Concentrations and compositions of metallic elements in commercially important marine species

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Preface

My dissertation concerns the metal compositions and concentrations of 5 commercially important marine species. This dissertation is presented in an article format. I have already submitted two articles for review; Regional Studies in Marine Science, and African Zoology.

Outline of dissertation: This dissertation is presented in four chapters; a short description of each follows:
Chapter 1: A general introduction to my study, concluded by the problem statement, aims, objectives, and hypotheses.

Chapter 2:
Concentrations and relative compositions of metallic elements differ between predatory squid and filter-feeding sardine from the Indian and South Atlantic oceans
This is the first article in this dissertation and has been submitted to Regional Studies in Marine Science for review in November 2018. The article was accepted for publication January 2020. This article focuses on the patterns of metal concentrations and compositions in sardine and chokka from the South Atlantic and Indian oceans. This is the first study to compare metal concentrations and compositions in muscle tissue of these species between the Indian and South Atlantic oceans.

Chapter 3:
Differences in metal compositions and concentrations of sympatric predatory fish and squid from the South Atlantic Ocean
This is the second article and has been submitted to African Zoology for review in November 2019. This article focuses on the metal concentrations and compositions of four commercial marine predators; chokka, hake, kingklip, and monkfish in the South Atlantic Ocean. This was the first study to compare the metal concentrations and compositions of these species off the coast of Southern Africa

Chapter 4: Discussion and conclusions
In this chapter, the results of Chapter 2 and 3 are combined, evaluated, discussed, and synthesized.

I contributed towards this dissertation and articles with literature studies, collecting data, sourcing materials, preparation of samples for analyses, data analyses and interpretation, constructing the tables and graphs, drafting and managing the manuscripts, and drafting the introduction and conclusion of the dissertation. The co-authors for the chapters contributed only at the latter stages with corrections and changes. I have been provided with permission to submit these articles for my dissertation by all co-authors of the articles

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Table of contents

Differences in metal compositions and concentrations of sympatric predatory fish and squid from the South Atlantic Ocean

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- I would like to thank my friends; without you, I would have surely lost my marbles long ago.
- To my family, these years were trying for us as a family. Thank you for your love and for supporting me fully in my endeavours no matter how ambitious.
- To God thank you for the talents and people you have gifted me with.

In loving memory of Ouma Miem and Roné Uren

Concentrations and compositions of metallic elements in commercially important marine species

Abstract

Metals have been in our environment since the genesis of the world and are ubiquitous in all environments. We as humans mine, utilize, and release these metals from the environment to a level that may affect chronically or acutely environment and human health. To that sense, information on metal concentrations are valuable in commercially marine species as they are an important food source to many communities. Fish and molluscs are also good bioindicators and biomonitors as they can indicate the health of the environment, they are found in. There is, however, an information gap about metal concentrations in the marine environment off the coast of South Africa.

In this study, sardines (*Sardinops sagax*) and chokka (*Loligo reynaudii*) from the Indian and the South Atlantic oceans, and hake (*Merluccius capensis*), kingklip (*Genypterus capensis*), and monkfish (*Lophuis vomerinus*) from the South Atlantic Ocean were analysed for metals using an inductively-coupled plasma mass spectrometry. These species, representing two trophic levels, are exploited in large quantities for human consumption. More knowledge on metal composition will shed light on the biological relationships between the species, possible impacts elevated metal concentrations might have on the fish, and on human and predator consumer health.

Metal concentrations and compositions of each species were established and compared. Significant differences in metal concentrations were found between sardine, chokka and the demersal species (hake, kingklip, and monkfish). As was expected, higher trophic levels (hake, kingklip and, monkfish) contained significantly higher concentrations of metals than lower trophic levels such as sardines. Chokka contained significantly lower concentrations of vanadium, cobalt, molybdenum, and manganese than sardines, hake kingklip, and monkfish, but significantly higher concentrations were found for boron, titanium, zinc, cadmium, rubidium, and strontium. These patterns could be caused by physiological regulation or lack of regulation of these metals in chokka. Demersal predators' metal concentrations and compositional patterns did not differ significantly between each other, probably due to a trophic overlap in diet.

Metal compositions or fingerprints were distinct between sardines and chokka from different oceans. This indicates metal compositional patterns could be used as a possible stock discrimination tool. Sardines and chokka metal compositions were distinct from the demersal predators. Demersal metal compositions overlapped, once again possibly caused by diet similarity. The effect of physical parameters (length and mass) on metal concentration was assessed for chokka, hake, kingklip, and monkfish. Negative regressions were found for most metals. However, a positive regression was found for chromium with kingklip length that could be cause for consumer (animal and human) concern. Compared with other studies from around the world cadmium, lead, and mercury concentrations were lower or on par in all species.

Cadmium, lead, and mercury concentrations in the muscle tissue of all species were assessed for human consumer safety. Concentrations were found to be below the limits set by South Africa and the European Union for these fish. Estimated daily intake and the total hazard quotient (for South

African adults) was calculated for cadmium, lead and mercury. All values were below the quotient of unity. Based on my study it can be concluded that the species studied would be safe for human consumption, but data from more fish from more sites would be needed for confirmation.

For the first time, sardines, chokka, hake, kingklip, and monkfish metal concentrations and compositions are compared. Also for the first time, metal concentrations and compositions are compared between sardine and chokka sampled from the South Atlantic and Indian oceans. This study provides valuable information on these commercial species and narrows the information gap currently present on commercial marine species off the South African coast. I also stress the need for more samples from more sites, the need to sample more species, the need to sample higher predators such as dolphins and sharks, and the possibility that metal compositions may be used in stock discrimination.

Keywords: Fish, squid, metals, cadmium, lead, mercury, South Atlantic Ocean, Indian Ocean

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Chapter 1: Introduction

1.1 What are metals?

Metals or metallic elements are defined as a solid substance has a metallic lustre, is malleable and ductile, conducts electricity, has basic oxides, and can form cations (Kotz et al., 2012). Not all metals fall perfectly into this mold and might be exceptions to the rule as there always is in chemistry.

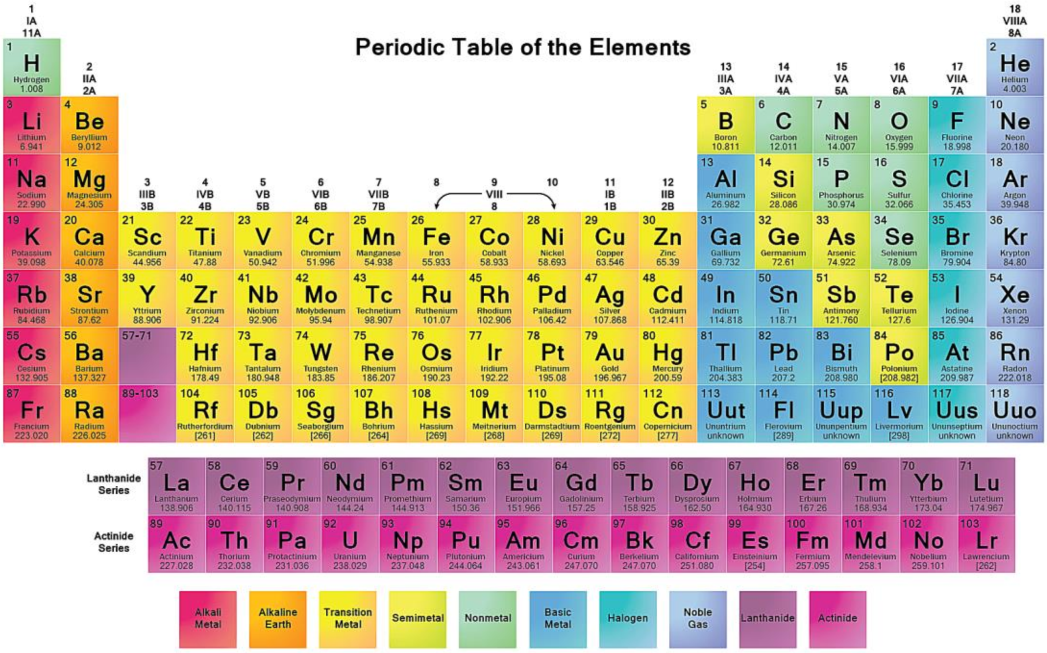

Figure 1: The periodic table of elements. Names and abbreviations of the elements that are also used in the text, their groups, and type of element is provided (Free use permitted; Helmenstine, 2013).

The periodic table (Figure 1) groups metals according to their atomic number and specific properties they share. Alkali metals and alkali earth metals are situated in the first and second group of the periodic table. These metals lose their electrons easily, becoming cations (Kotz et al., 2012). In this study, I will only be focusing on sodium (Na), potassium (K), and rubidium (Rb) from group one, and all of the second group metals except for radium (Ra) for analyses of muscle tissue content. Transitional metals make up most of the remaining periodic table and are placed in groups 3-12. Transition metals all have partially filled d-subshell orbits and can readily bind to form cations (Kotz et al., 2012). Included in the transition metals are rare earth metals such as uranium (U), and thorium (Th) that are both radioactive and naturally found in the environment. Transitional metals that were analysed in this study include titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), molybdenum (Mo), palladium (Pd), silver (Ag), cadmium (Cd), platinum (Pt), gold (Au), and mercury (Hg). In this study, aluminium (Al), thallium (Tl), lead (Pb), and bismuth (Bi) are the only post-transitional metals that were analysed. These metals all have characteristics of transitional metals but have a lower boiling point and are generally softer than all the transitional metals. Metalloids are the last group of "metals" as they are not by definition metals but have some metallic properties (Kotz et al., 2012). They are analysed as they are toxic to biota at high concentrations. Metalloids analysed in this study will include boron (B), arsenic (As), Antimony (Sb) and selenium (Se).

In ecotoxicology papers, metals are generally classified into three groups. ‘Heavy metals’ (Wang et al., 2019, Kumar et al., 2019; Swaleh et al., 2019) are generally referred to as metals that have a density > 5 g/cm^3 (Duffus, 2002). This definition, however, has been loosely used to describe other possibly toxic metals such as As which has a density of 1.97 g/cm^3 and in actuality is a metalloid. Heavy metals also have high toxicity such as Pb, Cd, and Hg. ‘Trace metals or elements’ are generally referred to as metals that are found naturally in the environment and may play a role in biology but can be toxic if high concentrations are accumulated (Gbogbo et al., 2019; Avigliano et al., 2019; Bouchoucha et al., 2019). Lastly, ‘metalloids’ can be highly toxic (Sara et al., 2018; Ali and Khan, 2019; Copat et al., 2019).

1.2 Sources of metals in the environment

Sources of metals in the environment range from natural to anthropogenic. Natural sources of metals in the environment tend to be of geological origin that includes weathering of metal-bearing rocks and volcanic eruptions (Garret, 2000). As the human population increases, industry and refinement of metals have led to an increase of anthropogenic metals. Major anthropogenic sources of metals in the environment are industrial and agricultural activities (O’Donoghue and Marshall, 2003; Greenfield et al., 2011; Wepener and Degger, 2012; Nel et al., 2015; du Preez et al., 2018). A wide range of metals is released during mining and extraction (Macklin et al., 2003). These metals can enter the atmosphere through smelting, refining, and other industrial processes, which will eventually be deposited through dry or wet deposition (Gromping et al., 1997). Domestic sewage and industrial effluents facilitate the release of metals to the environment through discharge as wastewater (Joshi et al., 2011). Metals can be added to agricultural soils through fertilizers. Eventually, these metals are leached into groundwater, possibly accumulating to high concentrations (Atafar et al., 2008). Combustion of fossil fuels is one of the major contributors of heavy metals such as Cr, Zn, Cd, Hg, and Pb in the environment (Zoller et al., 1974; Luo et al., 2015).

1.3 Essential and non-essential metals

Essential metals are metals essential to all living things. These metals can either be abundant in the environment such as magnesium (Mg), calcium (Ca), Na, K, and P or are trace metals (As, Co, Cr, Cu, Fe, Mn, Mo, Sb, Se, tin (Sn), Ti, V, tungsten (W), and Zn) that are found in small traces in most living things and in the physical environment (Rainbow, 2018). Metals (in their cation state) have high affinities to organic molecules. This leads to the incorporation of many of these metals in the key metabolic interactions they have today (Amiard et al., 2008). This is seen as Zn acts as the catalytic centre for many enzymes including carbonic anhydrase in the metabolic cycle (Rainbow, 2018). An interaction that is well known to the layperson is the incorporation of Fe in haemoglobin to transport oxygen through in most vertebrates. This affinity, however, also causes the basis of their toxicity. Although essential at lower concentrations, essential metals above certain thresholds can cause acute or chronic effects to biota.

Non-essential metals (metals not mentioned above) play no role in the biology of organisms but are still naturally found at comparatively low (for example Au, Pt, and Ag) and high concentrations (for example Cd, Rb, and Sr) in the environment (Rainbow, 2018). These elements may be toxic to organisms as these metals may have a high affinity for organic molecules in the body just like essential metals. Non-toxic and non-essential metals (such as Rb and Sr), as for essential metals, may have toxic thresholds where over acute and toxic effects can occur (Amiard et al, 2008). Some non-essential metals may be toxic at very lose doses (such as Cd and Pb (Béchard et al, 2007)) and are in some cases highly volatile as in the case of Hg (Bellas et al, 2001).

1.4 Metal toxicity

Metals are one of the oldest known toxicants - they are part of the earth's crust and have been part of human existence since our genesis. Unlike organic and organo-metallic contaminants such as POPs (persistent organic pollutants), PAHs (polycyclic aromatic hydrocarbons), and TBT (tributyltin), we cannot, except for rare instances, manufacture elemental atoms (Jan et al., 2008). We merely release and concentrate metals that were already available in the environment into confines that may eventually reach levels of concern. The nature of the elements affects many important factors such as bioabsorption, biotransformation, and toxicity (Pagnanelli et al., 2003). Humans may also alter the forms of metals that are not toxic and cause these metals to have increased toxic properties such as for TBT. The reason metals are such a prevalent contaminant is that unlike other toxicant molecules, metallic atoms cannot be broken down.

Biomagnification is defined as the uptake of substances or elements that is absorbed from the intestines of an organism (Gray, 2002). This limits the definition to only the secondary and tertiary consumer as this is the primary pathway for these organisms in the environment (Gray, 2002). Bioconcentration is defined as the direct uptake of a substance from the environment, such as from air for air-breathing organisms, or from water in the case of most aquatic organisms, through tissue such as skin, lungs, and gills (Mackay, 2000). Highly volatile compounds, for instance, maybe taken up from the air via lungs such as mercury (Hg). Bioaccumulation is a combination of biomagnification and bioconcentration.

Humans and most animal species take up and/or accumulate metals through their diet. Uptake and/or accumulation is affected, inter alia, by metal solubility, molecular species, pH, the secreted ligand in the guts, the rate of transport through mucous layers, and the presence and density of transport proteins of the organism (Jakimska et al., 2011).

Organisms have limited detoxification processes for metals such as chelation; metals normally become less toxic by being bound with organic molecules (chelating agent). Generally, the chelating agent contains S, N, or O (Sears, 2013). A chaperone protein called metallothionein (MT) is a well-known chelating agent that binds to metals which gives them less chance to react with sensitive molecules in the body (Newman, 2015). After binding of the metal to MT, the resulting chelate can then be stored in detoxifying organs such as the liver (Lobel et al., 1991), or transported out of the body (Gregus and Klaasen, 1986). Since metal atoms cannot be metabolized, they can only be stored or eliminated out of the body through excretion. Storing in the body can result in the accumulation of metals. Even if the storage of metals in organs is protective of the organism, the metals themselves may accumulate further into higher trophic levels with the potential to cause acute or chronic effects.

1.5 Effects of metals on organisms

Contaminants can affect the bodies of organisms on multiple levels simultaneously. Metals can produce a stress response in the body of organisms causing heat-shock and MT proteins to be activated to detoxify and protect the body. However, metals may cause severe harm to organisms as they can affect multiple biological processes. The following is a summary, based on Newman (2014):

- **Growth**

Growth is a sub-lethal effect that is tested in many studies. It is an easy endpoint for which to measure the effects of toxic elements or substances. Growth is governed by a host of biochemical and physiological factors making it a multifaceted endpoint to measure. Low concentrations of contaminants may already have an effect on growth and thus makes growth a very sensitive parameter to test. Metals have been proven to have negative effects on the growth of fish and molluscs (Cai et al., 2019; Ding et al., 2019; Russel et al.,2019; Xie et al., 2019; Zheng et al., 2019)

- **Development**

Contaminants can adversely affect the development of an organism through various stages such as the egg, embryo, larva, or juvenile. These effects can range from malformation, deficiencies of important compounds, delayed development, or death. Metal effects on the larvae and embryos of fish have been shown by many studies (Jezierska et al., 2008; Sierra-Marquesz et al., 2019; Gao et al; 2019; Reinhardy et al., 2019)

- **Developmental stability**

Metals may affect the capacity of an organism to develop a constant phenotype. This effect is directly linked to the survival and fitness of an organism. If a constant phenotype (such as colour and symmetry) is not achieved, reduced survival and reproduction is possible. Metals may cause the divergence from the ideal phenotype in fish and molluscs with deleterious effects to survival (Abdelhandy, 2016; Ding et al., 2016).

- **Reproduction**

Contaminants can also affect the reproductive systems of an organism decreasing (or increasing) the offspring it can produce threating the population of that organism. Reproductive effects such as endocrine disruption of metals on fish have been documented in various studies (Mukherjee et al., 1994; Athikesavan et al., 2006; Ackermann, 2008; Correia et al., 2010).

- **Physiology**

Contaminants commonly cause changes in physiology impairing normal homeostasis or normal functioning. This includes feeding, swimming, respiration, neuromuscular activity to name a few. There are numerous studies indicating the physiological effects metals have on fish (Benoit, 1976; Gill and Epple, 1993; Radi and Matkovics, 1998; Alwan et al., 2009;; Cai et al., 2019; Russel et al, 2019; Zebral et al., 2019).

- **Immunology**

Contaminants can affect the immune response of organisms either decreasing or increasing the immune system to a level such that the body is harmed by the resulting immune response. Effects on immunity have been observed pertaining to metals in fish (Liao et al, 2004; Datta et al., 2009; Vera-Candioti et al., 2011; Al-Ghamin, 2011)

- **Behaviour**

Behavioural toxicology is the study of altered behaviour caused by the exposure to an element or compound. This can include preference or avoidance, activity level, feeding, performance, learning, respiratory activity, etc. The effects of metals on behaviour have been extensively studied and demonstrated (Beyers and Farmer, 2001; Scott et al., 2003; Webber and Haines, 2003).

1.6 Molluscs as bioindicators

A bioindicator is an organism that allows the assessment of the health of the organism (Markert et al., 2003). However, a biomonitor is defined as an organism that contains quantifiable information on the health of the environment such as metal concentrations (Markert et al., 2003). A biomonitor is a bioindicator as well, but the obverse is not always the case, as a bioindicator cannot always meet the requirements of a biomonitor.

Bioindicators and biomonitors can be further classified as follows (Markert et al., 2003):

- Accumulation bioindicators and biomonitors are organisms that can accumulate single or multiple elements and compounds from their environment, for example, fish that accumulate POPs and metals.
- Effect or impact bioindicators and biomonitors are organisms that demonstrate effects in response to a certain substance or element or the mixture effect of substances and elements. These effects would include negative effects on growth, morphology, reproduction, and behaviour.
- Reaction indicators would be classified as a mixture of accumulation indicators and effect indicators.

Molluscs are well suited to act as bioindicators or biomonitors of an environment. Even though many of these characteristic listed will be shared with other groups of the animal kingdom, the molluscs have a unique combination, including (Oehlmann and Schulte-Oehlmann, 2003):

- Gastropods and bivalves are ubiquitously spread around the world and mediums (terrestrial, freshwater, and marine).
- Most molluscs, especially those living in the aquatic environment, have a wide distribution making them well-suited for large geographical studies. Certain genera are also cosmopolitan such as the mussel genus *Mylilus*.
- Molluscs can be keystone species in ecosystems; pollutants that may affect them may severely influence a whole food web or community.
- Certain gastropod and bivalve species have limited mobility as in the case of sessile adult mussel species. These organisms are thus a good representation of their habitat.
- Molluscs reproduce in various ways; contaminants may thus have different effects depending on the type of reproduction.

- Molluscs exhibit various life-cycle-strategies, especially with the longevity of species. Many cephalopods have lifespans as short as a year, while some bivalves and large marine snails may live for up to 400 years (Ridgway et al., 2011). This means that many generations can be studied in quick succession for short-lived species. With long-lived species, long-term studies can be undertaken.
- Most mollusc species are large enough to be easily handled, meaning that they can be used for laboratory and field studies.
- Compared with other taxa such as arthropods and vertebrates, molluscs have a limited ability to excrete pollutants (Langston et al., 1989). Consequently, molluscs may attain high concentrations of pollutants from the environment. Since there are limited ways for these organisms to excrete pollutants, a lower environmental concentration may affect them more severely than fish species.
- Molluscs are highly sensitive to environmental pollutants and stress. This can be indicated by the high number of mollusc species on “red lists” worldwide.
- The normal morphological and histological structure of organs and tissue of the mollusc are well documented in the literature, with physiology well documented for the laboratory species.
- Many species and genera of mollusc are consumed by humans, making information on pollutants crucial as it may affect human health.

1.7 Fish as bioindicators

Fish have been used widely for decades to assess the health of the ecosystems where they are present. Fish have characteristics that make them good bioindicators and biomonitors; the characteristics are listed as follows, based on Chovanec et al. (2003).

- Fish are highly sensitive to a wide range of contaminants and severe effects have been observed.
- Fish are cost-effective bioindicators and biomonitors.
- Many species are readily identifiable.
- Fish have always been the traditional organism of study for biomonitoring of aquatic ecosystems. This has led to a large body of knowledge on this group. This includes standardized methods of determining the concentrations and toxic effects of contaminants in the environment, handling, and breeding of model fish species.
- Different habitat requirements such as abiotic environmental variables and factors may affect the larval and juvenile stages of fish, providing various life stages for study (Mélard et al., 1996).
- Fish are known to migrate according to their habitat and prey preferences. Some species may also move from saltwater to fresh such as salmon (Hodgson and Quinn, 2002). Some fish may also migrate between different depths of the ocean; for example, hake that migrate from deep waters to the pelagic zone to find prey (Durholtz et al, 2015). This allows fish to be bioindicators of wide geographical and depth stratification.
- Fish species are generally large enough that analytical test can be performed on the different organs.
- Fish have long life spans, allowing in many cases accumulation of high concentrations of a contaminant. Fish can thus bioindicate the health of an ecosystem integrated over time.

- Fish are present in the ecosystem as primary, secondary, and tertiary consumers, making the contaminant information valuable as it may reflect the various trophic conditions of an aquatic environment.
- Historic analysis of fish is a boon of bioindicator data as pristine or less polluted conditions in the past can be referred to the current condition of the ecosystem that is being studied.
- Fish often offer top-down approaches to assess the changes in aquatic ecosystems. Conversely, fish may provide a bottom-up assessment as well because of their trophic diversity in an ecosystem.

Fish are useful bioindicators and biomonitors, however, problems may arise:

- Fisheries may cause different effects on a fish community such as stocking, species transfer, and overfishing. This may affect the conclusions of environmental studies.
- The characteristic of migration of fish may also be a negative factor as the source, duration, and time of exposure of a pollutant may not be readily known.

1.7 Conclusions

Metals are ubiquitous in the environment and the ocean is no exception. However, there is a gap on information on metal concentrations in the commercial marine species of South Africa. The species I chose for my study, sardine (*Sardinops sagax*), chokka (*Loligo reynaudii*), hake (*Merluccius capensis*), kingklip (*Genypterus capensis*), and monkfish (*Lophuis vomerinus*) all represent different links in a large food web and different biologies (Figure 2). Thus, differences in metal concentrations and compositions are expected. These species are all commercially caught and are an important part of the South African economy as export products (WWF, 2014). This study will provide metal concentration and compositional information on sardines, chokka, hake, kingklip, and monkfish.

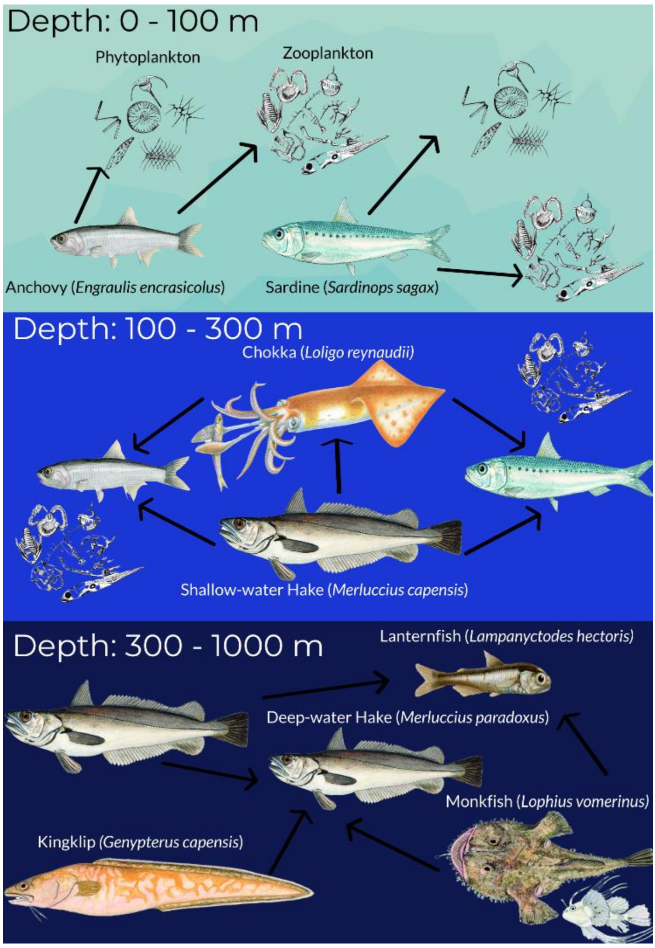

Figure 2: Simplified diagram of the food web and depth stratification of sardine, hake, kingklip, monkfish, and chokka in the South Atlantic Ocean (Free use permitted; Adapted from SAIAB, 2019).

1.8 Aims, objectives, and hypotheses

Aims:

1. To determine the metal concentrations in the muscle tissue of five commercially important marine species (sardine, chokka, hake, kingklip, and monkfish).

2. To determine metal concentration and compositional differences within and between these species from different oceans and sites, and explain any patterns uncovered.

3. To assess, if metal concentrations in these species pose any threat to human consumers.

Objectives:

Objectives for aim 1.

- To collect whole specimens of sardine, chokka, hake, kingklip, and monkfish
- To analyse the specimens for a wide range of metallic elements using ICP-MS

Objective for aim 2

- To compare the concentrations and compositions of metals between species using univariate and multivariate statistical analyses.

Objective for aim 3

- Compare the concentrations found in fish with established human consumer limits set by the South African government and European Union, and calculate estimated daily intake and target hazard quotients to assess human consumer safety.

Hypotheses:

Hypothesis 1.

- The metal concentrations of all five species (sardine, chokka, hake, kingklip, and monkfish) will differ significantly from each other due to differences in biology, location, and taxonomy.

Hypothesis 2.

- There will be a clear difference in metal concentrations and compositional patterns between the South Atlantic, Indian Ocean, and species due to a combination of underlying geological and oceanographic conditions, and overlying anthropogenic factors.

Hypothesis 3.

- All species will be safe for consumption by humans.

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Chapter 2:

Concentrations and relative compositions of metallic elements differ between predatory squid and filter-feeding sardine from the Indian and South Atlantic oceans

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Highlights

- First assessment of metal concentrations in sardine and squid from South Africa
- Concentrations differed between organisms from the South Atlantic and Indian Oceans
- A number of metals occurred at higher concentrations in the predatory squid
- 'Fingerprint' analyses indicate possible stock discrimination for sardine and squid
- No mean concentrations exceeded recommended human intake limits

Key words: South Atlantic Ocean; Indian Ocean; muscle tissue; cadmium; mercury; lead.

Abstract

Although metallic elements occur naturally, they can occur or accumulate in organisms at levels toxic to the organism and/or their consumers. Concentrations of twenty-nine metallic elements in muscle tissue from sardine *Sardinops sagax* and chokka squid *Loligo reynaudii* from South Atlantic and Indian Ocean waters off South Africa were established, for the first time, using inductively-coupled plasma mass spectrometry. Chokka showed significantly higher ($p < 0.05$) concentrations of B, Cr, Zn, As, Se, Rb, Sr, Cd, and Tl and significantly lower concentrations of V, Mn, Ti, and Mo compared to sardine. There were also significant differences in some metallic elements between the two oceans. Multivariate analyses indicated possible population structure of both species, suggesting that these analyses may be useful as a stock discrimination tool. Only two sardine samples contained quantifiable Hg. Based on South African estimated daily intake, total hazard quotient, and European Union limits for Hg, Cd, and Pb, we consider tissues from sardine and chokka in South African waters to be safe for human consumption.

1. Introduction

The aquatic ecosystem is both a receiver and a reservoir for pollutants such as metals and persistent organic pollutants (Bouwman et al., 2015; Newman and Watling, 2007; Smita Achary et al., 2017). The uptake of pollutants by aquatic biota occurs through a dissolved state in water flowing over the body and gills, the organism's diet, or combinations thereof (Fernandez et al., 2014). Metallic elements occur in the environment naturally, or elevated above natural background levels due to pollution from mining, industry, and agriculture (du Preez et al., 2018; Greenfield et al., 2011; Nel et al., 2015; O'Donoghue and Marshall, 2003; Wepener and Degger, 2012). Fish and cephalopods are good bio-indicators of metallic elements because they occupy many trophic levels, can accumulate pollutants over long lifespans, and in some cases, exhibit severe effects (Jiang et al., 2018; Mziray and Kimirei, 2016; Viera et al., 2011). Organisms at higher trophic levels also tend to accumulate higher concentrations of pollutants, potentially making them more susceptible to the effects of excess pollutants such as metals (Fernandez et al., 2014), and passing them on to yet higher trophic levels.

South Africa is bordered by two oceans; the Indian Ocean on the east and south coasts, and the South Atlantic Ocean on the west coast, with these two oceans adjoining in the region of Cape Agulhas (20°E; Hutchings et al., 2009, Fig 1.). The upwelling of cold, nutrient-rich water onto the west coast shelf in the austral spring and summer and due to south-easterly winds makes these waters in the South Atlantic Ocean highly productive. The south coast is impacted by the warm Agulhas Current at the shelf edge, shows shelf-edge and coastal upwelling, and is characterised by both easterly and westerly winds. Shelf waters are stratified in summer and mixed in winter. Circulation patterns are complex but create a clockwise eddy that circulates around a sub-surface cool ridge and acts as a retention area for phytoplankton and zooplankton (Hutchings et al*.,* 2009), and likely also nutrients and metals.

The sardine *Sardinops sagax* is an economically and ecologically important fish (Beckley and van der Lingen, 1999) for the South African small pelagic fishery. Annual catches of sardines since 1990 ranged between 51,000 and 374,000 metric tons, and it is mainly canned for human consumption, pet food, and bait (DAFF, 2016). Sardines are prey to many predators such as Cape fur seals *Arctocephalus pusillus*, sea birds, and squids (Huisamen et al., 2012; Connan et al., 2017; Bouwman et al., 2015). Chokka or the Cape Hope squid *Loligo reynaudii* also supports a commercial fishery for human consumption (mostly export) and bait.). Chokka are caught primarily with jigging from deck boats, and as by-catch in demersal trawls. Annual catches fluctuated between 2,700 and 13,300 metric tons between 2003 and 2015 (DAFF, 2016).

The South African sardine is primarily an indiscriminate filter feeder, feeding mostly on phytoplankton, zooplankton, and fish eggs (van der Lingen, 2002), making the sardine a primary and secondary consumer. Chokka are opportunistic predators during all life stages, with juveniles favouring crustaceans and adults feeding mostly on small pelagic fish and other cephalopods (Augustyn et al., 1994; Lipinski, 1992; Sauer and Lipinski, 1991). Because chokka feed on small pelagic fish as part of their diet, they occupy a higher trophic level than do sardines, as shown by stable isotope analyses; chokka had higher nitrogen stable isotope ratios ($\delta^{15}N$; an index of trophic level) (mean 13.8, trophic level 4.08) than did sardines (mean 11.1, trophic level 3.0; van der Lingen and Miller, 2011). While sardines would be exposed to and potentially accumulate metals via uptake from both water and ingestion of filtered food particles, chokka squid would be exposed to metals in water and metals accumulated by their prey (likely including sardines), potentially resulting in higher body burdens. However, physiologically important elements may be regulated to stay within physiological limits, resulting in narrower concentration variations than in elements that are not so regulated (Emsley, 2003).

The aim of this study was to establish metallic element concentrations and patterns in sardine and chokka muscle tissues from the South Atlantic and Indian Ocean coasts of South Africa. We compare the concentrations within and between species from the same ocean, and compare within species between the two oceans. We also assess levels of three major toxic metals (cadmium (Cd), lead (Pb) and mercury (Hg)) for human consumer safety.

2. Methods and Materials

2.1. Sardine and chokka collection sites

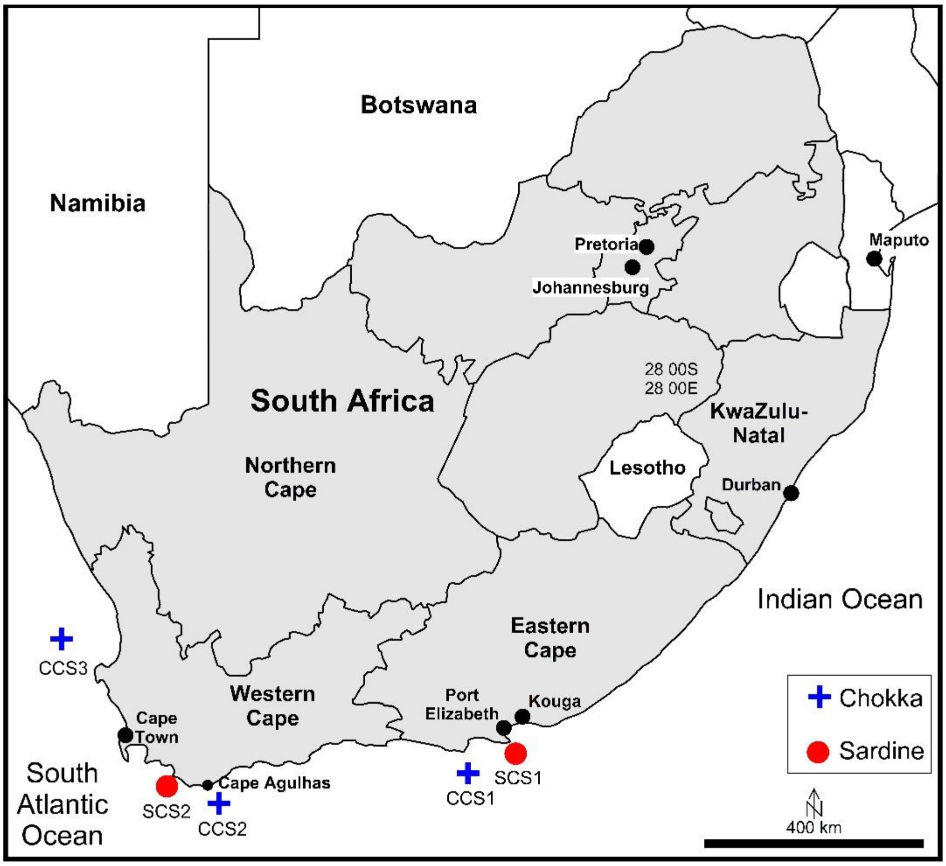

Figure 1: Map of sardine and chokka sample collection sites

Sardine and chokka samples were received from the South African Department of Agriculture, Forestry, and Fisheries (DAFF). The sardine samples (SCS1 and SCS2; bottom depths 39 m and 68 m, respectively) were sourced from commercial catches (Figure 1) in March 2017. Sardine standard length ranged from 150-195 mm, while mass ranged from 51-120 g. One of the chokka samples (CCS1, bottom depth 162 m) was sourced from a commercial catch made in the Indian Ocean in November 2017. The other two chokka samples (CCS2 and CCS3, bottom depths 192 m and 145 m, respectively) were sourced from demersal trawls conducted during a research survey off the west coast in February 2017. Chokka total length ranged from 160-320 mm, and mass ranged from 45-504 g.

Fish and chokka were chilled on ice after capture. Whole samples were selected randomly for analyses, and then frozen. The sardine and chokka samples were couriered frozen from Cape Town and Port Elizabeth to the laboratory at the North-West University (NWU) in Potchefstroom, South Africa, where they were received frozen and stored in -20°C freezers pending analysis. Ethical clearance was obtained from the North-West University.

2.2. Dissection of sardine and chokka

We thawed sardine and chokka samples at room temperature until they were in workable condition (still firm). Sardine muscle tissue and chokka mantle (without skin) samples were taken and weighed to obtain wet mass (approximately 10 g) from both dorso-lateral sides of the fish and squid, and transferred to 50 ml high-density polypropylene centrifuge tubes. The weighed samples were first frozen and then freeze-dried for three days at 8 kPa and -50°C. The freeze-dried samples were then weighed to obtain dry mass (approximately 2 g).

2.3. Analyses of sardine and chokka

Freeze-dried samples were analysed by Eco-Analytica at the North-West University (NWU). Accurately weighed tissue samples were dissolved in concentrated ni tric acid, and microwave-digested with an Ethos UP microwave unit, as per the United States Environmental Protection Agency method 3051A. The sample and acid were placed in a fluorocarbon polymer vessel, sealed, and heat-digested in the microwave unit for 35 minutes.

A standard reference material (SRM) (ERM-CE278K – marine mussel tissue) was used as quality control (QC) with the same digestion protocol. We analysed the microwave-digested solutions using an Agilent 7500ce, inductively-coupled-plasma mass-spectrometer (ICP-MS), optimised with an aqueous solution containing cerium (Ce), lithium (Li), ytterbium (Y), and thallium (Tl) (1 µg/L) to reduce interference (≤ 1.5%). Forward power was 1550 W, plasma gas flow was set at 15 l/min, the nebuliser gas flow was 1.2 l/min, and the sampling depth in the container was at 8 mm. We quantified twenty-nine elements.

For external calibration, we used ULTRASPEC-certified mixed multi-element standard solutions (De Bruyn Spectroscopic Solutions). Full calibration sets and QC-check standards were run. The QC check standard was a calibration standard of midrange concentration. Detection limits for each element analysed are indicated in table S1. Data are expressed as mg/kg dry mass (dm). Wet-mass based concentrations were calculated (mg/kg wm, see section 2.2) for comparisons with intake limits.

2.4. Statistical analysis

We tested data for normality using the Shapiro-Wilks normality test (GraphPad Prism 7.04; www.graphpad.com). Data that were not normally distributed were log-transformed. We used two-tailed t-tests to compare mean metal concentrations between the same species from different oceans, and between species from the same ocean. We used Kruskal-Wallis (unpaired, non-parametric, one-way ANOVA) to compare concentrations between the three chokka collection sites, using Dunn's test to correct for multiple comparisons.

For multivariate analyses, we used nonmetric-multidimensional-scaling (NMS) to ordinate the metal concentrations for species and collection sites (MjM Software PC-ORD version 7.03; www.pcord.com). The data were relativised per element to obtain proportional elemental compositions per sample (or 'fingerprints'). Four axes were allowed. We selected Gower-ignore-0 as the distance measure. The maximum number of iterations allowed was 200. Fifty runs of real data were used with random starting configurations. Monte Carlo tests were then performed with randomised data, also using 50 runs. We used convex hulls to visualise relative elemental compositions of species and collection sites.

3. Results

3.1 Concentrations

We analysed 18 sardines and 15 chokka from the South Atlantic, and 12 sardines and ten chokka from the Indian Ocean. Recoveries of certified elements were within 20% of SRM certified values. Beryllium, Ni, Ag, Bi and Au were below detection limits in sardine muscle. Beryllium, Ni, Au and Bi were below detection limits in chokka muscle. Summary results of the 14 elements that had significantly different concentrations in the same species between oceans are presented in Table 1. Summarised data for all elements analysed per species and sample site are presented in Table S1.

Table 1: Concentrations (mg/kg dm) of metallic elements in muscle tissue that had significantly different ($p < 0.05$) concentrations according to species and catch site

Element	Organism	Indian Ocean			Atlantic Ocean		
		n	Mean	SD	n	Mean	SD
B	Sardine	10	0.73	0.82	18	1.5	0.67
B	Chokka	10	5.2	2.8	15	5.5	1.1
Ti	Sardine	12	18	2.7	18	17	2.3
Ti	Chokka	10	21	1.2	15	23	2.1
V	Sardine	12	0.29	0.17	18	2.0	0.96
V	Chokka	10	0.12	0.078	15	0.039	0.028
Cr	Sardine	12	0.61	0.064	18	0.66	0.069
Cr	Chokka	10	1.6	0.21	15	1.6	0.18
Mn	Sardine	12	2.2	1.0	18	2.3	0.93
Mn	Chokka	10	2.6	1.3	15	1.1	0.16
Co	Sardine	12	0.078	0.032	18	0.083	0.036
Co	Chokka	10	0.027	0.0064	15	0.022	0.011
Zn	Sardine	12	27	13	18	21	4.0
Zn	Chokka	10	46	3.1	15	47	2.4
As	Sardine	12	2.7	0.60	18	4.1	0.95
As	Chokka	10	17	6.1	15	17	2.7
Se	Sardine	12	1.2	0.17	18	1.6	0.28
Se	Chokka	10	2.8	0.63	15	2.8	0.35
Rb	Sardine	12	3.6	0.26	18	3.1	0.25
Rb	Chokka	10	11	0.82	15	8.4	1.2
Sr	Sardine	12	5.1	2.9	18	5.7	1.7
Sr	Chokka	10	15	2.5	15	13	2.0
Mo	Sardine	12	0.056	0.025	18	0.49	0.22
Mo	Chokka	10	0.034	0.0079	15	0.038	0.011
Cd	Sardine	12	0.18	0.13	18	0.30	0.17
Cd	Chokka	10	1.8	1.3	15	2.7	2.2
Tl	Sardine	12	0.034	0.028	17	0.11	0.022
Tl	Chokka	10	0.21	0.085	14	0.21	0.044

3.2. Concentration differences between sardines from different oceans

Significant differences (un-paired, two-way t-tests; $p < 0.05$) were found between sardines from the two different oceans for mean concentrations of B, V, Cr, As, Se, Rb, Mo, Cd, and Tl (Table 1; Figures 2 and 3). Boron, V, Cr, Se, Mo, Cd, As, and Tl had significantly ($p < 0.05$) higher mean concentrations in sardines from the South Atlantic Ocean with only Rb showing significantly higher concentrations in fish from the Indian Ocean. Titanium, Mn, Zn, Co, and Sr mean concentrations were not significantly different between sardine from the two oceans (Figures 2 and 3).

3.3. Concentration differences between chokka from different oceans

We found significant differences (un-paired, two-way t-tests; $p < 0.05$) in mean concentrations between chokka from the Indian and South Atlantic oceans for Ti, V, Mn, Rb, and Sr. Chokka from the Indian Ocean had significantly higher mean concentrations of V, Rb, Mn, and Sr (Figures 2 and 3). Boron, Cr, Co, Zn, As, Se, Mo, Cd, and Tl showed no mean concentration difference between samples from the South Atlantic and Indian oceans.

3.4. Concentration differences between sardines and chokka per ocean

In both oceans, sardines had significantly (un-paired, two-way t-tests; $p < 0.05$) higher mean concentrations compared with chokka for V, Co, and Mo, and for Mn for the Atlantic Ocean only. Chokka had significantly higher (un-paired, two-way t-tests; $p < 0.05$) concentrations than sardines for B, Ti, Cr, Zn, As, Se, Rb, Sr, Cd, and Tl (Figures 2 and 3) in both oceans.

3.5. Multivariate analyses of relative elemental compositions of sardine and chokka per collection site

Two dimensions were needed to ordinate the relative elemental compositions in sardine muscle, with a final stress of 11.7, and a final instability of 0.00017, reached after 30 iterations (Figure 4). According to Clarke's rule of thumb (McCune and Grace, 2002), a final stress between 10 and 20 presents a usable picture for interpretation. Sardine sample site 1 (SCS1, Indian Ocean) had a convex hull that did not overlap with SCS2 from the South Atlantic Ocean. SCS1 was characterised by higher relative proportions of Th (thorium), Sb, Pt, and Pb, while SCS2 was characterised by higher relative proportions of B, Tl, Mo, Cd, and V.

Three dimensions were needed to ordinate the relative elemental compositions in chokka muscle tissue, of which we show only two (Figure 5). The third dimension explained 21.8% of the variability. The final stress was 9.89, and the final instability was < 0.0001, reached after 54 iterations. According to Clarke's rule of thumb (McCune and Grace, 2002), a final stress between 5 and 10 presents a good ordination with little risk of deriving false inferences. The chokka collection sites had distinctly different profiles, with especially CCS1 characterised by higher relative proportions of Mn, U, and V.

ANOVAs for the three chokka collection sites indicated significantly (Kruskal-Wallis, one-way ANOVA; $p < 0.05$) higher median concentrations of V, Mn, Rb, and Sr in CCS1. Significantly (Kruskal-Wallis, one-way ANOVA; $p < 0.05$) higher concentrations of Ti were found in CCS2 and CCS3. Other mean elemental concentrations were not significantly different between the chokka collection sites.

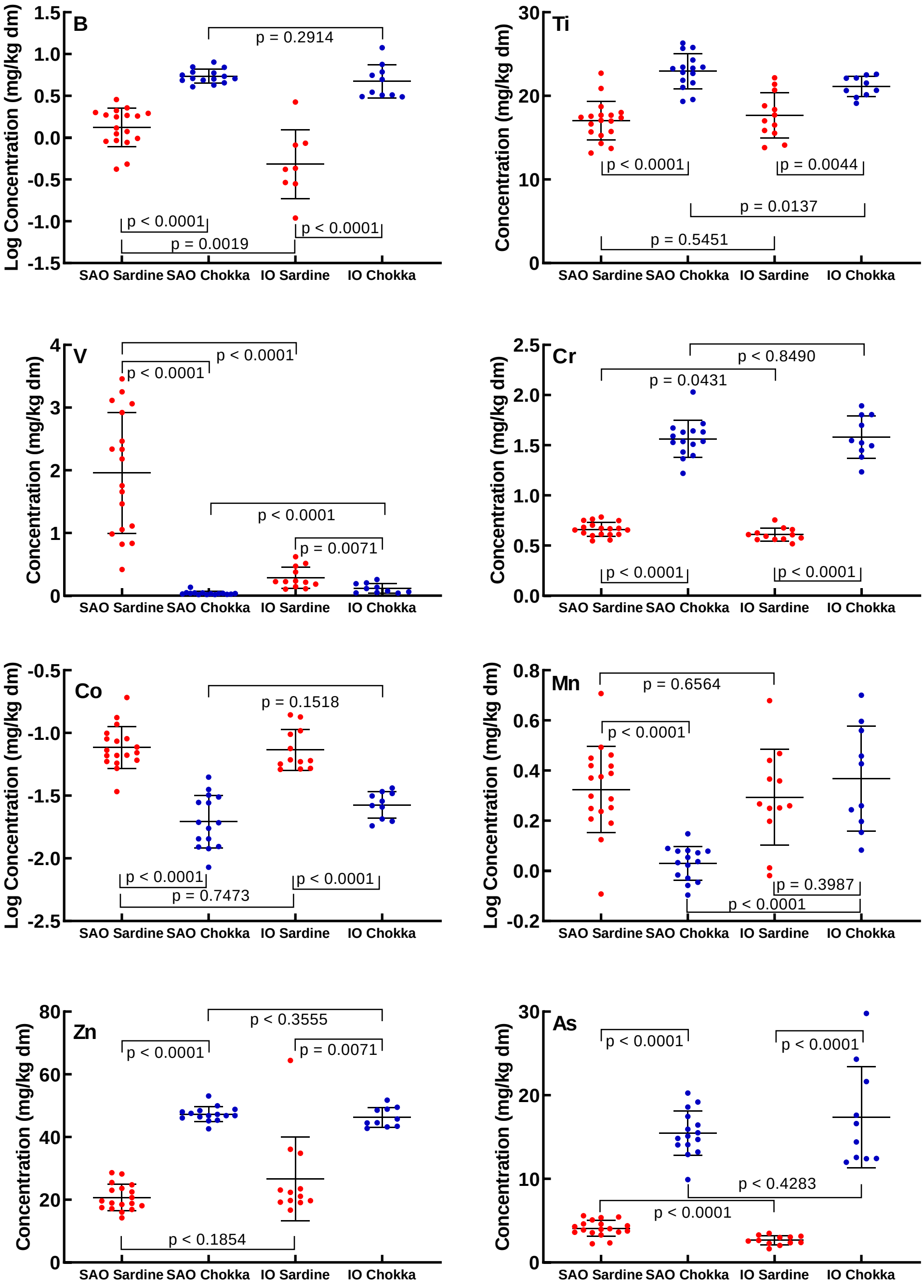

Figure 2: Scatterplots and results of two-way, unpaired t-tests between logical pairs for elements that showed significant differences: Between sardines (red symbols) from each ocean, between chokka (blue symbols) from each ocean, and between sardines and chokka from the same ocean. Means and standard deviations are indicated. SAO = South Atlantic Ocean; IO = Indian Ocean.

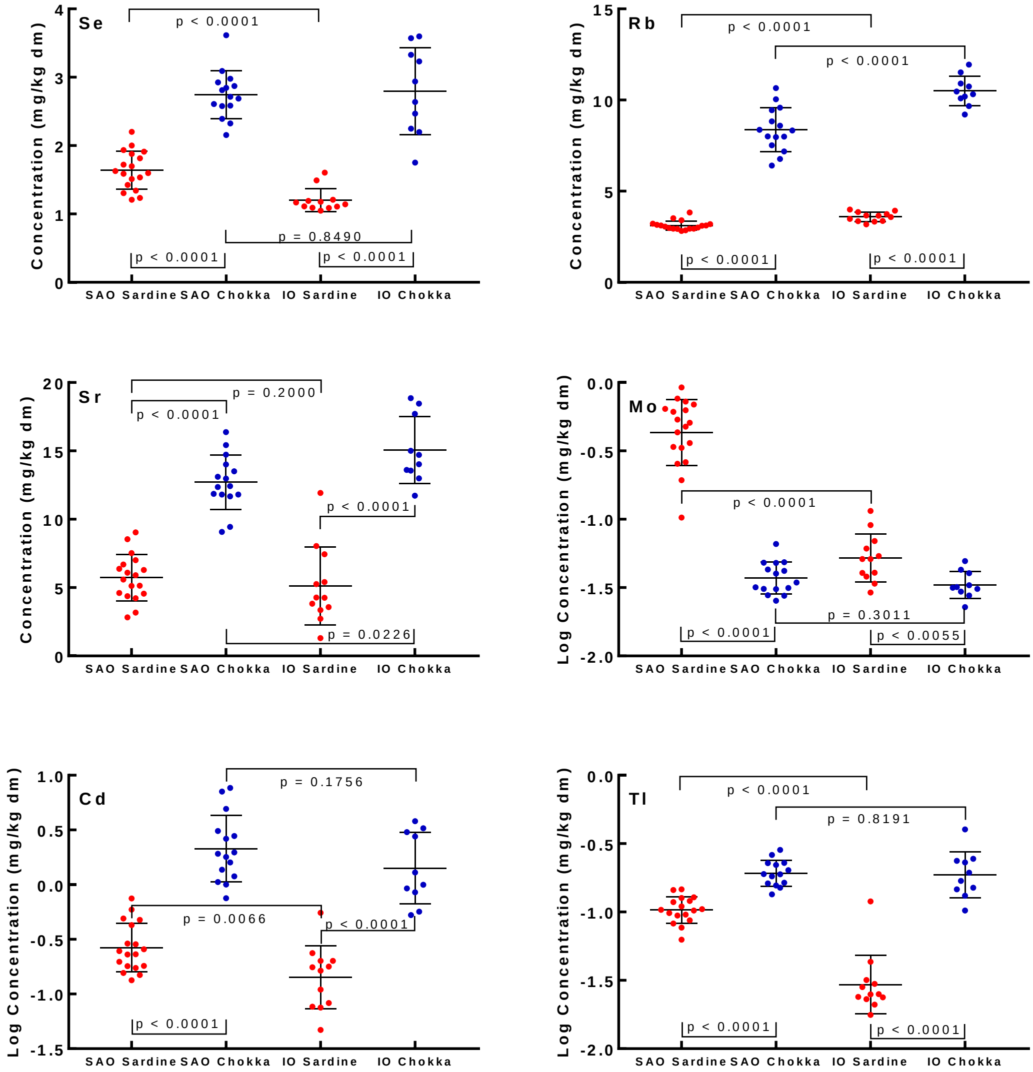

Figure 3: Scatterplots and results of two-way, unpaired, t-tests between logical pairs for elements that showed significant differences: Between sardines (red symbols) from each ocean, between chokka from each ocean, and between sardines and chokka from the same ocean. Means and standard deviations are indicated. SAO = South Atlantic Ocean; IO = Indian Ocean.

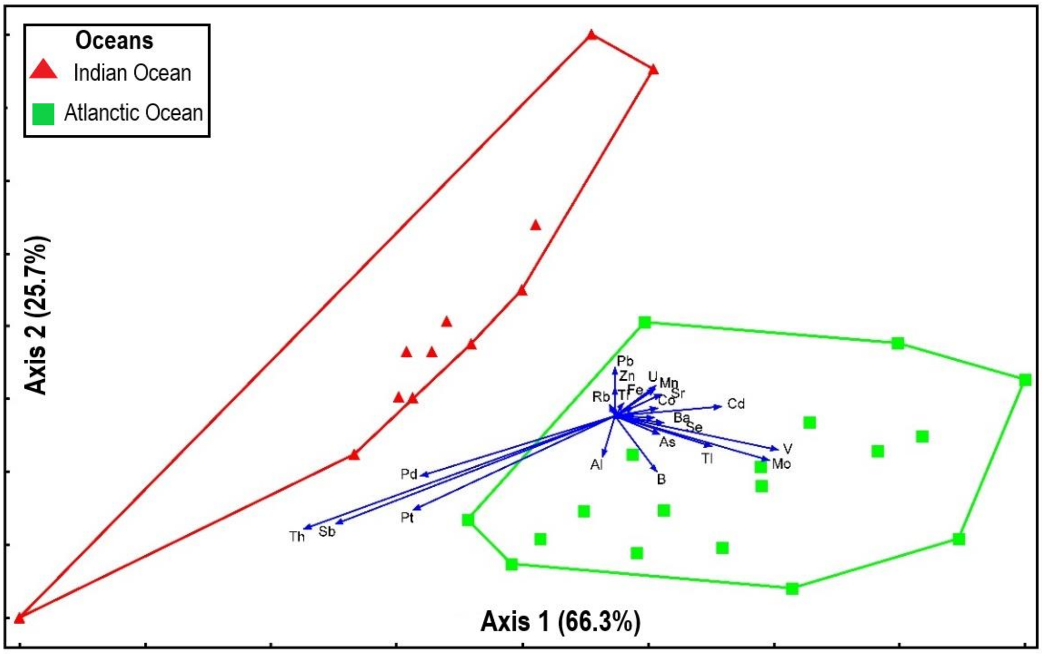

Figure 4: Nonmetric multidimensional scaled (NMS) ordination of relativized (using Gower-ignore-0 as distance measure) for sardine muscle tissue.

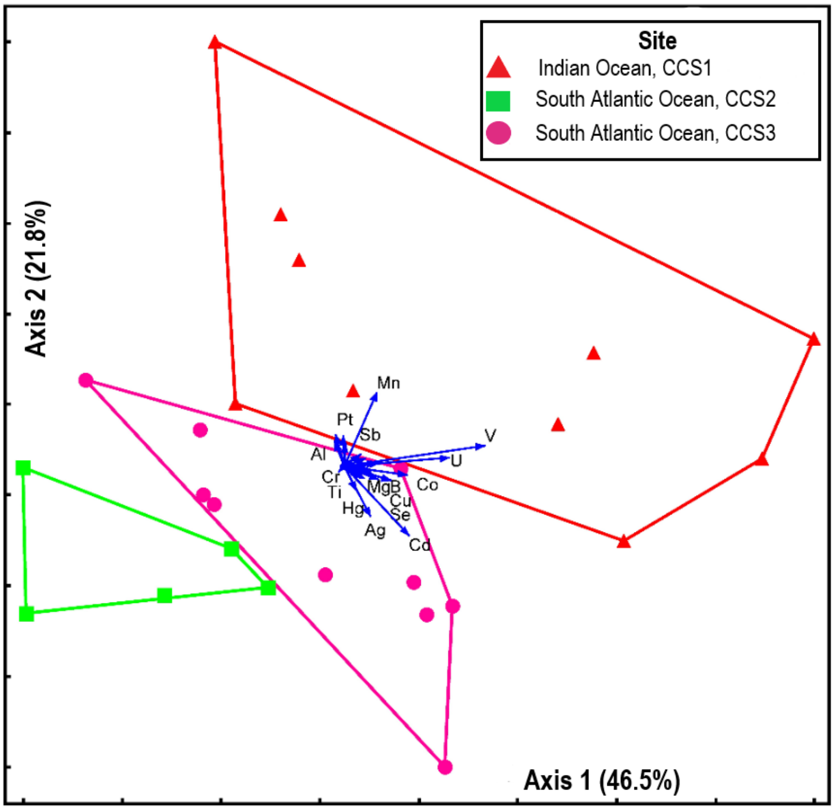

Figure 5: Nonmetric multidimensional scaled (NMS) ordination of relativized (using Gower-ignore-0 as distance measure) for chokka mantle tissue.

4. Discussion

4.1. Concentration differences between sardines from different oceans

Oceanographic conditions along the South African coast vary substantially both in time and space (Hutchings et al., 2009; Hampton et al., 2018) influencing the occurrence and numbers even of such widespread species as the sardine. Human activities may also influence the occurrence of various species, as will be discussed in section 4.3.

We found that sardine muscle tissue had significantly higher mean concentrations of Mo, Cd, Tl, B, V, Cr, As, and Se in the South Atlantic Ocean compared with the Indian Ocean, while the reverse was true for Rb (Figures 2 and 3). Metallic element concentrations in organisms can be influenced by age, sex, size, feeding habits, and habitats (Griboff et al., 2018). For instance, B is found in the ocean as boric acid (Wu et al., 2017) which occurs at higher concentrations in zooplankton than in phytoplankton (EPA, 1997).

SCS2 was in the South Atlantic Ocean, which is known for upwelling events during austral spring and summer (Hutchings et al., 2009). Upwelling is known to increase metallic elements (especially Cd) in water (Jiang et al., 2017) which can then be incorporated into fish muscle tissue and otoliths. This seasonal upwelling would result in increased plankton and hence more food for sardines (Bode et al., 2018). Since plankton take up metallic elements effectively from their environment (Sanders and Riedel, 1998), sardines at SCS2. may be exposed to higher concentrations via plankton compared with sardines from SCS1.

Sardines are primarily indiscriminate filter feeders. While phytoplankton is the most commonly-ingested prey, zooplankton provides most of the fish’s sustenance in both the South Atlantic and Indian oceans (van der Lingen, 2002). Spatial variability in sardine diet is apparent, with phytoplankton and smaller zooplankton more important in the South Atlantic Ocean (van der Lingen, 2002; Idris et al., 2016). This difference in diet, spatial differences in natural background concentrations arising from different oceanographic conditions and anthropogenic sources, may explain the differences in sardine muscle metallic concentrations between collection sites. Similar background concentrations (Newman and Watling, 2007) or physiological regulation (Emsley, 2003) may explain the lack of significant differences of other elements.

4.2. Concentration differences between chokka from different oceans

Significant differences were found for V, Mn, Rb, Sr and, Ti between chokka collection sites (Table 2). Vanadium, Mn, Rb, and Sr had significantly ($p < 0.05$) higher concentrations in the Indian Ocean, and Ti had a significantly ($p < 0.05$) higher concentration in the South Atlantic Ocean. As mentioned in section 4.1, the species included in this study are exposed to an ever-changing environment in the oceans around South Africa, and we show clear mean concentration differences. Apart from the natural background levels, the geographic distribution of anthropogenic sources (discussed in 4.3) of elements probably also explains the differences found.

4.3. Possible sources

4.3.1. Geological background

The predominant geology of the land mass close to the catch sites are dominated by the Cape Supergroup that can be subdivided into three groups the Table Mountain Group, the Bokkeveld Group, and the Witteberg Group (Shone and Booth, 2005). These groups are mostly made up sedimentary rocks containing quartz and quartz arenites (Shone and Booth, 2005). These groups would mostly contribute Si, Na, Ca, and Al to the surrounding waters as these elements that are the most abundant minerals found in quartz (Klein and Philpotts, 2013). The fairly uniform geology of the mainland does not provide an explanation for the differences in metal concentrations found in this study.

4.3.2. Anthropogenic sources

Table 2: Possible anthropogenic sources of metals that were significantly different between sites and species in oceans.

Metallic elements	Possible anthropogenic sources	References
B	Combustion of coal and biomass, soap, fertilizer, and flame retardants	Park and Schlesinger, 2002.
Ti	Mining, manufacturing of paints, fibre, and cosmetics	Reimann and de Caritat, 1998
V	Mining and the combustion of petroleum and coal	Schelsinger et al., 2017
Cr	Electroplating, paint, agriculture, combustion of fossil fuels and waste	He et al., 2005, Mzimela et al., 2014
Mn	Mining, dry cell batteries, and animal feed	Richir and Gobert, 2016
Zn	Agriculture (pesticide), wastewater, and galvanic industry	Neff, 2002, van Aswegen et al., 2019
As	Timber treatment, agriculture(pesticide) and burning of fossil fuels	Tchonwou et al., 2012, Lesch and Bouwman., 2018
Se	Mining, agriculture and combustion of fossil fuel	Wen and Carignan, 2007
Mo	Mining and agricultural runoff	Reimann and de Caritat, 1998
Cd	Batteries, paints, agricultural runoff, mining and the combustion of fossil fuels	Yuan et al., 2019
Tl	Mining and coal combustion	Karbowska, 2016

Natural processes are not the only factor that affects the elemental composition of the world's oceans. South Africa is a mineral-rich country (V, Mn, and Ti; Department of Mineral Resources; DMR, 2008a; DMR, 2013; DMR, 2008b), and a high proportion of her income is derived by the mining and processing of metals (Minerals Council of South Africa, 2018). Metals from combustion, consumer products, and other sources (Table 2) can enter rivers and eventually oceans through run-off and atmospheric deposition (AMAP, 2005; Odiyo, et al., 2005; Dallas and Day, 2004; Fatoki and Mathabatha, 2001). Terrestrial and freshwater pollution, which is widely reported in South Africa (Wepener and Degger, 2012; O'Donoghue and Marshall, 2003), could therefore have affected the concentrations found in our study.

For instance, the Port Elizabeth and Kouga harbours and associated estuaries are potential sources of metals to the Indian Ocean from industries and ore loading (Nel et al., 2015; Fig. 1). Hence, we expected higher metallic element concentrations at the nearby sardine and chokka collection sites, which, indeed, we found for Pb in sardines from SCS1 (Table S1).

Although naturally occurring in the ocean, Cd, As, Tl, Mo, and Se can be anthropogenically increased by mining and industry (Satarug et al., 2003; Oremland and Stolz, 2003; Kehrig et al., 2013; Karbowska, 2016). These elements occurred at higher concentrations in sardines collected in the South Atlantic. Cadmium was also higher for chokka there compared to the Indian Ocean. Although upwelling could have caused the higher concentrations, this does not dismiss the possible contribution from anthropogenic sources.

Large deposits of Mn are found in the North Cape (west coast) (DMR, 2013) which could have affected the concentrations found in our study. However, this was not the case as significantly higher concentrations of Mn were found in chokka from the Indian Ocean (CCS1). This could be due to the influence of Mn nodules found in the mid Indian Ocean (Bollman et al., 2010).

Our data therefore indicates that the concentrations found in our study cannot be attributed to either natural background or anthropogenic activity as major sources, but rather a combination of these two, the relative contributions of which we do not know. Indications of relative contributions may be done when comparing anthropogenic and natural halogenated organic compounds with metals in marine organisms from the two oceans (Bouwman et al., 2015; Aznar-Alemany et al., 2019; Van Aswegen et al., 2019; Wu et al., 2019).

4.4. Differences in concentrations between sardine and chokka

We found significantly higher ($p < 0.001$) concentrations of B, Ti, Cr, Zn, As, Se, Rb, Sr, Cd, and Tl in chokka when compared with sardines (Figures 2 and 3), suggesting accumulation in chokka from their prey. Loliginid species are believed to accumulate most of their metallic elements via the trophic pathway (Penicaud, 2017). Other prey items are likely to reflect similar local conditions, contributing towards consistently higher concentrations and accumulation in chokka, despite inter-ocean differences. However, sardines had significantly ($p < 0.001$) higher concentrations of Mn, Mo, V, and Co than chokka, suggesting physiological regulation by either or both. A contributing explanation could be that that Co and V accumulate in the brachial heart of loliginid species (Penicaud, 2017) and may cause these elements to be lower in the muscle tissue.

Manganese is incorporated into loliginid eggs (Penicaud, 2017) suggesting a possible reason why Mn concentrations were lower in the chokka mantle tissue as these samples were caught in the austral summer when chokka lay their eggs. Chokka could also incorporate these elements into the gladius – this, however, has not been tested before. Different tissues should be analysed in the future as loliginids build up stores of metallic elements in the brachial heart and the digestive gland (Penicaud, 2017). This information could be crucial in understanding the accumulation of metallic elements in squids in general.

To our knowledge, no study has been done on the relationship of metallic elements in muscle tissue between sardine and any squid in the Southern Hemisphere. Comparison with studies from elsewhere indicates similarities and differences (Table 2). Rubidium has been found at higher concentrations in higher trophic levels in aquatic ecosystems (Campbell et al., 2005a, b). Zinc has been shown to accumulate through trophic levels by a number of studies, some using stable isotope analyses (Trevizani et al., 2018; Tu et al., 2012; Campbell et al., 2005b; Quinn et al., 2003; Chen et al., 2000). Lower concentrations in higher trophic levels have been found for Mn (Griboff et al., 2018; Hao et al., 2013).

On the other hand, and contrary to our findings, other studies found lower concentrations in higher trophic levels for Zn (Besser et al., 2001; Jara-Marini et al., 2009; Borrell et al., 2016), Cd (Guo et al., 2016; Campbell et al., 2005b), Cr (Revenga et al., 2012), and As (Trevizani et al., 2018; Chen et al., 2000). Differences in food web structure, and probably differences in the physiologies of molluscs (chokka) and vertebrates (sardine), where invertebrate molluscs feed on vertebrates, are likely contributory factors. Little information is available about the possible accumulation B, Tl, Se and, Sr; more research on the trophic differences of these elements is required.

Our data are not directly comparable with the findings of others, as different trophic systems may react to metallic elements in different ways. Analysing more samples, more species from more trophic levels (e.g., plankton, sharks, and tuna) from more sites, and increasing the number of samples from different trophic levels within the same systems (the Indian and South Atlantic oceans in this case) would be needed for clarification.

4.5. Multivariate analyses of relative elemental compositions of sardine and chokka per collection site

Clear ‘fingerprint’ differences in metallic element compositions were seen between the two sardine sites (Figure 4). These differences can be attributed to many factors mentioned in section 4.1 and 4.3. Although we only had two sardine sampling sites, our data supports the hypothesis of multiple sardine stocks off South Africa. Multiple stocks are also implied by other studies that compared morphometric, meristic, genetic, and parasite load characteristics of sardines from the west, south, and east coasts (van der Lingen et al., 2015 and references therein; Weston et al., 2015; Idris et al., 2016; Teske et al., 2018; Groenewald et al., 2019). A recent study (Hampton et al., 2018) showed that otolith microchemistry of South African sardine juveniles differed between collection sites in the same ocean - most of the compositional differences were site specific and varied annually. Our study had a smaller sample size and only two collection sites and could not indicate if these differences were gradual (supporting a single stock assumption) or discontinuous (indicating multiple stocks) along the coast of South Africa. Larger samples sizes and more collection sites would confirm and be able to track different stocks using muscle tissue elemental concentrations as an easy alternative and/or complement to otolith microchemistry, morphometrics, and parasite composition.

Chokka collection sites had relative elemental composition profiles that did not overlap (Figure 5), and ANOVAs and t-tests showed that only a few elements (V, Mn, Rb, Ti) differed in mean concentration between the collection sites. These differences in compositions could be explained by different prey at the different collection sites, as prey is a crucial pathway of metallic elements in loliginids. Although spatial differences in metallic element compositions in chokka were not as large as those seen in sardines, the non-overlapping compositions (Figure 5) between chokka collection sites supports the hypothesis of multiple stocks in this species. Chokka from around southern Africa appear to be genetically uniform (Shaw et al., 2010) but show significant morphometric diversity (van der Vyver et al., 2015), leading to the hypothesis that chokka off southern Africa is a large and mobile metapopulation, that comprises three main geographic groups, two of which are off South Africa (Lipinski et al., 2016). Again, data from more samples from more sites would be needed to confirm this.

4.6. Comparisons with similar studies

Table 3: Global comparison of Hg, Pb and Cd (mg/kg wm) in sardine (*Sardina pilchardus* and *Sardinops sagax*) and chokka (*Loligo vulgaris* and *L. reynaudii*) with limits set by the European Union (EU) and the South African government for these metals.

Species	Location	Cd	Pb	Hg	Reference
Sardina pilchardus	Adriatic Sea	0.02	0.03	0.07	Storeli, 2008
Sardina pilchardus	North Atlantic Ocean	0.024	0.076	0.084	Chahid et al., 2014
Sardina pilchardus	Atlantic Ocean	0.0064	0.03	0.019	Viera et al., 2011
Sardina pilchardus	Mediterranean Sea	0.002-0.1	0.01-0.08	0.07-0.09	Falco et al., 2006
Sardinops sagax	Bass Strait (Australia)	0.096	0.013	0.034	Finger et al., 2017
Sardinops sagax	Bass Strait (Australia)	0.018	0.034	0.034	Finger et al., 2017
Sardinops sagax	Arabian Gulf	0.037	0.005	<LOQ	Tawfik, 2013
Sardinops sagax	**Indian Ocean (SCS1)**	**0.052**	**0.052**	**0.0049**	**This study**
Sardinops sagax	**South Atlantic Ocean (SCS2)**	**0.078**	**0.044**	**0.00057**	**This study**
EU limits		0.25	0.3	0.5	EC, 2018
RSA limits (Sardine)		1	0.5	0.5	South Africa, 2008
Loligo vulgaris	Adriatic Sea	0.26	0.03	0.02	Storeli, 2008
Loligo vulgaris	Mediterranean Sea	0.05-0.15	0.01-0.01	0.02-0.03	Falco et al., 2006
Loligo vulgaris	Pacific Ocean	0.29	0.04	<LOQ	Sangiuliano et al., 2017
Loligo vulgaris	Mediterranean Sea	0.3	0.05	0.11	Storeli, 2010
Loligo reynaudii	**Indian Ocean (CCS1)**	**0.38**	**0.04**	**0.03**	**This study**
Loligo reynaudii	**South Atlantic Ocean (CCS2)**	**0.72**	**0.036**	**0.056**	**This study**
Loligo reynaudii	**South Atlantic Ocean (CCS3)**	**0.66**	**0.04**	**0.05**	**This study**
EU limits (Chokka)		1	1	0.5	EC, 2018
RSA limits (Chokka)		3	4	0.5	South Africa, 2008

We selected three well-known toxic metals that are regularly reported in literature (Cd, Pb, Hg) and compared our findings with other studies (Table 3). Studies were chosen with similar taxa as this is the first time metallic elemental data is presented for *Loligo reynaudii,* and only the third study published on *Sardinops sagax*. *Loligo vulgaris* was chosen as the species to compare to chokka as *L. reynaudii* was previously classified as a subspecies of *L. vulgaris*. *Sardina pilchardus* was chosen as another comparative species for sardine because of its similar feeding habits as *Sardinops sagax* (Garrido and van der Lingen, 2014). Sardines from the South Atlantic Ocean (SCS2) and Indian Ocean (SCS1) collection sites had the second and third highest Cd, and third and second highest Pb concentrations of the studies that were compared. Only two of our sardine samples had quantifiable Hg, and these values were the lowest of all the studies we compared. Cadmium concentrations in chokka were the highest, and Hg the second highest, when compared with other studies, indicating either a high natural background, pollution, or both.

4.7. Seafood safety

Metal concentrations found in muscle tissue of sardine and chokka did not exceed the limits (despite marginal exceedances for Cd in two chokka samples) set by both the European Union

(EU) and the South African government (Table 3) for Pb, Hg and Cd. No other elements have similar standards except for Sn, but this is for canned fish only, hence not applicable here.
Estimated daily intakes (EDIs) and total hazard quotients (THQs) were calculated according to the formulae used by Beheary and El-Matary (2018). The average mass and lifespan of a South African resident was selected at 70 kg and 72.7 years, respectively. The daily intake of sardine was estimated to be 0.0006 kg/day, and the chokka daily intake was estimated to be at 0.00018 kg/day for South African residents (WWF, 2014). The THQ values (Table 4) were all orders of magnitude below the quotient of unity and would thus pose no threat to consumers based on our assumptions. For Cd, Pb, and Hg, sardine and chokka should be considered safe for human consumption and export, but more samples from more locations would add weight.

Table 4: Estimated daily intakes (EDIs) and total hazard quotients (THQs) for cadmium, lead and mercury measured in sardine and chokka from each catch site.

Catch sites	Cd EDI	Cd THQ	Pb EDI	Pb THQ	Hg EDI	Hg THQ
SCS1	0.0000043	0.0043	0.0000043	0.0012	0.00000040	0.00013
SCS2	0.0000064	0.0064	0.0000036	0.0010	0.000000047	0.000016
CCS1	0.00000094	0.00094	0.0000001	0.000029	0.000000074	0.000047
CCS2	0.0000018	0.0018	0.000000074	0.000021	0.00000014	0.00004
CCS3	0.0000016	0.0016	0.0000001	0.000029	0.00000012	0.028

5. Conclusions

Globally, marine fish and cephalopods are important food sources and are ecologically important. This is the first study to report on the metallic element composition in sardine muscle and chokka mantle tissues from the African coast, with previous studies only done on Hg in seafood in South African supermarkets (Bosch et al., 2016).
Different sites showed considerable differences in metallic element composition for sardines and chokka. Due to different oceanographic conditions around South Africa, with upwelling substantially more important on the west coast, the background concentrations of metals may vary, resulting in variable metal concentrations in fish and squid.

Although based on only two collection sites, metallic element concentration differences did not contradict a multiple stock assumption for sardine. Although spatial differences in chokka metallic element composition were not as marked as in sardine, our data also support a multiple stock hypothesis for chokka. Metallic elements could therefore be investigated as a stock discrimination tool for both of these species. Boron Cr, Zn, As, Se, Rb, Sr, Cd, and, Tl had significantly higher ($p < 0.0001$) concentrations in chokka than sardines, indicating possible accumulation by chokka. Vanadium, Mn, Ti, and, Mo concentrations were significantly lower ($p < 0.0001$) in chokka than sardines, indicating physiological regulation of these elements in chokka.

Chokka and sardines from South Africa, although based on a limited sample size, can be considered safe for human consumption in terms of the concentrations of Pb, Hg, and Cd. We recommend that future studies of the metallic element composition of South African marine biota increase the number of species from different trophic levels, increase the number of sites, and use larger sample sizes to investigate trophic level interactions and the utility of this method as a stock discrimination tool for fisheries management.

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Appendix A.

Table S1.

Mean concentrations (using two significant figures) of metallic elements in sardine (SCS) and chokka (CCS) muscle tissue per site (mg/kg dry mass) with the detection limit of each element analysed. Elements not mentioned in text are presented for archival purposes.

		SCS1		SCS2		CCS1		CCS2		CCS3	
	LOD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
B	0.0025	0.73	0.82	1.5	0.67	5.2	2.8	5.7	1.7	5.5	0.76
Mg	0.00003	1800	230	2100	220	3900	400	3400	270	3900	580
Al	0.0018	7.4	4.5	9.5	5.8	4.1	1.7	3.3	2.5	3.9	3.2
P	0.00094	11000	1800	11000	1400	12000	600	1300	1400	12000	800
K	0.0034	22000	2400	19000	2400	17000	1100	16000	1400	16000	900
Ti	0.000041	18	2.7	17	2.3	21	1.2	23	2.8	23	1.8
V	0.000029	0.30	0.17	2.0	0.96	0.10	0.077	0.020	0.0042	0.048	0.031
Cr	0.00089	0.61	0.064	0.66	0.069	1.6	0.21	1.6	0.10	1.6	0.22
Mn	0.000016	2.2	1.0	2.3	0.93	2.6	1.3	1.0	0.19	1.0	0.15
Fe	0.0011	50	10	53	12	10	1.3	8.0	2.7	9.0	1.8
Co	0.000008	0.079	0.032	0.083	0.036	0.027	0.0060	0.013	0.0039	0.026	0.010
Cu	0.000012	3.5	0.68	3.2	0.41	20	8.9	21	7.9	15	3.2
Zn	0.000076	27	13	21	4.2	46	3.1	46	1.9	48	2.4
As	0.000012	2.7	0.55	4.1	0.95	17	6.1	13	2.1	17	2.1
Se	0.00011	1.2	0.17	1.6	0.28	2.8	0.63	2.8	0.53	2.7	0.25
Rb	0.0000023	3.6	0.26	3.1	0.25	11	0.82	8.0	0.76	9.0	1.4
Sr	0.0000056	5.0	2.8	5.7	1.7	15	2.5	11	2.1	13	1.6
Mo	0.0000023	0.056	0.025	0.49	0.22	5.2	2.8	5.7	1.7	5.4	0.76
Pd	0.00000098	0.010	0.015	0.0020	0.0024	0.011	0.0021	0.0079	0.0024	0.012	0.0066
Ag	0.0000098	<LOQ	<LOQ	<LOQ	<LOQ	0.40	0.34	0.65	0.32	0.41	0.15
Cd	0.000032	0.18	0.13	0.30	0.17	1.8	1.3	1.6	0.70	3.3	2.5
Sb	0.0000040	0.0040	0.0020	0.0048	0.0040	0.0090	0.0029	0.0062	0.0011	0.0068	0.0017
Ba	0.000021	0.42	0.19	0.68	0.25	0.16	0.052	0.014	0.024	0.17	0.063
Pt	0.0000062	0.0040	0.0042	0.0012	0.0017	0.011	0.0072	0.011	0.0056	0.0074	0.0052

Hg	0.0000085	0.0049	0.061	0.00057	0.0065	0.15	0.12	0.28	0.27	0.025	0.081
Tl	0.0000093	0.026	0.0065	0.11	0.022	0.21	0.044	0.17	0.036	0.21	0.086
Pb	0.0000013	0.20	0.22	0.17	0.10	0.20	0.042	0.18	0.032	0.20	0.081
Th	0.0000023	0.0080	0.0098	0.0086	0.0067	0.0040	0.0011	0.0031	0.0012	0.0032	0.0014
U	0.0000011	0.0060	0.0054	0.0061	0.0033	0.0066	0.0041	0.0023	0.0019	0.0052	0.0017

Chapter 3:

Differences in metal compositions and concentrations of sympatric predatory fish and squid from the South Atlantic Ocean

Submitted to African Zoology

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Abstract

Organisms at higher trophic levels often contain higher concentrations of some toxicants such as metals, potentially making them more susceptible to the detrimental effects of these pollutants. The concentrations of thirty metals' concentrations were quantified in hake *Merluccius capensis*, kingklip *Genypterus capensis*, monkfish *Lophuis vomerinus* and chokka *Loligo reynaudii* from the South Atlantic Ocean of South Africa, using inductively-coupled plasma mass spectrometry. Nektobentic chokka differed significantly in metal concentrations and composition compared with demersal predators (hake, kingklip, and monkfish). Hake, kingklip, and monkfish metal concentrations and relative pattern compositions (fingerprints) were more similar to each than to chokka. Since the samples were all collected within an 80 km radius, the differences are likely due to a combination of factors such as diet, habitat (depth), and regulation of metals between cephalopods and fish rather than location. Based on South African estimated daily dietary intake, total hazard quotient, and European Union limits for Hg, Cd, and Pb, these four economically important species from the South African South Atlantic Ocean are safe for human consumption. The differences found between fish and squid suggest further investigation. Plankton, herbivorous marine species, and larger predators such as sharks and dolphins could be included in future studies to obtain a food web view regarding metals in the South Atlantic Ocean.

Keywords: Fish, squid, South Africa; muscle tissue; cadmium; mercury; lead.

1. Introduction

Organisms at higher trophic levels often have higher concentrations of some pollutants and elements, potentially making them more susceptible to toxic effects of elevated concentrations in their bodies (Fernandez *et al*., 2014). For marine top predators such as mammals, seabirds, or large pelagic fish, dietary intake represents the main pathway for the intake of metallic elements, secondary to direct uptake from the water. Metals can be increased in the environment by natural or anthropogenic sources such as mining, shipping, and agriculture (Du Preez et al., 2018; Greenfield et al., 2011; Nel et al., 2015; O'Donoghue and Marshall, 2003; Wepener and Degger, 2012). Fish are an important source of calories, proteins, fatty acids, vitamins, and minerals in a normal human diet (Elnabris et al., 2013). A seafood-based diet had been shown to contribute the most to the metal and other chemical contaminants in the human population (Bae et al., 2017), making it important to know what the concentrations of metals in commercial marine species are.

Two oceans border South Africa: The Indian Ocean on the east and south coasts, and the South Atlantic Ocean on the west coast (Hutchings et al., 2009, Figure 1.). The South Atlantic Ocean on the west coast of South Africa is characterized by the upwelling of cold, nutrient-rich waters in the austral spring and summer, resulting in a highly productive region. The increased biomass of zooplankton and phytoplankton increases the number of filter feeders, which in turn are prey for a wide variety of marine predators such as fish, squid, birds, dolphins, and seals. Almost no knowledge exists on the metal composition in piscivorous fish and squid from this region. The exploitation of filter-feeding fish and their predators support important fisheries in South Africa and Namibia, underscoring the importance of better understanding their metal compositions.

The aim of this study was to quantify and compare metal concentrations and compositional patterns in hake (*Merluccius capensis*), kingklip (*Genypterus capensis*), monkfish (*Lophuis vomerinus*), and chokka (*Loligo reynaudii*) muscle tissue from the west coast of South Africa. We took allometric influence such as length and mass that may affect concentrations into consideration. Three major toxic metals (cadmium (Cd), lead (Pb), and mercury (Hg)) were assessed and evaluated against other studies that analysed similar species. Human consumer safety of Cd, Pb, and Hg were assessed according to European and South African consumption limits, total hazard quotients (THQ), and estimated daily intakes (EDI). As far as we are aware, this study is the first of its kind on commercial marine species from the southern hemisphere and will therefore, provide important information on human consumer safety and the biology of the species considered.

2. Materials and methods

2.1. Species sampled

The Shallow-water Hake (*Merluccius capensis*, from hereon called 'hake') is an economic and ecological important demersal fish that is predatory during all life stages (Durholtz et al, 2015).

Hake feed on different prey depending on its size and habitat as explained in Figure 2. This species is important to the fisheries of South Africa, as its catch is second only to small pelagic fishes (WWF, 2014). Hake are caught by trawling which results in the bycatch of other species. Kingklip (*Genypterus capensis*), also known as the Cusk Eel (and a bycatch of hake trawling), is a species endemic to South African waters. It is a demersal ambush predator during all its life stages (Macpherson, 1983). This species feeds mostly on other demersal fish such as the Deep-water Hake (*Merluccius paradoxus*) (Macpherson, 1983).

The Cape Monkfish (*Lophuis vomerinus*, from hereon called 'monkfish') is an anglerfish, also endemic to South African waters, and, similar to the kingklip, also a bycatch of hake trawling. Monkfish are slow-growing and predatory during all life stages (Walmsley et al, 2010). Juvenile monkfish feed on small deep-sea filter feeders such as lanternfish (myctophids), while adults primarily feed on demersal species, mostly Deep-water Hake (Walmsley et al 2010).

Chokka or Cape Hope squid (*Loligo reynaudii*, hereafter called 'chokka'), which is another bycatch of hake trawling, is predatorial during all its life stages, favouring crustaceans when juveniles, changing to small pelagic fish and cephalopods as adults (Sauer and Lipinski, 1991; Lipinski, 1992; Augustyn et al., 1994).Ethical clearance was obtained from the Faculty of Natural and Agricultural Sciences of the NWU.

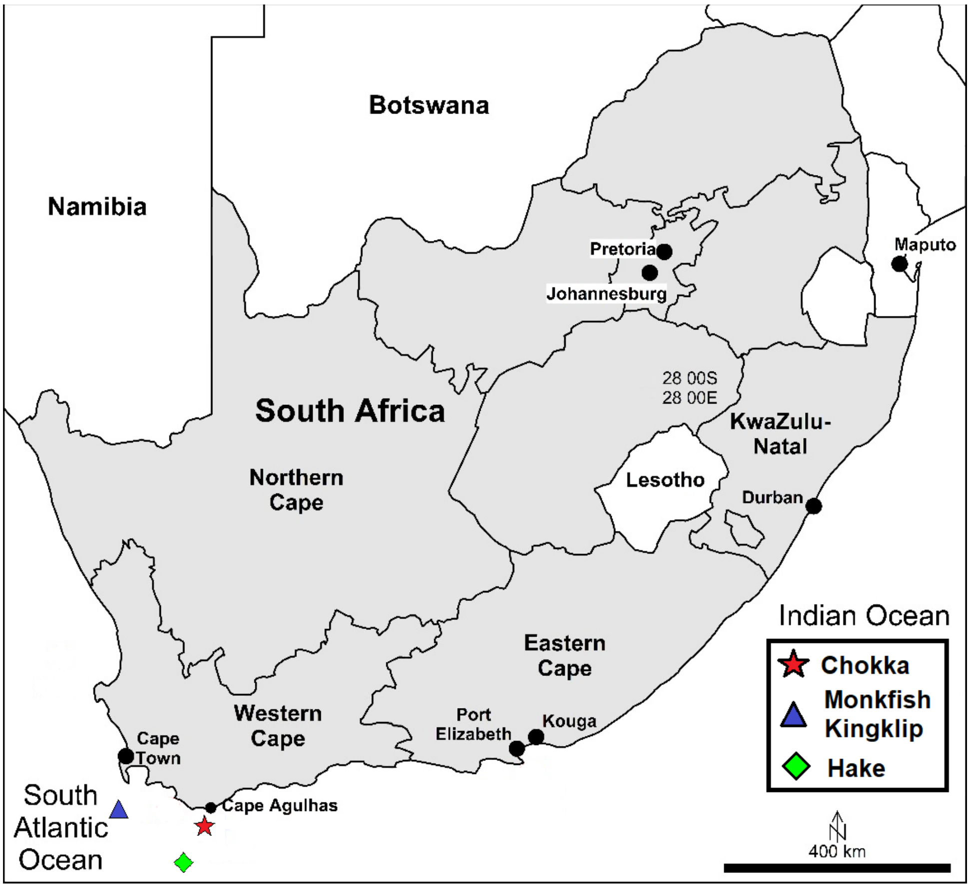

Figure 1: Map of the sampling sites

2.2 Sampling sites

The sampling sites were located within a radius of 80 km (Figure 1). Since the sites were in close proximity and all located on the Alphard Banks, we consider for the purposes of this study the animals caught as sympatric. Hake, kingklip, and monkfish samples were received gratis from Irvin and Johnson (I&J) fisheries. Chokka samples were received from the Department of Environment, Fisheries and Forestry of South Africa (DEFF). Monkfish and kingklip were caught at the same site in the South Atlantic Ocean at approximate depths of 470 m and 420 m, respectively (Figure 1). Hake were caught closer to Cape Agulhas, the point where the Indian Ocean and the South Atlantic Ocean meet, at an approximate depth of 367 m. Chokka were caught off the west coast of South Africa close to the hake site near Cape Agulhas at an approximate depth of 192 m. Whole samples were selected randomly, couriered frozen from Cape Town to the laboratory at the North-West University (NWU) in Potchefstroom, South Africa, where they were received frozen and stored in freezers.

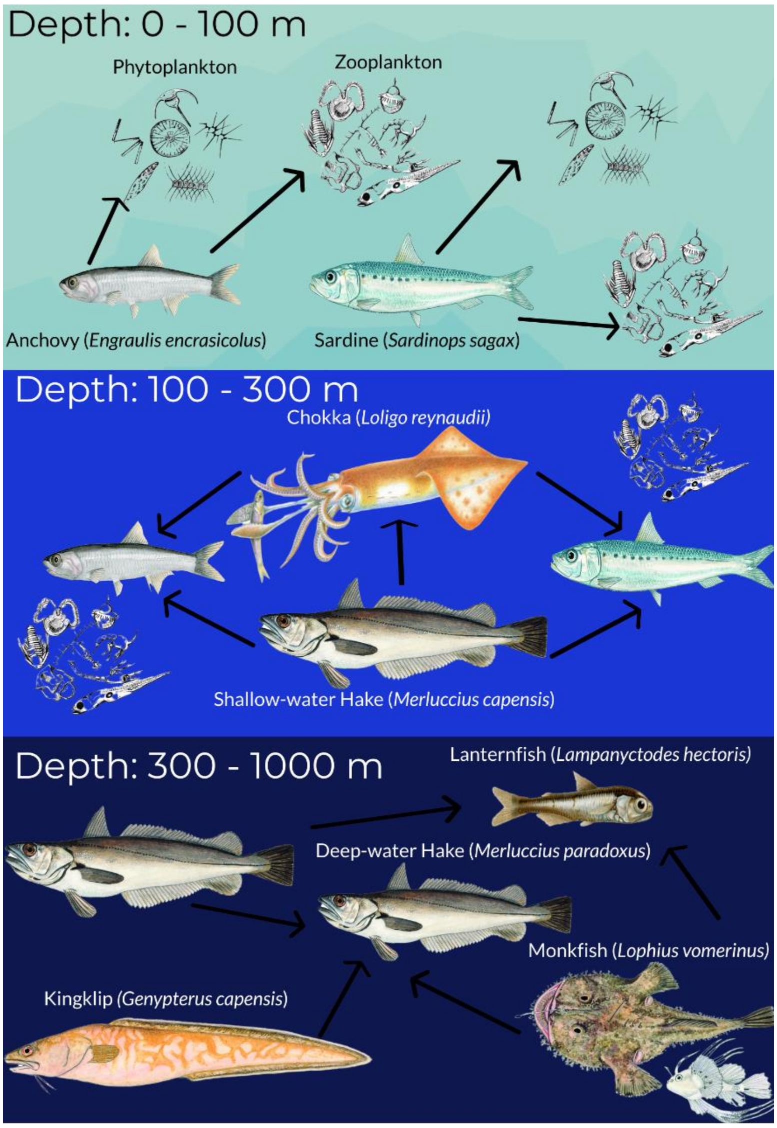

Figure 2: Simplified diagram of the food web and depth stratification of hake, kingklip, monkfish, and chokka in the South Atlantic Ocean (Free use permitted; Adapted from SAIAB, 2019).

2.3 Dissection and sample preparation

Fish and squid were defrosted until firm and in a workable condition, weighed, and measured for length. Ten specimens of each species were used. All work surfaces were cleaned with soap and water and rinsed with deionised water between samples to prevent contamination. Dissection tools were cleaned with acetone and hexane after each dissection to avoid contamination. The samples were dissected on wooden chopping boards. Muscle tissue and chokka mantle samples without skin were dissected from the left dorso-lateral side of the fish and squid. The sampled tissue never came into contact with the dissection boards. The tissue was weighed to obtain a wet-mass sample of approximately 10 g. Each sample was transferred to a 25 mL high-density polypropylene centrifuge tube. The weighed samples were frozen at -80°C and then freeze-dried for three days at 8 kPa and -50°C. The freeze-dried samples were then weighed to obtain accurate dry mass (approximately 200 mg). Freeze-dried samples were analysed by Eco-Analytica at the North-West University (NWU).

2.4 Chemical analyses

Accurately weighed, freeze-dried, samples were dissolved in concentrated nitric acid. The solutions were placed in a fluorocarbon polymer vessel, sealed, and heat-digested in an Ethos UP microwave unit, as per the United States Environmental Protection Agency method 3051A, for 35 minutes (USEPA, 2007). A standard reference material (SRM) (ERM-CE278K – mussel tissue) was used as quality control (QC) with the same digestion protocol.

The microwave-digested solutions were analysed using an Agilent 7500ce, inductively-coupled-plasma mass-spectrometer (ICP-MS), optimised with an aqueous solution containing cerium (Ce), lithium (Li), ytterbium (Y), and thallium (Tl) (1 µg/L) to reduce interference (≤ 1.5%). Forward power was 1550 W, plasma gas flow was set at 15 L/min, the nebuliser gas flow was 1.2 L/min, and the sampling depth in the container was at 8 mm. External calibration was done by using ULTRASPEC-certified mixed multi-element standard solutions (De Bruyn Spectroscopic Solutions). Full calibration sets and QC-check standards were run. The QC check standard was a calibration standard of midrange concentration. We quantified concentrations of 30 elements for each sample (see Table S1), as milligram per kilogram on a dry mass basis (mg/kg dm). For human consumer calculations, we used wet-mass based data.

2.5 Statistical analyses

We tested data for normality using the Shapiro-Wilks normality test (GraphPad Prism 8.0.2; www.graphpad.com). Since almost all datasets were not normally distributed, we used Kruskal-Wallis (unpaired, non-parametric, one-way ANOVA) to compare concentrations between hake, kingklip, monkfish, and chokka, using Dunn's test for multiple comparisons. We used linear regressions of the elemental concentrations against length and mass of the fish and squid sampled to investigate allometric associations. For multivariate analyses, we used unconstrained principal component analysis (PCA) and constrained (mass and length) redundancy analysis (RDA) to ordinate the metal concentrations (MjM Software PC-ORD version; www.pcord.com). Convex hulls were used to separate species. Each convex hull may be viewed as the metal ‘fingerprint’ for that species. The extent of overlap of the convex hulls indicates the similarity or difference between each species’ ‘fingerprint’.

3. Results

3.1 Samples

Kingklip and monkfish mean lengths and standard deviations were 44 cm ± 8.1 cm and 67 cm ± 5.4 cm, while their mean mass and standard deviations were 2237 g ± 909 g and 1679 g ± 672 g, respectively. Hake mean length and standard deviation was 44 cm ± 4.5 cm, with a mean mass and standard deviation of 858 g ± 132 g. Chokka mean mantle length and standard deviation was 14.8 cm ± 3 cm, and the mean mass and standard deviation was 98 g ± 54 g.

3.2 Analytical results

Recoveries of certified elements were within 20% of SRM certified values. Table S1 summarises the analytical results, and graphically presented in Figure 3. Nickel (Ni), bismuth (Bi), and gold (Au) concentrations were below the limit of detection (LOD) in chokka mantle.

3.3 Concentration differences between species

We found significant concentration differences (Kruskal-Wallis, one-way ANOVA; $p < 0.05$) between hake, kingklip, monkfish and chokka for boron (B), magnesium (Mg), phosphorous (P), titanium (Ti), vanadium (V), chromium (Cr), manganese (Mn), cobalt (Co), zinc (Zn), Cd, selenium (Se), rubidium (Rb), strontium (Sr), molybdenum (Mo), silver (Ag), palladium (Pd), antimony (Sb), platinum (Pt), mercury (Hg), thorium (Th), uranium (U), aluminium (Al), nickel (Ni), and bismuth (Bi) (Figure 3). We found significantly higher concentrations of B, Mg, copper (Cu), P, Zn, titanium (Ti), Cd, Rb, Sr, Ag, and U in chokka compared with any of the three fish species (Kruskal-Wallis, one-way ANOVA; Dunn’s multiple comparisons, $p < 0.05$) (Figure 3) and significantly lower concentrations of V, Se, Cr, Mn, iron (Fe), Co, Mo, Pd, Pt, Th, Al, Bi, Sb, Hg, arsenic (As), and Ni in chokka compared with any of the three fish species (Kruskal-Wallis, one-way ANOVA; Dunn’s multiple comparisons, $p < 0.05$) (Figure 3). We found significantly higher concentrations of Mg and As in hake compared with kingklip (Kruskal-Wallis, one-way ANOVA; Dunn’s multiple comparisons, $p < 0.05$) (Figure 3).

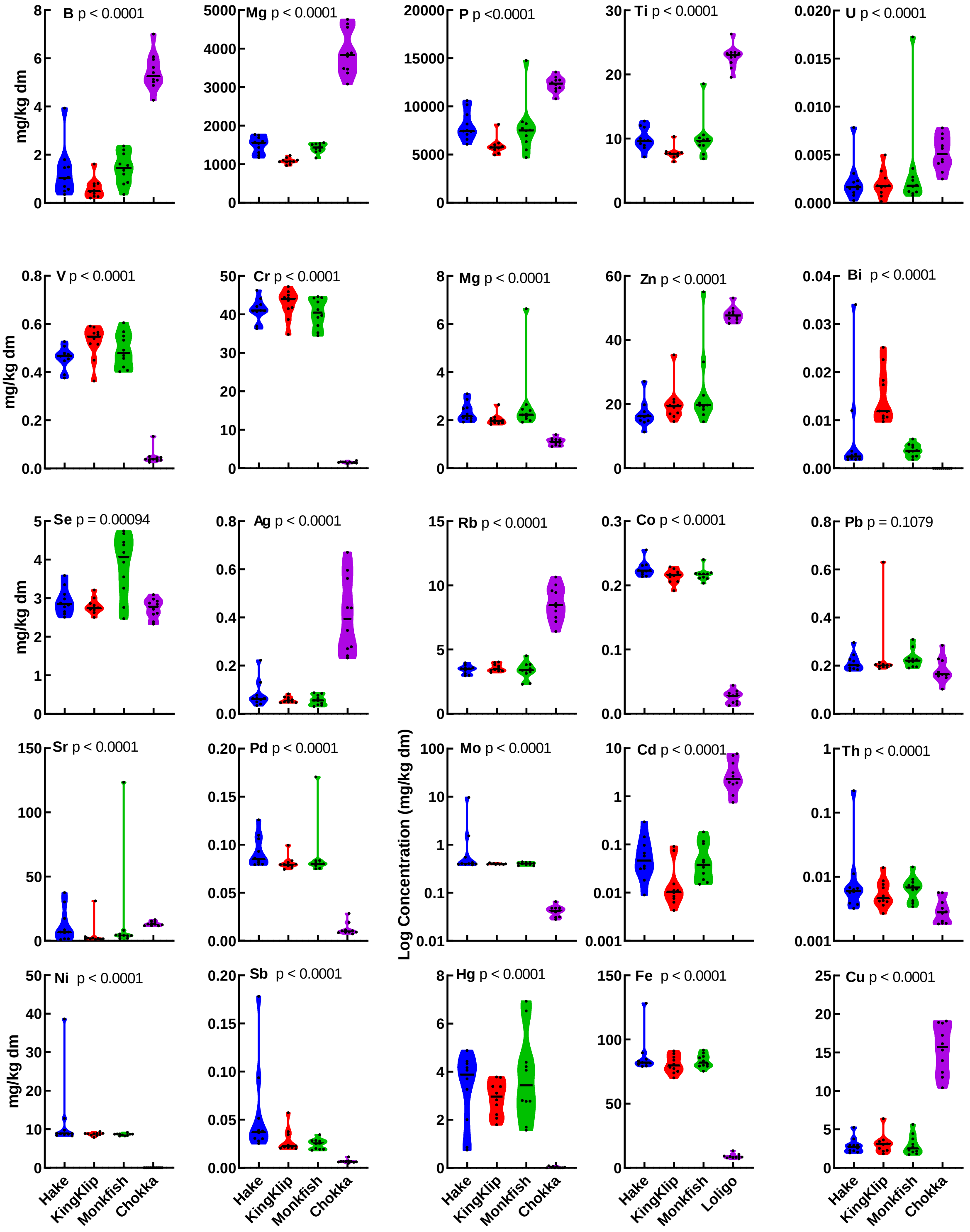

Figure 3: Violin plots of metal concentrations (mg/kg dm) and results of significance of Kruskal-Wallis one-way ANOVA between hake, kingklip, monkfish, and chokka. Medians are indicated by horizontal lines, and individual values by dots. Note that Mo, Cd, and Th concentrations are on a log scale. Detailed statistical information is in Table S1.

3.4 Associations between length and mass with metal concentrations

We found significantly negative linear regressions between Co, Pb, Cr, and U with hake mass, and Zn with monkfish length (Figure 4). Significant negative linear regressions were found for Se, Mn, Co, and Mo with chokka length (Figure 4). We found significantly negative linear regressions ($p < 0.05$) for Co and U with hake mass, Sb and monkfish length, and Se, V, and Co with chokka mass (Figure 5). Chromium and kingklip length had a significant positive regression (Figure 5).

3.5 Differences in metal composition patterns

The PCA indicated an overlap in metal compositional pattern between hake, kingklip, and monkfish (Figure 6). However, the chokka convex hull separated completely from the demersal predators according to their metal composition. Results of the PCA are provided in Tables S2 and S3. The first principal component (Axis 1) contributed 58.68% of the total variance, the second principal component (Axis 2) contributed 8.48%, and the third principal component (axis 3, not shown) contributed 7.1%. The demersal predators were characterized by higher proportions of Mo, Sb, V, Ni, Cr, Hg, Fe, Co, Pt, Pd, Al, Mn, and Se. Chokka were characterized by higher proportions of Cd, Rb, Cu, B, Ag, Zn, Ti, and U.

The RDA indicated a separation between hake, kingklip, monkfish, and chokka according to their mass, length and elemental composition (Figure 7). Two axes were needed to explain the variance observed. The first axis explained 83.7% of the total variance, and the second axis explained 16.3%. Hake was characterized by higher proportions of barium (Ba) and gold (Au). Kingklip was characterized by higher proportions of Sb, Th, Pt, Mn, Mo, Pd, Fe, Ni, Co, Al, Cr, Bi, V, and Hg. Monkfish was characterized by higher proportions of Se, and As. Chokka were characterized by higher proportions of Zn, Ti, Ag, B, Cu, Rb, Cd, and U.

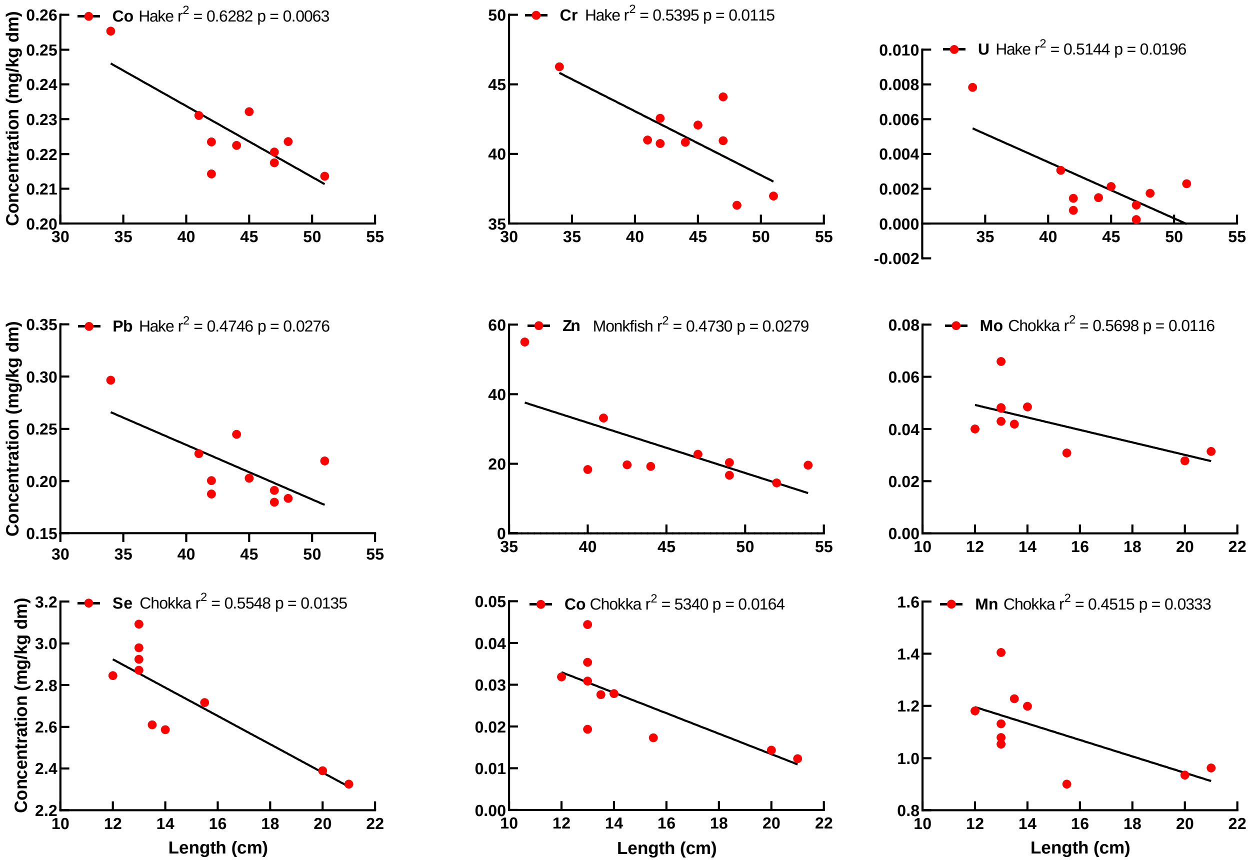

Figure 4: Significant linear regressions of metal concentrations with individual length (cm) (respective r^2 and p values indicated on each graph).

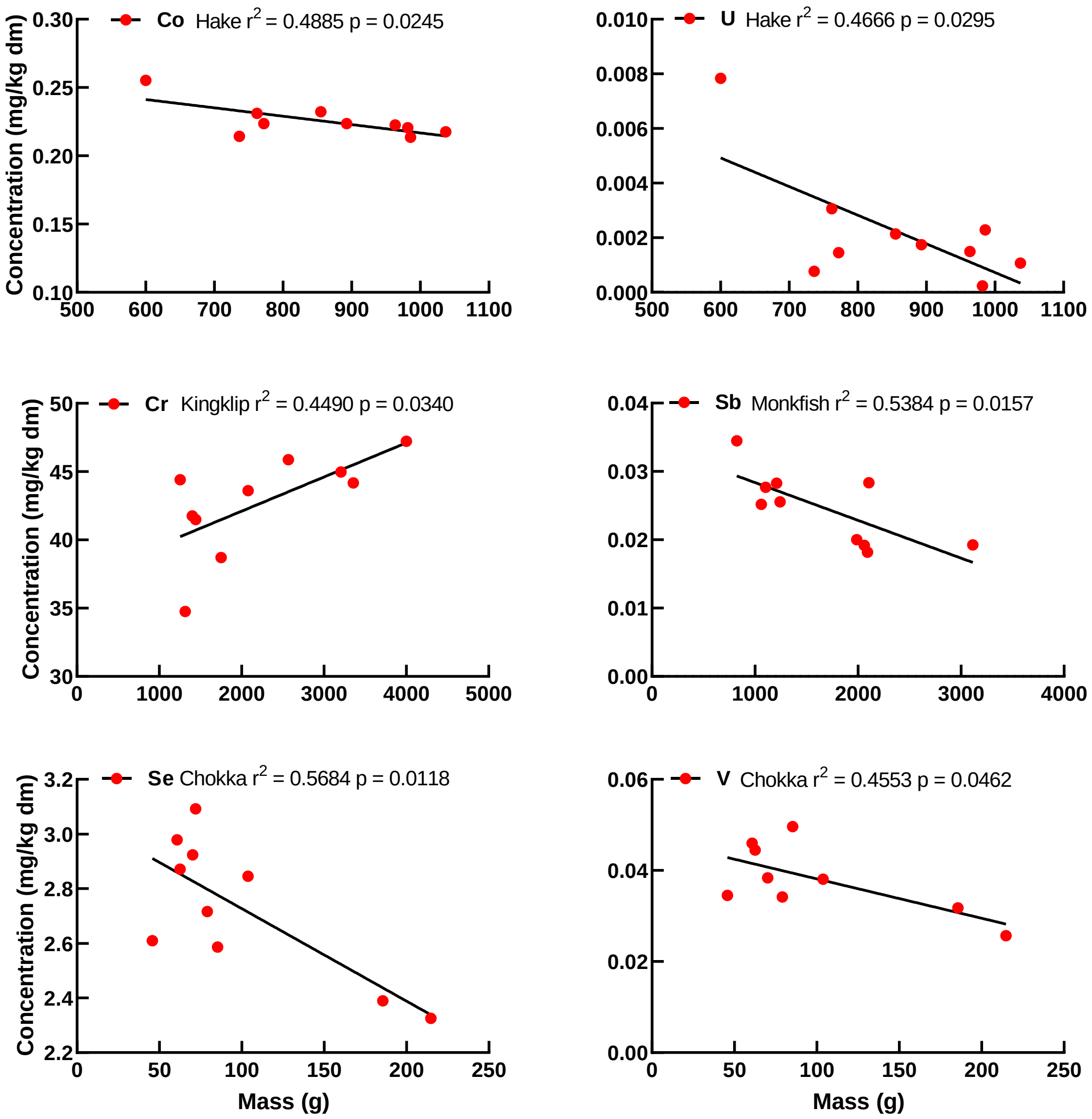

Figure 5: Significant linear regressions of metal concentrations and individual mass (g) (respective r^2 and p values are indicated on each graph).

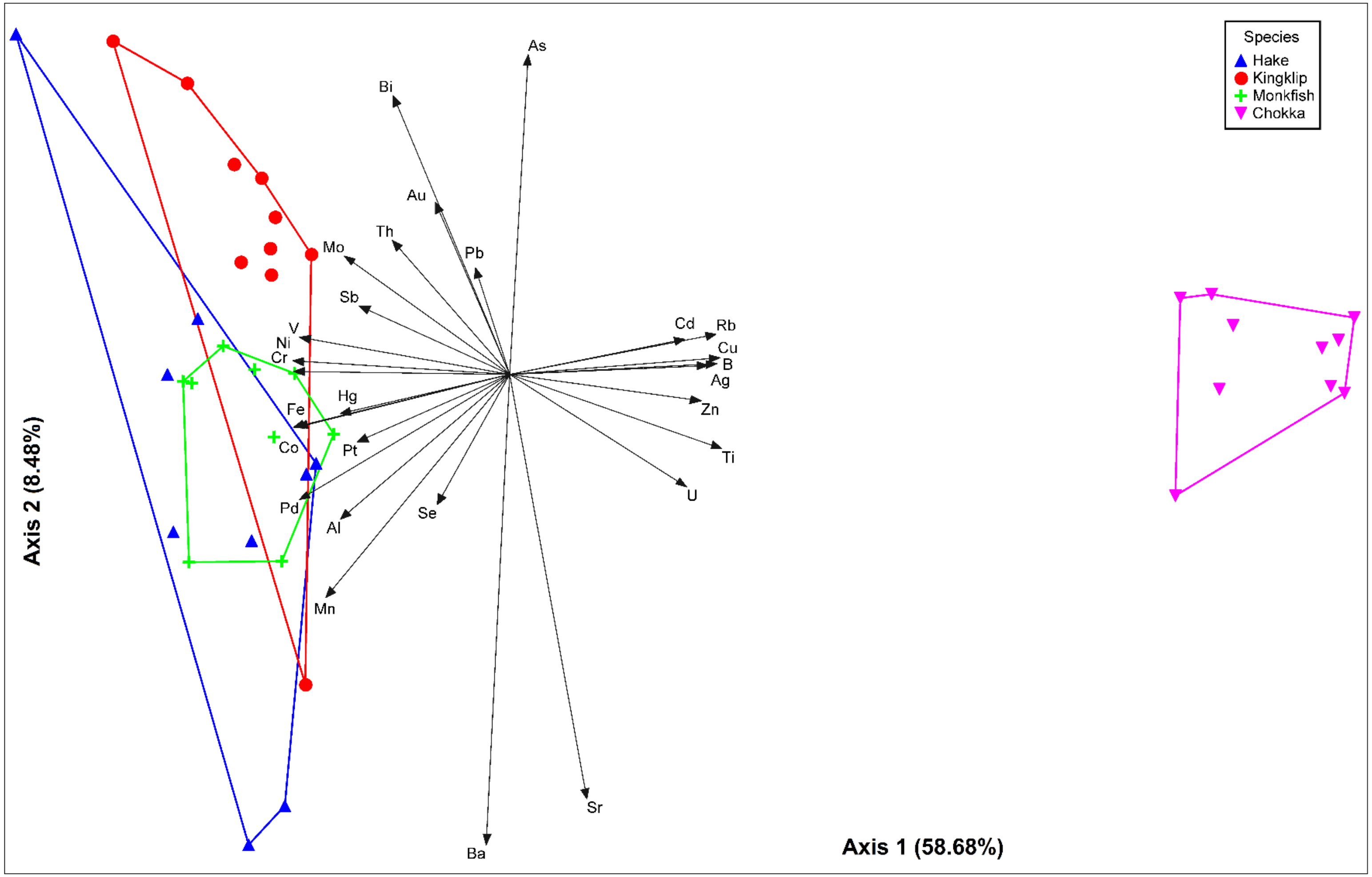

Figure 6: Principal component analysis (PCA) ordination of metals for hake, kingklip, monkfish and chokka muscle tissue. Convex hulls are shown for each species.

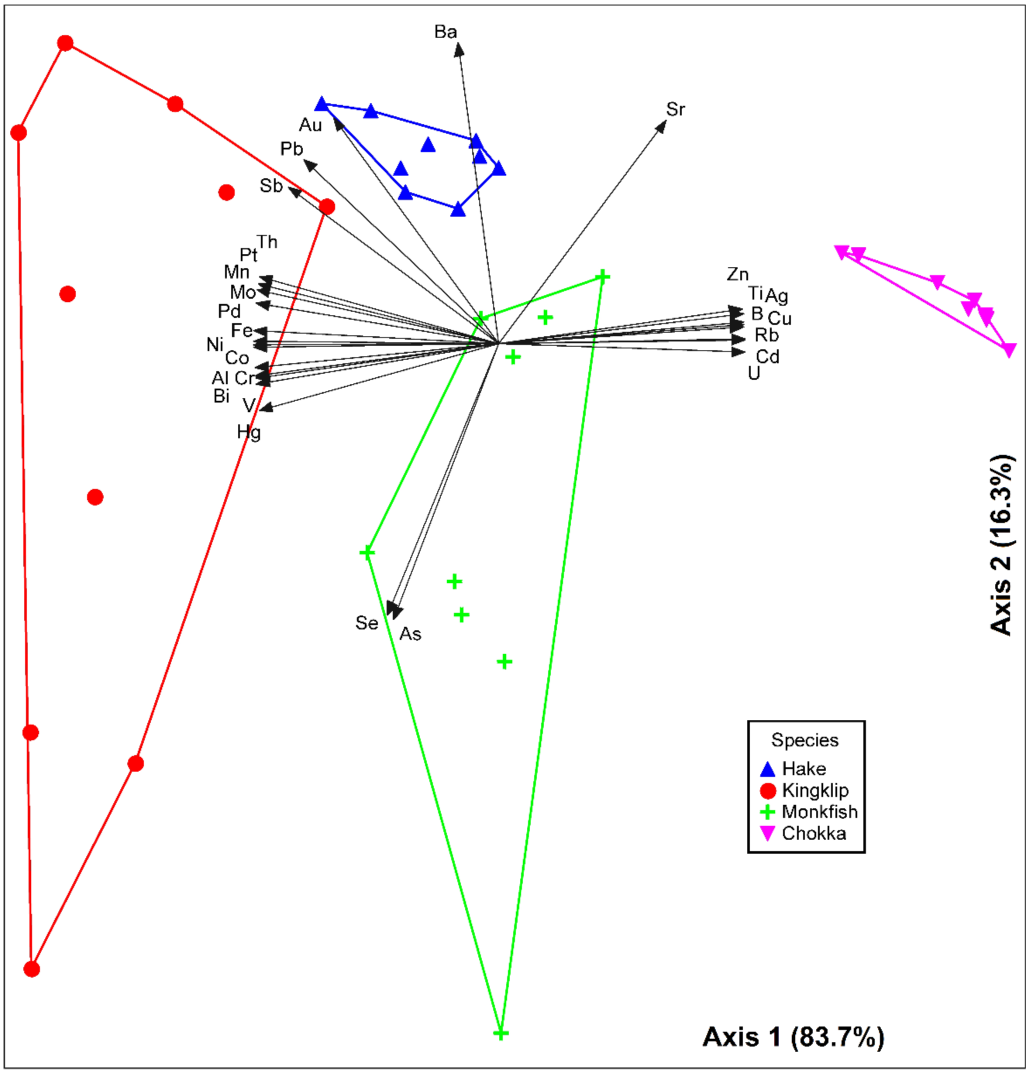

Figure 7: Redundancy analysis (RDA) ordination of metal concentrations for hake, kingklip, monkfish, and chokka muscle tissue, with mass and length, included as factors. Convex hulls are shown for each species.

4. Discussion

4.1 Differences in metal concentrations and compositions

4.1.1 Differences between chokka and the fish species

We found significant ($p < 0.05$) differences between hake, kingklip, monkfish, and chokka based on their metal composition and concentration (results section, and Figure 3). ANOVAs indicated significantly ($p < 0.05$) higher concentrations of B, Mg, Cu, P, Zn, Ti, Cd, Rb, Sr, Ag, and U in chokka compared with the demersal predators (Figure 3). The PCA analysis indicated metal compositional differences between the demersal species and chokka (Figure 6). The PCA findings strengthen the results obtained from the ANOVAs: chokka was characterized by having higher proportions of Cd, Rb, Cu, B, Ag, Zn, Ti, and U than the fish (Figure 6). Since the locations of the sampling sites were relatively close to each other, the differences and coincidences in metal concentrations and compositions of the species are likely due to a combination of differing habitat (depth), prey (Figure

2), and differences in physiological regulation of these metals (Omar et al., 2013; Giesy and Wiener, 2011; Mol and Ouboter, 2004; Uren et al., 2020 in press), but not location.

Many of these elements are known to increase in upwelling zones increasing the zooplankton and phytoplankton in the upper reaches of the ocean (Valdés et al., 2008). Cephalopods and especially loliginids (squids) are believed to accumulate most of their metals through their diet (Penicaud, 2017). Chokka mainly feed on anchovy and other small pelagic fish (Augustyn et al., 1994; Lipinski, 1992; Sauer and Lipinski, 1991) which, in turn, feed on zooplankton and phytoplankton (Van der Lingen et al., 2010) possibly explaining the concentrations we found. Cephalopods are known to accumulate high concentrations of Cu, Zn, Ag, and Cd (Mok et al., 2014), suggesting that cephalopods are not as sensitive to these elements or they regulate these elements differently from the sympatrically sampled fish species. Copper concentration differences would be explained by cephalopod physiology: cephalopod blood contains hemocyanin with Cu as their oxygen carrier (Brix et al., 1989). Similarly, the higher Fe concentrations in the fish could be explained by the haemoglobin and the Fe atom carrying the oxygen (Figure 3).

We found significantly ($p < 0.05$) lower concentrations of V, Se, Cr, Mn, Fe, Co, Mo, Pd, Pt, Th, Al, Bi, Sb, Hg, As, and Ni in chokka than the demersal predators (Figure 3), further supported by the PCA that integrated all the elements (Figure 6). The demersal species were characterized by a higher proportion of Mo, Sb, V, Ni, Cr, Hg, Fe, Co, Pt, Pd, Al, Mn, and Se (Figure 6). Chokka physiologically regulate Mo, V, and Co through storage in the brachial heart (Penicaud, 2017). Manganese is regulated by incorporation into their eggs. Some metals could be incorporated into the gladius of the chokka, as chitin absorbs metals effectively and have been applied industrially to do so (Anastopoulos et al., 2017, Zhou et al 2004). The distribution of the elements between the gladius and other organs could be investigated in future studies. We could trace no such studies in literature.

Palladium and Pt are part of the platinum group of metals and together with Bi and Au have been found to enter organisms as nanoparticles (Zhu et al., 2010; Bystrzejewska-Piotrowska et al., 2009; Luo et al., 2012). Higher concentrations of Hg and Se were expected in the demersal species as it is well known that Hg bioaccumulates in higher trophic levels (Bosch et al., 2016). Selenium generally has a positive ratio to Hg in most organisms as Se acts as a detoxifier of Hg (Leonzio et al., 1982; Cuvin-Aralar and Furness, 1991). We regressed the two elements, and found no significant association. Chromium, Al and Ni have been seen to increase in the top predators in different habitats (De Mora et al., 2004). We have found that this was the case in our study as well. Thorium and Sb are toxic (Cooper and Harrison, 2009; Peng et al., 2017), but information on quantification is limited in fish and should be investigated in the future.

4.1.2 Differences between hake, kingklip, and monkfish

ANOVAs only indicated Mg and As concentrations differing between hake and kingklip (Figure 3). The PCA (an unconstrained ordination) indicated an overlap of metal composition between hake, kingklip, and monkfish (Figure 6). This homogeneity was surprising as we expected more pronounced differences due to differing taxonomy and life histories. Monkfish is a slow-growing species that rely on ambushing their prey by burrowing into the sand with only their dorsal spine sticking out tipped with a lure (Walmsley et al., 2010). Kingklip is also an ambush predator found near rocky areas (Macpherson, 1983). However, hake migrate nightly from depths of 400 m to feed in the pelagic zone (Durholtz et al., 2015) causing this species to be more active than kingklip and monkfish.

Feeding habits according to literature and patterns observed from dissection could shed light on the reason these concentrations and compositions were close to the same (Figure 2). Hake from the west coast diet has been identified as feeding on mostly lanternfish (myctophids) when they are smaller (< 30 cm), medium hakes (30–50 cm) preyed mostly on Deep-water Hake, and large hake (> 50 cm) fed on primarily on other demersal species (Durholtz et al., 2015). Hake obtained for this study all fell in the medium range and thus we assume, would feed mostly on Deep-Water Hake (Durholtz et al., 2015). Monkfish and kingklip diets consist of other demersal fish (mostly deep-water hake) when they are mature (Walmsley et al., 2010; Macpherson, 1983) possibly explaining why these metal concentrations and compositions are so similar.

Dissection of the monkfish also revealed lanternfish in the stomach, indicating another overlap of diet with hake. Since monkfish and kingklip are caught as bycatch of the hake demersal trawling, it would be expected that these species also share a common habitat and depth (Figure 2).

4. 2 Effect of length and mass on metal concentrations

The RDA (a constrained ordination) indicated a difference in metal composition (non-overlap of the convex hulls) with fish length and mass as secondary variables (Figure 7). This suggests that allometric characteristics such as length and mass are associated with metal concentrations and compositions of these species. We found significant ($p < 0.05$) regressions between mass and length with metal concentrations (Figures 4 and 5), keeping in mind that heavier animals are normally also longer, and therefore co-variate. We found significant ($p < 0.05$) negative regressions for Co and U compared with hake mass, Sb compared with monkfish mass, and Se and V compared with chokka mass (Figure 5). We found a significant ($p < 0.05$) positive regression with Cr and kingklip mass (Figure 5).

In three instances element concentrations regresses negatively ($p < 0.05$) with both mass and length of the animal: Co and U for hake and Se for chokka (Figure 4). However, other elements such as Pb and Cr compared with hake length (but not mass) had a significant ($p < 0.05$) negative association based on regression analyses (Figure 4). Zinc compared with monkfish length was significantly ($p < 0.05$) negative, as was Mn, Co, and Mo compared with chokka length (Figure 4). We found that mass and length affect metal concentrations in hake and chokka the most, with most elements decreasing as the specimen size increases. Kingklip concentrations seem to be the least influenced by length and mass. However, the positive regression with Cr could be concerning as large specimens of kingklip may then harbour high concentrations of Cr (Figure 4).

Effects of allometry on metal concentrations may vary between species, habitat, the life stage of an organism, physiology, and diet. Different species would thus not follow the same allometric patterns. The general finding is that metals would have a negative slope if there was a significant association (Al-Yousuf et al., 2000; Canli and Atli, 2003; Farkas et al., 2003; McKinley et al., 2012 Merciai et al., 2014), and that the strength of association would be different between species, agreeing with what has been found in our study.

However, Kasimoglu (2014), contrary to our study, found significant ($p < 0.001$) positive regressions between Co, Cu, Fe, Mn, Ni, and Zn with fish length and mass, once again indicating that these associations would not be consistent between species and habitats. However, he did find that Cr increased significantly with kingklip size.

Arantes et al. (2016) found a positive regression with Hg and body size of fish. This is believed to be the general case for Hg in most species. However, no significant positive regression was found between Hg, length, and mass for any species in this study. This may be due to a sample size of ten specimens per species, or that the Hg concentrations might be too low to register such an effect.

4.3 Comparison with similar studies

We compared present mean Cd, Pb, and Hg concentrations (based on wet mass) from hake and monkfish with concentrations found in other studies with similar species (Table 1). These elements were selected as they are recognised toxic elements in seafood by regulators such as the European Union (EU) and the South Africa government. Information on kingklip is very limited as it is endemic to only Southern African waters. Keeping in mind that kingklip concentrations were much the same as hake and monkfish (Figure 3), we compared our mean data with hake and anglerfish data from elsewhere.

Hake and monkfish from South Africa had lower concentrations of Cd compared with other studies (Table 1). Chokka compared with other squid species had the second-highest concentration of Cd. Hake and monkfish had lower concentrations of Pb compared with other hake and anglerfish species (*Lophuis piscatorius*). Chokka had a lower Pb concentration compared with other studies. Hake from South Africa had the highest mean concentration of Hg compared with European hake data (*Merluccius merluccius*). The monkfish mean Hg concentration was much higher compared with European hake, and lower than European anglerfish. Concentrations of Hg were similar to another study on monkfish from the Namibian coast (Erasmus et al., 2019). Kingklip had a higher mean Hg concentration than European hake, and a lower concentration than anglerfish. Chokka mantle tissue had a lower mean Hg concentration than comparable studies.

Therefore, Hg concentrations in hake, kingklip, and monkfish, and Cd concentrations in chokka could be cause for concern to the health of these predators as the concentrations we found to be much higher than comparable studies. Future monitoring of Hg and Cd elements in these and other species should be considered.

Table 1: Comparison of mean Hg, Pb, and Cd concentrations (mg/kg wet mass) in hake (*Merluccius merluccius* and *M. capensis*), kingklip (*Genypterus capensis),* monkfish (*Lophuis piscatorius* and *L. vomerinus*), and chokka (*Loligo vulgaris* and *L. reynaudii*), and limits set by the European Union (EU) and the South African Government (RSA)**.**

Species	Location	Cd	Pb	Hg	Reference
Merluccius merluccius	Mediterranean Sea	0.00074	<LOQ	0.0076	Kalogeropoulos et al., 2012
Merluccius merluccius	Mediterranean Sea	0.00003	0.031	0.067	Pastorelli et al., 2012
Merluccius merluccius	Mediterranean Sea	NA	NA	0.30	Llull et al., 2017
Merluccius merluccius	Mediterranean Sea	0.005-0.01	0.01-0.013	0.12.0.29	Falco et al., 2006
Merluccius merluccius	Mediterranean Sea	0.01	0.542	NA	Iamiceli et al., 2015
Merluccius merluccius	Mediterranean Sea	0.019	<LOQ	NA	Ramos-Miras et al., 2019
Merluccius merluccius	Mediterranean Sea	LOD	0.07	0.20	Nadal et al., 2008
Merluccius merluccius	Adriatic Sea	0.002	0.023	0.373	Juresa and Blanusa, 2003
Merluccius merluccius	Marnara Sea	0.82	9.4	0.17	Aksu et al., 2011
Merluccius merluccius	North Atlantic Ocean	0.002	0.05	NA	Mormede and Davies., 2001
Merluccius merluccius	Adriatic Sea	0.0041-0.014	0.049-0.141	NA	Kljakovic Gaspic et al., 2002
Merluccius capensis	**South Atlantic Ocean**	**0.015**	**0.04**	**0.63**	**Current study**
Genypterus capensis	**South Atlantic Ocean**	**0.0048**	**0.048**	**0.58**	**Current study**
Lophuis piscatorius	North Atlantic	0.002	0.002	NA	Mormede and Davies, 2001
Lophuis piscatorius	Mediterranean Sea	NA	NA	0.74	Llull et al., 2017
Lophuis vomerinus	South Atlantic Ocean	NA	NA	0.12 – 0.647	Erasmus et al., 2019
Lophuis vomerinus	**South Atlantic Ocean**	**0.01**	**0.039**	**0.65**	**Current study**
RSA Limits		1.0	0.5	1.0	**South Africa, 2008**
EU Limits		0.05	0.3	1.0	**EU, 2018**
Loligo vulgaris	Mediterranean Sea	0.728	0.586	0.029	Pastorelli et al., 2012
Loligo vulgaris	Mediterranean Sea	0.05-0.15	0.01-0.01	0.05-0.15	Falco et al., 2006
Loligo vulgaris	Mediterranean Sea	0.068	LOD	0.01	Kalogeropoulos et al., 2012
Loligo reynauldii	**South Atlantic Ocean**	**0.66**	**0.04**	**0.005**	**Current study**
RSA Limits		1.0	0.5	1.0	**South Africa, 2008**
EU Limits		0.5	1.0	0.5	**EU, 2018**

<LOQ – Below limit of detection
*NA - Not analysed

4.4 Consumer safety

Mean Cd, Hg, and Pb wet mass concentrations in muscle tissue of hake, kingklip, monkfish, and chokka in this study did not exceed the human consumer limits (despite marginal exceedances for Cd in two chokka samples) set by the EU and the RSA (Table 1). These elements were selected as they are generally considered as the most dangerous metallic pollutants in seafood.

Table 2: Estimated daily human dietary intakes (EDIs) and total hazard quotients (THQs) for cadmium, lead and mercury measured in hake, kingklip, monkfish and chokka.

Species	Cd EDI	Cd THQ	Pb EDI	Pb THQ	Hg EDI	Hg THQ
Hake	0.0000047	0.0000047	0.000013	0.0000036	0.0002	0.000066
Kingklip	0.000000045	0.000000045	0.00000045	0.00000013	0.0000054	0.0000018
Monkfish	0.00000018	0.00000018	0.00000069	0.0000002	0.0000114	0.000266
Chokka	0.0000016	0.0016	0.0000001	0.000029	0.00000012	0.028

Estimated daily intakes (EDI) and total hazard quotients (THQs) were calculated according to the formulae in Wang et al (2012) and assumptions used by Uren et al,2020 (in press) (Table 2). The average mass and lifespan of a South African resident were selected as 70 kg and 70 years, respectively. The daily intake of hake was estimated to be 0.0042 kg/day, kingklip daily intake was estimated to be at 0.00013 kg/day and, monkfish daily intake was estimated at 0.00021 kg/day for South African residents (WWF, 2014). The THQ values were all below the quotient of unity and would pose no threat to consumers based on our assumptions (Table 2). The highest THQ was for Hg in chokka, still two order of magnitude below 1. For Cd, Pb, and Hg, hake, kingklip, monkfish, and chokka should be considered safe for human consumption and export, but more samples from more locations would add weight to this finding.

5. Conclusions and recommendations

Seafood is consumed daily worldwide and contains important nutrients and minerals needed for humanity. There is an information gap on metals in commercially important marine species in South Africa such as hake, sardine, etc. As far as we are aware of, this is the first study that compares metal concentrations in hake, kingklip, monkfish, and chokka from the southern hemisphere. Clear differences in metal concentration and composition were found between chokka and the demersal fish predators (hake, kingklip, and monkfish).

We found significantly higher concentrations of B, Mg, Cu, P, Zn, Ti, Cd, Rb, Sr, Ag, and U, and significantly lower concentrations of V, Se, Cr, Mn, Fe, Co, Mo, Pd, Pt, Th, Al, Bi, Sb, Hg, As, and Ni in squid (chokka) than in the demersal fish predators (Figure 3). PCA and RDA indicated distinctly different metal compositions between chokka and the fish species (Figures 6 and 7). Since the collection points were within a radius of 80 km, the differences in concentrations compositional are likely due to the combined influence of differences in diet, habitat (depth), life histories, and physiological regulation of metals between cephalopods and fish. PCA and ANOVAs did not indicate any substantial differences between hake, kingklip, and monkfish, but did separate chokka. These organisms, especially the fish, share common prey, possibly explaining the similar metal concentrations and compositions of the piscine species.

The RDA indicated metal composition differences between hake, kingklip, chokka, and monkfish possibly indicating that mass and length have an effect on metal concentrations (Figure 7). Linear regressions (Figures 4 and 5) of metal concentration with either length or mass indicated a decrease in metal concentrations as the size of the organisms increased, except for Cr in kingklip that increased with larger individuals. It seems therefore that there is allometric involvement with concentrations of some metals.

Although we analysed only ten samples of each species, we found that chokka, hake, kingklip, and monkfish would be safe for human consumption and export based on Cd, Pb, and Hg concentrations. In future studies, we recommend that more samples from different sites to investigate if the homogenous concentrations and compositions hold true for hake, kingklip, and monkfish around the coast of South Africa. The differences in elemental composition found between fish and squid are also an area of future investigation. Plankton, herbivorous marine species and larger predators such as sharks and dolphins could be included in future studies to gain an extended food web view regarding metals in the South Atlantic Ocean.

Acknowledgements

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Appendix A

Table S1: Mean concentrations (using two significant numbers) of metallic elements in hake, kingklip, monkfish and chokka muscle tissue per site (mg/kg dry mass) with the detection limit of each element analysed. Elements not mentioned in the text are listed for archival purposes.

		Hake		Kingklip		Monkfish		Chokka	
	LOD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
B	0.0025	1.3	1.1	0.61	0.42	1.4	0.66	5.4.	0.76
Mg	0.00003	1500	220	1100	77	1400	120	3900	580
Al	0.0018	16	4.4	12	1.6	17	6.5	3.9	3.2
Ti	0.000041	10	1.7	7.8	1	10	3.2	23	1.8
V	0.000029	0.46	0.047	0.53	0.07	0.49	0.071	0.048	0.031
Cr	0.00089	41	3	43	3.7	40	3.8	1.6	0.22
Mn	0.000016	2,3	0.4	2	0.24	2.7	1.4	1.1	0.15
Fe	0.0011	87	15	81	68	83	5.2	9	1.8
Co	0.000008	0.2	0.012	0.21	0.011	0.22	0.0097	0.026	0,010
Ni	0.000002	12	9.4	8.7	0.4	8.6	0.28	LOD	LOD
Cu	0.000012	3	1	3.2	1.3	3	1.3	15	3.1
Zn	0.000076	17	4.2	20	5.8	24	12	48	2.4
As	0.000012	8.5	4.1	20	7.4	15	6.2	17	2.1
Se	0.00011	2.9	0.34	2.8	0.2	3.8	0.8	2.7	0.25
Rb	0.0000023	3.4	0.33	3.6	0.29	3.4	0.67	8.6	1.4
Sr	0.0000056	12	13	4.7	9,3	16	38	13,4	1.63
Mo	0.0000023	1.5	2.9	0.4	0.0095	0.4	0.027	0.043	0.011
Pd	0.00000098	0.093	0.016	0.081	0.007	0.09	0.029	0.012	0.0066
Ag	0.0000098	0.08	0.057	0.057	0.012	0.06	0.021	0.41	0.16
Cd	0.000032	0.079	0.086	0.024	0.032	0.06	0.056	3.3	2.5
Sb	0.0000040	0.055	0.048	0.028	0.012	0.025	0.0053	0.0068	0.0017
Ba	0.000021	0.22	0.088	0.16	0.064	0.29	0.29	0.17	0.067
Pt	0.0000062	0.016	0.0036	0.015	0.0026	0.014	0.0018	0.0074	0.0055
Au	0.0000005	0.015	0.033	0.003	0.0074	0.003	0,0075	LOD	LOD
Hg	0.0000085	3.3	1.5	2.9	0.71	3.8	38	0.025	0.03
Pb	0.0000013	0.21	0.036	0.24	0.14	0.23	0.038	0.20	0.086
Bi	0.0000005	0.0065	0.01	0.015	0.0056	0.0038	0.0013	LOD	LOD
Th	0.0000023	0.027	0.068	0.0061	0.0033	0.007	0.0032	0.0032	0.0015
U	0.0000011	0.0022	0.0021	0.002	0.0014	0.0033	0.005	0.0052	0.0018

Appendix B

Table S2: Percentage variance explained by the first ten axes of the PCA

Axis	Eigenvalue	% of Variance	% Cumulative
1	16.431	58.683	58.683
2	2.373	8.477	67.160
3	1.983	7.083	74.243
4	1.462	5.221	79.463
5	1.100	3.930	83.393
6	0.949	3.390	86.784
7	0.738	1.968	89.418
8	0.551	1.968	91.386
9	0.453	1.619	93.005
10	0.421	1.505	94.510

Table S3: Eigenvectors for all elements in the first three axes.

Element	PCA1	PCA2	PCA3	Element	PCA1	PCA2	PCA3
B	0.94	0.019	0.12	Sr	0.35	-0.73	0.26
Al	-0.77	-0.25	-0.0048	Mo	-0.75	0.20	0.13
Ti	0.96	-0.13	0.10	Pd	-0.95	-0.22	0.044
V	-0.95	0.065	-0.11	Ag	0.90	0.015	0.082
Cr	-0.98	0.0042	-0.091	Cd	0.80	0.060	0.09
Mn	-0.84	-0.38	0.12	Sb	-0.68	0.12	0.24
Fe	-0.96	-0.092	-0.043	Ba	-0.11	-0.81	0.26
Co	-1	-0.091	-0.036	Pt	-0.69	-0.12	0.26
Ni	-0.99	0.024	-0.019	Au	-0.34	0.3	0.76
Cu	0.95	0.029	0.20	Hg	-0.77	-0.068	0.12
Zn	0.87	-0.045	0.15	Pb	-0.16	0.18	0.77
As	0.084	0.5519	0.11	Bi	-0.53	0.48	-0.15
Se	-0.33	-0.2240	0.022	Th	-0.53	0.23	0.55
Rb	0.94	0.0685	0.074	U	0.80	-0.19	0.19

Chapter 4: Discussion and conclusions

1. Overview

Metals are ubiquitous in the environment and accumulation of these metals may cause acute or chronic harm to organisms (Chapter 1). There is however, a gap in the information on metal concentrations and compositions of commercial marine species off the coast of Southern Africa. I chose sardine (*Sardinops sagax)*, chokka (*Loligo reynaudii*), hake (*Merluccius capensis*), kingklip (*Genypterus capensis*), and monkfish (*Lophuis vomerinus*) as vertebrate representatives occupying different trophic levels. The chokka is a predatorial representative of the phylum Mollusca (Figure 1).

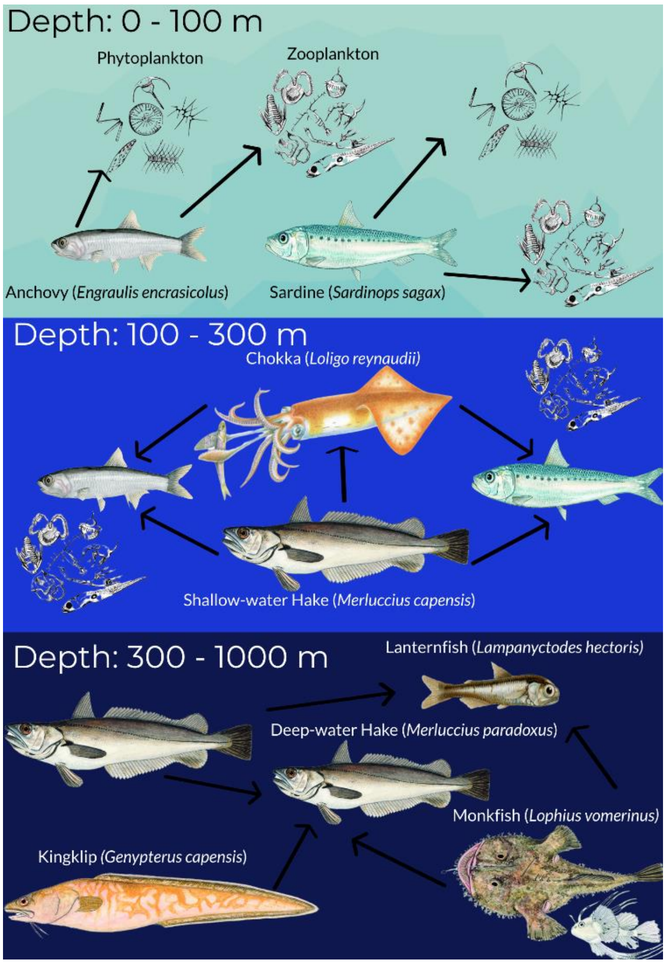

Figure 1: Simplified diagram of the food web and depth stratification of sardine, chokka hake, kingklip, and monkfish in the South Atlantic Ocean (Free use permitted; Adapted from SAIAB, 2019).

These species also range from a short lifespan (sardine) to a longer lifespan (monkfish) indicating the accumulation over different lifetimes (Yaremko, 1996; Walmsley et al., 2010). These animals are excellent bioindicators and biomonitors because of their unique characteristics explained in chapter one. Factors that would influence the concentrations in these species are plentiful such as diet, habitat, metabolism, depth, sample size, seasonality and in some cases, pollution from industries close to sample collection sites (Griboff et al., 2018). Metals can cause acute or chronic sub-lethal effects to fish and cephalopods as metals affect growth, development, development stability, immune system, behaviour, reproduction, and physiology, as explained in Chapter 1. Metals can also accumulate in a food web eventually possibly posing a threat to human health (Hayes, 1997). In this vein, we compared Cd, Pb, and Hg concentrations of species studied to limits set by the South African government and the European Union, and calculated the estimated daily intake (EDI) and total hazard quotient (THQ) of these elements.

2. Summary of chapters

Chapter 1 served as an introduction to metals and their toxicity. This chapter also explains the possible sub-lethal effects metals might have on organisms and includes a literature review of these effects in fish and squid. Molluscs and fish are also evaluated as bioindicators and biomonitors according to their unique mix of characteristics. Lastly, this chapter states the problem statement, aims, objectives, and hypotheses of this study.

Chapter 2 (written as an article manuscript and submitted to Regional Marine Science) focuses on the differences in metal concentration and composition in sardine and chokka from the South Atlantic and Indian oceans off the coast of South Africa. This chapter also evaluates the possible threat to consumers the Cd, Pb, and Hg concentrations could have. Significant differences were found between the species and oceans. Chokka muscle tissue contained significantly ($p < 0.05$) higher concentrations of B, Ti, Cr, Zn, As, Se, Rb, Sr, Cd, and Tl but significantly ($p < 0.05$) lower concentrations of V, Co, Mo, and Mn than the sardine. I found significantly ($p < 0.05$) different concentrations between the two oceans for both species indicating metal concentration could possibly be investigated as a stock discrimination tool. EDIs and THQs for sardine and chokka were calculated according to the average South African biometrics and diet. THQs for sardine and chokka were below the quotient of unity, providing an indication of safety to consumers. According to Cd, Pb, and Hg concentrations in chokka and sardine, muscle tissue would be safe for consumption by humans, although Cd concentrations should be monitored in chokka.

Chapter 3 (written as an article manuscript and submitted to African Zoology) focuses on the differences of metal concentrations and compositions in four marine predators (chokka, hake, kingklip, and monkfish). Significant differences were found for metal concentrations and compositions between chokka and the other predators. Surprisingly hake, kingklip and monkfish metal concentrations did not differ significantly and compositions were homogenous, suggesting a similarity in diet. Results from regressions with physical parameters indicted negative regression for most metals with only chromium indicating a positive regression with the length of kingklip. Estimated daily intake and THQ for chokka, hake, kingklip, and monkfish were calculated according to the average South African biometrics and diet. THQs for sardine and chokka were below the quotient of unity providing an indication of safety to consumers. Cadmium, Pb, and Hg concentrations found in chokka, hake, kingklip, and monkfish did not exceed limits set by the South African government and European Union. However, Cd should be monitored in chokka and Hg in monkfish.

3. Summary discussion according to hypotheses

3.1 The metal concentrations of all five species (sardine, chokka, hake, kingklip, and monkfish) will differ significantly from each other due to differences in biology, location, and taxonomy.

I analysed the concentrations of 30 different elements in sardines, chokka, hake, kingklip, and monkfish off the coast of South Africa. Differences in metal concentrations between chokka and sardines were discussed in Chapter 2, and concentration differences between chokka, hake, kingklip, and monkfish were discussed in Chapter 3. In this chapter, I combine and analyse data from both studies, using the same statistical methods as in chapters 2 and 3. The findings are summarised below:

- For sardines and chokka significant (un-paired, two-way t-tests; $p < 0.05$) differences were found within species from the South Atlantic and the Indian Ocean off the coasts of South Africa.

- Predators generally contain higher concentrations of metallic elements. I found that significantly higher concentrations of Ti, Cr, Co, Se, Pd, Ag, Sb, Pt, Hg, U, Al, Ni, and Bi (Kruskal-Wallis, one-way ANOVA; Dunn's multiple comparisons; $p < 0.05$) in higher trophic hake, kingklip, and monkfish compared with the lower trophic sardines.

- Patterns of concentrations were found for chokka in both studies; chokka contained significantly lower concentrations of V, Co, Mo, and Mn than sardines, hake, kingklip, and monkfish, but significantly higher concentrations were found in both studies for B, Ti, Zn, Cd, Rb, and Sr. This suggests effective regulation of these metals and possibly poor regulation in metals with higher concentrations. Molluscs lack an effective excretion mechanism for metals that may explain these higher concentrations found (Langston et al., 1989).

- The main pathway for the uptake of metals for most vertebrates and higher invertebrates such as cephalopods is through their diet (Gray, 2002). These species represent different biologies and trophic levels thus concentration differences were expected. However, hake, kingklip, and monkfish concentrations did not differ significantly, highlighting the probability of a shared diet and/or trophic overlap.

3.2 There will be a clear difference in metal concentrations and compositional patterns between the South Atlantic, Indian Ocean, and species due to a combination of underlying geological and oceanographic conditions, and overlying anthropogenic factors.

Chapter 2 compared the metal compositions of sardines and chokka of the South Atlantic and the Indian Ocean. Chapter 3 compared metal compositions of chokka, hake, kingklip, and monkfish, from the South Atlantic Ocean. The metal compositions of the five species were ordinated, together with comparable data I have available for anchovy (a small pelagic filter feeder similar to sardine (Figure 1)), resulting in Figure 2. According to Figure 2, and the information gathered in Chapter 2 the following conclusions can be made:

It was expected that metal compositions would differ as these species have different biologies and trophic levels. Hake, kingklip, and monkfish metal compositions overlapped (Figure 2). Surprisingly, sardine and anchovy did not overlap with each other despite having similar diets. The metal compositions of these two filter feeding fish also did not overlap with the predatorial fish. Thus, a trophic overlap and shared diet would not always cause a homogenous or differed metal composition. These similarities or differences may be attributed to factors such as life histories, geographical location, seasonality, etc.

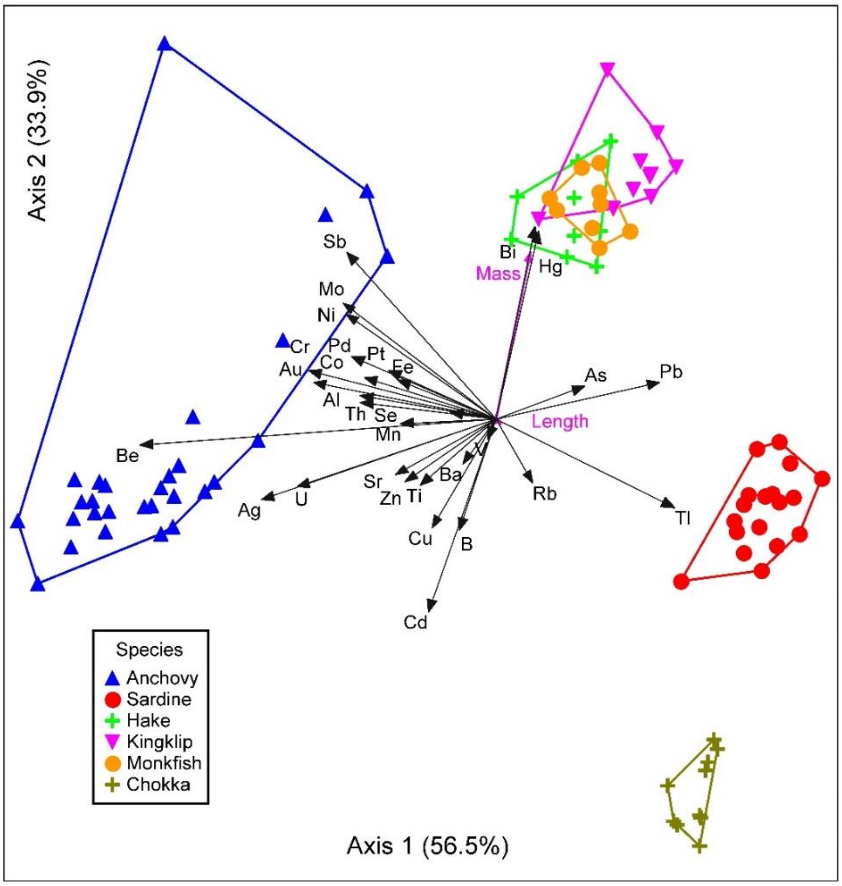

Figure 2: Nonmetric multidimensional scaled (NMS) ordination of relativized (using Gower- ignore-0 as distance measure) for sardine, anchovy, chokka, hake, kingklip, and monkfish muscle tissue. Three dimensions (third axis not shown) were needed to ordinate the relative elemental compositions in sardines, anchovy, chokka, hake, kingklip, and monkfish muscle, with a final stress of 4.3, and a final instability of < 0.00001, reached after 88 iterations.

Chapter 2 indicated differences in the metal compositions in muscle tissue for both chokka and sardine in the South Atlantic and Indian oceans. This suggests that the geographic location of samples greatly influences their metal compositions. The two oceans are different as explained in Chapter 2. Thus, metal concentrations and prey could differ between oceans resulting in the differences I found. For sardine and chokka, the existence of different stocks (van der Lingen et al., 2015; Lipinski et al., 2016) could provide another explanation into why these metal compositions would differ.

3.3 All species will be safe for consumption by humans.

I assessed the toxicity of Cd, Pb, and Hg, as these elements are toxic above certain concentrations (Hayes, 1997). Regulatory concentration limits for human consumption have been set by the South

African government and the European Union. Sardine, chokka, hake, kingklip, and monkfish Cd, Pb and Hg concentrations were all below the limits set by South African government and European Union (Table 1). Muscle tissue of all species analysed should therefore be considered safe for consumption and export.

To assure that the Cd, Pb, and Hg concentrations were below the limit that would pose a threat to consumers, EDIs and THQs (Table 2) were calculated for the consumption of each species, based on a typical South African consumer. The average mass and lifespan of a South African resident were selected as 70 kg and 70 years, respectively. All values were well below the quotient of unity concluding that muscle tissue of all species analysed would be safe for human consumption according to Cd, Pb, and Hg concentrations.

Table 1: Cd, Pb and Hg concentrations (mg/kg wet mass) in sardine, chokka, hake, kingklip, and monkfish and their limits established by South Africa (RSA) and European Union (EU)

Species	**Cd**	**Pb**	**Hg**	
Sardine (SAO)	0.078	0.052	0.0049	
Sardine (IO)	0.052	0.052	0,00057	
RSA Limits	**1**	**0.5**	**0.5**	**South Africa, 2008**
EU Limits	**0.25**	**0.3**	**0.5**	**EU, 2018**
Chokka (SAO)	0.66	0.04	0.005	
Chokka (SAO)	0.72	0.036	0.05	
Chokka (IO)	0.38	0.04	0.03	
RSA Limits	**3**	**1**	**0.5**	**South Africa, 2008**
EU Limits	**1**	**1**	**0.5**	**EU, 2018**
Hake	0.015	0.04	0.63	
Kingklip	0.0048	0.048	0.58	
Monkfish	0.01	0.039	0.65	
RSA Limits	**1**	**0.5**	**1**	**South Africa, 2008**
EU Limits	**0.05**	**0.3**	**1**	**EU, 2018**

Table 2: Estimated daily human dietary intakes (EDIs) and total hazard quotients (THQs) for cadmium, lead and mercury measured in sardines, chokka, hake, kingklip and monkfish.

Species	Cd EDI	Cd THQ	Pb EDI	Pb THQ	Hg EDI	Hg THQ
Sardine (IO)	0.0000043	0.0043	0.0000043	0.0012	0.00000040	0.00013
Sardine (SAO)	0.0000064	0.0064	0.0000036	0.0010	0.000000047	0.000016
Chokka (IO)	0.00000094	0.00094	0.0000001	0.000029	0.000000074	0.000047
Chokka (SAO)	0.0000018	0.0018	0.000000074	0.000021	0.00000014	0.00004
Chokka (SAO)	0.0000016	0.0016	0.0000001	0.000029	0.00000012	0.028
Hake (SAO)	0.0000047	0.0000047	0.000013	0.0000036	0.0002	0.000066
Kingklip (SAO)	0.000000045	0.000000045	0.00000045	0.00000013	0.0000054	0.0000018
Monkfish (SAO)	0.00000018	0.00000018	0.00000069	0.0000002	0.0000114	0.000266

***IO (Indian Ocean) and SAO (South Atlantic Ocean)**

4. Recommendations for future studies

I recommend future studies to keep in mind the following:

- More samples and sites of sardines, chokka, hake, kingklip, and monkfish should be included in future studies to assess if topography plays a major role in these metal concentration and compositional differences.
- Age of the fish analysed could be determined in future studies to determine the effect it has on metal concentrations and compositions.
- More species from the same and different trophic levels should be assessed to identify if these differences found in my study hold true in other species.
- Different seasonality should be sampled to investigate the effect and if these differences hold true in other seasons.
- Other tissues (such as the liver or gladius of chokka) should be analysed as it may contain crucial information and different concentrations and compositions than muscle tissue.
- Different life stages may be sampled where applicable to analyse patterns found in earlier stages of sardines, chokka, hake, kingklip, and monkfish.
- Cooked muscle tissue of these commercial species could be analysed in future studies to indicate if these metals become more available after preparation for consumption.
- Future studies can endeavour to investigate the effect of different sexes would have on metal concentrations and compositions.
- Risk assessment according to human consumption should be expanded to other toxic metal that may cause possible harm but are not as widely regulated and published on.
- Other toxicants could be included in future studies as their concentrations and compositions may vary between species and topography, similar to metals.

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