INSTITUTO POLITÉCNICO NACIONAL

CENTRO INTERDISCIPLINARIO DE CIENCIAS MARINAS

Accumulation trends, potential toxicity, and human health risk assessment of organochlorines in three commercial shark species from the western Gulf of California, Mexico

TESIS

QUE PARA OBTENER EL GRADO DE DOCTORA EN CIENCIAS MARINAS

PRESENTA:

Ángela Ángel-Moreno Briones

LA PAZ, BCS, MÉXICO JULIO 2023

TABLE CONTENTS

ABSTRACT 4
RESUMEN 5
CHAPTER 1. General introduction 6
1.1. Organochlorine compounds 6
1.1.1. Organochlorine pesticides 9
1.1.2. Polychlorinated biphenyls 14
1.2. Bioaccumulation of organochlorines in marine organisms 16
1.2.1. Factors influencing organochlorine accumulation 17
1.3. Organochlorines in elasmobranch species 20
1.3.1. Elasmobranchs as bioindicators of OC contamination 21
1.3.2. Potential effects of OCs in elasmobranchs 21
1.3.3. Health risk associated with OCs due to the elasmobranch human consumption 23
1.4. Organochlorines in the Gulf of California 23
1.5. Biology, ecology, and conservation status of the scalloped hammerhead, the Pacific sharpnose shark, and the Pacific angel shark 24
1.5.1. Scalloped hammerhead shark *Sphyrna lewini* 24
1.5.2. Pacific sharpnose shark *Rhizoprionodon longurio* 25
1.5.3. Pacific angel shark *Squatina californica* 26
1.6. Justification 27
1.7. Research objective 28
1.8. Specific objectives 28
1.9. Study area 28
CHAPTER 2. Organochlorine pesticides in immature scalloped hammerheads *Sphyrna lewini* from the western coast of the Gulf of California, Mexico: Bioaccumulation patterns and human exposure 30
2.1. Abstract 30
2.2. Introduction 31
2.3. Material and methods 33
2.3.1. Sample collection 33
2.3.2. Chemical and instrumental analyses 35
2.3.3. Statistical analysis 38
2.3.4. Human exposure risk assessment 38
2.4. Results and discussion 39
2.4.1. OCPs detections, levels, and profiles 39
2.4.2. Influence of biological parameters on the accumulation of OCPs 49

2.4.3. Human health assessment 58
2.5. Conclusion 59
CHAPTER 3. Levels and species-specific accumulation of organochlorines in three shark species from the western Gulf of California (Mexico) with different life history traits 60
3.1. Abstract 60
3.2. Introduction 61
3.3. Material and methods 64
3.3.1. Samples 64
3.3.2. Laboratory analyses and quality control 65
3.3.3. Data analysis 66
3.3.4. Potential toxicity of PCBs 67
3.4. Results and discussion 68
3.4.1. Biological parameters 68
3.4.2. Organochlorine levels and profiles 69
3.4.3. Species-specific accumulation trends 77
3.4.4. Inter-specific comparisons 81
3.4.5. Potential health risk assessment 84
3.5. Conclusions 89
CHAPTER 4. Are shark tacos healthy or toxic? A multiple contaminant risk-benefit assessment 91
4.1. Abstract 91
4.2. Introduction 92
4.3. Material and methods 94
4.3.1. Shark meat sampling 94
4.3.2. Organochlorine analyses 95
4.3.3. Expressions, data treatment and statistics 96
4.3.4. Health risk assessment 96
4.3.5. Health benefit-risk assessment 97
4.4. Results and Discussion 98
4.4.1. Organochlorine levels in shark muscle 98
4.4.2. Maximum allowable shark consumption rates 101
4.4.3. Health risk-benefit 103
4.5. Uncertainty analysis 104
4.6. Conclusions 105
CONCLUDING REMARKS 106
REFERENCES 108

ANNEX 1 132
ANNEX 2 135
ANNEX 3 140

ABSTRACT

The scalloped hammerhead (HH) *Sphyrna lewini*, the Pacific sharpnose shark (PS) *Rhizoprionodon longurio* and the Pacific angel shark (PA) *Squatina californica* are species of ecological and economical importance in the Gulf of California (GC). As tertiary consumers, they are prone to the accumulation of contaminants such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) through biomagnification, which can affect their health and that of humans consuming these species. The objective of this research was to characterize the accumulation of both types of organochlorines (OCs) in HH, PS, and PA from the western GC in the context of conservation and food safety. In total, forty of the forty-seven contaminants analyzed were identified, showing a greater influence of agricultural (OCPs) than industrial (PCBs) sources. Higher levels of contaminants were detected in liver than in muscle due to its high lipid content and the lipophilic nature of OCs. Contaminant levels were generally lower than those reported in shark species from other areas of the world. Interspecific differences in OC accumulation were associated mainly with differences in habitat use and liver lipid content, while intraspecific differences were related with life stage. The bioaccumulation of some OCs was observed in HH and PS, which indicates that exposure to contaminants is greater than their elimination capacity. Toxic equivalents (TEQs) were calculated from liver concentrations of dioxin-like PCBs, resulting well below the toxicity thresholds established for fish. The human risk of non-cancerous and cancerous effects associated with multiple OCs was calculated from the consumption of shark muscle following Environmental Protection Agency guidelines. According to the results, consumption of 2 or 3 servings per week (65 – 98 g/day) of any of the three species would pose no risk to human health, but ingestion of HH and PS at the consumption rates of different populations of GC fishers (up to 471 g/day) would imply cancer risk. The toxic risk associated with OCs from ingesting shark muscle was greater than the nutritional benefit associated with essential fatty acids (BRQ > 1). This study provides a baseline knowledge about the levels of OCs in commercially important sharks from the GC.

RESUMEN

El tiburón martillo (TM) *Sphyrna lewini*, el tiburón bironche (TB) *Rhizoprionodon longurio* y el tiburón angelito (TA) *Squatina californica* son especies de importancia ecológica y económica en el Golfo de California (GC). Como consumidores terciarios, son propensos a la acumulación de contaminantes como pesticidas organoclorados (POCs) y bifenilos policlorados (PCBs) mediante biomagnificación, lo que puede afectar su salud y la de sus consumidores. El objetivo de este trabajo fue caracterizar la acumulación de ambos tipos de organoclorados (OCs) en TM, TB y TA del osete del GC en el contexto de la conservación y la seguridad alimentaria. En total, se identificaron cuarenta de los cuarenta y siete contaminantes analizados, mostrando una mayor influencia de fuentes agrícolas (POCs) que industriales (PCBs). Se detectaron niveles más altos de contaminantes en el hígado que en el músculo debido a su alto contenido en lípidos y a la naturaleza lipofílica de los OCs. Los niveles de contaminantes fueron en general inferiores a los registrados en especies de tiburones de otras zonas del mundo. Las diferencias interespecíficas en la acumulación de OC se asociaron principalmente con diferencias en el uso del hábitat y en el contenido lipídico del hígado, mientras que las diferencias intraespecíficas se relacionaron con estadio de vida. En TM y TB se observó bioacumulación, lo que refleja que la exposición a OCs es superior a su capacidad de eliminación. Se calcularon equivalentes tóxicos (EQT) a partir de las concentraciones hepáticas de PCBs similares a las dioxinas, resultando inferiores a los umbrales de toxicidad establecidos para los peces. El riesgo humano de efectos cancerígenos y no cancerígenos asociados a múltiples OCs se calculó siguiendo las directrices de la Agencia de Protección Medioambiental. Según los resultados, el consumo de 2 o 3 raciones semanales (65 – 98 g/día) de cualquiera de las tres especies no presenta ningún riesgo para la salud humana, pero la ingesta de TM y TB a las tasas de consumo de pescadores del GC (hasta 471 g/día) implicaría riesgo de cáncer. El riesgo tóxico asociado a los OC por ingerir músculo de tiburón fue mayor que el beneficio nutricional asociado a los ácidos grasos esenciales (BRQ > 1). Este estudio proporciona un conocimiento de referencia sobre los niveles de OC en tiburones de importancia comercial del GC.

CHAPTER 1. General introduction

The present Doctoral Thesis, developed during the SARS-CoV-2 pandemic, was structured into article-based chapters, and written in English to facilitate its dissemination. Chapter 1 introduces the topic of pollution by organochlorine compounds and their accumulation in sharks, which addresses the background of this research as well as justifying the statement of our objectives. Each of the following three chapters corresponds to a scientific article written for publication in JCR journals, addressing one or more specific objectives. After Chapter 4, the concluding remarks are provided. At the time of the presentation of this thesis, Chapter 2 has been published in the journal Science of the Total Environment, and Chapter 3 had been submitted to the same journal.

1.1. Organochlorine compounds

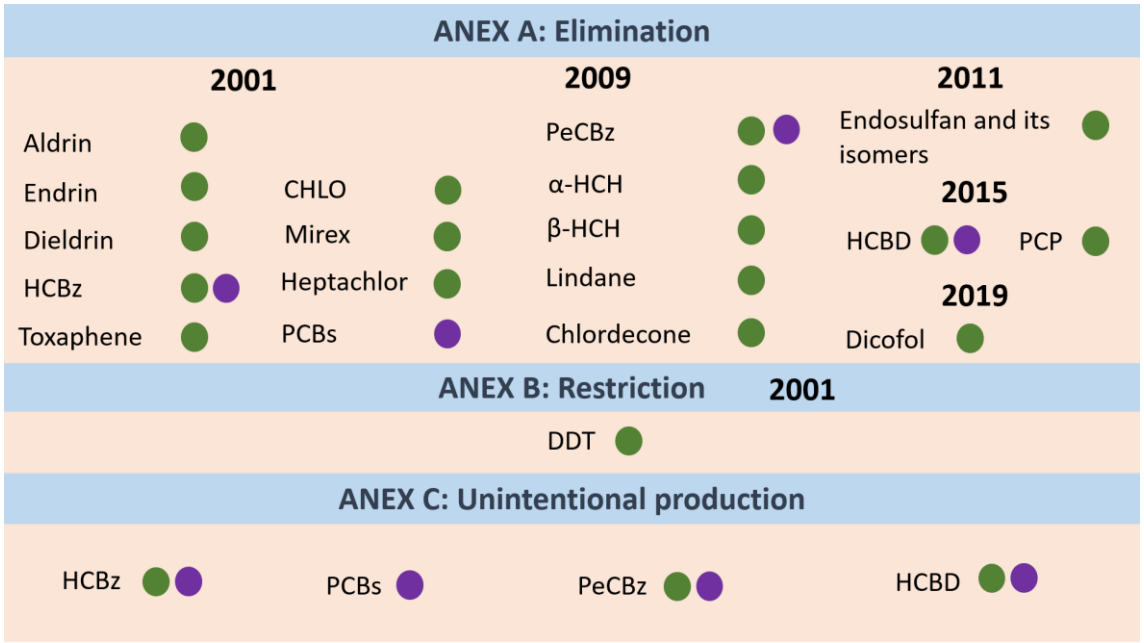
Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are organochlorine chemicals (OCs) that have different anthropogenic origins but share four basic characteristics: 1) they are compounds highly resistant to degradation, 2) they accumulate in the tissues of living organisms, 3) they are toxic to humans and wildlife, and 3) they have the ability to disperse (UNEP, 2017). The persistence, dispersion power, bioaccumulation and toxicity of OCs depend, in addition to the environmental conditions, on the physicochemical properties of each compound (Jacob and Cherian, 2013).

Due to these characteristics, OCPs and PCBs were included in the group of persistent organic pollutants (POPs) and different organizations began to regulate their use and manufacture before its intensive use during decades (Sparling, 2016). The Environmental Protection Agency (EPA) of the United States and other organizations cancelled many POPs between 1970 and 1980 at a national level. But it was not until 2001 that The Stockholm Convention, an international agreement promoted by the United Nations Environment Program (UNEP), banned or restricted the use and manufacture of POPs on a global scale. Since the entry into force of this treaty in 2004, 151 countries have committed to protect human health and the environment from POPs (Fiedler, 2003). Twelve

substances were initially included in the treaty, including nine OCPs (DDT, aldrin, dieldrin, endrin, heptachlor, mirex, chlordane, toxaphene and hexachlorobenzene) and PCBs, in addition to dioxins and furans. These pollutants were called the "dirty dozen" and have been considered the most toxic substances produced by humans (Ritter et al., 1995). To date, eighteen additional POPs have been added to the Stockholm Convention (UNEP, 2019b) (**Table 1.1**).

Table 1.1

Organochlorine compounds included in the Stockholm Convention. Green circles indicate pesticide use. Purple circles indicate industrial use.



Despite the regulation of many POPs taking place several decades ago, they are still ubiquitous in global ecosystems (Jepson and Law, 2016). Structurally, OCs are molecules with a ring-shaped hydrocarbon skeleton and multiple chlorine atoms. Their C-Cl bonds are highly stable, resulting in some having half-lives of years or decades, depending on the characteristics of the compound and environmental conditions. The persistence of the various OCs generally increases with molecular weight and the number of chlorine atoms. However, they eventually degrade abiotically and biotically. Their reactivity and bioavailability are influenced by the solid-water (K_{OC}) and octanol-air (K_{OA})

partition coefficients of the compounds. The higher these coefficients, the easier the pollutants will be to bind to particles in the environment, being less reactive and less bioavailable (Sparling, 2016).

The semi-volatility of POPs means that many are pollutants with high dispersive power. Volatility is primarily related to their vapor pressure, which reflects the tendency of the chemicals to be present in the atmosphere. The higher the vapor pressure of a compound, the more volatile it is and the greater its atmospheric dispersive power. Vapor pressure tends to be lower when the higher the molecular weight and degree of halogenation (Fiedler, 2003; Sparling, 2016).

The lipid solubility that characterizes many OCs and allows them to cross biological membranes and accumulate in living organisms is mainly determined by their high octanol-water partition coefficient (K_{OW}). This in turn, is directly related to the molecular size and number of chlorine atoms (Sparling, 2016).

The inherent toxicity of these organic contaminants is strongly influenced by their molecular structure (Fiedler, 2003; Sparling, 2016). OCs have the ability to bind to intracellular receptors for steroid hormones, interfering at various checkpoints of the hormone signaling pathways. Thus, they can inhibit or excessively potentiate (at the wrong time and in the wrong tissue) hormonal cascades (Mrema et al., 2013; Swedenborg et al., 2009). In addition, many OCs bind to cellular hydrocarbon receptors (AhR), producing the transcription of enzymes involved in phase I biotransformation, mainly CYP450 monooxygenases. The action of these enzymes can eventually produce an excess of free radicals or reactive oxygen species (ROS), causing oxidative stress (Henry, 2015). Oxidative stress is the main trigger for the induction of apoptosis and can lead to immunodeficiencies, autoimmune diseases, cancer and reproductive abnormalities (Mrema et al., 2013).

Many OCs can interfere with nerve impulses by different mechanisms of action. Some OCs as DDT and its metabolites act on the central nervous system (CNS) by binding to the sodium channels of neuronal axons. This interferes with the permeability of sodium and potassium ions, preventing the passage of the action potential (Costa, 2015). Other compounds, as cyclodienes and HCHs, act on the gamma-aminobutyric acid (GABA) receptors of neurons, which are important

neurotransmitters in the CNS. By binding to these receptors, they prevent the passage of the action potential producing convulsions, excitability and other neurological disorders that can lead to the death of animals (Costa, 2015; Sparling, 2016).

Genotoxicity is other biological effect caused by OCs, since they can enter the cell nucleus and bind with DNA producing adducts, which can alter gene expression, break chromosomes, damage DNA and cause mutations. It can result in tumors and different types of cancer. In addition, if the damage occurs in germ cells it is possible to affect fertility or cause genetic problems in subsequent generations (Sparling 2016).

The biochemical, subcellular, and cellular effects of OCs trigger effects at all higher levels of organization, including ecosystemic. This implies that efforts to monitor OCs concentrations in the abiotic and biotic environment continue to be necessary. However, since tissue concentrations of OCs tend to increase along trophic chains, top predators usually accumulate higher levels, increasing the risk of producing neurotoxicity, endocrine disruption, immune dysfunction, and cancer, among other effects (Mrema et al., 2013).

1.1.1. Organochlorine pesticides

OCPs were used for more than 60 years mainly in agriculture and pest control, and although a global decrease in their concentrations has generally been observed since their prohibition, they continue to be present in almost all abiotic and biotic compartments (Keogh et al., 2020; Muñoz-Arnanz and Jiménez, 2011).

Most OCPs are crystalline solids, and many are represented by different stereoisomers (chemicals with the same molecular weight and the same amount of carbon, hydrogen, oxygen and chlorine atoms, but with their atoms arranged differently in space). Because many were marketed as mixtures of compounds, and because many degrade into more persistent chemicals than the parent compound once released into the environment, there are numerous molecules resulting from the use of pesticides (Sparling, 2016).

1.1.1.1. DDT

Dichlorodiphenyltrichloroethane (DDT) is the best known and most widely used of the OCPs. During World War II it was used to control lice and the malaria-carrying mosquito; later its use was extended to agriculture (Turusov et al., 2002). In many countries it was used intensively from 1939 until it was restricted in the early 1970s (in other countries, such as Mexico, it was not restricted until 2000) (Sparling, 2016). During that time, it is estimated that approximately 4.5 x 10^6 tons of DDT were applied worldwide (Li and Macdonald, 2005). Since the Stockholm Convention came into force, its use is only allowed in some countries in Africa, Asia, India and Latin America for malaria and leishmaniasis control (ATSDR, 2019). However, DDT is still used clandestinely in antifouling paints in China (Xin et al., 2011) or in dicofol, an acaricide that contains up to 14% DDT in its formulation (Turgut et al., 2009). Another recent source is through the antineoplastic Lysodren (Ricking and Schwarzbauer, 2012).

The DDT has two isoforms: o,p' and p,p'. The main products of its transformation are DDE (dichlorodiphenyldichloroethylene) and DDD (dichlorodiphenyldichloroethane). Under anaerobic conditions, the metabolite that is mainly formed by dehalogenation of DDT is DDD, while under aerobic conditions it is DDE (Ricking and Schwarzbauer, 2012), its most stable metabolite. During anaerobic and aerobic transformation, the o,p' and p,p' substitutions on the aromatic rings remain unadulterated, with the p,p' configuration being more persistent (ATSDR, 2019).

According to the World Health Organization, the composition of DDT is: 77.1% p,p'-DDT, 14.9% o,p'-DDT, 4% p,p'-DDE, 0.3% p,p'-DDD, 0.1% o,p'-DDD, 0.1% o,p'-DDE and 3.5% of other compounds (WHO, 1989). DDD was also manufactured and used as an insecticide, but in much smaller quantities than DDT (ATSDR, 2019). Therefore, mainly six DDT residues with different physicochemical properties are found in the environment. In general, they are low volatile compounds with relatively low dispersing power (Fernandez and Grimalt, 2003; Sparling, 2016). DDT and DDE can persist for several decades in organisms due to their chemical stability, unlike DDD, which is more easily

excreted. The highest levels observed in human and animal tissues correspond to p,p'-DDE (ATSDR, 2019).

1.1.1.2. Drins

Aldrin and dieldrin were, after DDT, the most widely used pesticides from the late 1940s to the early 1970s (Beyer and Meador, 2011), being used primarily on cotton crops. Although its use was cancelled in 1970, the EPA lifted restrictions for termite and moth control in 1972, and it continued to be produced for this purpose until 1989. Endrin is a stereoisomer of dieldrin and was also marketed from 1951 to 1991 (ATSDR, 2022).

Aldrin has been shown to be non-toxic to insects but degrades rapidly to dieldrin in the environment and in organisms. The high molecular weight and lipophilicity of dieldrin are responsible for its high bioaccumulation power (Sparling, 2016).

Endrin, although it has one of the highest toxicities among the POCs (Sparling, 2016), is more rapidly metabolized and accumulates in lower concentrations in lipids than its steroisomer (Beyer and Meador, 2011). It can be found in small concentrations in the form of endrin aldehyde (an impurity and degradation product) or endrin ketone (a product of endrin photolysis) (ATSDR, 2022).

1.1.1.3. Endosulfan

Endosulfan was marketed globally since 1954 as a broad-spectrum agricultural pesticide, for the control of tsetse flies and livestock ectoparasites, and as a wood preservative (SEMARNAT, 2019). It was used as a substitute for DDT, aldrin and dieldrin, and was included in the Stockholm Convention in 2011, with its use restricted to certain crops until its total ban in 2016 (ATSDR, 2015a).

The technical grade of this pesticide contains 95% of the mixture of two diasteromers: α-endosulfan and β-endosulfan, in ratios ranging from 2:1 to 7:3. At solid-water and air-water interfaces their isomeric conversion occurs, mainly from β-endosulfan to α-endosulfan (Weber et al., 2010). In addition, by aerobic and anaerobic oxidation, endosulfan sulfate is formed. This is a metabolite more persistent than the parent compound and it have a similar toxicity (Yan et al., 2019).

Endosulfan has a low K_{OW}, so its solubility in water is higher than that of other OCPs, while its accumulation in fatty tissue is lower (Beyer and Meador, 2011). Nevertheless, it has long-range dispersing power (Weber et al., 2010). The α-endosulfan has more potent insecticidal properties and produces higher acute toxicity in mammals and aquatic organisms. However, β-endosulfan shows greater neurotoxicity and biomagnification power than α-endosulfan (Beyer and Meador, 2011; Yan et al., 2019).

1.1.1.4. Chlordane and heptachlor

Chlordane was used in agriculture, gardens, and construction, especially from 1970 onwards replacing other POCs. However, most of its applications were also soon restricted due to its persistence and lipophilicity, although it continued to be used until the late 1980s for termite control (SEMARNAT, 2019).

In a technical preparation of chlordane, about 150 different compounds could be identified, the most abundant being cis-chlordane (15%), trans-chlordane (15%), trans-nonachlor (9.7%), octachlordane (3.9%), heptachlor (3.8%) and cis-nonachlor (3.8%) (USEPA, 1997).

Oxychlordane is the major metabolite of both cis-chlordane and trans-chlordane, and heptachlor epoxide is the major metabolite of heptachlor. Moreover, heptachlor was also marketed as a pesticide in a formulation with approximately 72% heptachlor and the remaining related compounds (SEMARNAT, 2019).

The chlordane isomers were metabolized more rapidly than the nonachlor isomers, while oxychlordane tends to persist (ATSDR, 2018). Oxychlordane and trans-nonachlor tend to exhibit higher biomagnification power (Beyer and Meador, 2011). According to their inherent toxicity, they are ordered as follows: trans-nonachlor > cis- and trans-chlordane > heptachlor and heptachlor epoxide > cis-nonachlor (Beyer and Meador, 2011; Bondy et al., 2003).

1.1.1.5. Mirex and chlordecone

Mirex was produced in the United States from the late 1950s until 1975, although more than 90% of production was exported to Latin America, Europe and Africa. Its main use was as an industrial flame-retardant additive (in plastics, paints, paper, etc.), and to a lesser extent it was used as a pesticide (ATSDR, 2020). It

is a highly lipophilic compound that readily adheres to soils and sediments, which decreases its bioavailability and dispersing power (Sparling, 2016).

1.1.1.6. Hexachlorocyclohexane

Hexachlorocyclohexane (HCH) was used as an agricultural insecticide until it ceased production in the United States in 1976. Generally, technical grade HCH is a mixture of approximately 60-70% α-HCH; 5-12% β-HCH; 10-15% γ-HCH; 6-10% δ-HCH, and other minority compounds (Jackovitz and Hebert, 2015).

The only HCH isomer with insecticidal properties is γ-HCH, which was marketed since 1940 under the name lindane (99% γ-HCH) for veterinary, urban, agricultural and industrial use (ATSDR, 2005). The isomers α-HCH, β-HCH, γ-HCH are listed in the Stockholm Convention since 2009 (UNEP, 2019b), although all are persistent and toxic to animals (Jackovitz and Hebert, 2015).

According to Bala et al. (2012) they are ordered according to their bioaccumulation and biomagnification power as follows: β-HCH > α-HCH > γ-HCH > δ-HCH. However, the γ-HCH isomer exhibits the highest acute neurotoxicity, followed by α-, δ- and β- isomer. In contrast, under chronic exposure conditions they are ordered according to their toxicity as follows: β-HCH> α-HCH> γ-HCH> γ-HCH> δ-HCH (Jackovitz and Hebert, 2015).

1.1.1.7. Chlorobenzenes

Hexachlorobenzene (HCB) was used as a fungicide in many countries until 1984. It was also used in the production of pyrotechnic materials and synthetic rubber, among other applications. In addition, it is formed as an intermediate in the manufacture of other compounds, such as chlorinated hydrocarbons and some current and banned pesticides, such as mirex and lindane. Unintentional emissions of this compound continue to occur in coal, cement and biomass combustion processes and in the incineration of municipal hazardous or medical waste (ATSDR, 2015b).

For its part, pentachlorobenzene (PeCB) is a metabolite of HCB and was included in the Stockholm Convention in 2009. It was used as a fungicide, pesticide and flame retardant. It was also present as part of chlorobenzene mixtures added to

reduce the viscosity of PCBs. In addition, it arises in combustion processes, and it is found as an impurity in other pesticides (ATSDR, 2015b; UNEP, 2008).

1.1.1.8 Hexachlorobutadiene

The main source of hexachlorobutadiene (HCBD) is as a residual by-product of the manufacture of chlorinated hydrocarbons. It is also generated in most combustion processes as municipal solid waste and fuels. In addition, it was used as a solvent, hydraulic fluid, reagent in laboratories and, to a lesser extent, as a pesticide. Since 2015 it has been listed in Annexes A and C of the Stockholm Convention due to its persistence and toxicity (ATSDR, 2021).

1.1.1.9. Methoxychlor

Methoxychlor is a broad-spectrum insecticide that began to be marketed in 1948 as a replacement for DDT and was discontinued in many countries during the 1990s. However, it has continued to be widely used in several developing countries because, although it has been proposed for inclusion in Annex A of the Stockholm Convention, it is not yet listed there. It is a very hydrophobic compound with low volatility that tends to concentrate in sediment and biota (ECHA, 2020; UNEP, 2019a).

1.1.2. Polychlorinated biphenyls

PCBs were introduced in the United States in 1929, and for approximately 50 years about 1.3 x 10^6 tons were manufactured, most of which were used in the northern hemisphere (Breivik et al., 2007). They were used as insulating fluids in the electrical industry, in the manufacturing process of aluminum, copper, iron and steel; as flame retardants; as lubricants in the treatment of wood; in the manufacture of fabrics, paper, paints, plastics, among others. Currently, they can still be found, for example, in old electrical equipment, microscopes, and old buildings, as well as they can be unintentionally released from industrial processes (ATSDR, 2000; Mao et al., 2021). It is estimated that around 80% of the world's stockpiles have not yet been destroyed, and that at current PCB phase-out rates, many countries will not achieve the targets agreed in the Stockholm Convention by 2025 and 2028 (Desforges et al., 2018).

PCBs are a group of organic compounds consisting of a biphenyl molecule to which 1 to 10 chlorine atoms are attached at different positions. Therefore, there are 209 PCB congeners with different physicochemical characteristics, and a numbering system developed by Ballschmiter and Zell in 1980 is used to name them (ATSDR, 2000). However, although all 209 congeners can be synthesized in the laboratory, only about 130 have been identified in commercial mixtures, known and marketed as Aroclor (Giesy and Kannan, 2002).

Positions 2, 2', 6 and 6' are called "ortho" positions; positions 3, 3', 5 and 5' are called "meta" positions, and positions 4 and 4' are called "para" positions. Depending on the arrangement of the chlorine atoms in the biphenyl structure, PCBs are classified into coplanar (or dioxin-like) and non-coplanar (ATSDR, 2000; Giesy and Kannan, 2002). The dioxin-like PCBs (DL-PCBs) are congeners with free ortho substitutions of chlorine atoms (non-ortho PCBs: 77, 126 and 169), and those with only one chlorine atom in one of the ortho positions (mono-ortho PCBs: 77, 126 and 169). Non-ortho PCBs are considered to be the most toxic PCBs (ATSDR, 2000). Conversely, the non-coplanar congeners are those that have more than one chlorine atom in their ortho positions; they cannot assume a planar configuration, and the two benzene rings are at a 90° angle to each other (ATSDR, 2000).

Congeners of PCBs are also classified according to their degree of chlorination (monochlorobiphenyls, dichlorobiphenyls, trichlorobiphenyls, etc.), with those congeners having the same number of chlorine atoms being referred to as "homologues". Homologues with different substitution patterns are called isomers (ATSDR, 2000). Their water solubility, vapor pressure and ease of degradation decrease with increasing chlorination level (Sparling, 2016). PCBs can also be grouped in function on the number and position of hydrogen and chlorine atoms, which determines whether or not they can be metabolized by the cytochrome P450 enzymes (Buckman et al., 2007, 2006). Group I (congeners without vicinal hydrogen atoms, such as PCB 153, 180 and 189) and group II (congeners with vicinal hydrogen atoms only in the ortho and meta positions, such as PCB-138) are not metabolizables. Conversely, group III is only metabolized in some mammals, and groups IV (e.g., PCB-52) and V are metabolized in some fish (Buckman et al., 2007, 2006).

1.2. Bioaccumulation of organochlorines in marine organisms

In marine environments OCs can enter directly (through agricultural runoff, industrial spills) or indirectly, through atmospheric deposition and ocean currents (Li and Macdonald, 2005). Once in the sea, more hydrophilic OCs will be found in higher concentration in the water column than those more lipophilic (Miglioranza et al., 2013). However, due to the strong hydrophobicity of most OCs, they can be found concentrated in the surface microlayer up to 500 times relative to the concentrations in the underlying water column (Wurl et al., 2017). Here, water-air exchange, adsorption to particulate organic matter and uptake to phytoplankton occur. Thus, OCs can continue atmospheric transport, sink to deep water and reach sediments, or enter marine food webs. The balance between these processes depends on both contaminant properties and ecosystem conditions (productivity, solar radiation, temperature, wind speed, water pH, among others) (Wagner et al., 2019).

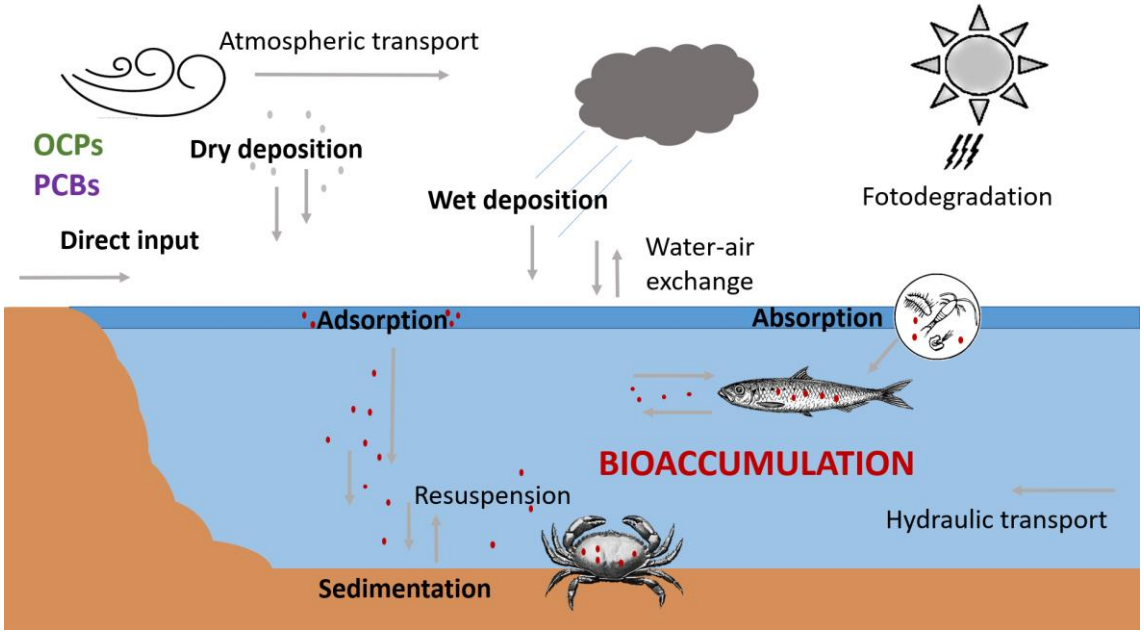


Fig. 1.1. Transport and environmental cycle of OCs.

Marine animals acquire higher concentrations of contaminants than those presented in their surrounding environment through the mechanism of bioaccumulation (Borgå et al., 2011; Daley et al., 2014). This mechanism is the net result between the absorption rate (via respiration, dermal diffusion and

feeding) and the elimination rate (via respiration, dermal diffusion, feces, metabolic biotransformation, reproductive discharge and growth dilution) (Arnot and Gobas, 2006; Russell et al., 1999). Other mechanisms such as ingestion or inhalation of microplastics with OCs adsorbed to their surface (Wright and Kelly, 2017), seasonal molts (Jaspers et al., 2007) or secretions (Van den Brink et al., 1998), can influence contaminant uptake and elimination rates.

A special case of bioaccumulation is biomagnification, a process in which an organism's concentration of contaminants exceeds those of its prey, because uptake from the diet occurs faster than elimination (Borgå et al., 2011). Through this mechanism, some OCs can biomagnify in apex predators, for which diet is the main route of exposure to these contaminants (Borgå et al., 2004; Leonards et al., 2008). Thus, many species of marine mammals, seabirds, and fish feeding at high levels in food webs, can accumulate elevated concentrations of OCs (Jepson and Law, 2016; Letcher et al., 2010; Mull et al., 2012; Tartu et al., 2017).

1.2.1. Factors influencing organochlorine accumulation

The bioaccumulation of OCs in marine organisms is determined by various physicochemical (which determine the bioavailability and bioaccumulation capacity of the compounds), biological and ecological factors. Therefore, it varies significantly between tissues, during the life of the individual, between individuals, species, and taxonomic groups (Borgå et al., 2004). Understanding the mechanisms influencing the accumulation of these pollutants in marine species is valuable in helping to establish risk assessments and management measures.

1.2.1.1. Physicochemical factors

Van der Oost et al. (2003) define bioavailability as the fraction of chemicals present in soil, sediment and water that can potentially be absorbed by the surfaces of an organism (excluding the digestive tract). This bioavailability is determined by the interaction between the characteristics of the compound (e.g., partition coefficients, vapor pressure) and those of the environment (e.g., solar radiation, dissolved organic matter, particulate organic matter, microbial activity) (Sparling, 2016). For example, more volatile and water-soluble compounds such as HCH, are more bioavailable to marine predators living on the surface; in

contrast, more hydrophobic compounds such as PCBs and DDTs, adsorb on suspended particles, sink and are therefore more bioavailable to benthic organisms (Lavoie et al., 2010). In areas with high phytoplankton productivity, OCs available for direct uptake by water decrease (Borgå et al., 2011).

As mentioned, the lipophilicity of OCs is the main factor influencing their bioaccumulation capacity. If a compound is sufficiently hydrophobic and recalcitrant (with a low rate of biotransformation) it will be biomagnified in trophic chains (Kainz and Fisk, 2009). It has been established that organic chemicals with log K_{OW} > 3 accumulate in organisms, and those very hydrophobic (log K_{OW} ≥ 5) can biomagnify (Sparling, 2016). However, poorly metabolizable and moderately hydrophobic substances (log K_{OW} 3-5), do not biomagnify in fish food webs, but do it in marine mammals and birds because their low elimination rates through lung respiration (Kelly et al., 2007). On the other hand, the size of organic molecules influences their ability to bioaccumulate, since larger molecules have greater difficulty in crossing biological membranes (ATSDR 2002b). However, if assimilated, they are also the most persistent and tend to remain in organisms for many years (Sparling, 2016).

1.2.1.2. Biological factors

The lipid content is an important factor influencing the OC accumulation. Due to the lipophilicity of OCs, they are strongly influenced by lipid dynamics in organisms, being stored mainly in the fatty tissues and organs (Borgå et al., 2004). Variations in the lipid content of an organism influence the concentration of OCs and their distribution between tissues (Borgå et al., 2004; Kainz and Fisk, 2009).

Although the affinity of different OCs can be variable depending on the lipid type, generally the increase of these contaminants is related to the content of triacylglycerols (Borgå et al., 2011). Thus, to assess exposure to OCs, it is advisable to carry out their analysis in fatty tissues (Bentzen et al., 2008b). Other tissues such as skin, blood, or muscle tend to accumulate lower concentrations of organochlorines (Boldrocchi et al., 2019; Boldrocchi et al., 2020; Nomiyama et al., 2011).

Other biological factor influencing the bioaccumulation process is the organism's biotransformation capacity. Biotransformation of xenobiotics is the enzyme-catalyzed process that contributes to their elimination by transforming them into more polar and water-soluble compounds, facilitating excretion and hindering biomagnification. This process is influenced by both the characteristics of the compound and the metabolic capacity of the organism. However, biotransformation may also result in a metabolite that is more bioaccumulative and more toxic than the parent compound (Borgå et al., 2004).

Continuous exposure during the lifetime of an organism usually implies increasing concentration of OCs, mainly the most recalcitrant ones. However, other age-related factors such as increased body size, reproduction, metabolic changes and variations in behavior (variations in diet or habitat use) may mask their effects (Borgå et al., 2004). Dilution of contaminants may also occur with the growth of organisms. This usually occurs during early life stages, if the animals' diet has fewer contaminants than the initial body burdens acquired by maternal transfer or if there is an increase in metabolic capacity (Borgå et al., 2004, 2011).

Another process related to body size and accumulation of OCs, as opposed to dilution by growth, is bioamplification. This term refers to the condition whereby an organism loses body mass at a faster rate than it can eliminate chemicals and occurs in some species during periods of time when their feeding rates decrease. Thus, even if their exposure to OCs is lower due to reduced intake, weight loss implies an increase in recalcitrant contaminants as a function of the animal's mass (Daley et al., 2014; Kainz and Fisk, 2009).

The main determinant in the different bioaccumulation of OCs between females and males is usually reproduction. However, in some species there are sex-specific trophic, habitat use or metabolic differences that may also influence differential accumulation of contaminants (Borgå et al., 2004; Lyons and Adams, 2014). Maternal discharge is a pollutant removal mechanism for adult females, and at the same time involves exposure to a certain number of compounds from birth. The magnitude of maternal discharge is influenced by numerous factors, as the mechanism of reproduction, the age of sexual maturity of the mother (Lyons et al., 2013), the number of previous reproductive events (Ylitalo et al., 2001) or litter size (Lyons and Adams, 2014).

1.2.1.3. Ecological factors

Trophic level is a determining factor in the accumulation of OCs, as well as diets rich in lipids (Lawson et al., 2020).

Habitat is also an important factor in the accumulation of OCs in predators, in relation to both contaminant bioavailability, prey availability and metabolic rates. For example, tropical food webs are more complex than temperate food webs, promoting greater diet diversity. Conversely, bioavailability in tropical systems may be affected by higher microbial activity and organic matter (Borgå et al., 2011). In turn, at high latitudes, the removal rates of aquatic organisms decrease, as do their feeding rates (Sparling, 2016). In addition, migratory animals may vary their OC accumulation profiles due to regional differences in exposure (Borgå et al., 2004).

1.3. Organochlorines in elasmobranch species

Many elasmobranchs are at the apex of marine food webs, which exposes them to relatively high levels of OCs through the biomagnification process (Kelly et al., 2007). In addition, their longevity, slow metabolism, low reproductive rates, and late maturity, together with its particular lipid metabolism, make them prone to significant accumulation of organic pollutants (Gelsleichter and Walker, 2010). This makes them potentially vulnerable to OC toxic effects, as well as good bioindicators of contamination (Alves et al., 2022; Miller et al., 2020; Muñoz-Arnanz et al., 2022). Nevertheless, due to the diversity of diets, sizes, and habitat uses among different elasmobranch species, there are important inter-specific differences in their ability to accumulate organochlorines (Gelsleichter and Walker, 2010). In addition, since many species are an important fishery resources, investigating contaminant levels in edible tissues is essential for food safety.

Elasmobranchs have large (up to 25% of body weight) lipid-rich livers involved in energy storage and buoyancy control (Leigh et al., 2017). Therefore, liver is the main accumulation organ of OCs, and its analyses allows estimating the maximum loads of an organism (Gelsleichter et al., 2005, 2006). Consequently,

the analysis of muscle and fins allows estimating the risk to human health associated with OCs, since elasmobranch meat consumption has increased significantly during recent decades, and the fins of some species of sharks and rays are a delicacy in some Asian countries (Dulvy et al., 2014; Mezzalira-Pincinato et al., 2022).

Among elasmobranchs, high trophic level sharks appear to be particularly prone to organochlorine accumulation (Tiktak et al., 2020). There is information from various shark species with different biological and ecological characteristics; however, species-specific accumulation trends are only characterized for a few shark species in discrete areas of the world (Consales and Marsili, 2021; Lyons et al., 2019b).

1.3.1. Elasmobranchs as bioindicators of OC contamination

Monitoring of OCs is an obligation of all countries that are signatories of the Stockholm Convention. However, the high cost of determining OCs in environmental samples implies that studies on this subject are limited (Muir and Sverko, 2006). Nevertheless, the monitoring of OCs concentration in different marine organisms has been conducted for decades as a tool to evaluate pollution and changes in the environment. Different shark species have been used for this purpose, since they have some characteristics that make them good bioindicators (high trophic position, relative longevity) and their sampling may be relatively accessible in places where they are a fishery resource (Páez-Osuna and Osuna-Martínez, 2011). However, the migratory habits of some species are a limiting factor when using the analysis of their pollutant concentrations to assess the degree of contamination of a restricted area.

1.3.2. Potential effects of OCs in elasmobranchs

The main premise in toxicology states that the toxicity of any substance depends on its dose (Sparling, 2016). Thus, marine predators (as many shark species) may present a high vulnerability to OCs, since their exposure can be up to one hundred times higher than that of lower trophic levels (Leonards et al., 2008). However, toxicity further depends on the distribution of the compounds in the

organism, their metabolism, and their interaction with tissues (Mrema et al., 2013).

Different approaches are used to assess the possible effects of OCs on elasmobranchs. The analysis of contaminant concentrations and subsequent comparison with toxicity threshold values established from laboratory tests conducted on surrogate species provides an estimate of the potential risks (Lyons and Adams, 2014). However, this approach has important limitations. Laboratory studies usually expose animals to high doses of a substance for a short period of time (acute exposure), making it difficult to extrapolate the effect of lower doses to chronic exposure (as occurs in nature) (Green and Larson, 2016). The comparison of results with other studies also implies that it is sometimes necessary to resort to comparisons between different species (with the highest possible degree of relatedness), since the works where biomarkers are analyzed are limited (Leonards et al., 2008). Differences in accumulation and vulnerability between species, age, health status, sex, and environmental variables also complicate risk assessment studies (Green and Larson, 2016; Leonards et al., 2008).

On the one hand, some studies analyze the relationship between the obtained concentrations of the different compounds with certain biomarkers (biological responses produced at the individual or lower level that demonstrate an alteration of the normal condition of the organism) (Leonards et al., 2008). Alves et al. (2016), through biomarker analysis in blue sharks, found a positive association between levels of dioxin-like PCBs with DNA damage in muscle and liver cells. They also found a strong negative association between these contaminants and antioxidant enzyme activity. The authors noted that this could ultimately produce effects on the swimming, feeding or reproductive behavior of sharks. Similarly, in the Greenland shark *Somniosus microcephalus* an association was observed between elevated plasma PCB levels and imbalances in vitamin A and E homeostasis (Molde et al., 2013). In batoids, OCs concentrations have also been related to biological effects. In the round skate *Urobatis halleri*, a correlation was found between levels of OCs, mainly PCBs, and effects on the immune system (Sawyna et al. 2017). For the same species, Lyons and Edwards (2018) published the first work to demonstrate the negative effect of PCB exposure on

embryo growth in elasmobranchs. They analyzed embryos collected in southern California, in an area highly exposed to PCB contamination (and with low exposure to other contaminants) and on Santa Catalina Island, with low anthropogenic influence. Embryos from the more exposed area weighed less at each developmental stage than reference embryos. In the Atlantic stingray *Dasyatis sabina*, it was determined that exposure to OCs could be a factor causing endocrine and immune alteration when correlations were observed between contaminant levels and serum steroid and white blood cell concentrations (Gelsleichter et al., 2006). However, it should be noted that free-ranging animals are exposed to mixtures of many contaminants, as well as other environmental stressors, so it is sometimes risky to claim that a physiological response is caused by only one pollutant (Green and Larson, 2016).

1.3.3. Health risk associated with OCs due to the elasmobranch human consumption

Despite their health benefits, the consumption of fish is one of the main routes of exposure to toxic chemicals for humans, and the risk of contamination tends to increase when consuming high trophic level species (Arrebola et al., 2018; Weitekamp et al., 2021).

Shark muscle is comparatively less prone to OCs accumulation than the muscle of many teleost fish due to their low lipid content (Lyons et al., 2021). However, due to the generalized high exposure risk of sharks, they may accumulate numerous chemicals that can act additively or synergistically causing adverse effects on the health of shark consumers (Kibria and Haroon, 2015; Souza-Araujo et al., 2021).

1.4. Organochlorines in the Gulf of California

Mexico had the highest OCPs consumption among all the Latin American countries (Wong et al., 2008). The continental coast of the GC has an important agricultural tradition, mainly in the states of Sonora and Sinaloa, and the Mexicali valley (Páez-Osuna et al., 2017). In contrast, in a large extent of the peninsular zone of Baja California, agricultural activity is reduced, and more so in the coastal

part of the GC (Botello et al., 2014; Páez-Osuna et al., 2017). However, substantial amounts of OCPs were used in the Mexicali Valley (in Baja California) to improve agricultural productivity (Sánchez-Osorio et al., 2017). In addition, OCPs were heavily used to combat vector-borne diseases in the peninsula

Regarding to the historical usage of PCBs in Mexico, it was low comparing to those in more industrialized countries (Breivik et al., 2002). In the Baja California peninsula, the major concentrations of these chemicals have been found in coastal areas with wastewater discharges from urban centers (Labrada-Martagón et al., 2011).

Although works on levels of OCs in the GC generally report low concentrations compared to other more impacted areas (García-Solorio et al. 2014; Niño-Torres et al., 2009), some references also warn about the continuous and recent contribution of banned compounds (Ponce-Vélez and Botello, 2018). Therefore, and due to its richness of species and the fact that it is the most important fishing region in Mexico, it is important to monitor OC levels in this region.

1.5. Biology, ecology, and conservation status of the scalloped hammerhead, the Pacific sharpnose shark, and the Pacific angel shark

More than 7500 tons of shark are caught annually in Baja California Sur, which represents about 50% of Mexico's shark production (CONAPESCA 2020). Three of the most frequently caught species are the scalloped hammerhead *Sphyrna lewini* (Griffith & Smith, 1834), the Pacific sharpnose shark *Rhizoprionodon longurio* (Jordan & Gilbert, 1882) and the Pacific angel shark *Squatina californica* Ayres, 1859 (Saldaña-Ruiz et al., 2017), for which there is information on the levels of inorganic contaminants (Bergés-Tiznado et al., 2015; Escobar-Sánchez et al., 2016; Frías-Espericueta et al., 2019). However, there is no information on the levels of organic contaminants such as OCPs and PCBs in these nor other commercially important shark species of the CG.

1.5.1. Scalloped hammerhead shark *Sphyrna lewini*

The scalloped hammerhead (HH) *Sphyrna lewini* is a tertiary coastal and semi-oceanic predator circumglobally distributed from the surface to nearly 300 m

depth (Compagno, 1982). The size range of neonates is 31-57 cm and females can reach more than 3 m in some areas of their distribution (Compagno, 1982). Piercy et al. (1987) reported a maximum age of 30.5 years for females and males of this species. The GC is a feeding, mating, and nursery area for this species. Adults are migratory and organize in schools around seamounts, feeding at night on pelagic prey (Torres-Rojas et al., 2013). Juveniles, on the other hand, inhabit shallow coastal bays (Duncan and Holland, 2006), where they feed mainly on benthic prey such as small fish, crustaceans, and squid (Torres-Rojas et al., 2013). In the Mexican Pacific, females reach sexual maturity at 220 cm total length (TL) and males at 180 cm. It is a viviparous species that present a gestation period of 10 months, having 6 - 40 embryos per litter (Bejarano-Álvarez et al., 2010). The HH is classified as “Critically Endangered” (Rigby et al., 2019), and it is estimated that populations from the GC have declined in recent years (Hoyos-Padilla et al., 2014).

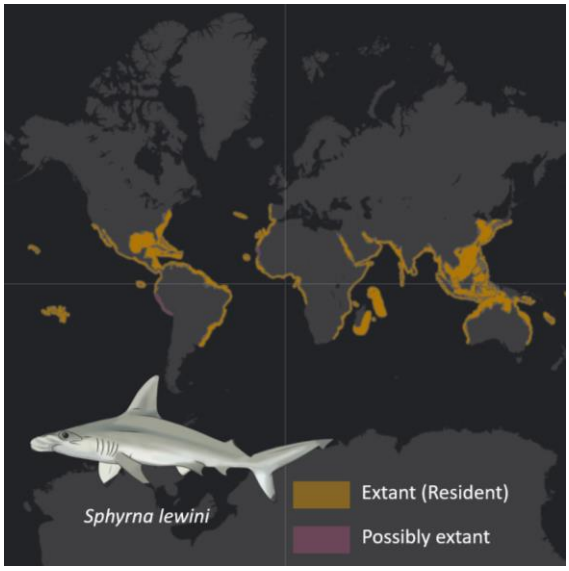


Fig. 1.3. Geographic distribution of scalloped hammerhead *Sphyrna lewini*. Source: Rigby et al. (2019).

1.5.2. Pacific sharpnose shark *Rhizoprionodon longurio*

The Pacific sharpnose shark (PS) *Rhizoprionodon longurio* is a short-lived (maximum age of 7.5 years, according to Corro-Espinosa, 2011) and a small size (up to 154 cm) species that lives on sandy and muddy bottoms close to the coast

(Compagno, 1982). Its distribution is from southern California to Peru. The PS exhibits seasonal migration patterns in the GC, although juveniles show fidelity to their breeding grounds (Márquez-Farias et al., 2005; Trejo-Ramírez, 2017). They are tertiary piscivorous predators, whose feeding habits may vary according to sex and ontogeny (Alatorre-Ramírez et al., 2013; Trejo-Ramírez, 2017). It is a viviparous species with litter sizes between 4 and 12 embryos, and with an annual reproductive cycle. In the GC, the mature size of males is 101 cm and that of females is 93 cm (Corro-Espinosa, 2011). The International Union for Conservation of Nature (IUCN) cataloged this species as “Vulnerable”, particularly to anthropogenic impacts such as pollution due to its markedly coastal habits (Smith et al., 2009).

Fig. 1.4. Geographic distribution of Pacific sharpnose shark *Rhizoprionodon longurio*. Source: Smith et al. (2009).

1.5.3. Pacific angel shark *Squatina californica*

The Pacific angel shark (PA) *Squatina californica* is usually found semi buried on shallow, sandy or muddy bottoms, although it can also be found up to 200 m (Compagno, 2005). This species is discontinuously distributed from Alaska to the Gulf of California, and from Ecuador to southern Chile, having a low dispersal capacity (Compagno 2005). The maximum age of PA is estimated in 35 years, and its size in the GC ranged from 23 to 100 cm TL (Cailliet et al., 1992; Márquez-Farías, 2020). In this place, females mature at a L_{50} of 74.4 cm, and males at 77.8

using aplacental viviparity as mechanism of reproduction and having litters of between 2 and 10 embryos per year (Márquez-Farías, 2020; Romero-Caicedo et al., 2016). They are selective, tertiary predators that mainly ambush demersal fishes (Escobar-Sánchez et al., 2011). Its conservation status has been assessed as “Near Threatened”, although in Baja California Sur landings have severely decreased (Cailliet et al., 2020).

Fig. 1.5. Geographic distribution of Pacific angel shark *Squatina californica*. Source: Caillet et al. (2020).

1.6. Justification

The bioaccumulation of contaminants is a central aspect of ecotoxicology, and understanding their dynamic processes is crucial for the protection of wildlife animals, ecosystems, and human health (Arnot and Gobas 2004; Daley et al., 2014; Kelly et al., 2004). The number of studies analyzing OC loads in elasmobranchs has increased in recent years providing useful information to carry out management policies. Assessing the potential risk of toxic effects of OCs on the health of elasmobranchs is challenging due to the lack of dose-response studies and the numerous environmental stressors to which they are exposed. However, it is essential for conservation and management strategies, especially for species threatened by other anthropogenic impacts, as it could allow recommendations on habitat protection and fishery regulation. Similarly, human risk assessment through the consumption of contaminated species is

necessary to ensure food safety. Thus, knowing the levels of organochlorine contaminants in the edible tissue of commercially important species could provide information to make recommendations on their consumption.

1.7. Research objective

The knowledge about the organochlorine levels in the species of ecological and economical relevance *Sphyrna lewini*, *Rhizoprionodon longurio* and *Squatina californica,* as well as the main factors that govern in their accumulation is essential to understanding the contaminant influence in those organisms and its ecosystem. The overall aim of this research is to characterize the accumulation of OCPs and PCBs in three of the most exploited shark species from the western Gulf of California, in the context of conservation and food security.

1.8. Specific objectives

The specific objectives of this research were to:

1) Characterize the OC exposure of HH, PS, and PA in the western coast of the Gulf of California.

2) Describe species-specific accumulation trends.

3) Infer the potential risk of toxic effects on these shark species.

4) Evaluate the potential risk associated with organochlorines to human health.

Each of the following chapters partially covers all the particular objectives. Chapters 2 and 3 address all the specific objectives, while Chapter 4 addresses objectives 1, 2, and 3.

1.9. Study area

The organisms of HH were obtained from artisanal fisheries in San Bruno (SB), El Saladito (SA), and El Manglito (MA), in Baja California Sur; PS samples were obtained from SB and SA captures, as well as PA individuals were sampled from El Saladito and El Manglito landings (**Fig. 1.6**).

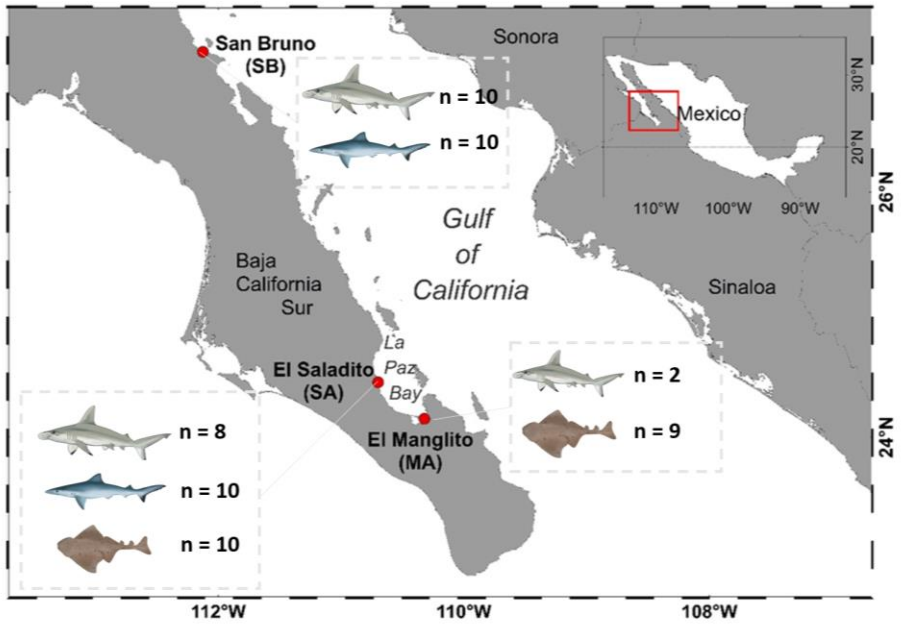


Fig. 1.6. Fishing camps locations where the samples were collected in Baja California Sur, Mexico, and number or individuals sampled in each locality.

San Bruno (27°09′37″ N, 112°09′31″ W) is a small town located in the municipality of Mulegé. It is about 20 km from the city of Mulegé and almost 30 km from Santa Rosalía, a district with a mining tradition. The municipality supports about 8% of the state's agricultural area (Graciano, 2013).

El Saladito (24°26′30″ N, 110°42'37'' W) and El Manglito (24°15’21’’ N, 110°32’82’’ W) are in La Paz Bay, in the municipality of La Paz. This bay communicates with the city of La Paz, capital of the state and with an important tourist activity. The planted area of this municipality constitutes 11% of that of the entire state (Graciano, 2013).

CHAPTER 2. Organochlorine pesticides in immature scalloped hammerheads *Sphyrna lewini* from the western coast of the Gulf of California, Mexico: Bioaccumulation patterns and human exposure

Published in: Science of the Total Environment
https://doi.org/10.1016/j.scitotenv.2021.151369

2.1. Abstract

Despite the intensive use of organochlorine pesticides (OCPs) in the proximity of the Gulf of California, there is no information regarding their levels in predatory shark species, which could be exposed to relatively high concentrations. In this area, neonates and juveniles of the critically endangered scalloped hammerhead *Sphyrna lewini* are caught for consumption, so the examination of the accumulation of OCPs is necessary for future conservation, as well as to assess the exposure to humans. Levels and accumulation patterns of 29 OCPs were analyzed in the liver and muscle of 20 immature scalloped hammerheads. Twenty-three compounds were detected in liver and 17 OCPs were found in muscle. In the latter tissue, only p,p'-DDE presented concentrations above the detection limit in all samples (0.59 ± 0.21 ng/g w.w.), while in the liver, DDTs were also the main group of pesticides (215 ± 317 ng/g w.w.), followed by ∑Chlordanes > ∑Chlorobenzenes > Mirex > HCBD > Others. One of the two analyzed neonates presented high concentrations of OCPs in the liver (1830 ng/g w.w.), attributed to a bioamplification process. No differences in accumulation of OCPs were found between juveniles of both sexes, where an increase in the concentration of various compounds related with size and age was observed. Additionally, juveniles under 2 years of age may undergo a growth dilution process. Our results suggest that the consumption of this species does not imply risks to human health (chronic or carcinogenic effects) associated with OCPs. Likewise, we recommend further monitoring due to the possible recent inputs of some OCPs (e.g., dicofol, median of ratio o,p'-DDT/p,p'-DDT = 0.7) into the environment.

Key words: Sharks, POPs, DDT, bioamplification, growth dilution.

2.2. Introduction

Organochlorine pesticides (OCPs) were used for more than 60 years in agriculture for the control of vector diseases, pest control, and several industrial processes (El-Shahawi et al., 2010). Despite their utility, they were classified as Persistent Organic Pollutants (POPs) and their use and manufacture were banned in many countries since the last third of the 20th century (Stockholm Convention, 2017). However, OCPs are still of high concern due to their persistence in the environment and their inherent toxicity to organisms (Olisah et al., 2019). Worldwide, DDT has been the most used OCP due to its high efficiency in the elimination of public health vectors such as malaria. In addition, other OCPs such as endosulfan, drins, or hexachlorocyclohexanes (HCHs) were applied intensively and extensively in many countries (Beckvar and Lotufo, 2011; Li and Macdonald, 2005). The mismanagement of obsolete stocks, the unintended production, and their use in some countries, along with their high environmental persistence and dispersal capacity, produce their ubiquity in marine ecosystems (Koenig et al., 2013; Muñoz-Arnanz and Jiménez, 2011; Sparling, 2016). In such environments, OCPs bioaccumulate and biomagnify in food webs (Li et al., 2021). Marine predators such as sharks tend to accumulate higher concentrations of OCPs in their tissues than those at lower trophic levels, which can be a risk to the health of organisms and to humans as top consumers (Lee et al., 2015; Weijs et al., 2015). Therefore, knowledge about the levels of OCPs in shark species of ecological and economic relevance is important, particularly in areas where there are no baseline studies (Consales and Marsili, 2021; Tiktak et al., 2020).

Organochlorine pesticides have different mechanisms of action in organisms. They can act on the endocrine and immune systems, and have also been linked with different types of cancer (Alharbi et al., 2018; Mrema et al., 2013). Thus, organisms in their early stages of life are particularly vulnerable to adverse effects given it can affect their growth and increase their vulnerability to infectious diseases (Daley et al., 2014; Mrema et al., 2013). However, toxicity studies of OCPs in elasmobranchs are still scarce and inconclusive (Gelsleichter et al., 2006; Sawyna et al., 2017). Moreover, the concentrations reported in their tissues are often higher than those related to the toxic effects in other taxa (Lyons and Adams, 2014; Mull et al., 2012; Weijs et al., 2015). The concentrations of OCP

can vary between shark species, geographical distribution, habitat, sex, or life stages (Borgå et al., 2004; Lee et al., 2015). The identification of species and/or population groups prone to the exposure and accumulation of pollutants facilitates the focus of risk assessment and management efforts (Consales and Marsili, 2021; Lyons et al., 2019b). Furthermore, OCPs concentrations vary among tissues, where the lipid-rich (≈ 80%) liver of sharks is the main accumulation organ (Pethybridge et al., 2010; Tiktak et al., 2020). In elasmobranchs, liver tissue analysis is carried out to estimate the maximum load of OCPs that can eventually cause toxicity when they are distributed to other tissues i.e., blood or brain (Gelsleichter et al., 2006, 2005). In muscle tissue, the lower concentrations of OCPs are related to a low lipid content (< 2%), in which its evaluation allows the assessment of the risk to human health (Boldrocchi et al., 2019; Pethybridge et al., 2010; Tiktak et al., 2020).

The Gulf of California (GC) exhibits high abundance of elasmobranch species. Approximately 80% of shark species of the Mexican Pacific occur in the GC (Galván-Magaña et al., 2019). It is one of the most biologically productive marine regions in the world and of great importance for fisheries in Mexico (Brusca, 2010; Lluch-Cota et al., 2007). Although it is considered a relatively pristine environment, its fauna has been exposed to OCPs (Páez-Osuna et al., 2017; Ponce-Vélez and Botello, 2018), given that the coast of the GC represents more than 30% of the area destined for irrigated agriculture in Mexico (SIAP, 2020). Despite the fact that Mexico signed the Stockholm Convention in 2001, its legislation on organochlorine pesticides is scarce and ambiguous (Bejarano-González et al., 2017; Ponce-Vélez and Botello, 2018). Although the drins, chlordecone, and mirex have been banned since 1991, compounds such as DDT, lindane, chlordane, and endosulfan were restricted until 2016 for the control of some vector, agricultural or household pests (Bejarano-González et al., 2017; CICOPLAFEST, 1991, COFEPRIS, 2021). The use of dicofol is actually restricted to some agricultural applications, and the rest of the OCPs do not have any specific regulation (COFEPRIS, 2021; Ponce-Vélez and Botello, 2018). Recent contributions of OCPs such as dicofol, chlordanes, endosulfan, or HCHs to the GC has been suggested in different studies, and in some cases the concentrations reported in commercial fish still constitute a potential risk to

human health (Fossi et al., 2017; Granados-Galván et al., 2015; Ponce-Vélez and Botello, 2018; Reyes-Montiel et al., 2013). In addition, exposure to OCPs has previously been reported in protected species from the GC, such as some marine mammals and the filter feeding whale shark *Rhincodon typus* (Fossi et al., 2017, 2014; Niño-Torres et al., 2009).

The scalloped hammerhead *Sphyrna lewini* does not have special protection in Mexico despite its critically endangered status and the reduction of its landings in the GC during recent decades (Bizzarro et al., 2009; Rigby et al., 2019; Saldaña-Ruiz et al., 2017). It is one of the most caught shark species in the artisanal fishery of the GC, where mainly neonates and juveniles are captured for their meat for local and national consumption (Bizzarro et al., 2009; Saldaña-Ruiz et al., 2017). As a long-lived, slow-growing tertiary consumer that inhabits coastal environments during its juvenile stage, it is susceptible to the accumulation of high concentrations of pollutants (Anislado-Tolentino et al., 2008; Borgå et al., 2004; Rosende-Pereiro et al., 2019; Torres-Rojas et al., 2013). The accumulation of inorganic contaminants in scalloped hammerheads from the GC has been documented in several studies (Bergés-Tiznado et al., 2015; García-Hernández et al., 2007; Hurtado-Banda et al., 2012; Ruelas-Inzunza et al., 2020; Ruelas-Inzunza and Páez-Osuna, 2005). However, there is no information regarding the levels of organic pollutants in this or in other elasmobranch species of fishery importance. The aims of the present study are to: 1) determine the concentrations of organochlorine pesticides (DDTs, chlordanes (CHLO), chlorobenzenes (CBz), HCHs, endosulfan, drins, mirex, hexachlorobutadiene (HCBD) and methoxychlor) in the liver and muscle of immature scalloped hammerheads; 2) identify their accumulation patterns; and 3) evaluate the possible risk associated with OCPs due to the human consumption of the muscle of the scalloped hammerhead.

2.3. Material and methods

2.3.1. Sample collection

In this study, liver and muscle samples were collected from 20 specimens of *S. lewini* caught by artisanal fishers operating nearby three fishing camps (El

Saladito, El Manglito, and San Bruno) from the southwestern coast of the GC, Mexico (**Fig. 2.1**).

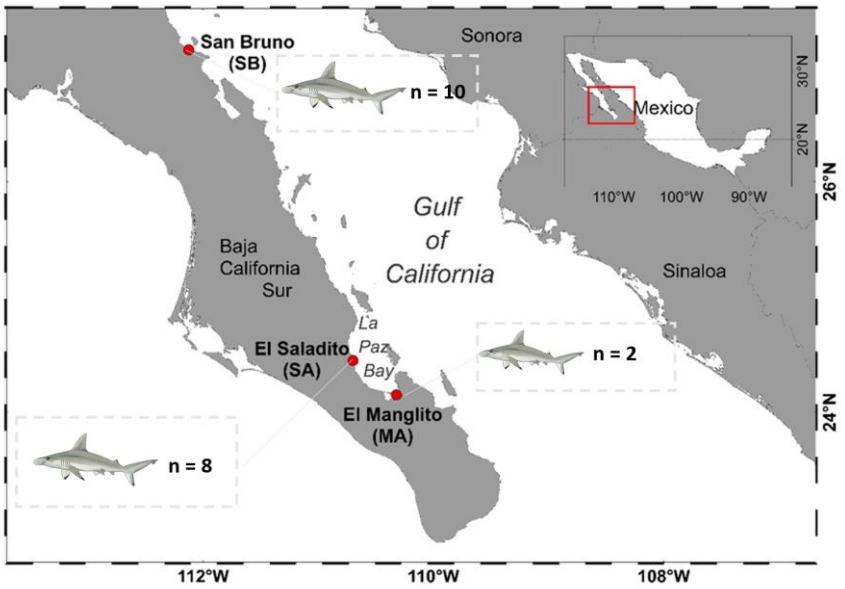


Fig. 2.1. Fishing camps locations where the HH samples were collected in Baja California Sur, Mexico.

Biological information such as total length (TL) and sex were recorded. Liver samples were collected from the posterior left lobe of the liver, while muscle samples were taken from the dorsal musculature, anterior to the first dorsal fin (Cornish et al., 2007; Lyons and Adams, 2014). Each sample was placed in a cleaned glass jar (4 h at 400 °C in a muffle furnace) with screw caps lined with pre-muffled aluminum foil. All samples (n = 40) were preserved in ice (a few hours after their collection) until later storage in a freezer at −20 °C

The obtained specimens during August 2018 in El Manglito (n = 2) were neonates according with the classification given by Duncan and Holland (2006). A total of 18 individuals caught in December 2018 in San Bruno (SB) (n = 10) and between December 2018 and February 2019 in El Saladito (SA) (n = 8) were classified as juveniles according to Bejarano-Álvarez et al. (2010). Due to the proximity of El Manglito and El Saladito, the samples collected in both fishing camps were considered as La Paz Bay (LPB) when appropriate.

Additionally, the age of the individuals was estimated according to the parameters of von Bertalanffy published by Anislado-Tolentino et al. (2008) for *S. lewini* from

the GC (females: $L\infty$ = 376 cm, K = 0.1 year-1, t_0 = -1.16 years; and males: $L\infty$ = 364 cm, K = 0.123 year-1, t_0 = 1.18 years). Sampling date, locality, sex, TL, and the age of each individual are provided in the Supplementary Material, **Table S2.1**.

2.3.2. Chemical and instrumental analyses

2.3.2.1. Determination of lipid content

Analytical procedures were conducted in the Institute of Oceanological Research (IIO) from the Autonomous University of Baja California. The lipid content of each sample was determined gravimetrically by extracting approximately 1 g w.w. (wet weight) with hexane (HPLC grade), using an accelerated solvent extractor (Thermo DIONEX ASE 350, Dionex, USA) equipped with 22 mL stainless steel extraction cells. The subsamples were homogenized with 2 g of diatomaceous earth. One piece of glass fiber filter, 4 g of white sand, the homogenate, and 3 g of diatomaceous earth were sequentially layered from the bottom of the ASE cell, filling the remaining volume with white sand. The ASE was programmed to run two static cycles at a temperature of 125 °C and a constant pressure of 1500 psi (ThermoScientific, 2012). The extracts were recovered in 60 mL glass vials and concentrated to ≈ 2 mL on a Genevac Rocket evaporator. The extracted lipids were then transferred to pre-weighed 5 mL aluminum trays and placed in the oven for the complete evaporation of the solvent. The gravimetric determination of lipids was carried out by weighting each tray until no weight variation was recorded. Additionally, the standard reference material NIST SRM-1946 (National Institute of Standards & Technology) was used to evaluate the recovery efficiency of the method.

2.3.2.2. OCPs extraction and cleanup

Approximately 0.4 g of liver tissue and 1 g of muscle tissue (w.w.) were extracted performing a single step of purification simultaneously (in-cell clean-up) by placing sorbents (activated overnight at 400 °C) inside a 34 mL ASE extraction cell. The samples were homogenized with 3 g of diatomaceous earth, adding 1 g of Dionex™ ASE™ Prep MAP Moisture Absorbent Polymer as a desiccant for the muscle samples. A fiber glass filter was placed in the bottom of the cells, which

were sequentially filled with 10 g of silica gel, a thin layer of white sand, the homogenate, and a thin layer of diatomaceous earth. In the case of the liver samples, 2 g of florisil were added in the cell before the homogenate for further purification of the fatty tissue. To fill the remaining empty part of the cell, white sand was added before spiking with recovery surrogates (TCMX and PCB_{209}, Ultra Scientific Inc.). The ASE 350 was programmed following the extraction conditions described by Galbiati et al. (2016), using a mixture of pesticide grade hexane/acetone (4:1, v/v) as extraction solvents. The extracts were collected in 250 mL glass bottles and concentrated to ≈ 1 mL on a Genevac Rocket evaporator.

The second cleaning step was performed by adsorption liquid chromatography, using activated or 3% deactivated sorbents (for muscle and liver samples, respectively) and pesticide-grade solvents. Due to the differences in their lipid content, different protocols were carried out for the purification of liver and muscle extracts. Muscle extracts were cleaned on glass columns filled with 3 g of florisil and 1 g of alumina (dry packing method). Following the conditioning of the column with 10 mL of hexane, extracts were eluted as follows: 10 mL of hexane, 20 mL of hexane/dichloromethane (70:30, v/v), and 25 mL of dichloromethane. Considering the high lipid content of liver samples, the extracts of this tissue were cleaned on a glass column (1 cm i.d., 30 cm length) packed from bottom to top with 12 cm of silica, 6 cm of alumina, and 3 cm of florisil previously immersed in hexane (slurry packing method). The column was conditioned with 20 mL of hexane and the elution volumes were: 15 mL of hexane, 40 mL of hexane/dichloromethane (70:30, v/v), and 30 mL of hexane/dichloromethane (60:40, v/v). After to the cleanup procedure, the purified extracts were concentrated on a Rocket Evaporator to ~ 1 mL, and the solvent exchanged to hexane and blown down with a gentle stream of nitrogen until it reached 0.1 mL. Prior to the instrumental analysis, the volumes were adjusted to 1 mL with hexane and the internal standards PCB_{30} and PCB_{205}) were added.

2.3.2.3. Instrumental analysis and quality control

An Agilent 7010A Triple Quadrupole GC/MS operated in Electron Ionization (EI) mode and multiple reaction monitoring (MRM) mode was used for the analyses

and quantification of 29 OCPs: DDTs (p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD and o,p'-DDD), chlordanes (heptachlor, heptachlor epoxide, oxychlordane, cis- and trans-chlordane, cis- and trans-nonachlor), chlorobenzenes (penta- and hexachlorobenzene); hexachlorocyclohexanes (α-, β-, δ-, and γ-HCH), endosulfan (α- and β- endosulfan and endosulfan sulfate), drins (aldrin, dieldrin, endrin, and endrin aldehide), mirex, hexachlorobutadiene (HCBD), and methoxychlor. The chromatographic separation was achieved by two capillary columns HP-5ms Ultra Inert (15 m x 250 µm x 0.25 µm). The oven was programmed as follows: 60 °C for 1 min, then 40 °C/min until 120 °C, and finally 5 °C/min until 285 °C for 0 min (Total time: 35.5 min). The ion source and quadrupoles were operated at 300 °C and 180 °C, respectively. The temperatures of the injection port and transfer line were set at 280 °C. Helium was used as the carrier gas at a constant flow rate of 1.4 mL/min. Helium and nitrogen were used as collision gases at a constant flow rate of 2.25 mL/min and 1.5 mL/min, respectively. Identification was performed by the retention times, masses, and relative abundance of the confirmation ions. Quantification of OCPs was done by a five-point calibration curve covering a concentration range of 1 to 100 ng/mL and using the internal standard method (PCB_{30} and PCB_{205}). MRM acquisition method was configured according to the Agilent Pesticides and Environmental Pollutants MRM database (G9250AA).

Laboratory blank samples and certified reference material (NIST SRM-1946) were extracted with each batch of 20 samples and analyzed on a regular basis. No evidence of contamination was observed in the procedural blanks. The recoveries of the certified reference material can been found in the Supplementary Material (**Table S2.2**). The limits of detection (LOD) were calculated as three times the standard deviation (SD) of seven measurements of the lowest point of the calibration curve. The range of LOD in liver was 0.23 - 8.62 ng/g w.w. and 0.09 - 3.45 ng/g w.w. in muscle. The recoveries in liver samples were 65 ± 15% (TCMX) and 98 ± 13% (PCB_{209}). The recoveries in muscle samples were 63 ± 13% (TCMX) and 93 ± 27% (PCB_{209}). The concentrations reported were not corrected for recoveries.

2.3.3. Statistical analysis

The concentrations (ng/g) of each analyte in every sample were calculated on a wet weight and lipid weight (l.w.) basis. Mean, standard deviation, and range were calculated to compare the results of OCPs residues detected above the detection limit. The non-detected (n.d.) compounds were considered as zero for purposes of the statistical analysis. Compounds were analyzed independently and also grouped in function of their chemical group as follows: ∑DDTs (p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD, and o,p'-DDD), ∑Chlordanes (heptachlor, heptachlor epoxide, cis- and trans-chlordane, cis- and trans-nonachlor), ∑Chlorobenzenes (penta- and hexachlorobenzene); ∑HCHs (α - and γ-HCH), and ∑Endosulfan (α-endosulfan and endosulfan sulfate). The ∑OCPs was calculated as the sum of all analytes detected above the detection limit and it was included for statistical analyses. Analytes and groups of compounds were used for statistical analyses when their concentrations were above the detection limit in more than 50% of the samples (Lee et al., 2015). Data were tested for normal distribution (Shapiro–Wilk). Subsequently, non-parametric tests were used to analyze differences (Wilcoxon-test) and correlations (Spearman's test) after removing the extreme outliers (data values that lied more than three times the interquartile range above the third quartile). Statistical significance was defined as $p < 0.05$. All calculations were performed using the statistical software R (version 3.6.3).

2.3.4. Human exposure risk assessment

Human health risk via consumption of scalloped hammerhead muscle was estimated according to the equations suggested by the United States Environmental Protection Agency (USEPA) for cancer and non-cancer effects. The estimated daily intake (EDI) of each OCP was calculated as follows:

$$EDI = (C \times D) / BW$$

Where C is the measured concentration of each chemical (ng/g w.w.) in muscle of *S. lewini*; D is the estimated daily intake of fish meat, and BW is the average adult body weight (females 60 kg, males 70 kg) (USEPA, 2000). The estimated daily intake of elasmobranch meat for the Mexican population is 0.98 g

(CONAPESCA, 2018). However, as shark meat is often marketed as fish fillets in Mexico (Oceana, 2019), the estimated mean of fish consumption per day can reach values of up to 35.9 g (CONAPESCA, 2018), which was also used in the estimates as an extreme value.

The Hazard Ratio (HR) for non-cancer health effects is the ratio between the EDI and the reference dose (RfD) of a given chemical:

$$HR = EDI / RfD$$

The RfD (ng/kg-day) of the different analytes are listed by the USEPA (2000). When HR > 1, potential human health risk is suggested.

The potential cancer risk (CR) was calculated according to the following equation:

$$CR = EDI \times CSF$$

Where CSF is the cancer slope factor proposed by USEPA (2000). CR < 10^{-6} is considered acceptable, CR between 10^{-6} and 10^{-4} is considered as some level of concern, and CR > 10^{-4} is considered unacceptable (USEPA, 2005).

Additionally, the Hazard Ratio for carcinogenic risk (HRcr) was calculated following the equation by Jiang et al. (2005):

$$HRcr = EDI / BMC$$

Where BMC is the benchmark concentration for cancer effects:

$$BMC = (Risk \times BW) / (Fish\ consumption \times CSF)$$

The risk is the probability of lifetime risk of cancer and is set as one in one million. The fish consumption is the daily consumption divided by the BW (0.51 g/kg-day). Values of HRcr > 1 indicate a potential human health risk (Jiang et al., 2005).

2.4. Results and discussion

2.4.1. OCPs detections, levels, and profiles

Organochloride pesticides were identified at various frequencies and levels in the liver and muscle tissue samples of the 20 immature individuals (**Table 2.1)**.

Table 2.1

Limits of detection (LOD), concentrations (mean ± SD) in ng/g w.w. and identification frequencies (IF, in %) of OCPs identified in liver and muscle tissue of 20 immature *S. lewini*. "n.d." = non detected; "b.d.l." = below detection limit. "<" indicates the limit of detection of each compound identified below it.

OCPs	LIVER			MUSCLE		
	LOD (ng/g w.w)	Concentration	IF (%)	LOD (ng/g w.w	Concentration	IF (%)
ΣDDTs		**215.2 ± 317.0**	**100**		**0.59 ± 0.21**	**100**
***p,p'*-DDT**	0.52	16.7 ± 55.5	100	0.21	n.d.	0
***o,p'*-DDT**	1.07	1.3 ± 1.1	60	0.43	< LOD	5
***p,p'*-DDE**	0.71	188.8 ± 243.7	100	0.28	0.59 ± 0.21	100
***o,p'*-DDE**	1.07	0.75 ± 0.88	45	0.43	n.d.	0
***p,p'*-DDD**	0.37	7.1 ± 24.1	100	0.15	< LOD	15
***o,p'*-DDD**	0.64	0.46 ± 0.58	40	0.26	n.d.	0
ΣCHLO		**22.7 ± 48.1**	**100**		**< LOD**	**65**
Heptachlor	0.50	0.61 ± 0.86	35	0.20	< LOD	50
Heptachlor epoxide	1.20	0.31 ± 0.63	20	0.48	n.d.	0
cis-chlordane	1.63	2.5 ± 3.8	60	0.65	< LOD	10
trans-chlordane	1.09	1.0 ± 1.4	40	0.44	< LOD	5
cis-nonachlor	8.62	12.9 ± 37.3	35	3.45	< LOD	5
trans-nonachlor	1.16	5.5 ± 6.2	100	0.47	< LOD	10
ΣCBz		**7.7 ± 4.7**	**100**		**0.02 ± 0.10**	**100**
Pentachlorobenzene	1.13	4.5 ± 1.6	100	0.45	< LOD	100
Hexachlorobenzene	1.07	3.1 ± 3.1	100	0.43	0.02 ± 0.10	100
Mirex	5.23	**11.2 ± 22.8**	**100**	2.09	**< LOD**	**40**
HCBD	1.58	**2.7 ± 0.59**	**100**	0.63	**< LOD**	**100**
ΣDrins		**1.2 ± 1.9**	**50**		**< LOD**	**5**
Endrin	0.99	< LOD	5	0.40	n.d.	0
Dieldrin	0.79	1.2 ± 1.9	50	0.32	< LOD	5
ΣHCHs		**0.76 ± 1.3**	**35**		**< LOD**	**45**
α-HCH	1.11	0.42 ± 0.67	30	0.44	< LOD	45
δ-HCH	1.08	0.34 ± 0.84	15	0.43	n.d.	0
ΣEndosulfan		**0.30 ± 0.98**	**10**		**n.d.**	**0**
α-endosulfan	1.68	0.10 ± 0.47	5	0.67	n.d.	0
Endosulfan sulphate	0.70	0.20 ± 0.89	5	0.28	n.d.	0
Methoxichlor	0.23	**< LOD**	**10**	0.09	**< LOD**	**10**
ΣOCPs		**261.8 ± 392.4**	**100**		**0.61 ± 0.29**	**100**
% Lipids		52.9 ± 10.9			0.22 ± 0.20	

A higher number of OCPs were identified in the liver compared to the muscle (23 and 17 OCPs, respectively) in which significantly higher concentrations were observed ($W = 400$, $p < 0.001$) (**Table 2.1**). This is mainly attributed to the

difference in lipid content in both tissues (liver: 52.9 ± 10.9%; muscle: 0.22 ± 0.20%) (Gelsleichter et al., 2005; Lyons et al., 2021). Nevertheless, no significant relationships were found between OCPs concentrations and the lipid contents (liver: Spearman's test, p = 0.078 – 0.80; muscle: r = 0.15, p = 0.52), as it was reported in other shark species (Chynel et al., 2021; Lyons et al., 2013).

Twenty-one of the pesticides identified in the liver were found in concentrations above the LOD. Considering all individuals, the ∑DDTs represented 81.0 ± 6.0 % of the total pesticides in this tissue, which is associated with its high capacity of bioaccumulation in lipid-rich tissue and its broad use, mainly in agriculture and in vector control (Li and Macdonald, 2005; Sparling, 2016). The second most abundant group of pesticides were the ∑Chlordanes (7.2 ± 4.9 %), followed by ∑Chlorobenzenes (4.6 ± 2.2 %), Mirex (4.5 ± 1.9 %), and HCBD (1.9 ± 1.0 %). Dieldrin, ∑HCHs, and ∑Endosulfan contributed altogether less than 1% to the ∑OCPs in the liver and presented low identification frequencies (≤ 50 %). In the neonate NN_F and the juvenile female SA_107F, hepatic concentrations of OCPs of 1,830.5 and 657.1 ng/g w.w., respectively, were detected. Thus, their liver concentrations were excluded from the statistical analysis of comparisons and correlations (Wilcoxon and Spearman's tests) in order to avoid the masking of patterns that could imply a misinterpretation of the general population (Beaudry et al., 2015; Lyons et al., 2013; Randhawa et al., 2015).

In the muscle, only p,p'-DDE (0.35 – 1.2 ng/g w.w.; min – max; n = 20) and hexachlorobenzene (0.45 ng/g w.w.; n = 1) were above the LOD (**Table 2.1**). The concentration of p,p'-DDE in the muscle was correlated with the concentration of this compound in the liver (r = 0.69, p < 0.001). However, given that all compounds in the liver were not detected in the muscle of the same individuals, the analysis of muscle tissue is not recommended as a proxy of internal organochlorines burden in *S. lewini* (Lyons et al., 2021). However, it can reflect the identity of the compounds that are contributing most to liver OCPs accumulation, and it is useful to evaluate the risk in humans associated to its consumption (Boldrocchi et al., 2019; Gelsleichter et al., 2005, Lyons et al., 2021). Additionally, it is a good indicator of the chronic exposure to organic contaminants, given it is a metabolically inert tissue whose lipid content does not fluctuate based on corporal condition and it has slow turnover rates compared to

the liver (Chynel et al., 2021; Cullen et al., 2019; Speers-Roesch and Treberg, 2010).

2.4.1.1. Differences between sampling areas

Juvenile scalloped hammerheads were captured (see **Fig. 2.1**. and Supplementary Material, **Table S2.1**) in two localities: San Bruno (SB: n = 10; 5 females, 5 males) and El Saladito (SA: n = 8; 4 females, 4 males). No statistical differences were found by age (W = 34; p = 0.62) or size (W = 28.5; p = 0.33) between individuals of both zones. However, higher concentrations of ∑Chlordanes (W = 13, p = 0.033) in the liver tissue of juveniles from SA were observed even excluding the individual SA_107F. In addition, significant differences in both zones were found between concentrations of p,p'-DDE (W = 14, p = 0.021) in the muscle tissue (**Fig. 2.2a**). The relative contribution percentages of chlordanes to ∑OCPs in the liver of the individuals captured in SB (4.6 ± 1.7%) were lower (W = 11, p = 0.019) than those of SA (8.0 ± 4.3%) (**Fig. 2.2b**). Due to the high residence of juvenile scalloped hammerhead in feeding and refuging coastal areas (Holland et al., 1993; Rosende-Pereiro and Corgos, 2018; Torres-Rojas et al., 2013), the differences found in OCPs levels and profiles could indicate local variations in exposure to these contaminants. Nevertheless, the complex migratory behavior of the scalloped hammerhead and their predation on migratory species make difficult to consider this species as a reliable bioindicator of a discrete geographic zone (Hoyos-Padilla et al., 2014; Torres-Rojas et al., 2013).

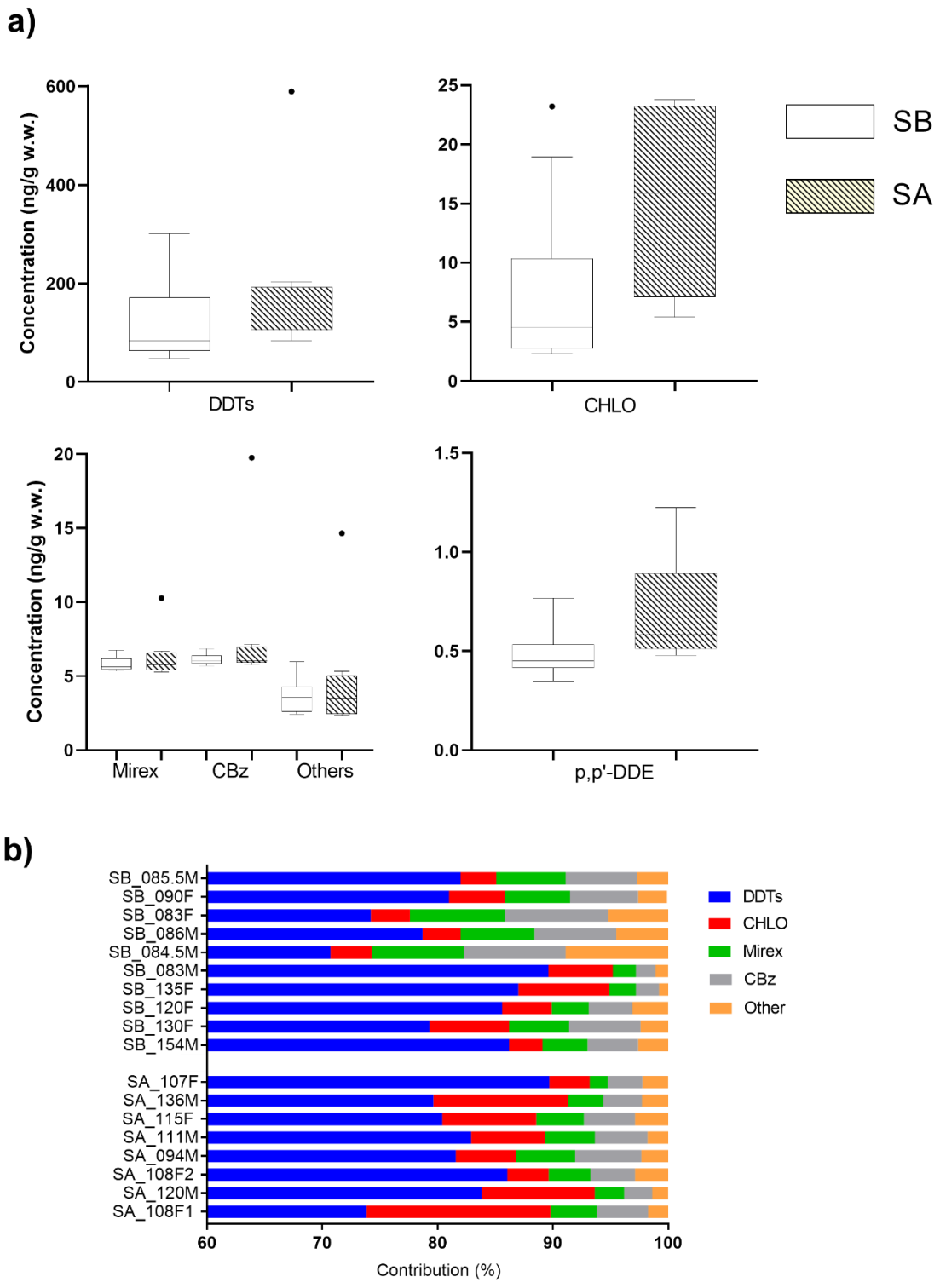


Fig. 2.2. a) Concentrations (in ng/g w.w.) of DDTs, chlordanes (CHLO), mirex, chlorobenzenes (CBz) and the sum of HCBD, dieldrin and HCHs (Others) in liver tissue and p,p'-DDE concentrations (ng/g w.w.) in muscle tissue of juveniles of *S. lewini* (SB: n = 10, SA: n = 8). The lower and upper ends of boxplot represent the 25th and 75th percentiles; the line indicates the median, while that the lower and

upper whiskers represent the minimum and maximum concentrations, respectively, excluding outliers. Outliers are represented with a black circle. **b)** Contribution (in %) of DDTs, chlordanes (CHLO), mirex, chlorobenzenes (CBz) and the sum of HCBD, dieldrin and HCHs (Others) to ∑OCPs in liver tissue of juveniles of scalloped hammerhead shark (n = 18). The code of each individual indicates the sampling place, the total length (TL, in cm) and sex (female: "F", male: "M").

2.4.1.2. DDTs

The levels of DDTs in the liver (215.2 ± 317.0 ng/g w.w.) and muscle (0.59 ± 0.21 ng/g w.w.) tissues observed in our work are below the average of concentrations (liver: 19500 ± 37100 ng/g w.w.; muscle: 10 ± 14 ng g/g w.w.) reported for worldwide elasmobranch species (Tiktak et al., 2020). These concentrations are highly variable between species and geographical distribution, evidencing important differences in species-specific accumulation (related with trophic level, metabolism, lipid content, etc.) and exposure. In addition, numerous factors such as sample size, population groups included or excluded from the analysis, and period of sampling, could influence in these variations and difficult the comparisons across studies (Genov et al., 2019).

The levels of DDTs in the liver tissue were 2011 ± 1188 ng/g l.w. in four young-of-the-year (YOY) scalloped hammerheads captured between 2006-2011 in Florida, U.S.A. (Lyons and Adams, 2014). Moreover, concentrations of DDTs were 406 ± 556 ng/g l.w. in the same tissue of six immature individuals captured in 2016-2017 from the Gulf of Aden (Boldrocchi et al., 2019). The similarity of the DDTs values between the Gulf of Aden and the present study (443 ± 702 ng/g l.w. n = 20), as well as the high concentrations reported for the YOYs in Florida, could be evidence of the global tendency of the decrement in the environmental OCPs concentrations since their regulation (Rigét et al., 2019). Nonetheless, it cannot be discarded that the sharks in Florida had been exposed to higher environmental levels of these contaminants. It is important to note that the current exposure of *S. lewini* by DDTs in the GC is comparable to that reported in other regions where the use of legacy chemicals has not been heavily regulated (Boldrocchi et al., 2019).

On a regional scale, DDTs concentrations in sharks were previously evaluated in skin biopsies of juvenile whale sharks (n = 12) from LPB. The levels in whale sharks (1.31 ± 1.76 ng/g w.w.) were higher than those found in the muscle tissue of the scalloped hammerheads (0.68 ± 0.24 ng/g w.w.) from the same locality (Fossi et al., 2017). It could be that the migratory, large, filter-feeding whale sharks have a greater chronic exposure to organic pollutants in the GC than the immature scalloped hammerhead. However, caution should be exercised in the comparisons between different matrices (skin biopsies vs. muscle), particularly when their lipid content is not reported (Boldrocchi et al., 2021; Genov et al., 2019). The information regarding OCPs in the GC has been primarily carried out in marine commercial species of the continental coast due to their extensive use in agriculture (Páez-Osuna et al., 2017; Ponce-Vélez and Botello, 2018). The concentrations of DDTs in the muscle of *S. lewini* were lower than the average concentration reported in the muscle of mullets (*Mugil cephalus*: 5.8 ng/g w.w) and snappers (*Lutjanus* spp.: 6.64 ng/g w.w.) in the continental coast of the GC, which is associated with its frequent use in potentially more contaminated environments (Borgå et al., 2004; Granados-Galván et al., 2015; Reyes-Montiel et al., 2013). In addition, the lean muscle of the scalloped hammerhead could be less prone to the accumulation of organochlorines than the muscle tissue of some teleost, due to the inability of elasmobranchs to oxidize lipids in that tissue (Lyons et al., 2021; Speers-Roesch and Treberg, 2010). Regarding the comparison with other top predators in the GC, the lipid weight concentrations of DDTs in the liver tissue of *S. lewini* were lower than those reported in the fatty tissue of sea lions (*Zalophus californianus*: 2300 ± 1600 ng/g l.w.) and odontocetes (whose average concentrations ranging on 1558 ± 818 ng/g l.w. in *Tursiops truncatus* and 557083 ± 372204 ng/g l.w. in *Orcinus orca*) (Fossi et al., 2014; Niño-Torres et al., 2009). Generally, marine mammals are more susceptible to the accumulation of OCPs due to their biological features (i.e., subcutaneous fat, pulmonary respiration, higher maternal transfer) (Borgå et al., 2004; Kelly et al., 2007; Lyons and Adams, 2014).

The p,p'- isomers of DDT and its metabolites were identified in all liver samples and had a predominance over the o,p'-isomers: p,p'-DDE (92.3 ± 6.0 %) > p,p'-DDT (4.0 ± 4.0 %) > p,p'-DDD (1.7 ± 1.9 %) > o,p'-DDT (1.2 ± 1.2 %) > o,p'-DDE

(0.5 ± 0.7 %) > o,p'-DDD (0.4 ± 0.5 %). The formulation of commercial DDT consists of a mixture of various compounds, mainly p,p'-DDT (≈ 77%) and o,p'-DDT (≈ 15%) (WHO, 1989). Once in the environment, p,p'-DDT eventually degrades to p,p'-DDD and its most persistent metabolite: p,p'-DDE (ATSDR, 2019; Ricking and Schwarzbauer, 2012). In general, the (p,p'-DDE + p,p'-DDD)/p,p'-DDT ratios are frequently used to estimate if there is recent application of the pesticide (Muñoz-Arnanz and Jiménez, 2011). Typically, ratios higher than 1, such as the interval observed in the liver of the scalloped hammerheads (4.8 - 57.3), suggest that the DDT contributions are not recent. Nevertheless, this information needs to be viewed with caution due to the numerous factors that influence in the persistence of technical DDT, both environmental and biological (including the metabolism of the sharks themselves) (ATSDR, 2019).

On the other hand, the ratio (o,p'-DDT/p,p'-DDT) is used to estimate if the determined residuals correspond to the recent application of dicofol, an organochlorine pesticide that is synthesized from technical DDT (Muñoz-Arnanz and Jiménez, 2011; Qiu et al., 2005). In the formulation of dicofol, the ratio (o,p'-DDT/p,p'-DDT) is higher than the observed for technical DDT (3 – 7, 0.2 – 0.3, respectively) (Eng et al., 2016; Pozo et al., 2017). Since the o,p'-DDT shows a shorter half-life in the environment than p,p'-DDT, it can be said that the greater this ratio is, the higher the contribution of DDT by dicofol (Muñoz-Arnanz and Jiménez, 2011). Except liver samples in which o,p'-DDT was no detected (n = 8), the median of the ratios was 0.74 (0.2 – 1.5; min – max; n = 12), which suggests a DDT contribution related to dicofol. This pesticide is still being used in Mexico, unlike other countries where its use and fabrication had been prohibited before its inclusion to the Stockholm Convention in 2019 (Stockholm Convention, 2017, 2019). In the present study, the contribution by dicofol was observed in the sharks collected in both areas. In the liver tissue of the juveniles from SB, the (o,p'-DDT / p,p'-DDT) ratios had a median value of 0.9 (0.4 – 1.5; min – max; n = 5); while the median for juveniles from SA was 0.7 (0.6 – 0.7; min – max; n = 6). As reported by Fossi et al. (2017) in their analysis of whale shark biopsies from LPB, the analyzed sharks could be exposed to dicofol coming from its application in the marinas and tourist complexes in La Paz City, Baja California Sur. However, an input by atmospheric transport from surrounding regions cannot be discarded,

given that elevated atmospheric concentrations of this pesticide have been recorded and suggest its recent agricultural application (Rauert et al., 2018). Likewise, the exposure to this compound could be related to different sources of contamination, by considering the dispersal capacity of dicofol and the mobility of juvenile scalloped hammerheads through the GC (Hoyos-Padilla et al., 2014; Li et al., 2015).

2.4.1.3. Chlordane compounds

In the case of chlordanes (22.7 ± 48.1 ng/g w.w.), the trans-nonachlor was the only compound quantified in all hepatic samples and of highest contribution to the ∑Chlordanes (52.7 ± 32.6%). The following compound in terms of contribution was cis-nonachlor (22.5 ± 31.7%), whose identification frequency was low (35%), but presented higher concentrations in those individuals in which it was detected (12.1 – 19.3 ng/g w.w.; min - max). The cis-chlordane represented 13.4 ± 15.0 %, followed by the trans-chlordane (5.2 ± 8.1 %) > heptachlor (4.1 ± 8.9%) > heptachlor epoxide (2.2 ± 5.2%).

About 150 different compounds can be identified in a technical preparation of chlordane, in which the most abundant are cis-chlordane (≈ 15%) and trans-chlordane (≈ 15%), followed by trans-nonachlor (≈ 10%), heptachlor (≈ 4%), and cis-nonachlor (≈ 4%) (USEPA, 1997). The chlordane isomers are less persistent than the isomers from nonachlor and they usually metabolize rapidly into oxychlordane, with a very high persistence and capacity to biomagnify (ATSDR, 2018; Beckvar and Lotufo, 2011). Nonetheless, the biotransformation capacity of cis- and trans-chlordane is practically exclusive of mammals and birds, which would explain the absence of oxychlordane in the tissues of the scalloped hammerhead (Fisk et al., 2002, 2001). Given immature individuals of *S. lewini* feed mainly on fishes and invertebrates, oxychlordane does not tend to incorporate through the diet as has been reported in other shark species (Fisk et al., 2002; Rosende-Pereiro et al., 2019; Torres-Rojas et al., 2010).

Apart from the differences in chlordane concentrations in liver samples (**Fig. 2.2**) between SB and SA, discrepancies were also observed in the accumulation patterns of metabolites from this group (**Fig. 2.3)**. In addition, it was found that the contribution of cis-chlordane to ∑Chlordanes was greater (W = 16.5, p =

0.032) in juveniles of SA (20.1 ± 15.8%) than in those observed in SB (7.9 ± 14.5%), suggesting differences in the exposure to chlordanes between the studied localities. For more than 30 years, chlordane was only authorized for its use in the control of termites in Mexico (Granados-Galván et al., 2015), which would explain its high distribution and incidence of its main primary molecules in urban centers with large populations.

Moreover, the presence of heptachlor in the liver (IF = 35%) and muscle (IF = 50%) of *S. lewini* suggest its possible recent application, either as part of the formulation of chlordane or as a pesticide. This is because heptachlor is one of the OCPs that degrades more rapidly in aquatic environments, in which studies requiring approximately two weeks to degrade to heptachlor epoxide, and three weeks to disappear completely (Granados-Galván et al., 2015).

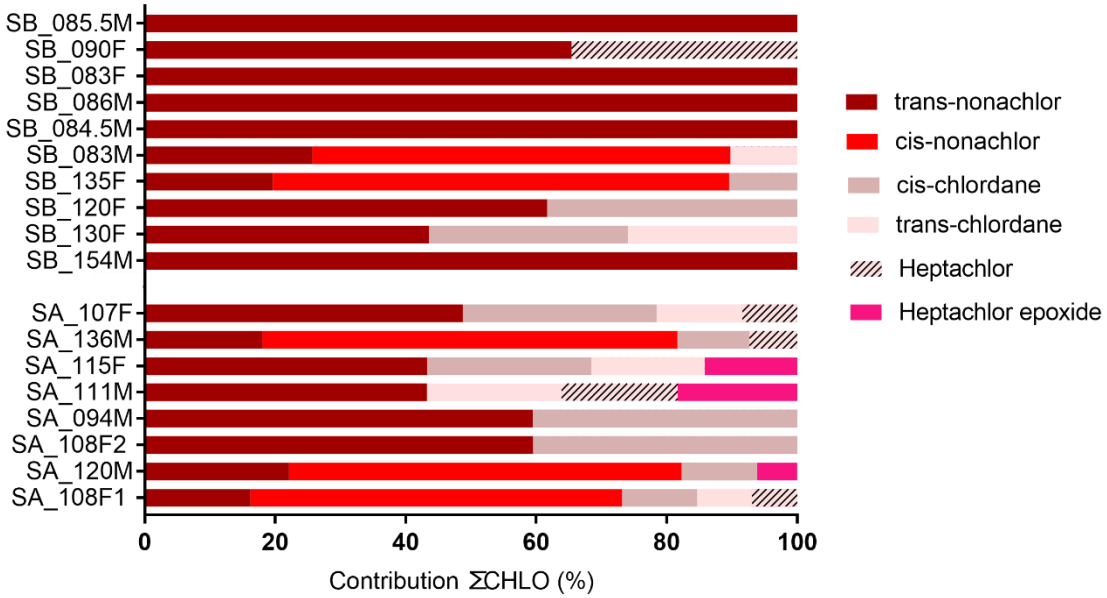


Fig. 2.3. Contribution (in %) of chlordanes related compounds to ∑Chlordanes in liver tissue of juveniles scalloped hammerheads (n = 18). The code of each individual indicates the sampling place, the total length (cm) and sex (female: “F”, male: “M”).

Moreover, the presence of heptachlor in the liver (IF = 35%) and muscle (IF = 50%) of *S. lewini* suggest its possible recent application, either as part of the formulation of chlordane or as a pesticide. This is because heptachlor is one of

the OCPs that degrades more rapidly in aquatic environments, in which studies requiring approximately two weeks to degrade to heptachlor epoxide, and three weeks to disappear completely (Granados-Galván et al., 2015).

2.4.1.4. Other OCPs

HCH was commercialized as a mixture of approximately 60 – 70% α-HCH; 5 – 12% β-HCH; 10 – 15% γ-HCH; 6 – 10% δ-HCH, as well as other minor compounds (Jackovitz and Hebert, 2015). Additionally, γ-HCH (lindane) was also marketed as a broad-spectrum pesticide. Commonly, α-HCH and lindane degrade to β-HCH, being the latter, the major compound detected in marine biota (i.e., in the fatty tissue of sea lions) due to its high resistance to enzymatic degradation (Niño-Torres et al., 2009; Phillips et al., 2005). Therefore, the absence of β-HCH in the scalloped hammerheads analyzed in our work could suggest that the organisms were recently exposed to the technical HCH. Within the drins, which were prohibited more than 30 years ago in Mexico, the only compound quantified above the detection limit in the scalloped hammerhead was dieldrin (SB = 1.3 ± 0.4 ng/g w.w., n = 4; LPB = 3.0 ± 2.6 n/g w.w., n = 6), whose presence could be associated to its elevated persistence and bioaccumulation capacity (Ponce-Vélez and Botello, 2018; Sparling, 2016). The elevated identification frequency of mirex (70%), chlorobenzenes (100%), and hexachlorobutadiene (100%), whose applications were mainly industrial, could be attributed to their environmental persistence and to the use of old manufacturing materials. Chlorobenzenes and hexachlorobutadiene can be produced non-intentionally during combustion processes (ATSDR, 2021, 2015b).

2.4.2. Influence of biological parameters on the accumulation of OCPs

The concentrations of distinct groups of OCPs in liver and muscle tissue by life stage and sex are reported in **Table 2.2**. In the female neonate (NN_F), concentrations of pesticides in the liver were quantified up to two orders of magnitude higher (in the case of mirex) than the male neonate (NN_M) and the juveniles. The juvenile female (SA_107F) evidenced concentrations of pesticides in the liver considerably higher compared to the rest of the juveniles, with extreme values of p,p'-DDE, p,p'-DDT, p,p'-DDD, trans-nonachlor, mirex, pentachlorobenzene, hexachlorobenzene, and hexachlorobutadiene.

Table 2.2

Hepatic concentrations of the organochlorine pesticide groups, and muscle concentrations of p,p'-DDE (mean ± SD) detected in neonates (n = 2), the juvenile SA_1071F, and the rest of juveniles (females: n = 8; males: n = 9) captured in La Paz Bay and San Bruno. Concentrations are expressed in ng/g wet weight and lipid weight (in parentheses). "n.d." = not detected

	Neonates		**Juveniles**		
OCPs	NN_F (n = 1)	NN_M (n = 1)	SA_107F (n = 1)	Females (n = 8)	Males (n = 9)
LIVER					
ΣDDTs	1457.7 (3250)	102.1 (226.8)	589.3 (1053)	123.2 ± 63.5 (265.0 ± 171.4)	129.9 ± 79.6 (245.4 ± 180.2)
ΣCHLO	223.3 (497.9)	31.9 (70.9)	23.1 (41.3)	10.7 ± 8.1 (23.9 ± 20.3)	10.0 ± 8.7 (20.4 ± 23.7)
ΣCBz	22.7 (50.6)	6.1 (13.5)	19.8 (35.3)	6.3 ± 0.50 (12.95 ± 2.71)	6.0 ± 0.33 (11.3 ± 3.3)
Mirex	107.8 (240.3)	7.7 (17.1)	10.3 (18.3)	5.9 ± 0.60 (12.2 ± 3.1)	5.8 ± 0.47 (10.8 ± 3.6)
HCBD	4.7 (10.4)	2.4 (5.3)	4.0 (7.2)	2.6 ± 0.19 (5.2 ± 1.0)	2.5 ± 0.15 (4.6 ± 1.3)
Dieldrin	5.9 (13.1)	n.d.	6.8 (12.1)	0.65 ± 0.73 (1.3 ± 1.6)	0.58 ± 0.71 (1.1 ± 1.4)
ΣHCHs	4.5 (10.0)	1.9 (4.1)	3.9 (7.0)	0.31 ± 0.57 (0.57 ± 1.1)	0.28 ± 0.55 (0.52 ± 1.0)
ΣEndosulfan	4.0 (8.9)	n.d.	n.d.	n.d.	0.23 ± 0.70 (0.38 ± 1.2)
ΣOCPs	1830.5 (4081.4)	152.1 (337.6)	657.1 (1186.2)	149.5 ± 69.3 (316.8 ± 182.1)	155.4 ± 86.7 (286.9 ± 196.0)
% Lipids	44.9	45.1	56.0	50.3 ± 10.4	56.7 ± 12.1
MUSCLE					
p,p'-DDE	0.75 (289.3)	0.48 (1044)	0.58 (422.8)	0.57 ± 0.13 (297.2 ± 158.6)	0.59 ± 0.29 (430.2 ± 316.4)
% Lipids	0.26	0.05	0.14	0.30 ± 0.29	0.17 ± 0.08

2.4.2.1. Neonates

Neonate sharks presented lower lipid content in the liver (44.9 ± 0.1%) than the juveniles (53.8 ± 11.1%), but the differences were not statistically significant ($W = 6$, $p = 0.17$). No relationship was observed between the percentage of lipids and length ($r = -0.19$, $p = 0.41$) or age of the individuals ($r = -0.18$, $p = 0.46$). The NN_F and NN_M had a considerable number of organochlorine pesticides in the hepatic tissue (18 and 16 OCPs identified, respectively). The scalloped hammerhead is a placental viviparous species that depends largely on maternal provisions during the first months of life (Rosende-Pereiro et al., 2019). However, it has the capacity to predate over crustaceans and small fish since its birth (Rosende-Pereiro et al., 2019). Based on the criteria of Duncan and Holland (2006), it was estimated that the male neonate was less than five days old, so its levels of OCPs are almost entirely attributed to those acquired by maternal transfer. Conversely, it is possible that the load of contaminants observed in the female neonate (5-14 days old) reflect the highest degree of incorporation of these compounds via feeding and bioconcentration, in addition to those acquired by maternal transfer.

It has been reported that the magnitude of the maternal transfer of organic compounds in sharks depends on numerous factors related to the biology and ecology of pregnant females (Borgå et al., 2004; Lyons et al., 2013; Lyons and Adams, 2014; Williams et al., 2020). Therefore, the pups from different litters could present significant differences in the concentrations of OCPs in the liver (Lyons and Adams, 2014). However, this does not seem to explain the large difference between the female neonate and the rest of the analyzed individuals from the present study (**Table 2.2**). If so, the mother of this individual would have the highest levels of DDTs reported for the species, if we consider the maternal transfer rate suggested by Lyons and Adams (2014). A possible explanation is that this individual (NN_F) could have undergone a process of bioamplification of contaminants. This process occurs when an organism loses corporal mass at a higher speed than it can eliminate chemicals, and it has been documented in invertebrates, teleosts, mammals, and birds during fasting periods, malnutrition, or high energy requirements (Daley et al., 2014). A considerable weight loss has been reported in a broad percentage of neonates of *S. lewini* caused by their

limited predator success (Duncan and Holland, 2006; Lowe, 2002), which could produce the bioamplification of pollutants in the liver. Additionally, a potential distribution of these pollutants from storage tissue to organs and other tissues (e.g., blood, brain) could be of concern due to the consequences it could have on the health of this species (Daley et al., 2014). However, more research would be needed to confirm this hypothesis, as no evidence of starvation was examined in this individual during sampling. The levels of ∑DDTs and ∑Chlordanes in the NN_F (1560 ng/g w.w.) were higher than those related to immunotoxic effects (314.5 ± 110.3 ng/g w.w.) in the round stingray *Urobatis halleri* (Sawyna et al., 2017). The concentrations of DDTs in this individual were also higher than the threshold values suggested for adverse effects (700 ng/g w.w.) in fish in early life stages (Beckvar et al., 2005).

Both neonates presented similar hepatic profiles of DDTs and chlordanes between them, but different than the juveniles (**Fig. 2.4**). The p,p'-DDE had a lower contribution to the ∑DDTs in neonates (75.7 ± 0.1%) than in juveniles (94.1 ± 2.1%); cis-nonachlor was the main compound in the contribution to ∑Chlordanes in neonates (67.9 ± 10.6%), while trans-nonachlor was the main contributor in juvenile sharks (57.0 ± 31.4%).

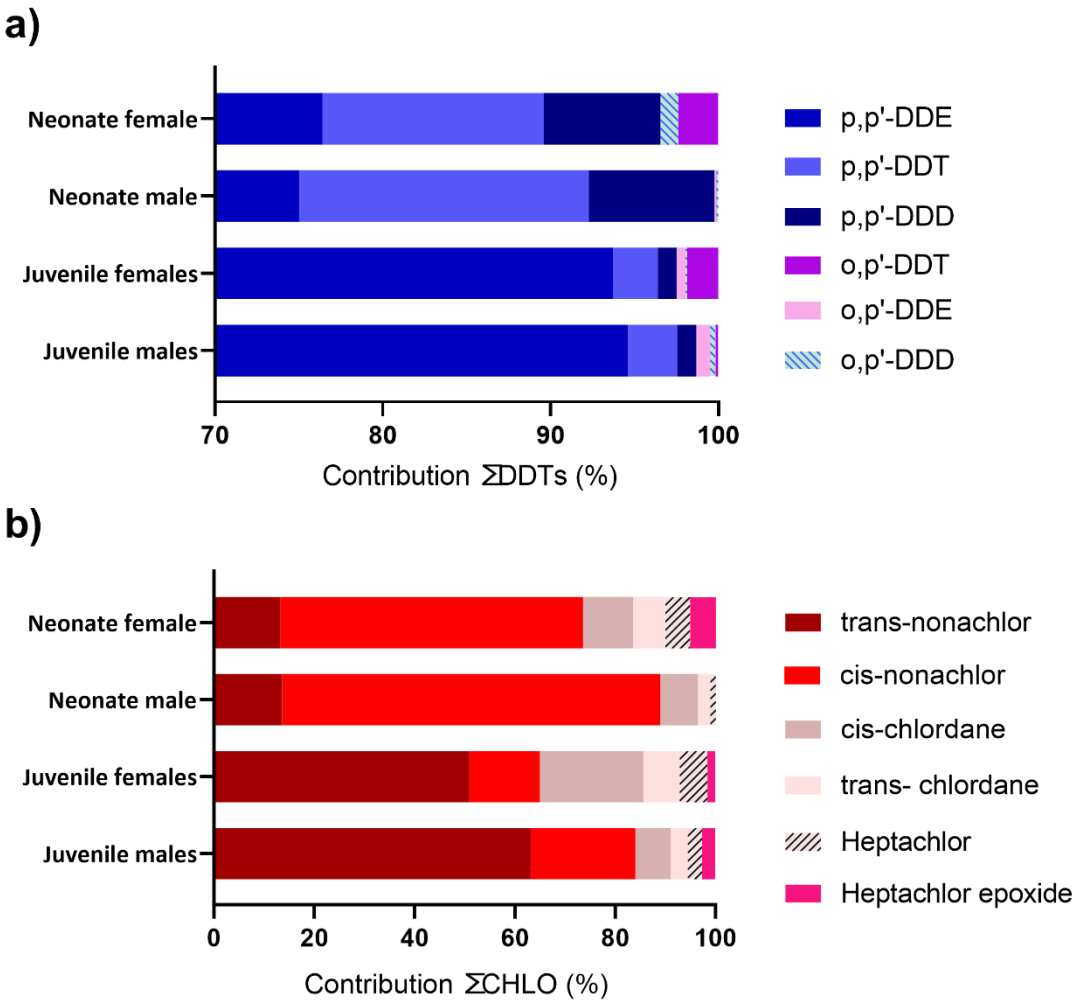


Fig. 2.4. Contribution (in %) of related compounds to ∑DDTs **(a)** and ∑Chlordanes **(b)** in liver tissue of both neonates and juveniles of *S. lewini* (n = 20).

It has been reported that the least recalcitrant compounds (i.e., with a lower lipophilicity and higher biotransformation rate) are those transferred in a greater proportion to embryos during maternal off-loading processes, while the most lipophilic tend to accumulate in the mother's liver (Lyons and Lowe, 2013; Pedro et al., 2017; Verreault et al., 2006;). This would explain the lower proportion of p,p'-DDE (W = 0, p = 0.011) and trans-nonachlor (W = 0, p = 0.026) in neonate individuals, as both compounds are more lipophilic than the rest of their congeners (Ricking and Schwarzbauer, 2012; Simpson et al., 1995).

2.4.2.2. Juveniles

2.4.2.2.1. Influence of sex on OCPs accumulation

In regards to male and female juveniles, no significant differences were observed in the accumulation of OCPs (wet weight basis) in the liver (DDTs: W = 36, p = 1; CHL: W= 31, p = 0.67; CBz: W = 28, p = 0.48; Mírex: W = 36, p = 1; HCBD: W = 30, p = 0.61) or muscle (p,p'-DDE: W = 33, p = 0.55), since sharks of both sexes have the same trophic level and feed on similar prey in the GC (Rosende-Pereiro et al., 2019; Torres-Rojas et al., 2013, 2010).

The elevated concentrations in the juvenile SA_107F and the variations in the accumulation of the groups of OCPs concentrations among juveniles of the same sex (**Table 2.2**) and size (**Fig. 2.2b**; **Fig. 2.3**) could be explained by: 1) differences in the trophic level and habitat use among immature individuals of the same population (Torres-Rojas et al., 2013); 2) metabolic differences that affect the biotransformation and elimination of organic compounds (Borgå et al., 2004); or 3) different degrees of exposure through maternal transfer that could result in variations in the accumulation of OCPs since birth (Lyons et al., 2013).

2.4.2.2.2. Influence of size and age on OCPs accumulation

A moderate relationship was observed between the total length of juveniles and the concentration increase of some OCPs (**Fig. 2.5**). When analyzing the relationship between the age of juveniles and the concentration of pesticides, a moderate correlation was observed only in the case of cis-chlordane ($r = 0.53$, $p = 0.027$) and hexachlorobenzene ($r = 0.72$, $p = 0.0012$) in the liver, as well as with p,p'-DDE in the muscle ($r = 0.48$, $p = 0.042$). However, due to the correlation between age and body size ($r = 0.91$, $p < 0.001$) deciphering the impact of each variable individually is difficult. The trend in the increasing concentrations in the sharks suggests that the elimination rate of these compounds is lower than the uptake rate, resulting in their accumulation over time (Borgå et al., 2004). In addition, many factors related with organism's increase in body size and age could influence the bioaccumulation process. The increase in body size produces a reduction in the surface – volume ratio and the respiration rate, which leads to a decrease in the elimination rate of pollutants by direct exchange with water (Borgå et al., 2004). Conversely, variations in habitat use or the increase in the

trophic level of juveniles hammerhead at larger sizes could produce an increment to OCPs exposure (Borgå et al., 2004; Torres-Rojas et al., 2013). Moreover, changes in dietary assimilation and ingestion rate related with growth could influence the bioaccumulation of pollutants (Zhang and Wang, 2007).

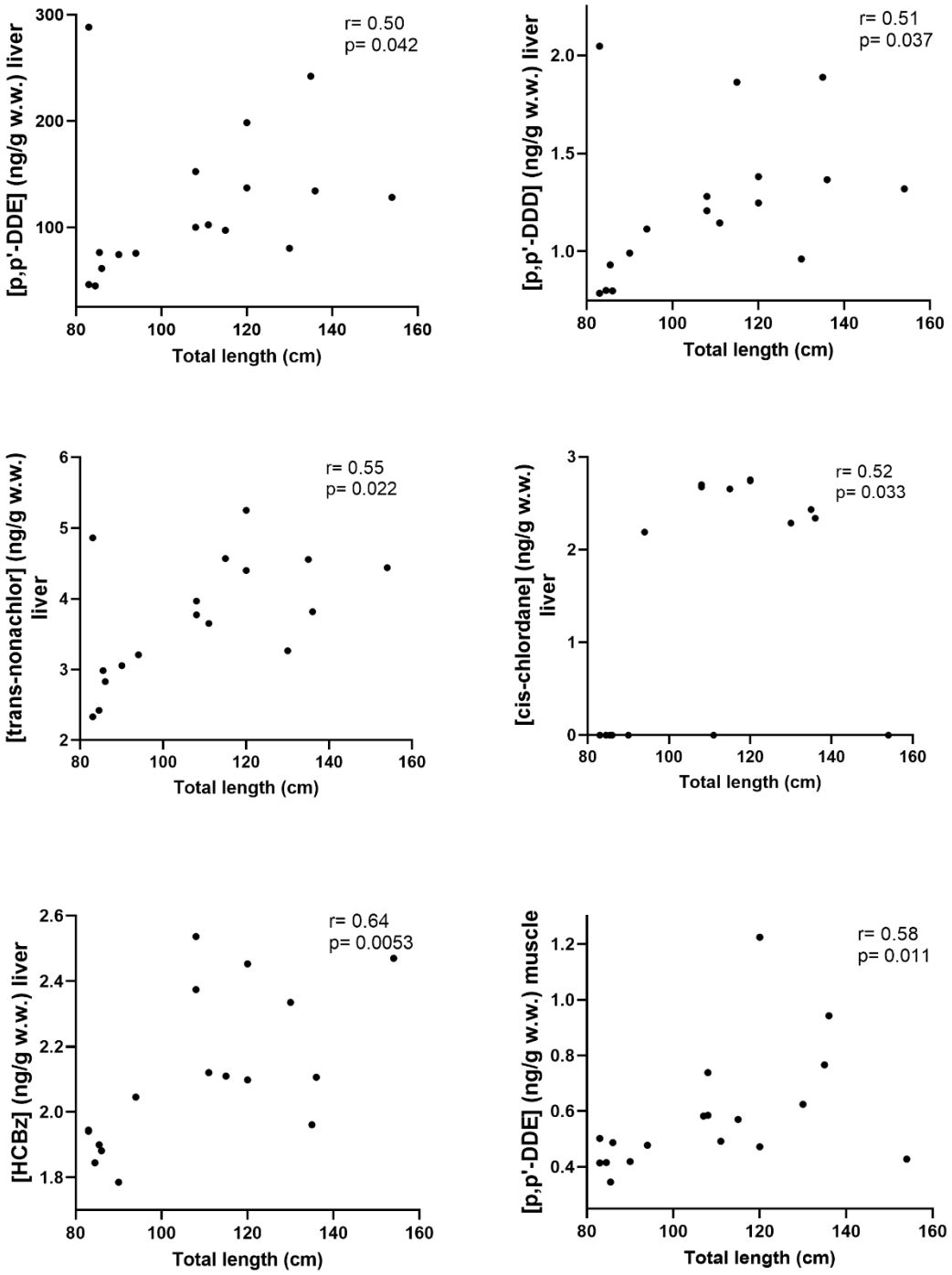


Fig. 2.5. Significant relationships between size (cm) and liver concentrations (ng/g w.w.) of the different compounds analyzed in juveniles of *S. lewini* (n = 17).

A significant increase was observed in the ratio (p,p'-DDE + p,p'-DDD)/p,p'-DDT) with length and age ($r = 0.79$, $p < 0.001$, in both cases) of the individuals analyzed in this study (Supplementary Material **Fig. S2.1**). The values of this ratio increase during the transport of DDTs through the trophic web (Borrell et al., 1995). An increment in the trophic level with the size of juvenile scalloped hammerheads has been previously suggested (Torres-Rojas et al., 2013), which would explain the relation between this and the increment in the ratio. Additionally, the metabolic activity of organisms could increase with the exposure to contaminants, resulting in higher concentrations of metabolites in the liver (Borrell and Aguilar, 1987). In this manner, the increment in trophic level and the high exposure to pesticides with size (**Fig. 2.5**) and age would produce an increase in the concentrations of the metabolites with respect to the original compound.

2.4.2.2.3. Growth dilution

During the first year of life of sharks, there is a decrease in the content of liver lipids as a result of the consumption of energy reserves provided by maternal nutrition (Lyons and Adams, 2014; Olin et al., 2013). This contrasts with the observed in juveniles ≤ 1 year (64.6 ± 11.2%) vs neonates (45.0 ± 0.14%), although the differences were not significant ($W = 8$, $p = 0.13$). This is possibly related to the fact that the juveniles ≤ 1 years would have refilled the energetic reserves spent during the first months of life as a consequence of their predatory success (Rosende-Pereiro et al., 2019). Moreover, the hepatic lipid content in juveniles < 2 years was significantly superior ($W = 13$, $p = 0.0015$) than that of the juveniles > 2 years. In addition, significant differences were observed ($p < 0.05$) in the concentration in lipid bases of all compounds, except for p,p'-DDT ($W = 50$, $p = 0.20$), between juveniles < 2 years and juveniles > 2 years (**Fig. 2.6**). Considering the OCPs concentrations in lipid bases of the NN_M (Fig. 5) and based on previous studies on juvenile sharks (Olin et al., 2013) it is possible that juveniles of *S. lewini* < 2 years experiment a process of growth dilution due to their elevated growth rates (Anislado-Tolentino et al., 2008). The diet of juveniles, which is mainly composed of benthic invertebrates and small species of fish (Rosende-Pereiro et al., 2019; Torres-Rojas et al., 2013), could present lower levels of OCPs with respect to those obtained through maternal reserves, which would produce a decrease in their concentration (Borgå et al., 2004). After this

stage (>2 years), the reduction in their growth rates (Anislado-Tolentino et al., 2008), as well as ontogenic variations in the diet because of their predatory abilities, speed, force, or the size of their mandible (Rosende-Pereiro et al., 2019; Torres-Rojas et al., 2013), could result in a greater accumulation of OCPs. Nevertheless, a larger sample size of neonates would be necessary to confirm this hypothesis.

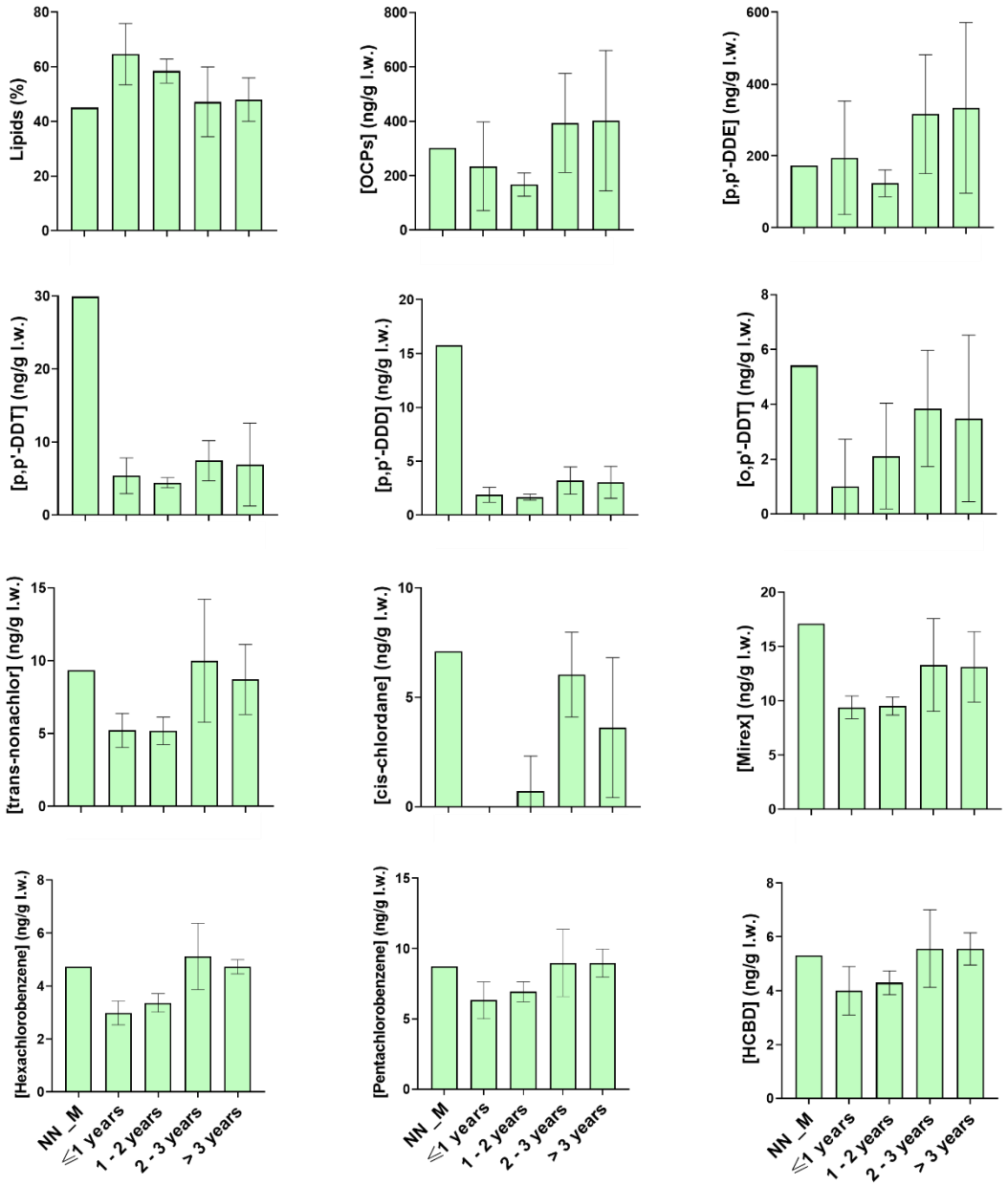


Fig. 2.6. Mean (± SD) of liver lipid content and OCPs concentrations (ng/g l.w.) of the neonate male (NN_M) and juveniles of *S. lewini* per age class: ≤ 1 year (n = 3), 1 – 2 years (n = 5), 2 – 3 years (n = 6), > 3 years (n = 3).

2.4.3. Human health assessment

The maximum concentration of p,p'-DDE (1.2 ng/g w.w.) and hexachlorobenzene (0.45 ng/g w.w.) in the muscle of *S. lewini* were below the concentration limits in fish established by international norms (2,000 - 5,000 and 100 - 500 ng/g, respectively) (FAO, 1983). Due to the low incidence of hexachlorobenzene in muscle tissue, no health risk estimations were carried out for the consumption of this compound. Conversely, given that the concentrations of p,p'-DDE in muscle were significantly different (W = 19; p = 0.019) between individuals captured in LPB and SB, the values of HR, HRcr, and CR were calculated considering the average per site (0.68 and 0.49 ng/g w.w., respectively) (**Table 2.3**). The values of HR for non-cancerous and cancerous effects were < 1, as well as CR values that were within the acceptable range (< 10^{-6}). Thus, the consumption of the muscle tissue of immature scalloped hammerheads does not imply health risks to the population associated to OCPs.

Table 2.3

Hazard ratio for non-cancer (HR) and cancer (HRcr) risk, and cancer risk factor (CR) calculated from p,p'-DDE concentrations in muscle tissue of *S. lewini* (from San Bruno and La Paz Bay). It was considered the average body weight of females and males, as well as the daily intake of elasmobranch and fish in Mexico.

Daily Intake	Population	Region	HR	CR	HRcr
Elasmobranch	Females	SB	1.6×10^{-5}	2.7×10^{-9}	7.4×10^{-7}
		LPB	2.3×10^{-5}	3.9×10^{-9}	1.6×10^{-6}
	Males	SB	1.4×10^{-5}	2.3×10^{-9}	4.7×10^{-7}
		LPB	2.0×10^{-5}	3.3×10^{-9}	6.7×10^{-7}
Fish	Females	SB	6.0×10^{-4}	10×10^{-8}	10×10^{-4}
		LPB	8.0×10^{-4}	1.4×10^{-7}	1.4×10^{-3}
	Males	SB	5.0×10^{-4}	8.5×10^{-8}	6.3×10^{-4}
		LPB	7.0×10^{-4}	1.2×10^{-7}	8.9×10^{-4}

2.5. Conclusion

This research includes the first information on organic pollutants in a target shark species from the GC. DDTs were the main group of pesticides detected in liver and muscle samples of the scalloped hammerhead. Moreover, the wide variety of compounds quantified in both analyzed tissues suggests that OCPs continue to be a potential threat due to their persistence and, possibly, to their recent application.

Although sex does not appear to significantly influence OCPs accumulation in immature scalloped hammerhead individuals, other biological and ecological factors such as size, age, and distribution, can exert an influence. The high levels observed in one of the two neonates of *S. lewini* could be a concern due to the vulnerability during early life stages to these pollutants. Therefore, it would be necessary to focus future research efforts in this sector of the population.

The low concentrations of OCPs in the edible tissue suggests that the consumption of scalloped hammerheads does not imply risks to human health associated with these chemicals. Nevertheless, since juvenile scalloped hammerheads are exposed to bioaccumulation of organic and inorganic pollutants in the GC, the consumption of larger individuals could pose some risk, mainly for coastal human populations with a high consumption of fishery products (Bergés-Tiznado et al., 2015; Ruelas-Inzunza et al., 2020).

Due to the high biodiversity of the GC and the threatened conservation status of some of the species that occur in the area (e.g., sea turtles, marine mammals, sharks), it is important to continue monitoring the abiotic and biotic OCPs concentrations in this region. The identification of the sources of these pollutants is also necessary to carry out mitigation actions to reduce the environmental levels and to determine effective management policies. Therefore, the need to expand the information on the impact of organic pollutants on elasmobranchs, and particularly on endangered species such as *S. lewini* is emphasized.

CHAPTER 3. Levels and species-specific accumulation of organochlorines in three shark species from the western Gulf of California (Mexico) with different life history traits

3.1. Abstract

Organochlorine compounds (OCs) such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) remain ubiquitous in marine ecosystems despite their prohibition or restriction, posing a risk to marine wildlife and humans. Their accumulation in liver tissue and its potential toxicity in three exploited shark species (scalloped hammerhead (HH): *Sphyrna lewini*, the Pacific sharpnose shark (PS): *Rhizoprionodon longurio*, and the Pacific angel shark (PA): *Squatina californica*) with different physiological and ecological features from the western Gulf of California (GC) was investigated. Forty of the 47 OCs analyzed were identified, evidencing a higher influence of agricultural than industrial sources considering the high DDTs/PCBs ratios. The DDTs was the main group in contribution to ∑OCs in the three species, while Hexa- and Hepta-CBs dominated the PCBs profiles. HH and PS had similar and significantly higher concentrations of ∑OCPs (wet and lipid weight) than PA, which is attributed to their migration to other regions of the GC more exposed to these pollutants than the study area. The levels of ∑PCBs (lipid weight) in the three species were comparable between them and relatively low. Overall, no intraspecific differences were found by sexes, but concentrations of many OCs were higher in larger individuals. HH and PS showed different OCs bioaccumulation trends, while no relationship between size and ∑OCs concentrations was observed in PA. Toxic equivalents (TEQs) of the three shark species were calculated from dioxin-like PCBs concentrations and were far below the established TEQs thresholds for fish. However, more information about possible effects of PCBs and OCPs in elasmobranch species is needed. This research provides the basis for monitoring organic contaminants in predatory sharks from the western GC and highlight the importance of further research on the environmental levels and sources of unintentionally produced organochlorines.

Key words: Marine pollution, DDT, PCBs, pesticides, bioaccumulation, toxic equivalent factors.

3.2. Introduction

The ocean is the final reservoir of anthropogenic pollutants, which are one of the major treats to marine wildlife as they can affect the individual and population health through different mechanisms of action (Desforges et al., 2018; Dietz et al., 2018; Islam and Tanaka, 2004). Within the complex mixture of chemical substances to which marine organisms are exposed, persistent organic pollutants (POPs) such as organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) are of great concern due to their toxicity, environmental persistence, its long-range transport, and their potential for bioaccumulation (Desforges et al., 2018; Olisah et al., 2019; Wang et al., 2022). Therefore, although their use and manufacture were cancelled or restricted decades ago, they continue to be detected at significant levels in marine biotic and abiotic matrices worldwide, posing a threat to animals and humans through the consumption of seafood products (Li et al., 2019; Megson et al., 2022; Routti et al., 2019; Sun et al., 2018).

The OCPs constitute an important part of POPs, and were intensively and extensively used for the control of pests affecting agriculture, domestic animals, residences, and human health from the 1940s to the early 2000s (Li and Macdonald, 2005; Rani et al., 2017). The best-known and most widely used OCP is the DDT, but numerous substances such as chlordane (CHLO), drins, mirex, chlorobenzenes (CBz) or endosulfan were globally used for pest control and to a lesser extent, in some industrial applications (Li and Macdonald, 2005; Xin et al., 2011). The technical composition of many OCPs, consists of chemical mixtures with different physicochemical properties, that eventually degrade to form products which can be more bioaccumulative and toxic than the parent compound (Li and Macdonald, 2005; Sparling, 2016). Therefore, numerous hazard organic compounds resulting from the manufacture and application of OCPs, are found in the environment. PCBs were marketed since 1929 to late 1970s mainly by the company Monsanto, who commercialized mixtures of PCBs named Aroclors, for many industrial applications such as insulating fluids in the electrical industry or as flame-retardants (Breivik et al., 2007; Jaspers et al., 2013). Of the 209 existing congeners of PCB, approximately 130 were found in the mixtures of Aroclors (Frame et al., 1996). However, usually only a subset of three to seven PCBs indicators (i-PCBs) are screened to estimate total PCB concentrations in

environmental and biological samples (Gandhi et al., 2015). In addition, the most toxic congeners, the twelve dioxin-like (DL) PCBs (DL-PCBs), are often investigated in biological matrices to assess health risks.

Despite the lack of precise data of POPs emissions, some estimations indicate that approximately 4.5 and 1.2 millions of tons of DDTs and PCBs, respectively, were applied worldwide before their inclusion in the Stockholm Convention of 2001 and its subsequent cancellation (Jaspers et al., 2013; Li and Macdonald, 2005). Factors such as the high stability of many OCs, the remobilization from environmental reservoirs, mismanagement of obsolete stocks, post-ban uses and unintentional productions, are responsible for the non-decline, and even increase, of their levels in some areas (Gardes et al., 2021; Mao et al., 2021; Muñoz-Arnanz and Jiménez, 2011; Rigét et al., 2019; Song et al., 2018; Valle et al., 2005; Vergara et al., 2019; Zhu et al., 2022). Thus, conducting research and monitoring of POPs is a legal obligation of the Stockholm Convention country signatory (Wang et al., 2022). One of the different approaches used for monitoring POPs in marine ecosystems is through the analysis of biota. This biomonitoring has certain advantages over the analysis of the abiotic environment, since it provides information on the bioavailability and bioaccumulation of pollutants. In addition, this approach allows the estimation of the potential risks of adverse effects (da Costa Filho et al., 2022). Moreover, the analyses of organic pollutants in animal tissues provides information on the habitat use and trophic ecology of species, since they integrate pollutants from their environment and prey (Dickhut et al., 2009; Lyons et al., 2019a, 2013).

The bioaccumulation is an intrinsic property of most OCs, linked to their lipophilicity and their high resistance to metabolism (Borgå et al., 2004; Fisk et al., 2001). OCs are absorbed by marine organisms mainly from the diet, and are stored mainly in the lipid rich tissues (e.g. blubber, liver) (Borgå et al., 2004; Kainz and Fisk, 2009). This implies that concentrations tend to increase with individuals growth and with trophic position, but they are also influenced by the lipid dynamics of the tissues (Borgå et al., 2004; Romero-Romero et al., 2017). However, maternal offloading is an important route of pollutants elimination in some species, then, adult females can reduce their OCs levels via parental investment (Lyons and Lowe, 2015; Murphy et al., 2018). In addition, ontogenetic

changes in habitat use, diet, or metabolism, among other physicochemical, biological and ecological factors, influence the complex process of uptake and elimination of pollutants (Borgå et al., 2004). Consequently, large intra- and interspecific differences in bioaccumulation capacity and, therefore, in potential susceptibility to adverse impacts can be observed (Renaguli et al., 2021; Vergara et al., 2019).

The global decline of shark populations has motivated in recent years toxicological research in this group, since pollutants could have been acting synergistically with overfishing and habitat loss during decades (Consales and Marsili, 2021; Correa et al., 2022; Muñoz-Arnanz et al., 2022). Besides their high position in marine food webs, the frequent use of coastal habitats of most shark species could increase their OCs exposure due to the proximity of sources (Islam and Tanaka, 2004). Many shark species have biological features such as long life span, slow growth rates, late age of sexual maturity and high hepatic lipid content, which renders them susceptible to the accumulation of organic pollutants (Borgå et al., 2004). This makes them potentially vulnerable to the toxic effects of OCs, as well as good bioindicators of contamination (Alves et al., 2022; Miller et al., 2020; Muñoz-Arnanz et al., 2022). Despite this, the effects of OCPs and PCBs on this group have not yet been well identified as they are in marine mammals (Consales and Marsili, 2021; Cullen et al., 2019). Likewise, the exposure to OCs and the species-specific accumulation patterns are just characterized in a few shark species, despite their importance in marine ecosystems and the need to identify their possible threats to conducting efficient risk assessment and management efforts (Consales and Marsili, 2021; Lyons et al., 2019b).

Three of the most exploited shark species in the western coast of the GC are mesopredators with divergent life history traits: the critically endangered scalloped hammerhead (HH) *Sphyrna lewini*; the vulnerable Pacific sharpnose shark (PS) *Rhizoprionodon longurio*, and the near threatened Pacific angel shark (PA) *Squatina californica* (Bizzarro et al., 2009; Cailliet et al., 2020; Pollom et al., 2020; Rigby et al., 2019). The HH is a relatively large-size and long-lived viviparous species which inhabits coastal environments when juveniles and offshore during its adult stage (Klimley et al., 1993). The PS and PA are small

size elasmobranch which inhabit close to the coast during their entire life cycle. However, the PS is a migratory viviparous species with a fast growth-rate that mature at a relatively early age, with a lifespan of ~7 years (Corro-Espinosa, 2011). On the other hand, the PA is an aplacental viviparous species with a low dispersal capacity, and a maximum age of 35 years (Cailliet et al., 1992).

There is a need to monitor the levels of organic pollutants in the GC to identify their possible sources, as well as to understand their bioaccumulation process in species of ecological and economical relevance in order to assess the potential risk of adverse effects. In this study, the liver of HH juveniles (n = 18) analyzed in Ángel-Moreno-Briones et al. (2022) for OCPs were screened for PCBs, and both types of OCs were analyzed in juveniles and adults PS (n = 20) and PA (n = 19). Therefore, the aims of this investigation were to: 1) assess the OCs contamination of the HH, the PS, and the PA from the western GC, 2) characterize liver accumulation trends among sexes and age classes, 3) determine the main factors influencing the interspecific differences in OCs accumulation, and 4) assess the potential risk of toxic effects through an indirect approach.

3.3. Material and methods

3.3.1. Samples

Sampling was performed according to the availability of the captures of the target species in artisanal fishing camps located in San Bruno (SB) and La Paz Bay (LPB) during September 2018-April 2019 (**Fig. 3.1**). Both sites are in the western GC, and due to the migratory behavior of HH and PS (Hoyos-Padilla et al., 2014; Kato and Carvallo, 1967), no distinction was made between individuals captured in both localities. Sharks were measured and sexed after fishery landings. Liver samples of HH (n = 18), PS (n = 20), and PA (n= 19) were collected from the posterior left lobe, stored in pre-muffled glass jars and kept in ice a few hours before freezing (-20°C) until further treatment.

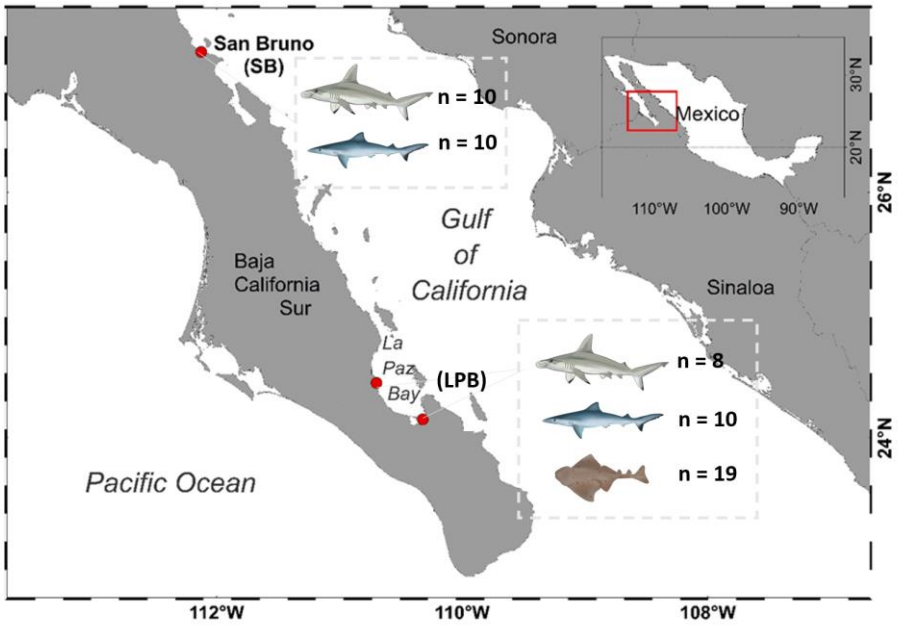


Fig. 3.1. Sampling locations in Baja California Sur, Mexico, and number of individuals sampled in each location.

3.3.2. Laboratory analyses and quality control

Chemical and instrumental analyses were performed at the Oceanological Research Institute from the Autonomous University of Baja California (Mexico) using the methodology described on Ángel-Moreno-Briones et al. (2022). Briefly, liver lipid content was extracted and determined gravimetrically (ASE 350, Dionex, USA) of ≈ 1 g w.w. with hexane HPLC-grade. Contaminant extraction and a first step of purification was performed simultaneously by placing sorbents (silica gel and florisil) inside a 34 mL ASE extraction cell. Recovery surrogates (TCMX and PCB_{209}, Ultra Scientific Inc.) were spiked before extraction with hexane/acetone (4:1, v/v) pesticide-grade. A second cleaning step was performed by a chromatographic column packed with silica and alumina deactivated (at 3%) and eluted with pesticide-grade solvents (hexane, dichloromethane). An Agilent 7010A Triple Quadrupole GC/MS operated in Electron Ionization (EI) mode and multiple reaction monitoring (MRM) was used for the detection and quantification of OCs. The target OCPs included 29 analytes: DDTs (p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD and o,p'-DDD), chlordanes (CHLOs: heptachlor, heptachlor epoxide, oxychlordane, cis- and trans-chlordane, cis- and trans-nonachlor), chlorobenzenes (CBz: penta- and

hexachlorobenzene); hexachlorociclohexenes (HCHs: α-, β-, δ-, and γ-HCH), endosulfan (α- and β- endosulfan and endosulfan sulfate), drins (aldrin, dieldrin, endrin and endrin aldehyde), mirex, hexachlorobutadiene (HCB), and methoxychlor. The 18 targeted PCBs include the most abundant congeners in the environment resulting of the major Aroclor mixtures (6 indicator PCBs: i6-PCBs), and the DL-PCBs (Gandhi et al., 2015). They were grouped in function of these classification and also in function of their number of chlorines: Trichlorobiphenyl (Tri-CB: PCB-28), Tetrachlorobiphenyls (Tetra-CBs: PCB-52, PCB-81, PCB-77), Pentachlorobiphenyls (Penta-CBs: PCB-101, PCB-123, PCB-118, PCB-114, PCB-105, PCB-126), Hexachlorobiphenyls (Hexa-CBs: PCB-153, PCB-138, PCB-167, PCB-156, PCB-157, PCB-169), and Heptachlorobiphenyls (Hepta-CBs: PCB-180, PCB-189). The retention times, masses and relative abundance of the confirmation ions were used as a criterion for the identification of OCs compounds. Quantification was done by a five-point calibration curve and using the internal standard method (PCB_{30} and PCB_{205}).

Quality assurance and quality control were performed by extracting blank samples and certified reference material (National Institute of Standards & Technology SRM-1946) with each batch of twenty samples. The limits of detection (LOD) were quantified as three times the SD (standard deviation) of six and four measurements of the lowest point of the calibration curve (for OCPs and PCBs, respectively). The range of LOD for OCPs was 0.20 – 3.99 ng/g, w.w. and for PCBs was 0.12 – 0.94 ng/g w.w. (Supplementary Material, **Table S3.1**). There were no signs of external contamination above de LOD in blanks. Percentage recoveries (mean ± SD) of TCMX and PCB_{209} were 65.2 ± 15.2% and 97.6 ± 13.1%, respectively. The obtained SMR values are reported in the Supplementary Material, **Table S3.2**. In this work, the concentrations reported were not corrected for recoveries.

3.3.3. Data analysis

Organochlorines concentrations were expressed as nanograms per gram (ng/g) of wet weight (w.w.) and by the lipid weight (l.w.) content. Lipid content was expressed as percentage (%). The concentrations of OCPs analytes were grouped in ΣDDTs, ΣCHLOs, ΣCBz, Mirex, and ΣOther OCPs (the sum of ΣDrins,

HCBD, ΣHCHs, ΣEndosulfan and Methoxychlor). PCBs were grouped in ΣHexa-CBs, ΣHepta-CBs and ΣOther PCBs (the sum of ΣTri-CBs, ΣTetra-CBs and ΣPenta-CBs). The sum of OCPs, PCBs, and OCs (ΣOCPs, ΣPCBs, and ΣOCs, respectively) were also used for statistical purposes. Only individual or summed compounds detected above the LOD in more than 60% of the samples of each species were considered for statistical treatment (Munschy et al., 2020), but all detections were considered for total amounts. The concentrations below detection limits (b.d.l.) were replaced by a proxy value corresponding to the LD/2 (George et al., 2021; Mikkonen et al., 2018). Non-detected (n.d.) compounds were treated as zero. Descriptive statistics were performed to measure the average concentrations, its range, and to determine the standard deviations (SD). Organochlorine profiles were calculated as the contribution of each contaminant group (ΣDDTs, ΣCHLOs, Mirex, ΣCBz, ΣOther OCPs, ΣHexa-CBs, ΣHepta-CBs, ΣOther PCBs) to ΣOCs, ΣOCPs, or ΣPCBs in each sample, and mean ± SD was calculated across samples within species. Statistical analyses were performed using non-parametric tests due to the small number of analyzed individuals and non-homogeneous nature of variances. Kruskal-Wallis followed by Dunn's post-hoc test was used for intra and interspecific comparisons of more than two groups, while Wilcoxon test was used for two groups comparison. Spearman's tests were applied to analyze correlations between: 1) OCs concentrations and liver lipid content of each species, 2) concentrations of different compounds, 3) OCs and total length (TL). In order to identify if the ontogeny accumulation follows a U-shape curve, there were used quadratic polynomial equations (Lyons et al., 2019b).

3.3.4. Potential toxicity of PCBs

The potential toxicity of PCBs was assessed from the dioxin-like toxic equivalents (TEQs) approach (Cullen et al., 2019). TEQs were calculated for DL-PCBs congeners (non-ortho: PCB-77, PCB-81, PCB-126, PCB-169, and mono-ortho: PCB-114, PCB-105, PCB-118, PCB-123, PCB-156, PCB-157, PCB-167, and PCB-189) as follows:

$$TEQ = [DL\text{-}PCB] \times TEF$$

Considering the toxic equivalent factors (TEFs) for fish proposed by Van Den Berg et al. (1998). The TEQs of the individuals analyzed were compared with tissue residue-based toxicity benchmarks (TRBs) assessed by Steevens et al. (2005) for early life stage fishes, since there are not toxicity benchmarks stablished for elasmobranchs.

3.4. Results and discussion

3.4.1. Biological parameters

Hammerheads ranged from 83 to 154 cm TL (between < 1 year and > 3 years) and were classified in two population groups: juvenile female (n = 9) and juvenile male (n = 9) (Anislado-Tolentino et al., 2008). PS ranged from 79 to 113 cm TL and were classified as juveniles (n = 11; 4 females and 7 males, < 3 years old) and adults (n = 9, 6 females and 3 males, < 7 years old) (Corro-Espinosa, 2011). PA ranged from 60 to 89 cm TL and were classified as juveniles (n = 5; 3 females and 2 males) and adults (n = 14; 6 females and 8 males, < 35 years old) (Cailliet et al., 1992; Romero-Caicedo et al., 2016). Due to until our knowledge there is no peer-reviewed data about the age and growth of PS and PA in the GC, it was not possible to calculate with certain accuracy the age of individuals of these species. No statistical differences were found in TL between males and females of any species ($W = 46 – 58.5$, $p > 0.05$). PA individuals were significantly smaller than individuals of PS and PA (Kruskal – Wallis: $\chi^2 = 23.48$, $df = 2$, $p < 0.001$).

Liver lipid content did not vary between sexes in any species ($p > 0.05$), and it was only weakly and inversely correlated with total length in PA ($r = - 0.46$, $p = 0.046$). There were not statistical differences in lipid content between juveniles and adults of PS and PA. However, it was observed high lipid content ($W = 13$, $p = 0.0015$) in HH individuals < 2 years than that of the individuals > 2 years, as it was reported previously by Ángel-Moreno-Briones et al. (2022). In addition, interspecific differences were found in liver lipid content: PS > HH > PA (Kruskal – Wallis: $\chi^2 = 40.08$, $df = 2$, $p < 0.001$). The liver of sharks acts as a buoyancy organ and as a storage site for energetic reserves, which can be used during periods of food limitation, migrations, and in reproduction (Davidson et al., 2014; Hoffmayer et al., 2006; Pethybridge et al., 2010; Wetherbee and Nichols, 2000). Therefore, it is difficult to identify the factors governing those variations in liver

lipid content within and among species, which could influence the accumulation of OCs (Borgå et al., 2004; Daley et al., 2014).

3.4.2. Organochlorine levels and profiles

Detection frequencies, ranges and mean concentrations of OCs are presented in **Table 3.1**. Levels of OCs classes quantified above detection limits in more than 60% samples of each species are order as follows: HH: DDTs > CHLO > CBz > Mirex > Other OCPs > Hepta-CBs > Hexa-CBs; PS: DDTs > CHLO > Mirex ~ Hexa-CBs > Hepta-CBs > Hexa-CBs > CBz ~ Other-PCBs and PA: DDTs > Hepta-CBs > Hexa-CBs.

Table 3.1
Levels (ng/g w.w.) and identification frequencies (IF) above detection limits (a.d.l.) of organochlorines, and lipid content (%) in liver tissue of *Sphyrna lewini*, *Rhizoprionodon longurio* and *Squatina californica*

	Sphyrna lewini			*Rhizoprionodon longurio*			*Squatina californica*		
	IF a.d.l.	Concentrations (ng/g w.w.)		**IF a.d.l.**	Concentrations (ng/g w.w.)		**IF a.d.l.**	Concentrations (ng/g w.w.)	
	(%)	Mean ± SD	Range (min - max)	**(%)**	Mean ± SD	Range (min - max)	**(%)**	Mean ± SD	Range (min - max)
OCPs	**100**	180.6 ± 140.2 (355.2 ± 278.6)	67.2 - 657.1 (110.1 - 1173.9)	**100**	153.9 ± 80.1 229.7 ± 110.7	42.3 - 337.5 (65.5 - 502.0)	**100**	6.5 ± 3.3 (24.3 ± 13.3)	1.7 ± 16.6 (12.0 - 71.7)
DDTs	**100**	152.5 ± 128.6 (299.0 ± 250.7)	47.5 - 589.3 (77.9 - 1052.8)	**100**	138.6 ± 72.9 207.0 ± 101.9	33.5 - 323. 7 (49.5 - 427.0)	**100**	5.2 ± 2.9 (19.6 ± 11.5)	1.2 - 14.6 (10.1 - 63.1)
CHLO	**100**	11.0 ± 8.5 (23.1 ± 21.4)	2.3 - 23.8 (3.8 - 77.3)	**75**	6.9 ± 8.6 (9.9 ± 11.9)	n.d.- 24.7 (n.d. - 30.7)	**0**	/	n.d. - b.d.l.
Mirex	**100**	6.1 ± 1.2 (11.8 ± 3.6)	5.3 - 10.3 (8.6 - 20.1)	**100**	6.4 ± 1.1 (9.7 ± 1.8)	5.3 - 8.6 (7.0 - 13.8)	**0**	/	n.d. - b.d.l.
CBz	**100**	6.9 ± 3.2 (13.4 ± 6.2)	5.7 - 19.8 (7.4 - 35.3)	**85**	1.5 ± 0.6 (2.2 ± 0.8)	b.d.l. - 3.1 (b.d.l. - 4.1)	**0**	/	n.d. - b.d.l.
Other OCPs	**100**	4.4 ± 3.0 (8.6 ± 6.2)	2.4 - 14.8 (3.9 - 26.4)	**25**		n.d. - 2.9 (n.d. - 4.0)	**5**	/	b.d.l. - 0.8
PCBs	**100**	8.1 ± 3.7 (16.0 ± 9.2)	5.4 - 18.4 (7.7 - 39.1)	**100**	13.4 ± 8.7 (20.7 ± 14.3)	5.6 - 33.9 (7.2 - 55.1)	**100**	3.1 ± 1.4 (13.3 ± 8.5)	1.4 - 5.6 (4.0 - 18.2)
Hexa-CBs	**100**	3.4 ± 2.9 (6.7 ± 6.3)	1.1 - 9.3 (2.2 - 20.0)	**100**	6.4 ± 6.4 (10.0 ± 10.5)	1.1 - 22.6 (1.4 - 36.6)	**79**	0.86 ± 0.37 (3.7 ± 2.2)	b.d.l. - 1.37 (b.d.l. - 7.7)
Hepta-CBs	**100**	4.0 ± 0.80 (7.8 ± 2.8)	2.3 - 5.5 (4.2 - 13.6)	**100**	5.6 ± 1.7 (8.5 ± 2.8)	3.4 - 13.6 (4.4- 23.7)	**100**	1.9 ± 1.0 (8.5 ± 6.6)	0.77 - 3.4 (2.3 - 27.4)
Other PCBs	**16**	/	b.d.l. - 1.3 (b.d.l. - 3.7)	**85**	1.5 ± 1.1 (2.3 ± 1.7)	b.d.l. - 4.0 (b.d.l. - 6.2)	**11**	/	n.d. - 1.36 (n.d. - 5.1)
Lipids		53.8 ± 11.1 (%)	30.8 - 77.2 (%)		66.7 ± 7.8 (%)	50.5 - 80.4 (%)		28.5 ± 11.6 (%)	8.2 - 46.7 (%)

Considering all samples (n = 57), 40 of the 47 targeted analytes were identified, reflecting the wide variety of OCs presented in the GC. As reported previously in fatty tissues of marine biota (Cresson et al., 2016; Voorspoels et al., 2004), the metabolite p,p'-DDE strongly correlated with ∑OCPs in the three species ($r > 0.92$, $p < 0.001$), as well as the congener PCB-153 correlated with ∑PCBs ($r > 0.85$, $p < 0.001$). This implies that both analytes are good proxies for general amounts of OCs.

Overall, contributions of OCPs to OCs were higher than PCBs contributions (**Table 3.1; Fig 3.2**). Indeed, ∑DDTs/∑PCBs ratio, used for characterizing the magnitude of agricultural or industrial sources to animal exposure, was above one in all liver samples of HH and PS, and in 12/19 samples of PA. This general predominance of DDTs over PCBs have been observed in other predators from the GC such as sea lions *Zalophus californianus* and orcas *Orcinus orca* (Fossi et al., 2014; Niño-Torres et al., 2009), reflecting a greater importance of agriculture than industrial activities around the study area. In fact, Mexico is among the top ten countries that had the highest use of DDT and other OCPs worldwide, while the use of PCBs was comparatively low (Barber et al., 2005; Breivik et al., 2002; Li and Macdonald, 2005).

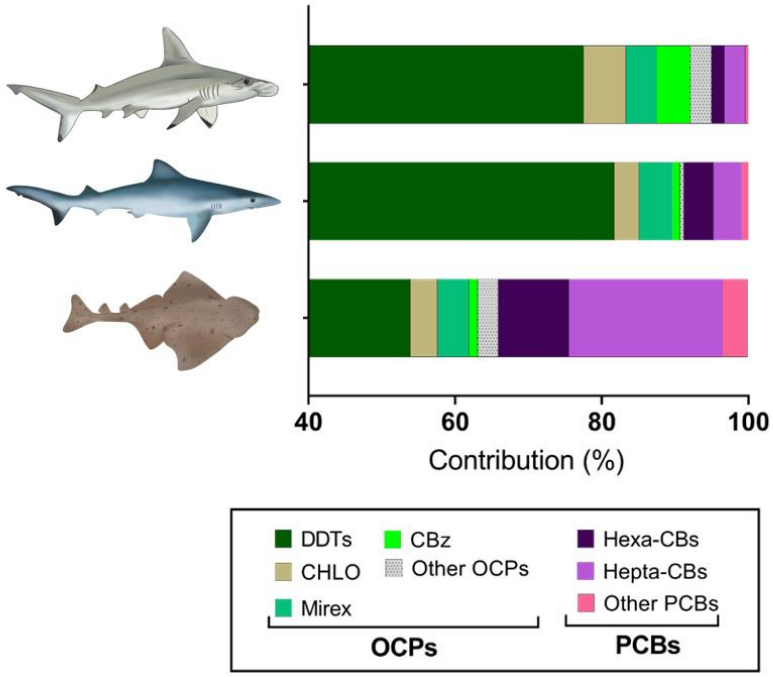


Fig. 3.2. Contribution (in %) of each organochlorine group to total OCs.

The correlation between different contaminants suggests their acquisition from the same source (Lavoie et al., 2010; Voorspoels et al., 2004). Unlike in PA and PS, concentrations of p,p'-DDE were highly correlated with PCB-153 in HH ($r = 0.93$, $p < 0.001$). This may indicate that PS and PA are exposed to these pollutants from different sources (dietary and/or environmental) or may reflect different biotransformation rates of both types of compounds.

3.4.2.1. Organochlorine pesticides

The ∑DDTs represented more than 80% of ∑OCPs in HH and PS, while in PA it was the only group of OCPs with concentrations above detection limits (**Table 3.1**). The predominance of DDTs among OCPs is commonly reported and is related mainly with its broad use in agriculture and human health (Li and Macdonald, 2005). In all species, the main DDTs related-compound was p,p´-DDE, accounting between 90 and 96% to ∑DDTs in HH, and between 79 and 97% in PS. In PA, the only DDT related-compounds detected were the p,p'-DDE and o,p'-DDE; however, the o,p'-DDE was only quantified in two individuals and their concentrations were below detection limit. Commercial DDT was composed mainly of p,p'-DDT (approximately 77%), whose main aerobic degradation product is its most persistent metabolite, p,p'-DDE (Ricking and Schwarzbauer, 2012; WHO, 1989). The predominance of p,p'-DDE is usually reported in the fatty tissues of marine predators, reflecting the degradation of DDT from its historical use (Chierichetti et al., 2021; Durante et al., 2016; Harley et al., 2019; Schlenk et al., 2005; Ylitalo et al., 2001).

Comparison of contaminant levels found in elasmobranchs from different areas of their distribution (**Table 3.2**) is often useful to obtain an approximation of the magnitude of contaminant exposure (Chynel et al., 2021; Muñoz-Arnanz et al., 2022).

Table 3.2

Concentrations of OCs (in ng/g l.w.) reported in the liver of *Sphyrna spp*, *Rhizoprionodon spp,* and *Squatina spp* found worldwide and in present study. '"n.a." = not analyzed; "b.d.l." = below detection limit; "P.S." = present study.

Species	n	Life stage	Sampling area	Sampling year	DDTs	Non-DDTs OCPs	PCBs	References
***Sphyrna* spp.**								
	18	Juveniles	West GC	2018-2019	299.0 ± 250.7	56.2 ± 31.3	16.0 ± 9.2	P.S.
S.lewini	6	Juveniles	Gulf of Aden	2016-2018	406 ± 545	n.a.	415 ± 272	a
	4	Juveniles	Florida	2006, 2009, and 2011	2011± 1188	581 ± 314*	4389 ± 3271**	b
***Rhizoprionodon* spp.**								
R. longurio	20	Juveniles and adults	West GC	2018-2019	207 ± 101.9	22.7 ± 12.4	20.7 ± 14.3	P.S.
R. acutus	5	Juveniles and adults	Gulf of Aden	2016-2018	53.1 ± 39.1	n.a.	1020 ± 267	a
R. lalandii	14	Juveniles	Brazil	2008	111 ± 40	b.d.l.	230 ± 132	c
***Squatina* spp.**								
S. californica	19	Juveniles and adults	West GC	2018-2019	19.6 ± 11.5	b.d.l.	13.3 ± 8.5	P.S.
	4	Adults	California	2010 and 2014	24557.0 ± 20170.9	397.6 ± 122.6	6098.9 ± 3598.8	d

*Considering only chlordanes.

** Considering only Hexa-CBs.

a = Boldrocchi et al. (2019)

b = Lyons and Adams (2014)

c = Cascaes et al. (2014)

d = Lyons and Lowe (2015)

DDTs levels found in HH juveniles from the GC (299.0 ± 250.7 ng/g l.w.) were similar than those reported in the same species in the Gulf of Aden (406 ± 545 ng/g l.w.) but lower than HH levels reported on Florida (2011 ± 1188 ng/g l.w.) (Boldrocchi et al., 2019; Lyons and Adams, 2014). In *R. longurio*, DDTs (207.0 ± 101.9 ng/g l.w.) were about two times higher than those reported in *R. lalandii* from Brazil (111 ± 40 ng/g. l.w.) and about four times higher than concentrations found in *R. acutus* from the Gulf of Aden (53.1 ± 39.1 ng/g l.w.) (Boldrocchi et al., 2019; Cascaes et al., 2014). However, is necessary to consider possible interspecific differences in the accumulation capacity even among related species (Lyons et al., 2013). Conversely, DDTs concentrations in PA liver from the GC (19.6 ± 11.5 ng/g l.w.) were three orders of magnitude lower than levels in liver samples of the same species from California (DDTs: 24557 ± 20171) (Lyons and Lowe, 2015). Comparisons across studies are challenging because differences in sample size, sampling periods, sexes, range of sizes, or population group of animals sampled can significantly influence the average concentrations (Genov et al., 2019). In addition, variability in analytical approaches followed in different laboratories and/or the lack of uniformity in data treatment can also influence the results (Genov et al., 2019; Muir and Sverko, 2006). However, it is likely that the wide variation of DDTs levels in sharks analyzed in the GC and the USA (Florida and California) is due to differences in the contamination status of the two areas, as it is estimated that the USA historically used almost three times more DDT than Mexico (Li and Macdonald, 2005). The GC could be equally or more contaminated by DDTs than areas of lower latitude as a result of a greater use of this pesticide (Li and Macdonald, 2005).

The group of CHLOs were found at concentrations above detection limits in all HH samples (see **Chapter 2**) and in 15/20 PS samples (**Table 3.1**). In PA, CHLOs were detected (at concentrations b.d.l.) in 6/19 samples. Chlordane related-compounds are usually the second group accounting to OCPs in the liver of elasmobranch species, which is related with its lipophilicity and wide use (Gelsleichter et al., 2005; Lyons and Adams, 2014; Mull et al., 2012). In Mexico, chlordane was imported from USA and used for agriculture and termiticide purposes (Alegria et al., 2008; Sánchez-Osorio et al., 2017; Wong et al., 2008). Mirex and CBz were also found at relatively significant concentrations in HH and

PS (**Table 3.1**). Those pesticides were also used in industrial applications (e.g. flame retardant additives), and moreover, CBz can be produced during combustion processes or during the manufacture of other pesticides (ATSDR, 2020; Barber et al., 2005). In fact, it is estimated that more than 30% of annual HCBz emissions during the 1990s came from waste combustion (Bailey, 2001; Hirano et al., 2007). Detailed information about other OCPs quantified in HH are reported in Ángel-Moreno-Briones et al. (2022). In PS, the only compound grouped in "Other OCPs" detected above detection limit was dieldrin.

A small number of studies have examined concentrations of non-DDTs OCPs in sharks, even though they are also included in the Stockholm Convention because of their high toxicity (Gelsleichter and Walker, 2010). Following a similar pattern to DDTs, the non-DDTs OCPs in HH and PA individuals from USA were higher than those found in the GC (**Table 3.1;** Lyons and Adams, 2014; Lyons and Lowe, 2015). However, the levels of the non-DDTs OCPs in *R. longurio* were higher than concentrations in *R. lalandii* from Brazil, in which these analytes were found at concentrations below detection limits (**Table 3.1;** Cascaes et al., 2014).

3.4.2.2. Polychlorinated biphenyls

The PCBs profiles were dominated by Hexa- and Hepta-CBs, which together accounted between 73 and 100% of ∑PCBs (**Fig. 3.2**). This is consistent with other studies on lipid-rich tissues of marine predators, as the hydrophobicity of the most chlorinated PCBs makes them highly bioaccumulative, which also favors its biomagnification in the trophic chain (Chierichetti et al., 2021; Cresson et al., 2016; Cullen et al., 2019; Kannan et al., 2004; Lippold et al., 2019). PCB-153 was the predominant congener in HH (2.5 ± 1.8 ng/g w.w.) and PS (4.4 ± 4.1 ng/g w.w.). This congener was followed by PCB-180 > PCB- 189 in HH (2.2 ± 0.5 and 1.9 ± 0.5 ng/g w.w., respectively), and by PCB-189 > PCB-180 in PS (2.9 ± 1.1 and 2.7 ± 1.4 ng/g w.w., respectively). These three congeners were the only ones identified at concentrations above the detection limit in all HH and PS individuals. In PA, the predominant congeners were PCB-180 > 189 > 153 (0.98 ± 0.97, 0.91 ± 0.37, and 0.82 ± 0.36 ng/g w.w., respectively), which were identified above detection limit in 10/19, 17/19, and 15 / 19 samples, respectively.

In the liver tissue of elasmobranchs, the congeners PCB-153 and PCB-180 usually dominate the overall PCBs (Cascaes et al., 2014; Correa et al., 2022; Gilbert et al., 2015). In addition to their lipophilicity and resistance to metabolization, the higher presence of those congeners in the fatty tissues of marine biota is associated with their high relative contribution to the most common mixtures of Aroclors (Frame et al., 1996; Gandhi et al., 2015). Conversely, the PCB-189 was contained in common Aroclor mixtures only as a trace level (Frame et al., 1996; Megson et al., 2019; Tuerk et al., 2005). Consequently, PCB-189 has not been found to be a main contributor in the PCB profiles of shark tissues (Cascaes et al., 2014; Corsolini et al., 2014; Cullen et al., 2019; Lyons et al., 2019a; Muñoz-Arnanz et al., 2022), as occurs in our study and in the whale shark biopsies from La Paz Bay (Fossi et al., 2017). The presence of this dioxin-like compound has been linked to unintentional production during waste combustion (Kim et al., 2004; Kim and Masunaga, 2005; Luthardt et al., 2002; Sakai et al., 2001), whose uncontrolled execution is a serious problem in the study area. This is supported by the fact that PCB-153 and PCB-180 correlated with each other in all three species ($r = 0.65 - 0.95$, $p < 0.001$), but not with PCB-189, reflecting different sources (Aroclors and non-Aroclors). Thus, our results, in line with recent work, highlight the need to pay more attention to non-Aroclor PCBs analysis in environmental samples, due to their continuous increase (Cui et al., 2013; Mastin et al., 2022; Megson et al., 2022, 2019).

In this study, ∑PCBs levels found in juveniles HH (16.0 ± 9.2 ng/g l.w.) were one order of magnitude lower than levels reported in the Gulf of Aden (415 ± 272 ng/g l.w.) (**Table 3.2**; Boldrocchi et al., 2019). Concentrations found in PS (22.9 ± 17.6 ng/g l.w.) were between one and two orders of magnitude lower than concentrations in *R. lalandii* from Brazil and *R. acutus* from the Gulf of Aden (1019 ± 267 and 230 ± 132 ng/g l.w., respectively) (**Table 3.2**; Boldrocchi et al., 2019). In addition, concentrations found in PA from the GC (13.3 ± 8.5 ng/g l.w.) were two orders of magnitude lower than levels in the same species from California (**Table 3.2**; Lyons and Lowe, 2015).

3.4.3. Species-specific accumulation trends

Because no relationship was found between liver lipid content and contaminant concentrations in any species, intraspecific trends in accumulation were analyzed on a wet weight basis (Koenig et al., 2013).

No significant differences in the accumulation of OCs by sex were observed in any species when all life stages were included (**Fig. 3.3)**.

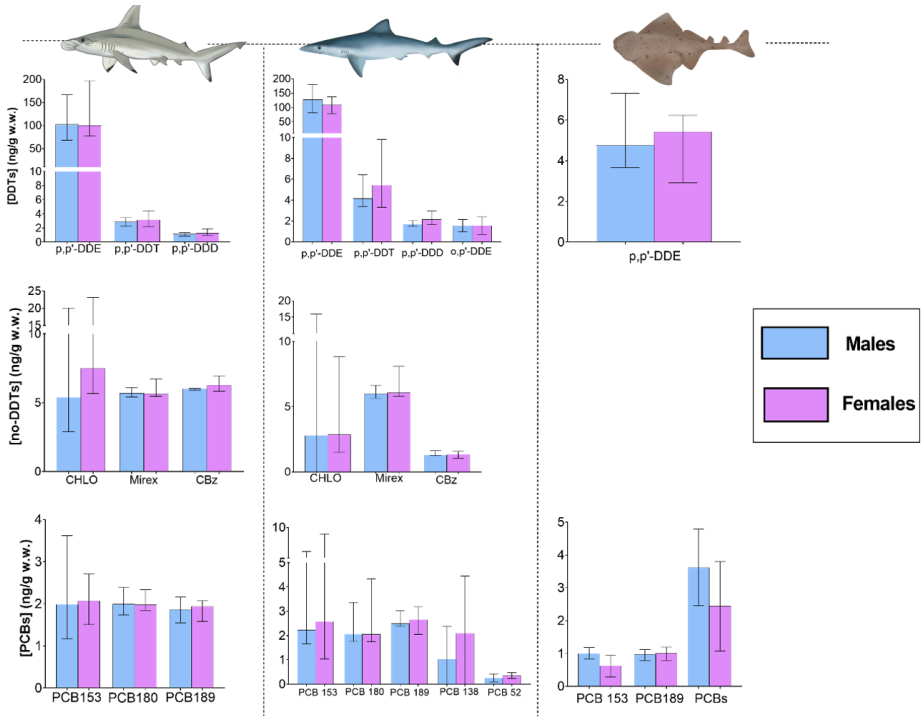


Fig. 3.3. Comparisons between median concentrations detected in male and female hammerhead *Sphyrna lewini*, Pacific sharpnose shark *Rhizoprionodon longurio* and Pacific angel shark *Squatina californica.* Error bars represented the interquartile range. Asterisk indicates significant differences ($p < 0.05$) between groups of individuals.

However, results by age class showed higher concentrations of some OCs groups and analytes in older than younger individuals of the three species (**Fig. 3.4**; Supplementary Material, **Fig. S3.1**), as well as the increase in some contaminant levels with TL (Supplementary Material, **Fig. S3.2**). Those observations are frequently reported in elasmobranch species (Chynel et al., 2021; Correa et al., 2022; Gilbert et al., 2015; Lyons et al., 2019b; Matulik et al.,

2017; Sawyna et al., 2017). Due to the high biological persistence of OCs, the lifetime exposition to them tends to produce their increase in concentrations with age (Borgå et al., 2004). Generally, organic chemicals with octanol-water partition coefficient (K_{OW}) > 10^5 are susceptible to bioaccumulation and biomagnification in fish due to their poor (if at all) potential of metabolization (Kelly et al., 2007). In addition, ontogenetic changes in diet, metabolism, feeding and growth rates, and habitat use, as well as reproduction, may influence in the exposition and elimination of different compounds (Borgå et al., 2004; Lyons et al., 2019b). Therefore, the observed results are due to a combination of numerous species-specific factors together with the physicochemical properties of the different chemicals analyzed.

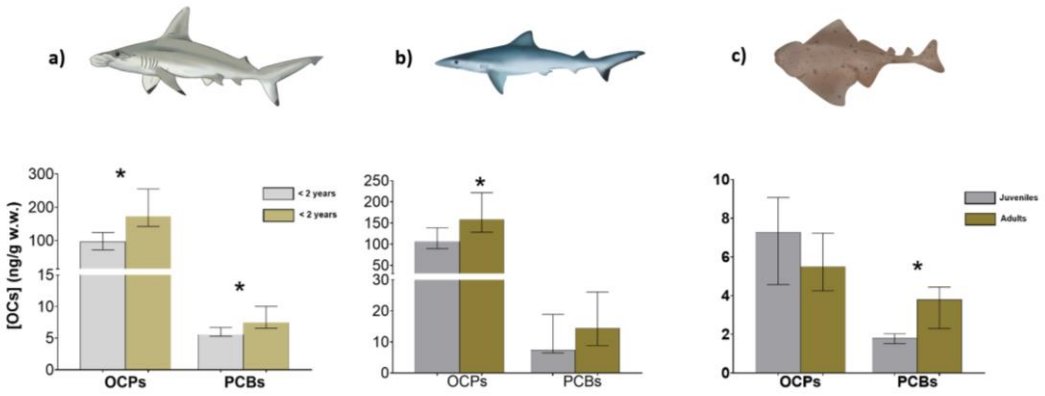


Fig. 3.4. Comparisons between ∑OCPs and ∑PCBs measured in: a) juvenile < 2 years vs. juvenile > 2 years scalloped hammerhead *Sphyrna lewini*; b) juveniles vs. adults Pacific sharpnose shark *Rhizoprionodon longurio;* and c) juveniles vs. adults Pacific angel shark *Squatina californica*. Error bars represented the interquartile range. Asterisk indicates significant differences ($p < 0.05$) between groups of individuals.

3.4.3.1. Sphyrna lewini

Correlations between the TL and the concentrations of ∑OCs ($r = 0.64$, $p < 0.05$) and various compounds when excluding the outlier (Ángel-Moreno Briones et al., 2022; Supplementary Material, **Fig. S3.2**) reflects that the exposition of juveniles HH to these chemicals is greater than their metabolization capacity (Borgå et al., 2004). However, significant relationships were not found between TL and ∑OCs

when analyzing females and males separately (**Fig. 3.5a**), probably due to the small sample size. The increase in trophic position of juveniles HH with growth (Torres-Rojas et al., 2013) is expected to influence the significant differences in the accumulation of many OCs between individuals younger and older than 2 years (Supplementary Material, **Fig. S3.1**). Therefore, older HH feeding at higher trophic levels may cause a greater exposure to compounds prone to biomagnification such as p,p'-DDE, p,p'-DDD, CHLOs, PCB-153 and PCB-180. In addition, significant higher DDT/PCBs ratio was found in older juveniles (W = 66, p = 0.0021), as well as a moderate increase with TL (r = 0.49, p = 0.037). This may be related with their migratory behavior, since older juveniles HH could increase their exposure to DDTs relative to PCBs when move through the Gulf of California (Hoyos-Padilla et al., 2014). However, it is needed to note the possible influence of the maternal signature of contaminants in younger juveniles HH, since maternal investment is an important via of acquisition of organic pollutants for young's-of-the-year (YOYs) of numerous shark species (Chynel et al., 2021; Lyons et al., 2013; Lyons and Adams, 2014; Lyons and Lowe, 2015; Marler et al., 2018; Mull et al., 2013).

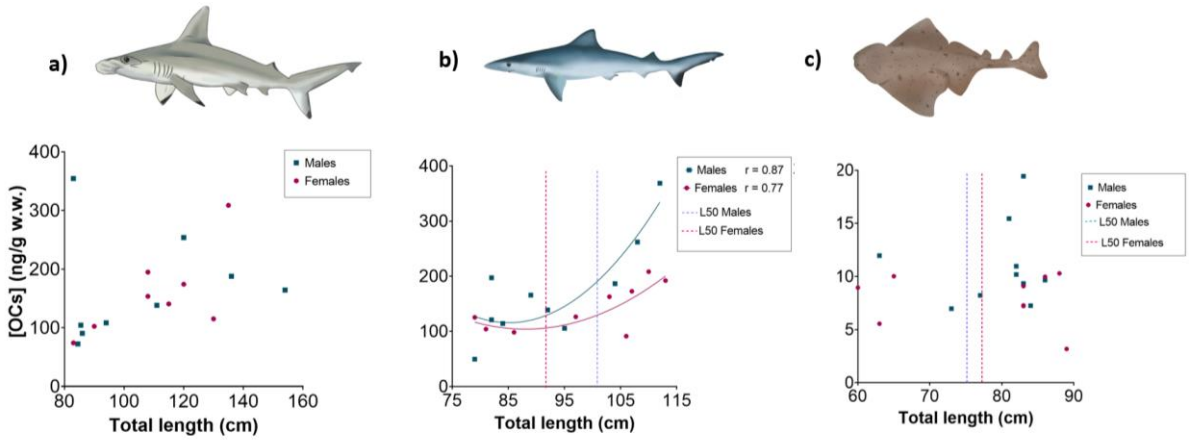


Fig. 3.5. Relationships between total length (cm) and liver concentrations (ng/g w.w.) of ∑OCs analyzed in males and females of scalloped hammerheads *Sphyrna lewini*, Pacific sharpnose shark *Rhizoprionodon longurio* and Pacific angel shark *Squatina californica*. Lines indicate that concentrations adjusted to a parabolic (in sharpnose sharks) increment with size. Vertical dashed line

represents the L50 of males (blue) and females (pink) assessed by sharpnose and angel sharks from the Gulf of California.

3.4.3.2. Rhizoprionodon longurio

Overall, the ontogenetic accumulation of ∑OCs in PS fit a parabolic curve when removing the juvenile outlier (R = 0.68, p = 0.0064, **Fig**. **3.5b**). This accumulation trajectory indicates a period of growth dilution in juveniles prior to the increase in concentrations after maturity, as was previously described in mako sharks *Isurus oxyrinchus* (Lyons et al., 2019b). The rapid growth of the species of the genus Rhizoprionodon during their first life stages (Baje et al., 2018; Corro-Espinosa et al., 2011; Corsso et al., 2021), would avoid the accumulation of OCs until its maturation (Borgå et al., 2004). When sharks mature, the reduction in their growth rates will contribute to the increase in OCs concentration (**Fig. 3.5b**). However, adults PS only presented significantly higher concentrations of p,p'-DDD, p,p'-DDD, and PCB-52 than juveniles (Supplementary Material, **Fig. S3.1**). This is possibly related with the lack of differences in diet between population groups, and the short life cycle of PS (Alatorre-Ramírez et al., 2013; Corro-Espinosa et al., 2011). Thus, the feeding at the same trophic level and the small temporal difference between individuals at different stages of development, may not allow reflecting statistical differences in many bioaccumulative contaminant loads. In addition, the maternal offload of OCs could influence in these results since most of adults were females.

Maternal transfer has been shown to be an important route of pollutant elimination for this species (Baró-Camarasa et al., 2022; Frías-Espericueta et al., 2014). In fact, maternal offload seems to be the reason of the less pronounced increase in OCs concentration with size in females than in males (**Fig. 3.5b**). In addition, despite the small sample size of PS adult males, and the unbalanced sex-ratio, significant differences were found in p,p'-DDE and CHLO (W = 17, p = 0.048, in both cases) between adult males (n = 3; 215.94 ± 88.21 and 19.70 ± 4.99 ng/g w.w., respectively) and adult females (n = 6; 116.94 ± 28.15 and 4.91 ± 7.36 ng/g w.w., respectively).

Environmental ontogenetic shifts could be also reflected in some accumulation trends of PS, such as the higher concentrations of the low-chlorinated PCB-52 in

adults (Supplementary Material, **Fig. S3.1**). While juveniles of PS remain foraging close to the coast, it has been suggested that adults conduct extensive seasonal migrations along the GC (Márquez-Farías et al., 2005). The PCB-52 is a low chlorinated and volatile congener with the ability to disperse far from its source of production, so animals frequenting offshore habitats may be more exposed to their accumulation than those restricted to the coastal environment (Webster et al., 2011).

3.4.3.3. Squatina californica

For PA, no significant correlation was found between ∑OCs and TL (**Fig. 3.5c**.). The only compound that slightly increase with size was PCB-153 ($r = 0.048$, $p = 0.040$). This general lack of bioaccumulation of OCs may reflect that the low exposure of this species is similar to its ability to eliminate them. However, significant differences in the accumulation of PCBs can be observed between juveniles and adults (Supplementary Material, **Fig**. **S3.1**), suggesting different degree of exposition to these pollutants. For example, the PCB-180 was no detected in any juvenile ($n = 5$), but it was the congener with the highest concentrations in adults (1.33 ± 0.90 ng/g w.w., detected in 10/14 adult samples above detection limit). In addition, the DDT/PCBs ratio was higher ($W = 9$, $p = 0.014$) in juveniles PA, suggesting that adults could frequent areas relatively more influenced by industrial sources than juveniles. Although PA does not make long-distance migrations, adults seem to move to greater depths in the coastal zone during the warm season to reproduce (Escobar-Sánchez et al., 2011; Galván-Magaña et al., 1989), which could explain these results. On the other hand, maternal transfer is the most likely explanation for the low concentration of PCBs ($W = 8$, $p = 0.043$) in adult females ($n = 6$; 2.75 ± 1.62 ng/g w.w.) compared to adult males ($n = 8$; 4.11 ± 0.63 ng/g w.w.), as this species has a high rate of OCs discharge to offspring (Lyons and Lowe, 2015).

3.4.4. Inter-specific comparisons

Due to the significant differences in liver lipid content between the three species (see **Section 3.1.**), interspecific comparisons were performed primary on a lipid-weight basis in order to reduce the effect of the lipid content variability on the results.

Marine predators accumulate pollutants through different pathways, but diet appears to be the main one for many chemicals (Borgå et al., 2004). The three sharks feed on a similar trophic level and share most prey items in the GC (Alatorre-Ramírez et al., 2013; Escobar-Sánchez et al., 2011; Torres-Rojas et al., 2013). This could be expected to result in a similar diet exposition to contaminants in the study area, if we neglect the influence of possible differences in consumption rates and intestine absorption efficiencies between species (Giesy and Kannan, 2002). However, it is assumed that most PA individuals were older than those of HH and PS (see **Section 3.1**.), which may result in a greater accumulation of contaminants over time if the exposure of the three species were similar (Borgå et al., 2004). In addition, the PA inhabits semi-buried in the bottom, that usually results in a higher exposure to hydrophobic compounds via gills and skin compared to organisms inhabiting the water column (Borgå et al., 2004; Li et al., 2021). Therefore, the lower concentrations of ΣOCPs in PA than HH and PS (**Fig. 3.6a, c**) were unexpected. The migratory behavior of HH and PS (Aldana-Moreno et al., 2019; Hoyos-Padilla et al., 2014; Márquez-Farías et al., 2005) and the low dispersal ability of PA (Ramírez-Amaro et al., 2017), seems to indicate that HH and PS are exposed to these pollutants in environments more impacted than the study area, such as the continental coast of the GC (Páez-Osuna et al., 2017). The different degree of pesticides exposition between species is also supported by a higher ratio of DDTs/PCBs in HH and PS compared to PA (Kruskal – Wallis: $\chi^2 = 36.5$, df = 2, $p < 0.001$). In addition, a lower number of analytes was detected in PA (18/47), suggesting that the other two species (HH: 35/47; PS: 32/47 organochlorines detected) integrate contaminants from a more diverse origin due to their mobility. On the other hand, the lack of significant differences in ΣPCBs lipid-normalized concentrations (**Fig. 3.6d**), suggests a similar degree of exposure to these pollutants between the three shark species. Furthermore, these results highlight the influence of the liver lipid content in the species-specific accumulation capacity of organochlorines, since HH and PS presented higher PCBs concentrations than PA when levels were compared on a wet weight basis (**Fig. 3.6b**).

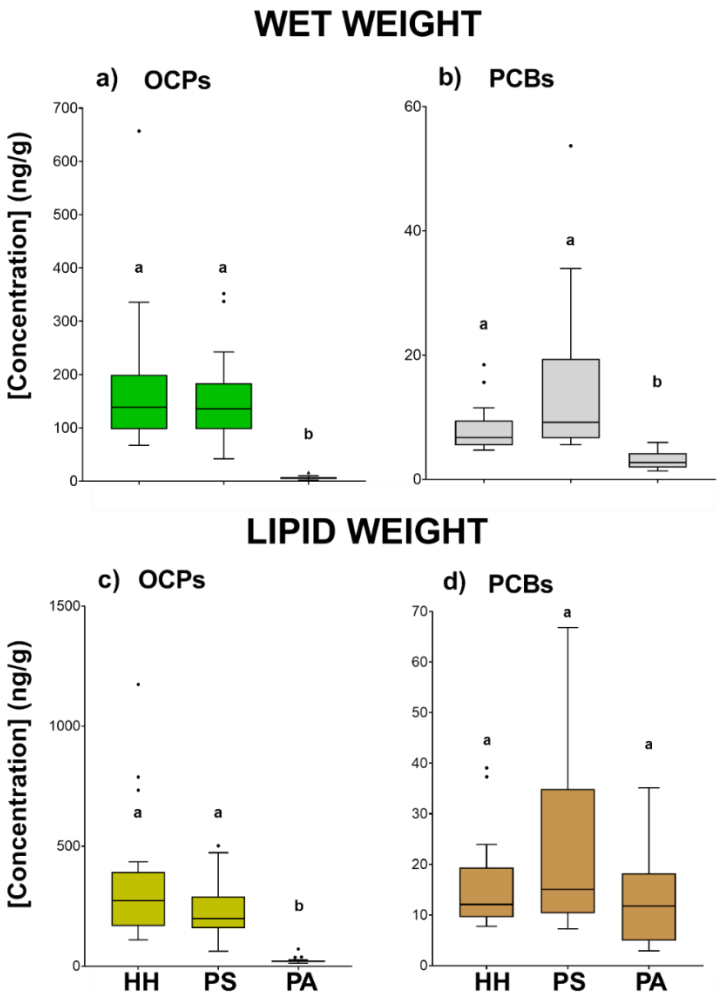


Fig. 3.6. Concentrations of ∑OCPs (3.6a, 3.6c) and ∑PCBs (3.6b, 3.6d) in hammerheads (HH) *Sphyrna lewini*, Pacific sharpnose sharks (PS) *Rhizoprionodon longurio*, and Pacific angel sharks (PA) *Squatina californica* on a wet weight (3.6a, 3.6b) and a lipid weight (3.6c, 3.6d) basis. The lower and upper ends of boxplot represent the 25th and 75th percentiles; the horizontal line represents the median, and the lower and upper whiskers indicate the minimum and maximum concentrations, respectively, excluding outliers. The black circles represent outliers data. Statistical differences are indicated with different letters.

Considering only the juvenile individuals, a lack of differences in ΣOCPs and ΣPCBs concentrations (l.w.) were found (W = 46, p = 0.077; W = 100, p = 0.98, respectively) between HH and PS. However, levels of CHLO, mirex, and CBz were higher in HH than in PS (W = 246-360, $p < 0.05$). In contrast to PS, the HH have a long life span and late maturity (Corro-Espinosa et al., 2011; Klimley et

al., 1993). Therefore, it is to be expected that longer-term exposure in HH adult females may result in higher concentrations of contaminants than in PS, which would be reflected in the magnitude of maternal offloading (Borgå et al., 2004; Lyons et al., 2013). Consequently, HH may born with a higher contaminant load than neonate PS, which would influence in the differences observed in OC concentrations between juveniles of both species (Lyons et al., 2019b). This fact does not seem to apply to PA. Despite its longevity and late maturity, adult PA have similar PCBs levels than adult PS ($W = 83$, $p = 0.22$) when comparing on a l.w. basis, reflecting a lower accumulation rate in angel sharks. In addition, juveniles PA presented less concentration of these pollutants than juveniles PS ($W = 6$, $p = 0.013$), which could be related with a lower magnitude of maternal offloading. Besides age-at-maturity, numerous factors are linked to maternal transfer, as reproductive strategy, mother feeding and habitat ecology, gestational length, and litter size (Lyons et al., 2013; Lyons and Adams, 2014; Marler et al., 2018; Weijs et al., 2015). Species with aplacental viviparity such as PA, tend to have lower maternal discharge rates of organic chemicals than placental ones such as HH and PS (Lyons and Adams, 2014; Weijs et al., 2015). Therefore, this factor seems to contribute to the observed differences.

3.4.5. Potential health risk assessment

Information about physiological effects of OCs in elasmobranch species is restricted to a few field studies that analyze the relationship between contaminant concentrations and different biomarkers (Gelsleichter et al., 2006; Sawyna et al., 2017). The main disadvantage is that free-ranging animals are subjected to multiple contaminants exposition as well as other environmental stressors. Therefore, is difficult to assess the relationship between the observed response and the levels of a given contaminant, considering that different stressors can have synergistic, additive or antagonistic effects (Gelsleichter et al., 2006; Green and Larson, 2016). Nevertheless, ecotoxicological information derived from correlations between contaminant loads and physiological responds is usually used to make inferences on potential risk effects through the extrapolation of OC accumulation data (Correa et al., 2022). Gelsleichter et al. (2006) found elevated steroids and blood leukocytes concentrations in a population of Atlantic stingrays *Dasyatis sabina* which were highly exposed to DDTs and cyclodienes (hepatic

concentrations of 80 ± 10 and 88 ± 3 ng/g w.w., respectively) compared to other populations of the same species. Mean concentrations of DDTs in HH and PS livers determined in the present study were generally higher (47.5 - 589.3 and 33.5 - 323. 7 ng/g w.w., respectively) than *D. sabina* levels, but ∑Cyclodienes (drins + endosulfan + CHLO) concentrations were more than two times lower. On the other hand, 11 and 10% of HH and PS liver samples, respectively, presented higher levels of DDTs + CHLO compared to the average concentrations linked with immunostimulation (314.5 ± 110.3 ng/g w.w.) in the round stingray *Urobatis halleri* from California (Sawyna et al., 2017). However, hepatic concentrations of PCBs found in the round stingray (3733 ± 610 ng/g w.w.) were between two and three orders of magnitude higher than HH, PS, and PA levels (**Table 3.1, Table 3.2**). Therefore, it is risky to state the probability of health impacts in HH, PS and PA based on OC concentrations found in stingrays, especially if we also consider that there is a great variability of toxic outcomes even in closely related species (Cooke, 1973; Zhou et al., 2010).

Another tool used to assess the likelihood of toxicity due to OCs is through the comparison of concentrations with threshold values stablished for other taxa, been the teleost group the most appropriate surrogate laboratory species for elasmobranchs given their taxonomic closeness (Cullen et al., 2019; Storelli and Marcotrigiano, 2001). The most protective threshold values established for biological effects in fish due to ΣDDTs (600 ng/g w.w.) and ΣPCBs (100 ng/g w.w.) were far above the concentrations measured in the three shark species analyzed (**Table 3.1, Table 3.2**) (Beckvar et al., 2005; Berninger and Tillitt, 2019). However, laboratory organisms are usually exposed to elevated concentrations of a single contaminant during short periods, which makes difficult to extrapolate the effects of lower concentrations of chemical mixtures at chronic exposures, as it usually occurs in the environment (Green and Larson, 2016; Letcher et al., 2010). In addition, considering the estimate that DDTs and PCBs have declined from marine biota by 2 - 8% each year since their historical peak in the 1980s (Lawson et al., 2020), it cannot be discarded that OCs have been harmful to sharks from the GC.

On the other hand, dioxin-like toxic equivalents (TEQs) approach have been used to assess the health risk due to DL-PCBs measured in bioaccumulation studies

on marine wildlife (Cullen et al., 2019; Keogh et al., 2020; Ross et al., 2004, 2000; Storelli et al., 2005). Among the 209 PCBs congeners synthetized, the DL-PCBs are the most toxicologically relevant ones, as they activate the Ah receptor (AhR) inducing a toxicity similar to 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (2, 3, 7, 8–TCDD), one of the most dangerous chemicals ever known (Giesy and Kannan, 2002). Consequently, DL-PCBs produce adverse effects on organisms at relatively smaller concentrations than non-dioxin-like PCBs (Non-DL-PCBs) (Giesy and Kannan, 2002). However, few papers report concentrations of the 12 DL-PCBs in sharks, as they usually focus on the analysis of i-PCBs (Tiktak et al., 2020). The TEQs of DL-PCBs calculated for HH, PS, and PA were estimated according to the TEFs recommended by the World Health Organization (WHO) based on in vitro and in vivo bioassays for multiple teleost species (Van Den Berg et al., 1998) (**Table 3.3**). Due to these values were between one and two orders of magnitude lower than the most protective tissue-residue benchmarks (TRBs) established for fish (57 pg/g l.w.), adverse effects produced by DL-PCBs are expected to be unlikely (Steevens et al., 2005). However, it is important to consider the uncertainty of the TEQ approach, since TEFs and TRBs of fish were established from studies that only evaluate mortality as the endpoint in organisms in early life stages (Cullen et al., 2019; Steevens et al., 2005; Van Den Berg et al., 1998). Therefore, TEQs approach do not consider sub-lethal effects, and may not be directly applicable in adult organisms (Cullen et al., 2019). Moreover, other DL- compounds such as polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) were not quantified in our study (as in many others), so the calculated TEQs could be underestimated since the contribution of these compounds is unknown (Giesy and Kannan, 2002; Tiktak et al., 2020).

Table 3.3

Toxic equivalent factors (TEFs) for fish proposed by Van Den Berg et al. (1998), concentrations (pg/g w.w.) and Toxic equivalencets (TEQs) of DL-PCBs found in the liver tissue of *Sphyrna lewini*, *Rhizoprionodon longurio* and *Squatina californica*.

		Sphyrna lewini		***Rhizoprionodon longurio***		***Squatina californica***	
	TEF	Concentration (pg/g w.w.)	TEQ	Concentration (pg/g w.w.)	TEQ	Concentration (pg/g w.w.)	TEQ
No-ortho							
77	0.0001	16	0.00155	75	0.00754	b.d.l.	/
126	0.005	b.d.l.	/	34	0.17	b.d.l.	/
Mono-ortho							
118	0.000005	b.d.l.	/	292	0.00146	b.d.l.	/
105	0.000005	b.d.l.	/	150	0.000748	b.d.l.	/
156	0.000005	76	0.000382	145	0.000727	b.d.l.	/
167	0.000005	16	0.00008	99	0.000493	b.d.l.	/
189	0.000005	1812	0.00906	2850	0.0142	930	0.00465
Total		1920	**0.0110**	3611	**0.195**	930	**0.00465**

Shark liver-derived products are manufactured worldwide for numerous applications due to their properties (Tiktak et al., 2020). Regionally, shark liver was a valuable product during World War II, when GC shark landings became important to supply the USA demand of shark oil as a source of vitamin A (Saldaña-Ruiz et al., 2017). Despite the collapse of the shark liver market in the area decades ago, liver oil is still used in some elasmobranch-fishing communities as a preventive against diseases (Saldaña-Ruiz et al., 2017). Therefore, humans are not only susceptible to contaminant exposure from eating shark meat, but also from pharmaceuticals or cosmetics products that contain shark liver oil (Muñoz-Arnanz et al., 2022; Tiktak et al., 2020). Thus, maximum limits (MLs) set by different agencies must be applied in the same way to ensure human safety (FDA, 2022). Considering both wet weight and lipid weight, none of the 57 liver samples analyzed in this work (see **Table 3.2**) exceeded the limits

established by the Environmental Protection Agency (EPA) and the Food and Drug Administration (FDA) for DDTs, chlordanes, mirex, drins, or PCBs (5000, 300, 100, 300 and 2000 ng/g, respectively) (FDA, 2022).

3.5. Conclusions

This investigation provided the first data about organochlorines in the PS and the PA from the western GC, including the levels of PCBs in juvenile HH sharks. Our results indicated a greater incidence of agriculture than industrial activities in the region, which is evidenced in the low levels of PCBs found in the three species comparing with worldwide levels. However, the relevant presence of non-intentionally produced OCs (hexachlorobenzene, mirex, PCB-189) highlights the necessity to identify and control the unintentional sources of these endocrine-disrupting and carcinogenic chemicals. Moreover, differences in OCPs concentrations between HH, PS, and PA demonstrate the importance of the proper species selection for monitoring studies in order to obtain reliable information on the environmental contamination. The PA, due to its low mobility, integrates pollutants from more restricted areas than the other two species analyzed. This allows to determine that the western coast of the GC is less contaminated by OCPs than surrounding areas in view of the low levels detected in PA.

The three shark species showed different intraspecific accumulation trends due to their different life history traits. Size and age in juvenile HH, as well as size, maturity stage, and sex in PS and PA seems to be factors influencing OC loads. This fact, together with the presence of outliers in our results, shows the limitations of research with small sample sizes in toxicological studies, as well as the importance of knowing the biological and ecological characteristics of the species for their interpretation.

Habitat use, lipid content, longevity and the strategy of reproduction are factors that determine the exposition and accumulation capacity of shark species. In view of the limited data on organic contaminants in GC sharks, it would be necessary to expand the information on this subject focusing efforts on threatened species with characteristics that make them more prone to their accumulation.

Since the research about pollutant effects on elasmobranchs is in their early stages of development, it is difficult to assess the potential risk of the three shark species analyzed. The interpolation of OCs levels in order to assess the probability of health impacts in HH, PS and PA from the GC have several

limitations associated, being risky to make conclusions in this regard. The levels of some OCPs in HH and PS were similar to those associated with toxic effects in other elasmobranchs species. Conversely, the TEQs of DL-PCB indicate no risk of health effects in any shark species when compared to established thresholds in fish.

CHAPTER 4. Are shark tacos healthy or toxic? A multiple contaminant risk-benefit assessment

4.1. Abstract

Seafood is an important source of essential fatty acids for human nutrition, but also of toxic chemicals which tend to be at higher concentrations in the edible tissue of top predator species. In the state of Baja California Sur (Mexico), the scalloped hammerhead shark (HH) *Sphyrna lewini* the Pacific sharpnose shark (PS) *Rhizoprionodon longurio* and the Pacific angel shark (PA) *Squatina californica* are between the most frequent shark species caught off the coast of the Gulf of California (GC) for consumption. Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) were analyzed in the muscle tissue of 20 HH, 20 PS and 19 PA in order to assess the health non-cancer and cancer risk due to multiple organochlorine (OC) contaminants following the Environmental Protection Agency guidelines. The risk-benefit assessment was done using the docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) concentrations obtained from published data. The results showed interspecific but not intraspecific differences in the OC concentrations (PS > HH > PA). The maximum allowable consumption rate of the three species for cancer risk were 105.6, 160, and 259.3 g/day for PS, HH and PA, respectively. Therefore, their consumption twice or three times per week does not pose a risk on human health associated with OCs. However, the lifetime high-frequency intake of HH and PS muscle may result in cancer effects for subsistence shark consumers. The risk of consumption of HH, PS and PA over weighted benefits of EPA and DHA intakes. Therefore, it is advisable to avoid its consumption.

Key words: Organochlorine pesticides, PCBs, DDT, maximum allowable fish consumption, scalloped hammerhead.

4.2. Introduction

Fish consumption provides essential fatty acids, high-quality proteins, vitamins, and minerals for human nutrition (Domingo, 2016). In fact, the main source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) for humans is fish (Domingo, 2016; Mahaffey, 2004). These polyunsaturated fatty acids of omega 3 family have numerous health benefits, such as neuroprotection, cardioprotection, and hepatoprotection, among other properties (Chen et al., 2022). Low dietary intakes of EPA and DHA have physiological effects for humans (e.g., reduced visual function and learning behavior) (Mahaffey, 2004). For this reason, global and national health organizations recommend frequent consumption of fish to satisfy the daily requirement of DHA and EPA (1000 mg/day) (Gladyshev et al., 2020). However, the meat content of EPA and DHA differ substantially between fish species, which implies differences in the nutritional value for humans (Gladyshev et al., 2018).

In contrast to its benefits, fish consumption is one of the most important routes of exposure to organic and inorganic toxic chemicals for humans (Arrebola et al., 2018; WHO and FAO, 2011; Weitekamp et al., 2021). Persistent organic pollutants (POP), namely organochlorine pesticides (OCPs) as DDT, chlordanes or chlorobenzenes and polychlorinated biphenyls (PCBs), are anthropogenic organic substances used intensively between 1930's and 1990's in agriculture, public health, and industries (Breivik et al., 2007; Li and Macdonald, 2005). These chemicals are found worldwide in every oceanic compartment due to its chemical stability, and they are considered of first concern by health agencies due to its bioavailability and inherent toxicity (Koenig et al., 2013; Olisah et al., 2019). Many OCPs and PCBs are known to affect the endocrine, reproductive, immune and neurologic systems, among other potential health effects such as cancer (Singh, 2016; Sparling, 2016). In addition, the combination of multiple co-ocurring contaminants in food may result in additive or synergetic adverse effects for human health, although this fact is usually not taken into account during the risk assessments (Pose-Juan et al., 2016).

Due to both the Food and Agriculture Organization (FAO) and The World Health Organization (WHO) point to the need to provide information about healthy consumption choices of fish, the benefit-risk assessment of the co-ingestion of

essential fatty acids and contaminants through fish consumption has received special attention during the last years (Anishchenko et al., 2017; Gladyshev et al., 2018; Milićević et al., 2022; Turyk et al., 2012; Zhang et al., 2012). The increase of both essential fatty acids and pollutant concentrations has been related with the increase in trophic position of fishes (Borgå et al., 2004; Ruess and Müller-Navarra, 2019). Because of essential fatty acids are not used as a source of energy in animals, they are incorporated into the body tissue of consumers without major modification, producing their retention as the trophic level increases (Ruess and Müller-Navarra, 2019). On the other hand, since the diet is the main route of exposition to many toxic chemicals, they tend to biomagnify in marine food webs (Borgå et al., 2011, 2004; Lourenço et al., 2017). Therefore, fish occupying high levels in the food webs, such as many shark species, could be prone to the accumulation of both healthy and toxic chemicals for humans.

Current shark and ray fishing is estimated at approximately in 1.5 million tons per year although most of their populations are in decline mainly because of it (Pierce, 2022; Worm et al., 2013). Small-scale shark fisheries are an important livelihood resource in many developing countries due to its low price compared with other fish species (Pierce et al., 2022). In northwestern Mexico, fish tacos are one of the most popular dishes in the states surrounding the Gulf of California (GC). Fish tacos are often prepared with different species of small to medium-sized sharks. Among the most caught shark species in the GC are the scalloped hammerhead (HH) *Sphyrna lewini*, the Pacific sharpnose shark (PS) *Rhizoprionodon longurio*, and the Pacific angel shark (PA) *Squatina californica*. Different studies have been carried out in the area to evaluate the possible risk associated to inorganic pollutants (mainly mercury, Hg) through the consumption of these three species (Escobar-Sánchez et al., 2016; Frías-Espericueta et al., 2019; Ruelas-Izunza et al., 2020). However, the toxic risk associated with organic contaminants, such as OCPs and PCBs is almost unexplored, as well as the risk-benefit associated with the intake of contaminants and essential fatty acids.

Therefore, our investigation was focused primary on the human health risk assessment by multiple organochlorine contaminants through the consumption of the three most exploited shark species from the western GC. We used a

dose/concentration addition approach due to the similar mode of action of the different pollutants analyzed (Pose-Juan et al., 2016; USEPA, 2000). Finally, we estimate the benefit-risk ratio of the human consumption of these species, basing on concentrations of EPA and DHA reported by Lladó-Cabrera (2020), Hg (published by Escobar-Sánchez et al. (2016) for PA captured in the study area) and new data on OCs in muscle tissue of HH, PS and PA from the GC.

4.3. Material and methods

4.3.1. Shark meat sampling

Samples from the dorsal musculature of HH (n = 20), PS (n =20), and PA (n = 19) were collected between 2018 and 2019 in two fishing camps of Baja California Sur (BCS), in the western coast of the GC (Mexico) (**Fig. 4.1**). Every sample was placed in a cleaned glass jars and preserved in ice until later storage in a freezer at −20 °C.

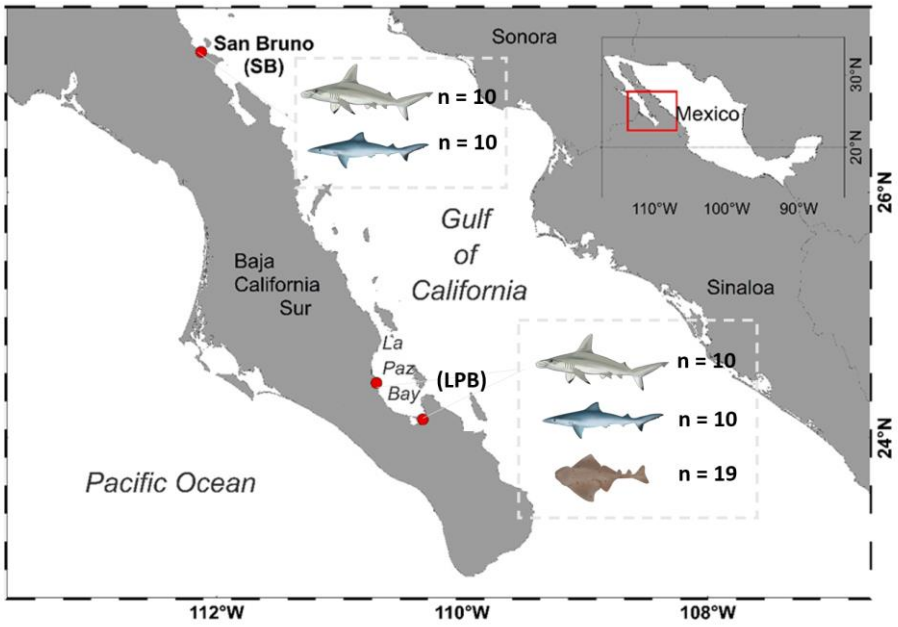


Fig. 4.1. Sampling locations in Baja California Sur, Mexico, and the number of individuals sampled in each location.

4.3.2. Organochlorine analyses

Laboratory analyses were conducted at the Institute of Oceanological Research (IIO) from the Autonomous University of Baja California following the methodology described on Ángel-Moreno-Briones et al. (2022). Muscle lipid content was determined gravimetrically after extraction with hexane HPLC-grade. Contaminant extraction was performed from ≈ 1 g of muscle using silica gel and florisil as sorbents, and pesticide-grade hexane/acetone (4:1, v/v), as solvents. Recovery surrogates (TCMX and PCB_{209}, Ultra Scientific Inc.) were spiked before contaminant extraction in ASE 350. The extracts were cleaned using adsorption chromatography columns packed with activated florisil and alumina. The organochlorines were eluted from the column with 10 mL of hexane, 20 mL of hexane/dichloromethane (70:30, v/v), and 25 mL of dichloromethane (pesticide-grade).

The detection and quantification of 29 OCPs and 18 PCBs was performed using an Agilent 7010A Triple Quadrupole GC/MS operated in Electron Ionization (EI) mode and multiple reaction monitoring (MRM). The OCPs targeted were: DDTs (p,p'-DDT, o,p'-DDT, p,p'-DDE, o,p'-DDE, p,p'-DDD and o,p'-DDD), CHLOs (heptachlor, heptachlor epoxide, oxychlordane, cis- and trans-chlordane, cis- and trans-nonachlor), CBz (penta- and hexachlorobenzene); hexachlorociclohexenes (HCHs: α-, β-, δ-, and γ-HCH), endosulfan (α- and β- endosulfan and endosulfan sulfate), drins (aldrin, dieldrin, endrin and endrin aldehyde), mirex, hexachlorobutadiene (HCB), and methoxychlor. The PCBs analyzed included: Trichlorobiphenyl (Tri-CB: PCB-28), Tetrachlorobiphenyls (Tetra-CBs: PCB-52, PCB-81, PCB-77), Pentachlorobiphenyls (Penta-CBs: PCB-101, PCB-123, PCB-118, PCB-114, PCB-105, PCB-126), Hexachlorobiphenyls (Hexa-CBs: PCB-153, PCB-138, PCB-167, PCB-156, PCB-157, PCB-169), and Heptachlorobiphenyls (Hepta-CBs: PCB-180, PCB-189). The identification was carried out using the retention times, masses and relative abundance of the confirmation ions. A five-point calibration curve performed for each targeted analyte were used for the quantification, using the internal standard method (PCB_{30} and PCB_{205}). Method blanks and reference material (National Institute of Standards & Technology SRM-1946) were included during the extraction of each set of twenty samples (see **ANNEX 2**, **Table S3.2**). The recoveries were 63 ± 14% (TCMX) and 93 ±

27% (PCB_{209}). The limits of detection (LOD) are reported in the Supplementary Material, **Table S4.1**.

4.3.3. Expressions, data treatment and statistics

Organochlorine concentrations were expressed as nanograms per gram of wet weight (ng/g w.w.). Only the compounds and chemical groups detected above the detection limit in more than 50% of the samples of each species were considered for statistical treatment. The concentrations below detection limits were replaced by a proxy value corresponding to the LOD/2 (George et al., 2021; Mikkonen et al., 2018). Non-detected (n.d.) compounds were treated as zero. Descriptive statistics were performed to measure the average concentrations, its range, and to determine the standard deviations. Bioaccumulation was determined analyzing the correlation between total length of organisms and pollutant concentration using Spearman's- test. Differences between species and population groups (sex, life stage) were analyzed using Wilcoxon-test. Differences between sample places were not tasted due to the migratory behavior of HH and PS (Kimley et al., 1989; Márquez-Farías et al., 2005) and proximity between both localities. Statistical significance was established at $p < 0.05$. All statistics were conducted in R (version 3.6.3).

4.3.4. Health risk assessment

To assess the possible risk associated to OCs thought the consumption of HH, PS and PA muscle we used the CR_{lim} approach. The CR_{lim} is the maximum allowable consumption rate (g/d), and represents the maximum lifetime daily consumption rate below which consumption of the species is not expected to cause adverse effects on human health (USEPA, 2000). The CR_{lim} was assessed for each species considering multiple contaminants for both non-carcinogenic and carcinogenic effects.

CR_{lim} for non-carcinogenic effects was calculated according to the equation proposed by USEPA (2000) and modified by (Yu et al., 2012):

$$CR_{lim} = \frac{BW}{\frac{C_1}{RfD_1} + \frac{C_2}{RfD_2} + \cdots + \frac{C_x}{RfD_x}}$$

CR_{lim} for carcinogenic effects was calculated following USEPA (2000):

$$\mathrm{CR}_{\mathrm{lim}} = \frac{\mathrm{ARL} * \mathrm{BW}}{(\mathrm{C}_1 + \mathrm{C}_2 + \cdots + \mathrm{C}_x) * \mathrm{CSF}}$$

where BW is the average weight of consumer population (kg), C is the concentration of a given analyte (1,2,…,x); RfD is the reference dose of each analyte (mg/kg per day); CSF is the cancer slope factor of each analyte (mg/kg per day)$^{-1}$, and ARL is the maximum acceptable individual lifetime risk level, established in 10^{-5} (unit less) (USEPA, 2000). In this study, the average population weight was set at 70 kg as recommended by the USEPA (2000), in the absence of precise data on the average population weight in BCS. The RfD and CSF of the different analytes are listed by USEPA (2000). The ARL is established in 10^{-5}.

4.3.5. Health benefit-risk assessment

In order to evaluate the benefit-risk by the consumption of the three shark species, we used the benefit-risk quotient (BRQ) according to Gladyshev et al. (2020):

$$BRQ = \frac{CR_{EFA}}{CR_{lim}}$$

where CR_{EFA} is the shark consumption rate of each shark species (g/day) needed to reach the recommended daily intake of essential fatty acids (EFA: EPA + DHA). When BRQ < 1, the benefit from shark consumption is higher than risk, while BRQ > 1 indicate risk.

The CR_{EFA} was calculated as follows:

$$\mathrm{CR}_{\mathrm{EFA}} = \frac{\mathrm{R}_{\mathrm{EFA}}}{\mathrm{C}_{\mathrm{EFA}}}$$

where R_{EFA} is the recommended daily intake of EFA (EPA + DHA, 1000 mg/day according to Gladyshev et al. (2020)), and C_{EFA} is the concentration of EFA determined in the muscle of each species. In this study, we used the average concentrations of EPA + DHA reported by (Lladó Cabrera, 2020) in muscle of HH (2.0 mg/g w.w.), PS (2.4 mg/g w.w.) and PA (1.7 mg/g w.w.) captured in the study area. These values were obtained from dry weight (d.w.) to wet weight (w.w.)

multiplying d.w. by 0.3, assuming a 70% moisture content (Kim et al., 2019; Suryaningsih et al., 2020).

4.4. Results and Discussion

4.4.1. Organochlorine levels in shark muscle

Overall, 35 of the 48 screened contaminants were identified in the muscle of sharks. However, only 6 analytes were found at concentrations above detection limits (a.d.l.) in more than 60% of individuals of at least one of the species: p,p'-DDE, hexachlorobencene, pentachlorobencene, heptachlor, hexachlorobutadiene, and PCB-189. Descriptive statistics of these pollutants and the lipid content of muscle tissue are reported in **Table 4.1**.

Table 4.1

Organochlorine concentrations and lipid content in muscle of the three shark species from the western coast of the Gulf of California. The numbers in parentheses correspond to the number of samples in which each analyte was detected.

	Risk values[a]		**Concentration (ng/g w.w.) and IF in shark muscle**		
Contaminant	RfD (ng/kg-d)	CSF $(mg/kg\text{-}d)^{-1}$	*S. lewini*	*R. longurio*	*S. californica*
p,p'-DDE	5×10^{-4}	0.34	0.59 ± 0.21 (20)	0.59 ± 0.16 (20)	b.d.l.[c]
HCBz	8×10^{-4}	1.6	0.36 ± 0.022 (10)	0.50 ± 0.032 (20)	b.d.l.
PeCBz	/	/	b.d.l.	0.74 ± 0.028 (20)	b.d.l.
HCBD	/	/	b.d.l.	0.63 ± 0.023 (20)	b.d.l.
Heptachlor	1.3×10^{-5} [b]	9.1[b]	0.17 ± 0.0052 (10)	0.39 ± 0.01 (9)	b.d.l.
PCB - 189	2×10^{-5}	1.6	0.37 ± 0.10 (10)	0.68 ± 0.22 (19)	0.51 ± 0.21 (17)
Lipid content (%)			0.22 ± 0.20	0.14 ± 0.10	0.18 ± 0.053

[a] Risk values for noncarcinogens (RfD) and carcinogens (CSF) reported in USEPA (2000).

[b] The values of RfD and CSF listed are for heptachlor in USEPA (2000).

[c] b.d.l.: below detection limit

The p,p'-DDE was the main compound presented in HH (0.59 ± 0.21 ng/g); the PeCBz was the main one quantified in PS (0.74 ± 0.028 ng/g), while PCB-189 was the only analyte detected at concentration a.d.l. in PA (0.18 ± 0.053 ng/g) (**Table 4.1**). Higher concentrations of Hg than OCs were found in previous studies on the three shark species (Supplementary Material, **Table S4.2**), which is attributed to the physico-chemical properties of the pollutants and the biochemical composition of shark tissue. The low detection frequencies and average concentrations (< 1 ng/g) of organochlorine compounds in muscle are partially explained by its low lipid content (**Table 4.1**). The three shark species can be classified as fishes with "very low fat in muscle" (< 2% of lipid content) according to Swapna (2010), which implies reduced absorption of lipophilic compounds (Borgå et al., 2004). Conversely, Hg has affinity for the thiol sulfhydryl groups of the muscle amino acids and tends to accumulate in this tissue (O'Bryhim et al., 2017).

The concentrations of DDT and PCBs quantified in all samples were far below the human safety tolerance levels in fish meat set by different international agencies (5000 and 2000 ng/g w.w, respectively) (ATSDR, 2012).

4.4.1.1. Comparisons with OC levels in muscle of worldwide sharks

Concentrations of p,p'-DDE in HH, PS and PA muscle (0.59 ± 0.21 ng/g, 0.59 ± 0.16 ng/g, and b.d.l., respectively) were far below the average value reported by Tiktak et al. (2020) based on the review of works on levels of DDT in muscle of worldwide elasmobranch species (11 ± 14 ng/g w.w.). Mexico was the Latin America country with the highest DDT use, but it was far below the amount used in other countries such as the USA (Li and MacDonald, 2005). This, together with the fact that most studies on OC concentrations in sharks focus on both coasts of the United States or traditionally polluted areas as the Mediterranean Sea, partially explains the low concentrations in our work compared to the global average. On the other hand, our studied species presented a low lipid content in muscle compared to other shark species such as the spiny dogfish *Squalus acanthias* (18.7 ± 3.6%), or the blacktip reef shark *Carcharhinus melanopterus* (4.2 ± 1.3%) (Lee et al., 2015), which could influence the observed results (Lyons et al., 2021). Conversely, levels of p,p'-DDE in HH and PS were similar to those reported in the lean muscle (< 1% of lipid content) of bull (*Carcharhinus leucas*:

0.58 ± 0.65 ng/g w.w.) and tiger sharks (*Galeocerdo cuvier*: 0.59 ± 0.58 ng/g w.w.) from Reunion Island, Southwest Indian Ocean (Chynel et al., 2021).

Non-DDT OCPs are not frequently analyzed in shark tissues despite its known adverse effects on the health of wild animals and humans, which difficult to evaluate the magnitude of the levels obtained in our study. But there is some information for comparison purposes. Levels of HCBz in the muscle of HH and PS were similar to those found in white shark *Carcharodon carcharias* from South Africa (0.30 ± 0.17 ng/g w.w.) (Schlenk et al., 2005) and higher than concentrations determined in *Sphyrna zygaena*, *Isurus oxyrinchus* and *Alopias superciliosus* from Brazil (b.d.l. - 0.06 ng/g w.w.) (Azevedo-Silva et al., 2009). The levels of heptachlor detected in HH and PS were similar than those determined in the bamboo shark *Chiloscyllium plagiosum* from China (b.d.l. - 0.50 ng/g w.w.) (Cornish et al., 2007). To the best of our knowledge, there is no information reporting levels of PeCBz nor HCBD in muscle of worldwide sharks.

Regarding PCBs, the levels found in the present study (∑PCBs = 1.49 ± 0.43, 1.88 ± 0.25, and 1.35 ± 0.35 ng/g w.w. in HH, PS and PA, respectively) are one order of magnitude lower than the average concentrations found in sharks worldwide (15 ± 14 ng/g w.w.) (Tiktak et al., 2020).

4.4.1.2. Inter-specific comparisons

In general, PS presented higher IF frequencies and concentrations of analytes than HH and PA. The inter-specific variability in contaminant accumulation found in muscle (**Table 4.1**, **Fig. 4.2**) was also observed in the liver tissue of these species and it is attributed to differences in the life history traits and habitat use (see **Chapter 3**).

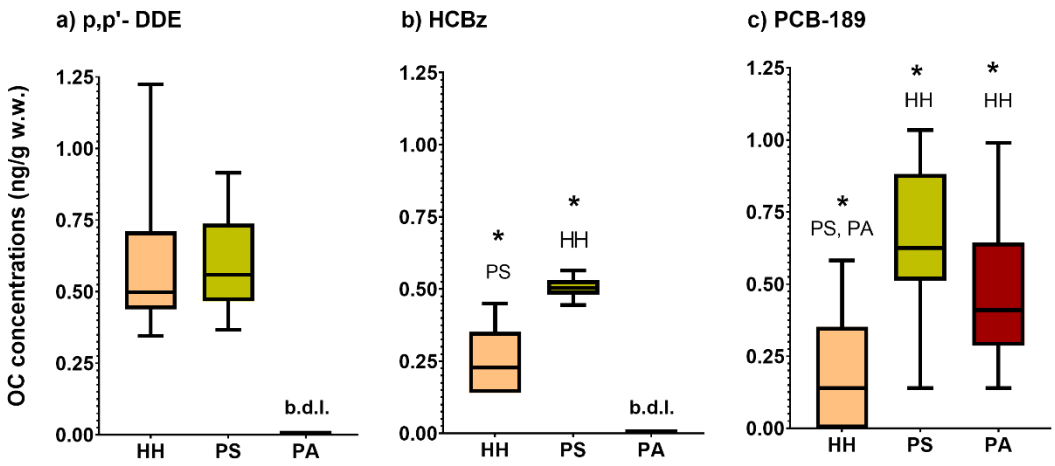


Fig. 4.2. Comparison between OC concentrations in muscle of the three shark species from the western coast of the Gulf of California. Asterisks represent significant differences.

4.4.1.3. Intra-specific accumulation trends

Differences in the accumulation of analytes were not found between females and males of each species, nor between juveniles and adults of PS and PA ($p > 0.05$). When examining the relationship between contaminant concentration and the size of individuals, the correlations were only statistically significant between p,p'-DDE and the TL of juveniles HH ($r = 0.58$, $p = 0.011$), as it was previously presented in **Chapter 2**.

4.4.2. Maximum allowable shark consumption rates

Table 4.2 presents the maximum daily consumption rate of the three species for non-cancer and cancer risk, considering multiple OC contaminants. For PA, the Hg average concentration (240 ng/g w.w.) published by Escobar-Sánchez et al. (2016) was incorporated in the assessment of non-cancer risk. Due to the muscle samples of both studies were taken in the same area (LPB), we can assume a low variability in mercury levels between the individuals analyzed by Escobar-Sánchez and those sampled for the present work.

Table 4.2

Maximum allowable consumption rates of *Sphyrna lewini*, *Rhizoprionodon longurio* and *Squatina californica* for non-cancer and cancer risk.

Species	CR_{lim} **No Cancer** Risk (g/day)		CR_{lim} **Cancer** Risk (g/day)
	OCs	OCs and Hg	OCs
S. lewini	847.0	/	160.0
R. longurio	657.9	/	105.6
S. californica	1037	28.4	259.3

Considering only organic pollutants, the values of CR_{lim} for cancer risk are more restrictive than those of non-cancer (**Table 4.2**). Therefore, the consumption limits should be set considering the CR_{lim} for cancer risk. Considering the cancer risk results, a lifetime intake higher than 105.6 g/day of PS, 160 g/day of HH, and 259.3 g/day of PA would be expected to be harmful to humans. Therefore, based on the CR_{lim} values for multiple organochlorines, the healthier shark species for human consumption is PA, followed by HH and PS (**Table 4.2**). However, taking into account the levels of PCBs found in PA and the concentrations of mercury analyzed by Escobar-Sánchez et al. (2016), a consumption rate higher than 28.4 g/day may imply risk of non-cancer effects on human health. It is important to note that the USEPA guidelines do not provide a CSF for Hg and therefore, cancer risk for this element could not be calculated.

Per capita fish consumption in Mexico, as well as worldwide, has increased in the last years (Shiffman and Hueter, 2017). However, the daily fish intake of the Mexican average population (35.9 g/day) and the estimated global consumption rate of fish (56.2 g/day) (CONAPESCA, 2018; Statista, 2022) are well below the CR_{lims} calculated for the three species captured in the GC considering only OCs (**Table 4.2**). Nevertheless, considering Hg concentrations in PA, the CR_{lim} for non-cancer effects is lower than the fish consumption rate of Mexican and global average population. In addition, it is important to consider that the population from coastal communities usually have higher rates of fish and shellfish consumption. For example, it was determined that women from small fishing villages from Sonora consumes an average of 307 g of seafood per day, founding the highest ingestion rate in women between 40 – 49 and 16 – 19 years old (471 and 469

g/day, respectively) (García-Hernández et al., 2018). Similarly, Zamora-Arellano et al. (2017) reported that the general population of Mazatlán (the coastal capital of Sinaloa) consume 207 g of fish products per day, while the fishing-related population consume 423 g/day. All these ingestion rates are above the CR_{lim} of cancer risk obtained in HH and PS. Regarding the CR_{lim} of cancer risk resulted in PA, it is still above the consumption rate of the general population from Mazatlán, but it is below the ingestion rates of people involved in fishing activities from Sonora and Sinaloa. Unfortunately, until our knowledge there are not studies about the consumption habits of BCS population, although it can be expected to exceed the Mexican average intake rate, mainly in fishing populations.

In Mexico it is recommended eating 2 – 3 servings of fish per week (Procuraduría Federal del Consumidor, 2017), which is equivalent to 64.9 – 97.3 g/day assuming a meal size of 227 g (USEPA, 2000). This recommendation would not pose a health risk to consumers if only OCs were taken into account, but it could pose a risk considering the levels of mercury in PS. Further studies should analyze both organic and inorganic pollutants in the muscle of HH, PS and PA in order to provide a better risk assessment.

4.4.3. Health risk-benefit

Considering a monospecific diet, to supply the recommended daily intake of EFA (1000 mg/g day) to prevent cardiovascular diseases, as well as inflammatory and neural disorders (Anishchenko et al., 2017) it would be necessary to eat 490.2 g/day of HH, 411.5 g/day of PS and 598.9 g/day of PA. Taking into account the CR_{lims} of cancer risk (**Table 4.1**), the BRQ > 1 in the three species (HH = 3.1; PS = 3.9, and PA = 2.3). Therefore, risk of consumption of HH, PS and PA from the eastern coast of BCS over weighted benefits of EPA and DHA intakes, even when only considering the concentrations of OCs. These results can be partially attributed to their low EFA content. Fayet-Moore et al. (2015) classified sharks as a moderate source of essential fatty acids for human nutrition, as well as other types of fish as whitings or tilapias. In contrast, species such as Atlantic salmon *Salmo salar* or Pacific saury *Cololabis saira* can reach EFA concentrations up to 20 times higher than those reported in the three shark species studied (Anischenko et al., 2017; Ansorena et al., 2010).

In view of our results, it would be recommendable to limit the consumption of HH, PS, and PA captured in the western coast of the GC.

4.5. Uncertainty analysis

The identification and discussion of uncertainty is an important step in the assessment of human health risks associated to fish consumption (Lee et al., 2021; More et al., 2019; USEPA, 2000; Wang et al., 2013; Yu et al., 2014).

In the current study, a wide range of contaminants was analyzed. However, some detected compounds as HCBD on PeCBz are not considered in the health risk equations because there are not RfD and CSF defined for them (USEPA, 2000). In addition, it has been reported that sharks from the GC are exposed to other pollutants as PAH or As, that were not included in our evaluation (Fossi et al., 2017; Frías-Espericueta et al., 2014). It is important to note that risk equations for multiple contaminants assume additive effects among them, but many pollutants could present synergistic or even antagonistic interactions, which is difficult to incorporate in risk assessments (Yu et al., 2014).

This research carried out a conservative approach as it has been considered more appropriate for the initial health-risk characterization, but it could imply certain overestimation of the exposure and risk (Jiang et al., 2005). For example, we used in the calculations of risk-benefit a recommended EFA daily intake of 1000 mg as suggested by Gladyshev et al. (2020), but a daily intake of 500 or 250 mg have been used by other authors (e.g., Zhang et al., 2012). When included Hg concentrations from published data in the non-cancer risk equations, we did not take into account that the absorbed portion of this element, monomethyl mercury (MeHg), represents around the 80% of total Hg (USEPA, 2000). In addition, digestible DDT from fish consumption is reported to be up to 36% of the DDTs concentrations presented in raw fish (Wang et al., 2011). In addition, tacos and other popular dishes are prepared with fried, grilled, boiled or steamed fish. We did not incorporate in the estimations the effects of marinating or cooking, which influence in the contaminant levels as well as in the nutritional composition of fish. Although the cooking process seems to significantly increase

Hg concentrations, it could imply a reduction of up to 50% of OC levels in fish flesh (Bayen et al., 2005; Domingo, 2011; Sobral et al., 2017).

The numerous uncertainties highlight the need to take the recommendations of this work with caution. More accurate recommendations about shark consumption will need the incorporation of more information about pollutant concentrations in edible tissue, individual variations within the human population exposed to them (e.g., average body weight) and more complex analyses.

4.6. Conclusions

This investigation provides initial valuable insights regarding on the human exposure to multiple organochlorines through the consumption of three of the most commercial shark species in BCS. Levels of DDTs and PCBs in the muscle of HH, PS, and PA were low compared with levels in other sharks worldwide, while the concentrations of non-DDT OCPs are difficult to assess due to the lack of background information. However, taking into account the multiple contaminants approach, lifetime consumption of HH and PS at subsistence fishermen rates could be harmful to humans.

Given that the toxic risk outweighs the nutritional benefit of consuming HH, PS, and PA captured in the western GC, fish consumers are advised to avoid these species in their diet.

CONCLUDING REMARKS

This investigation includes the first information on organic pollutants in liver and muscle tissue of three target shark species from the GC. Their concentrations of OCPs and PCBs in both tissues were generally lower than those reported worldwide for elasmobranch species, and in other marine predators from the Gulf of California. However, the wide variety of analytes detected in shark tissues suggests that OCPs PCBs continue to be a potential threat due to their persistence and, possibly, to their recent (intentionally or not) application. The generally higher levels of OCPs than PCBs indicated a greater incidence of agriculture than industrial activities in the region. The PA integrates pollutants from more restricted areas than the other two species analyzed due to its low mobility, which allows to determine that the western coast of the GC is less contaminated by OCPs than surrounding areas.

The metabolite p,p'-DDE was the main compound detected in liver samples of the three species (HH = PS > PA), as well as in the muscle tissue of HH. In muscle of PS, the main compound was PeCBz, and in PA, PCB-189. Habitat use, lipid content, longevity and the strategy of reproduction are factors that determine the exposition and accumulation capacity of shark species. Moreover, differences in OCPs concentrations between HH, PS, and PA demonstrate the importance of the proper species selection for monitoring studies in order to obtain reliable information on the environmental contamination. The three shark species showed different intraspecific accumulation trends due to their different life history traits. Size and age in juvenile HH, as well as size, maturity stage, and sex in PS and PA seems to be factors influencing OC loads. This fact, together with the presence of outliers in our results, shows the limitations of research with small sample sizes in toxicological studies, as well as the importance of knowing the biological and ecological characteristics of the species for their interpretation.

The hepatic levels of some OCPs in HH and PS were similar to those associated with toxic effects in other elasmobranchs species. The high levels observed in one of the two neonates of HH could be a concern due to the vulnerability during early life stages to these pollutants. Conversely, the TEQs of DL-PCB indicate no risk of health effects in any shark species when compared to established thresholds in fish. Therefore, we can conclude that the risk of potential health

effects due to OCs in PA is low, but it would be necessary to focus future research efforts in the potential risk of toxic effects in PS and HH, mainly in neonates of this species.

Regarding on the human exposure to multiple organochlorines through the consumption of muscle of the three shark species, its consumption twice or three times per week seems to not pose a risk to human health. However, the lifetime consumption of HH and PS at subsistence fishermen rates could suppose cancer risk. Considering the levels of OCs and the concentration of essential fatty acids in muscle of the three species, the toxic risk outweighs the nutritional benefit. Therefore, its consumption is not recommended.

REFERENCES

Alatorre-Ramírez, V.G., Galván-Magaña, F., Torres-Rojas, Y.E., 2013. Trophic habitat of the Pacific sharpnose shark, *Rhizoprionodon longurio*, in the Mexican Pacific. J. Mar. Biol. Assoc. United Kingdom 93, 2217–2224. https://doi.org/10.1017/S0025315413000957

Aldana-Moreno, A., Hoyos-Padilla, E.M., González-Armas, R., Galván-Magaña, F., Hearn, A., Klimley, A.P., Winram, W., Becerril-García, E.E., Ketchum, J.T., 2019. Residency and diel movement patterns of the endangered scalloped hammerhead *Sphyrna lewini* in the Revillagigedo National Park. J. Fish Biol. 1–6. https://doi.org/10.1111/jfb.14239

Alegria, H.A., Wong, F., Jantunen, L.M., Bidleman, T.F., Figueroa, M.S., Bouchot, G.G., Moreno, V.C., Waliszewski, S.M., Infanzon, R., 2008. Organochlorine pesticides and PCBs in air of southern Mexico (2002-2004). Atmos. Environ. 42, 8810–8818. https://doi.org/10.1016/j.atmosenv.2008.04.053

Alves, L.M.F., Lemos, M.F.L., Cabral, H., Novais, S.C., 2022. Elasmobranchs as bioindicators of pollution in the marine environment. Mar. Pollut. Bull. 176, 113418. https://doi.org/10.1016/j.marpolbul.2022.113418

Ángel-Moreno-Briones, Á., Hernández-Guzmán, F.A., González-Armas, R., Galván-Magaña, F., Marmolejo-Rodríguez, A.J., Sánchez-González, A., Ramírez-Álvarez, N., 2022. Organochlorine pesticides in immature scalloped hammerheads *Sphyrna lewini* from the western coast of the Gulf of California, Mexico: Bioaccumulation patterns and human exposure. Sci. Total Environ. 14. https://doi.org/10.1016/j.scitotenv.2021.151369

Anishchenko, O. V., Sushchik, N.N., Makhutova, O.N., Kalachova, G.S., Gribovskaya, I. V., Morgun, V.N., Gladyshev, M.I., 2017. Benefit-risk ratio of canned pacific saury (C*ololabis saira*) intake: Essential fatty acids vs. heavy metals. Food Chem. Toxicol. 101, 8–14. https://doi.org/10.1016/j.fct.2016.12.035

Anislado-Tolentino, V., Gallardo-Cabello, M., Amezcua-Linares, F., Robinson-Mendoza, C., 2008. Age and growth of the scalloped hammerhead shark, *Sphyrna lewini* (Griffith & Smith, 1834) from the Southern coast of Sinaloa, México. Hidrobiológica 18, 31–40.

Arnot, J.A., Gobas, F.A.P.C., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev. 14, 257–297. https://doi.org/10.1139/A06-005

Arnot, J.A., Gobas, F.A.P.C., 2004. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. Environ. Toxicol. Chem. 23, 2343–2355. https://doi.org/10.1897/03-438

Arrebola, J.P., Castaño, A., Esteban, M., Bartolomé, M., Pérez-Gómez, B., Ramos, J.J., 2018. Differential contribution of animal and vegetable food items on persistent organic pollutant serum concentrations in Spanish adults. Data from BIOAMBIENT.ES project. Sci. Total Environ. 634, 235–242. https://doi.org/10.1016/j.scitotenv.2018.03.283

ATSDR, 2000. Toxicological profile for polychlorinated biphenyls (PCBs). [WWW Document]. ATSDR's Toxicol. Profiles. URL https://www.atsdr.cdc.gov/toxprofiles/tp17.pdf

ATSDR, 2015a. Toxicological profile for endosulfan. [WWW Document]. ATSDR's Toxicol. Profiles. URL https://www.atsdr.cdc.gov/toxprofiles/tp41.pdf

ATSDR, 2015b. Toxicological profile for hexachlorobenzene. [WWW Document]. ATSDR's Toxicol. Profiles. URL https://www.atsdr.cdc.gov/toxprofiles/tp90.pdf

ATSDR, 2018. Toxicological profile for chlordane [WWW Document]. ATSDR's Toxicol. Profiles. URL https://www.atsdr.cdc.gov/ToxProfiles/tp31.pdf

ATSDR, 2019. Toxicological profile for DDT, DDE, and DDD [WWW Document]. ATSDR's Toxicol. Profiles. URL https://www.atsdr.cdc.gov/toxprofiles/tp35.pdf

ATSDR, 2020. Toxicological profile for mirex and chlordecone [WWW Document]. ATSDR's Toxicol. Profiles. URL https://www.atsdr.cdc.gov/toxprofiles/tp66.pdf

ATSDR, 2021. Toxicological profile for hexachlorobutadiene [WWW Document]. ATSDR's Toxicol. Profiles. URL https://www.atsdr.cdc.gov/toxprofiles/tp42.pdf

ATSDR, 2022. Toxicological profile for aldrin/dieldrin. [WWW Document]. ATSDR's Toxicol. Profiles. URL https://www.atsdr.cdc.gov/ToxProfiles/tp1.pdf

Azevedo-Silva, C.E., Azeredo, A., Cássia-Lima-Dias, A., Costa, P., Lailson-Brito, J., Malm, O., Daveé-Guimaraes, J.R., Machado-Torres, J.P., 2009. Organochlorine compounds in sharks from the Brazilian coast. Mar. Pollut. Bull. 58, 294–298. https://doi.org/10.1016/j.marpolbul.2008.11.003

Bailey, R.E., 2001. Global hexachlorobenzene emissions. Chemosphere 43, 167–182. https://doi.org/10.1016/S0045-6535(00)00186-7

Baje, L., Smart, J.J., Chin, A., White, W.T., Simpfendorfer, C.A., 2018. Age, growth and maturity of the Australian sharpnose shark *Rhizoprionodon taylori* from the Gulf of Papua. PLoS One 13, 1–17. https://doi.org/10.1371/journal.pone.0206581

Bala, K., Geueke, B., Miska, M.E., Rentsch, D., Poiger, T., Dadhwal, M., Lal, R., Holliger, C., Kohler, H.P.E., 2012. Enzymatic conversion of ε-hexachlorocyclohexane and a heptachlorocyclohexane isomer, two neglected components of technical hexachlorocyclohexane. Environ. Sci. Technol. 46, 4051–4058. https://doi.org/10.1021/es204143x

Barber, J.L., Sweetman, A.J., Van Wijk, D., Jones, K.C., 2005. Hexachlorobenzene in the global environment: Emissions, levels, distribution, trends and processes. Sci. Total Environ. 349, 1–44. https://doi.org/10.1016/j.scitotenv.2005.03.014

Baró-Camarasa, I., Marmolejo-rodríguez, A.J., Hara, T.M.O., Castellini, J.M., Murillo-Cisneros, D.A., Martínez-Rincón, R.O., Elorriaga-Verplancken, F.R., Galván-Magaña, F., 2022. Mercury maternal transfer in two placental sharks and a yolk-sac ray from Baja California Sur, Mexico. Mar. Pollut. Bull. 179. https://doi.org/10.1016/j.marpolbul.2022.113672

Baró-Camarasa, I., Marmolejo-Rodríguez, A.J., O'Hara, T.M., Elorriaga-Verplancken, F.R., Trejo-Ramírez, A., Martínez-Rincón, R.O., Galván-Magaña, F., 2020. Isotopic (δ15N) relationship of pregnant females and their embryos: Comparing placental and yolk-sac viviparous elasmobranchs. J. Fish Biol. 1–7. https://doi.org/10.1111/jfb.14625

Bayen, S., Barlow, P., Lee, H.K., Obbard, J.P., Bayen, S., Barlow, P., Lee, H.K., Obbard, J.P., Obbard, J.P., 2005. Effect of cooking on the loss of persistent organic pollutants from salmon. J. Toxicol. Environ. Heal. Part A. https://doi.org/10.1080/15287390590895126

Beckvar, N., Dillon, T.M., Read, L.B., 2005. Approaches for linking whole-body fish tissue residues of mercury or DDT to biological effects thresholds. Environ. Toxicol. Chem. 24, 2094–2105. https://doi.org/10.1897/04-284R.1

Beckvar, N., Lotufo, G.R., 2011. DDT and Other Organohalogen Pesticides in Aquatic Organisms, in: Beyer, N.W., Meador, J.P. (Eds.), Environmental Contaminants in Biota: Interpreting

Tissue Concentrations. Taylor & Francis Group, Boca Raton.

Bejarano-Álvarez, M., Galván-Magaña, F., Ochoa-Báez, R.I., 2010. Reproductive biology of the scalloped hammerhead shark *Sphyrna lewini* (Chondrichthyes: Sphyrnidae) off south-west Mexico. Aqua, Int. J. Ichthyol. 17, 11–20.

Bergés-Tiznado, M.E., Márquez-Farías, F., Lara-Mendoza, R.E., Torres-Rojas, Y.E., Bojórquez-Leyva, H., Páez-Osuna, F., 2015. Mercury and selenium in muscle and target organs of scalloped hammerhead sharks *Sphyrna lewini* of the SE Gulf of California: dietary intake, molar ratios, loads, and human health risks. Arch. Environ. Contam. Toxicol. 69, 440–452. https://doi.org/10.1007/s00244-015-0226-8

Berninger, J.P., Tillitt, D.E., 2019. Polychlorinated biphenyl tissue-concentration thresholds for survival, growth, and reproduction in fish. Environ. Toxicol. Chem. https://doi.org/10.1002/etc.4335

Beyer, N.W., Meador, J.P. (Eds.), 2011. Environmental Contaminants in Biota: Interpreting Tissue Concentrations. Taylor & Francis Group, Boca Raton.

Bizzarro, J.J., Smith, W.D., Hueter, R.E., Villavicencio–Garayzar, C.J., 2009. Activities and catch composition of artisanal elasmobranch fishing sites on the eastern coast of Baja California Sur, Mexico. Bull. South. Calif. Acad. Sci. 108, 137–151. https://doi.org/10.3160/0038-3872-108.3.137

Boldrocchi, G., Monticelli, D., Butti, L., Omar, M., Bettinetti, R., 2020. First concurrent assessment of elemental- and organic-contaminant loads in skin biopsies of whale sharks from Djibouti. Sci. Total Environ. 722, 137841. https://doi.org/10.1016/j.scitotenv.2020.137841

Boldrocchi, G., Monticelli, D., Omar, Y.M., Bettinetti, R., 2019. Trace elements and POPs in two commercial shark species from Djibouti: implications for human exposure. Sci. Total Environ. 669, 637–648. https://doi.org/10.1016/j.scitotenv.2019.03.122

Bondy, G., Armstrong, C., Coady, L., Doucet, J., Robertson, P., Feeley, M., Barker, M., 2003. Toxicity of the chlordane metabolite oxychlordane in female rats: Clinical and histopathological changes. Food Chem. Toxicol. 41, 291–301. https://doi.org/10.1016/S0278-6915(02)00229-6

Borrell, A., Aguilar, A., 1987. Variations in DDE percentage correlated to total DDT burden in the blubber of fin and sei whales. Mar. Pollut. Bull. 18, 70–74.

Borgå, K., Fisk, A.T., Hoekstra, P.F., Muir, D.C.G., 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. Environ. Toxicol. Chem. 23, 2367–2385. https://doi.org/10.1897/03-518

Borgå, K., Kidd, K.A., Muir, D.C.G., Berglund, O., Conder, J.M., Gobas, F.A.P.C., Kucklick, J., Malm, O., Powellkk, D.E., 2011. Trophic magnification factors: Considerations of ecology, ecosystems, and study design. Integr. Environ. Assess. Manag. 8, 64–84. https://doi.org/10.1002/ieam.244

Botello, A.V., Páez-Osuna, F., Mendez-Rodríguez, L., Betancourt-Lozano, S., Álvarez- Borrego, S., Lara-Lara, R., 2014. Pacífico Mexicano. Contaminación e impacto ambiental: Diagnóstico y tendencias. UAC, UNAM-ICMYL, CIAD-MAZATLÁN, CINBOR, CICESE. Mexico.

Breivik, K., Sweetman, A., Pacyna, J.M., Jones, K.C., 2007. Towards a global historical emission inventory for selected PCB congeners - A mass balance approach. 3. An update. Sci. Total

Environ. 377, 296–307. https://doi.org/10.1016/j.scitotenv.2007.02.026

Breivik, K., Sweetman, A., Pacyna, J.M., Jones, K.C., 2002. Towards a global historical emission inventory for selected PCB congeners - A mass balance approach: 1. Global production and consumption. Sci. Total Environ. 290, 181–198. https://doi.org/10.1016/S0048-9697(01)01075-0

Brusca, R.C., 2010. The Gulf of California: Biodiversity and conservation. University of Arizona Press. https://doi.org/10.1017/CBO9781107415324.004

Buckman, A.H., Brown, S.B., Small, J., Muir, D.C.G., Parrott, J., Solomon, K.R., Fisk, A.T., 2007. Role of temperature and enzyme induction in the biotransformation of polychlorinated biphenyls and bioformation of hydroxylated polychlorinated biphenyls by rainbow trout (*Oncorhynchus mykiss*). Environ. Sci. Technol. 41, 3856–3863. https://doi.org/10.1021/es062437y

Buckman, A.H., Wong, C.S., Chow, E.A., Brown, S.B., Solomon, K.R., Fisk, A.T., 2006. Biotransformation of polychlorinated biphenyls (PCBs) and bioformation of hydroxylated PCBs in fish. Aquat. Toxicol. 78, 176–185. https://doi.org/10.1016/j.aquatox.2006.02.033

Cagnazzi, D., Consales, G., Broadhurst, M.K., Marsili, L., 2019. Bioaccumulation of organochlorine compounds in large, threatened elasmobranchs off northern New South Wales, Australia. Mar. Pollut. Bull. 139, 263–269. https://doi.org/10.1016/j.marpolbul.2018.12.043

Cailliet, G.M., Chabot, C.L., Nehmens, M.C. & Carlisle, A.B., 2020. *Squatina californica.* [WWW Document] (amended version of 2016 assessment). The IUCN Red List of Threatened Species 2020: URL https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T39328A177163701.en (accessed 5.09.23).

Cailliet, G.M., Mollet, H.F., Pittenger, G.G., Bedford, D., Natanson, L.J., 1992. Growth and demography of the pacific angel shark (*Squatina californica*), based upon tag returns off california. Mar. Freshw. Res. 43, 1313–1330. https://doi.org/10.1071/MF9921313

Cascaes, M.J., Oliveira, R.T., Ubarana, M.M., Sato, R.M., Baldassin, P., Colabuono, F.I., Leonel, J., Taniguchi, S., Weber, R.R., 2014. Persistent organic pollutants in liver of Brazilian sharpnose shark (*Rhizoprionodon lalandii*) from southeastern coast of Brazil. Mar. Pollut. Bull. 86, 591–593.

Chen, J., Jayachandran, M., Bai, W., Xu, B., 2022. A critical review on the health benefits of fish consumption and its bioactive constituents. Food Chem. 369, 130874. https://doi.org/10.1016/j.foodchem.2021.130874

Chierichetti, M.A., Scenna, L.B., Ondarza, P.M., Giorgini, M., Di Giácomo, E., Miglioranza, K.S.B., 2021. Persistent organic pollutants and chlorpyrifos in the cockfish *Callorhinchus callorynchus* (Holocephali: Callorhynchidae) from Argentine coastal waters: Influence of sex and maturity. Sci. Total Environ. 796. https://doi.org/10.1016/j.scitotenv.2021.148761

Chynel, M., Munschy, C., Bely, N., Héas-Moisan, K., Pollono, C., Jaquemet, S., 2021. Legacy and emerging organic contaminants in two sympatric shark species from Reunion Island (Southwest Indian Ocean): Levels, profiles and maternal transfer. Sci. Total Environ. 751, 141807. https://doi.org/10.1016/j.scitotenv.2020.141807

CICOPLAFEST, 1991. Catálogo Oficial de Plaguicidas. Comisión Intersecretarial para el Control del proceso y uso de Plaguicidas, Fertilizantes, y Sustancias tóxicas.

COFEPRIS, 2021. Registro Sanitario de Plaguicidas y Nutrientes Vegetales. [WWW Document]

URL https://www.gob.mx/cofepris/acciones-y-programas/registro-sanitario-de-plaguicidas-y-nutrientes-vegetales (accessed 9.01.21).

CONAPESCA, 2018. Anuario Estadístico de Acuacultura y Pesca 2018. [WWW Document]. Anuario de la Comisión Nacional de Acuacultura y Pesca. URL https://www.conapesca.gob.mx/work/sites/cona/dgppe/2018/ANUARIO_2018.pdf (accessed 5.7.21).

CONAPESCA, 2020. Anuario Estadístico de Acuacultura y Pesca 2020. [WWW Document]. Anuario de la Comisión Nacional de Acuacultura y Pesca. URL https://www.conapesca.gob.mx/work/sites/cona/dgppe/2020/ANUARIO_2020.pdf (accessed 3.3.22).

Consales, G., Marsili, L., 2021. Assessment of the conservation status of Chondrichthyans: Underestimation of the pollution threat. Eur. Zool. J. 88, 165–180. https://doi.org/https://doi.org/10.1080/24750263.2020.1858981

Cooke, A.S., 1973. Shell thinning in avian eggs by environmental pollutants. Environ. Pollut. 4, 85–152. https://doi.org/10.1016/0013-9327(73)90009-8

Cornish, A.S., Ng, W.C., Ho, V.C.M., Wong, H.L., Lam, J.C.W., Lam, P.K.S., Leung, K.M.Y., 2007. Trace metals and organochlorines in the bamboo shark *Chiloscyllium plagiosum* from the southern waters of Hong Kong, China. Sci. Total Environ. 376, 335–345. https://doi.org/10.1016/j.scitotenv.2007.01.070

Correa, B., Paiva, L.G., Santos-neto, E., Vidal, L.G., Vianna, M., Lailson-Brito, J., 2022. Organochlorine contaminants in Rio skate (*Rioraja agassizii*), an endangered batoid species, from southeastern coast of Brazil. Mar. Pollut. Bull. 182. https://doi.org/10.1016/j.marpolbul.2022.114002

Corro-Espinosa, David, Márquez-Farías, J.F., Muhlia-Melo, A., 2011. Size at maturity of the Pacific sharpnose shark *Rhizoprionodon longurio* in the Gulf of California, Mexico. Ciencias Mar. 37, 201–214.

Corsolini, S., Ancora, S., Bianchi, N., Mariotti, G., Leonzio, C., Christiansen, J.S., 2014. Organotropism of persistent organic pollutants and heavy metals in the Greenland shark *Somniosus microcephalus* in NE Greenland. Mar. Pollut. Bull. 87, 381–387. https://doi.org/10.1016/j.marpolbul.2014.07.021

Corsso, J.T., Gadig, O.B.F., Caltabellotta, F.P., Barreto, R., Motta, F.S., 2021. Age and growth of two sharpnose shark species (*Rhizoprionodon lalandii* and *R. porosus*) in subtropical waters of the south-western Atlantic. Mar. Freshw. Res. 72, 398–410. https://doi.org/https://doi.org/10.1071/MF19379

Costa, L.G., 2015. The neurotoxicity of organochlorine and pyrethroid pesticides, 1st ed, Handbook of Clinical Neurology. 31, 135-148. https://doi.org/10.1016/B978-0-444-62627-1.00009-3

da Costa Filho, B.M., Duarte, A.C., Santos, T.A.P.R., 2022. Environmental monitoring approaches for the detection of organic contaminants in marine environments: A critical review. Trends Environ. Anal. Chem. 33, e00154. https://doi.org/10.1016/j.teac.2022.e00154

Cresson, P., Claire, M., Marco, F., Dufour, J., Bouchoucha, M., Elleboode, R., Sevin, K., Mah, K., 2016. Variability of PCB burden in 5 fish and sharks species of the French Mediterranean continental slope. Environ. Pollut. 212, 374–381. https://doi.org/10.1016/j.envpol.2016.01.044

Cui, S., Qi, H., Liu, L.Y., Song, W.W., Ma, W.L., Jia, H.L., Ding, Y.S., Li, Y.F., 2013. Emission of unintentionally produced polychlorinated biphenyls (UP-PCBs) in China: Has this become the major source of PCBs in Chinese air? Atmos. Environ. 67, 73–79. https://doi.org/10.1016/j.atmosenv.2012.10.028

Cullen, J.A., Marshall, C.D., Hala, D., 2019. Integration of multi-tissue PAH and PCB burdens with biomarker activity in three coastal shark species from the northwestern Gulf of Mexico. Sci. Total Environ. 650, 1158–1172. https://doi.org/10.1016/j.scitotenv.2018.09.128

Daley, J.M., Paterson, G., Drouillard, K.G., 2014. Bioamplification as a bioaccumulation mechanism for Persistent Organic Pollutants (POPs) in wildlife, in: Whitacre, D.M. (Ed.), Reviews of Environmental Contamination and Toxicology. Springer International Publishing, pp. 107–146. https://doi.org/10.1007/978-3-319-01327-5

Davidson, B.C., Nel, W., Rais, A., Namdarizandi, V., Vizarra, S., Cliff, G., 2014. Comparison of total lipids and fatty acids from liver, heart and abdominal muscle of scalloped (*Sphyrna lewini*) and smooth (*Sphyrna zygaena*) hammerhead sharks. SpringerPlus, 3(1), 1-8.

Desforges, J.P., Hall, A., McConnell, B., Rosing-Asvid, A., Barber, J.L., Brownlow, A., De Guise, S., Eulaers, I., Jepson, P.D., Letcher, R.J., Levin, M., Ross, P.S., Samarra, F., Víkingson, G., Sonne, C., Dietz, R., 2018. Predicting global killer whale population collapse from PCB pollution. Science 80 (361), 1373–1376. https://doi.org/10.1126/science.aat1953

Desforges, J.P.W., Sonne, C., Levin, M., Siebert, U., De Guise, S., Dietz, R., 2016. Immunotoxic effects of environmental pollutants in marine mammals. Environ. Int. 86, 126–139. https://doi.org/10.1016/j.envint.2015.10.007

Dickhut, R.M., Deshpande, A.D., Cincinelli, A., Cochran, M.A., Corsolini, S., Brill, R.W., Secor, D.H., Graves, J.E., 2009. Atlantic bluefin tuna (*Thunnus thynnus*) population dynamics delineated by organochlorine tracers. Environ. Sci. Technol. 43, 8522–8527. https://doi.org/10.1021/es901810e

Dietz, R., Desforges, J.P., Gustavson, K., Rigét, F.F., Born, E.W., Letcher, R.J., Sonne, C., 2018. Immunologic, reproductive, and carcinogenic risk assessment from POP exposure in east Greenland polar bears (*Ursus maritimus*) during 1983–2013. Environ. Int. 118, 169–178. https://doi.org/10.1016/j.envint.2018.05.020

Domingo, J.L., 2016. Nutrients and chemical pollutants in fish and shellfish. Balancing health benefits and risks of regular fish consumption. Crit. Rev. Food Sci. Nutr. ISSN 8398. https://doi.org/10.1080/10408398.2012.742985

Domingo, J.L., 2011. Influence of cooking processes on the concentrations of toxic metals and various organic environmental pollutants in food : A review of the published literature. Crit. Rev. Food Sci. Nutr. 51(1), 37–41. https://doi.org/10.1080/10408390903044511

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., Carlson, J.K., Davidson, L.N., Fordham, S. V, Francis, M.P., Pollock, C.M., Simpfendorfer, C.A., Burgess, G.H., Carpenter, K.E., Compagno, L.J., Ebert, D.A., Gibson, C., Heupel, M.R., Livingstone, S.R., Sanciangco, J.C., Stevens, J.D., Valenti, S., White, W.T., 2014. Extinction risk and conservation of the world's sharks and rays. Elife 3, 1–34. https://doi.org/10.7554/elife.00590

Durante, C.A., Santos-Neto, E.B., Azevedo, A., Crespo, E.A., Lailson-Brito, J., 2016. POPs in the south latin America: Bioaccumulation of DDT, PCB, HCB, HCH and mirex in blubber of common dolphin (*Delphinus delphis*) and Fraser's dolphin (*Lagenodelphis hosei*) from Argentina. Sci. Total Environ. 572, 352–360.

https://doi.org/10.1016/j.scitotenv.2016.07.176

Duncan, K.M., Holland, K.N., 2006. Habitat use, growth rates and dispersal patterns of juvenile scalloped hammerhead sharks *Sphyrna lewini* in a nursery habitat. Mar. Ecol. Prog. Ser. 312, 211–221. https://doi.org/10.3354/meps312211

ECHA 2020. Methoxychlor draft risk profile. [WWW Document] Persistent Organic Pollutants Review Committee. URL https://echa.europa.eu/documents/10162/b65a738e-b50f-64e5-cbb0-f2711d49c25e

El-Shahawi, M.S., Hamza, A., Bashammakh, A.S., Al-Saggaf, W.T., 2010. An overview on the accumulation, distribution, transformations, toxicity and analytical methods for the monitoring of persistent organic pollutants. Talanta 80, 1587–1597. https://doi.org/10.1016/j.talanta.2009.09.055

Eng, A., Su, K., Harner, T., Pozo, K., Sinha, R.K., Sengupta, B., Loewen, M., 2016. Assessing dicofol concentrations in air: retrospective analysis of global atmospheric passive sampling network samples from agricultural sites in India. Environ. Sci. Technol. Lett. 3, 150–155. https://doi.org/10.1021/acs.estlett.6b00041

Escobar-Sánchez, O., Galván-Magaña, F., Abitia-Cárdenas, L.A., 2011. Trophic level and isotopic composition of δ13C and δ15N of pacific angel shark, *Squatina californica* (Ayres, 1859), in the southern Gulf of California, Mexico. J. Fish. Aquat. Sci. 6, 141–150. https://doi.org/10.3923/ifas.2011.141.150

Escobar-Sánchez, O., Ruelas-Inzunza, J., Moreno-Sánchez, X. G., Romo-Piñera, A. K., & Frías-Espericueta, M. G., 2016. Mercury concentrations in pacific angel sharks (*Squatina californica*) and prey fishes from Southern Gulf of California, Mexico. Bull. Env. Cont. Tox. 96, 15-19.

FAO, 1983. Compilation of legal limits for hazardous substances in fish and fishery products. Food and Agriculture Organization of the United Nations, Rome.

Fayet-Moore, F., Baghurst, K., & Meyer, B. J., 2015. Four models including fish, seafood, red meat and enriched foods to achieve Australian dietary recommendations for n-3 lcpufa for all life-stages. Nutrients, 7(10), 8602-8614.

FDA, 2022. Fish and Fishery Products Hazards and Controls Guidance [WWW Document]. Department of health and human services. URL https://www.fda.gov/media/80637/download (accessed 1.01.22)

Fernández, P., Grimalt, J.O., 2003. On the global distribution of persistent organic pollutants. Chimia (Aarau). 57, 514–521. https://doi.org/10.2533/000942903777679000

Fiedler, H., 2003. Persistent organic pollutants (the handbook of environmental chemistry). Springer, Berlin.

Fisk, A.T., Hobson, K.A., Norstrom, R.J., 2001. Influence of chemical and biological factors on trophic transfer of Persistent Organic Pollutants in the Northwater Polynya marine food web. Environ. Sci. Technol. 35, 732–738.

Fisk, A.T., Tittlemier, S.A., Pranschke, J.C., Norstrom, R.J., 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland sharks. Ecology 83, 2162–2172. https://doi.org/10.1890/0012-9658(2002)083[2162:UACASI]2.0.CO;2

Fossi, M.C., Panti, C., Marsili, L., Maltese, S., Coppola, D., Jimenez, B., Muñoz-Arnanz, J., Finoia,

M.G., Rojas-Bracho, L., Urban, R.J., 2014. Could feeding habit and migratory behaviour be the causes of different toxicological hazard to cetaceans of Gulf of California (Mexico)? Environ. Sci. Pollut. Res. 21, 13353–13366. https://doi.org/10.1007/s11356-014-2574-8

Fossi, M.C., Baini, M., Panti, C., Galli, M., Jiménez, B., Muñoz-Arnanz, J., Marsili, L., Finoia, M.G., Ramírez-Macías, D., 2017. Are whale sharks exposed to persistent organic pollutants and plastic pollution in the Gulf of California (Mexico)? First ecotoxicological investigation using skin biopsies. Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol. 199, 48–58. https://doi.org/10.1016/j.cbpc.2017.03.002

Frame, G.M., Cochran, J.W., Bøwadt, S.S., 1996. Complete PCB Congener distributions for 17 aroclor mixtures determined by 3 HRGC systems optimized for comprehensive, quantitative, congener-specific analysis. HRC J. High Resolut. Chromatogr. 19, 657–668. https://doi.org/10.1002/jhrc.1240191202

Frías-Espericueta, M.G., Cardenas-Nava, N.G., Márquez-Farías, J.F., Osuna-López, J.I., Muy-Rangel, M.D., Rubio-Carrasco, W., Voltolina, D., 2014. Cadmium, copper, lead and zinc concentrations in female and embryonic pacific sharpnose shark (*Rhizoprionodon longurio*) tissues. Bull. Environ. Contam. Toxicol. 93, 532–535. https://doi.org/10.1007/s00128-014-1360-0

Frías-Espericueta, M.G., Ruelas-Inzunza, J., Benítez-Lizárraga, R., Escobar-Sánchez, O., Osuna-Martínez, C.C., Delgado-Alvarez, C.G., Aguilar-Juárez, M., Osuna-López, J.I., Voltolina, D., 2019. Risk assessment of mercury in sharks (*Rhizoprionodon longurio*) caught in the coastal zone of Northwest Mexico. J. Consum. Prot. Food Saf. 14, 349–354. https://doi.org/10.1007/s00003-019-01232-6

Galbiati, F., Chiesa, L., Labella, G., Pavlovic, R., Arioli, F., Panseri, S., 2016. Determination of Persistent Organic Pollutants in Fish Tissues by Accelerated Solvent Extraction and GC-MS/MS. Thermo Scientific, Milan.

Galván-Magaña, F., Nienhuis, H.J., Klimley, P.A., 1989. Seasonal abundance and feeding habits of sharks of the lower Gulf of California, Mexico. Calif. Fish Game 75, 74–84.

Gandhi, N., Bhavsar, S.P., Reiner, E.J., Chen, T., Morse, D., Arhonditsis, G.B., Drouillard, K.G., 2015. Evaluation and interconversion of various indicator PCB schemes for ΣPCB and dioxin-like PCB toxic equivalent levels in fish. Environ. Sci. Technol. 49, 123–131. https://doi.org/10.1021/es503427r

García-Hernández, J., Cadena-Cárdenas, L., García- De la Parra, L.M., Márquez-Farías, F., 2007. Total mercury content found in edible tissues of top predator fish from the Gulf of California, Mexico. Toxicol. Environ. Chem. 89, 507–522.

García-Hernández, J., Ortega-Vélez, M.I., Contreras-Paniagua, A.D., Aguilera-Márquez, D., Leyva-García, G., Torre, J., 2018. Mercury concentrations in seafood and the associated risk in women with high fish consumption from coastal villages of Sonora, Mexico. Food Chem. Toxicol. 120, 367–377. https://doi.org/10.1016/j.fct.2018.07.029

Gardes, T., Portet-koltalo, F., Debret, M., Copard, Y., 2021. Historical and post-ban releases of organochlorine pesticides recorded in sediment deposits in an agricultural watershed, France. Environ. Pollut. 288, 117769. https://doi.org/10.1016/j.envpol.2021.117769

Gelsleichter, J., Manire, C.A., Szabo, N.J., Cortés, E., Carlson, J., Lombardi-Carlson, L., 2005. Organochlorine concentrations in bonnethead sharks (*Sphyrna tiburo*) from four Florida estuaries. Arch. Environ. Contam. Toxicol. 48, 474–483. https://doi.org/10.1007/s00244-003-0275-2

Gelsleichter, J., Walker, C.J., 2010. Pollutant exposure and effects in sharks and their relatives. Sharks Their Relat. II Biodiversity, Adapt. Physiol. Conserv. 491–537. https://doi.org/10.1201/9781420080483

Gelsleichter, J., Walsh, C.J., Szabo, N.J., Rasmussen, L.E.L., 2006. Organochlorine concentrations, reproductive physiology, and immune function in unique populations of freshwater Atlantic stingrays (*Dasyatis sabina*) from Florida's St. Johns River. Chemosphere 63, 1506–1522. https://doi.org/10.1016/j.chemosphere.2005.09.011

Genov, T., Jepson, P.D., Barber, J.L., Hace, A., Gaspari, S., Centrih, T., Lesjak, J., Kotnjek, P., 2019. Linking organochlorine contaminants with demographic parameters in free-ranging common bottlenose dolphins from the northern Adriatic Sea. Sci. Total Environ. 657, 200–212. https://doi.org/10.1016/j.scitotenv.2018.12.025

George, B.J., Gains-Germain, L., Broms, K., Black, K., Furman, M., Hays, M.D., Thomas, K.W., Simmons, J.E., 2021. Censoring Trace-Level Environmental Data: Statistical Analysis Considerations to Limit Bias. Environ. Sci. Technol. 55, 3786–3795. https://doi.org/10.1021/acs.est.0c02256

Giesy, J.P., Kannan, K., 2002. Dioxin-like and non-dioxin like effects of polychlorinated biphenyls: Implications for risk assessment. Lakes Reserv. Res. Manag. 7, 139–181. https://doi.org/10.1046/j.1440-1770.2002.00185.x

Gilbert, J.M., Baduel, C., Li, Y., Reichelt-Brushett, A.J., Butcher, P.A., McGrath, S.P., Peddemors, V.M., Hearn, L., Mueller, J., Christidis, L., 2015. Bioaccumulation of PCBs in liver tissue of dusky *Carcharhinus obscurus*, sandbar *C. plumbeus* and white *Carcharodon carcharias* sharks from south-eastern Australian waters. Mar. Pollut. Bull. 101, 908–913. https://doi.org/10.1016/j.marpolbul.2015.10.071

Gladyshev, M.I., Anishchenko, O. V., Makhutova, O.N., Kolmakova, O. V., Trusova, M.Y., Morgun, V.N., Gribovskaya, I. V., Sushchik, N.N., 2020. The benefit-risk analysis of omega-3 polyunsaturated fatty acids and heavy metals in seven smoked fish species from Siberia. J. Food Compos. Anal. 90, 103489. https://doi.org/10.1016/j.jfca.2020.103489

Gladyshev, M.I., Sushchik, N.N., Tolomeev, A.P., Dgebuadze, Y.Y., 2018. Meta-analysis of factors associated with omega-3 fatty acid contents of wild fish. Rev. Fish Biol. Fish. 28, 277–299. https://doi.org/10.1007/s11160-017-9511-0

Graciano, J. C., 2013. Uso del agua y agricultura de exportación en Baja California Sur. Perspectivas desde el agro para el desarrollo regional [Master Thesis]. Universidad Autónoma de Baja California Sur, Mexico.

Granados-Galván, I.A., Rodríguez-Meza, D.G., Luna-González, A., González-Ocampo, H.A., 2015. Human health risk assessment of pesticide residues in snappers (Lutjanus) fish from the Navachiste Lagoon complex, Mexico. Mar. Pollut. Bull. 97, 178–187. https://doi.org/10.1016/j.marpolbul.2015.06.018

Green, A., Larson, S., 2016. A review of organochlorine contaminants in nearshore marine mammal predators. J. Environ. Anal. Toxicol. 06. https://doi.org/10.4172/2161-0525.1000370

Harley, J.R., Gill, V.A., Lee, S., Kannan, K., Santana, V., Burek-Huntington, K., O'Hara, T.M., 2019. Concentrations of organohalogens (PCBs, DDTs, PBDEs) in hunted and stranded Northern sea otters (*Enhydra lutris kenyoni*) in Alaska from 1992 to 2010: Links to pathology and feeding ecology. Sci. Total Environ. 691, 789–798. https://doi.org/10.1016/j.scitotenv.2019.07.040

Henry, T.B., 2015. Ecotoxicology of polychlorinated biphenyls in fish: A critical review. Crit. Rev. Toxicol. 45, 643–661. https://doi.org/10.3109/10408444.2015.1038498

Hirano, T., Ishida, T., Oh, K., Sudo, R., 2007. Biodegradation of chlordane and hexachlorobenzenes in river sediment. Chemosphere 67, 428–434. https://doi.org/10.1016/j.chemosphere.2006.09.087

Hoffmayer, E.R., Parsons, G.R., Horton, J., 2006. Seasonal and interannual variation in the energetic condition of adult male Atlantic sharpnose shark *Rhizoprionodon terraenovae* in the northern Gulf of Mexico. J. Fish Biol. 68, 645–653. https://doi.org/10.1111/j.1095-8649.2006.00942.x

Hoyos-Padilla, E.M., Ketchum, J.T., Klimley, A.P., Galván-Magaña, F., 2014. Ontogenetic migration of a female scalloped hammerhead shark *Sphyrna lewini* in the Gulf of California. Anim. Biotelemetry 2, 1–9. https://doi.org/10.1186/2050-3385-2-17

Hurtado-Banda, R., Gómez-Álvarez, A., Márquez-Farías, J.F., Córdoba-Figueroa, M., Navarro-García, G., Medina-Juárez, L.Á., 2012. Total mercury in liver and muscle tissue of two coastal sharks from the Northwest of Mexico. Bull. Environ. Contam. Toxicol. 88, 971–975. https://doi.org/10.1007/s00128-012-0623-x

Islam, S., Tanaka, M., 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. Mar. Pollut. Bull. 48, 624–649. https://doi.org/10.1016/j.marpolbul.2003.12.004

Jackovitz, A.M., Hebert, R.M., 2015. Wildlife Toxicity Assessment for Hexachlorocyclohexane (HCH), in: Williams, M., Reddly, G., Quinn, M., Johnson, M. (Eds.), Wildlife Toxicity Assessments for Chemicals of Military Concern. Elsevier, Oxford, pp. 473–497. https://doi.org/10.1016/b978-0-12-800020-5.00027-2

Jacob, J., Cherian, J., 2013. Review of environmental and human exposure to persistent organic pollutants. Asian Soc. Sci. 9, 107–120. https://doi.org/10.5539/ass.v9n11p107

Jaspers, V., Megson, D., O'Sullivan, G., 2013. POPs in the Terrestrial Environment, in: Environmental Forensics for Persistent Organic Pollutants. Elsevier B.V. https://doi.org/10.1016/B978-0-444-59424-2.00007-4

Jepson, P.D., Law, R.J., 2016. Persistent pollutants, persistent threats. Science 80 (352), 1388–1389.

Jiang, Q.T., Lee, T.K.M., Chen, K., Wong, H.L., Zheng, J.S., Giesy, J.P., Lo, K.K.W., Yamashita, N., Lam, P.K.S., 2005. Human health risk assessment of organochlorines associated with fish consumption in a coastal city in China. Environ. Pollut. 136, 155–165. https://doi.org/10.1016/j.envpol.2004.09.028

Kainz, M.J., Fisk, A.T., 2009. Integrating lipids and contaminants in aquatic ecology and ecotoxicology, in: Arts, M.T., Brett, M.T., Kainz, M. (Eds.), Lipids in Aquatic Ecosystems. Springer Science & Business Media, pp. 93–113. https://doi.org/10.1007/978-0-387-89366-2

Kannan, K., Kajiwara, N., Watanabe, M., Nakata, H., Thomas, N.J., Stephenson, M., Jessup, D.A., Tanabe, S., 2004. Profiles of polychlorinated biphenyl congeners, organochlorine pesticides, and butyltins in southern sea otters and their prey. Environ. Toxicol. Chem. 23, 49–56. https://doi.org/10.1897/03-53

Kato, S., Carvallo, A.H., 1967. Shark tagging in the Eastern Pacific Ocean, 1962-65. In P.W. Gilbert, R.F. Mathewson, & D.P. Rall, (Eds.). Sharks, skates and rays. John Hopkins Press, Baltimore,

pp. 93–109.

Kelly, B.C., Gobas, F.A.P.C., McLachlan, M.S., 2004. Intestinal absorption and biomagnification of organic contaminants in fish, wildlife, and humans. Environ. Toxicol. Chem. 23, 2324–2336. https://doi.org/10.1897/03-545

Kelly, B.C., Ikonomou, M.G., Blair, J.D., Morin, A.E., Gobas, F.A.P.C., 2007. Food web-specific biomagnification of persistent organic pollutants. Science (80-.). 317, 236–239. https://doi.org/10.1126/science.1138275

Keogh, M.J., Taras, B., Beckmen, K.B., Burek-huntington, K.A., Ylitalo, G.M., Fadely, B.S., Rea, L.D., Pitcher, K.W., 2020. Organochlorine contaminant concentrations in blubber of young Steller sea lion (*Eumetopias jubatus*) are influenced by region, age, sex, and lipid stores. Sci. Total Environ. 698, 134183. https://doi.org/10.1016/j.scitotenv.2019.134183

Kim, K.S., Hirai, Y., Kato, M., Urano, K., Masunaga, S., 2004. Detailed PCB congener patterns in incinerator flue gas and commercial PCB formulations (Kanechlor). Chemosphere 55, 539–553. https://doi.org/10.1016/j.chemosphere.2003.11.056

Kim, K.S., Masunaga, S., 2005. Behavior and source characteristic of PCBS in urban ambient air of Yokohama, Japan. Environ. Pollut. 138, 290–298. https://doi.org/10.1016/j.envpol.2005.03.011

Kim, S.W., Han, S.J., Kim, Y., Jun, J.W., Giri, S.S., Chi, C., Yun, S., Kim, H.J., Kim, S.G., Kang, J.W., Kwon, J., Oh, W.T., Cha, J., Han, S., Lee, B.C., Park, T., Kim, B.Y., Park, S.C., 2019. Heavy metal accumulation in and food safety of shark meat from Jeju island, Republic of Korea. PLoS One 14, 1–18. https://doi.org/10.1371/journal.pone.0212410

Klimley, A.P., Cabrera-Mancilla, I., Castillo-Geniz, J.L., 1993. Horizontal and vertical movements of the scalloped hammerhead shark, *Sphyrna lewini*, in the southern Gulf of California, Mexico. Ciencias Mar. 19, 95–115.

Koenig, S., Huertas, D., Fernández, P., 2013. Legacy and emergent persistent organic pollutants (POPs) in NW Mediterranean deep-sea organisms. Sci. Total Environ. 443, 358–366. https://doi.org/10.1016/j.scitotenv.2012.10.111

Labrada-Martagón, V., Tenorio Rodríguez, P.A., Méndez-Rodríguez, L.C., Zenteno-Savín, T., 2011. Oxidative stress indicators and chemical contaminants in East Pacific green turtles (*Chelonia mydas*) inhabiting two foraging coastal lagoons in the Baja California peninsula. Comp. Biochem. Physiol. - C Toxicol. Pharmacol. 154, 65–75. https://doi.org/10.1016/j.cbpc.2011.02.006

Lavoie, R.A., Champoux, L., Rail, J.F., Lean, D.R.S., 2010. Organochlorines, brominated flame retardants and mercury levels in six seabird species from the Gulf of St. Lawrence (Canada): Relationships with feeding ecology, migration and molt. Environ. Pollut. 158, 2189–2199. https://doi.org/10.1016/j.envpol.2010.02.016

Lawson, T.M., Ylitalo, G.M., O'Neill, S.M., Dahlheim, M.E., Wade, P.R., Matkin, C.O., Burkanov, V., Boyd, D.T., 2020. Concentrations and profiles of organochlorine contaminants in North Pacific resident and transient killer whale (*Orcinus orca*) populations. Sci. Total Environ. 722, 137776. https://doi.org/10.1016/j.scitotenv.2020.137776

Lee, C.C., Chang, W.H., Hung, C.F., Chen, H.L., 2021. Fish consumption is an indicator of exposure to non-dioxin like polychlorinated biphenyls in cumulative risk assessments based on a probabilistic and sensitive approach. Environ. Pollut. 268, 115732. https://doi.org/10.1016/j.envpol.2020.115732

Lee, H.K., Jeong, Y., Lee, S., Jeong, W., Choy, E.J., Kang, C.K., Lee, W.C., Kim, S.J., Moon, H.B., 2015. Persistent organochlorines in 13 shark species from offshore and coastal waters of Korea: species-specific accumulation and contributing factors. Ecotoxicol. Environ. Saf. 115, 195–202. https://doi.org/10.1016/j.ecoenv.2015.02.021

Leigh, S.C., Papastamatiou, Y., German, D.P., 2017. The nutritional physiology of sharks. Rev. Fish Biol. Fish. 27, 561–585. https://doi.org/10.1007/s11160-017-9481-2

Leonard J.V. Compagno, 1982. Carcharhiniformes. Sharks of the world, pp. 547–554.

Leonards, P.E.G., Hattum, B. Van, Leslie, H., 2008. Assessing the risks of persistent organic pollutants to top predators: A review of approaches. Integr. Environ. Assess. Manag. 4, 386–398.

Letcher, R.J., Bustnes, J.O., Dietz, R., Jenssen, B.M., Jørgensen, E.H., Sonne, C., Verreault, J., Vijayan, M.M., Gabrielsen, G.W., 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. Sci. Total Environ. 408, 2995–3043. https://doi.org/10.1016/j.scitotenv.2009.10.038

Li, H., Jiang, W., Pan, Y., Li, F., Wang, C., Tian, H., 2021. Occurrence and partition of organochlorine pesticides (OCPs) in water, sediment, and organisms from the eastern sea area of Shandong Peninsula, Yellow Sea, China. Mar. Pollut. Bull. 162, 111906. https://doi.org/10.1016/j.marpolbul.2020.111906

Li, X., Dong, S., Wang, P., Su, X., Fu, J., 2019. Polychlorinated biphenyls are still alarming persistent organic pollutants in marine-origin animal feed (fishmeal). Chemosphere 233, 355–362. https://doi.org/10.1016/j.chemosphere.2019.05.250

Li, Y.F., Macdonald, R.W., 2005. Sources and pathways of selected organochlorine pesticides to the Arctic and the effect of pathway divergence on HCH trends in biota: A review. Sci. Total Environ. 342, 87–106. https://doi.org/10.1016/j.scitotenv.2004.12.027

Lippold, A., Bourgeon, S., Aars, J., Andersen, M., Polder, A., Lyche, J.L., Bytingsvik, J., Jenssen, B.M., Derocher, A.E., Welker, J.M., Routti, H., 2019. Temporal trends of Persistent Organic Pollutants in Barents sea polar bears (*Ursus maritimus*) in relation to changes in feeding habits and body condition. Environ. Sci. Technol. 53, 984–995. https://doi.org/10.1021/acs.est.8b05416

Lladó Cabrera, D., 2020. Partición de recursos tróficos por tres especies de tiburones en bahía de La Paz, B.C.S. [Master Thesis]. Instituto Politécnico Nacional, Mexico.

Lluch-Cota, S.E., Aragón-Noriega, E.A., Arreguín-Sánchez, F., Aurioles-Gamboa, D., Jesús Bautista-Romero, J., Brusca, R.C., Cervantes-Duarte, R., Cortés-Altamirano, R., Del-Monte-Luna, P., Esquivel-Herrera, A., Fernández, G., Hendrickx, M.E., Hernández-Vázquez, S., Herrera-Cervantes, H., Kahru, M., Lavín, M., Lluch-Belda, D., Lluch-Cota, D.B., López-Martínez, J., Marinone, S.G., Nevárez-Martínez, M.O., Ortega-García, S., Palacios-Castro, E., Parés-Sierra, A., Ponce-Díaz, G., Ramírez-Rodríguez, M., Salinas-Zavala, C.A., Schwartzlose, R.A., Sierra-Beltrán, A.P., 2007. The Gulf of California: Review of ecosystem status and sustainability challenges. Prog. Oceanogr. 73, 1–26. https://doi.org/10.1016/j.pocean.2007.01.013

Lourenço, P.M., Serra-Gonçalves, C., Ferreira, J.L., Catry, T., Granadeiro, J.P., 2017. Plastic and other microfibers in sediments, macroinvertebrates and shorebirds from three intertidal wetlands of southern Europe and west Africa. Environ. Pollut. 231, 123–133. https://doi.org/10.1016/j.envpol.2017.07.103

Lowe, C.G., 2002. Bioenergetics of free-ranging juvenile scalloped hammerhead sharks (*Sphyrna lewini*) in Kāne'ohe Bay, Ō'ahu, HI. J. Exp. Mar. Bio. Ecol. 278, 141–156.

Luthardt, P., Mayer, J., Fuchs, J., 2002. Total TEQ emissions (PCDD/F and PCB) from industrial sources. Chemosphere 46, 1303–1308. https://doi.org/10.1016/S0045-6535(01)00277-6

Lyons, K., Adams, D.H., 2014. Maternal offloading of organochlorine contaminants in the yolk-sac placental scalloped hammerhead shark (*Sphyrna lewini*). Ecotoxicology 24, 553–562. https://doi.org/10.1007/s10646-014-1403-7

Lyons, K., Adams, D.H., Bizzarro, J.J., 2021. Evaluation of muscle tissue as a non-lethal proxy for liver and brain organic contaminant loads in an elasmobranch, the bonnethead shark (Sphyrna tiburo). Mar. Pollut. Bull. 167, 112327. https://doi.org/10.1016/j.marpolbul.2021.112327

Lyons, K., Wynne-Edwards, K.E., 2018. Legacy polychlorinated biphenyl contamination impairs male embryonic development in an elasmobranch with matrotrophic histotrophy, the round stingray (*Urobatis halleri*), Environmental Toxicology and Chemistry. https://doi.org/10.1002/etc.4255

Lyons, K., Carlisle, A., Preti, A., Mull, C., Blasius, M., O'Sullivan, J., Winkler, C., Lowe, C.G., 2013. Effects of trophic ecology and habitat use on maternal transfer of contaminants in four species of young of the year lamniform sharks. Mar. Environ. Res. 90, 27–38. https://doi.org/10.1016/j.marenvres.2013.05.009

Lyons, K., Kacev, D., Preti, A., Gillett, D., Dewar, H., 2019a. Organic contaminants as an ecological tool to explore niche partitioning: A case study using three pelagic shark species. Sci. Rep. 9, 1–7. https://doi.org/10.1038/s41598-019-48521-6

Lyons, K., Kacev, D., Preti, A., Gillett, D., Dewar, H., Kohin, S., 2019b. Species-specific characteristics influence contaminant signatures across ontogeny in three pelagic shark species. Environ. Sci. Technol. 53, 6997–7006. https://doi.org/10.1021/acs.est.8b07355

Lyons, K., Lowe, C.G., 2015. Organochlorine contaminants and maternal offloading in the lecithotrophic Pacific angel shark (*Squatina californica*) collected from southern California. Mar. Pollut. Bull. 22. https://doi.org/10.1016/j.marpolbul.2015.05.019

Mahaffey, K.R., 2004. Fish and shellfish as dietary sources of methylmercury and the o -3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: Risks and benefits. Environ. Res. 95, 414–428. https://doi.org/10.1016/j.envres.2004.02.006

Mao, S., Liu, S., Zhou, Y., An, Q., Zhou, X., Mao, Z., Wu, Y., 2021. The occurrence and sources of polychlorinated biphenyls (PCBs) in agricultural soils across China with an emphasis on unintentionally produced PCBs. Environ. Pollut. 271. https://doi.org/10.1016/j.envpol.2020.116171

Marler, H., Adams, D.H., Wu, Y., Nielsen, C.K., Shen, L., Reiner, E.J., Chen, D., 2018. Maternal transfer of flame retardants in sharks from the western north Atlantic ocean. Environ. Sci. Technol. 52, 12978–12986. https://doi.org/10.1021/acs.est.8b01613

Márquez-Farías, J.F., 2020. Length at maturity of the pacific angel shark (*Squatina californica*) in the artisanal elasmobranch fishery in the Gulf of California in Mexico. Fish. Bull. 118, 359–364. https://doi.org/10.7755/FB.118.4.5

Márquez-Farías, J.F., Corro-Espinosa, D., Castillo-Géniz, J.L., 2005. Observations on the biology of the Pacific sharpnose shark (*Rhizoprionodon longurio*, Jordan and Gilbert, 1882), captured in Southern Sinaloa, México. J. Northwest Atl. Fish. Sci. 35, 107–114.

https://doi.org/10.2960/j.v35.m506

Mastin, J., Harner, T., Schuster, J.K., South, L., 2022. A review of PCB-11 and other unintentionally produced PCB congeners in outdoor air. Atmos. Pollut. Res. 13, 101364. https://doi.org/10.1016/j.apr.2022.101364

Matulik, A.G., Kerstetter, D.W., Hammerschlag, N., Divoll, T., Hammerschmidt, C.R., Evers, D.C., 2017. Bioaccumulation and biomagnification of mercury and methylmercury in four sympatric coastal sharks in a protected subtropical lagoon. Mar. Pollut. Bull. 116, 357–364. https://doi.org/10.1016/j.marpolbul.2017.01.033

Megson, D., Benoit, N.B., Sandau, C.D., Chaudhuri, S.R., Long, T., Coulthard, E., Johnson, G.W., 2019. Evaluation of the effectiveness of different indicator PCBs to estimating total PCB concentrations in environmental investigations. Chemosphere 237, 124429. https://doi.org/10.1016/j.chemosphere.2019.124429

Megson, D., Brown, T., Jones, G.R., Robson, M., Johnson, G.W., Tiktak, G.P., Sandau, C.D., Reiner, E.J., 2022. Polychlorinated biphenyl (PCB) concentrations and profiles in marine mammals from the North Atlantic Ocean. Chemosphere 288, 132639. https://doi.org/10.1016/j.chemosphere.2021.132639

Mezzalira-Pincinato, R.B., Gasalla, M.A., Garlock, T., Anderson, J.L., 2022. Market incentives for shark fisheries. Mar. Policy 139, 105031. https://doi.org/10.1016/j.marpol.2022.105031

Miglioranza, K.S.B., Gonzalez, M., Ondarza, P.M., Shimabukuro, V.M., Isla, F.I., Fillmann, G., Aizpún, J.E., Moreno, V.J., 2013. Assessment of Argentinean Patagonia pollution: PBDEs, OCPs and PCBs in different matrices from the Río Negro basin. Sci. Total Environ. 452–453, 275–285. https://doi.org/10.1016/j.scitotenv.2013.02.055

Mikkonen, H.G., Clarke, B.O., Dasika, R., Wallis, C.J., Reichman, S.M., 2018. Evaluation of methods for managing censored results when calculating the geometric mean. Chemosphere 191, 412–416. https://doi.org/10.1016/j.chemosphere.2017.10.038

Milićević, T., Romanić, S.H., Popović, A., Mustać, B., Đinović-Stojanović, J., Jovanović, G., Relić, D., 2022. Human health risks and benefits assessment based on OCPs, PCBs, toxic elements and fatty acids in the pelagic fish species from the Adriatic Sea. Chemosphere 287. https://doi.org/10.1016/j.chemosphere.2021.132068

Miller, A., Elliott, J.E., Wilson, L.K., Elliott, K.H., Drouillard, K.G., Verreault, J., Lee, S., Idrissi, A., 2020. Influence of overwinter distribution on exposure to persistent organic pollutants (POPs) in seabirds, ancient murrelets (*Synthliboramphus antiquus*), breeding on the Pacific coast of Canada. Environ. Pollut. 259, 113842. https://doi.org/10.1016/j.envpol.2019.113842

Molde, K., Ciesielski, T.M., Fisk, A.T., Lydersen, C., Kovacs, K.M., Sørmo, E.G., Jenssen, B.M., 2013. Associations between vitamins A and E and legacy POP levels in highly contaminated Greenland sharks (*Somniosus microcephalus*). Sci. Total Environ. 442, 445–454. https://doi.org/10.1016/j.scitotenv.2012.10.012

More, S.J., Hardy, A., Bampidis, V., Benford, D., Hougaard Bennekou, S., Bragard, C., Boesten, J., Halldorsson, T.I., Hernández-Jerez, A.F., Jeger, M.J., Knutsen, H.K., Koutsoumanis, K.P., Naegeli, H., Noteborn, H., Ockleford, C., Ricci, A., Rychen, G., Schlatter, J.R., Silano, V., Nielsen, S.S., Schrenk, D., Solecki, R., Turck, D., Younes, M., Benfenati, E., Castle, L., Cedergreen, N., Laskowski, R., Leblanc, J.C., Kortenkamp, A., Ragas, A., Posthuma, L., Svendsen, C., Testai, E., Dujardin, B., Kass, G.E.N., Manini, P., Zare Jeddi, M., Dorne, J.L.C., Hogstrand, C., 2019. Guidance on harmonised methodologies for human health, animal

health and ecological risk assessment of combined exposure to multiple chemicals. EFSA J. 17. https://doi.org/10.2903/j.efsa.2019.5634

Mrema, E.J., Rubino, F.M., Brambilla, G., Moretto, A., Tsatsakis, A.M., Colosio, C., 2013. Persistent organochlorinated pesticides and mechanisms of their toxicity. Toxicology 307, 74–88. https://doi.org/10.1016/j.tox.2012.11.015

Muir, D., Sverko, E., 2006. Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance: A critical appraisal. Anal. Bioanal. Chem. 386, 769–789. https://doi.org/10.1007/s00216-006-0765-y

Mull, C., Blasius, M., O'Sullivan, J., Lowe, C., 2012. Heavy metals, trace elements, and organochlorine contaminants in muscle and liver tissue of juvenile white sharks, *Carcharodon carcharias*, from the southern California bight, in: Domeier, M.L. (Ed.), Global Perspectives on the Biology and Life History of the White Shark. Taylor & Francis Group, Boca Raton, pp. 59–76. https://doi.org/10.1201/b11532-7

Mull, C.G., Lyons, K., Blasius, M.E., Winkler, C., Sullivan, J.B.O., Lowe, C.G., 2013. Evidence of Maternal Offloading of Organic Contaminants in White Sharks (Carcharodon carcharias). PLoS One 8, 2–9. https://doi.org/10.1371/journal.pone.0062886

Muñoz-Arnanz, J., Bartalini, A., Alves, L., Lemos, M.F., Novais, S.C., Jiménez, B., 2022. Occurrence and distribution of persistent organic pollutants in the liver and muscle of Atlantic blue sharks: Relevance and health risks. Environ. Pollut. 309, 119750. https://doi.org/10.1016/j.envpol.2022.119750

Muñoz-Arnanz, J., Jiménez, B., 2011. New DDT inputs after 30 years of prohibition in Spain. A case study in agricultural soils from south-western Spain. Environ. Pollut. 159, 3640–3646. https://doi.org/10.1016/j.envpol.2011.07.027

Munschy, C., Vigneau, E., Bely, N., Héas-moisan, K., Olivier, N., Pollono, C., Hollanda, S., 2020. Legacy and emerging organic contaminants: Levels and profiles in top predator fish from the western Indian Ocean in relation to their trophic ecology. Environ. Res. 188, 109761. https://doi.org/10.1016/j.envres.2020.109761

Murphy, S., Law, R.J., Deaville, R., Barnett, J., Perkins, M.W., Brownlow, A., Penrose, R., Davison, N.J., Barber, J.L., Jepson, P.D., 2018. Organochlorine contaminants and reproductive implication in cetaceans: A case study of the common dolphin, Marine Mammal Ecotoxicology. 3-38. https://doi.org/10.1016/B978-0-12-812144-3.00001-2

Niño-Torres, C.A., Gardner, S.C., Zenteno-Savín, T., Ylitalo, G.M., 2009. Organochlorine pesticides and polychlorinated biphenyls in California sea lions (*Zalophus californianus californianus*) from the Gulf of California, México. Arch. Environ. Contam. Toxicol. 56, 350–359. https://doi.org/10.1007/s00244-008-9181-y

Nomiyama, K., Uchiyama, Y., Horiuchi, S., Eguchi, A., Mizukawa, H., Hirata, S.H., Shinohara, R., Tanabe, S., 2011. Organohalogen compounds and their metabolites in the blood of Japanese amberjack (*Seriola quinqueradiata*) and scalloped hammerhead shark (*Sphyrna lewini*) from Japanese coastal waters. Chemosphere 85, 315–321. https://doi.org/10.1016/j.chemosphere.2011.06.092

Oceana, 2019. Fraude y sustitución en la comida del mar [WWW Document]. URL https://gatoxliebre.org/wp-content/uploads/2019/03/Cuadernillo-resultados-GxL-WEB.pdf (accessed 5.7.21).

O'Bryhim, J.R., Adams, D.H., Spaet, J.L.Y., Mills, G., Lance, S.L., 2017. Relationships of mercury

concentrations across tissue types, muscle regions and fins for two shark species. Environ. Pollut. 223, 323–333. https://doi.org/10.1016/j.envpol.2017.01.029

Olin, J.A., Beaudry, M., Fisk, A.T., Paterson, G., 2013. Age-related polychlorinated biphenyl dynamics in immature bull sharks (*Carcharhinus leucas*). Environ. Toxicol. Chem. 33, 35–43. https://doi.org/10.1002/etc.2402

Olisah, C., Okoh, O.O., Okoh, A.I., 2019. Global evolution of organochlorine pesticides research in biological and environmental matrices from 1992 to 2018: A bibliometric approach. Emerg. Contam. 5, 157–167. https://doi.org/10.1016/j.emcon.2019.05.001

Páez-Osuna, F., Ávarez-Borrego, S., Ruiz-Fernández, A.C., García-Hernández, J., Jara-Marini, M.E., Bergés-Tiznado, M.E., Piñón-Gimate, A., Alonso-Rodríguez, R., Soto-Jiménez, M.F., Frías-Espericueta, M.G., Ruelas-Inzunza, J.R., Green-Ruiz, C.R., Osuna-Martínez, C.C., Sánchez-Cabeza, J.A., 2017. Environmental status of the Gulf of California: A pollution review. Earth-Science Rev. 166, 181–205. https://doi.org/10.1016/j.earscirev.2017.01.014

Páez-Osuna, F., Osuna-Martínez, C., 2011. Biomonitors of coastal pollution with reference to the situation in the mexican coasts: A review on the utilization of organisms [Biomonitores de la contaminación costera con referencia a las costas mexicanas: Una revisión sobre los organismos utilizados]. Hidrobiologica 21, 229–238.

Pedro, S., Boba, C., Dietz, R., Sonne, C., Rosing-Asvid, A., Hansen, M., Provatas, A., McKinney, M.A., 2017. Blubber-depth distribution and bioaccumulation of PCBs and organochlorine pesticides in Arctic-invading killer whales. Sci. Total Environ. 601–602, 237–246. https://doi.org/10.1016/j.scitotenv.2017.05.193

Pethybridge, H., Daley, R., Virtue, P., 2010. Lipid composition and partitioning of deepwater chondrichthyans: inferences of feeding ecology and distribution. Mar. Biol. 157, 1367–1384. https://doi.org/10.1007/s00227-010-1416-6

Phillips, T., Seech, A., Lee, H., Trevors, J., 2005. Biodegradation of hexachlorocyclohexane (HCH) by microorganisms. Biodegradation 16, 363– 392. https://doi.org/10.1007/s10532-004-2413-6

Pierce, S.J., 2022. Human impacts on sharks and rays [WWW Document]. IUCN Species Survival Commission.URLhttps://www.cms.int/sites/default/files/publication/cms_sharksmos4_inf.7_human%20impacts%20on%20sharks%20and%20rays_e_0.pdf

Piercy, A. N., Carlson, J. K., Sulikowski, J. A., & Burgess, G. H. (2007). Age and growth of the scalloped hammerhead shark, *Sphyrna lewini*, in the north-west Atlantic Ocean and Gulf of Mexico. Marine and Freshwater Research, 58(1), 34-40. https://doi.org/10.1071/MF05195

Pollom, R., Avalos, C., Bizzarro, J., Burgos-Vázquez, M.I., Cevallos, A., Espinoza, M., González, A., Mejía-Falla, P.A., Morales-Saldaña, J.M., Navia, A.F., Pérez Jiménez, J.C., Sosa-Nishizaki, O. & Velez-Zuazo, X., 2020. *Rhizoprionodon longurio*. [WWW Document]. The IUCN Red List of Threatened Species 2020. URL https://dx.doi.org/10.2305/IUCN.UK.2020-3.RLTS.T161662A124524022 (accessed 5.09.23).

Ponce-Vélez, G., Botello, A. V., 2018. Plaguicidas organoclorados en organismos costeros y marinos de los litorales mexicanos: Una revisión. Rev. Int. Contam. Ambient. 34, 81–98. https://doi.org/10.20937/RICA.2018.34.ESP02.07

Pose-Juan, E., Fernández-Cruz, T., Simal-Gándara, J., 2016. State of the art on public risk assessment of combined human exposure to multiple chemical contaminants. Trends Food Sci. Technol. 55, 11–28. https://doi.org/10.1016/j.tifs.2016.06.011

Pozo, K., Oyola, G., Estellano, V.H., Harner, T., Rudolph, A., Prybilova, P., Kukucka, P., Audi, O., Klánová, J., Metzdorff, A., Focardi, S., 2017. Persistent Organic Pollutants (POPs) in the atmosphere of three Chilean cities using passive air samplers. Sci. Total Environ. 586, 107–114. https://doi.org/10.1016/j.scitotenv.2016.11.054

Procuraduría Federal del Consumidor, 2017. Pescados y mariscos. [WWW Document]. Gobierno. URL https://www.gob.mx/profeco/documentos/pescados-y-mariscos?state=published (accessed 5.04.20).

Qiu, X., Zhu, T., Yao, B., Hu, J., Hu, S., 2005. Contribution of dicofol to the current DDT pollution in China. Environ. Sci. Technol. 39, 4385–4390. https://doi.org/10.1021/es050342a

Ramírez-Amaro, S., Ramírez-Macías, D., Vázquez-Juárez, R., Flores-Ramírez, S., Galván-Magaña, F., Gutiérrez-Rivera, J.N., 2017. Population structure of the Pacific angel shark (*Squatina californica*) along the northwestern coast of Mexico based on the mitochondrial DNA control region. Ciencias Mar. 43, 69–80. https://doi.org/10.7773/cm.v43i1.2692

Rani, M., Shanker, U., Jassal, V., 2017. Recent strategies for removal and degradation of persistent & toxic organochlorine pesticides using nanoparticles: A review. J. Environ. Manage. 190, 208–222. https://doi.org/10.1016/j.jenvman.2016.12.068

Rauert, C., Harner, T., Schuster, J.K., Eng, A., Fillmann, G., Castillo, L.E., Fentanes, O., Ibarra, M.V., Miglioranza, K.S.B., Rivadeneira, I.M., Pozo, K., Aristizábal Zuluaga, B.H., 2018. Air monitoring of new and legacy POPs in the Group of Latin America and Caribbean (GRULAC) region. Environ. Pollut. 243, 1252–1262. https://doi.org/10.1016/j.envpol.2018.09.048

Renaguli, A., Fernando, S., Holsen, T.M., Hopke, P.K., Adams, D.H., Balazs, G.H., Jones, T.T., Work, T.M., Lynch, J.M., Crimmins, B.S., 2021. Characterization of halogenated organic compounds in pelagic sharks and sea turtles using a nontargeted approach. Environ. Sci. Technol. 55, 16390–16401. https://doi.org/10.1021/acs.est.1c03798

Reyes-Montiel, N.J., Santamaría-Miranda, A., Rodríguez-Meza, G.D., Galindo-Reyes, J.G., González-Ocampo, H.A., 2013. Concentrations of organochlorine pesticides in fish (*Mugil cephalus*) from a coastal ecosystem in the southwestern Gulf of California. Biol. Environ. Proc. R. Irish Acad. 113. https://doi.org/10.3318/BIOE.2013.25

Ricking, M., Schwarzbauer, J., 2012. DDT isomers and metabolites in the environment: an overview. Environ. Chem. Lett. 10, 317–323. https://doi.org/10.1007/s10311-012-0358-2

Rigby, C.L., Dulvy, N.K., Barreto, R., Carlson, J., Fernando, D., Fordham, S., Francis, M.P., Herman, K., Jabado, R.W., Liu, K.M., Marshall, A., Pacoureau, N., Romanov, E., Sherley, R.B., Winker, H., 2019. *Sphyrna lewini* [WWW Document]. The IUCN Red List of Threatened Species 2019. URL https://www.iucnredlist.org/es/species/39385/2918526#bibliography (accessed 5.18.21).

Rigét, F., Bignert, A., Braune, B., Dam, M., Dietz, R., Evans, M., Green, N., Gunnlaugsdóttir, H., Hoydal, K.S., Kucklick, J., Letcher, R., Muir, D., Schuur, S., Sonne, C., Stern, G., Tomy, G., Vorkamp, K., Wilson, S., 2019. Temporal trends of persistent organic pollutants in Arctic marine and freshwater biota. Sci. Total Environ. 649, 99–110. https://doi.org/10.1016/j.scitotenv.2018.08.268

Ritter, L., Solomon, K.R., Forget, J., Stemeroff, M., Leary, C.O., 1995. Persistent organic pollutants: An assessment report on: DDT, aldrin, dieldrin, endrin, chlordane, heptachlor, hexachlorobenzene, mirex, toxaphene, polychlorinated biphenyls, dioxins and furans. Int. Program. Chem. Saf. https://doi.org/10.1016/j.chemosphere.2011.09.039

Romero-Caicedo, A.F., Galván-Magaña, F., Hernández-Herrera, A., Carrera-Fernández, M., 2016. Reproductive parameters of the Pacific angel shark *Squatina californica* (Selachii: Squatinidae). J. Fish Biol. 88, 1430–1440. https://doi.org/10.1111/jfb.12920

Romero-Romero, S., Herrero, L., Fernández, M., Gómara, B., Acuña, J.L., 2017. Biomagnification of persistent organic pollutants in a deep-sea, temperate food web. Sci. Total Environ. 605–606, 589–597. https://doi.org/10.1016/j.scitotenv.2017.06.148

Rosende-Pereiro, A., Corgos, A., 2018. Pilot acoustic tracking study on young of the year scalloped hammerhead sharks, *Sphyrna lewini*, within a coastal nursery area in Jalisco, Mexico. Lat. Am. J. Aquat. Res. 46, 645–659. https://doi.org/10.3856/vol46-issue4-fulltext-2

Rosende-Pereiro, A., Flores-Ortega, J.R., González-Sansón, G., Corgos, A., 2019. Stomach content and stable isotopes reveal an ontogenetic dietary shift of young-of-the-year scalloped hammerhead sharks (*Sphyrna lewini*) inhabiting coastal nursery areas. Environ. Biol. Fishes 103, 49–65. https://doi.org/10.1007/s10641-019-00932-0

Ross, P.S., Ellis, G.M., Ikonomou, M.G., Barrett-Lennard, L.G., Addison, R.F., 2000. High PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca*: Effects of age, sex and dietary preference. Mar. Pollut. Bull. 40, 504–515. https://doi.org/10.1016/S0025-326X(99)00233-7

Ross, P.S., Jeffries, S.J., Yunker, M.B., Addison, R.F., Ikonomou, M.G., Calambokidis, J.C., 2004. Harbor seals (*Phoca vitulina*) in British Columbia, Canada, and Washington State, USA, reveal a combination of local and global polychlorinated biphenyl, dioxin, and furan signals. Environ. Toxicol. Chem. 23, 157–165. https://doi.org/10.1897/03-85

Routti, H., Atwood, T.C., Bechshoft, T., Boltunov, A., Ciesielski, T.M., Desforges, J.P., Dietz, R., Gabrielsen, G.W., Jenssen, B.M., Letcher, R.J., McKinney, M.A., Morris, A.D., Rigét, F.F., Sonne, C., Styrishave, B., Tartu, S., 2019. State of knowledge on current exposure, fate and potential health effects of contaminants in polar bears from the circumpolar Arctic. Sci. Total Environ. 664, 1063–1083. https://doi.org/10.1016/j.scitotenv.2019.02.030

Ruelas-Inzunza, J., Amezcua, F., Coiraton, C., Páez-Osuna, F., 2020. Cadmium, mercury, and selenium in muscle of the scalloped hammerhead *Sphyrna lewini* from the tropical Eastern Pacific: variation with age, molar ratios and human health risk. Chemosphere 242. https://doi.org/10.1016/j.chemosphere.2019.125180

Ruelas-Inzunza, J.R., Páez-Osuna, F., 2005. Mercury in fish and shark tissues from two coastal lagoons in the Gulf of California, Mexico. Bull. Environ. Contam. Toxicol. 74, 294–300. https://doi.org/10.1007/s00128-004-0583-x

Ruess, L., Müller-Navarra, D.C., 2019. Essential biomolecules in food webs. Front. Ecol. Evol. 7, 1–18. https://doi.org/10.3389/fevo.2019.00269

Russell, R.W., Gobas, F.A.P.C., Haffner, G.D., 1999. Role of chemical and ecological factors in trophic transfer of organic chemicals in aquatic food webs. Environ. Toxicol. Chem. 18, 1250–1257. https://doi.org/10.1897/1551-5028(1999)018<1250:ROCAEF>2.3.CO;2

Sakai, S.I., Hayakawa, K., Takatsuki, H., Kawakami, I., 2001. Dioxin-like PCBs released from waste incineration and their deposition flux. Environ. Sci. Technol. 35, 3601–3607. https://doi.org/10.1021/es001945j

Saldaña-Ruiz, L.E., Sosa-Nishizaki, O., Cartamil, D., 2017. Historical reconstruction of Gulf of California shark fishery landings and species composition, 1939–2014, in a data-poor

fishery context. Fish. Res. 195, 116–129. https://doi.org/10.1016/j.fishres.2017.07.011

Sánchez-Osorio, J.L., Macías-Zamora, J.V., Ramírez-Álvarez, N., Bidleman, T.F., 2017. Organochlorine pesticides in residential soils and sediments within two main agricultural areas of northwest Mexico: Concentrations, enantiomer compositions and potential sources. Chemosphere 173, 275–287. https://doi.org/10.1016/j.chemosphere.2017.01.010

Sawyna, J.M., Spivia, W.R., Radecki, K., Fraser, D.A., Lowe, C.G., 2017. Association between chronic organochlorine exposure and immunotoxicity in the round stingray (*Urobatis halleri*). Environ. Pollut. 223, 42–50. https://doi.org/10.1016/j.envpol.2016.12.019

Schlenk, D., Sapozhnikova, Y., Cliff, G., 2005. Incidence of organochlorine pesticides in muscle and liver tissues of South African great white sharks *Carcharodon carcharias*. Mar. Pollut. Bull. 50, 208–211. https://doi.org/10.1016/j.marpolbul.2004.11.032

SEMARNAT, 2019. Fichas técnicas: Contaminantes Orgánicos Persistentes [WWW Doument]. Residuos COP, Manejo Ambientalmente Adecuado. URL http://www.residuoscop.org/movil/documentos (accessed 3.01.21)

Shiffman, D.S., Hueter, R.E., 2017. A United States shark fin ban would undermine sustainable shark fisheries. Mar. Policy 85, 138–140. https://doi.org/10.1016/j.marpol.2017.08.026

SIAP, 2020. Anuario Estadístico de la Producción Agrícola [WWW Document]. URL https://nube.siap.gob.mx/cierreagricola/ (accessed 2.03.21).

Simpson, C.D., Wilcock, R.J., Smith, T.J., Wilkins, A.L., Langdon, A.G., 1995. Determination of octanol-water partition coefficients for the major components of technical chlordane. Bull. Environ. Contam. Toxicol. 55, 149–153. https://doi.org/10.1007/BF00212402

Singh, Z., 2016. Toxic effects of organochlorine pesticides: A review. Am. J. Biosci. 4, 11. https://doi.org/10.11648/j.ajbio.s.2016040301.13

Singh, S., Li, S.S., 2012. Epigenetic effects of environmental chemicals bisphenol A and phthalates. Int. J. Mol. Sci. 13, 10143–10153. https://doi.org/10.3390/ijms130810143

Sobral, M.M.C., Cunha, S.C., Faria, M.A., Ferreira, I.M., 2017. Domestic cooking of muscle foods: Impact on composition of nutrients and contaminants. Compr. Rev. Food Sci. Food Saf. https://doi.org/10.1111/1541-4337.12327

Song, S., Xue, J., Lu, Y., Zhang, H., Wang, C., Cao, X., Li, Q., 2018. Are unintentionally produced polychlorinated biphenyls the main source of polychlorinated biphenyl occurrence in soils? Environ. Pollut. 243, 492–500. https://doi.org/10.1016/j.envpol.2018.09.027

Souza-Araujo, J., Souza-Junior, O.G., Guimarães-Costa, A., Hussey, N.E., Lima, M.O., Giarrizzo, T., 2021. The consumption of shark meat in the Amazon region and its implications for human health and the marine ecosystem. Chemosphere 265, 129132. https://doi.org/10.1016/j.chemosphere.2020.129132

Sparling, D.W., 2016. Ecotoxicology essentials: environmental contaminants and their biological effects on animals and plants, 1st ed. Elsevier, London.

Speers-Roesch, B., Treberg, J.R., 2010. The unusual energy metabolism of elasmobranch fishes. Comp. Biochem. Physiol. - A Mol. Integr. Physiol. 155, 417–434. https://doi.org/10.1016/j.cbpa.2009.09.031

Statista 2022. Average annual per capita consumption of seafood worldwide from 2014 to 2020 [WWW Document]. URL https://www.statista.com/statistics/820953/per-capita-

consumption-of-seafood-worldwide/ (accessed 10.05.22).

Steevens, J.A., Reiss, M.R., Pawlisz, A. V., 2005. A methodology for deriving tissue residue benchmarks for aquatic biota: a case study for fish exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin and equivalents. Integr. Environ. Assess. Manag. 1, 142–151. https://doi.org/10.1897/IEAM_2004a-014.1

Stockholm Convention, 2017. In: UNEP/POPS/POPRC.13/7/Add.1 (Ed.), Report of the Persistent Organic Pollutants Review Committee on the work of its thirteenth meeting. United Nations Environment Programme, Rome.

Stockholm Convention, 2019. In: UNEP/POPS/COP.9/30 (Ed.), Report of the Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants on the work of its ninth meeting. Risk management evaluation on dicofol. United Nations Environment Programme, Geneva.

Storelli, M.M., Marcotrigiano, G.O., 2001. Persistent organochlorine residues and toxic evaluation of polychlorinated biphennyls in sharks from the Mediterranena sea (Italy). Mar. Pollut. Bull. 42, 1323–1329. https://doi.org/10.1007/s002449910029

Storelli, M.M., Storelli, A., Marcotrigiano, G.O., 2005. Concentrations and hazard assessment of polychlorinated biphenyls and organochlorine pesticides in shark liver from the Mediterranean Sea. Mar. Pollut. Bull. 50, 850–855. https://doi.org/10.1016/j.marpolbul.2005.02.023

Sun, S.X., Hua, X.M., Deng, Y.Y., Zhang, Y.N., Li, J.M., Wu, Z., Limbu, S.M., Lu, D.S., Yin, H.W., Wang, G.Q., Waagbø, R., Frøyland, L., Zhang, M.L., Du, Z.Y., 2018. Tracking pollutants in dietary fish oil: From ocean to table. Environ. Pollut. 240, 733–744. https://doi.org/10.1016/j.envpol.2018.05.027

Suryaningsih, W., Supriyono, S., Hariono, B., Kurnianto, M.F., 2020. Improving the quality of smoked shark meat with ozone water technique. Earth Environ. Sci. 411. https://doi.org/10.1088/1755-1315/411/1/012048

Swapna, H.C., 2010. Lipid classes and fatty acid profile of selected Indian fresh water fishes. J. Food Sci. Technol. 47, 394–400. https://doi.org/10.1007/s13197-010-0065-6

Tartu, S., Bourgeon, S., Aars, J., Andersen, M., Polder, A., Thiemann, G.W., Welker, J.M., Routti, H., 2017. Sea ice-associated decline in body condition leads to increased concentrations of lipophilic pollutants in polar bears (Ursus maritimus) from Svalbard, Norway. Sci. Total Environ. 576, 409–419. https://doi.org/10.1016/j.scitotenv.2016.10.132

ThermoScientific, 2012. Accelerated Solvent Extraction - Applications Summary [WWW Document]. URL www.thermoscientific.com/dionex (accessed 5.7.21).

Tiktak, G.P., Butcher, D., Lawrence, P.J., Norrey, J., Bradley, L., Shaw, K., Preziosi, R., Megson, D., 2020. Are concentrations of pollutants in sharks, rays and skates (Elasmobranchii) a cause for concern? A systematic review. Mar. Pollut. Bull. 160, 111701. https://doi.org/10.1016/j.marpolbul.2020.111701

Torres-Rojas, Y.E., Páez-Osuna, F., Hernández-Herrera, A., Galván-Magaña, F., Aguiñiga-García, S., Villalobos-Ortíz, H., Sampson, L., 2013. Feeding grounds of juvenile scalloped hammerhead sharks (*Sphyrna lewini*) in the south-eastern Gulf of California. Hydrobiologia 726, 81–94. https://doi.org/10.1007/s10750-013-1753-9

Trejo-Ramírez, A., 2017. Caracterización de la bahía de La Paz como una posible área de crianza del tiburón bironche, *Rhizoprionodon longurio* [Master Thesis]. Instituto Politécnico

Nacional, Mexico.

Tuerk, K.J.S., Kucklick, J.R., McFee, W.E., Pugh, R.S., Becker, P.R., 2005. Factors influencing persistent organic pollutant concentrations in the Atlantic white-sided dolphin (*Lagenorhynchus acutus*). Environ. Toxicol. Chem. 24, 1079–1087. https://doi.org/10.1897/04-120R.1

Turgut, C., Gokbulut, C., Cutright, T.J., 2009. Contents and sources of DDT impurities in dicofol formulations in Turkey. Environ. Sci. Pollut. Res. 16, 214–217. https://doi.org/10.1007/s11356-008-0083-3

Turusov, V., Rakitsky, V., Tomatis, L., Ubiquity, D.D.D.T., 2002. Dichlorodiphenyltrichloroethane (DDT): Ubiquity, persistence, and risks. Res. Rev. 110, 125–128.

Turyk, M.E., Bhavsar, S.P., Bowerman, W., Boysen, E., Clark, M., Diamond, M., Mergler, D., Pantazopoulos, P., Schantz, S., Carpenter, D.O., 2012. Risks and benefits of consumption of great lakes fish. Environ. Health Perspect. 120, 11–18. https://doi.org/10.1289/ehp.1003396

UNEP, 2019a. Proposal to list methoxychlor in Annex A to the Stockholm Convention on Persistent Organic Pollutants [WWW Document]. URL https://doi.org/10.2307/2668517 (accessed 6.20.20).

UNEP, 2019b. Report of the Conference of the Parties to the Stockholm Convention on Persistent Organic Pollutants on the work of its ninth meeting [WWW Document]. URL https://doi.org/10.2307/2668517 (accessed 6.20.20).

UNEP, 2017. The 16 new POPs. An introduction to the chemicals added to the Stockholm Convention as Persistent Organic Pollutants by the Conference of the Parties.

UNEP, 2008. Perfil de riesgo del pentaclorobenceno. [WWW Document]. URL http://chm.pops.int/Portals/0/ aspx?d=UNEP-POPS-POPRC.3-20-Add.7 (accessed 6.22.20).

USEPA, 1997. Toxicological Review of chlordane (technical). https://doi.org/http://www.epa.gov/iris/toxreviews/0070tr.pdf

USEPA, 2000. Guidance for assessing chemical contaminant data for use in fish advisories. Risk Assessment and fish consumption limits, US Environmental Protection Agency.

USEPA, 2005. Guidelines for Carcinogen Risk Assessment. Risk Assessment Forum, US Environmental Protection Agency.

Valle, M.D., Jurado, E., Dachs, J., Sweetman, A.J., Jones, K.C., 2005. The maximum reservoir capacity of soils for persistent organic pollutants: implications for global cycling. Environ. Pollut. 134, 153–164. https://doi.org/10.1016/j.envpol.2004.07.011

Van Den Berg, M., Birnbaum, L., Bosveld, A.T.C., Brunström, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T., Larsen, J.C., Van Leeuwen, F.X.R., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Wærn, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs, PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792. https://doi.org/10.1289/ehp.98106775

Van den Brink, N.W., Van Franeker, J.A., De Ruiter-Dijkman, E.M., 1998. Fluctuating concentrations of organochlorine pollutants during a breeding season in two antarctic seabirds: Adelie penguin and southern fulmar. Environ. Toxicol. Chem. 17, 702–709.

https://doi.org/10.1897/1551-5028(1998)017<0702:FCOOPD>2.3.CO;2

Van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ. Toxicol. Pharmacol. 13, 57–149. https://doi.org/10.1016/S1382-6689(02)00126-6

Vergara, E.G., Hernández, V., Munkittrick, K.R., Barra, R., Galban-Malagon, C., Chiang, G., 2019. Presence of organochlorine pollutants in fat and scats of pinnipeds from the Antarctic Peninsula and South Shetland Islands, and their relationship to trophic position. Sci. Total Environ. 685, 1276–1283. https://doi.org/10.1016/j.scitotenv.2019.06.122

Verreault, J., Villa, R.A., Gabrielsen, G.W., Skaare, J.U., Letcher, R.J., 2006. Maternal transfer of organohalogen contaminants and metabolites to eggs of Arctic-breeding Glaucous gulls. Environ. Pollut. 144, 1053–1060. https://doi.org/10.1016/j.envpol.2005.10.055

Voorspoels, S., Covaci, A., Maervoet, J., De Meester, I., Schepens, P., 2004. Levels and profiles of PCBs and OCPs in marine benthic species from the Belgian North Sea and the Western Scheldt Estuary. Mar. Pollut. Bull. 49, 393–404. https://doi.org/10.1016/j.marpolbul.2004.02.024

Wagner, C.C., Amos, H.M., Thackray, C.P., Zhang, Y., Lundgren, E.W., Forget, G., Friedman, C.L., Selin, N.E., Lohmann, R., Sunderland, E.M., 2019. A global 3-D ocean model for PCBs: Benchmark compounds for understanding the impacts of global change on neutral persistent organic pollutants. Global Biogeochem. Cycles 33, 469–481. https://doi.org/10.1029/2018GB006018

Wang, D., Yu, Y., Zhang, X., Zhang, D., Zhang, S., Wu, M., 2013. Organochlorine pesticides in fish from Taihu Lake, China, and associated human health risk assessment. Ecotoxicol. Environ. Saf. 98, 383–389. https://doi.org/10.1016/j.ecoenv.2013.07.012

Wang, H., Zhao, Y., Man, Y., Wong, C.K.C., Wong, M., 2011. Oral bioaccessibility and human risk assessment of organochlorine pesticides (OCPs) via fish consumption, using an in vitro gastrointestinal model. Food Chem. 127, 1673–1679. https://doi.org/10.1016/j.foodchem.2011.02.035

Wang, Z., Adu-Kumi, S., Diamond, M.L., Guardans, R., Harner, T., Harte, A., Kajiwara, N., Klánová, J., Liu, J., Moreira, E.G., Muir, D.C.G., Suzuki, N., Pinas, V., Seppälä, T., Weber, R., Yuan, B., 2022. Enhancing scientific support for the Stockholm Convention's implementation: An analysis of policy needs for scientific evidence. Environ. Sci. Technol. 56, 2936–2949. https://doi.org/10.1021/acs.est.1c06120

Weber, J., Halsall, C.J., Muir, D., Teixeira, C., Small, J., Solomon, K., Hermanson, M., Hung, H., Bidleman, T., 2010. Endosulfan, a global pesticide: A review of its fate in the environment and occurrence in the Arctic. Sci. Total Environ. 408, 2966–2984. https://doi.org/10.1016/j.scitotenv.2009.10.077

Webster, L., Russell, M., Walsham, P., Phillips, L.A., Hussy, I., Packer, G., Dalgarno, E.J., Moffat, C.F., 2011. An assessment of persistent organic pollutants in Scottish coastal and offshore marine environments. J. Environ. Monit. 13, 1288–1307. https://doi.org/10.1039/c1em10100e

Weijs, L., Briels, N., Adams, D.H., Lepoint, G., Das, K., Blust, R., Covaci, A., 2015. Maternal transfer of organohalogenated compounds in sharks and stingrays. Mar. Pollut. Bull. 92, 59–68. https://doi.org/10.1016/j.marpolbul.2014.12.056

Weitekamp, C.A., Phillips, L.J., Carlson, L.M., DeLuca, N.M., Cohen Hubal, E.A., Lehmann, G.M.,

2021. A state-of-the-science review of polychlorinated biphenyl exposures at background levels: Relative contributions of exposure routes. Sci. Total Environ. 776, 145912. https://doi.org/10.1016/j.scitotenv.2021.145912

Wetherbee, B.M., Nichols, P.D., 2000. Lipid composition of the liver oil of deep-sea sharks from the Chatham Rise, New Zealand 125, 511–521.

WHO, 1989. Environmental health criteria for DDT and its derivatives: Environmental aspects. World Health Organization, Geneva.

WHO, FAO, 2011. Report of the joint FAO/WHO expert consultation on the risks and benefits of fish consumption [WWW Document]. World Health Organization. URL https://apps.who.int/iris/handle/10665/44666 (accessed 03.23.30)

Williams, R.S., Curnick, D.J., Barber, J.L., Brownlow, A., Davison, N.J., Deaville, R., Perkins, M., Jobling, S., Jepson, P.D., 2020. Juvenile harbor porpoises in the UK are exposed to a more neurotoxic mixture of polychlorinated biphenyls than adults. Sci. Total Environ. 708, 134835. https://doi.org/10.1016/j.scitotenv.2019.134835

Wong, F., Alegria, H.A., Jantunen, L.M., Bidleman, T.F., Salvador-Figueroa, M., Gold-Bouchot, G., Ceja-Moreno, V., Waliszewski, S.M., Infanzon, R., 2008. Organochlorine pesticides in soils and air of southern Mexico: Chemical profiles and potential for soil emissions. Atmos. Environ. 42, 7737–7745. https://doi.org/10.1016/j.atmosenv.2008.05.028

Worm, B., Davis, B., Kettemer, L., Ward-paige, C.A., Chapman, D., Heithaus, M.R., Kessel, S.T., Gruber, S.H., 2013. Global catches , exploitation rates , and rebuilding options for sharks. Mar. Policy 40, 194–204. https://doi.org/10.1016/j.marpol.2012.12.034

Wright, S.L., Kelly, F.J., 2017. Plastic and Human Health: A Micro Issue? Environ. Sci. Technol. 51, 6634–6647. https://doi.org/10.1021/acs.est.7b00423

Wurl, O., Ekau, W., Landing, W.M., Zappa, C., 2017. Sea surface microlayer in a changing ocean-A perspective. Elem. Sci. Antrhropocene 5, 1432–1437. https://doi.org/10.1109/TCOMM.2005.855022

Xin, J., Liu, X., Liu, W., Jiang, L., Wang, J., Niu, J., 2011. Production and use of DDT containing antifouling paint resulted in high DDTs residue in three paint factory sites and two shipyard sites, China. Chemosphere 84, 342–347. https://doi.org/10.1016/j.chemosphere.2011.04.005

Yan, J., Zhu, W., Wang, D., Teng, M., Yan, S., Zhou, Z., 2019. Different effects of α-endosulfan, B-endosulfan, and endosulfan sulfate on sex hormone levels, metabolic profile and oxidative stress in adult mice testes. Environ. Res. 169, 315–325. https://doi.org/10.1016/j.envres.2018.11.028

Ylitalo, G.M., Matkin, C.O., Buzitis, J., Krahn, M.M., Jones, L.L., Rowles, T., Stein, J.E., 2001. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (*Orcinus orca*) from Prince William Sound, AK. Sci. Total Environ. 281, 183–203. https://doi.org/10.1016/S0048-9697(01)00846-4

Yu, Y., Wang, X., Yang, D., Lei, B., Zhang, Xiaolan, Zhang, Xinyu, 2014. Evaluation of human health risks posed by carcinogenic and non-carcinogenic multiple contaminants associated with consumption of fish from Taihu Lake, China. Food Chem. Toxicol. 69, 86–93. https://doi.org/10.1016/j.fct.2014.04.001

Yu, Y., Zhang, D., Zhang, X., 2012. Correct equations for calculating the maximum allowable fish consumption rate for human health risk assessment considering the noncarcinogenic

effects of multiple contaminants in fish. Environ. Sci. Technol. 46, 10481–10482. https://doi.org/10.1021/es3033082

Zamora-Arellano, N.Y., Ruelas-Inzunza, J., García-Hernández, J., Ilizaliturri-Hernández, C.A., Betancourt-Lozano, M., 2017. Linking fish consumption patterns and health risk assessment of mercury exposure in a coastal community of NW Mexico. Hum. Ecol. Risk Assess. 23, 1505–1521. https://doi.org/10.1080/10807039.2017.1329622

Zhang, L., Wang, W.X., 2007. Size-dependence of the potential for metal biomagnification in early life stages of marine fish. Environ. Toxicol. Chem. 26, 787–794. https://doi.org/10.1897/06-348R.1

Zhang, D.P., Zhang, X.Y., Yu, Y.X., Li, J.L., Yu, Z.Q., Wu, M.H., Fu, J.M., 2012. Tissue-specific distribution of fatty acids, polychlorinated biphenyls and polybrominated diphenyl ethers in fish from Taihu Lake, China, and the benefit-risk assessment of their co-ingestion. Food Chem. Toxicol. 50, 2837–2844. https://doi.org/10.1016/j.fct.2012.05.043

Zhou, H., Qu, Y., Wu, H., Liao, C., Zheng, J., Diao, X., Xue, Q., 2010. Molecular phylogenies and evolutionary behavior of AhR (aryl hydrocarbon receptor) pathway genes in aquatic animals: Implications for the toxicology mechanism of some persistent organic pollutants (POPs). Chemosphere 78, 193–205. https://doi.org/10.1016/j.chemosphere.2009.09.012

Zhu, M., Yuan, Y., Yin, H., Guo, Z., Wei, X., Qi, X., Liu, H., Dang, Z., 2022. Environmental contamination and human exposure of polychlorinated biphenyls (PCBs) in China : A review. Sci. Total Environ. 805, 150270. https://doi.org/10.1016/j.scitotenv.2021.150270

ANNEX 1

Supplementary Material

Table S2.1

Sampling data and biological information of the 20 immature individuals of *S. lewini* collected in San Bruno, El Saladito and El Manglito. The age was calculated according to Anislado-Tolentino et al. (2008). The life stage was assigned according to Duncan and Holland (2006) and Bejarano-Álvarez et al. (2016). TL = Total length.

Organism code	Sampling area	Sampling date	Sex	TL (cm)	Age (months)	Age group	Life stage
SB_154M	San Bruno	15/12/2018	M	154	39.5	> 3	Juvenil
SB_130F	San Bruno	15/12/2018	F	130	37.0	> 3	Juvenil
SB_120F	San Bruno	15/12/2018	F	120	32.2	2 - 3	Juvenil
SB_135F	San Bruno	15/12/2018	F	135	39.5	> 3	Juvenil
SB_083M	San Bruno	15/12/2018	M	83	11.1	≤ 1	Juvenil
SB_84.5M	San Bruno	15/12/2018	M	84.5	11.6	≤ 1	Juvenil
SB_086M	San Bruno	15/12/2018	M	86	12.1	1 - 2	Juvenil
SB_083F	San Bruno	15/12/2018	F	83	16.0	1 - 2	Juvenil
SB_090F	San Bruno	15/12/2018	F	90	18.9	1 - 2	Juvenil
SB_85.5M	San Bruno	17/12/2018	M	85.5	12.0	≤ 1	Juvenil
SA_108F1	El Saladito	30/01/2019	F	108	26.7	2 - 3	Juvenil
SA_120M	El Saladito	30/01/2019	M	120	24.9	2 - 3	Juvenil
SA_107F	El Saladito	14/02/2019	F	107	26.3	2 - 3	Juvenil
SA_108F2	El Saladito	22/02/2019	F	108	26.7	2 - 3	Juvenil
SA_94M	El Saladito	12/12/2018	M	94	15.0	1 - 2	Juvenil
SA_111M	El Saladito	12/12/2018	M	111	21.3	1 - 2	Juvenil
SA_115F	El Saladito	12/12/2018	F	115	29.9	2 - 3	Juvenil
SA_136M	El Saladito	12/12/2018	M	136	31.5	2 - 3	Juvenil
NN_F	El Manglito	23/08/2018	F	48	2.5	0	Neonate
NN_M	El Manglito	23/08/2018	M	51	0.6	0	Neonate

Table S2.2

Values of the standard reference material NIST SRM-1946 (National Institute of Standards & Technology) used to evaluate the recovery efficiency of the method data.

Compound	Concentration in ng/g w.w			
	Certified values	SRM 1	SRM 2	SRM 3
α-HCH	**5.72 ± 0.65**	5.64	4.28	5.74
hexachlorobenzene	**7.25 ± 0.83**	6.75	6.68	7.09
γ-HCH	**1.14 ± 0.18**	-	-	1.35
oxychlordane	**18.9 ± 1.5**	15.81	15.06	16.87
heptachlor epoxide	**5.50 ± 0.23**	4.99	4.83	5.49
o,p'-DDE*	**1.04 ± 0.29**	1.37	1.69	1.29
cis-chlordane	**32.5 ± 1.8**	26.86	27.11	31.82
trans-chlordane	**8.36 ± 0.91**	8.13	7.07	7.71
trans-nonachlor	**99.6 ± 7.6**	99.46	91.61	98.90
p,p'-DDE	**373 ± 48**	329.45	314.26	336.73
dieldrin	**32.5 ± 3.5**	25.29	25.93	27.84
o,p'-DDD	**2.20 ± 0.25**	1.37	1.69	1.29
o,p'-DDT*	**22.3 ± 3.2**	16.33	13.68	14.85
cis-nonachlor	**59.1 ± 3.6**	63.28	66.12	59.96
p,p'-DDD	**17.7 ± 2.8**	18.76	21.13	19.42
p,p'-DDT	**37.2 ± 3.5**	32.79	34.78	37.31
Mirex	**6.47 ± 0.77**	7.29	7.85	8.20

***Reference values

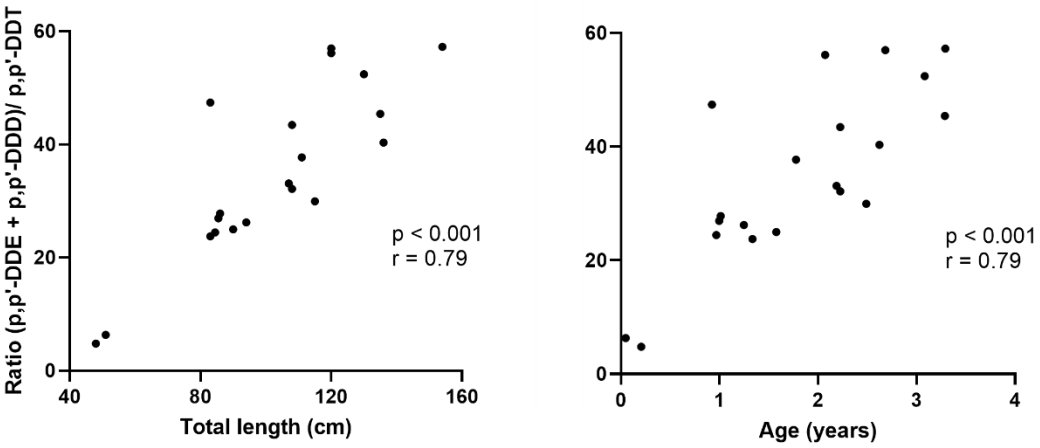


Fig. S2.1. Relationships between the ratio (p,p'-DDE + p,p'-DDD)/p,p'-DDT) in liver tissue and (a) the total length (cm) and (b) the age (years) of all individuals of *S. lewini*.

ANNEX 2

Supplementary Material

Table S3.1

Limits of detection (LOD) in ng/g w.w. of each targeted analyte.

OCs	**LOD (ng/g w.w.)**
OCPs	
p,p'-DDT	0.51
o,p'-DDT	0.73
p,p'-DDE	0.45
o,p'-DDE	0.91
p,p'-DDD	0.36
o,p'-DDD	0.45
Heptachlor	0.48
Heptachlor epoxide	0.89
cis-chlordane	1.37
trans-chlordane	0.86
cis-nonachlor	4.91
trans-nonachlor	0.81
Pentachlorobenzene	0.97
Hexachlorobenzene	1.07
Mirex	3.99
HCBD	1.42
Endrin	0.99
Dieldrin	0.79
Aldrin	0.46
Endrin aldehyde	1.45
α-HCH	0.93
β-HCH	0.52
γ-HCH	0.35
δ-HCH	1.03
α-Endosulfan	0.59
β-Endosulfan	0.95
Endosulfan sulphate	0.61
Oxychlordane	0.68
Methoxichlor	0.20
PCBs	
PCB-28	0.64
PCB-52	0.24
PCB-81	0.70
PCB-77	0.11
PCB-101	0.33
PCB-123	0.70
PCB-118	0.94
PCB-114	0.50

PCB-105	0.48
PCB-126	0.21
PCB-153	0.57
PCB-138	0.35
PCB-167	0.27
PCB-156	0.12
PCB-157	0.70
PCB-169	0.50
PCB-180	0.16
PCB-189	0.76

Table S3.2

Values of the standard reference material NIST SRM-1946 (National Institute of Standards & Technology) used to evaluate the recovery efficiency of the method data.

Compound	Concentration in ng/g w.w	
	Certified values	Measured values
OCPs		
α-HCH	5.7 ± 0.65	**5.2 ± 0.82**
hexachlorobenzene	7.2 ± 0.83	**6.8 ± 0.22**
γ-HCH	1.1 ± 0.18	**1.3****
oxychlordane	18.9 ± 1.5	**15.9 ± 0.91**
heptachlor epoxide	5.5 ± 0.23	**5.1 ± 0.34**
o,p'-DDE*	1.0 ± 0.29	**1.4 ± 0.21**
cis-chlordane	32.5 ± 1.8	**28.6 ± 2.8**
trans-chlordane	8.4 ± 0.91	**7.6 ± 0.53**
trans-nonachlor	99.6 ± 7.6	**96.7 ± 4.4**
p,p'-DDE	373.0 ± 48.0	**326.8 ± 11.5**
dieldrin	32.5 ± 3.5	**26.4 ± 1.3**
o,p'-DDD	2.2 ± 0.25	**1.5 ± 0.2**
o,p'-DDT*	22.3 ± 3.2	**15.0 ± 1.3**
cis-nonachlor	59.1 ± 3.6	**63.1 ± 3.1**
p,p'-DDD	17.7 ± 2.8	**19.8 ± 1.2**
p,p'-DDT	37.2 ± 3.5	**35.0 ± 2.3**
Mirex	6.5 ± 0.77	**7.8 ± 0.46**
PCB congeners		
52	8.1 ± 1.0	**7.0 ± 0.36**
101	34.6 ± 2.6	**29.1 ± 1.7**
77	0.33 ± 0.02	**0.44 ± 0.12**
118	52.1 ± 1.0	**31.9 ± 0.41**
153	170.0 ± 9.0	**176.5 ± 0.74**
105	19.9 ± 0.9	**17.4 ± 3.2**
138	115.0 ± 13.0	**103.8 ± 11.7**
126	0.38 ± 0.02	**0.25 ± 0.03**
156	9.5 ± 0.5	**9.5 ± 0.22**
180	74.4 ± 4.0	**67.7 ± 3.1**
169	0.11 ± 0.01	**NA**

* Reference value

** Only MRM 3

NA = Not available

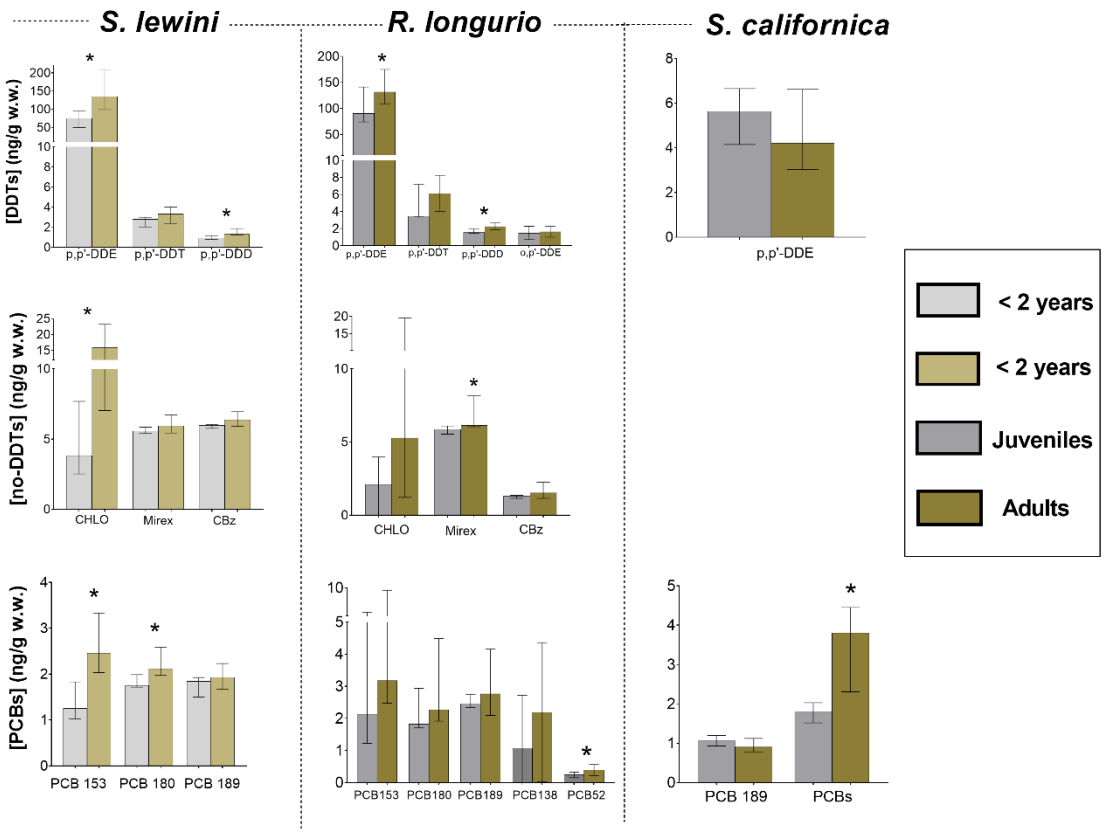


Fig. S3.1. Comparisons between median concentrations detected in juvenile < 2 years vs. juvenile < 2 years scalloped hammerhead *Sphyrna lewini*, and juveniles vs. adults Pacific sharpnose shark *Rhizoprionodon longurio* and Pacific angel shark *Squatina californica.* Error bars represented the interquartile range. Asterisk indicates significant differences ($p < 0.05$) between groups of individuals.

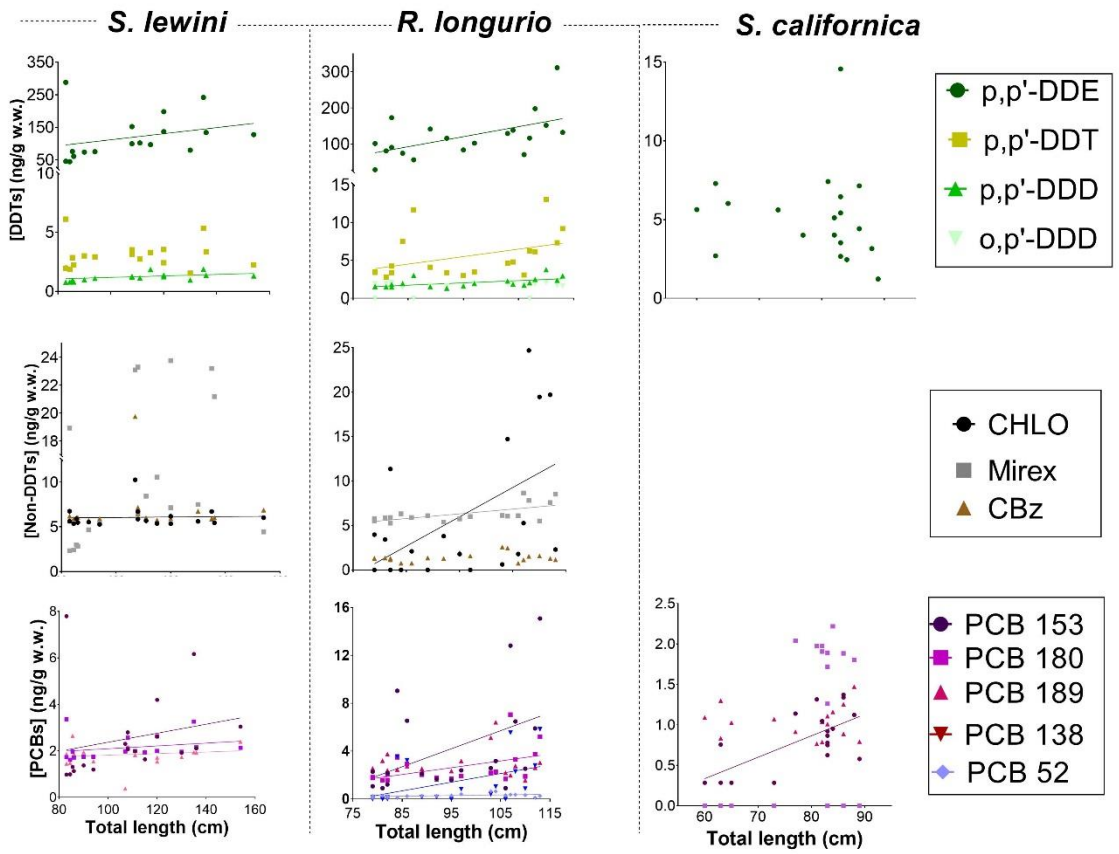


Fig. S3.2. Relationships between total lenght (cm) and liver concentrations (ng/g w.w.) of the different compounds analyzed in scalloped hammerheads *Sphyrna lewini*, Pacific sharpnose shark *Rhizoprionodon longurio* and Pacific angel shark *Squatina californica*. Lines indicate statistically significant correlation between variables.

ANNEX 3

Supplementary Material

Table S4.1

Limits of detection (LOD) in ng/g w.w. of OCPs and PCBs.

OCs	**LOD (ng/g w.w.)**
OCPs	
p,p'-DDT	0.21
o,p'-DDT	0.43
p,p'-DDE	0.28
o,p'-DDE	0.43
p,p'-DDD	0.15
o,p'-DDD	0.26
Heptachlor	0.20
Heptachlor epoxide	0.48
cis-chlordane	0.65
trans-chlordane	0.44
cis-nonachlor	3.45
trans-nonachlor	0.47
Pentachlorobenzene	0.45
Hexachlorobenzene	0.43
Mirex	2.09
HCBD	0.63
Endrin	0.40
Dieldrin	0.32
α-HCH	0.44
γ-HCH	0.43
α-Endosulfan	0.67
Endosulfan sulphate	0.28
Methoxichlor	0.09
PCBs	
PCB-28	0.31
PCB-52	0.43
PCB-81	0.30
PCB-77	0.6
PCB-101	0.24
PCB-123	0.74
PCB-118	0.42
PCB-114	0.25
PCB-105	0.14
PCB-126	0.73
PCB-153	0.30
PCB-138	0.33
PCB-167	0.33

PCB-156	0.34
PCB-157	0.41
PCB-169	0.26
PCB-180	0.22
PCB-189	0.28

Table S4.2

Concentrations of Hg in muscle of *Sphyrna lewini*, *Rhizoprionodon longurio* and *Squatina californica* from different locations of the Gulf of California. "b.d.l." = below detection limit.

Species	n	Hg concentration (ng/g w.w.)	GC location	References
Sphyrna lewini	40	630 ± 40 (mean ± SE)	Southeastern	Bergés-Tiznado et al. (2015)
	12	50 - 1490 (min - max)	Eastern	Hurtado-Banda et al. (2012)
	1	1200	Southeastern	Ruelas-Inzunza et al. (2005)
	22	b.d.l. - 3540	Various	García-Hernández et al. (2007)
Rhizoprionodon longurio	15	539 - 2330 (min - max)	Southeastern	Frías-Espericueta et al. (2019)
	26	104 - 3360 (min - max)	Eastern	Hurtado-Banda et al. (2012)
Squatina californica	94	240 ± 270 (mean ± SE)	Southwestern	Escobar-Sánchez et al. (2016)