



Heavy metal and organic compound bioaccumulation in bronze whaler sharks (*Carcharhinus brachyurus*) along the coastline of South Africa

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Presented in fulfilment of the requirements for the degree of
MASTER OF SCIENCE (MSc) IN BIOLOGICAL SCIENCES
by full dissertation

June 2023

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Acknowledgements

Alhamdulillah robil alamin! I express my gratitude to Almighty God for guiding me through this remarkable journey of my life. I extend my sincere appreciation to the MasterCard Foundation Scholars Program for providing me with the opportunity and funding to achieve this goal. Over the past two years, I have had the privilege of working with lovely and exceptional individuals who have positively impacted my Masters thesis in various ways. It is my pleasure to express my gratitude to you all.

Firstly, I would like to express my deepest gratitude to my supervisor, Prof. Justin O'Riain. Thank you for accepting to supervise me and for providing me with the freedom to conduct my research work. Your unwavering support, constructive feedback, and enthusiasm throughout the various phases of my study were instrumental in its success. To my co-supervisor, Dr Vincent Naude, I am truly grateful for your invaluable assistance. You have been more than just a "co-supervisor" to me, providing motivation and guidance from the project's conception to its completion. I really appreciate your help.

Acquiring shark samples for my research proved to be one of the most challenging aspects of the project, and I would like to thank the following individuals and institutions who came through with that:

- Dr. Charlene Da Silva, thank you for assisting me in obtaining samples and offering me an internship where I could gain practical marine knowledge and research experience.
- Dr. Matt Dicken and Ms. Kristina Naidoo from the KwaZulu Natal Sharks Board (KZNSB) for their support in acquiring samples from the East Coast of South Africa, enabling me to conduct a comparative analysis. Thank you to the latter for assisting in sample preparation and shipping.
- Sarah Waries, Toby Rogers, and the entire SharkSpotters team for providing samples, facilitating my engagement in shark and coastal education programs, and connecting me to helpful individuals.
- Dr. Greg Hofmeyr, thank you for providing samples for my research.

My iCWild family, particularly Zoë Woodgate, deserves a special mention for their support with data analysis and for providing a vibrant, secure, and enjoyable work environment. Thank you!

To my fiancée, Sherifah, I am grateful for your love, prayers, and emotional support, which have been invaluable to me. May we remain together forever.

I express my heartfelt appreciation to my parents, Mr. and Mrs. Adebowale, for their unwavering love, prayers, and support throughout my academic journey, not just over the past two years. I cannot thank you enough.

Finally, to my siblings and all my friends who have supported and cheered me on throughout my academic journey, I love you all. You are all amazing.

Abstract

Anthropogenic activities may release harmful contaminants into the environment which are subsequently ingested and gradually bioaccumulated up the food-web. As apex predators, sharks are prone to heavy metal and persistent organic pollution, being especially vulnerable to such exposure over long lifespans, making these species indicators of systemic pollution in marine ecosystems. As tons of shark meat is harvested annually for consumption, the risk of human exposure to these harmful bioaccumulated pollutants cannot be overemphasized. In this study, we examined heavy metal and persistent organic pollutant concentrations in the muscle tissue of 41 bronze whaler sharks (*Carcharhinus brachyurus*) sampled along the southern and eastern regions of the South African coastline. The concentrations of 10 heavy metals (Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Pb, and Zn) and 8 congeners of polychlorinated biphenyls (PCB 28, 52, 101, 118, 138, 153, 180, 194) were analysed with inductively coupled plasma optical emission (ICP-OES) and gas chromatography coupled with low-resolution mass spectrometry (GC-LRMS), respectively. Average concentrations of mercury (2.53 ± 0.44 mg/kg), arsenic (16.60 ± 1.38 mg/kg) and chromium (0.31 ± 0.07 mg/kg) exceeded the World Health Organisation and other internationally recognised regulatory maximum limits for human consumption, while lead (0.14 ± 0.09 mg/kg) and zinc (13.70 ± 1.74 mg/kg) was close to the permissible limit. Aluminium, cadmium, copper, iron, manganese, and zinc were well below these regulatory limits, including those set by the Department of Health in South Africa and all PCB congener concentrations were below detectable limits. There were no significant differences in heavy metal concentration between sexes, except for chromium which was significantly higher in male sharks. We found that heavy metal concentrations varied significantly with shark size and sampling region. Mercury, chromium, and iron concentrations correlated positively and significantly (Hg: $r = 0.78$; Cr: $r = 0.60$; Fe: $r = 0.47$) with shark size (i.e., total length and body weight) while manganese had a strong negative correlation ($r = -0.42$). Cadmium, chromium, iron, and mercury concentrations were significantly higher in both adult (>230 cm) and sub-adults (130–230 cm) than in juvenile sharks (<130 cm) while manganese and aluminium concentrations were significantly higher in juvenile sharks. Mercury, iron, cadmium, and chromium concentrations were significantly higher in sharks sampled on the eastern coast while aluminium and manganese were higher in sharks from the southern coast of South Africa. Significantly positive and negative correlations were also found between heavy metals, suggesting underlying and systemic interactions between these pollutants. Our results underscore the ecological threat of heavy metal pollution along the South African coastline and the potential toxicity of consuming such shark meat from small-scale fisheries (i.e., high levels of mercury, arsenic and chromium toxicity have lethal effects). Potential sources of these heavy metal and organic pollutants include improper sewage treatment, dysfunctional wastewater treatment plants, and mining activities both inland and along South African coastline. Building on these study findings alongside existing literature and international policy, we suggest several recommendations to reduce such pollution and promote shark health and conservation in South Africa. Furthermore, detailed guidelines on safe shark meat consumption and more stringent environmental policies around waste-water management should be considered by the Departments of Health and Forestry, Fisheries, and the Environment in South Africa.

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A mature female bronze whaler shark showing the distinctive large and pointed pectoral fins, 1st and 2nd spineless dorsal fins, long and pointed snout, round and moderately large eyes, five pairs of fairly long gill slits, and the long heterocercal tail with a grey to bronze-coloured on the dorsal side and white on the ventral side.

Introduction

Pollution in aquatic systems

Heavy metal and organic compound contamination of the environment may have cascading negative effects on biodiversity and ecological functionality, as well as human health and livelihoods (Jitar *et al.*, 2015). Since the industrial revolution (1760–1840), global anthropogenic activities such as agriculture, mining, and both chemical and industrial development have been increasingly recognised as a primary source of pollution in all aquatic environments (Görür *et al.*, 2012; Meyer & Verbruggen, 2012; Daffonchio *et al.*, 2013). Such pollution, often in the form of industrial or agricultural wastewater that is discharged into the environment with little or no treatment, negatively impacts both freshwater and marine ecosystems and threatens the organisms that are reliant on these resources (Zahran & Willis, 2003).

Heavy metal pollution

Metal pollutants are created through chemical and agricultural industry, where poor product (e.g., spillage and inadequate recycling) and waste management (e.g., landfill, agricultural run-off and sewage dumping) lead to significant quantities of metals being released into the environment (Santos *et al.*, 2005; Yang *et al.*, 2012). Potentially toxic elements, though some are essential to life, such as arsenic (As), cadmium (Cd), lead (Pb), manganese (Mn), mercury (Hg), zinc (Zn), chromium (Cr), and copper (Cu) can be found in polluted run-off water which ultimately flows to the ocean (Field *et al.*, 1976). Heavy metals are highly insoluble in water and tend to settle in the substrate (Yang *et al.*, 2014), where they are absorbed and bioaccumulated throughout the aquatic food chain (Alrabie *et al.*, 2019).

Persistent organic pollution

Human activities are also the primary source of many organic compound pollutants (Rhind, 2009) which can be toxic, persistent and whose chemical structure is highly stable over extended periods and can travel great distances in both air and water (Walker, 2009). Organic pollutants can occur as manufactured chemicals in pesticides, electronic devices, dyes, plastics or as by-products from chemical and industrial manufacturing processes, such as incomplete combustion (Lee, 2010; USEPA, 2012). Among the most disconcerting pollutants are persistent organic pollutants (POPs), which have bioaccumulative properties, and include several first-generation organochlorines such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT; Carey, 1998). PCB has up to 209 known congeners or 'chemical compounds' which bioaccumulate and can be deleterious even at low concentrations (Carey, 1998). Large quantities of DDT waste can still be found in poorly managed terrestrial stockpiles and aquatic dumpsites throughout Africa (Ikem *et al.*, 2002; Osibanjo *et al.*, 2002), including South Africa (Whyllie *et al.*, 2003), contributing to pollution and posing a serious threat to the aquatic environment and both human and animal health (Jitar *et al.*, 2015).

Pollutant distribution and persistence

While the production of many of these hazardous chemicals has recently ceased in most countries, their remnants may still be detected in the environment (Whyllie *et al.*, 2003). Heavy metals and POPs

are both ubiquitous, as they are effectively transported over great distances by air currents (Buehler *et al.*, 2004), drainage channels, rivers, floodwaters (Rawn *et al.*, 1999; Buehler *et al.*, 2004), ocean currents (Bidleman *et al.*, 1995) and migrating animals (Lötter & Bouwman, 1997; Wahlström, 2003). Thus, heavy metals and POPs are often transported to areas where they have never been utilised or are not generally tested for (Ballschmiter *et al.*, 2002) and have been detected in multiple sources across ecosystems from snowballs (Risebrough *et al.*, 1976; Tanabe *et al.*, 1983) to penguins (Schneider *et al.*, 1985; Luke & Johnstone, 1989). By channeling industrial or agricultural wastewater into hydrodynamic features (e.g., drainage channels, rivers, and lakes), these pollutants ultimately accumulate in marine coastal waters and subsequently in the tissues of exposed species (EMA, 1998). In addition to being hydrophobic, and highly persistent, these compounds are often found in fatty tissues (lipophilic) of animals and even humans, where their concentrations become biomagnified, contributing to higher levels of contaminant throughout the global food-web (UNEP, 2008; Fitzgerald & Wikoff, 2014).

The delayed chemical and metabolic decay of heavy metal and synthetic organochlorine pollution combined with the general inability of animals to metabolise or excrete such pollutants from muscular organs and fatty tissues poses a health risk to wildlife and humans (Masindi & Muedi, 2018; Nicolopoulou-Stamati *et al.*, 2016; Balabanova, 2021; Table S1). For instance, an assessment of pollution in the river fish of the Choco region in Western Colombia, which is globally known for its rich biodiversity, found that mercury levels in all sampled fish musculature exceeded the World Health Organisation (WHO) limits of 0.05 µg/g (Palacios-Torres *et al.*, 2018). Similarly, organophosphate pesticide pollution (OPP) of an aquatic body in Bangladesh, exceeded concentrations of 9.1 chlorpyrifos/L in water and 51 g of diazinone/kg (dry-weight) in sediments, indicating both acute and chronic OPP threats to freshwater species as evaluated through Risk Quotient (RQ) models (Sumon *et al.*, 2018). A study in West Bengal, northeast India also recorded high levels of accumulated organochlorines, such as DDT and HCH (i.e., Hexachlorocyclohexane) with concentrations ranging from 0.038 to 58 ng/g, and PCBs, which are toxic to aquatic species, ranging from 0.032 to 1.4 ng/g (Guzzella *et al.*, 2005).

The physiological and behavioural costs of such bioaccumulation have become increasingly well-studied, and the findings are concerning (Table S1). For example, Nwani *et al.*, (2013), observed disruptions in coordinated behaviour which included erratic swimming, decreased opercular movement, and excessive mucus secretion in the body and gills of African catfish (*Clarias gariepinus*) after exposure to POPs. While in marine species, such as the common bottlenose dolphin (*Tursiops truncatus*), organochlorines have also been linked to reproductive and immunological abnormalities (Schwacke *et al.*, 2012). The human body also reacts negatively to such toxic substances. In addition to causing mental disorders, long-term heavy metal and POP exposure can damage blood constituents and impair the lungs (Jaishankar *et al.*, 2014), while mercury, lead or cadmium can damage nucleic acids, driving mutations and mimicking hormones that alter the endocrine and reproductive systems which in extreme cases results in cancerous growths (Jarup, 2003).

Bioaccumulation in apex predators

Heavy metal and POP pollution is common in marine ecosystems, with organisms at higher trophic levels in these food-webs being most severely impacted by toxic levels of bioaccumulation

(Compagno, 1990). Toxic effluent settles on the ocean floor and is ingested by primary consumers such as zooplankton (Dinoflagellate spp.); however, due to delayed chemical and metabolic decay or the inability to metabolise or excrete such pollutants from muscular organs and fatty tissues, these pollutants accumulate in the secondary trophic level species that feed on them. Secondary trophic levels such as krill (*Euphausiacea* spp.), are then consumed by tertiary trophic level species such as salmon (*Salmonidae* spp.) or tuna (*Thunnini* spp.), which are ultimately consumed by apex predators (e.g., large sharks, billfish, dolphins, toothed whales, and large seals). These pollutants accumulate and become more concentrated as they move up this trophic ladder, making predatory apex species particularly effective indicators of pollution for entire marine ecosystems (Cortés, 1999). Such detrimental effects of heavy metal and POP pollution are often amplified at higher trophic levels due to the bioaccumulated trophic chain height and volume of prey species harvested by apex predators (Ali & Khan, 2018; Strid *et al.*, 2007; Mansour, 2009) and humans (Jaishankar *et al.*, 2014).

Sharks as apex predators

Sharks are cartilaginous fish that typically reside in coastal and estuarine waters and are among the top predators in most marine environments (Cortés, 1999; White, 2012). Examples include tiger (*Galeocerdo cuvier*; Endo *et al.*, 2008), great white (*Carcharodon carcharias*; Mull *et al.*, 2010), thresher (*Alopias vulpinus*; Suk *et al.*, 2009), hammerhead (*Sphyrna* spp.; Escobar-Sánchez *et al.*, 2010), six gill (*Hexanchus* spp.; Hornung *et al.*, 1993), sleeper (*Somniosus* spp.; Hornung *et al.*, 1993) and requiem sharks, such as spinner (*Carcharhinus brevipinna*; Sumpton *et al.*, 2010), big nose (*Carcharhinus altimus*; Turan *et al.*, 2020), pigeye (*Carcharhinus amboinensis*; Knip *et al.*, 2011), blacktip (*Carcharhinus limbatus*; Powell & Powell, 2001), dusky (*Carcharhinus obscurus*; Benavides *et al.*, 2011), bull (*Carcharhinus leucas*; Maljkovic, 2011) and bronze whaler sharks (*Carcharhinus braychurus*; O'Connell, 2007; Benavides, 2011). Most sharks are opportunistic predators, feeding on a variety of marine species ranging from crustaceans and cephalopods to fish (Baremore *et al.*, 2010), other sharks (Ebert, 1986) and occasionally large marine mammals depending on seasonal and local opportunity (e.g., whales Stevens, 1973; dolphins Stillwell & Kohler, 2011; seals Wetherbee *et al.*, 2012). Larger pelagic fish species, such as mackerel and tuna, have also been discovered in shark stomach contents (Lopez *et al.*, 2010, Klarian *et al.*, 2018). In contrast, reports have also claimed that Ganges sharks (*Glyphis gangeticus*) feed heavily on rays (*Dasyatidae* spp.) in the Bay of Bengal, such rays are primarily bottom-dwelling (Roberts, 2006). By opportunistically feeding on a vast array of species (including on other sharks), and at different trophic levels and throughout the water column, these apex predators bioaccumulate the net effect of pollution exposure throughout many marine ecosystems (Kibria & Haroon, 2015).

Sharks and pollution

Sharks present some the highest known concentrations of toxic contaminants in their tissues as they feed on demersal species that graze on pollution-adsorbing sediments, and on other polluted fish at lower trophic level that in turn may feed on bottom-dwelling organisms (Ross, 2000; Kelly *et al.*, 2007). Additionally, shark anatomy and physiology allow for large volumes of water to flow over their highly vascularised respiratory membranes, increasing the likelihood of absorbing toxic substances (Weijs *et al.*, 2015). Sharks also have relatively long lifespans (20–100 years, depending on the

species) and slow growth rates allowing the accumulation of toxins over time (Storelli *et al.*, 2003; 2005). Even in unpolluted areas, sharks commonly present with high concentrations of heavy metals (Serrano *et al.*, 2000; Strid *et al.*, 2007; Mársico *et al.*, 2007; e.g., mercury, cadmium and lead) and micropollutants (e.g., POPs) compared to other aquatic organisms including mammals (Pethybridge *et al.*, 2010; Barrera-Garcia *et al.*, 2012). Their wide global range and ecological significance, make sharks an ideal sentinel species for the assessment of pollution in the marine environment (Marcovecchio *et al.*, 1991; Vas, 1991) and the negative impacts of such pollution on humans who consume contaminated fish (Serrano *et al.*, 2000; Strid *et al.*, 2007).

Humans, as global 'apex predators', also disproportionately bioaccumulate toxic compounds via numerous metabolic processes (Engwa *et al.*, 2018). Humans may be directly exposed to heavy metals and POPs by consuming contaminated food or water, which may be transferred to the placenta and foetus during pregnancy (Ming-Ho, 2005). These toxins have detrimental effects when they bind to nucleic acids and proteins in the body, damaging their macromolecular structure and altering biological activities, often with detrimental effects, such as those affecting development, respiration and reproduction (Jaishankar *et al.*, 2014; Mansour, 2009).

Monitoring marine pollution

While heavy metal and POP contamination is well studied in many fish species, such research is comparatively rare for marine mammals and cartilaginous fish such as rays and sharks (Schlenk *et al.*, 2005; Gelsleichter *et al.*, 2005; Silva *et al.*, 2009). Most marine pollution impact studies involving sharks have been derived from the by-catch of commercial fishing activities, and therefore have included a higher proportion of smaller, lesser-known species such as the spiny dogfish, *Squalus acanthias* (De Boeck *et al.*, 2001; Eyckmans *et al.*, 2013). Larger (> 5m) species are dangerous to handle once caught (Poisson *et al.*, 2021) and many large shark species are formally protected and therefore less likely to be declared when caught by commercial fishermen (Lack & Sant, 2008). Longline techniques typically capture pelagic species and thus, inshore species are less likely to be available for opportunistic research (Gilman *et al.*, 2008; Pethybridge *et al.*, 2011; Alves *et al.*, 2016; Córdova-Zavaleta *et al.*, 2018). Artisanal fishers also occasionally catch sharks including bronze whaler sharks in the Ain El-Ghazala lagoon on the Mediterranean (Rafi & El-Mor, 2015) and by 'trek' fishermen who use nets along the inshore region of False Bay in Cape Town (Lamberth *et al.*, 1995). Valuable shark samples for research can also be provided as bycatch for shark bite mitigation measures, such as in the KwaZulu-Natal Province of South Africa, where great white and bronze whaler sharks are captured in large-mesh gillnets which are used to kill large sharks and so reduce the probability of shark attacks at recreational beaches (Cliff *et al.*, 1989). Alternatively, researchers may fish for targeted shark species. For instance, whale sharks (*Rhincodon typus*) are caught in the inshore waters of La Paz Bay, California, where they are tagged with Global Positioning System (GPS) devices and photographed for individual identification, sexed, measured and muscle biopsied for biomarker analysis (Ramírez-Macías *et al.*, 2007; 2012; Fossi *et al.*, 2017). These shark tissue samples, derived from various sources, may then be used for a variety of research purposes (e.g., isotopic, and genetic research) including tests for heavy metal and POP contamination.

Measuring heavy metal toxicity

Heavy metal concentrations (including arsenic, cadmium, lead, manganese, mercury, zinc, chromium and copper) can be measured in the tissues of animals using a variety of techniques, following the principles of Atomic Absorption Spectroscopy (AAS), Inductively Coupled Plasma – Optical Emission Spectroscopy (ICP-OES) and other electroanalytical procedures (Helaluddin *et al.*, 2016). AAS quantitatively measures the concentration of elements present in a liquified sample of known weight and volume, following the principle that when energised to the gas phase, elements absorb light at very specific wavelengths which gives the technique excellent specificity and detection limits (Beaty & Kerber, 1993). Similarly, the ICP-OES principle measures the amount of emitted light at each wavelength and uses this information to calculate the concentration of each heavy metal in a sample using an established element-specific calibration curve (Brown & Milton, 2005).

For example, the concentrations of heavy metals were assessed in fish from the Kelantan River, Malaysia (Hashim *et al.*, 2014), by analysing dorsal muscle tissue in a graphite furnace Atomic Absorption Spectrometer (AAS, Analyst 800, Perkin Elmer, Massachusetts, USA; Perkin, 1996). Concentrations of cadmium, nickel and lead were determined through AAS (using high-purity argon), where samples were spiked (in triplicate) with varying known concentrations of these heavy metals to establish a recovery-repeatability test and calibration curve to verify these analytical methods for unknown samples. AAS results for cadmium, nickel and lead concentrations were then expressed as $\mu\text{g/g}$ dry weight and converted to mg/kg for published comparability (Hashim *et al.*, 2014). Similarly, heavy metal (cadmium, chromium, zinc, lead and copper) contamination in the gill, liver, and muscle tissues of Cyprinids (*Barbus sharpeyi* and *B. xanthopterus*) was determined in the Tigris River of Iraq. Again, heavy metal concentration was assessed using AAS (Perkin-Elmer model 5000, Perkin-Elmer Corp, Waltham, MA, USA; Hashim *et al.*, 2014). The accuracy and precision of the AAS were verified using standard reference materials (MA-A-2/TM) provided by the International Atomic Energy Agency (IAEA; Zschunke, 2013). The absorption wavelengths and detection limits for these heavy metals were determined as 228.8 nm and 0.002 ppm for cadmium; 425.4 nm and 0.002 ppm for chromium; 217.0 nm and 0.001 ppm for lead; 213.9 nm and 0.001 ppm for zinc; and 324.7 nm and 0.02 ppm for copper (Mensoor & Said, 2018). Heavy metal contamination (i.e., aluminium, arsenic, cadmium, copper, iron, lead, and zinc) has also been assessed in tilapia (*Oreochromis placidus*) from lake Taman Mutiara, Malaysia, where muscle tissues were analysed by ICP-OES. Finally, heavy metal contamination (i.e., zinc, copper, lead, and cadmium) has also been analysed in *G. gangeticus* muscle tissues using Inductively Coupled Plasma – Mass Spectrometry (ICP-MS; Table S1).

Measuring POP toxicity

POPs such as PCB, DDT, HCH, PBDE (polybrominated diphenyl ethers), HCB (hydrochlorobenzene) and their various congeners can be analysed using Gas Chromatography–Mass Spectrometry (GC-MS). Gas Chromatography (GC) separates chemical components such as organic molecules in the gas phase while Mass Spectrometry (MS) detects and quantifies these components by recording the relative light spectrum of each chemical component within a sample. POPs are classified into three groups; 1) industrial chemical compounds such as PCBs, 2) by-products of industrial processes or

those resulting from improper disposals, such as polychlorinated dibenzodioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), HCB and emerging POPs such as PBDEs; and 3) chemicals used as pesticides and agriculture such as aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, mirex, and toxaphene (Bowman, 2004). POPs generally exist as families of congeners, meaning that each is an individual member of a POP chemical family based on the same carbon skeleton but differing by the number of substituents, such as 1-chloronaphthalene, 1,4-dichloronaphthalene, 1,3,8-trichloronaphthalene, which are all congeners of Polychlorinated Naphthalene (PCN). The number of congeners in a typical congeneric family, created by substitution with only one type of substituent (i.e., chlorine), usually reaches a hundred or more. For such families of congeners, numbers of possible congeners are known and are published in recognised literature or as online resources. For instance, there are 135 congeners of PCDFs, 209 congeners of PCBs and 209 congeners of PBDEs. (Haraczyk *et al.*, 2010). The choice of congeners to be analysed is justified by their 1) relative toxicity, 2) prevalence in the existing literature and 3) rank in the Stockholm Convention on Persistent Organic Pollutants (SCPOP; Lakhmanov *et al.*, 2020). Prior to analysis, POPs may be isolated from a wide range of environmental sources, including soils, animal tissues, water, or other samples using different extraction techniques such as the Accelerated-Solvent Extraction (ASE), Microwave-Assisted Extraction (MAE), Liquid-Liquid Extraction (LLE), Solid-Phase Extraction (SPE) and the Soxhlet Extraction Technique (SET), which is one of the most widely used liquid-solid extraction procedures (Hess *et al.*, 1995; Castro & Garcia-Ayuso, 1998). Each extraction method has various merits and limitations (Muir & Sverko, 2006; LeDoux, 2011; Asensio-Ramos *et al.*, 2012).

Practical and published examples of POP analysis

The concentrations of HCB and different congeners of DDT, PCB, and PBDE were tested in whale shark muscle tissue collected in La Paz Bay (CA), USA (Fossi *et al.*, 2017). Gas Chromatography linked Low-Resolution Mass Spectrometry (GC-LRMS) was used to quantify HCB, DDT, PCB, and PBDE congener contamination expressed in $\text{nm} \pm \text{SD}$ converted to ppm by volume (Das *et al.*, 2015). Using similar extraction techniques multiple POP (PCBs, PBDEs, DDXs, HCB, CHLs) and MeO-PBDEs (methoxylated polybrominated diphenyl ethers) classes were investigated in the liver tissues of Atlantic stingrays (*Dasyatis sabina*), as well as bonnethead (*Sphyrna tiburo*), lemon (*Negaprion brevirostris*) and bull sharks collected from the south-eastern coastline of the USA (Weijjs *et al.*, 2015). PBDE, MeO-PBDE and CHL contamination was measured by GC-ECNIMS (Gas Chromatography linked Electron Capture Negative Ion Mass Spectrometry). PCBs and organochlorine pesticides such as DDT and HCH have also been measured in wild-caught fish by the Food and Agriculture Organisation (FAO) Major Fishing Areas (MFA 37.1.1) in the western Mediterranean Sea (Panseri *et al.*, 2019). POP contamination (i.e., PCBs, HCBs, and DDTs) has also been investigated in Northern pike (*Esox ucius*) and Salmon species including pink salmon (*Oncorhynchus gorbuscha*), Arctic char (*Salvelinus alpinus*), humpback whitefish (*Coregonus pidschian*) and Navaga (*Eleginus nawaga*) in the Russian Arctic (Lakhmanov *et al.*, 2020; Table S1).

These examples would suggest that heavy metal and POP contamination in marine environments are relatively well documented. This is however an emerging field and the downstream effects of such contamination, detected at higher trophic levels with ecosystem-wide ramifications including human consumption, remain poorly understood. Researchers and environmental monitors have

recorded elevated levels of metal toxicity in coastal fish including Atlantic salmon (*Salmo salar*), Japanese medaka (*Oryzias latipes*) and European flounder (*Platichthys floesus*; Nirmala *et al.*, 1999; Vigano *et al.*, 2001; Muirhead *et al.*, 2006; Lerner *et al.*, 2007; Nakayama & Oshima, 2008) which was attributed to sewage, factory and stormwater discharges, many of which exceed WHO/FAO permissible limits and European Economic Community limits (Chan, 1995). Further studies have documented the detrimental effects of organochlorine contaminants on reproduction and development in marine fish species (Hose, 1989; Fry, 1995; Tielmans, 1999; Toft *et al.*, 2004; Kleanthi *et al.*, 2008). Unfortunately, available information regarding the toxic and sub-lethal effects of heavy metal and POP contamination in shark species remains limited. Understanding the long-term impacts of such contamination is essential to the development of continuous monitoring and pollution mitigation strategies across marine ecosystems (Mull *et al.*, 2013).

Heavy metal correlation

Assessing relationships or correlations between heavy metals in fish is crucial because these metals can interact in complex ways, affecting their relative toxicity and impact on ecological health. Some heavy metals, such as zinc and selenium, are essential micronutrients for proper physiological functioning in fish and humans, providing antioxidant defences that minimise the impact of other metals, such as lead and cadmium, which can both cause oxidative stress which can severely damage cells and tissues. Previous studies have identified correlations between various heavy metals in fish, which could be negative, depending on whether the metals compete for binding sites, or positive if they accumulate in tandem, potentially influencing their relative absorbance and physiological retention. For instance, it has been demonstrated that mercury and selenium concentrations in the muscle and liver may be positively correlated, as selenium has a detoxifying effect on mercury (Branco *et al.*, 2007; Carvalho *et al.*, 2005). Similarly, zinc has also been shown to have additive effects on arsenic toxicity associated with impaired neurological function in arsenic-exposed fish (Milton *et al.*, 2004; Zeng *et al.*, 2005; Roy & Bhattacharya, 2006).

Bronze whaler sharks globally

Bronze whaler or copper sharks of the Carcharhinidae family (i.e., whaler sharks) are large (up to 3.25 m in the wild), globally distributed marine predators which play a significant ecological role in most coastal and anti-tropical environments (Garrick 1982; Compagno *et al.*, 2005). After orcas (*Orcinus orca*) and great white sharks, a suite of large (3–5 m) sharks, including bronze whalers, occupy the top trophic levels in many food-webs. Commercially, these sharks are harvested for food (e.g., skin, fins, liver, and oil; Musick, 2005) and medicine for humans (Musick, 2005b; Rao *et al.*, 2000), while their flesh and fins are also used to produce fishmeal for livestock feed (Compagno, 1990). Bronze whaler fins form part of the global shark fin soup trade (both legal and illegal), having featured in many shark fin markets (Clarke *et al.*, 2006; Roy *et al.*, 2015), including the Hong Kong market, where 50 % of worldwide shark fin trade is believed to occur (Fong & Anderson, 2000; Field *et al.*, 2009). Shark derivatives are also consumed for their high concentration of vitamin A, while liver oil is utilised as a lubricant in the tanning and textile industries (Kibria & Haroon, 2015). Bronze whalers are classified among "Vulnerable" species of sharks on the International Union for Conservation of Nature (IUCN) Red List (Huvneers *et al.*, 2020) due to threats of overexploitation, climate change, fisheries bycatch, and pollution exposure (Miller *et al.*, 2014). Bronze whalers, like other apex

predators, are exposed to heavy metal, POP and microplastic pollution throughout their global distribution.

Bronze whaler sharks in South Africa

In South Africa, bronze whaler sharks are caught as part of the commercial fishing industry and are also captured by other non-target fisheries as bycatch, including shark gill-netting in KwaZulu Natal on the east coast of the country. High levels of international demand have resulted in bronze whaler shark meat, liver, oil, and fins being harvested commercially as a by-product of shark gill-netting since the early 19th century in South Africa (Kroese & Sauer 1998; Sauer *et al.*, 2002). Bronze whalers in South Africa are predominantly caught as bycatch by tuna and swordfish pelagic longline fisheries established in the region (Duffy & Gordon, 2003; Ebert *et al.*, 2013; Irigoyen & Trobbiani, 2016) from whence they are exported to Australia and throughout Asia (Prestowitz 1996; Shivji *et al.*, 2002), especially to centres of high demand such as Hong Kong (Duffy & Gordon, 2003; Da Silva & Burgener, 2007). Numerous studies have shown that these and other shark species are directly threatened by unsustainable rates of harvest (Beerkircher *et al.*, 2002; Baum & Myers 2004). This combined with their conservative life history parameters contributes to range-wide population declines (Castro *et al.*, 1999), making them one of 19 shark species threatened by either directed fishing operations or bycatch (IUCN, 2007). Bronze whaler sharks are likely exposed to heavy metal, POP and microplastic pollutants in marine ecosystems adjacent to highly developed and industrialised areas (Sumpter, 2009) with direct links to the marine environment through stormwater runoff and waste-water disposal. Such anthropogenic activities contribute to poor water quality and subsequent risk to key species throughout these marine systems (Bredenhand, 2005; Dabrowski *et al.*, 2000; DWAF, 1995). Harmful contaminants, if left untreated, are released into waste-water drainage systems, which ultimately flow into the aquatic environment where they accumulate in the benthic zone and bioaccumulate in the tissues of aquatic species throughout the food-web (River Health Programme, 2003; Jebali, 2007).

Marine species pollution in South Africa

A recent investigation of systemic pollution along the coastline surrounding the City of Cape Town (CoCT) revealed that minimal water quality standards were not met by over half of the 49 testing locations throughout False Bay (Kretzmann, 2020). Most locations between Muizenberg and Strandfontein and from Monwabisi down to Gordon's Bay were classified as having 'poor' water quality. Most of these locations have continuously failed to meet annually assessed safety levels due to the influx of polluted stormwater from inland sources since 2015. In addition to the highly contaminated runoff from the Cape Flats communities (with limited access to basic sanitation), False Bay is also inundated with effluent from the overburdened Strandfontein, Zandvliet and Gordon's Bay waste-water treatment facilities. Industrial and pharmacological chemicals found in fish landed at Kalk Bay, an iconic fishing village along the False Bay coastline, have further corroborated the extent of this pollution (Kretzmann, 2020). Considering that more than 100,000 tons of shark meat are traded annually for human consumption across the globe, assessing local levels of pollution, such as these, are important to public health and regulatory considerations (Eriksson & Clarke, 2015). There have been relatively frequent assessments of such heavy metal and POP contamination in fish species, but such assessments are comparatively rare for marine mammals and sharks (Gelsleichter

et al., 2005; Schlenk *et al.*, 2005; Silva *et al.*, 2009).

Hypothesis statement

Based on the observed elevated levels of pollutants in other pelagic sharks, such as great white sharks, within the same region, it is hypothesized that muscle tissue from *C. brachyurus* will exhibit high levels of these pollutants. Additionally, considering variations in terrestrial activities, specifically coal-fired power stations, cement production, and artisanal gold mining operations in the North-east versus the South coast of South Africa, it is hypothesized that *C. brachyurus* sampled in the East coast will present higher toxic contaminant levels in their muscular tissues compared to those sampled in the South coast. Furthermore, recognizing the phenomenon of bioaccumulation in aquatic organisms, where fish tend to accumulate more toxic contaminants in their muscular tissues as they age, it is hypothesized that adult *C. brachyurus* will possess higher levels of toxic contaminants than sub-adult and juvenile sharks.

Aims and objectives

Given the detrimental impacts of heavy metal and POP pollution on people and wildlife, it is necessary to assess the level of these contaminants in sentinel species. This study aimed to examine the bioaccumulation concentration of 10 heavy metals and PCBs congeners in the muscular tissue samples of bronze whaler sharks sampled along the southern and eastern regions of the South African coastline. My objectives included understanding whether: 1) there are heavy metal and PCB levels in bronze whaler sharks along the South African coastline that exceed WHO/FAO recommended permissible limits, 2) heavy metal and PCB levels differ between the coastal regions where sharks were sampled and 3) between sharks of different sexes and sizes, 4) there are correlations between metal concentrations and shark biological parameters such as total body length (TBL) and body weight (BW) and, 5) there is a correlation between the bioaccumulated heavy metals, and between heavy metals and PCBs.

Methods

Study area

The study area theoretically encompasses the entirety of bronze whaler shark distribution along the coastline of southern Africa, stretching approximately 3,650 km from central Namibia to Mozambique. The west coast is adjacent to the Atlantic Ocean, which is characterised by the cold, nutrient-rich Benguela upwelling system which transitions to the warmer, subtropical Agulhas system of the Indian Ocean to the East (Figure 1).

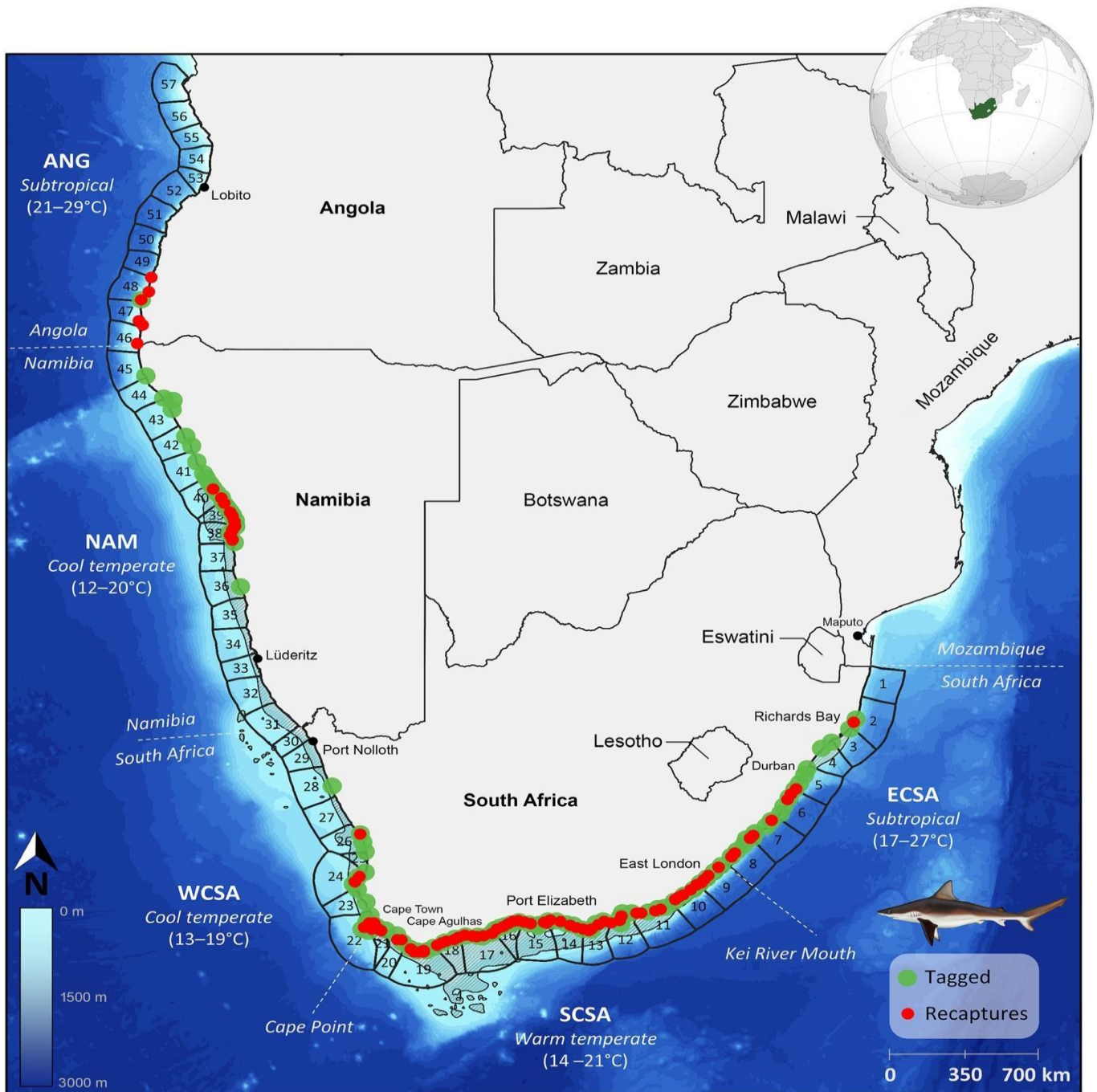


Figure 1. The southern African coastline and neritic zone, indicating known bronze whaler shark range across the four recognised biogeographic regions (from Rogers *et al.*, 2022).

The coastline and neritic zone are further subdivided into four recognised biogeographic regions: the northern cool-temperate region of Namibia (NAM; 1,500 km; 12–20 °C; Potts *et al.*, 2015), the cool-temperate of West Coast of South Africa (WCSA) ranging from the Orange River mouth to Cape Point (900 km; 13–19 °C; Smit *et al.*, 2013), the warm-temperate south coast of South Africa (SCSA) ranging from Cape Point to Great Kei River mouth (1,300 km; 14–21 °C), and the East Coast of South Africa (ECSA) which ranges from the Great Kei River mouth to the Mozambiquan border (850 km; 17–27 °C; Schumann 1998, Sink *et al.*, 2005).

Recent research has proposed that bronze whaler sharks can be broadly categorised into two populations on the southern African coastline. A population moving from the WCSA through the SCSA to the ECSA, and the other ranging between Namibia and southern Angola (Rogers *et al.*, 2022). There is trans-boundary movement between the populations suggesting that they are not geographically discrete (Walter & Ebert 1991; Rogers *et al.*, 2022). During migrations, individual sharks have been recorded to travel up to 1,320 km and they have also been recorded from the surf-zone (5–10 m) to a depth of about 100m (330 ft; Rogers *et al.*, 2022).

Sample collection

Bronze whaler sharks were collected from the tagging and recapture research activities of the Oceanographic Research Institute's Cooperative Fish Tagging Project (ORI-CFTP; Dunlop *et al.*, 2013, Rogers *et al.*, 2022) and through opportunistic strandings or wash-ups in Kwazulu-Natal, from the South to the East coast of South Africa (Figure 2), from December 2021 – August 2022. Samples were collected opportunistically as part of long-term biodiversity management research from natural stranding events or small-scale fisheries quota and bycatch, which did not require institutional ethical approval. The total length and weight of each shark was measured, and shark sex was determined by the presence of claspers. Geo-location data for each sample was recorded (Table S2). Approximately 10 g of muscle tissue was taken from each individual and placed in sterile polythene bags and kept on ice before long-term storage at –15°C. As samples were obtained through a network of collaborators, no particular preference was given to the part of the shark where samples were collected other than from the primary musculature. Samples were later analysed for heavy metal and PCB contamination through accredited commercial laboratories.

Heavy metal concentrations

Heavy metal analyses were conducted through Waterlab Pty. Ltd. (23B De Havilland Crescent, Persequor Techno Park, Pretoria; reports: 105890; 110459; 112440; 113643). The concentration of ten heavy metal pollutants (i.e., aluminium, arsenic, cadmium, chromium, copper, iron, mercury, manganese, lead and zinc) were determined for each sample by acid digestion in a closed vessel device, temperature-controlled microwave heating and inductively coupled plasma optical emission spectrometry (ICP-OES). All glassware used in sample processing was soaked in 10 % nitric acid (HNO₃) for 12 minutes and rinsed thoroughly with deionised water (diH₂O). Freshly frozen samples were dried to 1g (dry mass) at 40°C in an extractor oven, before adding 6ml of HNO₃ (65 %) and 2ml of 30 % hydrogen peroxide (H₂O₂). After allowing the solution to homogenize, the reaction was then heated in a microwave at 200 °C for 15 mins before cooling to room temperature. The detection

limits for each analyte were calculated as three times the standard deviation of the blank measurements based on 1 g sample and were as follows: aluminium [0.100 mg/l], arsenic [0.001 mg/l], cadmium [0.001 mg/l], chromium [0.001 mg/l], copper [0.001 mg/l], iron [0.025 mg/l], mercury [0.001 mg/l], manganese [0.025 mg/l], lead [0.001 mg/l], and zinc [0.001 mg/l]. Quality control measures included the use of duplicate blanks and internationally certified reference material (CRM) (DORM-4 fish protein, National Research Council of Canada) in each analytical run of 20 samples. Recoveries of analytes from DORM-4 fish protein ranged between 91.5 %–103.9 % and showed quantitative agreement with the Certified Values, thus validating the accuracy of the procedure. Final heavy metal concentrations were presented in mg/kg.

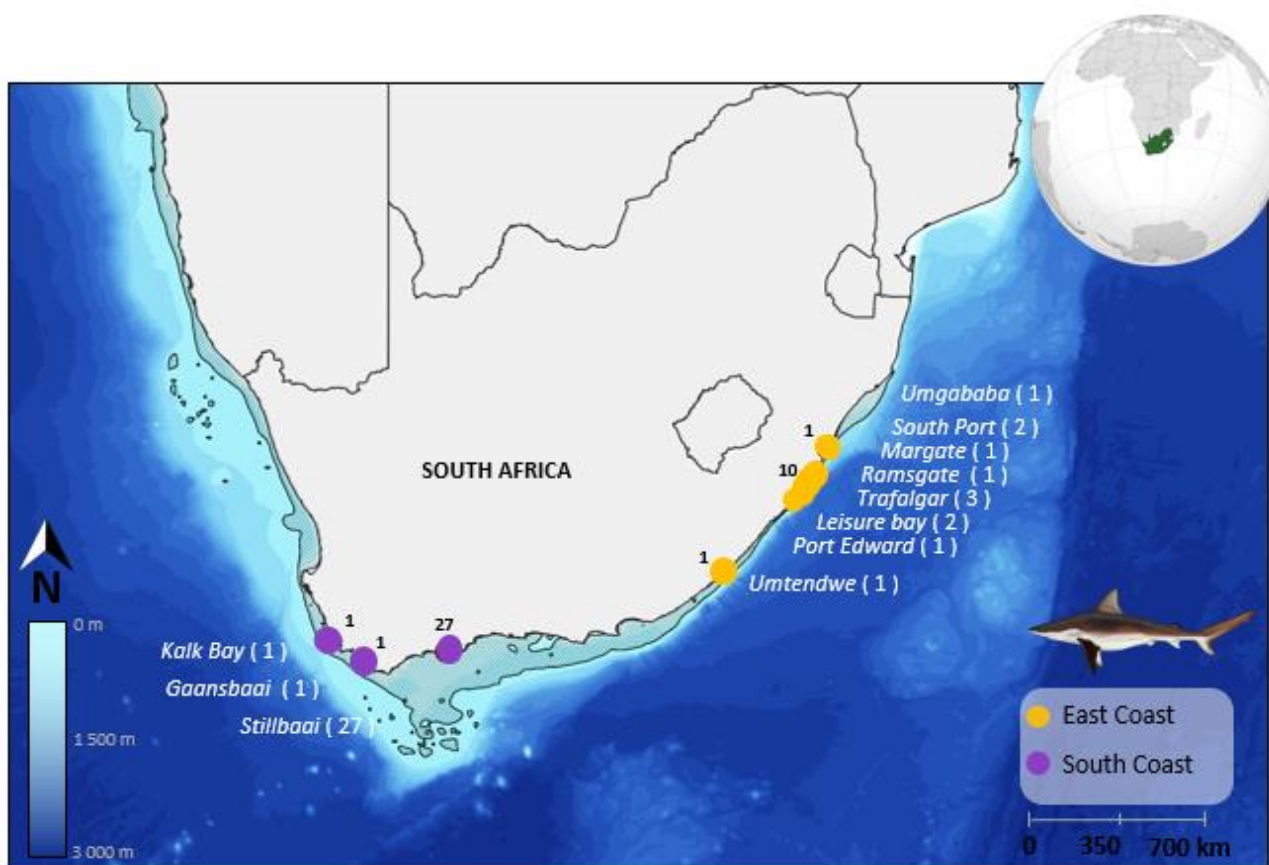


Figure 2. Map showing sample collection sites (filled circles) in two different geographical regions (ECSA– solid yellow circles and SCSA – solid purple circles) along the South African Coastline.

Persistent organic pollutants (POPs)

POP analyses were conducted through the Food and Drug Assurance (FDA) laboratory (Pretoria, South Africa), where eight PCB congeners (i.e., 28, 52, 101, 118, 138, 153, 180, 194) were assessed in all fish muscle samples following Gui *et al.* (2014), to represent the total PCB concentration in the sample. Before chemical analysis, the dried samples were ground down for 10 minutes in an automatic agate mortar and stored in refrigeration pipes at -80 °C. Muscle samples of about 20 g were added with ¹³C-labelled standards (ISs), allowed to rest for 2 hours, homogenised with anhydrous Na₂SO₄, and extracted for nine hours using a Soxhlet apparatus with a 50 % mixture of acetone and n-hexane. Each extract was concentrated to 20 ml using a rotary evaporator; a 2 mL

aliquot was used for lipid content determination by gravimetry. Clean-up was carried out by filtration through Extrelut® impregnated with concentrated sulphuric acid and a silica gel layer (di Domenico *et al.*, 1992). The extract was analysed for PCB 28, 52, 101, 118, 138, 153, 180, 194 and relative quantification was performed by gas chromatography coupled with low-resolution mass spectrometry (GC-LRMS). A procedural blank was run together with three to five samples. Reliable measurements were allowed above the limit of determination of 0.1 ng/g with a repeatability in the order of $|\pm 10\%$ (extended uncertainty, $|\pm 20\%$). The recovery rates of internal standards (ISs) were accepted within 80–120 %.

Statistical analysis

All statistical analyses were performed using R statistical software v4.0. (R Core Team, 2023). Bronze whaler shark samples were grouped by sex (male; female), total body length as a proxy for age class (juveniles < 130 cm; sub-adults 130–230 cm; adults > 230 cm; following Walter & Ebert [1991], Cliff & Dudley [1992], and Lucifora *et al.* [2005]), and coastal region (SCSA; ECSA). All concentration data were checked for homogeneity of variances using the Levene's test and normality using both the Shapiro-Wilk test and the Q-Q plot, where these were not normally distributed, data were log-transformed. Multivariate analyses of variance (MANOVA) were used to determine whether concentrations differed significantly by shark sex, size, and sampling region, with Dunn's post-hoc multiple comparison tests applied among these predictor variables. A boxplot was also performed to make statistical comparison between bronze whalers age classes, sex, and sampling region. Spearman's correlation was then used to examine the relationship between the pollutant concentrations. Heavy metal and PCB contamination levels from this study were then compared to an informal review of published studies and globally recommended permissible limits.

Results

Sampled shark characteristics

A total of 41 bronze whaler sharks ($n_{\text{female}} = 21$; $n_{\text{male}} = 20$) were sampled along the South African coastline ($n_{\text{SCSA}} = 29$; $n_{\text{ECSA}} = 12$) between December 2021 and August 2022 (Table S2). Mean shark total body length and mass were 140.81 ± 71.11 cm (74.4–300) and 31.8 ± 41.89 kg (1.9–133) respectively (Table 1). Shark body length and mass did not differ significantly by sex, but by sampling region ($P < 0.001$), being 2.3x longer and 8.4x heavier on average along the ECSA than the SCSA. In both sampling regions, shark body length and weight showed a significant ($P < 0.001$) positive correlation ($r_{\text{SCSA}} = 0.97$; $r_{\text{ECSA}} = 0.96$), allowing a combined measure of overall shark ‘size’ (Figure 3) and approximate age categorisation (following Walter & Ebert [1991], Cliff & Dudley [1992], and Lucifora *et al.* [2005]) in subsequent analyses.

Table 1. Total body length and mass of bronze whaler sharks by sex and sampling region.

Aspect		\bar{x} (SE)	t (df)	P	95 % C.I.
Total body length (cm)	Overall ($n = 41$)	140.81 \pm 11.11	-	-	-
Sex	Female ($n = 21$)	139.00 \pm 14.77	0.16 (37)	.873	(-49.37, 42.10)
	Male ($n = 20$)	142.70 \pm 17.06			
Sampling region	SCSA ($n = 29$)	102.20 \pm 7.79	12.97 (35)	<0.001	(-152.40, -111.10)
	ECSA ($n = 12$)	234.00 \pm 6.53			
Total body mass (kg)	Overall ($n = 41$)	31.80 \pm 6.54	-	-	-
Sex	Female ($n = 21$)	27.57 \pm 8.11	0.66 (36)	.517	(-35.59, 18.22)
	Male ($n = 20$)	36.25 \pm 10.49			
Sampling region	SCSA ($n = 29$)	10.13 \pm 4.75	9.33 (23)	<0.001	(-90.45, -57.62)
	ECSA ($n = 12$)	84.17 \pm 6.35			

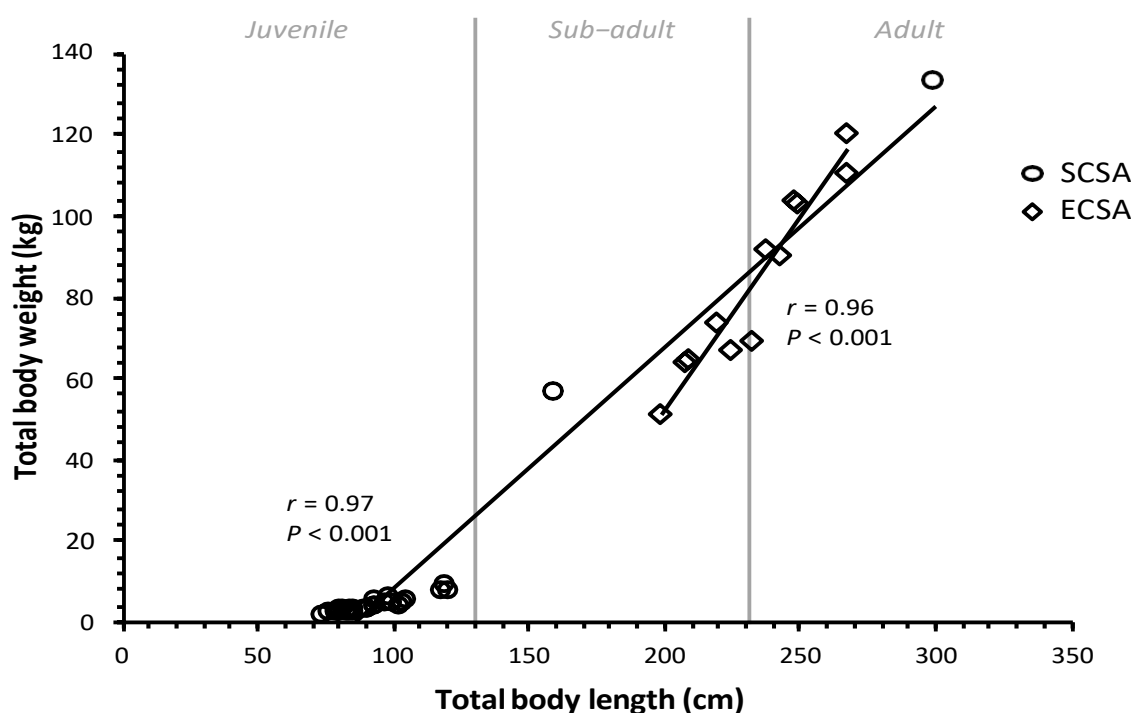


Figure 3. Correlation between bronze whaler shark total body length and mass as a proxy for age class in both the Eastern Coastline of South Africa (ECSA) and the Southern Coastline of South Africa (SCSA).

Table 2. Heavy metal concentrations in bronze whaler sharks sampled at different sites along the South African coastline (see Figure 2) of the South African coastline. Samples are displayed as those that exceed the recommended permissible limit (red cells), those below the limit but above the sample mean (yellow cells), those below the limit considered safe (green cells) and no limit available in the literature (blue cells).

		Heavy metal concentration (mg/kg)									
Regulatory limits		-	3.00	0.05-1.00	0.25	5.00	100.00	0.40-1.00	20	0.20-2.00	25.00
ID	Collection site	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Pb	Zn
1	Stilbaai	11.40	15.92	0.00	0.25	0.83	18.04	1.12	1.24	0.00	6.39
2	Stilbaai	12.02	17.92	0.00	0.16	0.79	14.88	1.02	1.44	0.00	7.47
3	Stilbaai	157.35	47.36	0.00	0.24	0.70	19.11	1.38	1.21	0.00	6.21
4	Stilbaai	14.42	20.02	0.00	0.35	0.90	20.17	1.05	1.47	0.10	7.34
5	Stilbaai	5.57	6.10	0.00	0.09	0.41	9.39	1.91	1.01	0.00	3.99
6	Stilbaai	7.72	13.63	0.00	0.14	0.49	12.52	0.96	1.35	0.00	3.09
7	Stilbaai	26.86	15.24	0.00	0.13	0.46	7.88	1.88	2.19	0.00	4.22
8	Ramsgate	6.26	33.00	0.00	0.77	0.41	11.00	5.24	0.00	0.00	15.00
9	Umgababa	16.00	28.00	0.00	1.03	0.31	21.00	3.02	0.10	0.08	13.00
10	Stilbaai	9.81	11.80	0.00	0.19	0.47	3.57	1.84	1.76	0.00	4.62
11	Kalk bay	10.56	29.21	0.00	0.44	0.93	10.80	3.69	0.89	0.00	3.87
12	Stillbaai	3.94	9.22	0.00	0.06	0.83	1.03	1.41	0.97	0.13	4.88
13	Stilbaai	4.73	13.25	0.00	0.09	0.48	6.95	2.46	1.80	0.00	3.55
14	Leisure bay	14.00	8.40	0.00	0.92	0.50	23.00	5.38	0.43	0.00	13.00
15	Trafalgar	56.00	6.55	0.07	1.22	0.55	69.00	9.51	0.15	0.08	16.00
16	Margate	5.36	12.00	0.24	0.89	0.56	23.00	5.07	0.00	0.07	16.00
17	Port Edward	6.78	8.00	0.00	0.72	0.38	16.00	4.95	0.00	0.00	15.00
18	Umtendwe	8.48	14.40	0.00	2.54	0.52	26.03	4.93	0.52	0.00	11.67
19	Trafalgar	0.00	15.55	0.00	0.14	0.96	1.85	5.20	0.35	0.00	12.31
20	South Port	247.01	10.49	0.07	0.00	0.73	7.01	3.55	0.65	0.05	14.53
21	South Port	2.20	7.18	0.11	0.39	1.09	36.48	13.77	0.72	0.06	18.88
22	Trafalgar	0.00	14.24	0	0.22	0.52	22.75	5.96	0.56	0.05	9.81
23	Gansbaai	85.59	21.30	0.11	0.40	2.12	371.47	4.20	1.70	0.07	29.39
24	Leisure bay	8.47	14.87	0.10	0.08	0.62	15.25	4.96	0.75	0.00	12.97
25	Stillbaai	24.53	13.03	0.00	0.00	0.23	14.13	0.24	0.87	0.00	11.50
26	Stillbaai	37.20	15.67	0.00	0.00	0.48	2.00	0.39	0.76	0.05	13.39
27	Stillbaai	12.63	19.30	0.00	0.00	0.48	2.59	0.63	0.39	0.00	5.94
28	Stillbaai	30.22	11.65	0.00	0.00	0.34	11.93	0.78	0.85	0.08	14.09
29	Stillbaai	11.06	13.27	0.00	0.12	0.13	7.46	0.54	0.48	0.17	8.36
30	Stillbaai	15.01	18.31	0.00	0.00	0.29	2.02	1.02	0.72	0.17	7.74
31	Stillbaai	17.03	15.09	0.00	0.13	1.12	2.09	0.77	1.11	0.16	22.44
32	Stillbaai	238.81	19.30	0.00	0.00	0.65	3.19	0.35	0.76	3.56	12.86
33	Stillbaai	304.42	23.02	0.00	0.00	1.52	0.00	0.36	0.63	0.16	20.15
34	Stillbaai	19.92	17.99	0.00	0.17	0.71	0.00	0.37	0.85	0.25	35.20
35	Stillbaai	42.65	14.46	0.00	0.00	0.67	0.00	0.52	0.54	0.00	11.81
36	Stillbaai	59.67	9.35	0.00	0.08	0.88	0.00	0.41	0.70	0.09	14.35
37	Stillbaai	21.34	44.21	0.00	0.18	0.53	0.00	0.50	0.00	0.00	12.61
38	Stillbaai	20.74	13.65	0.00	0.18	0.75	0.00	0.82	1.03	0.26	70.48
39	Stillbaai	14.71	19.26	0.00	0.12	0.78	0.00	0.72	0.26	0.06	13.96
40	Stillbaai	14.91	12.10	0.00	0.15	0.62	13.15	0.29	0.61	0.07	15.15
41	Stillbaai	21.40	6.99	0.00	0.14	0.62	0.00	0.60	0.82	0.00	18.20
Mean (± SE)		39.67 (10.77)	16.6 (1.38)	0.02 (0.01)	0.31 (0.07)	0.67 (0.05)	20.18 (8.90)	2.53 (0.44)	0.80 (0.08)	0.14 (0.09)	13.70 (1.74)

DOH (Bosch *et al.*, 2016): AS | WHO-FAO (Lopez *et al.*, 2013): Cd, Hg, Pb | JECFA (Kim *et al.*, 2019): Cd, Hg, Pb | KFDA/EU (Lopez *et al.*, 2013): Cd, Hg, Pb | SCF (Turan *et al.*, 2021): Cr, Cu, Fe, Zn | UNEP (Kim *et al.*, 2019): Hg | CODEX (Kim *et al.*, 2019): Pb.

Heavy metal toxicity

Heavy metal concentrations in bronze whaler sharks indicated that arsenic concentrations exceeded recommended permissible limits in all samples (Table 2), being on average, 5.5x higher than the maximum threshold. Mercury concentrations were higher than the recommended permissible limit in 59 % of samples, while all but two of the remaining samples were within potentially dangerous toxicity levels. Chromium concentrations were higher than the permissible limit in 29 % of samples, while 37 % of the remaining samples were within potentially dangerous toxicity levels. All but one sample had iron concentrations below the recommended permissible limit. Zinc concentrations were below the recommended limit in 27 % of samples, with 66 % exceeding potentially dangerous toxicity levels, and 7 % surpassing the recommended permissible limit. 71 % of samples had safe levels of lead concentration, 27 % exhibited potentially dangerous toxicity levels, and only one sample had lead levels higher than the recommended limit. Cadmium concentrations were within potentially dangerous toxicity levels in 15 % of samples while all samples were well below the recommended permissible limits for copper and manganese. While levels of aluminium toxicity varied greatly across samples, an extensive literature review (Table S1) did not indicate the recommended permissible limit for this heavy metal. Aluminium had the highest mean concentration (39.67mg/kg) followed by Iron (20.18 mg/kg), then arsenic (16.60 mg/kg), with cadmium (0.02mg/kg) having the lowest, in the sample population.

Correlation between heavy metals in bronze whaler sharks

The relationships between the concentrations of 10 heavy metals in bronze whaler sharks were analysed (Table 3) revealing both strong positive ($r \geq 0.4$) and strong negative ($r \leq -0.4$) relationships for select metals (Shipley *et al.*, 2021). Strong positive correlations were found between iron and both cadmium ($r = 0.47$) and chromium ($r = 0.63$), between mercury and cadmium ($r = 0.47$), chromium ($r = 0.63$), and iron ($r = 0.64$), and between zinc and both cadmium ($r = 0.40$) and lead ($r = 0.48$). A strong negative correlation was observed between mercury and aluminium ($r = -0.54$). Significant positive correlations were observed also between lead and aluminium ($r = 0.32$), as well as between zinc and both aluminium ($r = 0.35$) and copper ($r = 0.31$). Further significant negative correlations were observed between chromium and aluminium ($r = -0.33$), as well between zinc and manganese ($r = -0.37$).

Table 3. Spearman correlation matrix examining relationships between heavy metal concentrations found in the muscle tissue of bronze whaler sharks ($n = 41$) sampled along the South African coastline. Bold numbers indicate significance while cell colour denotes positive (red) and negative (blue) correlations with darker shading indicating stronger correlation coefficients.

Spearman Correlation	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Pb	Zn
Al	-	0.161	0.968	<0.05	0.395	0.102	<0.001	0.547	<0.05	<0.05
As	0.22	-	0.153	0.805	0.229	0.470	0.215	0.924	0.833	0.484
Cd	0.01	-0.23	-	0.193	0.114	<0.01	<0.01	0.433	0.552	<0.05
Cr	-0.33	0.04	0.21	-	0.623	<0.001	<0.001	0.254	0.295	0.328
Cu	0.14	0.19	0.25	0.08	-	0.450	0.978	0.181	0.162	<0.05
Fe	-0.26	-0.12	0.47	0.63	-0.12	-	<0.001	0.945	0.232	0.749
Hg	-0.54	-0.20	0.49	0.62	0.00	0.64	-	0.320	0.055	0.781
Mn	0.10	0.02	-0.13	-0.18	0.21	-0.01	-0.16	-	0.841	<0.05
Pb	0.32	0.03	0.10	-0.17	0.22	-0.19	-0.30	-0.03	-	<0.01
Zn	0.35	-0.11	0.40	0.16	0.31	-0.05	-0.04	-0.37	0.48	-

POP toxicity

The concentrations of eight PCB congeners (i.e., 28, 52, 101, 118, 138, 153, 180, and 194) were analysed in the first batch of bronze whaler shark samples collected ($n_{\text{female}} = 7$; $n_{\text{male}} = 4$; $n_{\text{SCSA}} = 9$; $n_{\text{ECSA}} = 2$). PCB concentrations were not detectable at the 20 $\mu\text{g}/\text{kg}$ detection limit in any of these samples (Table S2), and given the prohibitive cost of these analyses, no further samples were assayed for organic pollutants.

Toxicity by shark age, sex, and sampling region

Heavy metal concentrations varied significantly by shark age ($F_{56} = 5.87$, $P < 0.001$) and sampling region ($F_{27} = 4.69$, $P < 0.001$) (Table 4). Significant age-based differences in toxicity level were evident for aluminium ($H_2 = 6.27$, $P < 0.05$), cadmium ($H_2 = 13.80$, $P < 0.01$), chromium ($H_2 = 14.31$, $P < 0.001$), iron ($H_2 = 16.12$, $P < 0.001$), mercury ($H_2 = 27.00$, $P < 0.001$), and manganese ($H_2 = 8.86$, $P < 0.05$). Only chromium concentrations differed significantly ($H_1 = 7.44$, $P < 0.001$) by sex. While aluminium ($H_1 = 5.52$, $P < 0.05$), cadmium ($H_1 = 9.32$, $P < 0.01$), chromium ($H_1 = 10.14$, $P < 0.01$), iron ($H_1 = 11.32$, $P < 0.001$), mercury ($H_1 = 23.73$, $P < 0.001$), and manganese ($H_1 = 14.32$, $P < 0.001$) concentrations all differed significantly by sampling region.

Table 4. Multivariate analysis of variance (MANOVA) exploring variation in heavy metal concentrations in bronze whaler sharks ($n = 41$) sampled along the South African coastline and key biological variables including shark age and sex, as well as sampling region. Probabilities in bold indicate significance.

Variable	MVS	Metals									
		<i>Al</i>	<i>As</i>	<i>Cd</i>	<i>Cr</i>	<i>Cu</i>	<i>Fe</i>	<i>Hg</i>	<i>Mn</i>	<i>Pb</i>	<i>Zn</i>
Age	<0.001	<0.05	.086	<0.01	<0.001	.791	<0.001	<0.001	<0.05	.441	.165
Sex	.060	.686	.764	.433	<0.001	.498	.082	.886	.774	.967	.999
Sampling Region	<0.001	<0.05	.144	<0.01	<0.01	.519	<0.001	<0.001	<0.001	.307	.076

Iron, cadmium, and chromium concentrations were significantly higher in adult (iron: $Z_{27} = -3.85$, $P < 0.001$; cadmium: $Z_{27} = -3.44$, $P < 0.01$; chromium: $Z_{27} = -3.37$, $P < 0.01$) than juvenile sharks. Mercury concentrations were significantly higher in both sub-adults ($Z_{27} = -3.76$, $P < 0.001$) and adults ($Z_{27} = -4.28$, $P < 0.001$) than juvenile sharks, while juvenile sharks had significantly higher aluminium concentrations (aluminium: $Z_{27} = 2.49$, $P < 0.05$) than sub-adults, and higher manganese concentrations (manganese: $Z_{27} = 2.54$, $P < 0.01$) than adult sharks. Chromium concentrations were significantly higher in male than female sharks ($Z_{21} = 2.73$, $P < 0.01$). Iron, mercury, cadmium, and chromium concentration were significantly higher in the ECSA (iron: $Z_{12} = 3.37$, $P < 0.001$; mercury: $Z_{12} = 4.87$, $P < 0.001$; cadmium: $Z_{12} = 3.05$, $P < 0.01$; chromium: $Z_{12} = 3.18$, $P < 0.01$) than sharks sampled along the SCSA. Whereas aluminium and manganese concentrations were significantly higher in the ECSA (aluminium: $Z_{12} = -2.35$, $P < 0.05$; manganese: $Z_{12} = -3.78$, $P < 0.001$) than sharks sampled along the SCSA

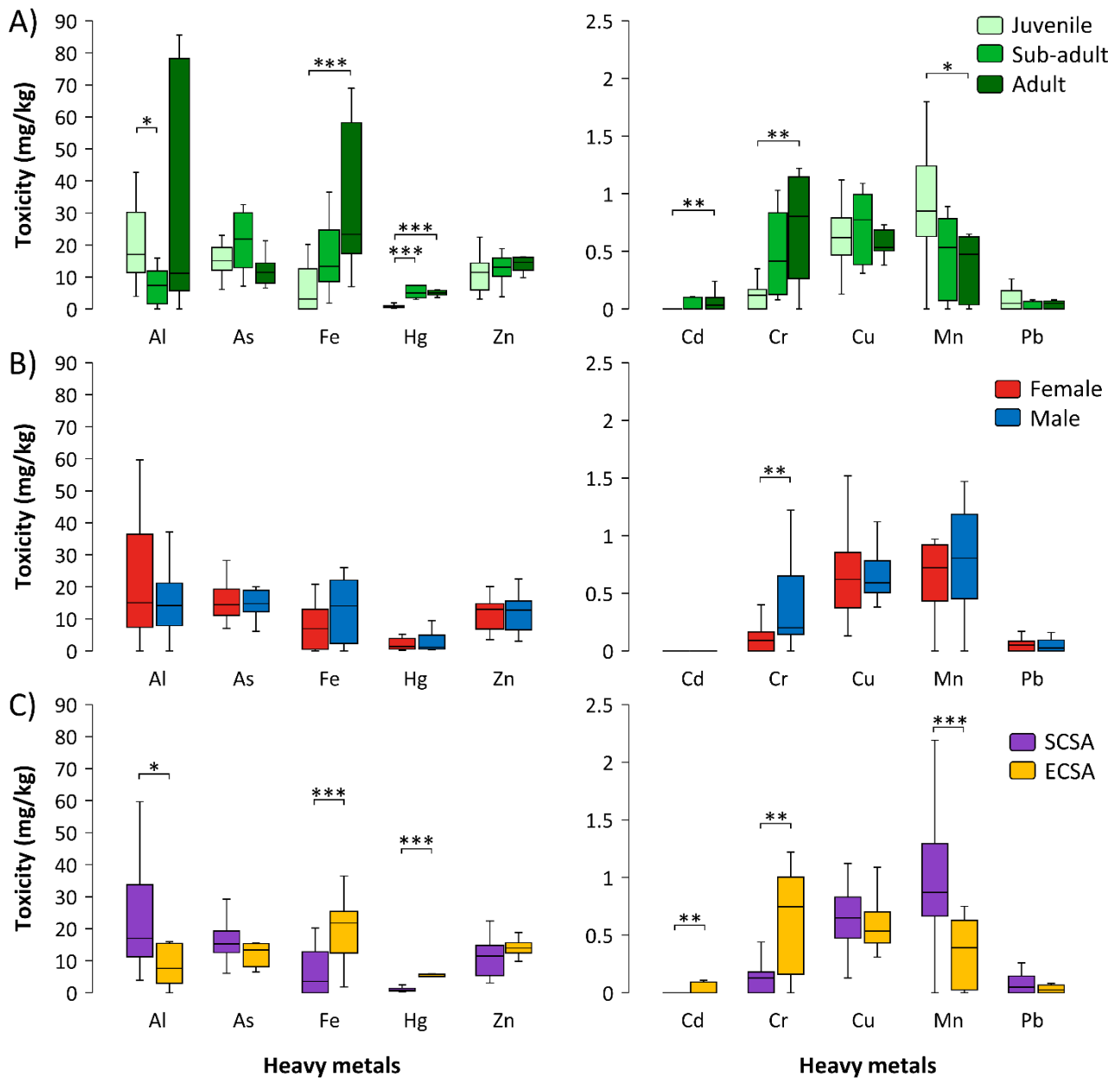


Figure 4. Mean (\pm SE) heavy metal toxicity in the muscle tissue of bronze whaler sharks ($n = 41$) sampled along the South African coastline, by: A) age (juvenile, <130cm, light green; sub-adult, 130–230cm, green; adult, >230cm, dark green), B) sex (female, red; male, blue), and C) sampling region (South Coast of South Africa, SCSA, purple; East Coast of South Africa, ECSA, gold). The right axis is scaled visually for lower relative concentrations (0-2mg/kg). Significance (* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$).

Discussion

Heavy metals in bronze whalers and other shark species worldwide

This study represents the first assessment of heavy metal and persistent organic pollutant levels in bronze whaler sharks along the Southern African coastline. The concentration of 10 heavy metals (aluminium, arsenic, cadmium, chromium, copper, iron, lead, manganese, mercury, and zinc) were analysed in 41 bronze whaler shark samples from the southern (SCSA) and eastern (ECSA) regions of the Southern African coastline. In addition, the concentrations of eight polychlorinated biphenyl congeners (28, 52, 101, 118, 138, 153, 180, 194) were analysed in 11 bronze whaler sharks from the SCSA. Heavy metal concentrations were compared to international recommended regulatory maximum limits and explored in relation to shark size (i.e., both total body length and total body weight), as well as shark age, sex, and sampling region. This assessment was also done to understand the interactions between all bioaccumulated heavy metals. Metals with the highest concentrations in bronze whaler sharks were aluminium, iron, and arsenic. The mean concentrations of these metals were similar to levels found in bronze whaler sharks from other regions (Table 5) with iron (8.22 ± 7.54 mg/kg) and arsenic (6.98 ± 3.29 mg/kg) both having high concentrations in bronze whalers from Jeju Island, Korea (Kim *et al.*, 2019). These metals also occurred at high concentrations in other shark species in South African waters including smooth hound sharks, in Langebaan bay (aluminium: 1.34 ± 0.52 mg/kg; iron: 3.54 ± 1.54 mg/kg, and arsenic: 28.31 ± 18.79 mg/kg) (Bosch *et al.*, 2016) and Great white sharks sampled from five different regions (Algoa Bay, False Bay, Gansbaai, Mossel Bay, and Struisbaai) along the South African coastline, (iron: 1485.80 ± 862.93 mg/kg and arsenic: 833.43 ± 781.09 mg/kg, Merly *et al.*, 2019).

Regulatory limits of heavy metal concentration

Various shark heavy metal assessment studies have compared findings to the regulatory maximum limits set by institutions and governments to determine the toxicity level of these metals for shark and human health. In this study, the mean values of Mercury, Arsenic and Chromium often exceeded the toxic limit for human consumption. (Table 2). For mercury, only 24 sharks (59 %) had levels above the maximum limit, while the remaining 41 % had levels close to the maximum allowable limit. However, mean mercury concentration (2.53 mg/kg) for all bronze whaler sharks sampled exceeded the maximum regulatory limit. Similar heavy metal concentrations have been reported for bronze whalers, other Carcharhinid species, and other sharks. For instance, Kim *et al.* (2019) reported mean mercury concentrations of 1.85 ± 0.83 mg/kg, in bronze whaler sharks' samples from Jeju Island, Korea which is higher than the recommended maximum limit (1.0 mg/kg). McKinney *et al.* (2016) reported "above limit" mercury levels (4.98 ± 0.88 mg/kg) in bronze whaler sharks, and other Carcharhinid species from Kwazulu-Natal in the Eastern Coastal region of South Africa. Erasmus *et al.* (2020) also reported exceedingly high levels of Total mercury in Pigeye shark (4.52 mg/kg), Spinner sharks (3.10 ± 1.41 mg/kg), Bull shark (13.15 mg/kg), Blacktip shark (1.63 mg/kg), Dusky sharks (2.16 ± 0.65 mg/kg), and Tiger sharks (3.72 ± 1.18 mg/kg). However, mean mercury levels (0.96 ± 0.69 mg/kg) found in Smooth hound sharks from Langebaan Bay, South Africa coastline (WCSA) were below the maximum allowable limit. The latter finding may reflect the primarily benthic feeding strategy of smooth hound sharks which limits mercury biomagnification

compared to the more pelagic feeding bronze whaler sharks.

Table 5. Concentration (mg/kg) of heavy metal in different shark species from various regions of the world.

Species	n	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Pb	Zn	Location	References
Bronze whaler sharks	41	39.67	16.60	0.02	0.31	0.67	20.18	2.53	0.80	0.14	13.7	South Africa	This study
Bronze whaler sharks	6	–	6.98	–	0.12	–	8.22	1.85	–	–	–	Jeju Island, Korea	Kim <i>et al.</i> , 2019
<i>Smooth hound sharks</i>	30	1.34	28.31	0.04	0.09	0.31	3.54	0.96	0.09	0.04	4.38	Langebaan Lagoon, South Africa	Bosch <i>et al.</i> , 2016
Bronze whaler sharks	5	–	–	–	–	–	–	4.98	–	–	–	Indian Ocean, South Africa	McKinney <i>et al.</i> , 2016
<i>Smooth hound sharks</i>	7	–	–	–	–	–	–	0.23	–	–	–	South coast, South Africa	Erasmus <i>et al.</i> , 2022
<i>Squatina aculeata</i>	1	–	7.54	–	–	0.02	52.97	6.85	0.05	–	17.26	North-Eastern Mediterranean Coast, Turkey	Turan <i>et al.</i> , 2021
<i>Somniosus microcephalus</i>	18	–	–	4.07	–	–	–	2.60	–	0.12	–	North-eastern coast, Greenland	Corsolini <i>et al.</i> , 2014
<i>Centrophorus granulosus</i>	16	–	–	43.8	–	–	–	–	–	260.1	–	Macaronesian islands, Spain	Lozano-Bilbao <i>et al.</i> , 2018
<i>Centrophorus squamosus</i>	44	–	–	120.36	–	–	–	78	–	98.18	–	Macaronesian Islands, Spain	Lozano-Bilbao <i>et al.</i> , 2018
<i>Carcharhinus acronotus</i>	3	–	0.07	0.15	2.58	4.23	–	–	1.52	0.10	104.17	Bahamas	Shiple <i>et al.</i> , 2021
<i>Carcharhinus perezii</i>	24	–	7.31	0.12	2.64	4.90	–	–	0.97	0.37	80.13	Bahamas	Shiple <i>et al.</i> , 2021
<i>Carcharhinus leucas</i>	1	–	1.38	0.22	0.09	2.73	–	–	0.16	0.14	37	Bahamas	Shiple <i>et al.</i> , 2021
<i>Galeocerdo cuvier</i>	7	–	1.00	0.14	0.74	3.46	–	–	1.77	0.10	41.3	Bahamas	Shiple <i>et al.</i> , 2021
<i>Ginglymostoma cirratum</i>	5	–	3.75	0.26	1.53	6.41	–	–	0.64	0.11	88	Bahamas	Shiple <i>et al.</i> , 2021
<i>Negaprion brevirostris</i>	2	–	0.31	0.23	2.31	30.88	–	–	2.31	0.13	64.14	Bahamas	Shiple <i>et al.</i> , 2021
<i>Sphyrna lewini</i>	1	–	–	0.02	–	0.37	–	0.76	–	0.14	3.8	West coast, Bougainville Island, Papua New Guinea	Powell & Powell, 2001
<i>Rhizoprionodon acutus</i>	40	–	–	0.06	–	5.54	–	1.15	–	0.10	22.50	Northern basin of Persian Gulf	Adel <i>et al.</i> , 2017
<i>Carcharhinus albimarginatus</i>	81	–	–	1.26	–	1.71	31.12	3.34	–	–	8.54	Ishigaki Island, Japan	Endo <i>et al.</i> , 2016
<i>Alopias pelagicus</i>	34	–	–	47.14	–	–	–	0.65	–	–	–	Baja California Sur, Mexico	Lara <i>et al.</i> , 2020
<i>Sphyrna lewini</i>	10	–	640.88	1.72	–	–	–	186.36	–	15.07	–	Trinidad and Tobago	Mohammed & Mohammed, 2017
<i>Carcharhinus porosus</i>	12	–	793.08	2.57	–	–	–	116.88	–	19.31	–	Trinidad and Tobago	Mohammed & Mohammed, 2017
<i>Prionace glauca</i>		–	–	–	–	–	–	0.05	–	2	–	Southeastern Pacific Ocean, Chile	Lopez <i>et al.</i> , 2013
<i>Isurus oxyrinchus</i>	69	–	–	–	–	–	–	0.04	–	0.92	–	Southeastern Pacific Ocean, Chile	Lopez <i>et al.</i> , 2013
<i>Carcharhinus dussumieri</i>	40	–	–	0.11	–	7.49	–	0.03	–	0.12	3.47	Northern basin of Persian Gulf	Adel <i>et al.</i> , 2016

All sharks sampled had arsenic concentrations higher than the regulatory limit (3.0 mg/kg) with an average concentration of 16.60 mg/kg recorded. High arsenic levels are not uncommon in shark muscular tissue with concentrations exceeding the regulatory limits in sharks at different trophic levels and in different regions. According to De Gieter *et al.* (2002) and Smale & Compagno, (1997), demersal sharks feeding on benthic organisms and smaller fish are more exposed to and accumulate more arsenic than pelagic fish. For example, the two demersal species, the Sawback Angel Shark (*Squatina aculeata*) in the North-Eastern Mediterranean, and Smooth hound sharks, from the South African coastline, had high levels of arsenic (7.54 ± 0.66 mg/kg and 28.31 ± 18.79 mg/kg, respectively, Turan *et al.*, 2021). However, the generality of this statement has been called into question with exceptionally high mean arsenic concentration (833.43 ± 781.09 mg/kg) reported for *Carcharodon carcharias* near the Korean peninsula. Similarly, both *Carcharhinus porosus* and *Sphyrna lewini* from the waters off Trinidad exhibited exceptionally high arsenic concentrations 762.7–5791.1 mg/kg, and 855.5–2309.1 mg/kg respectively. The results also revealed high arsenic levels in the pelagic bronze whalers which can accumulate in prey species from natural sources in the environment, such as geologic formations and mineral deposits. Additionally, their elevated arsenic levels may be due to their release into the environment through copper mining activities in southern Africa. Bronze whaler sharks may also have a high tolerance to arsenic like the other pelagic sharks mentioned above, and this may allow them to accumulate high levels of this contaminant.

Average chromium concentration from sharks sampled in this study was 0.31 mg/kg which exceeds the maximum regulatory limit (0.25 mg/kg). Chromium was not as prevalent as the other metals that were found to exceed the regulatory limits in the study. Specifically, only about 12 sharks (29 %) had chromium levels higher than the permissible limit while 15 sharks (37 %) had chromium levels close to the maximum limits. A study conducted by Turan *et al.* (2022) found that bronze whaler sharks had higher concentrations of chromium than other shark species, including smooth-hound sharks and blue sharks in the Mediterranean Sea. Bronze whaler sharks inhabit shallow coastal waters and estuaries (Rogers *et al.*, 2022), where they feed on a variety of prey, including fish, squid, and crustaceans, and thus they may be exposed to higher levels of chromium compared to other sharks that inhabit deeper waters and have a more limited diet. That said, the mean chromium level (0.12 mg/kg) for bronze whaler sharks sampled from Jeju Island, Korea, was lower than in this study and below the regulatory maximum limits. High levels of chromium have also been found in sharks from other regions, for instance Caribbean reef sharks and blacknose sharks (*Carcharhinus acronotus*) in the Bahamas had concentrations of 2.641 ± 3.272 mg/kg and 2.577 ± 2.156 mg/kg, respectively (Shipley *et al.*, 2021). Additionally, mean chromium concentration in great white sharks sampled along the South African coast showed higher chromium levels (2.88 ± 1.2 mg/kg, Merly *et al.*, 2019), while smooth hound sharks in the same region were lower at 0.09 ± 0.22 mg/kg (Bosch *et al.*, 2016).

Sex-specific heavy metal concentrations in bronze whaler sharks

Factors such as metabolic activities (Al-Yousuf *et al.*, 2000), growth rates (Endo *et al.*, 2013), feeding patterns (Barrera-Garcia *et al.*, 2012) as well as maturity stages (Lopez *et al.*, 2013) may cause an inconsistent accumulation of some toxic elements between the sexes of a species. Heavy metal accumulation in marine top predators may vary by sex, albeit not always at statistically significant

levels. For instance, in blue fin tuna caught in the Straits of Messina, higher levels of cadmium, copper, mercury, manganese and lead were detected in the liver and muscle tissues of females, whereas only zinc showed a higher concentration in males. Cadmium concentration has been shown to vary between sexes in sharks with male Pacific sharp nose shark (*Rhizoprionodon longurio*; Martínez-Ayala *et al.*, 2022) having significantly higher levels. Although the precise cause of higher accumulation of certain metals (such as chromium, arsenic, and mercury) in one sex of bronze whaler sharks remains unclear, it is likely influenced by various life history factors, including their size, diet, habitat use and metabolic rate. In my study, only chromium had a significantly higher concentration in male sharks (Figure 4B). Male bronze whaler sharks tend to feed more heavily on cephalopods and other benthic prey, which may contain higher levels of chromium compared to other types of prey (Walter & Ebert, 1991). Male bronze whaler sharks in Jeju Island, Korea had higher copper levels while females had higher concentrations of arsenic. Female fish tend to accumulate higher levels of heavy metals than males because they have higher metabolic rates and therefore may absorb and accumulate more heavy metals from their diet or the environment (Varol *et al.*, 2022). However, this depends on the specific heavy metal in question and the species (Bervoets & Blust, 2003). For instance, mercury concentration was significantly higher in male silvertip sharks (*C. albimarginatus*) and dogfish sharks (*Mustelus manazo*), while the same metal was significantly higher in female white cheek shark (*C. dussumieri*) and in female Mako sharks (Lopez *et al.*, 2013). By contrast, no significant difference in blood metal levels were observed between male and female great white sharks caught along the southern Africa coastline (Merly *et al.*, 2019), or milk sharks from the Persian Gulf (Adel *et al.*, 2016).

Heavy metal accumulation variation by shark age

Bioaccumulation of toxins can be magnified over time and thus several studies have found that larger (i.e., older) sharks tend to have accumulated more toxins than their smaller counterparts (Delahaut *et al.*, 2020). In addition, as sharks mature, their dietary habits shift towards consuming larger prey (Simpfendorfer *et al.*, 2001; Lucifora *et al.*, 2008; Dicken *et al.*, 2017), which has been shown to correlate with increased bioaccumulation of toxic metals (Lowe *et al.*, 1996; Shipley *et al.*, 2021). In support of these theoretical predictions a study on smooth tooth blacktip sharks (*Carcharhinus leiodon*) found that adults had higher levels of mercury, lead, and arsenic than juveniles (Moore *et al.*, 2015). Mercury concentrations in adult blue sharks than juveniles (Escobar-Sánchez *et al.*, 2011) while adult silky shark (*Carcharhinus falciformis*) had higher levels of cadmium than juveniles, while adult silky shark (*Carcharhinus falciformis*) had higher levels of cadmium than juveniles (Terrazas-López *et al.*, 2016).

The results show that both adult (TL > 230 cm) and sub-adult (TL 130–230 cm) bronze whaler sharks exhibited significantly higher concentrations of cadmium, chromium, iron, and mercury, than juveniles (TL ≤ 130 cm) while the latter had significantly higher levels of manganese and aluminium (Figure 4A). This finding is unusual and while contrary to theoretical predictions is not unprecedented. For example, Endo *et al.* (2008) found that juvenile sandbar shark (*Carcharhinus plumbeus*) had significantly higher levels of cadmium and zinc than adults which they attributed to differences in both the feeding habits and habitat preference of the juveniles. Specifically juvenile sandbar shark tends to feed on smaller fish, such as sardines, anchovies, and squid and inhabit

shallower waters, which are often more polluted with heavy metals than the deeper waters where adult sharks spend more time.

Another study on dusky sharks reported higher concentrations of arsenic in juveniles compared to adults (Gilbert *et al.*, 2015) which they attributed to juveniles having a greater proportion of crustaceans in their diets (Medved *et al.*, 1985, Bornatowski *et al.*, 2014). It is unclear why juvenile sharks in this study have elevated levels of aluminium and manganese compared to adults but similar to the above studies must relate to differences in both diet and habitat use. Juvenile sharks have a strong affinity for the SCSA compared to sub-adult and adult sharks (Rogers *et al.*, 2022). The SCSA is an important nursery region for bronze whaler sharks (Kohler & Turner, 2001; Dicken *et al.*, 2009; Hussey *et al.*, 2009; Dunlop *et al.* 2013) and serves as an important summer parturition area for them, where they prey on seasonally abundant chokka squid (*Loligo reynaudii*) along the SCSA (Smale 1991; Roberts & Sauer, 1994; Lipiński *et al.*, 2020). Manganese and aluminium are the most prevalent metals in toxic effluent discharge to this region, and they originate in the manufacturing sector (i.e., manganese-based fungicides in vineyards, and manganese as an alloying agent) and in bentonite used for wine finning in the agriculture sector, wherein high levels of aluminium are released in water (Jackson *et al.*, 2013; Elumalai *et al.*, 2017; van Wyk *et al.*, 2021). Although difficult to prove a link between potential terrestrial sources of metals and their presence in sharks the significantly higher aluminium and manganese levels found in juvenile sharks and their preference for coastal regions adjacent to land where these metals are commonly used in manufacturing and agriculture suggests a positive link worth further investigation.

Evidence of bioaccumulation in sharks

Relationships between metal concentrations and phenotypic variables (e.g., total body length and body weight) are common in sharks (Endo *et al.*, 2008, 2016; Escobar-Sánchez *et al.*, 2011; Bosch *et al.*, 2016; Merly *et al.*, 2019). The findings revealed a strong positive relationship between mercury, iron, chromium with shark size. Cadmium also exhibited a positive relationship, although it was not statistically significant. Zinc and copper demonstrated a weak positive relationship, with correlation coefficients of 0.098 and 0.199, respectively (Figure S1). These results are consistent with the phenomenon of biomagnification in sharks, where toxic metal concentrations increase with age in sharks. This has been well documented for mercury (Van den Broek & Tracey, 1981; Storelli *et al.*, 2002; Kraepiel *et al.*, 2003; Campbell *et al.*, 2010), where certain mercury species such as methylmercury readily bind to thiol groups of proteins whose content increases with fish age (Branco *et al.*, 2007) and combined with a slow rate of elimination results in an increase in concentration over time and consequently with increasing fish size (Spry & Wiener, 1991). Previous studies have reported similar positive correlations between metal concentration and body size in shark species such as *Galeocerdo cuvier*, *Squalus megalops* and *M. mustelus* (Endo *et al.*, 2008; Escobar-Sánchez *et al.*, 2011; Bosch *et al.*, 2016). A significant strong negative relationship was also observed between shark size and manganese concentration (Figure S1). This observation corroborates my result that juveniles had significantly higher manganese concentration than both adult and sub-adult sharks which suggests that not all heavy metals biomagnify in sharks and in fact the converse may happen for some metals. Similar negative correlations between heavy metals and shark size have been reported in other studies (Canli & Atli, 2003; Erasmus *et al.*, 2004),

which was attributed to size-dependent variability in metabolic activity. As fish grow, metabolic activity decreases, resulting in higher accumulation of some metals in younger fish with faster metabolic rates (Canli & Atli, 2003).

Geographical distribution of bronze whaler sharks along the South African coastline

Bronze whaler sharks exhibit seasonal movement between the five biogeographic regions of the South African coastline and in particular between the SCSA and ECSA (Rogers *et al.*, 2022). Bronze whaler sharks' movement between these regions is strongly influenced by the annual "sardine" run, from the cool waters of the Agulhas Bank in SCSA to the ECSA (Cliff & Dudley, 1992). Bronze whaler sharks have been recorded moving from offshore habitats to the inshore waters to take advantage of this seasonal abundance of prey, travelling large distances in the process and exposing themselves to potential pollution sources on both the southern and eastern coastlines. Similar seasonal movement in bronze whaler sharks has been reported in Argentinian and Australian temperate waters (Lucifora *et al.*, 2005; Rogers *et al.*, 2013). For bronze whaler sharks moving into the ECSA the risk of mortality has historically been high because of the gill nets deployed to kill large sharks and hence protect recreational inshore ocean users along the KwaZulu–Natal (KZN) coastline (Huvneers *et al.*, 2020). This mortality may explain the substantially smaller bronze whaler sharks sample size (n = 12) collected from the ECSA compared to the SCSA (n = 29) despite the recent efforts to remove the gill nets during the annual sardine run.

Geographical variation in heavy metal concentration in bronze whaler sharks

Heavy metal concentrations in the same shark population can vary significantly depending on the geographical region where the sharks were sampled within a country's coastline (Mohammed & Mohammed, 2017; Adel *et al.*, 2018; Merly *et al.*, 2019). This variation can be attributed to differences in the distribution of industrial, pharmaceutical, and agricultural operations on land, and the adequacy of wastewater treatment plants at removing pollutants before the water enters the coastal environment. This study compared bronze whaler sharks sampled from the east and south coastlines of South Africa and show that sharks sampled from the East coast had significantly higher concentrations of chromium, mercury, iron, and cadmium, while those from the south coast had significantly higher concentrations of aluminium and manganese (Figure 4C). While it is exceptionally difficult to attribute specific activities on land with metal levels in sharks, the south coastline has on average less industrial and mining activity than the east coast, including provinces such as KZN and Gauteng (Lehohla, 2011; Tindall *et al.*, 2014). High levels of chromium, mercury, iron, and cadmium found in sharks from ECSA might be from the untreated waste generated from both mining activities and chemicals used in the agricultural sector such as pesticides and herbicides. Mercury in particular are emitted into the atmosphere from anthropogenic activities such as coal-fired power stations, cement production and small artisanal gold mining operations in South Africa (Walters *et al.*, 2011), where about 18 coal-fired power stations account for 84 % of the country's electricity production (Belelie *et al.*, 2019; EMBER, 2022). These activities are primarily located in the north-east region of the country (the Mpumalanga Highveld region) where the dominant atmospheric transport pattern flows over this mercury pollution hotspot towards the east coast of southern Africa (Freiman and Piketh, 2003) and is so dominant that it has been linked with iron fertilization in

the South Indian Ocean (Piketh et al., 2000). This study supports the mercury findings of Erasmus *et al.*, 2022 who revealed that the south coast elasmobranch species had generally lower total mercury concentrations compared to the east coast species.

The Western Cape which borders the SCSA is recognised as an important crop production region where the use of fertilizers, phytosanitary treatments, machinery, piping, fining agents, and additives may all contribute to toxic metals being released into the environment (Pohl, 2007; Blanka, 2011). High levels of aluminium are released into the environment when bentonite is used for wine fining. Additionally, the consistent use of manganese-based fungicides in vineyards is another significant contributor to the excessive amounts of manganese being released into rivers and ultimately the ocean (Stafilov & Karadjova, 2009). The Western Cape is also home to the country's second-largest manufacturing sector, which includes the production of batteries and steel, both activities known to contribute significantly to environmental contamination by aluminium and manganese. Manganese is used as an alloying agent in the production of steel and can be released into the air and water from furnaces, ladles, and other equipment. Additionally, manganese is used in the production of batteries, particularly alkaline batteries, and can be released into the environment during its use and improper disposal. The production of aluminium-based products such as metal sheets, cans, and foils may also lead to its high concentration in rivers and lakes in the Western Cape province, which is ultimately deposited into the South Atlantic marine body.

Heavy metal correlations in bronze whaler sharks

Very few studies have investigated the relationship between metal concentrations in sharks, and the patterns identified are often inconsistent among species due to potential differences in metabolism (Burger *et al.*, 2002; Rejomon *et al.*, 2010; Merely *et al.*, 2019). For instance, Bosch *et al.* (2016) did not find any correlation among metals in smooth hound sharks, while Kim *et al.* (2019) discovered a significant relationship between mercury and lead in bronze whaler sharks. In this study, aluminium was negatively correlated with mercury and chromium, and a similar trend was found with zinc and manganese. Toxic metals tend to exhibit negative correlations when competing for binding sites in fish (Pagenkopf, 1983; Playle, 1998). The impact of their interactions varies depending on the binding site's function. For example, metallothioneins bind to heavy metals, detoxify, and sequester them, and prevent them from interacting with other cellular components. Heavy metals can attach to DNA, disrupting its replication and transcription, resulting in genetic mutations and cell death. Additionally, metals bind to calcium channels, which regulate the flow of calcium ions into cells, and interrupt their operation, leading to cellular toxicity.

Similar accumulation behaviours, detoxification processes, and comparable input sources may lead to positive metal correlations (Barrera-García *et al.*, 2013; Dhanakumar *et al.*, 2015). For instance, it is believed that metallothioneins induced by high zinc and copper levels can interact with and detoxify metal ions like cadmium, mercury, lead, and silver (Roesijadi, 1992; Palmiter, 1998). In this study, it was discovered that mercury was positively correlated with iron, chromium, and cadmium, while zinc was positively correlated with copper, cadmium, and aluminium in the muscles of bronze whaler sharks (Table 3). As a result, these micronutrient metals may provide some protection against heavy metal toxicity (Roesijadi, 1992; Palmiter, 1998). Notably, cadmium has a unique relationship with zinc, as it may interact with zinc, to enhance its accumulation,

toxicity, and biomagnification in food chains, as reported from metal toxicity research in Gamtoos and Kromme estuaries, South Africa. Additionally, significant positive correlations were observed between iron with cadmium and chromium, suggesting that they might be introduced into the marine environment through similar geochemical pathways (e.g., mine dust deposition, organic matter from runoff, or suspended sediment), and between lead with aluminium and zinc. Although establishing the cause and implications of metal correlations in wild sharks is difficult, this descriptive approach indicates that a direct experimental study investigating the biological and environmental factors that influence metal correlations in sharks or lack thereof is necessary.

Study limitations

A major limitation of this study was the small sample size ($n = 41$) which while similar to published studies (Endo *et al.*, 2008 [tiger sharks ($n = 52$)]; Lopez *et al.*, 2013 [Shortfin mako shark $n = 69$]; Torres *et al.*, 2014 [tope shark (*Galeorhinus galeus*, $n = 124$)]; Endo *et al.*, 2016 [Silvertip sharks ($n = 81$)]), is much lower than that required to generalise to this species in this part of its global distribution. In addition, within population comparisons linked to sex, size and region of capture were limited by low sample sizes. Another limitation was not being able to compare the concentration of heavy metals in other shark tissues such as the liver, gonads, fins, gills, or kidney. Previous studies have shown that the liver may accumulate higher concentrations of heavy metals due to the high abundance of fats and being the main site for heavy metal bioaccumulation, storage, and detoxification (Endo *et al.*, 2008). It would be informative to conduct a study where metal concentration in other bodily tissues is compared to levels present in the muscular tissues, most importantly the liver. Additionally, we were unable to test for the speciation of each concentrated metal to understand their level of toxicity, such as arsenic. Total arsenic analysis in fish may not be a true representative of the arsenic concentration in muscular tissue, and arsenic speciation research may be necessary to determine a more accurate measure of arsenic toxicity in sharks. The study was also unable to test for other persistent organic pollutants that have previously been observed in sharks (Weijs *et al.*, 2015; Alves *et al.*, 2016; Fossi *et al.*, 2017) because of the prohibitive costs of running such analyses within South Africa. We were only able to test concentrations of 8 PCB congeners on the 1st bronze whaler sharks sample batch ($n = 11$). Other persistent organic pollutants such as DDT, HCBs, and PBDEs were not tested for in this study, but would have been highly informative.

Implications for conservation

Sharks are important apex predators in the marine ecosystem, playing a significant role in regulating the structure and function of communities (Heithaus *et al.*, 2008). However, their long-term exposure to heavy metals and persistent organic pollutants may adversely affect their functionality and may eventually lead to mortality in extreme cases. This and other factors such as direct and indirect over-exploitation have contributed to their population decline and inherent vulnerabilities to anthropogenic threats (Gallagher *et al.*, 2012). For instance, the bronze whaler shark population have reportedly reduced by 55–99 % in the past 71 years in South Australia, Southwest Atlantic, Northwest Pacific, and Peru (Reid *et al.*, 2011, Gibbs *et al.*, 2019, Liao *et al.*, 2019). Likewise in South Africa, the bronze whaler shark population has, together with other economically important sharks,

substantially decreased within the past decade. Lethal gill netting off the Kwazulu-Natal coastline (Huveneers *et al.*, 2020) and the high number of bronze whaler shark strandings observed on beaches in the Eastern Cape in recent years (Sgqolana, 2022) have contributed to mortality of adults in this population. Declines in the abundance of this and other large marine predators could impact marine biodiversity and drive negative trophic cascades in the marine ecosystem (Ferretti *et al.*, 2010). Therefore, understanding heavy metal and POP toxicity levels of this species is crucial due to their ecological significance and economic importance.

Potential sources of pollution

Various sources of heavy metal and POP pollutants in marine ecosystems have been reported, including industrial and domestic waste waters (Adewumi *et al.*, 2010; Gani *et al.*, 2021; Ojemaye & Petrik, 2019, 2022; Petrik *et al.*, 2017), refineries, mining activities, and landfills (Castro-González & Méndez-Armenta, 2008; Adel *et al.*, 2016; Gusso-Choueri *et al.*, 2018). The discharge of untreated or partially treated sewage into South African aquatic bodies has been identified as a cause of POP pollution in its ocean system by various studies (Petrik *et al.*, 2017; Ojemaye & Petrik, 2019, 2022; Gani *et al.*, 2021; Olisah *et al.*, 2021; Marcu *et al.*, 2023). The effectiveness, efficiency, and integrity of wastewater treatment plants (WWTPs) established across South African municipalities to properly treat effluents before releasing them into marine ecosystems have been challenged by numerous findings (Taljaard, 2006; Petrik *et al.*, 2017; Swartz *et al.*, 2018a, 2018b; Herbig & Meissner, 2019; Ojemaye & Petrik, 2019, 2022). For example, Swartz *et al.* (2018) found that several WWTPs in the Western Cape discharge effluents that are inadequately treated into False Bay, one of the largest bays along the SCSA. Herbig & Meissner (2019) also noted that many WWTPs in South Africa are poorly managed and do not comply with national discharge standards. As suggested by Swartz *et al.* (2018a, 2018b) and Taljaard (2006), these compounds escape the WWTP and are discharged into freshwater systems which lead to the marine environment. In addition, De Klerk's (2019) survey shows that 51 out of 108 WWTPs across South Africa do not treat wastewater satisfactorily, and 55 % of the WWTPs that discharge effluents along the Vaal River in the Mpumalanga and Free State Provinces are not in compliance with the national discharge regulations. The same can be said of the WWTPs in Eastern and Western Cape provinces. In Cape Town, Western Cape, various studies have clearly shown the inefficiency of these WWTPs, with exceedingly high levels of organic pollutants (e.g., perfluoroalkyl compounds) have been found in fish species, seaweeds, sediment, and seawater in the marine environment along the SCSA. Although no levels of PCBs were detected in my samples, it may not be a true representation of the levels of this organic compound in bronze whaler sharks since I only sampled a subset (27 %) of the total sharks collected. However, it is worth noting that high levels of these compounds have been detected in sharks in this region and other geographical areas (Table 6), in estuarine water, sediments, and biota from South Africa, as shown in Table 7, and in fish species in various Cape Town bays along the SCSA. Over time, bronze whaler shark may also accumulate significant levels of these compounds. Ojemaye *et al.* (2020) reported high levels of persistent organic pollutants (i.e., pharmaceuticals and personal care products, perfluoroalkyl compounds and industrial chemicals) in muscular tissues of fish species, including snoek (*Thyrsites atun*), bonito (*Sarda orientalis*), panga (*Pachymetopon blochii*) and Hottentot (*Pterogymnus lanarius*) obtained from Kalk Bay harbour, Cape Town.

The above fish species are all confirmed bronze whaler shark's prey items and toxins in these fish species may bioaccumulate when ingested. Diclofenac compound and other organic toxic compounds were also found in sediments, seawater, seaweeds, and organisms (e.g., limpets, mussels, sea urchins, sea snails, and starfish) tested in WWTP effluent sites around False Bay (Ojemaye & Petrik, 2022). Swartz *et al.* (2018b) found high levels of these toxic chemicals in analysed samples from False Bay, despite its remoteness from the seven Cape Town WWTP sites. Moreover, the City of Cape Town (2018) confirmed that the WWTPs are not designed to remove persistent organic pollutants from their effluent, and therefore, these pollutants are released back into the environment when effluent is discharged into rivers, estuaries, surf-zones, and so on, as is the city's practice (Ojemaye & Petrik, 2022). Furthermore, the poor state of sanitation in many parts of South Africa may enhance the spread of persistent organic compounds present in sewage effluents into the environment (Segura *et al.*, 2015; Madikizela *et al.*, 2017; City of Cape Town, 2019). Reclaimed water, used for irrigation in agriculture, washing of cars, and so forth, is subsequently deposited in the marine environment through stormwater drains (Brown *et al.*, 1991). The cumulative effect of these sources may enhance the pollution levels in South African aquatic bodies.

Mining activities also generate large amounts of heavy metal-laden waste (mine tailings) which are released into the environment (Mujuru, 2016). These toxin-rich waste sources pose a huge threat to ecosystems by causing widespread contamination in terrestrial, freshwater, and marine habitats (Morris *et al.*, 2003; Navarro *et al.*, 2008; Mileusnić *et al.*, 2014). For example, in 2000, large quantities of cyanide were reported to have leached into water bodies during gold mining and processing in the Ore district of Baia Mare, in Romania. This leaching led to harmful levels of lead, nickel, copper, and zinc being detected in the aquatic environment causing rapid high mortality in aquatic organisms and animals living close to the poisoned rivers (Soldán *et al.*, 2001).

Heavy metals from mining activities find their way into farmlands, accumulating in food crops and subsequently being deposited into the ocean via agricultural runoff and erosion. Vegetables grown in farmlands close to mining sites in Eastern China were found to contain lead and cadmium in concentrations exceeding the permitted limits (Li *et al.*, 2006). Even in Namibia, a country that shares the southern African Coastline, severe metal contamination in soil and high metal accumulation in crops were detected in an abandoned mining district (Mapani *et al.*, 2010). Heavy metals are artificially produced in all mining stages (e.g., land excavation, drilling, quarrying, and conveying of raw materials, milling, grinding, ore concentration and tailing disposal) and from cleaning and stock storage operations, as well as leachate infiltration. Ultimately waste from all these processes seeps into drainage lines which accumulate in rivers that mostly lead to the ocean (Antwi-Agyei *et al.*, 2009; Van Hook, 1979).

Table 6. PCB presence and concentrations in other shark species globally

Shark Species	n	Scientific name	ΣPCBs (µg/g)	Tissue	Geographical area	References
Dusky shark	42	<i>Carcharhinus obscurus</i>	30.5	Muscle	South Africa	Beaudry <i>et al.</i> , 2015
Great White shark	53	<i>Carcharodon carcharias</i>	20.4	Muscle	South Africa	Beaudry <i>et al.</i> , 2015
Whale shark	12	<i>Rhincodon typus</i>	11.42 ±8.60	Biopsy	La Paz Bay	Fossi <i>et al.</i> , 2017
Tiger shark	42	<i>Galeocerdo cuvier</i>	480	Liver	Southern coast of Japan	Haraguchi <i>et al.</i> , 2009
Silvertip shark	8	<i>Carcharhinus albimarginatus</i>	280	Liver	Southern coast of Japan	Haraguchi <i>et al.</i> , 2009
Sandbar shark	1	<i>Carcharhinus plumbeus</i>	280	Liver	Southern coast of Japan	Haraguchi <i>et al.</i> , 2009
Bull shark	8	<i>Carcharhinus leucas</i>	51,700	Liver	Southeastern USA	Weijs <i>et al.</i> , 2015
Greenland shark	18	<i>Somniosus microcephalus</i>	31.1 ±11.1	Muscle	Greenland	Corsolini <i>et al.</i> , 2014
Blue shark	20	<i>Prionace glauca</i>	1.22 ±1.12	Muscle	Southwest of Portugal	Alves <i>et al.</i> , 2016
Mediterranean basking Shark	6	<i>Cetorhinus maximus</i>	1483.06	Muscle	Mediterranean Sea (Italy)	Fossi <i>et al.</i> , 2014
Lemon shark	12	<i>Negaprion brevirostris</i>	1950	Liver	Southeastern USA	Weijs <i>et al.</i> , 2015
Bonnetheads shark	19	<i>Sphyrna tiburo</i>	91,550	Liver	Southeastern USA	Weijs <i>et al.</i> , 2015
Brazilian sharpnose shark	14	<i>Rhizoprionodon lalandii</i>	1019 ±267	Liver	Southeastern coast of Brazil	Cascaes <i>et al.</i> , 2014
Gulper shark	25	<i>Centrophorus granulosus</i>	28.3 ±11.3	Muscle	Mediterranean Sea (Italy)	Storelli & Marcotrigiano, 2001
Kitefin shark	64	<i>Dalatias licha</i>	1827 ±349	Liver	Mediterranean Sea (Italy)	Storelli <i>et al.</i> , 2005
Whitespotted bamboo Shark	8	<i>Chiloscyllium plagiosum</i>	2.0901	Liver	Southern waters of Hong Kong, China.	Cornish <i>et al.</i> , 2007
Small spotted dogfish	58	<i>Scyliorhinus canicula</i>	1292 ±577	Liver	Mediterranean Sea (Italy)	Storelli <i>et al.</i> , 2006

Table 7. Range of concentration of PCBs in biotic and abiotic components in South Africa estuaries

Estuaries	Range	Biotic and abiotic component	Reference
Buffalo River	185–2329 ng/L	Surface water	Yahaya <i>et al.</i> , 2019
Swartkops	14.8–2915.6 ng/L	Surface water	Olisah <i>et al.</i> , 2019
Sundays	3.2–713.7 ng/L	Surface water	Olisah <i>et al.</i> , 2019
Durban Bay	6–110 ng/g dw	Sediments	Vogt <i>et al.</i> , 2018
uMngeni River	<MDL–21 ng/g dw	Sediments	Vogt <i>et al.</i> , 2018
Swartkops	70–3800 ng/g dw	Sediments	Olisah <i>et al.</i> , 2020
Sundays	80–1710 ng/g dw	Sediments	Olisah <i>et al.</i> , 2020
Swartkops	<LOQ–195 ng/g ww	Fish tissues (<i>P. commersonii</i>)	Nel <i>et al.</i> , 2015
Swartkops	<LOQ–91 ng/g ww	Fish tissues (<i>Lichia amia</i>)	Nel <i>et al.</i> , 2015
Swartkops	<LOQ–198 ng/g ww	Fish tissues (<i>A. japonicas</i>)	Nel <i>et al.</i> , 2015

LOQ – limit of quantification; ww – wet weight; dw – dry weight; MDL – method detection limit.

Numerous reports have documented excessive levels of heavy metal pollution stemming from mining activities throughout South Africa (Kotzé *et al.*, 1999). Gold mining is a major industrial activity that contributes significantly to the growth and development of the country. However, it also poses a threat to humans, freshwater, and marine organisms through the production of mine tailings which are widely transported to the oceanic systems through rivers. In the Gauteng Province, mine tailings have been shown to contain high concentrations of toxic metals, such as arsenic and zinc (Weissenstein & Sinkala, 2011). For example, at the old Princess gold mine in Johannesburg high levels of lead, arsenic, and other heavy metals (Sherene, 2010) were detected while in Potchefstroom, Aucamp & van Schalkwyk (2003) reported contamination of soils with arsenic, chromium, and copper in abandoned gold mine tailings. In abandoned New Union Gold Mine Tailings of Limpopo Nelushi *et al.* (2013) detected high concentrations of Chromium while Kotzé *et al.* (1999) found that mining activities in the Olifants River estuary in South Africa have contributed to the accumulation of heavy metals in sediments and marine organisms such as mussels, which can ultimately lead to the accumulation of heavy metals in sharks. Although the majority of these mines are from provinces far inland (i.e., landlocked) such as Gauteng, Northwest and even from Limpopo, these heavy metals are carried by rivers such as Klip river (McCarthy *et al.*, 2007), Jukskei river (Rimayi *et al.*, 2019), into the crocodile and Olifants rivers that run through Kruger National Park and on to the Indian ocean.

Other potential sources of heavy metals and POP to this coastal system are wastewater from companies manufacturing car and gadget batteries (lead acid), Iron and steel wire products, as well as from tanking and car wash activities. Heavy metal levels found in this study may also be from the wastewater from these companies as several studies have reported the presence of heavy metals in wastewater from battery manufacturing industries globally and in South Africa. Vu *et al.* (2019) reported a lead concentration (3–15 mg/l) higher than the recommended level in battery wastewater from Korea. Similarly, Ribeiro *et al.* (2018) reported exceedingly high iron (344 ±96 mg/l), zinc (60 ±17 mg/l) and lead (22 ±15 mg/l) concentrations in Lead acid battery industry wastewaters in Brazil. Locally, for example, a study by Agoro *et al.* (2020) found high levels of lead,

cadmium, and chromium in wastewater from battery manufacturing industries in Durban, on the east coast of South Africa. Metal (i.e., iron and steel) producing industries have also been shown to release wastewater containing toxic heavy metals. Metal manufacturers undertake processes such as acid pickling of metal surfaces to remove the oxide layer, rust, encrustations, inorganic contaminants or other impurities from ferrous metals, copper, or precious metals, resulting in wastewater with high heavy metal concentrations (Collivignarelli *et al.*, 2019). For instance, Jooste *et al.* (2014) investigated the levels of heavy metals in water, sediment, and fish from the Olifants River system, which flows to the east coast and is impacted by metal manufacturing activities in the Mpumalanga Province of South Africa. The findings showed elevated levels of heavy metals, including copper, zinc, and cadmium, in the water, sediment, and fish samples, which were attributed to metal manufacturing activities in the region. Tekere *et al.* (2016) also reported toxic concentrations of copper and zinc in carwash effluents in Gauteng Province of South Africa, mainly derived from brake pads and tyres. These studies demonstrate the significant impacts of mining and manufacturing on wastewater, and untreated or partially treated sewage, which are discharged into rivers and ultimately end up in the ocean impacting organisms throughout the food chain and in particular apex predators through bioaccumulation.



Figure 5. Acid mine drainage generated in surface tailings deposits in the West Rand (Coetzee, 2013).

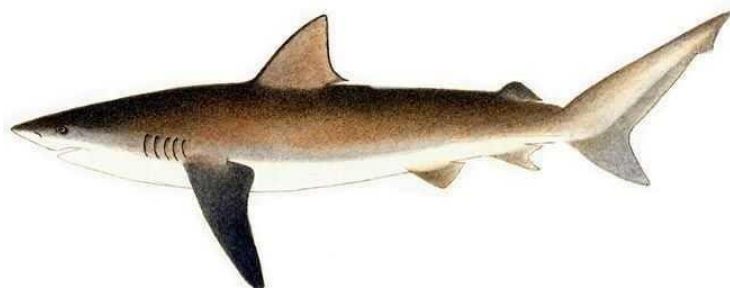
Conclusions

The presence of high levels of heavy metals in sharks can have significant implications for shark health and conservation efforts. High levels of heavy metals in sharks can be an indicator of pollution and can be used to track changes in environmental conditions and inform high-level policy to curb pollution. Statutory authorities and regulatory agencies can use this information to identify sources of pollution and take action to reduce or eliminate them. For shark conservation efforts, high levels of heavy metals are a concern because it can have negative effects on their development, health, and reproduction. This can ultimately lead to declines in shark populations, which can have cascading effects on the health of marine ecosystems.

It is important to continue monitoring the levels of heavy metals in sentinel species such as sharks and to take steps to reduce environmental pollution and so protect their natural habitats. Elevated levels of heavy metal contamination in sharks can have negative consequences on the health of people who consume them as well. Previous studies have reported developmental and birth defects, and detrimental effects on the nervous system and its functions associated with the consumption of fish with high levels of toxins (Grandjean *et al.*, 2010).

In this study, all sampled bronze whaler sharks had levels of arsenic, mercury, and chromium above internationally recommended maximum regulatory limits. This indicates that any bronze whaler shark caught in bays along the southern Africa coastline is likely to contain levels of these metals that make them potentially dangerous for human consumption. It is worth noting that although bronze whaler sharks' consumption is restricted in South Africa, the species is heavily harvested, processed and exported to Australia where it is in high demand and consumed in shark fillets (Duffy & Gordon, 2003; Da Silva & Burgener, 2007; Soest, 2016).

However, it is important to note that the presence of organic arsenic in fish meat, which accounts for most arsenic found, is not considered toxic (De Gieter *et al.*, 2002; WHO, 2011) and is removed by the kidney shortly after intake (WHO, 2011). Similarly, the toxic form of mercury, methylmercury, accounts for only a small amount of the total mercury concentration in fish. Therefore, the mercury and arsenic concentrations found in bronze whaler sharks in this study may not necessarily indicate toxic levels of the metal in the sample. Future research on mercury and arsenic speciation is necessary to estimate the safety of commercial fish meat more accurately in South Africa with regards to total mercury and arsenic contaminants and toxicity. Nonetheless, fishing, and consuming bronze whaler sharks should be limited in the area due to the elevated levels of heavy metals found in this study.



Recommendations

The results of this study lead to several recommendations that could be implemented to reduce heavy metal accumulation in bronze whaler sharks and promote their conservation in South Africa. First, improving water quality by reducing pollution and enhancing water quality in bays and coastal areas where bronze whaler sharks are found. This can be achieved through the regulation of industries and controlling runoff from agricultural and mining activities. In this regard, it is of great concern that waste water treatment plants and stormwater systems along the SCSA and ECSA have been shown to be increasingly defunct and transporting high levels of pollutants into the ocean (Petrik *et al.*, 2017; Ojemaye & Petrik, 2019, 2022; Gani *et al.*, 2021; Aina, 2023; du Plessis, 2023). The water infrastructure, particularly in provinces along the ECSA has steadily decayed over the past two decades due to poor maintenance, lack of human capacity, financial resources, and poor water governance, allowing the deposition of untreated and polluted sewage into various water courses such as the Umgeni River, and are ultimately deposited to the ocean (du Plessis, 2023). This crisis has been compounded by South Africa’s energy crisis – the planned nationwide power cuts known locally as “load shedding”, which can damage the pumps in sewage pump stations or cause sewage overflow due to prolonged power outages (Aina, 2023; du Plessis, 2023).



Figure 6. Dead fish in the Umgeni River as a result of high levels of pollution from failing sewerage infrastructure in Kwazulu-Natal (Carnie, 2023).

The recent flood incident in Kwazulu-Natal has also worsened the situation, by damaging these already ailing and inefficient wastewater treatment infrastructure and stormwater systems leading to immense sewage pollution eroding into aquatic systems and causing high mortality in fish (du Plessis, 2023). A large-scale fish mortality reported in the Isipingo Beach lagoon and Umgeni River mouth (du Plessis, 2023), and reported strandings of up to 50 bronze whaler sharks on Eastern Cape beaches (Sgqolana, 2022) are evidence of the precarious state of waste water infrastructure in South Africa.

Water quality in most beaches along the ECSA is presently rated poor and in some exceptional cases, critical, such as in Umgeni River which empties itself into the Indian Ocean via the Blue Lagoon beach (Mardon & Stretch, 2004). This has led to frequent closures of these beaches and negatively impacted their revenue generation (du Plessis, 2023). The high pollution levels at beaches in this region have also affected their international status as none of the beaches was rewarded the blue flag status award for the 2022-2023 year (Carnie, 2022; Aina, 2023). The blue flag status is awarded to beaches with very low and safe toxic pollutant levels that are safe for tourism purposes.



Figure 7. Bronze whaler sharks strandings in Winterstrand in the Eastern Cape of South Africa (Sgqolana, 2022)

A continued flow of untreated waste from chemical and manufacturing industries, agriculture and wastewater treatment plants into rivers, streams and the ocean are a major concern and can affect public health and aquatic ecosystems and cause major financial losses, especially for the tourism sector. Immediate action must be taken to resolve the issue and prevent further damage to the

environment and public health. There must be a comprehensive strategy in place to address the ongoing issue of water infrastructure decay and the deposition of sewage into water courses.

Secondly, promoting sustainable seafood practices by choosing seafood that has been sustainably harvested. This can reduce the impact of fishing on the marine ecosystem and promote healthy shark populations. Thirdly, improving the technology and infrastructure of wastewater treatment plants. This will remove many pollutants, including heavy metals, before releasing the water back into rivers or directly into the marine environment. Given the high levels of residency and site fidelity of bronze whaler sharks (Rogers *et al.*, 2022), regular monitoring of heavy metal concentrations in this species can help to identify areas of concern and track changes in heavy metal bioaccumulation over time. In addition, educating the public by raising awareness about the risks associated with consuming bronze whaler sharks or other marine organisms with high levels of heavy metal contamination will greatly help reduce demand for these products and promote conservation efforts. These recommendations can be achieved through collaboration between government agencies, research institutions, NGOs, and the public to raise awareness and implement policies that protect the marine environment and promote sustainable practices. By implementing these recommendations, the South African government and people can help reduce heavy metal and POP pollution in the environment and so reduce the bioaccumulation of pollutants in bronze whaler sharks and other marine apex predators.

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Appendix

Table S1. Literature review of publications that have reported on organic chemicals and metals that have been sampled for in different fish species in different parts of the world. The review includes the main aims of the publication and the main findings.

S/N	Location	Species	Sample size	Organic chemicals and metals	Aims	Finding	Reference
1	La Paz Bay, Gulf of California (Mexico)	<i>Rhiodon typus</i>	12	PCB, DDT, PBDE HCB.	To determine the POP and plastic pollution level in whale shark in the Gulf of California (Mexico)	PCBs > DDTs > PBDEs > HCB. Mean concentration values of 8.42 ng/g w.w. were found for PCBs, 1.31 ng/g w.w. for DDTs, 0.29 ng/g w.w. for PBDEs and 0.19 ng/g w.w. for HCB.	Maria <i>et al.</i> 2017
2	Southwest Portugal	<i>Prionace glauca</i>	20	PCB, PBDE, HCB, Al, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Ag, Cd, Pb, and Hg.	To find suitable biomarkers for future marine pollution biomonitoring studies by correlating biochemical responses with tissue contaminant body burden in blue sharks	Concentrations of some contaminants in sharks' tissues were found to be above the legally allowed limits for human consumption	Luis <i>et al.</i> , 2016
3	South-eastern USA	<i>D. sabina</i> , <i>S. tiburo</i> , <i>N. brevirostris</i> , <i>C. leucas</i>	53	PCBs, PBDEs, DDXs, HCB, CHLs	To determine the bioaccumulation of organohalogenated compounds in sharks and rays from the south-eastern USA	The lowest levels of POPs were found in Atlantic stingrays and the highest levels were found in bull sharks.	Weijis <i>et al.</i> , 2015
4	Lower Gangetic delta region of Indian	<i>Glyphis gangeticus</i>	10	Zn, Cu, Pb and Cd	To assess the heavy metal bioaccumulation in <i>Glyphis gangeticus</i>	The accumulation of heavy metals followed the order Zn > Cu > Pb > Cd.	Das <i>et al.</i> , 2015
5	Trinidad and Tobago	<i>S. lewini</i> n=10 <i>C. porosus</i> n=12	22	Hg, As, Cd and Pb	To investigate the levels of heavy metals (Hg, As, Pb and Cd) in two shark species;	Hg levels ranged between 74–1899 µg/kg in <i>S. lewini</i> and 67–3268 µg/kg in <i>C. porosus</i> . As levels ranged between 144–2309 µg/kg in <i>S. lewini</i>	Azad and Terry, 2017

					<i>Sphyrna lewini</i> and <i>Carcharhinus porosus</i>	and 762–6155 µg/kg in <i>C. porosus</i> . Cd levels ranged between 0.27–27.29 mg/kg in <i>S. lewini</i> and 0.6–29.89 mg/kg in <i>C. porosus</i> . Pb levels ranged between 0.14 and 208.81 mg/kg in <i>S. lewini</i> while <i>C. porosus</i> levels ranged between 0.30 and 459.94 mg/kg.	
6	Jeju Island Republic of Korea	<i>C. brachyurus</i> n=1 <i>C. obscurus</i> n=1 <i>I. oxyrinchus</i> n=1 <i>T. scyllium</i> n=1 <i>M. manazo</i> n=1 <i>C. umbratile</i> n=1	6	Cr, Fe, Cu, Zn, As, Se, Cd, Sn, Sb, Pb, Hg and MeHg	Assessment of heavy metal concentration in the muscle tissues of shark species	Concentrations of all metals, except for As, were below the regulatory maximum levels	Kim <i>et al.</i> , 2019
7	Northeast Atlantic Ocean	<i>Prionace glauca</i>	12	Zn, Cu, Pb and Cd	To assess the heavy metal bioaccumulation in blue sharks and other marine species	All metals analyzed were below the detectable limits	Stevens and Brown., 1974
8	East Coast of South Africa	<i>C. carcharias</i>	3	DDT, HCB	To evaluate liver and muscle tissues from white sharks collected off the coast of South Africa for DDT, its derivatives, and other persistent organochlorine pesticides.	Concentrations of DDT and other POPs in liver were significantly higher than the muscle due to higher lipid contents in the liver (70 %) relative to the muscle (0.1 %).	Schlenk <i>et al.</i> , 2005
9	Southwest Indian Ocean	tiger sharks n=21 bull sharks n=18	39	30 PCBs, 5 DDTs, HCB, Dieldrin, Aldrin, isodrin and mirex	Assessment of POP contamination in tiger and bull sharks from Re-union islands	Results showed that organic contaminant levels in the species were lower than those of other shark species in the Southern hemisphere.	Chynel <i>et al.</i> , 2021
10	Coastal Bahamas	<i>C. perezii</i> , tiger shark, <i>C. acronotus</i> , <i>G. cirratum</i> and lemon sharks	36	Cd, Pb, Cr, Mn, Co, Cu, Zn, As, Ag and THg	Assessment of heavy metals in the muscle tissue of coastal sharks from the Bahamas	Caribbean reef sharks exhibited some of the highest metal concentrations compared to the five other species and peaked in the	Shiple <i>et al.</i> , 2021

						concentrations of Pb, Cr, Cu were observed	
11	Southeast-South coast of Brazil	<i>P. glauca</i>	9	Al, Zn, Cr and Cu	To determine metallic trace elements Al, Zn, Cr and Cu in <i>Prionace glauca</i>	Cu, Zn and Al concentration was well below the maximum limits permitted by Brazilian and International agencies, Cr average (0.14mg/kg) was above the limits permitted by Brazilian legislation (0.10 mg/kg)	Vignatti <i>et al.</i> , 2018
12	South Pacific waters	<i>P. glauca</i> n=39 <i>I. oxyrinchus</i> n=69	108	Hg and Pb	To determine the levels of heavy metals in oceanic shark population in South Pacific waters	<i>P. glauca</i> showed greater values of Pb than <i>I. oxyrinchus</i> (p<0.001). large specimens of both species showed high metal concentration and are harmful for consumption especially with the high contributions of Pb in tissues of <i>P. glauca</i> and <i>I. oxyrinchus</i> while sexes showed no statistical differences (p>0.05)	Lopez <i>et al.</i> , 2013
13	Southern Brazilian coast	Blue shark, shortfin mako shark and hammerhead shark (<i>S. zygaena</i>)	39	Hg	To assess Hg accumulation in three shark species inhabiting the Southern Brazilian coast	Heavy metal concentration in 15 out of the 39 fish (38 %) analyzed exceeded the 0.5 $\mu\text{g}\cdot\text{g}^{-1}$ limit recommended by the WHO	Mársico <i>et al.</i> , 2007
14	Southern California	White sharks	20	PCBs and DDTs	To estimate the potential contribution of maternally derived contaminants to observed levels in white sharks from Southern California	POP levels in white sharks were higher than estimated by modelled dietary accumulation with mean liver concentrations of ΣDDTs and ΣPCBs approximately 3-fold higher than modelled levels.	Mull <i>et al.</i> , 2013
15	Baja California Peninsula, Mexico	Silky sharks(<i>C. falciformis</i>) n=15, Blue shark n=21, hammer	91	Hg	Mercury bioaccumulation assessment in the muscle tissue of shark species in the Baja California Peninsula	Hg levels of all shark species exceeded the Mexican government limit for human consumption with <i>P.</i>	Maz-Courrau <i>et al.</i> , 2011

		head n=31, Shortfin mako sharks n=24				<i>glauca</i> having the highest value of 1.96 ± 1.48 µg/g Hg d.w.	
16	South-east Brazilian coast	Brazilian sharpnose shark (<i>R. lalandei</i>) n=45 Caribbean sharpnose shark (<i>R. porosus</i>) n=12 Small eye smooth hound (<i>M. higmani</i>) n=23	80	Hg	Assessment Hg concentrations in three small shark species in the Southeast, Brazilian coast	Concentration ranges were lower compared with values reported for other large shark species of the Southwestern Atlantic Ocean. significant positive correlation occurred between Hg concentrations and individual size, which suggests biomagnification occurrence	Lacerda <i>et al.</i> , 2000
17	Western Equatorial Atlantic Ocean	Night Shark (<i>C. signatus</i>) n=38	38	Hg	Assessment of the total Hg in muscle tissue of <i>Carcharhinus signatus</i> sampled along the western equatorial Atlantic Ocean	average total Hg conc. were consistently greater than the maximum limit for human consumption established by the Brazilian Health Ministry for carnivorous fishes (1000 µg. kg ⁻¹ w.w.).	Ferreira <i>et al.</i> , 2004
18	Japan	Silvertip sharks (<i>C. albimarginatus</i>) n=81	81	Hg, Cd, Zn, Cu and Fe	Analyses of Hg, Cd, Zn, Cu and Fe concentrations in liver samples of silvertip sharks	Hg concentrations increased with increased body length. However, these increases were more prominent in the liver than in the muscle samples and appeared to occur after maturation.	Endo <i>et al.</i> , 2016
19	Sweden	Greenland shark (<i>S. microcephalus</i>)	-	PCBs, DDTs, PBDEs and pentabromoethylbenzene (PBEB)	Organohalogenated compound OHCs determination in a high trophic Arctic shark species, Greenland shark	The highest concentrations were observed for the DDTs, ranging up to 26 µg/g	Strid 2010
20	Khor Musa, South Sudan	Bamboo shark (<i>Chiloscyllium punctatum</i>)	-	Hg, Zn, Cu, Cd and Pb	Assessment of heavy metals in the liver and muscle tissues of Bamboo shark	Liver: Zn>Cu>Hg>Pb> Cd Muscle: Zn > Hg > Cu. Only Hg level is higher than standard limits.	Hashemi <i>et al.</i> , 2018

Table S2. Heavy metal concentrations (mg/kg) and biological data of the sampled sharks.

Sample #	Region	Location	Total Length (cm)	Mass (kg)	Sex	Latitude	Longitude	Al	As	Cd	Cr	Cu	Fe	Hg	Mn	Pb	Zn
BWS01	SCSA	Stillbaai	99.9	4.4	M	-34.371	21.440	11.4	15.92	0	0.25	0.83	18.04	1.12	1.24	0	6.39
BWS02	SCSA	Stillbaai	76.7	2.2	M	-34.371	21.440	12.02	17.92	0	0.16	0.79	14.88	1.02	1.44	0	7.47
BWS03	SCSA	Stillbaai	79.1	2.1	M	-34.371	21.440	157.35	47.36	0	0.24	0.7	19.11	1.38	1.21	0	6.21
BWS04	SCSA	Stillbaai	82.3	2.8	M	-34.371	21.440	14.42	20.02	0	0.35	0.9	20.17	1.05	1.47	0.1	7.34
BWS05	SCSA	Stillbaai	81.4	2.5	M	-34.371	21.440	5.57	6.1	0	0.09	0.41	9.39	1.91	1.01	0	3.99
BWS06	SCSA	Stillbaai	74.4	1.9	M	-34.371	21.440	7.72	13.63	0	0.14	0.49	12.52	0.96	1.35	0	3.09
BWS07	SCSA	Stillbaai	94	5.0	F	-34.371	21.440	26.86	15.24	0	0.13	0.46	7.88	1.88	2.19	0	4.22
BWS08	ECSA	Ramsgate	220.2	73.5	F	-30.889	30.349	6.26	32.59	0	0.77	0.41	11.47	5.24	0	0	14.98
BWS09	ECSA	Umgababa	199	51	F	-30.142	30.830	15.94	28.25	0	1.03	0.31	20.81	3.02	0.1	0.08	13.2
BWS10	SCSA	Stillbaai	81.1	2.8	F	-34.371	21.440	9.81	11.8	0	0.19	0.47	3.57	1.84	1.76	0	4.62
BWS11	SCSA	Kalk Bay	160	56	M	-34.130	18.449	10.56	29.21	0	0.44	0.93	10.8	3.69	0.89	0	3.87
BWS12	SCSA	Stillbaai	118.3	7.9	F	-34.371	21.440	3.94	9.22	0	0.06	0.83	1.03	1.41	0.97	0.13	4.88
BWS13	SCSA	Stillbaai	94.3	4.1	F	-34.371	21.440	4.73	13.25	0	0.09	0.48	6.95	2.46	1.8	0	3.55
BWS14	ECSA	Leisure bay	238	92	M	-31.026	30.240	13.91	8.4	0	0.92	0.5	23.23	5.38	0.43	0	13.43
BWS15	ECSA	Trafalgar	268.2	120	M	-30.958	30.301	55.94	6.55	0.07	1.22	0.55	68.9	9.51	0.15	0.08	16.29
BWS16	ECSA	Margate	250	103	M	-30.860	30.370	5.36	12.49	0.24	0.89	0.56	23.5	5.07	0	0.07	15.83
BWS17	ECSA	Port Edward	243	90.5	M	-31.052	30.230	6.78	7.99	0	0.72	0.38	15.56	4.95	0	0	14.63
BWS18	ECSA	Umtendwe	248	104	M	-32.741	28.287	8.48	14.4	0	2.54	0.52	26.03	4.93	0.52	0	11.67
BWS19	ECSA	Trafalgar	209	65	F	-30.958	30.301	0	15.55	0	0.14	0.96	1.85	5.2	0.35	0	12.31

BWS20	ECSA	South Port	233	69.5	F	-30.700	30.492	247.01	10.49	0.07	0	0.73	7.01	3.55	0.65	0.05	14.53
BWS21	ECSA	South Port	208	64	F	-30.700	30.492	2.2	7.18	0.11	0.39	1.09	36.48	13.77	0.72	0.06	18.88
BWS22	ECSA	Trafalgar	267.2	110.5	M	-30.958	30.301	0	14.24	0	0.22	0.52	22.75	5.96	0.56	0.05	9.81
BWS23	SCSA	Gansbaai	300	133	F	-34.574	19.342	85.59	21.3	0.11	0.4	2.12	371.47	4.2	1.7	0.07	29.39
BWS24	ECSA	Leisure bay	224.7	67	F	-31.026	30.240	8.47	14.87	0.1	0.08	0.62	15.25	4.96	0.75	0	12.97
BWS25	SCSA	Stillbaai	80.5	2.5	F	-34.371	21.440	24.53	13.03	0	0	0.23	14.13	0.24	0.87	0	11.5
BWS26	SCSA	Stillbaai	90.8	3.1	M	-34.371	21.440	37.2	15.67	0	0	0.48	2	0.39	0.76	0.05	13.39
BWS27	SCSA	Stillbaai	84.9	2.5	F	-34.371	21.440	12.63	19.3	0	0	0.48	2.59	0.63	0.39	0	5.94
BWS28	SCSA	Stillbaai	86.4	2.9	F	-34.371	21.440	30.22	11.65	0	0	0.34	11.93	0.78	0.85	0.08	14.09
BWS29	SCSA	Stillbaai	90.8	3.1	F	-34.371	21.440	11.06	13.27	0	0.12	0.13	7.46	0.54	0.48	0.17	8.36
BWS30	SCSA	Stillbaai	121.5	7.6	F	-34.371	21.440	15.01	18.31	0	0	0.29	2.02	1.02	0.72	0.17	7.74
BWS31	SCSA	Stillbaai	105.4	5.2	M	-34.371	21.440	17.03	15.09	0	0.13	1.12	2.09	0.77	1.11	0.16	22.44
BWS32	SCSA	Stillbaai	98.1	4.3	M	-34.371	21.440	238.81	19.3	0	0	0.65	3.19	0.35	0.76	3.56	12.86
BWS33	SCSA	Stillbaai	104	4.7	F	-34.371	21.440	304.42	23.02	0	0	1.52	0	0.36	0.63	0.16	20.15
BWS34	SCSA	Stillbaai	99.6	6.2	M	-34.371	21.440	19.92	17.99	0	0.17	0.71	0	0.37	0.85	0.25	35.2
BWS35	SCSA	Stillbaai	93.7	3.5	F	-34.371	21.440	42.65	14.46	0	0	0.67	0	0.52	0.54	0	11.81
BWS36	SCSA	Stillbaai	89.6	3.0	F	-34.371	21.440	59.67	9.35	0	0.08	0.88	0	0.41	0.7	0.09	14.35
BWS37	SCSA	Stillbaai	120.1	8.8	M	-34.371	21.440	21.34	44.21	0	0.18	0.53	0	0.5	0	0	12.61
BWS38	SCSA	Stillbaai	84.9	2.9	M	-34.371	21.440	20.74	13.65	0	0.18	0.75	0	0.82	1.03	0.26	70.48
BWS39	SCSA	Stillbaai	103.1	3.9	F	-34.371	21.440	14.71	19.26	0	0.12	0.78	0	0.72	0.26	0.06	13.96
BWS40	SCSA	Stillbaai	86.4	2.6	M	-34.371	21.440	14.91	12.1	0	0.15	0.62	13.15	0.29	0.61	0.07	15.15
BWS41	SCSA	Stillbaai	83.7	2.4	F	-34.371	21.440	21.4	6.99	0	0.14	0.62	0	0.6	0.82	0	18.2

Table S3. PCB concentrations ($\mu\text{g}/\text{kg}$) and biological data of the sampled sharks.

Sample #	Region	Location	Total Length (cm)	Mass (kg)	Sex	Latitude	Longitude	PCB-28	PCB-52	PCB-101	PCB-118	PCB-138	PCB-153	PCB-180	PCB-194
BWS01	SCSA	Stillbaai	99.9	4.4	M	-34.371	21.440	0	0	0	0	0	0	0	0
BWS02	SCSA	Stillbaai	76.7	2.2	M	-34.371	21.440	0	0	0	0	0	0	0	0
BWS03	SCSA	Stillbaai	79.1	2.1	M	-34.371	21.440	0	0	0	0	0	0	0	0
BWS04	SCSA	Stillbaai	82.3	2.8	M	-34.371	21.440	0	0	0	0	0	0	0	0
BWS05	SCSA	Stillbaai	81.4	2.5	M	-34.371	21.440	0	0	0	0	0	0	0	0
BWS06	SCSA	Stillbaai	74.4	1.9	M	-34.371	21.440	0	0	0	0	0	0	0	0
BWS07	SCSA	Stillbaai	94	5.0	F	-34.371	21.440	0	0	0	0	0	0	0	0
BWS08	ECSA	Ramsgate	220.2	73.5	F	-30.889	30.349	0	0	0	0	0	0	0	0
BWS09	ECSA	Umgababa	199	51	F	-30.142	30.830	0	0	0	0	0	0	0	0
BWS10	SCSA	Stillbaai	81.1	2.8	F	-34.371	21.440	0	0	0	0	0	0	0	0
BWS11	SCSA	Kalk Bay	160	56	M	-34.130	18.449	0	0	0	0	0	0	0	0

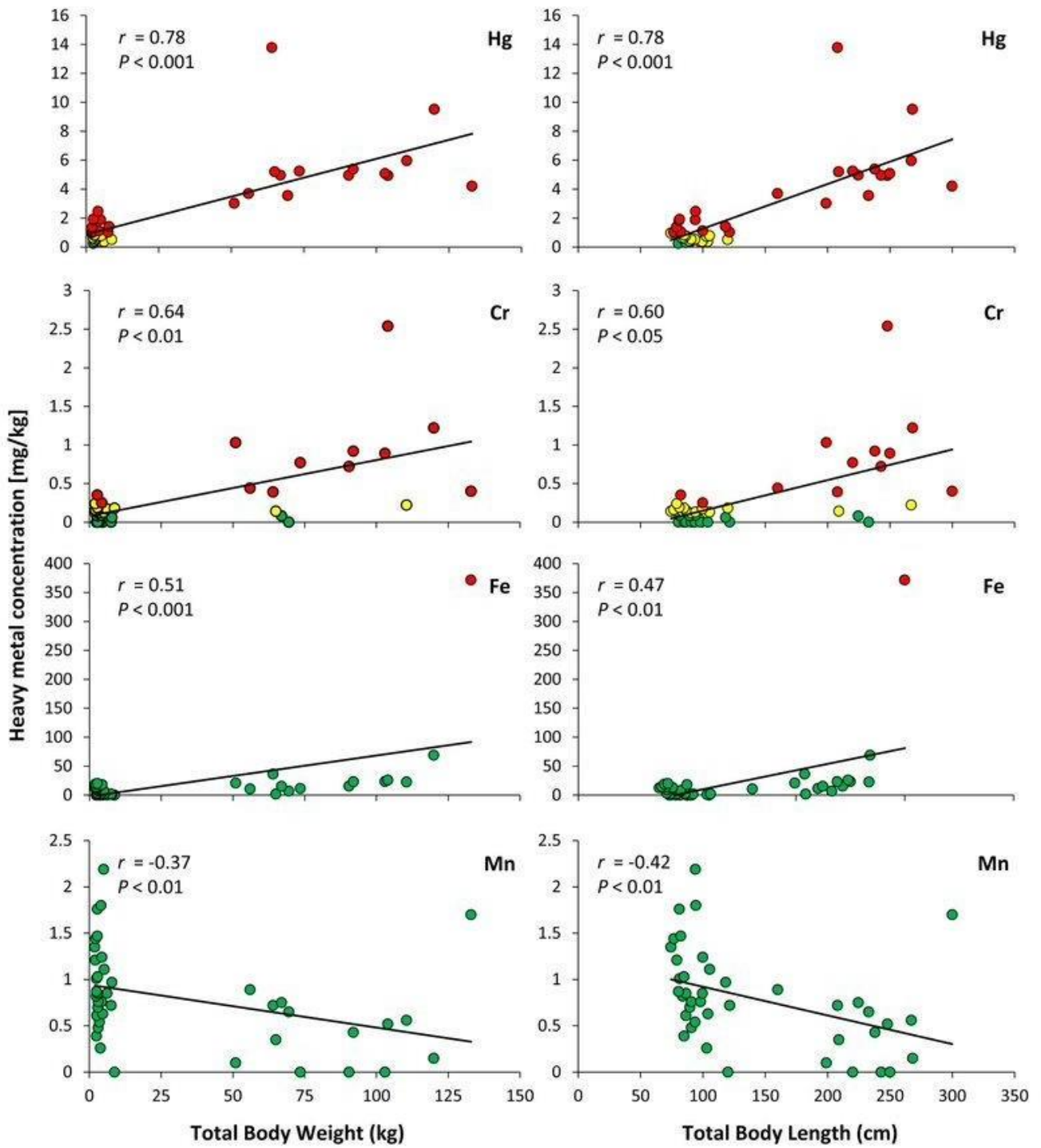


Figure S1. Correlation between metal concentration and Total body size in bronze whaler sharks

List of Acronyms

CODEX	CODEX Alimentarius collection of food standards
DOH	Department of Health (South Africa)
EU	European Union
FAO	Food and Agricultural organization
JECFA	Joint FAO/WHO Expert Committee on Food Additives
KFDA	Korea Food and Drug Administration
SCF	Scientific Committee on Food
UNEP	United Nations Environment Program
POP	Persistent Organic Pollutant
PCB	Polychlorinated Biphenyls
SCSA	South coast of South Africa
ECSA	East coast of South Africa
WCSA	West coast of South Africa
NAM	Namibia
DDT	Dichlorodiphenyltrichloroethane
HCH	Hexachlorocyclohexane
HCB	hydrochlorobenzene
WHO	World Health Organization
AAS	Atomic Absorption Spectroscopy
ICP-OES	Inductively Coupled Plasma – Optical Emission Spectroscopy
GC-MS	Gas Chromatography–Mass Spectrometry
PBDE	polybrominated diphenyl ethers
PCDD	polychlorinated dibenzodioxins
PCDF	polychlorinated dibenzofurans
PCN	Polychlorinated Naphthalene
SCPOP	Stockholm Convention on Persistent Organic Pollutants
LLE	Liquid-Liquid Extraction
SET	Soxhlet Extraction Technique
MAE	Microwave-Assisted Extraction
ASE	Accelerated-Solvent Extraction
SPE	Solid-Phase Extraction
CHL	Chlordane
GC-ECNIMS	Gas Chromatography linked Electron Capture Negative Ion Mass Spectrometry
MeO-PBDE	methoxylated polybrominated diphenyl ethers
GC-LRMS	Gas Chromatography linked Low-Resolution Mass Spectrometry
FAO	Food and Agriculture Organization
MFA	Major Fishing Areas
Dw	dry weight