and risks to Batoids can be found in Bezerra et al., (2019).

There are currently no toxic thresholds for tolerable concentrations of pollutants in elasmobranchs. Studies have suggested that pollutants, such as Hg and Cd, can alter the reproductive physiology of sharks, rays and skates (Molde et al., 2013; Mull et al., 2013; Bendall et al., 2014; Rumbold et al., 2014; Terrazas-López et al., 2016; Bezerra et al., 2019). Elasmobranchs have also been shown to maternally offload a wide range of pollutants to their offspring (Bezerra et al. 2019; Olin et al., 2014; Gilbert, Baduel, et al., 2015; Lyons and Lowe, 2015; Weijs et al., 2015; van Hees and Ebert, 2017). This poses a significant health risk to developing embryos and shark pups as they start their life with higher concentrations of pollutants and will continue to bioaccumulate these contaminants throughout their lifetime (De Boeck et al., 2010; Mull et al., 2013; Olin et al., 2014; Frías-espericueta et al., 2015; Lyons and Adams, 2015; McKinney et al., 2016). One recent study indicated that white sharks (*Carcharodon carcharias*) did not exhibit physiological responses (i.e. no change in enzymatic conditions and leukocyte counts) that would usually be expected when

organisms are exposed to high concentrations of heavy metals (Merly et al., 2019). This suggests that some species may be more tolerant to pollutant exposure or are able to biotransform and eliminate organic pollutants (e.g. DDTs and PCBs) more effectively than other species (Corsolini et al., 2014). More studies are needed to assess the risks of pollutants in elasmobranchs to accurately identify any adverse health effects and improve our understanding of the fate and transport of pollutants inside these organisms.

Due to the absence of toxic threshold of ΣPCBs in sharks, concentrations in this study were compared to the "applied" toxic threshold of ΣPCBs in marine mammals as set by Jepson et al. of at lowest 9 mg kg^{-1} and at highest 41 mg kg^{-1} (lipid weight) (Helle et al., 1976; Jepson et al., 2016). Short fin mako sharks and bull sharks exceed the lowest toxicity threshold, with concentrations in their muscle and liver tissue exceeding 37 mg kg^{-1} lipid weight. Studies carried out on marine mammals and teleost fish have found an association between exposure to pollutants and neurological disorders, structural damage to organs and gills, reduced fertility, reproductive developmental effects, oxidative stress, and cancer (Tanabe et al., 1983; Evans, 1987; Blocksom et al., 2010; Pandey, Govind and Madhuri, 2014; Sharma et al., 2014; Jepson et al., 2016; Desforges et al., 2018; Cagnazzi et al., 2019). More research is needed to confirm if elasmobranchs exhibit the same physiological effects that have been established in marine mammals and teleost fish.

More attention has been paid to the risks towards humans who consume shark meat rather than how pollutants affect the organisms themselves. The large amount of resources, funding, time, and planning required, as well as the shy and migratory behaviour of some species, make sampling for elasmobranchs incredibly

difficult. This may also explain the opportunistic nature of some studies. The negative portrayal of sharks in the media and in movies such as 'Jaws', 'The Shallows', 'Sharknado' (series of films) and 'The Meg' has made gathering support for their conservation extremely difficult (Reynolds et al., 2005; Simpfendorfer et al., 2011; Friedrich et al., 2014). Seeking funding to carry out pollutant monitoring programs for elasmobranchs is challenging and funding may support projects on species that are often associated with a positive public perception such as marine mammals, sea birds and turtles, rather than sharks, rays and skates.

Determining the exposure risk in elasmobranchs is difficult as there are differences among taxonomic groups, but also among orders, families and species. The high concentrations found in this study suggest that elasmobranchs could be negatively impacted, though to date research on the health impacts of pollutant exposure in elasmobranchs has typically been less extensive than in humans. Establishing baseline thresholds for pollutants in elasmobranchs poses a significant challenge; nevertheless, they currently represent one of the most vulnerable and at-risk taxa (Dulvy et al., 2014; IUCN, 2020) and therefore there is an urgent need to fully understand their susceptibility to pollutant exposure. The urgency is further underlined by the current rapid loss of species, which is driven by existing threats including overfishing, habitat loss, and climate change.

Table 4-1 Mean ± SD THg, Cd, ΣPCB and ΣDDT concentrations in the muscle and liver tissue of superorder Selachimorpha and Batoidea expressed in ng g^{-1} on a wet weight basis.

Pollutant	Basis	Tissue	Selachimorpha	Batoidea	Sig
THg	Wet Weight	Muscle	1670 ± 2580	598 ± 546	***
		Liver	538 ± 1150	498 ± 666	NS
Cd	Wet Weight	Muscle	272 ± 634	97 ± 142	*
		Liver	4710 ± 10800	8220 ± 25000	*
ΣPCBs	Wet Weight	Muscle	15 ± 14	1	NS
		Liver	6820 ± 9970	625	NS
ΣDDTs	Wet Weight	Muscle	11 ± 14	0.28	NS
		Liver	2140 ± 38100	89	NS

Significant differences in pollutant concentrations between Selachimorpha and Batoidea were indicated at * <0.05, ** <0.01, *** <0.001. NS = p > 0.05.

4.4.7. Human health risks

4.4.7.1. Human consumption

The consumption of shark is probably best recognised through the shark fin trade (e.g. shark fin soup), though other important exposure pathways are through the use of traditional Chinese medicine (e.g. gill plates), intake of dietary supplements (e.g. liver oil and cartilage supplements) and use of cosmetic products (Wong et al., 2009; Liu et al., 2013; Dulvy et al., 2014; Fields et al., 2015; Zeng et al., 2016; Cardeñosa et al., 2017; Steinke et al., 2017; Almerón-Souza et al., 2018; Ferretti et al., 2020). Cases of mislabelling and species substitution are becoming increasingly prevalent, with evidence also showing an increased occurrence of mislabelling in ray and skate species (Barbuto et al., 2010; Filonzi et al., 2010; Bornatowski et al., 2014; Dulvy et al., 2014; Zeng et al., 2016; Staffen et al., 2017; Almerón-Souza et al., 2018;

Wainwright et al., 2018; Hellberg et al., 2019; Hobbs et al., 2019; Pazartzi et al., 2019). This could be due to the advances in genetic tools for species identification (Barcaccia et al., 2016), but also the monetary incentives from selling shark, ray or skate meat as more highly valued and expensive species (e.g. tuna, swordfish, mackerel and bonito), as elasmobranchs often represent lower market values and are caught as by-catch (Filonzi et al., 2010). The decrease in landings for commercial bony fish may also put a strain on commercial fisheries (Mullon et al., 2005; Pinsky et al., 2011), resulting in an increase in fraudulent sales of other fish, such as sharks, rays and skates.

Food fraud and product mislabelling have occurred throughout history (Spink and Moyer, 2011; Johnson, 2014): a well-known case is the 'horse meat scandal' (2013), where horse meat was sold as beef (Walker et al., 2013). Food mislabelling is of great concern to the safety of consumers as they may be exposed to allergens (or in the case of sharks, high concentrations of pollutants), without their knowledge. Recent studies have found shark meat in countries where shark is not known to be a primary fish source. Examples of mislabelling include the UK where shark was sold as cod in fish and chip shops (Hobbs et al., 2019) and substitution of threatened sharks (CITES) as non-threatened species in Brazil, Greece, and the USA, amongst others (Bornatowski et al., 2014; Almerón-Souza et al., 2018; Hellberg et al., 2019; Pazartzi et al., 2019; Bernardo et al., 2020). Mislabelling and substitution thus represents not only a threat to vulnerable species of sharks, but also to the consumers of sharks and shark-based products.

4.4.7.2. Hazard quotients

Hazard quotients were calculated based on the recommended weekly and monthly intake (where applicable) for THg, Cd and ΣDDTs (Table 4-2). The minimum and maximum consumption limits were based on the most vulnerable and most-at-risk individuals; females and children. The adult weight was based on a woman of 75 kg, and the children's weight based on a two-year-old female of 13.4 kg and 11-year-old female of 47.5 kg. The average serving size of 113 g (four ounces) of fish was based on the US EPA's advice for adults and children (aged 11); for children aged two the average serving size was 28 g (EPA, 2020). People consume fish between one to three times per week, though young children on average consume only one serving per week (U.S. Environmental Protection Agency, 2011; EPA, 2020). Exposure risk was calculated using the average pollutant concentrations in the muscle tissue of sharks, as this was considered to be the most likely tissue type to be consumed.

Table 4-2 Hazard Quotients (HQ) were calculated for Cd, THg and ΣDDT indicating the minimum and maximum risk humans would have from consuming shark meat one to three times per week.

	Hazard Quotient		
	Hg	Cd	ΣDDT
Adult (Female aged 20 yrs or over eating 3x per week)	10.8	0.5	0.00071
Adult (Female aged 20 yrs or over eating 1x per week)	3.6	0.164	0.000236
Child (11 yr old female eating 3x per week)	17	0.776	0.00112
Child (11 yr old female eating 1x per week)	5.69	0.258	0.000373
Child (2 yr old female eating 3x per week)	15	0.68	0.00099
Child (2 yr old female eating 1x per week)	5	0.228	0.000329

4.4.7.2.1. Mercury (Hg)

Mercury can be present in the environment in several different forms (organic, inorganic and elemental); within the literature the majority of studies reported on THg and many did not report separately on methylmercury (MeHg) as it makes up 70-100% of total mercury in elasmobranchs (Storelli et al., 2003; Krystek and Ritsema,

2005; Pethybridge et al., 2010; de Carvalho et al., 2014; Rumbold et al., 2014; Alves et al., 2016; Torres et al., 2016; Mohammed and Mohammed, 2017; Chouvelon et al., 2018). The provisional tolerable weekly intake (PTWI) for humans was based on MeHg and was used to calculate the safe consumption limit of mercury in shark muscle tissue for adults and children (FAO and WHO, 2006; EPA, 2020) (Table 4-2). There is currently no scientific consensus on the PTWI of MeHg: the EFSA and WHO recommend a higher PTWI of 1.3 and 1.6 ng g^{-1} of body weight (bw) $week^{-1}$ respectively, whilst US EPA recommends a more conservative PTWI of 0.7 ng g^{-1} of bw $week^{-1}$ (FAO and WHO, 2006; EFSA, 2012; EPA, 2020). Hazard quotients were derived based on the US EPA's PTWI given the human health implications of over consumption of mercury (Table 4-2). Hazard quotients were calculated using the mean concentration of Hg in shark muscle tissue was 1670 ng g^{-1} on a wet weight basis (Table 4-2).

4.4.7.2.2. Cadmium

The European Food Safety Authority (EFSA), World Health Organisation (WHO) and the Food and Agricultural Organisation (FAO) set PTWI of Cd from food at 2.5 ng g^{-1} of bw $week^{-1}$ for all age groups (FAO and WHO, 2013; EFSA, 2011). The mean Cd level in shark muscle tissue was 272 ng g^{-1} on a wet weight basis. This value was used to determine hazard quotas for adults and children aged between two and 11 years old (Table 4-2). Although there is less risk of consuming shark muscle meat, Cd concentrations in the liver were much higher (maximum 4710 ng g^{-1}) and therefore shark products should be consumed with caution as Cd is especially toxic to kidneys, accumulating over time leading to renal dysfunction (Figure 4-5) (EFSA, 2011).

4.4.7.2.3. DDTs

The provisional tolerable daily intake (PTDI) of ΣDDT as set by the FAO and WHO is 10 ng g^{-1} of bw (70 ng g^{-1} of bw $week^{-1}$) (WHO, 1961) which was confirmed at the Joint Meeting of Pesticide Residues (JMPR) (FAO and WHO) in 2001 (WHO and FAO, 2000). The mean concentration of ΣDDT in shark muscle tissue was 11 ng g^{-1}. Maximum and minimum hazard quotients for children (aged two and 11 years old) and adults were less than 0.01, which indicated that there is a limited risk from exposure to DDT in shark meat when consumed one to three times per week Table 4-2). Similarly to Cd, concentrations of ΣDDT were higher in the liver of sharks, especially in Lamniformes (41,000 ng g^{-1}), and therefore shark products should be consumed with caution (Figure 4-6).

4.4.7.2.4. PCBs

It's challenging to accurately identify the risks to human health posed by PCBs based on the available data in the literature. The health risks from PCBs are calculated using the 12 DL-PCB and PCDD/Fs (FAO and WHO, 1991; van den Berg et al., 1998, 2006; WHO, 2010; Megson et al., 2019) however only three studies out of the 41 on PCBs reported concentrations of all 12 DL-PCBs, with the rest of the data being based on a subset of PCBs (e.g. i7 PCBs) or a "total" PCB concentration (ΣPCB) calculated using anywhere between seven to 55 PCBs (Supplementary Material 3). This is possibly because the aim of many of these studies was to undertake a baseline screening assessment rather than undertake a detailed human and animal health risk assessment. Studies that reported on the 12 DL-PCBs observed high concentrations (wet weight basis) in the liver (43 ± 6 pg g^{-1}) and muscle tissue (36 ± 6 pg g^{-1}) of Greenland sharks (Corsolini et al., 2014), and in the liver (45972 ± 43967 pg g^{-1}) and

muscle tissue (103 ± 77 pg g^{-1}) of blue sharks (Alves et al., 2016). Corsolini et al. (2014) reported a WHO_{2005} toxic equivalence (TEQ) of 5.23 pg TEQ g^{-1} in the muscle tissue of Greenland sharks and Alves et al. (2016) 0.0140 pg TEQ g^{-1} (wet weight) in the muscle tissue of blue sharks (van den Berg et al., 2006).

Due to the limited of the data available, only a preliminary human risk assessment could be undertaken. Hazard quotients were calculated based on the EFSA's conservative TWI of 2 pg TEQ kg^{-1} of bw $week^{-1}$ (Knutsen et al., 2019). This indicated that adults and children would be exposed to over three times more dioxins and DL-PCBs when consuming muscle tissue from Greenland sharks (Adult HQ = 3.9x, Child aged 11 HQ = 6.2x and Child aged two HQ = 5.5x). Although the total DL-PCB concentration was greater in muscle tissue from blue sharks, when this was converted to a TEQ risk assessment, it indicated that there was a lower risk (HQ = 0.1) from consuming blue shark meat. As POPs are more lipophilic, dioxins and DL-PCBs accumulate in higher concentrations in the liver than in muscle tissue, therefore there may be a significant risk from consuming products derived from the liver that should be investigated (e.g. liver oil capsules, and skin care products that are put directly onto skin). In addition, PCBs are just a subset of dioxin-like-compounds (DLCs) which exhibit the same toxic mode of action. Therefore, to properly assess health risks future studies should also consider determining concentrations of other dioxins and DLCs such as polychlorinated naphthalene (PCNs), polybrominated dibenzo-p-dioxins and dibenzofurans (PBDD/Fs) and mixed halogenated dioxins/furans (PxDD/Fs). This assessment indicated that there may be a significant risk to a human health from consuming DLCs in shark meat. However, more studies that focus on determining

DLCs in sharks and shark-based products are needed to accurately assess the human health risks.

4.4.7.3. Human health recommendations

The data gathered from this review indicated that humans should avoid consuming shark meat (specifically muscle tissue) as they would be exposed to high levels of mercury. Although there were no observed risks from Cd or DDT in muscle tissue, the higher concentrations in the liver suggest that shark products should be consumed with caution. One serving of shark meat (113 g for adults and 11-year-olds; 28 g for 2-year-olds) would expose adults and children to over three times the maximum recommended mercury consumption limit, and could lead to them experiencing toxic effects (Table 4-1) (Mohammed and Mohammed, 2017; EPA, 2020). Similar findings were observed by the US EPA and in numerous other studies reporting on mercury in sharks (Gomes Ferreira et al., 2004; Burger and Gochfeld, 2011; Escobar-Sánchez et al., 2011; Lopez et al., 2013; Lyons et al., 2013; Olmedo et al., 2013; Vélez-Alavez et al., 2013; Man et al., 2014; Nalluri et al., 2014; Teffer et al., 2014; Corsolini et al., 2014; de Carvalho et al., 2014; Gilbert, Reichelt-Brushett, et al., 2015; Kiszka et al., 2015; Alves et al., 2016; Biton-Porsmoguer et al., 2018; Cagnazzi et al., 2019). Although the US EPA's recommendations of avoiding shark meat are in line with this study, our data indicates that their current limit of 980 ng g^{-1} may be underestimating the risk as average mercury concentrations in sharks exceed this value by 66% (1670 ng g^{-1}).

It should also be noted that this value was an average for all sharks. People consuming sharks from the orders Carcharhiniformes and Lamniformes would be at greater risk as the average mercury concentration in these species exceeded 4000 ng

g^{-1}. This is concerning as species belonging to these elasmobranch orders have the highest economic value and so are one of the most targeted group of sharks in the international fin and meat trade. Species include blue sharks (28 studies), silky sharks (*Carcharhinus falciformis*) (6 studies), dusky sharks (*C. obscurus*) (9 studies), sandbar sharks (*C. plumbeus*) (6 studies), tiger sharks (*Galeocerdo cuvier*) (6 studies), hammerheads (*Sphyrna* spp.), bull sharks (*C. leucas*) (14 studies), short fin mako sharks (22 studies), thresher sharks (*Alopias* spp.) (12 studies), and oceanic white tips (*C. longimanus*) (5 studies) (Clarke et al., 2006; Worm et al., 2013; Gray and Kennelly, 2018; Ferretti et al., 2020).

The concentrations of mercury in sharks are greater than other regularly consumed fish species, such as marlin (490 ng g^{-1}), king mackerel (730 ng g^{-1}), swordfish (1000 ng g^{-1}), and bigeye tuna (690 ng g^{-1}) (EPA, 2020). There is evidence that humans that live in coastal areas, especially those who work in the fishing industry, eat twice as much fish as the general population; these groups are therefore likely to be at a greater risk than the general population (Svensson et al., 1995; Leng et al., 2009). Limited biomonitoring studies on these groups have revealed elevated concentrations of POPs (e.g. PCBs and PCDD/Fs) and trace elements (e.g. methylmercury) in their blood and semen (Svensson et al., 1995; Chien et al., 2002; Kiviranta et al., 2002; Toft et al., 2006; Rignell-Hydbom et al., 2007; Cheng et al., 2009). As these individuals are most at risk of pollutant exposure, it is crucial that they are aware of these threats. Any programs that are put into place to outline the health risks to consumers should acknowledge the importance of elasmobranchs for their livelihood and work to provide alternatives for communities that depend on fishing.

Although the focus of this study has been on the consumption of shark muscle tissue, it is important to acknowledge a potential exposure pathway from products deriving from shark liver, including shark liver oil, as well as a potential risk from consuming products deriving from rays and skates (Bezerra et al., 2019). The elevated concentrations of Cd, DDT, and PCBs within the liver of sharks suggest that, if anything, risks to human health are exacerbated when shark liver rather than shark muscle is considered. In some cases, for example for PCBs and DDTs in Carcharhiniformes, Lamniformes and Rajiformes, concentrations were higher than in muscle tissue, which highlights the risk from consuming any elasmobranch product. The consumption of elasmobranchs is thus a global health concern, especially in commonly traded species, such as smooth and scalloped hammerheads, short fin mako and blue sharks with the highest concentrations. The risks associated with the consumption of elasmobranch products makes it essential that governments, regulators, and seafood inspectors identify and track products that are sold in their country as well as the products that are imported and exported.

4.5. Knowledge gaps and future recommendations

This review was performed on 176 studies focussing on pollutants in elasmobranchs published between 1999 and 2019. Elevated concentrations were observed for common pollutants such as Hg, Cd, DDT, and PCBs, although very little is known about emerging toxic pollutants such as PFAS, dioxin-like-compounds, and halogenated flame retardants. Even for commonly reported pollutants, the limited number of studies indicates that there is a huge gap in our knowledge on the health impacts of pollutant exposure in sharks, rays and skates. With their diverse and complicated life history, comparing elasmobranchs to other taxa such as marine

mammals and bony fish could mean we are not accurately assessing their health risks. Most of the studies that discussed the potential health risks in elasmobranchs found that there was little or no evidence to prove these risks, though the high concentrations found in this study suggest that their health could be greatly impacted. There was also a greater focus on the risk to humans and so there is a critical need to understand the effect of these contaminants in elasmobranchs. Global trends and long-term changes in pollutant concentrations (i.e. further evidence of bioaccumulation) could not be inferred as there was not much consistency between species and pollutants studied. We suggest the development of a database, such as the Global Biodiversity Information Facility (GBIF, 2020), where all data on pollutants in elasmobranchs can be collated to determine trends over time, between species, taxa, gender, age, size, geographic location, etc.

Future studies should aim to focus their research on areas that have received less attention (e.g. the South Pacific, Indian Ocean and Red Sea) in order to accurately identify the global threats to elasmobranchs. This is especially crucial as in combination with threats from pollutant exposure, overfishing, habitat loss, and climate change, there may be an accelerated loss of already vulnerable species. There is also a need for biomonitoring programs that aim at providing long term information on the bioaccumulation and exposure risks of pollutants in elasmobranchs, as well as the threats for humans that consume elasmobranch related products.

Supplementary Information

Supplementary information for this chapter can be found online at Science Direct (https://www.sciencedirect.com/science/article/pii/S0025326X20308195#s0140) and includes:

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

4.6. References

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Chapter 5

5. Trace elements in the muscle tissue of five species of sharks (bigeye thresher, blue shark, pelagic thresher, silky shark, and smooth hammerhead shark) commercially landed at artisanal fish markets in Ecuador

Chapter Overview

This chapter presents the results of an investigation to measure fourteen trace elements (Al, As, Cd, Co, Cr, Fe, Hg, Ni, Mn, Mo, P, Pb, S and Zn) in five commercially important species of sharks: bigeye thresher shark (*Alopias superciliosus*), blue shark (*Prionace glauca*), pelagic thresher shark (*A. pelagicus*), silky shark (*Carcharhinus falciformis*) and smooth hammerhead shark (*Sphyrna zygaena*). Thirty-six samples were collected from the five shark species landed at three artisanal fisheries in Ecuador. Human risk assessments were carried out for Arsenic (As), cadmium (Cd) and mercury (Hg) to assess the risk of consuming shark to children and adults.

Author Contributions

As lead author I designed the project, collected the samples in Ecuador, carried out the lab work, collected the data, performed the analysis, and wrote the manuscript. Other co-authors provided support and assistance with sample collection, data interpretation and revising the manuscript. David McKendry ran the samples on the

ICP-OES and assisted with data analysis. This chapter will be streamlined for submission for publication in Marine Pollution Bulletin in early 2024.

5.1. Abstract

This study looked at fourteen trace elements in the edible muscle tissue of five shark species landed at artisanal fish markets in Ecuador. Concentrations of Hg in shark muscle samples (*n* = 36) ranged from 0.19 mg kg^{-1} (w.w.) in smooth hammerhead (*Sphyrna zygaena*) sharks to 3.81 mg kg^{-1} (w.w.) in silky sharks (*Carcharhinus falciformis*), no significant differences in metal concentrations were observed between species. Hg concentrations greatly exceeded the safe regulatory limit of consumption set by the EPA, and therefore children and adults may experience negative health effects even after consuming as little as one portion of shark a week. As shark is not only consumed in Ecuador but globally, this poses a risk to local fishing communities and international consumers of shark meat. This is further exacerbated in the case of mislabelling and species substitution that occurs on a global scale.

Key Words: *Sharks; Marine Pollution; Trace Elements; Mercury (THg); Human Health Risk; Conservation*

5.2. Introduction

Pollution threatens the fate of marine ecosystems and the organisms which inhabit it, with long lasting and potentially irreversible toxic effects (Islam and Tanaka, 2004). Marine ecosystems are exposed to a range of pollutants including metals, persistent organic pollutants (POPs), crude oil and marine debris (e.g. marine litter, ghost nets or plastics) (Islam and Tanaka, 2004; Henry, 2015; Jambeck et al., 2015; Rochman et al., 2015; Tiktak et al., 2020). Heavy metals, namely arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) remain among the well-studied pollutants in the marine environment (Ansari et al., 2004; Islam and Tanaka, 2004), with many long-term biomonitoring studies focussing on these pollutants specifically (Alves et al., 2016; Bezerra et al., 2019; Tiktak et al., 2020).

Although many metals are naturally occurring, human activities have greatly increased their concentrations in the environment, including the more toxic heavy metals such as Pb, Cd, As and Hg. Heavy metals and trace elements enter the marine environment as by-products of human activities such as sewage sludge, domestic and municipal waste, agricultural and surface runoff (e.g. fertilisers and pesticides), mining, industrial processes (e.g. oil, paints, leather, paper and batteries), or naturally by volcanic activity and forest fires (Ansari et al., 2004; Islam and Tanaka, 2004; Singh et al., 2011). Even when found in low concentrations, heavy metals can have negative impacts on marine fauna and humans including immunosuppression, reproductive developmental effects, carcinogenic, mutagenic and teratogenic effects, and endocrine disruption (Das et al., 2002; Storelli et al., 2002; Zheng et al., 2007; Iavicoli et al., 2009; Jakimska et al., 2011; Singh et al., 2011). Heavy metals do not degrade over time and therefore bioaccumulate and biomagnify in living organisms, this is

especially concerning for animals that occupy top levels of the food chain that accumulate high concentrations of pollutants leading to potential potentially irreversible toxic effects (Das et al., 2002). Humans face risk from high concentrations of pollutants when consuming animals from the top of the food chain, i.e., pelagic fish (tuna, swordfish, marlin) and sharks (Davis et al., 2002; Olmedo et al., 2013; Araújo and Cedeño-Macias, 2016).

Exposure to pollutants such as methylmercury, have been linked to various types of cancer, liver and kidney damage, immunosuppression, reproductive defects, and endocrine disruption in humans (Vračko et al., 2007; Zheng et al., 2007; Kim et al., 2013; Knutsen et al., 2019). Exposure to pollutants puts vulnerable members of the population, for example pregnant women and young children, at greater risk of exposure to the health risks associated with these contaminants (Patandin et al., 1999; Bruce-Vanderpuije et al., 2019). Twenty seven percent (>1.5 billion people) of the world's population lives within 100 km of the coast (Fleming et al., 2008; Kummu et al., 2016). It's important to assess the heavy metal exposure in seafood as many coastal countries and communities depend on fishing as a source of income, and seafood also serves as a crucial nutritional source(FAO, 2010). This puts them at greater risk of the negative health effects associated with exposure to high concentrations of these pollutants (Johansen et al., 2004; Fleming et al., 2006; Zheng et al., 2007; Brunner et al., 2009; Sharma et al., 2014). Ensuring the safety and quality of seafood is not only an economic concern but also a public health imperative (FAO, 2010).

Sharks are often not considered a "primary" food source in many non-coastal regions, though shark derived products are consumed worldwide, and are especially

important for coastal communities that depend on fishing as their main source of food and income (Johansen et al., 2004; Fleming et al., 2006; FAO, 2010). Shark can be found in shark fin soup, traditional medicine (e.g. gill plates), dietary supplements (e.g. cartilage supplements and liver oil), beauty products (e.g. mascara and lipstick) as well as in pet food (Caballero et al., 2012; Kibria and Haroon, 2015; Hammerschlag et al., 2016; Steinke et al., 2017; Wainwright et al., 2018; Cardeñosa, 2019; Hellberg et al., 2019; Zhang et al., 2021; Cardeñosa et al., 2022; Prasetyo, Cusa, et al., 2023; Prasetyo, Murray, et al., 2023). Shark meat is also often consumed worldwide and has been found to be mislabelled either as bony fish or other elasmobranch species (e.g., threatened as non-threatened species) globally including Brazil, Indonesia, Singapore, United Kingdom, Italy and Greece (Blaber et al., 2009; Barbuto et al., 2010; Sembiring et al., 2015; Staffen et al., 2017; Cardeñosa, 2019; Hobbs et al., 2019; Barbosa et al., 2020; Tiktak et al., 2020; Filonzi et al., 2021; Villate-Moreno et al., 2021). As sharks have been found with high concentrations of heavy metals as well as a wide variety of POPs (Weijs et al., 2015; Alves et al., 2016; Bezerra et al., 2019; Cagnazzi et al., 2019; Tiktak et al., 2020), it puts consumers at increased risk of exposure to pollutants. Shark species that are most consumed include species such as blue sharks, thresher sharks (*Alopias* spp.), scalloped hammerheads (*S. lewini*), silky sharks (*C. falciformis*) and short fin mako sharks *(Isurus oxyrinchus*) (Bonfil, 1997; Clarke et al., 2006a; Martínez-Ortiz et al., 2015; García Barcia et al., 2023). These species have also been found with the highest concentrations of heavy metals, with Hg often exceeding the tolerable daily intake (TDI) limit for both adults and children (Tiktak et al., 2020; Goyanna et al., 2023). Recent studies have shown the potential

for these species not only to be used as a biomonitor of marine contamination, but also as a gauge of risk of overexposure to pollutants through consumption.

The most likely route of exposure to pollutants in marine organisms is through their diet (Das et al., 2002). Apex predators such as killer whales, sharks, and other pelagic fishes (e.g. tuna and billfish) are especially susceptible to high concentrations of pollutants due to their high trophic position. Pollutants have been well-studied in cetaceans and bony fish (Cockcroft et al., 1993; Dorneles et al., 2013; Bartalini et al., 2022; Goyanna et al., 2023), but less attention has been paid to sharks (Bezerra et al., 2019; Tiktak et al., 2020). Sharks are part of a group of cartilaginous fish, elasmobranchs, which include sharks, rays and skates. Elasmobranchs have been around for over 420 million years, representing one of the most ecologically diverse taxa on the planet, that are present in every ocean. They occupy top positions in the food chain and so play a crucial role in the top-down control of coastal and oceanic ecosystem structure and function (Ebert et al., 2013; Dulvy et al., 2014).

Despite their important role in marine ecosystems, elasmobranchs face growing pressure from overfishing and other anthropogenic threats, with over 1/3 of all elasmobranchs threatened with extinction (Dulvy et al., 2021; Pacoureau et al., 2021). Sharks share similar traits to that of large, bodied mammals in that they often mature late, reproduce slowly, and have limited offspring. Its these traits in combination with their high trophic position that puts them at a higher risk of exposure from pollutants (Tiktak et al., 2020), though little is understood about the effects pollutants have on sharks.

Controlled experiments and long-term biomonitoring studies on pollutants are difficult to establish for pelagic shark species as they cannot be kept in captivity,

are large, difficult to catch/high catch mortality rates and are highly mobile (i.e., travel over large distances). These types of studies also require a significant number of resources, expertise, time, and money. Though, as it's crucial to study pollutants in sharks, fisheries may provide an alternative method of monitoring pollutant concentrations in these difficult shark species (i.e., large pelagic sharks) such as blue sharks, thresher sharks, mako sharks and hammerheads, as samples are often available (Ardura et al., 2013; Boldrocchi et al., 2019; Tiktak et al., 2020).

The Eastern Tropical Pacific (ETP) remains an important research area for sharks as they are locally consumed as well as traded internationally. The ETP also serves as a hotspot for many shark species, which often overlaps with shark fisheries, and makes up one of the 27 global fishing areas (FAO, 2015). Ecuador is home to a diverse array of shark species. They have been traditionally sold and consumed in fish markets along the coast of Ecuador, as well as their fins being sold and traded internationally (Dominguez and Cobeña, 2019). In 2007, the Ecuadorian government (Ministerio de Producción, Comercio Exterior, Inversiones y Pesca (MPCEIP)) took steps to curb shark finning and directed shark fisheries under the program "Acción Tiburón" with hopes to reduce the illegal trade. However, they maintained the sale of incidentally whole caught sharks (fins and body), with the exception of the Galápagos Marine Reserve, where sharks are fully protected (Executive Decree 486 of 2007, cited in (Hearn et al., 2022)). Additional efforts to protect sharks included prohibiting the fishing of hammerhead (Sphyrnidae) and oceanic whitetip sharks (*Carcharhinus longimanus*) in 2020 (Ministerial Agreement MCEIP-SRP-2020-0084-A, cited in (Hearn et al., 2022)). Ecuador is one of the few countries that has a national action plan for sharks, which was proposed as a tool to strengthen and consolidate

the National Action for the Conservation and Management of Sharks in Ecuador (PAT), which has been carried out since 2006 (Gobierno del Ecuador, 2020; Rosero and Rosero, 2020).

Despite the Ecuadorian governments' efforts to reduce shark fishing, over one million shark landings were reported in Ecuador between 2008 and 2012. Shark species listed in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) namely pelagic thresher, bigeye thresher, silky shark and smooth hammerhead shark (*S. zygaena*) accounted for 3.84% of the total landings (combined total including teleost fish and elasmobranchs) in Ecuador between 2008 and 2012 (Martínez-Ortiz et al., 2015). Pelagic thresher sharks were the most frequently landed elasmobranch representing 4.8% of the total catch, followed by blue shark (1.8%) and silky sharks (0.9%). Bigeye thresher and smooth hammerhead sharks only accounted for 0.1% and 0.5% (respectively) of the total catch (15,515,357 counts in total) (Martínez-Ortiz et al., 2015). Research on pollutants in these species from the TEP and Pacific Ocean are limited, with studies mainly focussing on the Atlantic Ocean and Mediterranean Sea (Tiktak et al., 2020). Over the last three years however there has been an increase in the number of studies conducted in the ETP (Álvaro-Berlanga et al., 2021). For bigeye thresher sharks there have only been three studies to date (Maurice et al., 2021; Li et al., 2023) determining trace element concentrations in their tissue despite them being a globally important species for the international fin trade, as well for seafood consumption locally (e.g. in Ecuador) (Clarke et al., 2006b; Martínez-Ortiz et al., 2015).

The main aim of this study was to determine trace element and heavy metal concentrations in five commercially important shark species (bigeye thresher shark, blue shark, pelagic thresher shark, silky shark, and smooth hammerhead shark) landed at artisanal fish markets in Ecuador, and to evaluate the potential risks to human health and ecological impacts associated with the consumption of these species. Specifically this study will 1) quantify the concentrations of 14 trace elements (As, Cd, Co, Cr, Cu, Fe, Hg, Ni, Mn, Mo, P, Pb, S and Zn) in the muscle tissue from five commercially important shark species in Ecuador, 2) compare concentrations of trace elements presented in this study to the literature, 3) investigate potential factors contributing to accumulation of trace elements in shark species, such as diet, geographic location, habitat and trophic level, 4) evaluate the potential human health risks from As, Cd and Hg associated with consuming shark meat based on the regulatory limits as set by the Environment Agency (EA), Joint FAO/WHO Expert Committee on Food Additives (JECFA), and EPA (EPA) (EA, 2009; JECFA, 2021; EPA, 2022) respectively, 5) compare the concentrations of trace elements in different shark species and assess if certain species pose a higher risk to human health than others, and 6) provide recommendations on trace metal contamination in commercially important shark species in Ecuador, which can inform future monitoring and conservation management efforts.

5.3. Material and Methods

5.3.1. Chemicals

Nitric acid, PrimarPlus™ (~68%) was used for trace metal analysis, Fisher Chemical™ and, HCl (37%) was used for THg analysis (Fisher Scientific, UK). Ultrapure

water (18.2 mΩ deionised H20) was obtained from a Type 1 Ultrapure water system (Avidity Science, USA).

For analyses, all preparation of standards and samples, and clean-up, were done in a laminar flow cabinet with an HEPA and carbon filter on both the inlet and exhaust. Extractions for metals were carried out using MARS 6 Microwave Digestion System (CEM Corporation, UK).

5.3.2. Sample Collection

White muscle tissue samples from five species of sharks: bigeye thresher (*n* = 6), blue shark (*n* = 9), pelagic thresher (*n* = 9), silky shark (*n* = 6) and smooth hammerhead (*n* = 6) were opportunistically collected at three artisanal markets between June and July 2018 (Santa Rosa, Salinas: *n* = 12, Playita Mia, Manta: *n* = 13 and Esmeraldas: *n* = 12) [CITES Permit: No. 21EC000010]. The sampling sites included Mercado de Mariscos Santa Rosa of Salinas in the province of Santa Elena (coordinates: 2° 13' 0" South, 80° 58' 0" West), Playita Mía in Manta, province of Manabí (coordinates: 0° 57' 10" South, 80° 48' 45" West) and Puerto Pesquero Artesanal de Esmeraldas situated in the province of Esmeraldas (coordinates: 0.9682° North, 79.6517° West). A total of 36 tissue samples were collected. Muscle tissue was collected from the main body of the shark, between the head and dorsal fin. Two additional samples (*P. glauca*, *n = 1*; *Scyliorhinus canicula*, *n = 1*) were collected from a fish market in Manchester, UK for method development.

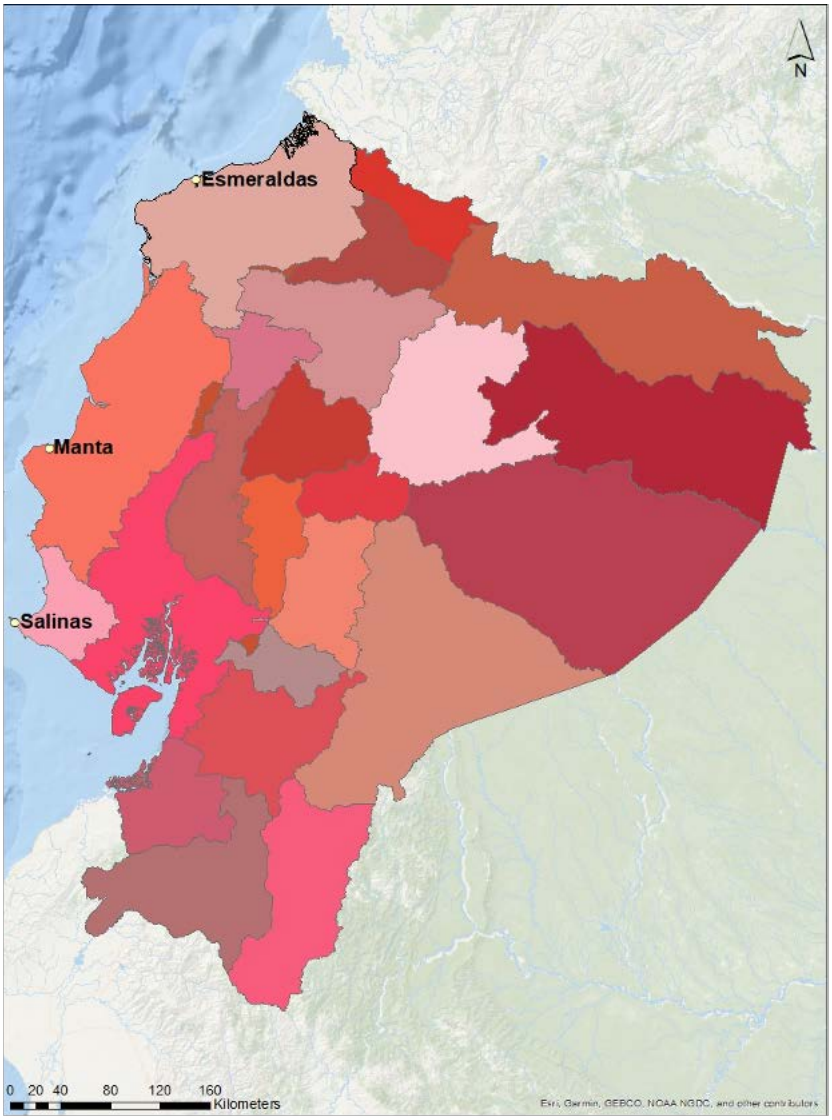

Figure 5-1 Map of the three study sites Salinas, Manta, and Esmeraldas in Ecuador.

Figure 5-2 The three study sites in Ecuador (a) Santa Rosa, Salinas (b) Playita Mia, Manta, and (c) Esmeraldas. In (a) species of blue sharks (*Prionace glauca*) and short fin mako sharks (*Isurus oxyrinchus*) can be seen, and in (b), blue sharks and silky sharks (*Carcharhinus falciformis*).

Sharks were identified using identification sheets and help from experts (fishery inspectors and analysts), study species can be found in Supplementary Information (Figure S 5-1). The identification guides included the main characteristics of the species, taxonomic classification from order to species (including fin identification), biological and ecological information such as diet, reproduction, behaviour and habitat, and aspects of fisheries, uses and marketing. Where possible, the sex of each shark was determined by the presence or absence of claspers. The

total length was measured from the tip of the shark's snout to the tip of the caudal fin, the fork length from the tip of the snout to the fork in the caudal fin (tail) where the upper and lower lobes of the caudal fin come together (Compagno, 1984; Zafeiraki et al., 2019). All measurements were rounded to the nearest centimetre (cm). Many sharks were landed without fins, including caudal fin, anal fins, pelvic fins, and claspers (for males only) and therefore it was not always possible to measure total body length, or determine gender.

Muscle tissue samples of approximately 20 g (wet weight; w.w.) were taken from the main body of each shark, wrapped in foil, and stored in glass amber SEPTA jars at -80 °C until further chemical analysis. All information on samples collected in this study can be found in SI 1. Aliquots of ~3 g (w.w.) were taken from the 20 g (w.w.) for trace metal analysis.

5.3.3. Sample Extraction

Total concentrations of 14 trace elements; arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), molybdenum (Mo), nickel (Ni), phosphorous (P), sulfur (S), zinc (Zn), and total mercury (THg) were analysed using ICP-OES. Approximately 3 g of (wet) tissue was freeze dried for 24 hours using a freeze-dryer (Buchi Lyovapor L-200 Freeze Dryer, UK). Approximately 0.5 g of freeze-dried tissue was accurately weighed out and placed in 55 mL MARSXpress vessels (CEM Corporation, UK). Nine millilitres of 68 % nitric acid (Fisher Scientific, UK) and 1 mL of 18.2 mΩ deionised H20 was added to the samples and placed in MARS 6 Microwave Digestion System using MARSXpress

vessels for microwave digest (CEM Corporation, UK) at 15-minute ramp to 175 °C, 30-minute hold at 175 °C (samples were cooled down for ~40 minutes).

5.3.4. Instrumental Analysis

After digestion, samples were made up to 100 mL with 18.2 mΩ deionised H20. ICP-OES was conducted with an iCAP 7000 ASX520 (Thermo Scientific, UK). A separate aliquot of 4.5 mL of each digested sample in nitric acid was mixed with 0.5 mL of 37% HCl (Fisher Scientific, UK) and analysed for THg using ICP-OES mercury hydride technique with an iCAP 7000 ASX520 (Thermo Scientific, UK). Multi Analyte Custom Grade Solution standards (Inorganic Ventures, USA) were used at a concentration of 0.02 to 20 ppm for calibration (Pearson's R > 0.998) of trace elements, specifically Mo, Cr, Mn, Co, Ni, Zn, Cd, As and Pb used a calibration of 0.02 to 4 ppm, Cu, P and S 0.05 to 10 ppm, and Fe 0.1 to 20 ppm (Table S 5 2). Mercury Standard for AAS (Fluka Analytical, Sigma, UK) was used for a standard calibration of THg ranging from 0.2 ppb to 20 ppb, and 10 to 80 ppb for calibration (Pearson's R > 0.999) of high range samples (Table S 5-3). Sample standards were made up in 10% HCl (Fisher Scientific, UK). All results are presented in mg kg^{-1} expressed in dry weight (d.w.) and wet weight (w.w.).

5.3.5. Data Quality

The limits of quantification (LOQ) were determined for each of the trace elements, and for standard and high range THg. Ten samples were repeated for a higher range for THg, for details of samples see Table S 5-3. All concentrations were above the detection limit (DL) of the instrument, and therefore all samples were included for upstream analysis. Procedural blanks were run after every 10 samples,

and these were used for blank corrections. Satisfactory linearity was observed of R^2 > 0.998 for all trace elements between 0.2 and 20 ppm, and 0.2 to 20 ppb for standard range Thg, and 10 to 80 ppb for high range THg ppm. Limits of Quantification (LOQ) varied by element and ranged from 0.000144 to 0.230 mg kg^{-1} in Cd and Cu respectively. Concentrations for Al were removed from the data set as samples were originally wrapped in foil as they were stored for use for not just trace elements but for a wider study on trace level POPs. A small aliquot was cut to remove any surfaces of tissue that were touching the Al, but for quality purposes these were removed from the data set. The wrapping of the samples should have little impact on sample concentrations as they were only touching, and effort was made to cut a small piece of tissue from the centre. Larger samples could not be taken due to the confines of sampling, transport and permits in Ecuador.

5.3.6. Data Analyses

Moisture content (%) in the sharks ranged from 62.3% to 81.6%, with a mean of 74.9 ± 3.75%. The moisture content (%) of each sample was used to calculate the w.w. of each concentration. All values are reported in mg kg^{-1} for d.w. and w.w.

The following equation was used to convert trace element concentrations to mg kg^{-1} and THg samples to µg kg^{-1}:

Concentration in tissue[a] (mg kg^{-1} or µg kg^{-1}) = ICP-OES Concentration * (1/mass of the sample in g) * Dilution Factor[b]

[a]Concentration in dry tissue

[b]A dilution factor of 100 was used for all trace elements, except for THg where dilution factor of 111 was used.

Threshold values that are defined by regulatory guidelines of health agencies are reported in w.w., and therefore the As, Cd and THg concentrations measured in d.w. in this study were transformed to w.w. based on the following formula:

(1) Dry content:

Dry content (%) = 100% - Moisture content (%)

(2) Wet weight:

Wet weight (%) = Dry weight (%) / Dry content (%) * 100

Shapiro-Wilk test and Bartlett Tests were carried out to evaluate the assumptions of parametric tests (normality and homogeneity of variance, respectively). Transformation of data were carried out for the data to fulfil these assumptions (logarithmic transformation for all metal concentrations (w.w.)) followed by parametric tests. Where parametric tests were not possible, non-parametric tests were used. Statistical tests were only carried out on the w.w. concentrations. Where data was normally distributed, a one-way ANOVA test followed by a Tukey post-hoc test was used to assess differences in metal concentrations between species. Where data was not normally distributed a Kruskal-Wallis rank-rum test and pairwise multiple comparison test was used to assess differences in metal concentrations between species (R version 3.6.1 (2019-07-05) (R Development Core Team, 2014)).

5.3.7. Human Health Risk Assessment

Hazard quotients (*HQ*) were calculated for the heavy metals considered toxic to human health, namely As, Cd, and THg. *HQ's* for Pb could not be calculated as all concentrations were below <LOD indicating no risk to humans from consumption. The *HQ* of the metals mentioned above were based on the recommended Provisional

Tolerable Daily, Weekly, or Monthly Intake (PTDI, PTWI or PTMI) as set by JECFA and the EPA respectively (JECFA, 2021; EPA, 2022).

The following equation as set by the EPA (EPA, 2022) was used to calculate hazard quotients (*HQ*) for consumption of shark meat risk to humans for As, Cd and THg:

(1) Amount consumed per week:

WR = C x F

(2) Exposure dose:

D = WR / bw

(3) Hazard Quotient (HQ)

***HQ* = D / RfD**

Where: **bw** = Body Weight (kg), **C** = Concentration (mg kg^{-1}), **D** = Exposure Dose (mg kg^{-1} per day), **F** = Average Filet (g), ***HQ*** = Hazard Quotient, **RfD** = Reference Dose (mg kg^{-1} per day) and **WR** = mean weekly consumption rate of the species of interest (mg kg^{-1} per week)

The RfD for chronic oral exposure to methylmercury is = 0.1 µg kg^{-1} bw per day. Therefore, 7 days (1 week) x 0.1 µg kg^{-1} bw per day = 0.7 µg kg^{-1} bw per week (taken from EPA's Integrated Risk Information System (IRIS) (EPA, 2022). We used the conservative estimate as set by the EPA, as the WHO recommends 1.6 µg kg^{-1} bw per week which is more than double the amount(FAO and WHO, 2013; EPA, 2022).

The RfD for Cd set by JECFA is 25 µg/kg bw per month (JECFA, 2021) which works out as 6.25 µg kg^{-1} bw per week.

The toxicological framework report by the EA (2009) set a RfD for arsenic as 0.003 µg kg^{-1} bw per day. This was used to calculate the PTWI = 7 days x 0.003 µg kg^{-1} bw per day = 0.02 µg kg^{-1} bw per week (EA, 2009).

5.4. Results and Discussion

5.4.1. General Overview

This study provides valuable insight on trace metal and mercury concentrations in some of the most frequently caught sharks in Ecuador, as well as globally (Clarke et al., 2006a; Martínez-Ortiz et al., 2015; Fields et al., 2017; Cardeñosa et al., 2018, 2021, 2022; Van Houtan et al., 2020). A total of 36 samples from five shark species (bigeye thresher shark, blue shark, pelagic thresher, silky and smooth hammerhead shark) were collected at three artisanal fish markets in Ecuador (Table 5 1).

Table 5-1 Number, total body length (mean ± SD), trophic level (mean ± SD)[d], gender, and age (juvenile, subadult or adult) of sharks sampled. Thirty-six sharks were sampled in total[a].

Common Name	**Species**	***n***	**IUCN Red List Status**[b]	**Trophic Level (TL)**[d]	**Total Length (cm)**	**Female**	**Male**	**Juvenile**	**Subadult**	**Adult**
Bigeye thresher shark	*Alopias superciliosus*	6	VU	4.50 ± 0.430	258 ± 20.0	3	3	0	0	6
Blue shark	*Prionace glauca*	9	NT	4.25 ± 0.250	189 ± 44.7	4	5	1	1	7
Pelagic thresher shark[c]	*Alopias pelagicus*	9	VU	4.52 ± 0.370	197 ± 461	8	0	4	0	2
Silky shark	*Carcharhinus falciformis*	6	NT	4.33 ± 0.200	188 ± 64.0	4	2	2	0	4
Smooth hammerhead shark	*Sphyrna zygaena*	6	VU	4.47 ± 0.290	101 ± 24.9	5	1	4	2	0

[a]Data for reference samples *Prionace glauca* and *Scyliorhinus canicula* (UK) were not included in this table.

[b]IUCN Red List Status from 2023

[c]Unknown gender: Pelagic thresher shark (*n* = 1)

[d]TL based on mean from several studies, see Table S 5-1 for data

5.4.2. Concentrations of Total Mercury (THg)

Mean concentrations of THg ranged from 0.19 mg kg^{-1} (w.w.) in smooth hammerhead sharks to 3.81 mg kg^{-1} (w.w.) in silky sharks (

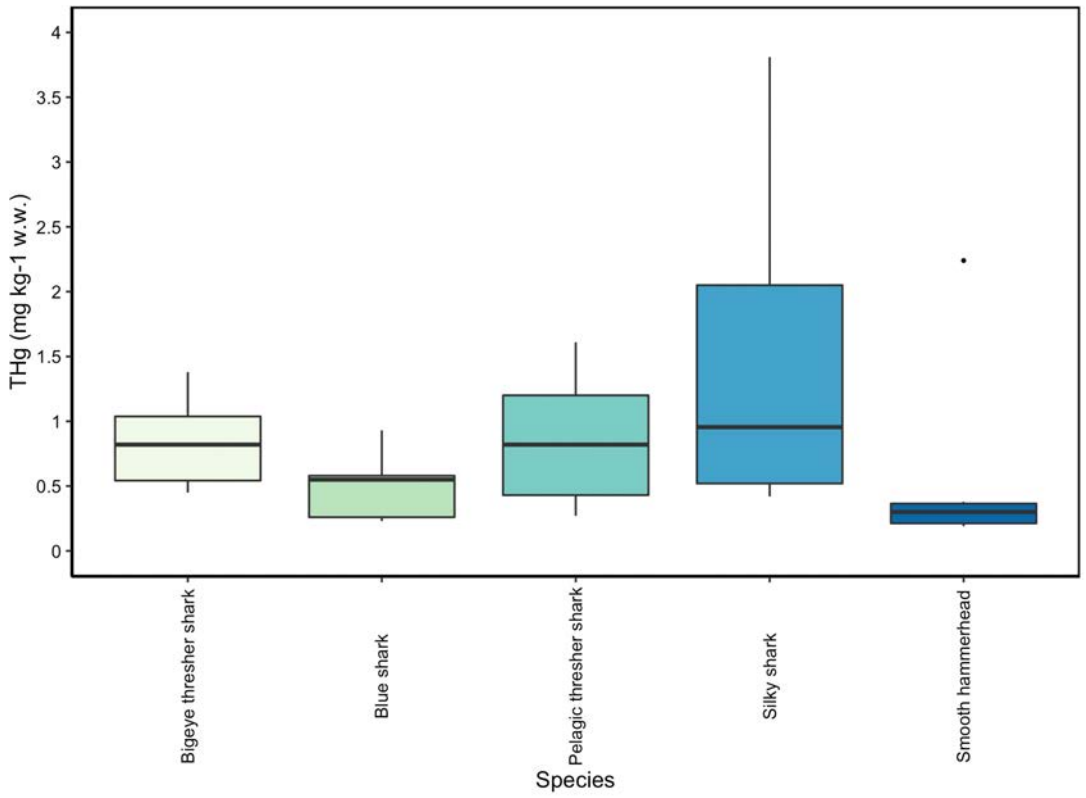

Figure 5-3). Existing data on pollutants in bigeye threshers is very limited. Only three studies have reported on THg concentrations in bigeye thresher sharks. Previous studies reported slightly higher mean concentrations of THg (4.12 and 6.04 mg kg^{-1} d.w. respectively) in bigeye thresher sharks from similar regions than what was measured in our study (3.74 mg kg^{-1} d.w.) (Maurice et al., 2021; Li et al., 2023), note that d.w. was referenced here because one of the studies only provided data in this format. Given the scarcity of studies reporting on pollutants in bigeye thresher sharks, it becomes challenging to infer whether these concentrations are comparable to other sharks, and to assess the effects they have. Bigeye thresher sharks are an important species commercially despite their CITES listing and Vulnerable status

(CITES, 2023; IUCN, 2023) This study highlights critical gaps in our understanding of mercury in bigeye thresher sharks and emphasises the need for ongoing monitoring.

Concentrations of THg were highest in silky sharks which are one of the most consumed and traded species internationally (Clarke et al., 2006b; Martínez-Ortiz et al., 2015; Fields et al., 2017). The concentrations of THg observed in the silky sharks presented in this study were comparable to those caught in the same area, namely Galápagos Marine Reserve and ETP (1.76 and 2.14 mg kg^{-1} w.w.) respectively (Maurice et al., 2021; Li, Hussey, et al., 2022). Our study provides valuable data to the limited body of research on sharks in the ETP, contributing to a greater understanding of these species in one of the world's key elasmobranch hotspots.

Concentrations in the pelagic thresher sharks reported in our study (0.834 ± 0.466 mg kg^{-1} w.w.) were consistent with those reported in previous studies on pelagic thresher sharks (0.36 to 4.93 mg kg^{-1} w.w.) from the Pacific Ocean, namely near Mexico and Colombia (Maurice et al., 2021; Li, Pethybridge, et al., 2022; Li et al., 2023). Pelagic thresher sharks from the ETP tend to have a higher trophic position (~5.0) than their Atlantic equivalents (Calle-Morán and Galván-Magaña, 2020), this may be because they feed on squid and bony fish that already have higher concentrations of THg in their tissue.

Our study reveals that THg concentrations observed in the smooth hammerhead sharks fall in the range previously reported in the same region area, where concentrations range between 0.196 to 8.25 mg kg^{-1} (w.w.) (Figure 5-3). Most smooth hammerheads in this study (*n* = 4) were juveniles, implying that concentrations could increase substantially as they reach adulthood, possibly exceeding previously reported concentrations. It is concerning that the majority were

also females (*n* = 5), as they have the potential to transfer pollutants to their future offspring once they reach sexual maturity, thus posing an increased threat to future populations. Both smooth and scalloped hammerheads are covered by the Ministerial Agreement No. 116 in Ecuador as established by the Ministerio de Agricultura, Ganadaría, Acuacultura y Pesca (MAGAP) in 2013 which states that a maximum catch per trip of five hammerheads (juveniles up to 150 cm TL) can be allowed as by catch. Concentrations of THg could therefore be much higher in adult smooth hammerheads due to bioaccumulation. Although now illegal to fish in Ecuador, smooth hammerheads still make up a large portion of catches globally, and at the time of collection were the fourth most caught shark species in Ecuador, making up 0.5% of total catches between 2008 and 2012 (Martínez-Ortíz et al., 2007; MAGAP, 2013; Martínez-Ortiz et al., 2015; Estupiñán-Montaño et al., 2017).

THg concentrations were not significantly different between species ($F = 2.516$, $df = 4$, $p = 0.0615$; Figure 5 3), which may be as they share similar trophic levels ranging between 4.25 to 5.2. These species are known to frequent similar pelagic or semi pelagic environments, where their habitats and foraging grounds overlap. Their dietary references also overlap as they consume similar prey. Despite being distinct species with varying ecological niches, their overlapping trophic positions and dietary preferences likely contribute to their similarity in THg concentrations.

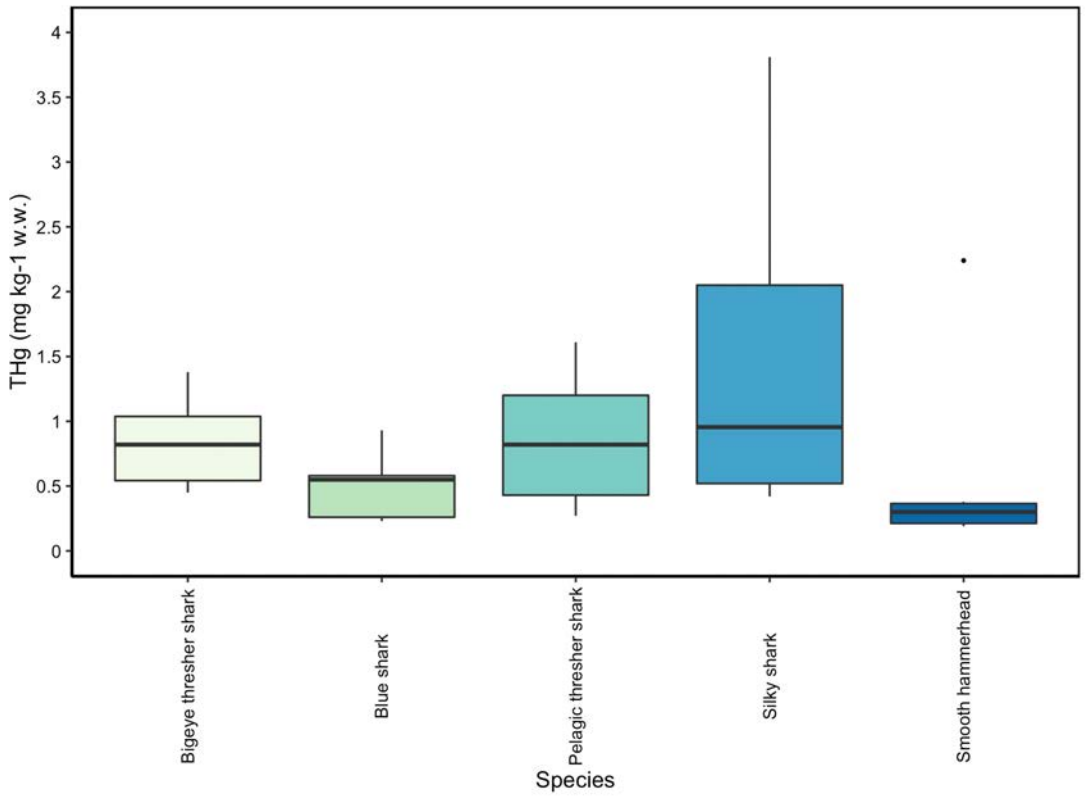

Figure 5-3 Total mercury concentrations of muscle tissue in five species of sharks landed at artisanal fisheries in Ecuador: bigeye thresher shark (*Alopias superciliosus*), blue shark (*Prionace glauca*), pelagic thresher shark (*A. pelagicus*), silky shark (*Carcharhinus falciformis*) and smooth hammerhead shark (*Sphyrna zygaena*). Concentrations are expressed in mg kg^{-1} on wet weight (w.w.) basis.

5.4.3. Human Health Risks

5.4.3.1. Hazard Quotients (HQs)

Hazard Quotients (HQs) were calculated based on regulatory maximum daily, weekly, or monthly intakes (where applicable) for heavy metals THg, Cd and As. Minimum and maximum consumption limits were calculated for the most vulnerable and most-at-risk individuals, pregnant females, and children. Weights for adults were based on the average weight of a pregnant woman of 75 kg and the children's weight on a two-year-old female of 13.4 kg and 11-year-old female of 47.5 kg (Tiktak et al., 2020; EPA, 2022). Serving of "shark" was based on the average serving of fish: 1 x a

week = 113 g and, 3 x a week = 339 g as suggested by the FDA (EPA, 2020, 2022). The average (minimum and maximum) serving of fish for adults from coastal communities were also included from six studies: minimum = 172 g and maximum serving = 1201 g (Svensson et al., 1995; Jiang et al., 2005; Hajeb et al., 2008; Rantakokko et al., 2008; Turunen et al., 2008; García-Hernández et al., 2018; Çamur et al., 2021).The mean concentration (mg kg^{-1} w.w.) for each species were used for calculating the exposure risks for each pollutant (more information can be found in SI 1). A *HQ* <1 indicated that the exposed population is unlikely to experience the adverse effects from consumption, whereas a *HQ* >1 meant there would be a high risk of adverse effects experienced from overexposure to the pollutant, with the likelihood increasing as the *HQ* increases (Krishna et al., 2014).

5.4.3.1.1. Total Mercury (THg)

The provisional tolerable weekly intake (PTWI) for humans was based on methylmercury (MeHg), as within the literature mercury is often reported as THg, and MeHg makes up 70% to 100% of THg in elasmobranchs (Storelli et al., 2003, 2022; Krystek and Ritsema, 2005; Pethybridge et al., 2010; de Carvalho et al., 2014; Rumbold et al., 2014; Alves et al., 2016; Torres et al., 2016; Mohammed and Mohammed, 2017; Mille et al., 2018; Tiktak et al., 2020). The *HQ* for each of the sharks was calculated using the most conservative regulatory limit as reported by the US EPA in 2011 (0.7 µg kg^{-1} of bw per $week^{-1}$) (U.S. Environmental Protection Agency, 2011; EFSA, 2012; EPA, 2022). Exposure risk of Hg was calculated for each species and is summarised in Table 5-2.

Table 5-2 Hazard quotients (*HQ*) were calculated for THg indicating the minimum (1 serving per week) and maximum (3 servings per week) risk adults (general and coastal population) and children (2-year-old's and 11-year-old's) would have from the consumption of shark meat.

Species	Adult (general, 75 kg)		Adult (coastal)		11-year-old child (47.5 kg)		2-year-old child (13.4 kg)	
	1 x serving (113 g)	3 x servings per week (339 g)	Minimum serving (172 g)[a]	Maximum serving (1201 g)[b]	1 x serving per week (113 g)	3 x servings per week (339 g)	1 x serving a week (28 g)	3 x serving a week (84 g)
Bigeye thresher shark	1.81	6.14	2.75	8.77	2.85	8.56	2.51	7.52
Blue shark	1.05	3.58	1.61	5.11	1.67	5	1.46	4.39
Pelagic thresher shark	1.79	6.07	2.72	8.67	2.82	8.46	2.48	7.43
Silky shark	3.21	10.9	4.88	15.57	5.06	15.19	4.45	13.3
Smooth hammerhead	1.29	4.39	1.97	6.27	2.04	6.12	1.79	5.37

HQ reference dose: US EPA, (2020) 0.7 µg kg^{-1} bw pw^{-1}

[a]The minimum serving of fish in coastal adults is based on one serving per day (g)

[b]The maximum serving of fish in coastal adults is based on the average amount of fish consumed in a week (g)

5.4.3.1.2. Arsenic

Currently there is no PTWI for arsenic as it occurs naturally at elevated concentrations in some food sources, and therefore establishing a PTWI may not be practical or necessary because it is difficult to further regulate or limit exposure in these cases. Although a reference dose may not be applicable to As from certain food sources because of existing background concentrations, guidelines and recommendations are still in place for the oral uptake of As. The health-orientated reference dose established in a toxicological framework report by the EA was 0.02 µg kg^{-1} bw per week (EA, 2009). This reference dose was established to ensure minimal excess lifetime cancer risk associated with exposure. This RfD was used to calculate *HQs* for As (Table 5-3).

The HQ results for As indicated that there was risk of overexposure to As from as little as one portion of shark a week for adults and children Table 5-3. Arsenic has been linked to causing various complications in different human organ systems, including the integumentary, nervous, respiratory, cardiovascular, hematopoietic, immune, endocrine, hepatic, renal, reproductive, and developmental issues (Mohammed Abdul et al., 2015). Some of the effects that are caused by arsenic exposure are diabetes, bone marrow depression, skin lesions, encephalopathy and peripheral neuropathy (Mohammed Abdul et al., 2015).

Table 5-3 Hazard quotients (*HQ*) were calculated for As indicating the minimum (1 serving per week) and maximum (3 servings per week) risk adults (general and coastal population) and children (2-year-old's and 11-year-old's) would have from the consumption of shark meat.

Species	Adult (general, 75 kg)		Adult (coastal)		11-year-old child (47.5 kg)		2-year-old child (13.4 kg)	
	1 x serving per week (113 g)	3 x servings per week (339 g)	Minimum serving (172 g)[a]	Maximum serving (1201 g)[b]	1 x serving per week (113 g)	3 x servings per week (339 g)	1 x serving per week (28 g)	3 x serving per week (84 g)
Bigeye thresher shark	197	592	300	2,100	312	935	274	821
Blue shark	555	1,670	845	5,900	877	2,630	770	2,310
Pelagic thresher shark	166	497	252	1,760	262	785	230	690
Silky shark	576	1,730	877	6,125	910	2,730	799	2,398
Smooth hammerhead	304	911	462	3,230	479	1,440	421	1,263

HQ RfD = 0.02 µg kg^{-1} bw pw^{-1}

[a]The minimum serving of fish in coastal adults is based on one serving per day (g)

[b]The maximum serving of fish in coastal adults is based on the average amount of fish consumed in a week (g)

5.4.3.1.3. Cadmium (Cd)

The provisional tolerable monthly intake (PTMI) for Cd as set by the WHO is 25 µg/kg bw per month(JECFA, 2021) which works out as 6.25 µg kg^{-1} bw per week.

HQs were calculated using the PTMI and mean Cd concentrations in the five shark species (Table 5-4).

The *HQ* results for Cd indicated that there was no risk of overexposure to Cd as *HQ's* were <1, even when consumed a maximum of three times a week (339 g) or in the case of adults living near the coast that may consume up to or over 1201 g a week.

Table 5-4 Hazard quotients (*HQ*) were calculated for Cd indicating the minimum (1 serving per week) and maximum (3 servings per week) risk adults (general and coastal population) and children (2-year-old's and 11-year-old's) would have from the consumption of shark meat (JECFA, 2021).

	Adult (general, 75 kg)		**Adult (coastal)**		**11-year-old child (47.5 kg)**		**2-year-old child (13.4 kg)**	
Species	**1 x serving per week (113 g)**	**3 x servings per week (339 g)**	**Minimum serving (172 g)[a]**	**Maximum serving (1201 g)[b]**	**1 x serving per week (113 g)**	**3 x servings per week (339 g)**	**1 x serving per week (28 g)**	**3 x serving per week (84 g)**
Bigeye thresher shark	0.0297	0.0890	0.0451	0.315	0.0468	0.145	<0.01	0.771
Blue shark	<0.01	0.014	<0.01	0.0487	<0.01	0.0217	<0.01	0.119
Pelagic thresher shark	0.0158	0.0473	0.0240	0.168	0.0249	0.0747	<0.01	0.410
Silky shark	0.0230	0.0689	0.0350	0.244	0.0363	0.109	<0.01	<0.01
Smooth hammerhead	0.0113	0.0338	0.0172	0.120	0.0178	0.0534	<0.01	<0

HQ RfD = 6.25 µg kg^{-1} bw pw^{-1}

[a]The minimum serving of fish in coastal adults is based on one serving per day (g)

[b]The maximum serving of fish in coastal adults is based on the average amount of fish consumed in a week (g)

A *HQ* value less than 0.01 were indicated as <0.01

The data from this study indicate that humans should avoid consuming shark meat from the ETP as all the sharks exceeded the maximum regulatory limit of Hg (Table 5-2) when consuming as little as one portion (28 g to 113 g) of shark a week for adults and children. Consuming over the maximum regulatory limit could expose the population to toxic health effects such cancer, liver and kidney damage, immunosuppression, reproductive defects, and endocrine disruption in humans (Vračko et al., 2007; Zheng et al., 2007; Kim et al., 2013; Knutsen et al., 2019). The mean concentration of Hg in this study was 0.82 mg kg^{-1} (w.w.), with the FDA reporting a concentration of 0.98 mg kg^{-1} in sharks which is comparable to the concentration of THg found in our study. Their advice to the public is also to "avoid" consumption of shark (EPA, 2022). As Ecuador has a large coastal community where fish may make up a large proportion of their diet (especially in coastal regions), they would be exposed to extremely high concentrations of Hg if shark is consumed as little as once a week. As the sharks from this study are not only consumed commonly within Ecuador, but also globally, it paints a very concerning picture. Humans are exposed to concentrations of Hg that pose a significant health risk when consuming shark.

Although the advice is to avoid consuming shark meat, consumers may be unaware that they are consuming it as shark is often mislabelled and sold under umbrella terms such as "white fish", "caçao", "pescado blanco", "toyo", "flake"

(Almerón-Souza et al., 2018; Hobbs et al., 2019; Barbosa et al., 2020; Rodrigues Filho et al., 2020; Cundy et al., 2023; Khalil et al., 2023; Sharrad et al., 2023). There is a growing need for seafood transparency throughout the whole supply chain as this would decrease the risk of mislabelling and thus the risk of overexposure to pollutants such as Hg and toxic POPs. Consumers should be given a choice of what seafood they want to consume, and this is only possible with effective labelling. WWF indicated that the total value of the global shark fin and meat trade was $4.1 billion USD between 2012 to 2019 (Niedermüller et al., 2021). Ecuador exported 21,176 tonnes of shark meat between 2012 to 2019. The most threatened shark species containing the highest concentrations of pollutants still make up the majority of the international trade (Clarke et al., 2006a; Cardeñosa et al., 2018, 2022; Tiktak et al., 2020).

5.4.4. Concentrations of Trace Elements

Thirteen trace elements (As, Cd, Co, Cr, Fe, Ni, Mn, Mo, P, Pb, S, and Zn) were analysed in the edible muscle tissue of five shark species (bigeye thresher, blue shark, pelagic thresher, silky shark, and smooth hammerhead shark) landed at artisanal fish markets in Ecuador (Table 5-5). Concentrations of Cd, Co, Fe, Ni, Mn, Mo, P, Pb, and Z did not significantly differ between the five shark species, whereas concentrations of As, Cr, Cu, and S did (Supplementary Information; Table S 5-4 & Table S 5-5). For statistical tests on trace elements see Table S 5-4.

Table 5-5 Mean concentrations of 13 trace elements in the muscle of five shark species from this present study. Values are expressed in mg kg^{-1} on a wet (w.w.) and dry (d.w.) weight basis. The numbers at the top represent mean values ± SD, and the values in parentheses represent the concentration ranges.

Metal	LOQ	Bigeye thresher shark (*n* = 6)		Blue shark (*n* = 9)		Pelagic thresher shark (*n* = 9)		Silky shark (*n* = 6)		Smooth hammerhead (*n* = 6)	
		w.w.	**d.w.**	**w.w.**	**d.w.**	**w.w.**	**d.w.**	**w.w.**	**d.w.**	**w.w.**	**d.w.**
As	0.006	2.62 ± 1.67 (1.28 – 5.69)	11.4 ± 6.55 (5.69 -22.3)	7.37 ± 3.29 (2.90 -12.5)	31.5 ± 14.6 (14.9 - 57.4)	2.20 ± 1.13 (1.02 –4.52)	8.64 ± 3.87 (4.26 - 15.5)	7.65 ± 5.28 (3.82 –18.1)	27.6 ± 19.2 (13.2 - 4.9)	4.03 ± 2.68 (3.82 –18.1)	14.4. ± 9.65 (8.89 - 33.8)
Cd	0.0001	0.123±0.160 (0.009-0.192)	0.554±0.695 (0.037-1.83)	0.019±0.083 (0.007-0.036)	0.084±0.043 (0.027-0.175)	0.065±0.054 (0.009-0.173)	0.279 ± 0.248 (0.025-0.793)	0.095±0.064 (0.070-0.192)	0.331±0.208 (0.025-0.664)	0.047±0.037 (0.011-0.112)	0.163±0.121 (0.043-0.374)
Co	0.0002	0.004±0.001 (0.003-0.005)		0.015±0.017 (0.041–0.058)		0.008±0.0127 (<LOD-0.039)		0.013±0.018 (<LOD-0.046)		0.003±0.0423 (<LOQ-0.012)	
Cr	0.0006	0.560±0.010 (0.045–0.069)	0.247±0.044 (0.18-0.299)	0.218 ± 0.237 (0.070–0.794)	0.967 ± 1.00 (0.262 - 3.28)	0.966±0.0329 (0.065-0.168)	0.397 ± 0.166 (0.222-0.731)	0.495 ± 0.875 (0.040 -2.25)	1.67 ± 3.02 (0.148 - 7.78)	0.149 ± 0.104 (0.052 - 0.349)	0.520 ± 0.33 2 (0.192 - 1.15)
Cu	0.320	<LOQ	0.715 ± 0.281 (0.430 - 1.22)	<LOQ	0.721 ± 0.862 (<LOQ - 2.89)	0.512 ± 0.553 (<LOQ – 1.93)	1.87 ± 1.91 (0.581 - 6.66)	<LOQ	0.795 ± 0.329 (0.392 - 1.14)	<LOQ	0.863 ± 0.266 (0.387 - 1.18)
Fe	0.016	8.98 ± 9.07 (1.70 – 26.2)	38.7 ± 36.1 (7.95 - 102)	25.2 ± 36.7 (2.6 -117)	124 ± 201 (10.2 - 637)	15.8 ± 21.7 (4.55 – 72.2)	67.4 ± 96.7 (15.6 - 315)	31.9 ± 43.2 (1.54 – 118)	112 ± 149 (5.68 - 409)	6.16 ± 4.86 (3.40 – 16.0)	22.0 ± 17.5 (11.2 - 57.5)

Ni	0.001	0.934 ± 1.97 (<LOQ – 4.12)	0.022 ± 0.045 (<LOQ - 0.088)	4.95 ± 6.30 (<LOQ – 18.4)	0.117 ± 0.159 (<LOQ-0.485)	3.20 ± 3.783 (<LOQ – 9.37)	0.073 ± 0.091 (<LOQ -0.229)	3.13 ± 10.3 (<LOQ – 21.2)	0.080 ± 0.697 (<LOQ - 0.402)	<LOQ	<LOQ
Mn	0.0002	0.011 ± 0.010 (0.038–0.254)	0.490 ± 0.393 (0.151–0.997)	0.702 ± 1.60 (0.064 – 4.97)	3.65 ± 8.78 (0.295 - 27.0)	0.175 ± 0.220 (0.053–0.746)	0.737 ± 0.969 (0.182 - 3.25)	0.677 ± 0.842 (0.119 – 2.12)	2.11 ± 2.32 (0.446 - 5.63)	0.482 ± 0.695 (0.139 – 1.90	1.64 ± 2.26 (0.520 - 6.23)
Mo	0.0008	0.013 ± 0.011 (0.002–0.033)	0.053 ± 0.045 (0.012–0.131)	0.702 ± 1.60 (0.064 – 4.97)	0.003 ± 0.032 (<LOQ-0.104)	0.007 ± 0.009 (0.065–0.168)	0.032 ± 0.041 (0.003- 0.130)	0.007 ± 0.004 (0.003–0.014)	0.025 ± 0.015 (0.073 0.050)	0.007 ± 0.007 (0.010–0.017)	0.027 ± 0.024 (0.004 -0.062)
P	0.009	1,750 ± 275 (1,310 – 2050)	7,660 ± 786 (6,770 - 8930)	1,820 ± 462 (982 – 2560)	7,810 ± 1,780 (3,910-9,210)	2,200 ± 516 (1,070-2,720)	8,710 ± 1,580 (4,910-9,850)	3,970 ± 4,320 (1,550-12,700)	12,500±10,500 (6,980-33,800)	3,740 ± 3,520 (2,030-10,900)	13,000 ± 11,300 (7,330-35,800)
Pb	0.007	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
S	0.115	1,760 ± 295 (1,370 – 2,130)	7750 ± 1,180 (6,100 - 9,480)	1,980 ± 446 (985 – 2,320)	8,530 ± 1,800 (3,920-9,880)	2,020 ± 351 (1,500–2,470)	8,900 ± 1,420 (6,250-10,600)	2,600 ± 474 (1,960 – 3,260)	9,170 ± 774 (8,070 -10,100)	2,770 ± 487 (2,160–3,430)	9,860 ± 1,700 (7,870 -12,300)
Zn	0.190	23.3 ± 14.8 (6.00 – 49.3)	21.6 ± 12.0 (6.29 - 42.9)	33.1 ± 16.5 (14.7 - 63.1)	26.9 ± 11.9 (15.2 - 47.0)	19.3 ± 15.4 (7.08 – 50.0)	22.5 ± 17.3 (7.90 - 50.6)	69.5 ± 65.0 (15.3 – 183)	70.5 ± 51.5 (12.1 - 155)	37.1 ± 21.6 (16.2 – 76.1)	34.4 ± 17.4 (12.7 - 59.5)

Note: values less than the LOQ were written as <LOQ. LOQ values varied per metal.

5.4.5. Implications for Sharks

Sharks are exposed to pollutants through various pathways in their marine environments, with the main route of exposure being through their diet. Sharks feed on a wide range of marine organisms, including teleost fish, cephalopods, elasmobranchs, crustaceans, and carcasses of large cetaceans (Cortés, 1999; Barnett et al., 2010; Kiszka et al., 2015). Their prey often contains elevated concentrations of pollutants through bioaccumulation as concentrations increase as they transfer up the food chain, becoming increasingly more concentrated in the tissue of top predators like sharks (Suk et al., 2009; Lara et al., 2020, 2022). The sharks represented in the present study are pelagic, oceanic shark species that have dynamic and diverse dietary preferences. They're opportunistic predators that feed on a variety of prey and have been found to actively seek regions of concentrated prey that are spatiotemporally variable (Bizzarro et al., 2017). There is significant dietary, and habitat overlap among the five shark species described in the present study, which may explain the similar concentrations of trace elements found in their tissue.

Sharks inherit the accumulated pollutants when they consume their prey, and over time these pollutants become more concentrated in their tissue. As a result of mercury's long persistence and high mobility in marine ecosystems, it has been shown to have an age-related accumulation and strong biomagnification in the food web (Islam and Tanaka, 2004). Bioaccumulation amplifies the pollutant concentrations up the marine food chain, posing health risk to the sharks themselves, and ultimately to humans who consume seafood at the top of the food chain (Kibria and Haroon, 2015).

Large pelagic shark species exhibit life traits like that of large-bodied mammals, characterized by their long lifespans, late maturation, and limited offspring production (Fisk

et al., 2002; McKinney et al., 2016; Matulik et al., 2017; Cagnazzi et al., 2019). This further allows for the bioaccumulation of pollutants, which puts shark at higher risk of experiencing adverse health effects from their prolonged exposure to pollutants. Sharks have also been shown to transfer pollutants to their offspring through maternal transfer. This poses a substantial health risk to their young, who now start their life with heightened concentrations of pollutants. This is especially concerning in species that have very few offspring such as smooth hammerhead, silky and thresher sharks, as shark pups may have a lower chance of survival (Parsons et al., 2020).

Trace elements such as As, Cd, Cr, Pb and Hg can interfere with the physiological process that generates reactive oxygen species (ROS). If cellular antioxidant defences fail to balance the production of ROS, it can lead to oxidative damage which includes lipid peroxidation, protein degradation, and DNA disruption. This oxidative stress can lead to cell death (Sies, 1997; Gastell and De Alejo, 2000; Sies et al., 2010; Barrera-García et al., 2012, 2013). The relationship between antioxidant defences and trace elements, specifically heavy metals, is well understood in fish that have been exposed to chemical pollutants (Van der Oost et al., 2003; Martínez-Álvarez et al., 2005). Lamniformes and other active swimming sharks appear to have higher antioxidant defences, protecting them from exercise-induced oxidative damage (Filho and Boveris, 1993; López-Cruz et al., 2010). High concentrations of these toxic metals in sharks may disrupt the production of ROS, leading to the health issues outlined above.

Other studies, for example in great white sharks (*Carcharodon carcharias*), have found that heavy metals (i.e. Hg and As) have little effect on their physiology. The sharks did not exhibit physiological responses (i.e. no change in enzymatic conditions and leukocyte counts) that would usually be expected in fish that have been exposed to high concentrations of heavy metals (Merly et al., 2019). This suggests that some species can withstand higher

concentrations of pollutants in their blood, tissue and vital organs (i.e., from bioaccumulation, or are able to biotransform and eliminate pollutants more effectively than other species (Corsolini et al., 2014; Cresson et al., 2016).

The health risks trace metals pose to sharks is still relatively unknown, and to date there is still no toxic threshold for any contaminant in sharks. While the literature has extensively documented the risks posed to cetaceans and fish, it's important to recognise that sharks possess distinct physiological traits. For example, they are cold blooded, the have evolved along a separate lineage to fish, they don't have a swim bladder, they have cartilaginous skeleton. Drawing comparisons among the three taxonomic groups is difficult and warrants caution. What affects cetaceans and fish may not necessarily apply to sharks. Consequently, there is a growing need for more comprehensive research, such as long-term biomonitoring studies, to assess the impact of pollutants on sharks. These studies are crucial for identifying the potential adverse health risks and enhance our understanding of how pollutants are processed, transported, and transformed within in sharks.

5.5. Conclusion and Future Recommendations

The concentrations of 14 trace elements were measured in the muscle tissue of bigeye thresher sharks, blue sharks, silky sharks, and smooth hammerhead sharks from Ecuador. Ecuador is an elasmobranch hotspot and the ETP is one of the FAO's 27 major fishing areas. Data on these species from the ETP is still relatively limited, especially for bigeye thresher sharks. At the time of this study, research on pollutants in sharks from the ETP was limited, however, over the last three years, there has been a noticeable increase in interest in the number of studies conducted. The five study shark species are large pelagic sharks that occupy tertiary positions in the food chain (Trophic Level > 4), making them more likely to accumulate high concentrations of pollutants, which was especially true for THg and As. The health risks

of heavy metals in sharks are still relatively unknown, and monitoring populations remains a conservation challenge. Nevertheless, further research is required to fully understand the health implications heavy metals as well as toxic persistent organic pollutants have on sharks.

Human health risk assessments indicated that meat consumption of all five shark species should be avoided as concentrations of THg may represent a significant health risk to the consumer. Consuming as little as one portion of shark meat poses a significant human health risk to both adults and children. This risk is further exacerbated for adults that consume fish daily, for example humans that live near the coast and where seafood might make up most of their diet (protein source).

5.6. References

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Supplementary Information

Figure S 5-1 Five study species of sharks (*n* = 36) landed at the three artisanal markets in Ecuador, where (a) bigeye thresher shark (*Alopias superciliosus*), (b) blue shark (*Prionace glauca*), (c) pelagic thresher shark (*A. pelagicus*), (d) silky shark (*Carcharhinus falciformis*) and (e) juvenile smooth hammerhead sharks (*Sphyrna zygaena*). Photo credit Guuske Tiktak.

Table S 5-1 Trophic level data for five sharks (bigeye thresher shark, blue shark, pelagic thresher shark, silky shark, and smooth hammerhead shark) taken from 15 studies.

Authors and Year	Bigeye thresher shark	Blue shark	Pelagic thresher shark	Silky shark	Smooth hammerhead shark
(Cortés, 1999)	4.2	4.1		4.2	4.2
(Post, 2002)			4.2		
(Hussey et al., 2010)			4.7		
(Kim et al., 2012)			4		
(Li et al., 2014)	4.53	4.17		4.07	
(Bornatowski et al., 2014)					4.2
(Hernandez-Aguilar et al., 2016)		4.05			
(Li et al., 2016)	5.2	4.7	4.7	4.6	4.7
(Bizzarro et al., 2017)	4.09			4.19	4.23
(Flores-Martínez et al., 2016)				4.2	
(Estupiñán-Montaño et al., 2017)				4.57	
(Estupiñán-Montaño et al., 2019)					4.7
(Froese and Pauly, 2019)	4.5	4.4	4.5	4.5	4.9
(Garcia Barcia et al., 2020)		4.08		4.29	4.33
(Calle-Morán and Galván-Magaña, 2020)			5		
Mean	**4.50**	**4.25**	**4.52**	**4.33**	**4.47**
SD	**0.43**	**0.25**	**0.37**	**0.20**	**0.29**

Table S 5-2 Calibration of trace elements using Multi Analyte Trade Solution Standards (Inorganic Ventures, USA) at a concentration of 0.02 to 20 ppm.

	Cal1	Cal2	Cal3	Cal4	Cal5
Mo202.030{467}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Cr267.716{126}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Mn257.610{131}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Fe240.488{140}(Axial)	0.100ppm	0.500ppm	1.000ppm	10.000ppm	20.000ppm
Co228.616{447}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Ni231.604{446}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Cu324.754{104}(Axial)	0.050ppm	0.250ppm	0.500ppm	5.000ppm	10.000ppm
Zn206.200{463}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Cd228.802{447}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Cd214.438{457}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Al396.152{85}(Axial)	0.100ppm	0.500ppm	1.000ppm	10.000ppm	20.000ppm
P178.284{489}(Axial)	0.050ppm	0.250ppm	0.500ppm	5.000ppm	10.000ppm
S182.034{485}(Axial)	0.050ppm	0.250ppm	0.500ppm	5.000ppm	10.000ppm
As189.042{478}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Pb220.353{453}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Zn213.856{458}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm
Zn202.548{466}(Axial)	0.020ppm	0.100ppm	0.200ppm	2.000ppm	4.000ppm

Calibration details

	Hg0.2	Hg0.5	Hg1	Hg2	Hg5
Hg 184.950 {482} (Axial)	0.200 ppb	0.500 ppb	1.000 ppb	2.000 ppb	5.000 ppb

	Hg10	Hg20	Hg0.2	Hg0.5	Hg1
Hg 184.950 {482} (Axial)	10.000 ppb	20.000 ppb	0.200 ppb	0.500 ppb	1.000 ppb

	Hg2	Hg5	Hg10	Hg20
Hg 184.950 {482} (Axial)	2.000 ppb	5.000 ppb	10.000 ppb	20.000 ppb

Figure S 5-2 Calibration of standard range mercury using Mercury Standard for AAS (Fluka Analytical, Sigma, UK) at a concentration of 0.2 to 20 ppb.

Calibration details

	Hg10	Hg20	Hg40	Hg60	Hg80
Hg 184.950 {482} (Axial)	10.000 ppb	20.000 ppb	40.000 ppb	60.000 ppb	80.000 ppb

Figure S 5-3 Calibration of high range mercury using Mercury Standard for AAs (Fluka Analytical, Sigma, UK) at a concentration of 10 to 80 ppb.

Table S 5-3 Samples rerun for higher calibration of THg.

Sample No.	Species	THg (mg kg^{-1} w.w.)
1	*Alopias pelagicus*	1.61
4	*Carcharhinus falciformis*	1.36
7	*Alopias superciliosus*	1.07
14	*Scyliorhinus canicula* (REFF UK)	1.08
18	*Alopias pelagicus*	1.31
22	*Alopias pelagicus*	1.20
30	*Sphyrna zygaena*	2.24
32	*Carcharhinus falciformis*	3.81
33	*Alopias superciliosus*	0.94
37	*Alopias superciliosus*	1.38

Reff UK = reference sample taken from the UK (mainly used for method development)

Table S 5-4 Statistical results on trace metal analyses performed to assess differences in concentrations between the five species of sharks (bigeye thresher shark, blue shark, pelagic thresher shark, silky shark and smooth hammerhead shark). One-way Anova's were carried out for data that was normally distributed (As, Cd, THg and Z after log transformation), and non-parametric test Kruskal-Wallis rank-sum were carried out for data that was not normally distributed. Post-hoc tests can be found in Table S 5-5.

Metals	Test	Output	Results
As	Anova	$F = 9.561$, $df = 4$, $p < 0.001$	***
Cd	Anova	$F = 1.989$, $df = 4$, $p = 0.121$	NS
Co	Kruskal Wallis	$X^2 = 7.6144$, $df = 4$, $p = 0.1068$	NS
Cr	Kruskal Wallis	$X^2 = 12.394$, $df = 4$, $p < 0.05$	*
Cu	Anova	$F = 3.94$, $df = 4$, $p < 0.05$	*
Hg	Anova	$F = 2.516$, $df = 4$, $p = 0.0615$	NS

Fe	Anova	F = 0.534, df = 4, p = 0.712	NS
Ni	Anova	F = 0.544, df = 4, p = 0.705	NS
Mn	Kruskal Wallis	X^2 = 39.097, df = 4, p = 0.3591	NS
Mo	Anova	F = 1.331, df = 4, p = 0.1938	NS
P	Anova	F = 1.319, df = 4, p = 0.294	NS
Pb	NA	NA	NA
S	Anova	F = 1.7797, df = 4, p <0.001	***
Zn	Anova	F = 2.516, df = 4, p = 0.0615	NS

Significant differences: * = 0.05, ** = 0.01, *** = 0.001 and **** = <0.001

NA = Not Applicable, for example if values were <0.001

NS = Not Significant (p > 0.05)

Table S 5-5 Post-hoc tests were carried out on trace metals if significant differences were found (Table S 5-4).

Species	**As**	**Cr**	**Cu**	**S**
Blue shark + bigeye thresher shark	<0.01	<0.05	NS	NS
Pelagic thresher + bigeye thresher shark	NS	NS	NS	NS
Silky shark + bigeye thresher shark	<0.01	NS	NS	<0.05
Smooth hammerhead + bigeye thresher shark	NS	NS	NS	<0.01
Pelagic thresher + blue shark	<0.001	1	<0.01	NS
Silky shark + blue shark	NS	1	NS	NS
Smooth hammerhead + blue shark	NS	1	NS	<0.01
Silky shark + pelagic thresher shark	<0.001	1	NS	NS
Smooth hammerhead + pelagic thresher shark	NS	1	NS	<0.05
Smooth hammerhead + silky shark	NS	1	NS	NS

NS = Not Signficant (p > 0.05)

Table S 5-6 Recommended weekly intake for different members of the population including children (2- and 11-year-olds), adult, adult (coastal) and fishermen.

Target Group	Sex	Age	Mean Weight (kg)	Minimum serving (g)	Maximum servings (g)
Children	Female	2	13.4	28	84
Children	Female	11	47.5	113	339
Adult	Female	>18	75	113	339
Adult (coastal)	Mixed	>16	-	172[a]	1201[b]
Fishermen	Male	>18	-	162[a]	1133[b]

For general population adults and children, the minimum serving is once a week and maximum serving is three times a week.

[a]Based on maximum serving of fish (g) from six studies (Svensson et al., 1995; Jiang et al., 2005; Hajeb et al., 2008; Turunen et al., 2008; García-Hernández et al., 2018; Çamur et al., 2021)

[b]Maximum serving of fish from six studies (g) /seven days = minimum serving (g), as fish is often consumed daily.

Table S 5-7 Raw data expressed in mg kg^{-1} (w.w.) for trace elements Cu, Zn, Mo, Cr, Mn, Fe, Co, Ni, Cd, Al, P, S, As and Pb.

Sample No.	Scientific Name	Cu	Zn	Mo	Cr	Mn	Fe	Co	Ni	Cd	Al	P	S	As	Pb
1	*Alopias pelagicus*	0.430	11.028	0.028	0.120	0.093	8.961	0.010	9.140	0.173	5.508	1070.045	2063.969	1.25098806	0.084
2	*Alopias superciliosus*	0.102	32.324	0.016	0.055	0.041	1.697	0.003	2.364	0.012	5.050	1686.522	1552.738	3.20082478	-0.079
3	*Alopias superciliosus*	0.136	5.957	0.014	0.045	0.038	3.659	0.003	-0.545	0.020	4.291	2010.493	2058.920	1.82996194	-0.072
4	*Carcharhinus falciformis*	0.186	183.086	0.007	0.069	0.189	22.166	0.013	3.261	0.084	15.594	1551.771	1960.149	5.43551259	-0.088
5	*Alopias pelagicus*	0.203	8.737	0.009	0.083	0.053	6.524	-0.001	-0.899	0.035	8.224	2538.483	1855.343	1.56660519	-0.089
6	*Prionace glauca*	0.589	43.078	0.019	0.320	4.965	116.997	0.058	18.488	0.022	88.535	1672.646	1600.344	2.89793951	0.052
7	*Alopias superciliosus*	0.185	32.545	0.006	0.051	0.064	4.258	0.005	0.937	0.172	18.258	1632.090	1373.810	1.28252112	-0.060
8	*Prionace glauca*	0.115	27.198	0.006	0.074	0.169	15.228	0.011	1.714	0.020	18.302	2151.777	2321.994	9.60132941	-0.080

9	*Carcharhinus falciformis*	0.336	27.439	0.005	0.070	0.119	7.068	0.003	1.032	0.064	231.080	2418.683	2147.555	5.15656886	-0.090
10	*Carcharhinus falciformis*	0.325	96.410	0.014	0.073	0.175	18.435	0.004	2.627	0.084	31.579	2093.631	2687.629	18.1026026	-0.114
11	*Alopias pelagicus*	0.199	15.493	0.003	0.085	0.190	22.576	0.012	4.757	0.038	16.641	2093.733	1716.910	1.9279881	-0.071
12	*Sphyrna zygaena*	0.131	33.328	0.004	0.349	1.896	3.400	0.012	-5.733	0.112	109.125	10880.418	2795.993	3.16338622	-0.023
13	*Prionace glauca*	0.060	21.460	0.006	0.244	0.064	3.623	0.004	4.730	0.010	24.957	2007.519	2138.034	12.5027066	-0.059
15	*Sphyrna zygaena*	0.313	37.667	0.017	0.123	0.241	16.018	0.001	1.148	0.055	44.848	2261.847	3433.534	9.4328658	-0.028
16	*Sphyrna zygaena*	0.258	18.292	0.014	0.086	0.150	4.700	0.002	3.043	0.022	20.343	2030.678	2532.582	2.6625945	-0.119
17	*Alopias pelagicus*	1.934	41.540	0.002	0.065	0.180	9.600	0.000	-0.292	0.037	205.683	2126.229	2363.069	1.92294031	-0.089
18	*Alopias pelagicus*	0.593	17.260	0.007	0.114	0.086	4.779	0.003	1.831	0.105	4.268	2361.985	1498.680	1.02278989	-0.081
19	*Prionace glauca*	0.096	14.657	0.005	0.074	0.128	3.607	0.005	0.735	0.017	11.951	2559.388	2317.503	9.6507893 6	-0.129

20	*Alopias pelagicus*	0.510	49.908	0.012	0.168	0.746	72.201	0.039	9.373	0.114	96.678	1844.736	2430.351	3.20124842	-0.062
21	*Prionace glauca*	0.198	49.180	0.006	0.794	0.308	25.995	0.021	12.226	0.016	116.113	1787.358	2200.892	6.61651727	-0.017
22	*Alopias pelagicus*	0.302	11.921	0.001	0.091	0.094	7.203	0.002	2.093	0.057	5.122	2358.989	1704.775	1.49205463	-0.097
25	*Prionace glauca*	0.104	15.049	0.003	0.105	0.096	2.599	0.009	2.784	0.023	2.958	1871.285	2302.549	5.08054197	-0.085
26	*Prionace glauca*	0.245	63.075	0.004	0.191	0.285	39.936	0.015	2.635	0.036	135.069	1319.457	1813.108	5.868006	-0.082
27	*Prionace glauca*	0.039	38.881	0.003	0.087	0.155	14.730	0.009	2.344	0.007	14.865	981.772	985.431	3.74326002	-0.070
28	*Alopias pelagicus*	0.246	7.075	0.003	0.078	0.080	5.618	0.001	1.624	0.023	16.928	2723.407	2091.170	2.93654597	-0.119
30	*Sphyrna zygaena*	0.282	76.147	0.005	0.137	0.175	4.459	0.002	0.186	0.059	32.773	2096.109	3229.871	3.72635905	-0.132
31	*Sphyrna zygaena*	0.253	16.210	0.001	0.052	0.139	3.686	0.000	2.483	0.011	19.909	2141.684	2443.935	2.76361164	-0.084
32	*Carcharhinus falciformis*	0.328	77.160	0.008	2.250	1.301	118.316	0.046	21.189	0.192	197.542	2747.206	2808.167	3.82439379	-0.090

33	*Alopias superciliosus*	0.160	31.949	0.002	0.049	0.190	11.395	0.004	-0.552	0.106	9.757	1306.047	1827.836	2.39919866	-0.093
34	*Carcharhinus falciformis*	0.118	15.305	0.005	0.040	0.159	1.543	-0.003	1.454	0.007	2.019	2241.129	2747.075	7.7504613	-0.132
35	*Prionace glauca*	0.074	25.543	-0.004	0.070	0.152	3.960	0.006	-1.074	0.020	7.911	2002.990	2129.131	10.3594839	-0.123
36	*Carcharhinus falciformis*	0.174	17.670	0.003	0.465	2.121	23.854	0.014	-10.812	0.141	48.027	12747.261	3259.297	5.60757331	-0.117
37	*Alopias superciliosus*	0.169	17.739	0.005	0.069	0.077	6.705	0.004	-0.725	0.421	9.155	2054.462	1636.605	1.31799677	-0.113
38	*Sphyrna zygaena*	0.360	40.849	0.003	0.149	0.292	4.667	0.004	-2.488	0.022	5.295	3043.705	2161.999	2.44209788	-0.067
39	*Alopias superciliosus*	0.346	49.345	0.033	0.067	0.254	26.192	0.005	4.124	0.009	15.162	1812.298	2126.261	5.69350152	-0.074
40	*Alopias pelagicus*	0.188	10.987	0.001	0.065	0.053	4.550	0.003	1.179	0.007	3.370	2718.467	2470.824	4.52374772	-0.111

Table S 5-8 Raw data expressed in mg kg^{-1} (w.w.) for trace elements Cu, Zn, Mo, Cr, Mn, Fe, Co, Ni, Cd, Al, P, S, As and Pb

Sample No.	Scientific Name	Cu	Zn	Mo	Cr	Mn	Fe	C0	Ni	Cd	Al	P	S	As	Pb
1	*Alopias pelagicus*	1.97	50.62	0.13	0.55	0.43	41.12	0.04672696	0.23	0.79	25.28	4911.03289	9472.71	5.74	0.39
2	*Alopias superciliosus*	0.43	19.44	0.07	0.26	0.19	7.95	0.01413088	0.07	0.06	23.65	7898.58426	7272.03	14.99	-0.37
3	*Alopias superciliosus*	0.49	6.29	0.06	0.18	0.15	14.63	0.01031527	-0.01	0.08	17.16	8038.04406	8231.66	7.32	-0.29
4	*Carcharhinus falciformis*	0.75	155.08	0.03	0.31	0.85	99.71	0.05946963	0.08	0.38	70.15	6980.35839	8817.37	24.45	-0.40
5	*Alopias pelagicus*	0.71	8.50	0.03	0.32	0.21	25.25	-0.0020987	-0.02	0.14	31.82	9822.18312	7178.90	6.06	-0.35
6	*Prionace glauca*	2.89	34.04	0.10	1.74	27.04	637.21	0.31616927	0.49	0.12	482.19	9109.81538	8716.03	15.78	0.29
7	*Alopias superciliosus*	0.74	24.33	0.02	0.23	0.28	18.89	0.02051721	0.02	0.76	81.02	7242.11101	6096.04	5.69	-0.27
8	*Prionace glauca*	0.44	18.73	0.03	0.31	0.72	64.80	0.04470575	0.04	0.08	77.88	9155.94587	9880.23	40.85	-0.34
9	*Carcharhinus falciformis*	1.14	28.23	0.02	0.26	0.45	26.55	0.01111368	0.02	0.24	868.08	9086.04169	8067.52	19.37	-0.34
10	*Carcharhinus falciformis*	1.05	96.92	0.05	0.26	0.63	66.08	0.01318343	0.05	0.30	113.19	7504.39756	9633.52	64.89	-0.41
11	*Alopias pelagicus*	0.84	12.93	0.02	0.40	0.89	105.39	0.05543506	0.11	0.18	77.68	9773.86368	8014.80	9.00	-0.33
12	*Sphyrna zygaena*	0.39	40.59	0.01	1.15	6.23	11.17	0.0382601	-0.09	0.37	358.59	35753.7325	9187.81	10.40	-0.07
13	*Prionace glauca*	0.25	18.91	0.03	1.12	0.30	16.62	0.01870707	0.11	0.05	114.50	9210.68811	9809.50	57.36	-0.27
15	*Sphyrna zygaena*	1.01	40.40	0.06	0.44	0.87	57.47	0.00390029	0.02	0.20	160.90	8114.65467	12318.23	33.84	-0.10
16	*Sphyrna zygaena*	0.84	16.09	0.05	0.31	0.54	16.96	0.00565075	0.06	0.08	73.43	7329.51596	9141.08	9.61	-0.43
17	*Alopias pelagicus*	6.66	41.08	0.01	0.25	0.69	36.68	-0.0018574	-0.01	0.14	785.93	8124.47007	9029.45	7.35	-0.34
18	*Alopias pelagicus*	2.23	14.77	0.03	0.48	0.36	19.92	0.01286622	0.04	0.44	17.79	9845.40841	6246.91	4.26	-0.34

Sample No.	Scientific Name	Cu	Zn	Mo	Cr	Mn	Fe	C0	Ni	Cd	Al	P	S	As	Pb
19	*Prionace glauca*	0.31	15.22	0.02	0.26	0.45	12.83	0.0169644	0.01	0.06	42.50	9102.12632	8241.90	34.32	-0.46
20	*Alopias pelagicus*	2.00	43.80	0.05	0.73	3.25	314.68	0.17189871	0.21	0.50	421.37	8040.14954	10592.51	13.95	-0.27
21	*Prionace glauca*	0.73	46.96	0.03	3.28	1.27	107.30	0.08573851	0.25	0.07	479.28	7377.57988	9084.50	27.31	-0.07
22	*Alopias pelagicus*	1.09	12.37	0.00	0.36	0.38	28.78	0.00638871	0.04	0.23	20.46	9423.60407	6810.17	5.96	-0.39
25	*Prionace glauca*	0.37	15.21	0.01	0.41	0.38	10.17	0.03629008	0.05	0.09	11.58	7325.24441	9013.45	19.89	-0.33
26	*Prionace glauca*	1.08	39.73	0.02	0.93	1.39	194.68	0.07285569	0.07	0.17	658.44	6432.07885	8838.53	28.61	-0.40
27	*Prionace glauca*	0.14	33.48	0.01	0.35	0.62	58.67	0.03509384	0.05	0.03	59.20	3910.22354	3924.80	14.91	-0.28
28	*Alopias pelagicus*	0.75	7.90	0.01	0.26	0.27	18.93	0.00177019	0.03	0.08	57.04	9176.88881	7046.48	9.90	-0.40
30	*Sphyrna zygaena*	0.91	59.53	0.02	0.49	0.63	15.99	0.00849804	0.00	0.21	117.54	7517.91068	11584.26	13.36	-0.48
31	*Sphyrna zygaena*	0.85	12.65	0.00	0.19	0.52	13.70	9.6005E-05	0.05	0.04	74.01	7961.33224	9084.90	10.27	-0.31
32	*Carcharhinus falciformis*	1.02	74.25	0.03	7.78	4.50	408.97	0.15937721	0.40	0.66	682.81	9495.82104	9706.54	13.22	-0.31
33	*Alopias superciliosus*	0.75	18.64	0.01	0.25	0.99	59.08	0.02121023	-0.02	0.55	50.59	6771.95631	9477.47	12.44	-0.48
34	*Carcharhinus falciformis*	0.39	12.09	0.02	0.15	0.58	5.68	-0.0098003	0.03	0.03	7.43	8251.41685	10114.21	28.54	-0.49
35	*Prionace glauca*	0.29	19.69	-0.02	0.30	0.66	17.16	0.02460751	-0.03	0.09	34.28	8678.78904	9225.35	44.89	-0.53
36	*Carcharhinus falciformis*	0.42	56.35	0.01	1.24	5.63	63.32	0.03755964	-0.10	0.38	127.49	33838.7661	8652.10	14.89	-0.31
37	*Alopias superciliosus*	0.66	17.94	0.02	0.30	0.34	29.15	0.0172757	-0.02	1.83	39.80	8930.37616	7114.03	5.73	-0.49
38	*Sphyrna zygaena*	1.18	37.04	0.01	0.54	1.06	17.00	0.01454391	-0.05	0.08	19.28	11083.782	7873.01	8.89	-0.24
39	*Alopias superciliosus*	1.22	42.92	0.13	0.26	1.00	102.61	0.02138756	0.09	0.04	59.40	7100.09813	8330.12	22.31	-0.29
40	*Alopias pelagicus*	0.58	10.83	0.00	0.22	0.18	15.59	0.01088154	0.02	0.03	11.54	9312.32821	8464.01	15.50	-0.38

Table S 5-9 Raw THg concentrations expressed in mg kg^{-1} on a dry (d.w.) and wet (w.w.) weight basis.

Sample No.	Scientific Name	Species	THg (d.w.)	THg (w.w.)
1	*Alopias pelagicus*	Pelagic thresher shark	7.392	1.61068962
2	*Alopias superciliosus*	Bigeye thresher shark	2.295	0.48999968
3	*Alopias superciliosus*	Bigeye thresher shark	2.812	0.70337462
4	*Carcharhinus falciformis*	Silky shark	6.113	1.35890339
5	*Alopias pelagicus*	Pelagic thresher shark	3.602	0.93080576
6	*Prionace glauca*	Blue shark	1.257	0.23071515
7	*Alopias superciliosus*	Bigeye thresher shark	4.732	1.06632563
8	*Prionace glauca*	Blue shark	1.127	0.26489449
9	*Carcharhinus falciformis*	Silky shark	2.076	0.55261511
10	*Carcharhinus falciformis*	Silky shark	1.837	0.51252477
11	*Alopias pelagicus*	Pelagic thresher shark	1.238	0.26516012
12	*Sphyrna zygaena*	Smooth hammerhead	1.24	0.37746773
13	*Prionace glauca*	Blue shark	2.679	0.58387526
14	*Scyliorhinus canicula* (REFF UK)	Small spotted catshark	4.35	1.08071548
15	*Sphyrna zygaena*	Smooth hammerhead	1.136	0.31676071

Sample No.	Scientific Name	Species	THg (d.w.)	THg (w.w.)
16	*Sphyrna zygaena*	Smooth hammerhead	0.688	0.19051947
17	*Alopias pelagicus*	Pelagic thresher shark	2.032	0.53185225
18	*Alopias pelagicus*	Pelagic thresher shark	5.464	1.31073457
19	*Prionace glauca*	Blue shark	0.911	0.25616354
20	*Alopias pelagicus*	Pelagic thresher shark	3.586	0.82282308
21	*Prionace glauca*	Blue shark	3.826	0.92694859
22	*Alopias pelagicus*	Pelagic thresher shark	4.813	1.20472092
25	*Prionace glauca*	Blue shark	0.915	0.23380716
26	*Prionace glauca*	Blue shark	2.723	0.55850532
27	*Prionace glauca*	Blue shark	2.209	0.55451912
28	*Alopias pelagicus*	Pelagic thresher shark	1.366	0.40534664
29	*Prionace glauca* (REFF UK)	Blue shark	2.647	0.49192326
30	*Sphyrna zygaena*	Smooth hammerhead	8.037	2.24093711
31	*Sphyrna zygaena*	Smooth hammerhead	1.046	0.28145317
32	*Carcharhinus falciformis*	Silky shark	13.155	3.80591543
33	*Alopias superciliosus*	Bigeye thresher shark	4.866	0.93846287
34	*Carcharhinus falciformis*	Silky shark	1.564	0.42490749

Sample No.	Scientific Name	Species	THg (d.w.)	THg (w.w.)
35	*Prionace glauca*	Blue shark	3.658	0.84428238
36	*Carcharhinus falciformis*	Silky shark	6.057	2.28158963
37	*Alopias superciliosus*	Bigeye thresher shark	6.002	1.38078036
38	*Sphyrna zygaena*	Smooth hammerhead	0.694	0.19045271
39	*Alopias superciliosus*	Bigeye thresher shark	1.744	0.4451161
40	*Alopias pelagicus*	Pelagic thresher shark	1.466	0.42795364

Table S 5-10 Biological data on the five shark species, including scientific name, species, family, order, sex, total length (cm), fork length (cmn), width (cm) and age, where available. Some data was not recorded as sharks were landed without heads or fins.

Sample No.	Scientific Name	Species	Family	Order	Sex	Total Length (cm)	Fork Length (cm)	Width (cm)	Age
1	*Alopias pelagicus*	Pelagic thresher shark	Alopiidae	Lamniformes	Female				
2	*Alopias superciliosus*	Bigeye thresher shark	Alopiidae	Lamniformes	Female	265.00	165.00	92.00	Adult
3	*Alopias superciliosus*	Bigeye thresher shark	Alopiidae	Lamniformes	Male	235.00	150.00	86.00	Adult
4	*Carcharhinus falciformis*	Silky shark	Carcharhinidae	Carcharhiniformes	Female	190.00	159.00		Adult
5	*Alopias pelagicus*	Pelagic thresher shark	Alopiidae	Lamniformes	Female	150.00			Juvenile
6	*Prionace glauca*	Blue shark	Carcharhinidae	Carcharhiniformes	Male				Adult
7	*Alopias superciliosus*	Bigeye thresher shark	Alopiidae	Lamniformes	Female				Adult

Sample No.	Scientific Name	Species	Family	Order	Sex	Total Length (cm)	Fork Length (cm)	Width (cm)	Age
8	*Prionace glauca*	Blue shark	Carcharhinidae	Carcharhiniformes	Female	218.00	164.00		Adult
9	*Carcharhinus falciformis*	Silky shark	Carcharhinidae	Carcharhiniformes	Male				Juvenile
10	*Carcharhinus falciformis*	Silky shark	Carcharhinidae	Carcharhiniformes	Female	155.00	125.00		Adult
11	*Alopias pelagicus*	Pelagic thresher shark	Alopiidae	Lamniformes	Female	249.00	234.00		Adult
12	*Sphyrna zygaena*	Smooth hammerhead	Sphyrnidae	Carcharhiniformes	Female				Subadult
13	*Prionace glauca*	Blue shark	Carcharhinidae	Carcharhiniformes	Male				Subadult
14	*Scyliorhinus canicula* (REFF UK)	Small spotted catshark	Scyliorhinidae	Carcharhiniformes	NA	NA	NA	NA	NA
15	*Sphyrna zygaena*	Smooth hammerhead	Sphyrnidae	Carcharhiniformes	Female	138.00	106.00		Juvenile

Sample No.	Scientific Name	Species	Family	Order	Sex	Total Length (cm)	Fork Length (cm)	Width (cm)	Age
16	*Sphyrna zygaena*	Smooth hammerhead	Sphyrnidae	Carcharhiniformes	Male	87.00	67.00	30.00	Juvenile
17	*Alopias pelagicus*	Pelagic thresher shark	Alopiidae	Lamniformes	Female	168.00	98.00		Juvenile
18	*Alopias pelagicus*	Pelagic thresher shark	Alopiidae	Lamniformes	Female		123.00		Juvenile
19	*Prionace glauca*	Blue shark	Carcharhinidae	Carcharhiniformes	Female	200.00	161.00		Adult
20	*Alopias pelagicus*	Pelagic thresher shark	Alopiidae	Lamniformes					
21	*Prionace glauca*	Blue shark	Carcharhinidae	Carcharhiniformes	Male	209.00	172.00		Adult
22	*Alopias pelagicus*	Pelagic thresher shark	Alopiidae	Lamniformes	Female		116.00		Juvenile
25	*Prionace glauca*	Blue shark	Carcharhinidae	Carcharhiniformes	Female	100.00	95.00		Juvenile

Sample No.	Scientific Name	Species	Family	Order	Sex	Total Length (cm)	Fork Length (cm)	Width (cm)	Age
26	*Prionace glauca*	Blue shark	Carcharhinidae	Carcharhiniformes	Male	215.00	167.00		Adult
27	*Prionace glauca*	Blue shark	Carcharhinidae	Carcharhiniformes	Male				Adult
28	*Alopias pelagicus*	Pelagic thresher shark	Alopiidae	Lamniformes	Female				
29	*Prionace glauca* (REFF UK)	Blue shark	Carcharhinidae	Carcharhiniformes	NA	NA	NA	NA	NA
30	*Sphyrna zygaena*	Smooth hammerhead	Sphyrnidae	Carcharhiniformes	Female				Subadult
31	*Sphyrna zygaena*	Smooth hammerhead	Sphyrnidae	Carcharhiniformes	Female	89.00	68.00		Juvenile
32	*Carcharhinus falciformis*	Silky shark	Carcharhinidae	Carcharhiniformes	Male	280.00	155.00		Adult
33	*Alopias superciliosus*	Bigeye thresher shark	Alopiidae	Lamniformes	Male				Adult

Sample No.	Scientific Name	Species	Family	Order	Sex	Total Length (cm)	Fork Length (cm)	Width (cm)	Age
34	*Carcharhinus falciformis*	Silky shark	Carcharhinidae	Carcharhiniformes	Female	108.00	88.00	52.00	Juvenile
35	*Prionace glauca*	Blue shark	Carcharhinidae	Carcharhiniformes	Female	194.00	161.00		Adult
36	*Carcharhinus falciformis*	Silky shark	Carcharhinidae	Carcharhiniformes	Female	208.00	177.00		Adult
37	*Alopias superciliosus*	Bigeye thresher shark	Alopiidae	Lamniformes	Female				Adult
38	*Sphyrna zygaena*	Smooth hammerhead	Sphyrnidae	Carcharhiniformes	Female	89.00	68.00		Juvenile
39	*Alopias superciliosus*	Bigeye thresher shark	Alopiidae	Carcharhiniformes	Male	273.00	160.00		Adult
40	*Alopias pelagicus*	Pelagic thresher shark	Alopiidae	Carcharhiniformes	Female	222.00	125.00	80.00	Adult

Reff UK = reference samples for method development.

Chapter 6

6. Conclusion and Future Work

6.1. Conclusions

The main aim of this thesis was to use genetic and chemical techniques to aid elasmobranch (sharks, rays, and skates) conservation. In **Chapter 1** we set out the following specific aims, which have been addressed in the following format;

- The first aim of this thesis was to explore methods for identifying elasmobranchs and elasmobranch-related-products in the international trade and provide a comprehensive and up-to-date synthesis of the existing evidence on the mislabelling of elasmobranchs across the world **(Chapter 2).**

We successfully conducted a systematic review identifying elasmobranchs and their related products within the international trade, and the extent of mislabelling of elasmobranchs globally. Our review comprised of 85 relevant studies, where 38 reported instances of mislabelling. The main findings revealed that 35% elasmobranchs caught were classified as threatened by the IUCN Red List and 9.48% were listed in either CITES Appendix I, II or III at the time of study. Around 44.7% of studies focussed on mislabelling, revealing that 11.3% of samples were mislabelled and 10.1% were labelled using umbrella terms such as “Cação” and “Flake”. Blue sharks (*Prionace glauca*) were the most frequently mislabelled species, and they were also the most common species sold under umbrella terms. Species listed as threatened made up 48.7% of mislabelled elasmobranchs and 53.7% of species

labelled using umbrella terms. Thirteen percent of mislabelled and 9.26% of umbrella labelled elasmobranch species originated from CITES-listed species. Mislabelling was widespread, with a significant number of studies conducted in Europe (*n* = 14) and South America (*n* = 14). Conversely, very few studies were conducted in Africa and in major elasmobranch trading hubs like China, Indonesia, Singapore, Vietnam etc.

This review sheds a light on the prevalence of mislabelling in elasmobranchs, highlighting the potential threats to biodiversity and conservation efforts. Mislabelling misinforms consumers who might unknowingly purchase and consume threatened species or products with high concentrations of pollutants under false pretences, reducing their ability to make informed decisions and potentially supporting unstainable practices. Mislabelling also undermines conservation efforts by concealing true numbers caught and sold, impeding accurate assessments of population declines and conservation priorities. This understanding can influence policy changes, trade regulations, and raise public awareness to mitigate the risks posed by mislabelling, ultimately aiding in the safeguarding and sustainable management of elasmobranch populations. The issue of mislabelling in the global context has mainly been conducted as “snapshot research”, but rather than just focussing on a single market or a few cities, mislabelling requires a global approach. Currently it’s academics, NGOs and government bodies acting in isolation doing snapshot research at specific points of time or specific locations which only gives us a glimpse of the issue, but not the whole picture. This issue requires a more unified approach to enable more effective efforts to improve shark conservation.

We are aiming to publish this chapter, **Chapter 2**, in Biological Conservation in 2025, with the aim to aim to raise awareness of the issue of mislabelling within the elasmobranch trade, as well as putting pressure on regulatory bodies to address this issue and regulate the trade

of elasmobranchs and elasmobranch-related products more effectively. We also hope that by publishing the review it will encourage further studies to be carried out in key understudied areas or markets, providing a more comprehensive global assessment of mislabelling in elasmobranchs.

- The second aim of this thesis was to develop a rapid, on-site identification tool in the form of a Lab-on-a-Chip (LOC) for genetic analysis of three CITES-listed sharks: bigeye thresher (*Alopias superciliosus*), pelagic thresher (*A. pelagicus*) and short fin mako shark (*I. oxyrinchus*) belonging to the order Lamniformes **(Chapter 3).**

We developed a paper-based lab-on-a-chip (LOC) that successfully identified the three CITES-listed sharks bigeye and pelagic thresher, and shortfin mako shark for intended use at market source. The LOC combined DNA amplification and visualisation using LAMP to give a simple "yes" or "no" answer with a colour change from pink to yellow if the target species of shark was present. We successfully incorporated a positive and negative control onto the chip to validate the accuracy of the method by confirming correct species identification and ruling out potential errors or contamination. The LOC serves as proof of concept (PoC) for field-based species identification, eliminating the need for expensive laboratory equipment and specialist facilities. The LOC's design allows for global application intended for use by non-scientifically trained personnel and in regions where there is limited access to specialist facilities and are prone to mislabelling and where elasmobranchs are traded the most. We carried out a workshop in Manta, Ecuador where ministerial officials were given a demonstration on the use of the LOC. Participants knowledge of LOC technology increased and all attendees responded positively that the LOC would be beneficial for the use of identifying sharks, as well as other CITES-listed species such as mobula rays. Although our LOC is a PoC, it has the

potential to enhance the monitoring and regulation of the trade in shark and shark-related-products, aiding in the conservation of elasmobranchs. It's essential that we involve and empower local stakeholders in conservation as it significantly enhances our capacity to challenge greater conservation issues and ultimately leads to the safeguarding of our ocean's biodiversity.

The work carried out in this chapter was conducted with support from two funded grants totalling ~£50,000 from the Save Our Seas Foundation (SOSF) and National Geographic Society (NGS) that I prepared with support from my supervisor (K. Shaw). I carried out the preparation and delivery of the workshop in Ecuador in 2022. I conducted the workshop in Spanish, a language I learnt during my PhD studies to be able to engage with important stakeholders in Ecuador thereby increasing the impact of my research within local communities. Additionally, I presented the methods of the LOC with faculty members and biology students at Universidad de San Francisco de Quito. I presented this research at microTAS in October 2021 and Sharks International in October 2022. I was selected to present a poster on the LOC at the House of Commons for STEM Britain in March 2022. This chapter was published in PLOS ONE in April 2024.

- The third aim of this thesis was to assess and evaluate the concentrations of pollutants in elasmobranchs across the world by systematically gathering and analysing the available scientific literature **(Chapter 4).**

This review included a total of 176 relevant studies on pollutants in elasmobranchs. Most studies (63%) focussed on trace elements, with fewer articles on POPs, (micro)plastics, and other pollutants. Despite the relatively good global coverage of studies, most were carried out in the North Atlantic Ocean, North Pacific Ocean, and Mediterranean Sea with areas such as

the South Pacific (including Eastern Tropical Pacific), South Atlantic and Indian Ocean (including the Red Sea and Persian Gulf) receiving proportionately less attention despite being important global hotspots for elasmobranchs. Underrepresenting these areas might hinder our understanding of pollutant exposure in elasmobranchs, particularly concerning due to the overlap of large-scale commercial fisheries with these critical zones, potentially posing a threat to human health through the consumption of seafood originating from these areas.

The highest concentrations of pollutants were found in sharks occupying top trophic levels (Carcharhiniformes and Lamniformes), many of which are threatened and/or CITES-listed. A human health risk assessment revealed consuming shark as little as once a week would put children and adults over the maximum mercury (Hg) intake levels as recommended by the US EPA. This presents a threat to local fishing communities and global consumers of shark-derived products, alongside those subject to the widespread mislabelling of elasmobranchs. This review identified major gaps in the literature which have already started to be addressed by other authors. Broader screening studies are required to determine the risk of emerging pollutants to elasmobranchs, while more robust studies are recommended to evaluate the potential health risks for humans.

This review was published in Marine Pollution Bulletin in 2020, and now has over 80 citations. The impact of this review is complex as it highlights the significant risks to both elasmobranchs and human consumers of elasmobranchs and their related products. Unveiling the potential health risks associated with the consumption of shark meat, it stresses the need for stricter monitoring and regulation of elasmobranch products within the global trade.

- The final aim of this thesis was to determine trace element and heavy metal concentrations in five commercially important shark species, bigeye thresher (*A. superciliosus*), pelagic thresher (*A. pelagicus*), silky shark (*Carcharhinus falciformis*), blue

shark (*Prionace glauca*) and smooth hammerhead shark (*Sphyrna zygaena*) landed at artisanal fish markets in Ecuador, and to evaluate the potential risks to human health and ecological impacts associated with the consumption of these species **(Chapter 5).**

The concentration of fourteen trace elements were measured in the muscle tissue from five commercially important shark species bigeye and pelagic thresher, silky shark, blue shark, and smooth hammerhead shark landed at three artisanal markets in Ecuador. Ecuador is one of FAO's major fishing areas and is an important elasmobranch hotspot. Mercury concentrations greatly exceeded the safe consumption limits established by the US EPA, suggesting potential adverse health effects for children and adults even after consuming as little as one portion of shark a week. As shark is consumed all over the world, it poses a risk not only to local fishing communities but also to international consumers of shark meat. This risk is amplified, particularly in the case of mislabelling that occurs on a global scale. Our knowledge of elasmobranchs from the Eastern Tropical Pacific (ETP) is still limited, and therefore additional research is necessary to fully understand the health effects of heavy metals as well as toxic POPs on elasmobranch populations. We aim to send this chapter off for publication in Marine Pollution Bulletin (where **Chapter 4** was published) in early 2024.

Understanding the effects pollutants have on elasmobranch populations helps us identifying further threats to their health and survival. This research can help guide conservation strategies, facilitating the advancement and implementation of focussed measures to alleviate the risks associated with pollutant exposure and protect elasmobranch populations. It may also promote the need for stricter regulations and policies aimed at reducing pollution levels, ultimately protecting these important marine species and their ecosystems.

6.2. Future work

The work in this thesis has contributed to the field of elasmobranch conservation, specifically targeting threats from overexploitation and pollution. As the LOC was a PoC, taking the application to the next level will require additional work. The initial steps would be to develop the method further by simplifying the method and reducing the production costs by bulk producing it. Once further tests have been carried out, we would trial the LOC in Ecuador with our existing stakeholders, such as the Viceministerio de Acuacultura y Pesca, WWF, customs officials, and environmental police (aimed at tackling wildlife crime). Ecuador would be a great location to trial the LOC as we already have good relations with local stakeholders, and their government is very committed to tackling the illegal wildlife trade especially with the implementation of a new National Action Plan for Sharks. Samples for the species we need to target for the LOC are also readily available and so access to samples would not be a problem. We would record the feedback and data on the LOC from this trial on a database, allowing us to develop the LOC further based on these results. Facilitating knowledge exchange with key stakeholders is crucial to empower local communities to lead conservation efforts, reducing the reliance on international input. Ultimately, the main aim would be to provide these communities with the necessary methods and resources, allowing them to undertake the conservation work within their country. Once we have shown that the LOC works well in one location (Ecuador), we can implement it on a global basis and engage with the wider community so that everyone is working in unison towards a common goal, rather than us carrying out snapshot research that's frequently done in this field.

We carried out initial workshops with these stakeholders in Ecuador and constructive feedback revolved around inclusion of additional CITES-listed species but not just restricted to sharks, e.g., mobula rays (Mobulidae), and further development on the SOP for the LOC.

For future work we would aim to include additional CITES-listed sharks, especially as hammerheads (Sphyrnidae) and Carcharhinid (Carcharhinidae) sharks are now listed in CITES. Some of these species are genetically very similar, and thus developing LAMP species-specific primers would be a challenge, therefore it may be easier to develop primers that target those groups rather than individual species. As many of the elasmobranchs are now listed in CITES, the LOC could be used as a comprehensive screening test. In cases where specific species need confirmation, this could be confirmed in a lab with sequencing facilities. This approach would still minimise the cost significant as only a few samples would need to be sequenced. This efficiency would be particularly advantageous when elasmobranchs need to be identified in huge seizures from airports, fin warehouses or Illegal, Unreported and Unregulated (IUU) vessels. Primers for mobula rays can also be developed, this will target mislabelling, as well as the ray fin and gill raker trade. We would aim to have better special coverage with the LOC, as it is important not just to focus on rolling the LOC out in Ecuador but also other countries where the elasmobranch trade is prevalent, for example South Africa, Hong Kong, and Indonesia.

It would be great to carry out analysis on emerging and highly toxic POPs such as PFAS, PCDD/Fs, OC's, UV chemical filters, as our systematic review on pollutants in elasmobranchs identified that very few studies had looked at these POPs. Future work would aim to analyse these POPs in the tissue of the five sharks (bigeye and pelagic thresher, blue shark, silky and smooth hammerhead shark) from **Chapter 5**. The edible tissue of sharks is important but as POPs tend to bioaccumulate in the liver as they have a highly lipophilic nature. Future work would include liver samples, to look more at concentrations of pollutants in squalene and other roots rather than meat as squalene is used in many products, including cosmetics (e.g., lipstick, mascara) as well as in vitamin and supplements (e.g., liver oil).

6.3. Summary Statement

Throughout my thesis, I have successfully explored key aspects of elasmobranch conservation, including mislabelling, international trade and pollutant exposure. By implementing meta-analysis and systematic reviews, I investigated patterns of mislabelling and pollutant exposure in elasmobranchs. I also applied innovative techniques, such as the LOC, to identify threatened and CITES-listed sharks in the field and used chemical analysis to assess pollutant concentrations in the tissue of commercially important and threatened sharks. This multidisciplinary approach has provided valuable insights into the conservation challenges facing elasmobranchs and has produced novel tools to address these key challenges. The main findings of my thesis were:

1. A systematic review on 85 studies that focussed on identifying elasmobranchs and related products in the trade, and mislabelling revealed that over 20% of elasmobranchs were either mislabelled or sold under umbrella terms, often concealing threatened or CITES-listed species, particularly blue sharks. Nearly half of the mislabelled species were threatened, and over half of those sold under umbrella terms were also threatened, highlighting the urgent need for stronger enforcement and more research in key trading hubs.
2. Created a novel paper-based LOC that accurately identifies three CITES-listed sharks (bigeye thresher, pelagic thresher, and shortfin mako) at market sources. The LOC can be used by non-scientifically trained personnel and doesn't require the need for expensive reagents, equipment or specialist facilities. It's particularly valuable in areas where there may be suspected high rates or mislabelling and can be used at border control during seizure of shark products (e.g. dried fins),

enhancing enforcement efforts where traditional methods and resources may be limited.

3. A total of 176 studies were included in a systematic review of pollutants in elasmobranchs. Sharks belonging to the top of the food chain had the highest concentrations of pollutants, specifically within the orders Carcharhiniformes and Lamniformes. The review also highlighted significant gaps in our knowledge regarding emerging toxic pollutants such as PFAS, dioxin-like-compounds, and halogenated flame retardants. Additionally, it identified critical areas that require further research, particularly in the South Pacific, Indian Ocean and Red Sea.
4. A human health risk assessment conducted in Chapter 4 and 5 revealed that individuals consuming shark meat as little as once a week may be exposed to mercury concentrations that exceed the guidelines set by the US EPA. This poses considerable risks not only to local fishing communities and international consumers of shark products but also to those impacted by the widespread mislabelling of elasmobranch products.

Elasmobranchs are frequently mislabelled and are therefore prevalent in our food chains and consumed by the public often without their knowledge. Just one portion of mislabelled shark meat can put children and adult over the toxic threshold for Hg, and possibly other emerging toxic persistent organic pollutants (POPs). Though our understanding of POPs in sharks is limited, their potential threat to humans that consume shark products, particularly liver products that accumulate lipophilic POPs, remains significant. Gathering support for elasmobranch conservation can be challenging as they often lack the endearing qualities of “charismatic” or commonly favoured species as they are not “cute” and “cuddly”. However, the human health angle provides an extra layer of significance from a public perspective. It

would be very costly and therefore near impossible to tackle the elasmobranch meat trade using only traditional genetic techniques, but our PoC LOC demonstrates that cost-effective, rapid, and field-based alternatives could make tackling this issue achievable in the near future. By focussing our efforts on enhancing these genetic approaches, we can increase our capacity for species identification, ensuring that conservation efforts and policy makers are well informed. As a result, this empowers us to address the illegal trade and mislabelling of elasmobranchs effectively, making a significant impact on both human health and elasmobranch conservation.

In conclusion, my thesis highlights the pressing need for strengthened legislation, policy reforms, and governance, alongside targeted conservation strategies, to effectively address the key threats facing elasmobranchs. By addressing the widespread issue of mislabelling and pollutant exposure in elasmobranchs, along with developing the LOC, my research will contribute to the development of more effective management practices. Future studies should focus on understanding the physiological and ecological impacts of pollutants, especially emerging toxic pollutants, and encourage the implementation community-driven initiatives that raise awareness and promote sustainable fishing practices. In order to protect future elasmobranch populations, collaboration between scientists, policymakers, governments, and local communities is essential. This multidisciplinary approach can facilitate the development of effective conservation strategies and ensure that we create long-lasting solutions that mitigate the threats elasmobranchs face and ensure their survival for generations to come.