

Trophic-level investigations of microplastics in marine pelagic fish and squid

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Declarations

- The samples were collected by the South African Department of Environment, Forestry and Fisheries and donated for the studies.
- I developed and tested the extraction and measurement methods, prepared the samples, conducted the extractions and measurements, conducted the data analyses, interpreted the data, and drafted all the chapters.
- I consulted co-authors for clarification purposes only.
- Both manuscript chapters are ready for submission.
- This work is my own and has been checked for plagiarism.
- I received permission from my supervisor and co-authors to submit these manuscripts as part of my dissertation for my degree, *Magister Scientiae* in Environmental Sciences at North-West University.

DEDICATION

In loving memory of Babs Swart and Dewald Hattingh

ACKNOWLEDGEMENTS

I thank God, for my abilities and for the exceptional individuals He has put in my path to journey with me.

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Preface

My dissertation concerns microplastic contamination of two ecologically and economically important marine species, the anchovy (*Engraulis encrasicolus*), and chokka squid (*Loligo reynaudii*) from the South Atlantic and Indian oceans off the coast of South Africa. Chapters 2 and 3 are in manuscript format to be submitted to accredited scientific journals such as *Marine Pollution Bulletin* and *Marine Environmental Research*.

Outline of dissertation: This dissertation comprises four chapters, a brief description of each chapter as follows:

Chapter 1: This chapter serves as a brief introduction to microplastics (MPs), explaining where MPs originate from, how they end up in marine environments, and how they may affect organisms. The differences between the two oceans sampled for this study are explained to better understand the environment of the two organisms. A brief explanation on both the organisms that are used for this experiment is provided. It is important to note that I am working on MPs incorporated into tissue, not MPs found in the gastrointestinal tracts as has been the case in almost all other studies. This chapter also presents the aims, objectives and hypotheses for this study.

Chapter 2: This chapter focuses on extraneously incorporated MPs in European anchovy (*Engraulis encrasicolus*) muscle tissue from the South Atlantic and Indian oceans. The Indian Ocean was shown in four other studies to have up to three times more MP particles per litre of water than the South Atlantic Ocean. However, the anchovy from the South Atlantic Ocean had significantly higher MP concentrations in muscle tissue than those from the Indian Ocean ($p = 0.0009$) by a factor of almost 2.5. Dilution by growth was the probable cause for the lower MP concentrations in the muscle tissue as the anchovy migrate and grow, migrating and growing from the South Atlantic to the Indian Ocean, taking the MPs incorporated in their bodies with them. I estimate that 3.3×10^{11} MP particles could be transported to the Indian Ocean from the South Atlantic Ocean in this manner. To the best of my knowledge, this is the first description of bio-facilitated transfer of MPs between oceanic systems. The MP concentrations found in this study was in the same range as the few comparative studies. Anchovy is a mid-trophic organism and is vital to the transfer of energy from lower to higher trophic levels. It is possible that they transfer MPs to predators and to humans that consume anchovy or their derived products.

Chapter 3: This chapter focuses on extraneous MPs incorporated in the mantle, digestive gland, and gills of the chokka squid (*Loligo reynaudii*) in the South Atlantic and Indian oceans. Four other studies showed that the Indian Ocean has up to three times more MP particles per litre than the South Atlantic Ocean. The mantle of the South Atlantic Ocean chokka squid had significantly higher MP concentrations than the Indian Ocean by a factor of three ($p < 0.001$), the reverse of the known water concentrations. There were significant differences between some of the organs, with the gills of chokka squid from both oceans having the highest concentrations. Dilution by growth is the probable cause for lower MP concentrations in the Indian Ocean chokka squid mantle. The chokka squid is an important species as both a predator and a prey organism. Humans also consume chokka squid, although most of the chokka squid caught in South African waters are exported to European countries. Chokka squid are thus a potential pathway of MPs to their predator and human consumers. Human consumption by this route is negligible compared with other sources of intake.

Chapter 4: This chapter will serve to collate the data in Chapters 2 and 3, along with the additional analysis in Chapter 4, to discuss and synthesize conclusions based on the relevant data.

Abstract

Microplastics (MPs) are ubiquitous in marine environments, with a large number of species constantly being exposed to contamination. Fish ingest MPs, and much research has gone into the reporting on MP ingestion by different species from different environments. There are, however, very few studies on MPs that have discoursed on MPs translocated into the tissues of marine organisms.

Each environment provides a new perspective on how MPs contaminate biota. There is, however, a lack of information on conspecifics in two adjacent oceans, each ocean having different MP concentrations and MP characteristics. South Africa is bordered by two oceans, the South Atlantic Ocean associated with the cold Benguela Current, and the Indian Ocean associated with the warm Agulhas Current. The two oceans are uniquely different, making them ideal for comparative studies on shared species. Available water data show that the Indian Ocean has two-to-three-times higher concentrations of MPs than the South Atlantic Ocean.

In this study, two species occurring in both oceans were sampled from both oceans: anchovy (*Engraulis encrasicolus*) and chokka squid (*Loligo reynaudii*). These two commercially important species make up a large proportion of South Africa's fisheries industry. Samples were analysed for MPs that have translocated into their tissues. I compared the MP concentration differences in the two organisms between the two oceans. This was done vide two manuscripts, one for each species. Microplastics from 2.39 μm to 5000 μm were found in the muscle tissue of anchovy, and mantle tissue, gills, and digestive glands of the chokka squid, regardless of ocean.

Anchovy from the Indian Ocean showed lower concentrations of MPs in their muscle tissue compared with South Atlantic Ocean anchovy. The South Atlantic Ocean anchovy were younger and filter feed instead of particulate feeding, compared with the larger anchovy from the Indian Ocean, possibly explaining the difference in MP concentrations. Fibre lengths and colours were largely the same between the two oceans, only fragment lengths had noteworthy differences. There is therefore a difference in how fibres and fragments are translocated to muscle tissue.

Anchovy migrate from the South Atlantic Ocean to the Indian Ocean, growing as they travel. At the same time, they carry with them incorporated MPs in their tissue. Assuming 1 million tonnes of anchovy migrating west to east every year, approximately 3.3×10^{11} MPs are transported from one ocean system to another. As far as I know, this is the first recognition of bio-facilitated transport of MPs between ocean systems.

Chokka squid from the Indian Ocean were significantly larger than South Atlantic Ocean chokka squid due to the colder temperature of the South Atlantic Ocean, affecting the growth of the chokka squid found there. MP concentrations in gills were consistently higher than the digestive gland, and lowest in mantle tissue. All three organs in the South Atlantic Ocean chokka squid had higher MP concentrations than the Indian Ocean chokka squid organs. Chokka squid from the South Atlantic Ocean had significantly higher MP concentrations in their mantle tissue than the Indian Ocean chokka squid. Fibre lengths increased in tandem with squid size in both oceans, therefore fibres from the Indian Ocean squid were longer, though only significantly so in the digestive gland. I cannot explain this phenomenon. The three organs of squid from the same ocean had no difference in the fibre lengths found in them. Considering all the results, three possible reasons were argued as to why chokka squid from a region with lower MP concentration in its waters had higher MP concentrations in their mantle, compared with squid from the Indian Ocean with higher MP concentrations. However, none of the reasons were satisfactory.

After analysing the MPs in chokka squid and anchovy separately, I compared the data between the two species within each ocean. South Atlantic Ocean anchovy had 50% higher

MP concentrations than chokka squid from the same ocean in their respective muscle tissue. The difference in MP concentrations may be attributed to the different trophic levels of the organisms. Available data suggests that organisms of lower trophic levels are more likely to have higher levels of MP contamination, as MPs do not biomagnify as do organic chemical pollutants. However, this was not the case in the Indian Ocean anchovy and chokka squid — there were no differences in MP concentrations. I have no plausible explanation for this dichotomy. There was therefore also no evidence of trophic transfer of MPs from anchovy to chokka squid in either ocean. Human consumption of either species do not constitute a human health hazard.

The dominant type of MP in both organisms and both oceans were fibres. The chokka squid from the South Atlantic Ocean had significantly longer fibres in their mantle than the anchovy from the same ocean ($p = 0.0172$). I posit that the differences in depths the two species inhabit may be the cause for the difference in fibre lengths. However, and again, this was not found in the Indian Ocean — no significant differences in fibre lengths between the two species. I cannot explain this phenomenon.

Blue was the dominant colour of MPs in both oceans and species, suggesting either that blue was the most abundant colour or that blue was preferentially taken up. There was no significant difference in MP colour proportions of fibres between chokka squid and anchovy in the South Atlantic Ocean, but the proportion of colours differed in the Indian Ocean. Again, I cannot explain this.

Both anchovy and chokka squid showed dilution by growth in terms of MP concentrations. I suggest the MPs in the tissues of anchovy and chokka squid are poor biomonitors for MPs in seawater.

My findings, though, will also be useful in other ways:

- I confirmed translocation of MPs to various organs of two different phyla, inviting more research into translocation mechanisms, given the expected increase in MP concentrations in the two oceans.
- I identified inter-ocean transfer of MPs.
- The differences in muscle tissue concentrations and characteristics of MPs in anchovy and chokka squid organs can be used as a tracer to track stocks and migration of marine organisms.

Keywords: anchovy, squid, microplastic, Indian Ocean, South Atlantic Ocean, trophic transfer

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Chapter 1

Trophic-level investigations of microplastics in marine pelagic fish and squid

1. Introduction

1.1 Plastic

1.1.1 Plastic, the basics

Plastic has become an integral part of modern human society due to its cost-effective manufacturing, durability, and versatility (Arienzo et al., 2021; Ryan, 2015). The versatility of plastic is fitting as it is derived from the Greek word 'Plastikos' which means fit for moulding (Liddell et al. 1968). The most common kinds of plastic are polyethylene, (PE; which can be high-density or low-density), polypropylene (PP), polyvinyl chloride (PVC), polyurethane (PUR), polystyrene (PS), and polyethylene terephthalate (PET) (GESAMP, 2015; Group of Experts on the Scientific Aspects of Marine Environmental Protection). These six polymers make up 80% of most manufactured plastics (GESAMP, 2015). Plastics can usually be divided into two main categories: Thermoplastic, which is plastic that can be deformed using heat, and thermoset plastic which cannot be deformed by heat (GESAMP, 2015). Examples of thermoplastic include, PE, PP, and PS. Thermoset plastics include PUR, melamine, Bakelite, and epoxy resins (GESAMP, 2015; Schwarz et al., 1995). All plastic is made from monomers turned into polymers via chemical methods of polymerisation (Arienzo et al., 2021). The three methods of polymerisation are polymerisation, polycondensation, and polyaddition, by bonding small bi-or multi-functional molecular compounds (Schwarz et al., 1995). The annual production of plastic has increased exponentially (Ryan, 2015) and production will continue to increase.

1.1.2 Recycling and disposal

Once these products have served their purpose they need to be disposed of appropriately. Methods to effectively dispose of plastic include re-use of the plastic, recycling, and landfills. Recycling can also be seen as an implementation of industrial ecology in which theoretically there is no waste, only products (Frosch & Gallopoulos, 1989). Recycling is done with plastics that are fairly pristine but the addition of other materials and the weathering of the plastic make it more challenging, as it results in a product of lower quality (Hopewell et al., 2009). Plastic is most commonly recycled using one of four methods; primary (mechanical), secondary (mechanical), tertiary (chemical), and quaternary (recovery of energy). The first two methods are known as mechanical recycling and involve breaking the plastic down into flakes that are then melted down to create new, recycled products. The products can be of either equal quality as the original (primary) or lower quality (secondary) (Hopewell et al., 2009). The second method is chemical recycling, which turns the plastic into monomers of the same quality as virgin plastic (Arienzo et al., 2021). Finally, there is quaternary, which is the use of plastic as a source of fuel to recover the energy equivalent to the use of coal (Geyer et al., 2017; Padmanabhan et al., 2022). Burning plastics are, however, an environmental risk as certain plastics such as polyvinylchloride can release harmful chemicals into the environment when burned (Gilpin et al., 2005). Landfills are also used to dispose of plastic by burying it so that it cannot pollute natural environments. However, a drawback of this method is that none of the

plastics or energy are recovered (Hopewell et al., 2009). There are more methods of plastic disposal, although the aforementioned methods are the most common

1.1.3 Ineffective plastic disposal

Due to the sheer volume of plastic requiring disposal, it is often poorly executed (Sparks & Immelman, 2020; Verster & Bouwman, 2020; Vilakati et al., 2020) resulting in large quantities of improperly disposal of plastic. Due to its durability, plastics can persist in environments for years, and their low density means they can easily be dispersed by water or wind. (Ryan et al., 2009). The improperly disposal of plastic often ends up in natural environments such as rivers, and eventually oceans (Verster & Bouwman, 2020).

1.1.4 Harmful effects of plastic

The high tolerance of plastics to natural degradation as a result of their stable chemical structure means that plastics persist in environments for a long time (Barnes et al., 2009; Hopewell et al., 2009). Plastic pollution can affect the organisms in the polluted area in a variety of ways. The large pieces of plastic can be ingested or cause entanglement as seen in whales (Borunda, 2021; Lusher et al., 2018), sharks (Barreto et al., 2019; Bernardini et al., 2018; Cliff et al., 2002; Smith, 2018), sea turtles (Schuyler et al., 2014), sea birds (Derraik, 2002), and other marine organisms (Scheld et al., 2016; Thiele et al., 2021). Plastic ingestion can cause false satiation, intestinal blockage, and eventual death of the afflicted organism (Jacobsen et al., 2010; Lusher et al., 2015). Floating plastic can also become vectors of potentially invasive organisms that grow on the surface of the plastic (Audrézet et al., 2021; Barnes et al., 2009; García-Gómez et al., 2021; Rech et al., 2018). Plastic is, however, not impervious to degradation; UV radiation, abrasion, and wave action can cause larger pieces of plastic to fragment and become smaller, these pieces are then known as microplastics (GESAMP, 2019).

1.2 Microplastic

1.2.1 Microplastic description

Microplastics (MPs) are any piece of plastic that is 5 mm to 0.1 μm in any given dimension and make up around 92% of all marine plastics (Arienzo et al., 2021; Eriksen et al., 2014). There are two ways in which MPs are categorised, primary MPs which are manufactured small, and secondary MPs which are a result of larger plastics being broken down as explained *supra* (GESAMP, 2015). Microplastics are then further categorised by their shape into one of three major types. The three major types of plastics are known as fibre, film, and fragment. Fibres are filaments of plastic, films are thin and flat, and fragments are particles that are irregularly shaped (GESAMP, 2019). There are many environments with MPs contamination although it is specifically the marine environment that is considered to have a ubiquitous spread of MPs (Ivar do Sul et al., 2013; Arienzo et al., 2021).

1.2.2 Microplastic sources

There are many sources of MPs in marine environments such as rivers, maritime activities, atmospheric transport, aquaculture, and littering (GESAMP, 2015). Rivers can become polluted with MPs via agriculture (Chen et al., 2020), wastewater treatment plants (Murphy et al., 2016), informal settlements (de Villiers, 2018), and stormwater runoff (Rathbone et al., 1998).

1.2.3 Microplastic effects

Microplastic is an insidious pollutant in marine environments that has a wide range of concentrations and effects on organisms. Concentrations of MPs in organisms vary from one study to another due to no standardised techniques for MP extraction and no analysis being available to researchers (Fossi et al., 2018; Cole et al., 2011). Organisms studied by researchers have different feeding methods (Li et al., 2021) and habitats (Markic et al., 2018; Weber et al., 2018) which may have effects on the uptake of MPs and how the organisms react to the MPs. Whilst the effects of MPs vary greatly between studies, some effects are evidenced more commonly than others, such as false satiation, oxidative stress, and decreased growth (Batel et al., 2016; Cole et al., 2015; Mallik et al., 2021; Naidoo & Glassom, 2019; Sussarellu et al., 2016; Welden & Cowie, 2016). All the aforementioned effects have the potential to cause decreased chances of survival in organisms, which in turn can affect multiple trophic levels. Predators that rely on the organisms affected by MPs are thus in turn also affected by the decreased populations (Sussarellu et al., 2016; Welden & Cowie, 2016). Lower population sizes are, however, not the only problem for aquatic predators as they themselves are also affected by MPs, either via ingestion and inhalation from their ambient or by trophic transfer (Compa et al., 2018; del Carmen Alejo-Plata et al., 2019; Farrell & Nelson, 2013; GESAMP, 2015; Gong et al., 2021).

1.2.4 Microplastics and co-contaminants

Trophic transfer is the process by which pollutants can move from one trophic level to another, and trophic transfer of MPs has been observed (Abdolahpur Monikh et al., 2022; Athey et al., 2020; Chae et al., 2018; Farrell & Nelson, 2013; Hussain, 2001; Nelms et al., 2018; Santana et al., 2017). Trophic transfer of chemicals is also observed in incurred additives and accumulated pharmaceuticals, metals, and persistent organics pollutants (POPs) (Gao et al., 2021; Hasegawa et al., 2021; Mazzoni et al., 2018; Xie et al., 2017; Zeng et al., 2018). Microplastics may already contain incorporated additives and accumulate various pollutants from their surroundings such as POPs, metals, and pharmaceuticals (Hasegawa et al., 2021; Imran et al., 2019; Joo et al., 2021; Koelmans et al., 2016; Lin et al., 2020; Liu et al., 2020; O'Donovan et al., 2018; Teuten et al., 2009; Velzeboer et al., 2014; Wang et al., 2020). Microplastics is suspected of being a vector of additives and pollutants to predatory species (Amelia et al., 2021). However, the transfer of chemicals via MPs are orders of magnitude lower than when compared with other routes of bioaccumulation (GESAMP, 2015; Herzke et al., 2016; Lohmann, 2017).

Though MPs may not transfer POPs and metals to organisms in significant amounts, co-contaminants such as pathogens may be transported over long distances using MPs as a vector (Viršek et al., 2017). Ballast water from ships have been theorised to transport microbes rafting on MPs to areas in which the microbes may proliferate and become problematic to the organisms found there (Naik et al., 2019). Certain microbes can also use MPs along with other co-contaminates to become antibiotic-resistant. Microbes such as bacteria, viruses, and xenobiotics are harmful, not only to the organisms in the marine environment, but also may affect the humans that rely on those environments for nutrients (Kirstein et al., 2016; Naik et al., 2019).

1.2.5 Microplastic exposure to humans

Microplastics are continuously ingested by humans through either the food containing MPs (Catarino et al., 2018; GESAMP, 2020; Henry et al., 2019) or via atmospheric fallout of MPs

landing on the food whilst eating (Catarino et al., 2018). Humans have not shown any adverse effects from MP contamination yet, though increased exposure to MPs or pre-existing ailments may result in more deleterious effects (GESAMP, 2020; Henry et al., 2019).

1.3 The South Atlantic and Indian oceans

1.3.1 Description of the two oceans

The route of MP exposure to humans via their food starts with the location of where some of the food originates, such as the ocean (Figure 1). South Africa is bordered by two distinct oceans, the Indian Ocean which has the warm Agulhas Current, and the South Atlantic Ocean with the cold Benguela Current (Branch, 2010).

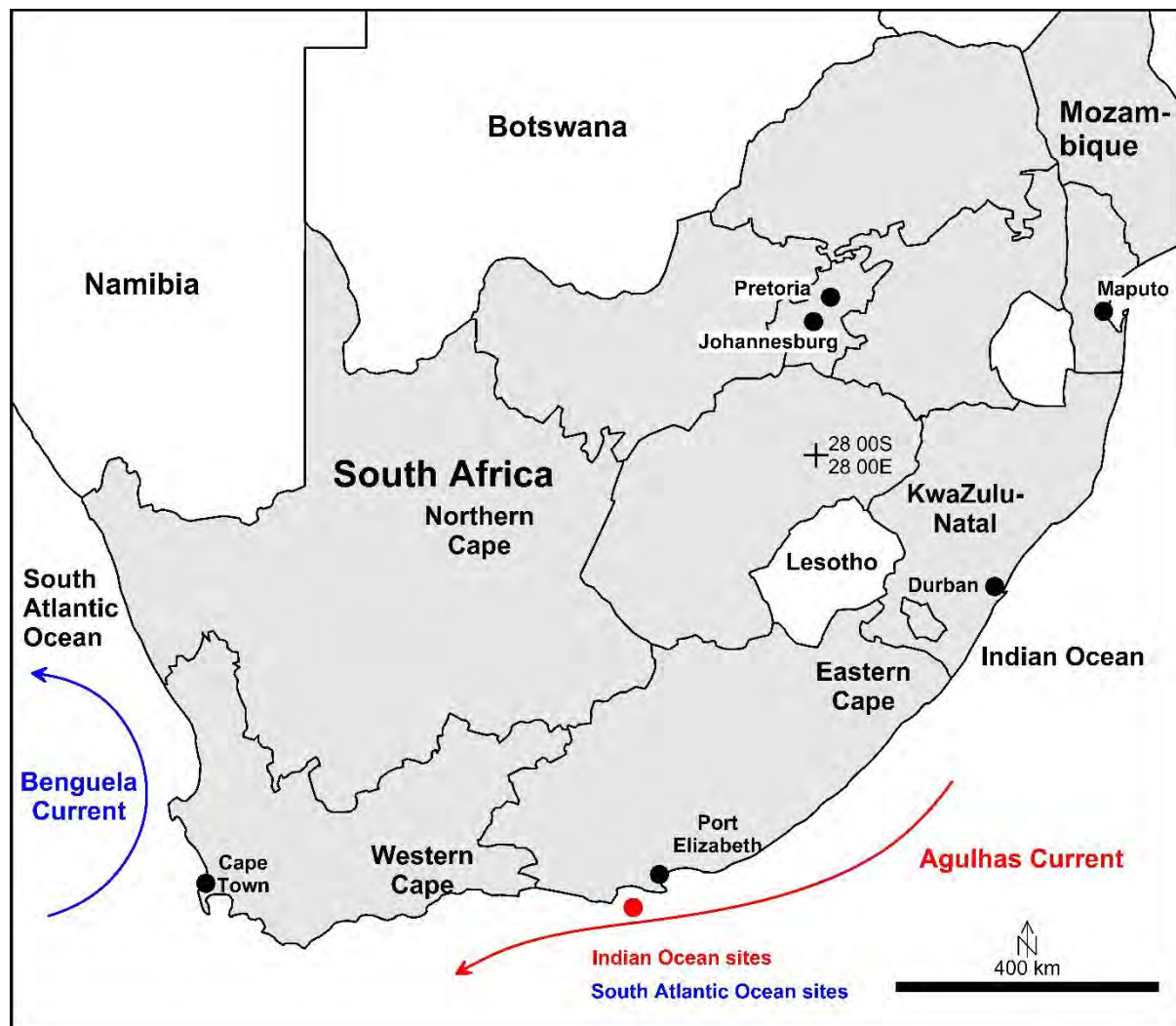


Figure 1: Map of South Africa and associated oceans, with the approximate directions of the two currents.

1.3.2 Differences in the two oceans

Microplastic concentrations in the waters of these oceans differ, with the Indian Ocean having higher MP concentrations than the South Atlantic Ocean (GESAMP, 2015; Isobe et al., 2021; Rahman, 2019; Tanhua et al., 2020). The two large estuaries of Durban Bay and Richards Bay are contributing factors to the higher MP concentrations in these waters (Nel et al., 2017). The Benguela Current also has a large associated estuary, the Orange River. However, the

Benguela Current pushes the Orange River plume northwards (Garrison & Ellis, 2015), away from the sampling sites used in this study. The Benguela Current also has a major upwelling zone (Lima et al., 2021) which can influence the dispersion of MPs to other regions (Lima et al., 2021) and potentially lower the concentration of MPs in the South Atlantic Ocean. These two oceans, with their distinct differences in temperature and MP concentration, whilst having some of the same species, make the coasts of South Africa ideal for MP research regarding comparative MP uptake and tissue incorporation analysis. One of the species found in both the South Atlantic Ocean and the Indian Ocean is anchovy (*Engraulis encrasicolus*).

1.4 Anchovy

E. encrasicolus is a small, pelagic fish that is an important source of food for many marine predators. The fish are mid-trophic and thus vital for the transfer of energy to higher trophic levels (Bakun, 2006; Bakun, 2010; Cury, 2000). Anchovy is normally found in coastal waters between the surface waters and a depth of 200 metres in the epipelagic zone. Anchovies employ two methods of feeding; filter-feeding and particulate feeding (James & Findlay, 1989). Younger anchovy prefer to filter feed on phytoplankton (James & Findlay, 1989), the older anchovy prefer selective feeding, 'biting' on zooplankton (copepods and euphausiids) (James & Findlay, 1989). Due to the fine branchial basket of the anchovy, they retain particles as small as 200 µm (James & Findlay, 1989).

Anchovies are the target of some of South Africa's largest commercial fisheries in terms of landed catch (DFFE, 2020; Department of Forestry, Fisheries, and the Environment). Anchovy is not consumed by South Africans regularly due to its small size and unpleasant smell to some (Backteman, 2010), though it may become an alternative and sustainable source of protein in the future. Other regions such as Peru have already begun using anchovies as a source of food for humans (Backteman, 2010). Many species of anchovy are used as fishmeal for animals, especially poultry, in various regions (Avila & Balloun, 1974; Thiele et al., 2021). Microplastic contamination in anchovies could therefore reach more than just marine animals.



Figure 2: The European anchovy (*Engraulis encrasicolus*) and the chokka squid (*Loligo reynaudii*). (Drawings provided by artist Ilze Weldon, 2022)

1.5 Chokka squid

Another ecologically and economically important species of both oceans is the Cape Hope squid (*Loligo reynaudii*) (DFFE, 2020). The Cape Hope squid, also known as chokka squid, reaches maturity at a mantle length of 12.5 cm for males and 16 cm for females (Zantsi, 2013). Chokka squid are nekto-benthic, meaning that they spend most of their time near the bottom of the ocean (SANBI, 2022; South African National Biodiversity Institute).

The chokka squid has a complex distribution that is influenced by many biological and environmental factors (DFFE, 2020). Chokka found along the coasts of South Africa are all genetically the same stock, however, there are morphological differences between regions. It is speculated that the colder waters of the South Atlantic Ocean may result in the squid there being slower to mature, but larger when fully matured (Olyott et al., 2007). Temperature affects growth rates in cephalopods (Forsythe et al., 1994; Forsythe, 2004). The chokka squid from the Indian Ocean on the southern and eastern coasts of South Africa reach maturity faster but are smaller in comparison to the South Atlantic Ocean chokka squid when fully matured (Olyott et al., 2007). Chokka squid are opportunistic predators and have a wide range of prey; the adults mostly feed on polychaetes, crustaceans, teleost fish such as anchovy (Augustyn et al., 1994; Lipinski, 1992; Sauer & Lipinski, 1991) and cephalopods, including other chokka squid (Augustyn, 1990; SANBI, 2022).

Chokka squid is an ecologically and economically important species, it is the most important commercially harvested cephalopod in South Africa, generating in excess of 480 million rand in successful years (DFFE, 2020). The chokka squid is used as bait but is also consumed by humans, and is also exported to Europe, most notably Italy (DFFE, 2020). Chokka squid are caught either as bycatch from hake directed demersal trawl fishing or by jig fishing (DFFE, 2020). Thorough research has been conducted on chokka squid; their early life history and adult ecology are relatively well known, but there is no research on MP contamination regarding chokka squid. The importance of this species both economically and ecologically cannot be understated and research on the potential threats of MP contamination on these squids should be invested in.

1.6 Transfer of microplastics from anchovy to chokka squid

Anchovy and chokka squid occur in both the South Atlantic Ocean and the Indian Ocean. Chokka squid and anchovy are therefore affected by the same ambient MP concentrations from both oceans, allowing cross-ocean comparisons between the two species. The chokka squid is also a predator of anchovy, it is thus possible for some of the MPs from anchovy to transfer to chokka squid via trophic transfer. Teleost fish such as anchovy are frequently eaten by chokka squid, especially at night, making trophic transfer all the more likely (Sauer & Lipiński, 1991).

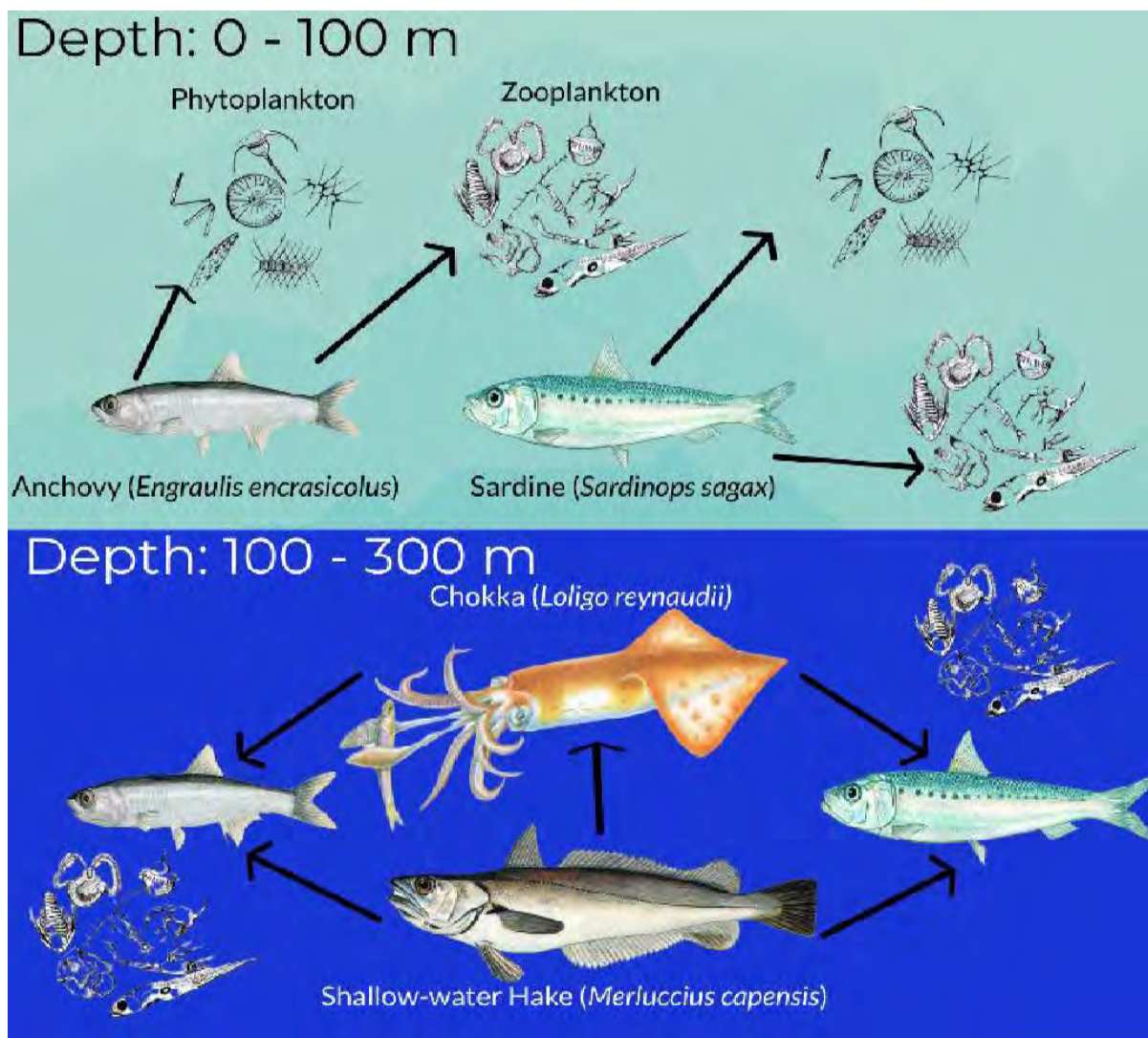


Figure 3: Simplified diagram of the food web and depth stratification of sardine, anchovy, hake, and chokka squid in the South Atlantic and Indian oceans (Adapted from SAIAB, 2019; The South African Institute for Aquatic Biodiversity).

1.7 Aims, hypotheses, and objectives

Based on the above, the aims, hypotheses, and objectives are presented.

Overall aim: Measure and compare MP concentrations and characteristics in anchovy and chokka squid from the Indian and Atlantic oceans.

Aim 1

To measure and compare MP concentrations and characteristics in anchovy muscle tissue from the South Atlantic and Indian oceans.

Hypotheses for Aim 1

- I. Anchovy from the South Atlantic Ocean will have higher MP concentrations than anchovy from the Indian Ocean, as the Indian Ocean has a higher concentration of MPs.
- II. MP characteristics in muscle tissue of anchovy will differ between the Indian and Atlantic oceans based on an assumed difference in MP characteristics.

Objectives for Aim 1

- I. To collect whole specimens of anchovy
- II. To analyse the muscle tissue of anchovy and measure and characterise MPs

Aim 2

To measure and compare MP concentrations and characteristics in chokka squid organs from the South Atlantic and Indian oceans.

Hypotheses for Aim 2

- I. Chokka squid from the Indian Ocean will have higher MP concentrations in their organs than chokka squid from the South Atlantic Ocean, due to the higher MP concentrations in Indian Ocean waters
- II. Microplastic characteristics will differ between oceans and organ, as each organ is exposed to MPs differently and due to an assumed difference in MP characteristics between oceans

Objectives for Aim 2

- I. To collect whole species of chokka squid
- II. To sub-sample organs and measure and analyse MP concentrations and characteristics using microscopy

Aim 3

To compare the MP concentrations and characteristic differences between chokka and anchovy muscle tissue within and between oceans.

Hypotheses for Aim 3

- I. There will be differences in MP concentrations and characteristics in muscle tissue of the two species from same the ocean
- II. Both species will have higher MP concentrations in the Indian Ocean as there is higher MP concentrations in its water
- III. Chokka squid from both oceans will have higher MP concentrations than anchovy based on trophic transfer

Objectives for Aim 3

- I. Comparing data from Chapters 2 and 3, with analyses done for Chapter 4
- II. Observing differences and similarities between the two species and ocean to draw conclusions from

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Chapter 2

Billions of microplastics, translocated to muscle tissue of anchovy (*Engraulis encrasicolus*), is transported from the South Atlantic Ocean to the Indian Ocean

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Abstract

Microplastic pollution in marine environments is well-documented, but with a paucity of comparative data of microplastic pollution between oceans. In this study, muscle tissue of the European anchovy (*Engraulis encrasicolus*), sampled from two different oceans along the coast of South Africa, and was tested for microplastic (fibres and fragments) content. Our samples are from the South Atlantic Ocean associated with the cold Benguela Current, and the Indian Ocean, with its associated warm Agulhas Current. Available microplastic water concentrations show two-to-three times lower concentrations in the South Atlantic Ocean compared with the Indian Ocean. However, the South Atlantic Ocean anchovy had a significantly higher mean microplastic concentration in muscle tissue of 1.4 ± 1.1 n/g compared with 0.58 ± 0.46 n/g in Indian Ocean anchovy. There were no differences in fibre lengths, fragment dimension, or colour proportions between fish from the two oceans. The anchovy from the South Atlantic Ocean were younger, smaller, and filter feeding. While they migrate eastwards around the coast towards the Indian Ocean, they grow larger and become selective feeders. Selective feeding and dilution by growth may explain the lower microplastic concentrations in muscle tissue, but other oceanographic and biological factors may also be in play such as upwelling on the west coast (South Atlantic Ocean) and changing rates in translocation of the microplastics with age. Colour and size characteristics between the two oceans were largely the same. Despite demonstrating that microplastics translocate to muscle tissue of anchovy, human ingestion of microplastics via anchovy muscle tissue is negligible and unlikely to pose any threat. However, transfer of microplastics to predators need more investigation. An estimate of 3.3×10^{11} microplastic particles could be transported to the Indian Ocean from the South Atlantic Ocean every year in anchovy muscle tissue. Fish may not be the best indicator of oceanic concentrations of microplastics, unless their biology is well understood.

Keywords: Microplastics; translocation; Indian Ocean: South Atlantic Ocean: biologically facilitated transport

1. Introduction

Due to the ineffective management of plastic and waste, much of it ends up in the environment (Verster & Bouwman 2020). Thus, with the increasing production rate of plastic (Geyer et al., 2017), its persistence in the environment, multiple sources (GESAMP, 2015; Verster & Bouwman, 2020), and ongoing poor waste management (Sparks & Immelman, 2020; Verster & Bouwman 2020; Vilakati et al., 2020), plastic pollution is now recognised as a serious and pressing environmental issue. However, plastics in the environment are not limited to large pieces of plastic such as shopping bags and plastic bottles; there is another, far more insidious threat, in the form of microplastics.

Microplastics (MPs) are smaller than five mm in its longest dimension (Barnes et al., 2009; Eriksen et al., 2014). Microplastics are estimated to comprise approximately 92% of all oceanic plastic pollution (Eriksen et al., 2014). Microplastics can be characterised in two ways, depending on how they are produced. Primary MPs are those purposefully manufactured as small-scale; secondary MPs result from larger plastics breaking down into smaller pieces (GESAMP, 2015; Group of Experts on the Scientific Aspects of Marine Environmental Protection). Generally, plastic is broken down by UV radiation, wave action, and abrasion (GESAMP, 2019). The smaller MPs are more difficult to detect and are difficult to remove from an environment once established. Microplastic can have a range of adverse effects on organisms, with many research articles reporting deleterious effects (Cowger et al., 2020; Foley et al., 2018; Gall & Thompson, 2015; GESAMP, 2019). A significant number of adverse effects have been observed in fish, such as false satiation, impaired reproduction, oxidative stress, and reduced growth and survival (Cole et al., 2015; Naidoo & Glassom, 2019; Sussarellu et al., 2016; Welden & Cowie, 2016). Interference in reproduction and the aforementioned adverse effects may result in a reduction in fish stocks.

Fish is an important economic resource and ecological component as a food source for humans and livestock, and prey for predators. Peruvian, Japanese, European, and South African anchovy are used as fishmeal (Thiele et al., 2021). Anchovy catches in South Africa ranged between 130 000 to 200 000 tonnes per year from 2000 to 2020 (excluding 2013, which was a particularly low yield year) (DFFE, 2020; Department of Forestry, Fisheries, and the Environment). Many communities depend on fisheries for a living and there are increasing concerns regarding the effect of marine litter on fisheries (Fossi et al., 2018; van der Lingen et al., 2006). The effects of MPs on humans are currently poorly understood, and this aspect deserves more in-depth research (Aragaw, 2021; Henry et al., 2019; Naidoo et al., 2020). To date, MP exposure in humans have revealed no known negative effects, although it is possible that particle ingestion and/or inhalation could be detrimental to human health via inflammation (GESAMP, 2020; Henry et al., 2019). Inhalation of plastic particles, resulting in inflammation of the lungs has become a key research focus (Blackburn & Green, 2021; Valavanidis et al., 2013).

More than 890 fish species have been reported to have MPs or other contaminants in their gastrointestinal tracts (Li et al., 2021). Little is known about MPs in fish muscle tissue, other than that there is a great variation in concentrations. This variability is likely due to a combination of location, feeding behaviour, extraction and analytical techniques used (Fossi et al., 2018; Cole et al., 2011). However, it is not only the MPs in the gastrointestinal tract, but also those that have translocated from fish skin, gills and the gastrointestinal tract (GIT) to other tissues that require consideration (Dong et al., 2023; Gong et al., 2021). Translocation of MPs is the process by which MPs move from the area it was taken in such as from the gills

or GIT, to other parts of the body (Hussain, 2001). This process may occur via endocytosis, transcytosis through the intestinal epithelial cells, persorption at villous tips, and paracellular uptake (Hirt & Body-Malapel, 2020; Powell et al., 2010). Factors that may affect MP uptake and translocation to other tissues are the type, size, and feeding methods of the fish (Collard et al., 2017; Li et al., 2021; Su et al., 2019; Setälä et al., 2016).

Predatory species preying on fish can be heavily impacted on by decreased populations of their prey, which may be affected by MPs. Not only are aquatic predators exposed to MPs in the ambient, but trophic transfer from their prey with MPs in their GIT and tissue should also be considered (Athey et al., 2020; Chae et al., 2018; Farrell & Nelson, 2013; Nelms et al., 2018; Santana et al., 2017). Trophic transfer refers to the process of contaminants moving from one trophic level to another; this can happen to various contaminants such as metals, persistent organic pollutants (POPs) and microplastics (Gao et al., 2021; Mazzoni et al., 2018; Zeng et al., 2018). MPs also adsorb different pollutants, such as POPs, metals, and pharmaceuticals (Guo et al., 2020; Hasegawa et al., 2021; Li et al., 2020; Lin et al., 2020; Liu et al., 2020; Rochman et al., 2014; Zhou et al., 2020). POPs have been found in plastic pellets in the sands along the South African coast (Ryan et al., 2012). Consequently, passively accumulated and concentrated pollutants in MPs may increase the concentration of pollutants that are transferred to higher trophic levels (Barboza et al., 2020a). However, the transfer of POPs and metals to organisms from plastic seems to be limited, with the magnitude of other routes of bioaccumulation probably being larger (Bakir et al., 2016; GESAMP, 2015; Herzke et al., 2016; Koelmans et al., 2016; Lohmann, 2017).

The South African anchovy (*Engraulis encrasicolus*) is an important source of food for many organisms, as they are mid-trophic level organisms, transferring energy from lower to higher trophic levels (Bakun, 2006; Bakun, 2010; Cury, 2000). These small, pelagic fish are found in coastal waters between the surface and a depth of ca. 200 m (the epipelagic zone). In terms of landed catch, they are the target of South Africa's largest commercial fisheries (DFFE, 2020). The anchovy employs both filter- and particulate-feeding and are able to ingest and retain MPs due to their relatively fine branchial basket that may retain particles as small as 200 μm (James & Findlay, 1989). Understanding MP contamination of anchovies will assist in clarifying the spread of MPs in marine ecosystems and how this contamination may affect various higher trophic organisms, including human consumption.

South Africa is bounded by the South Atlantic Ocean and the Indian Ocean (Figure 1). Anchovy distribution covers both oceans. As discussed *supra*, the two oceans differ in temperature with the South Atlantic Ocean having the cold Benguela Current and the Indian Ocean having the warmer Agulhas Current (Branch, 2016). Anchovy spawn close to Cape Agulhas, where the eggs and larvae are then moved to the productive west coast by currents (Hutchings et al., 1998).

The advantage of our study design is that we can analyse conspecific biota from both the Indian and South Atlantic Oceans (Figure 1) thereby providing us with two largely independent sample regions. Although each ocean has its own complex current system, fish do move between the two systems (Hutchings et al., 1998). We analysed South African anchovy from both oceans as opposed to many other studies that focused on MPs in GIT and gills (Hosseinpour et al., 2021; Neves et al., 2015; Savoca et al., 2020). An important point of reference for this study is Bakir et al. (2020) who documented MP occurrence in the stomachs of South African anchovy from the same region, finding more MPs in the Indian than South Atlantic Ocean fish. This may provide a stable indication of intake of MPs while considering the uptake and translocation of MPs to tissues. MPs translocated to muscle should be more stable than stomach contents as MPs in the stomach are subject to recency of feeding and

avoidance during and after catching, particularly in smaller fish (Staniland et al., 2001). As with POPs, the tissue concentrations of plastics are also persistent in the animal and may therefore reflect life-time exposure and uptake.

We predict that the MP content and characteristics between the two oceans will differ in the muscle tissue of the anchovy. We also predict that, as for metals in hake (*Merluccius capensis*), monkfish (*Lophius vomerinus*), and chokka squid (*Loligo reynaudii*) from the same region (Uren et al., 2020), there will be a decline in MP concentrations in terms of fish size. The aims of this study were therefore to determine the presence and concentrations of MPs in anchovy muscle tissue from both oceans, compare MP characteristics (size and colour), calculate and assess trophic transfer to humans, and assess whether the anchovy would be an accurate indicator of sea water pollution.

2. Materials and Methods

2.1 Collection of samples

Anchovy were caught off both the Indian and South Atlantic Ocean coasts of South Africa by the Department of Forestry, Fisheries, and the Environment using mid-water trawls conducted during the 2019 pelagic recruit survey between June and July that covered the inshore shelf off the South African west and south coasts (Figure 1). Some of these sites correspond to sites sampled for anchovy to measure MPs in the GIT of a variety of small pelagic fish species (Bakir et al., 2020). Fifty-eight and forty-five samples from the South Atlantic and Indian oceans, respectively, were analysed (Figure 1). The coordinates of the sampling sites are provided in Table S1 (Supplemental materials). The samples were kept frozen until analysis at the North-West University laboratories in Potchefstroom.

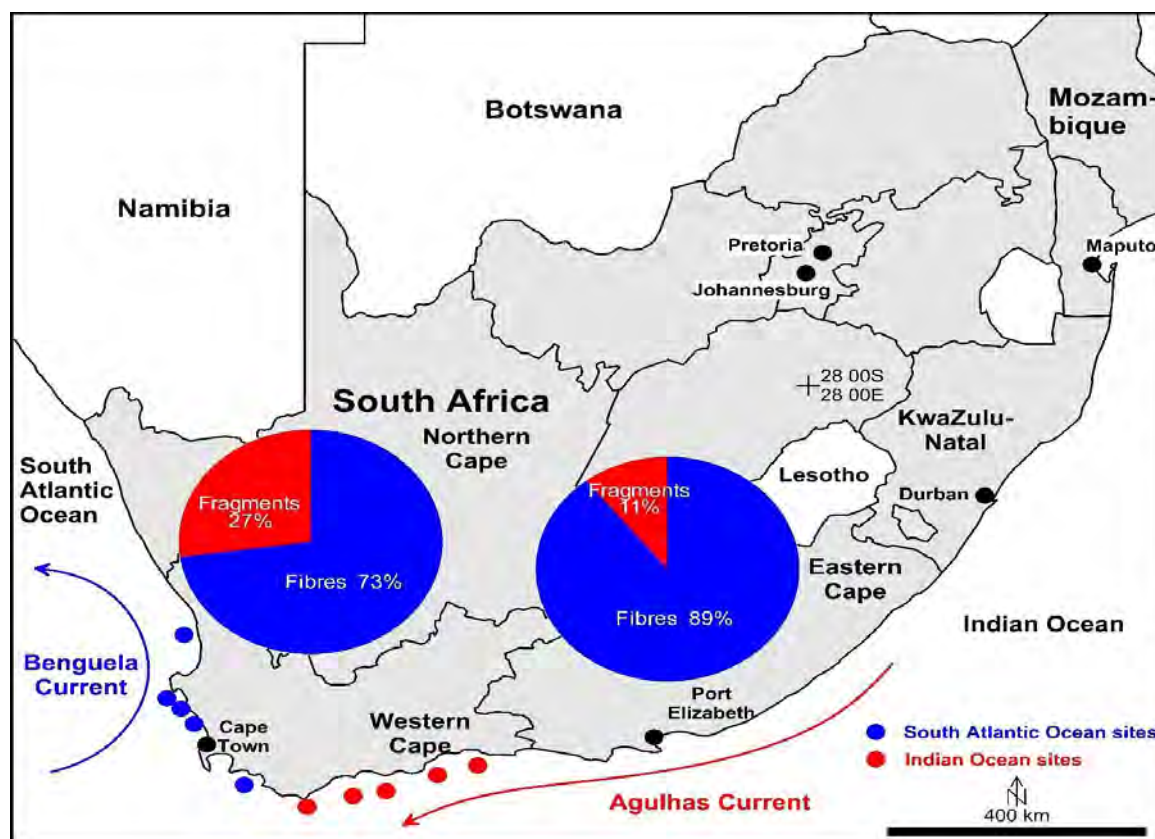


Figure 1: Locations of the anchovy collection sites, with the approximate flows of the two major currents. The percentage composition of fibres vs. fragments in muscle tissue for the two oceans are shown as pie charts. The Chi-square test for differences in fibre and fragment composition was $p < 0.0001$.

2.2 Dissection and sample preparation

All dissection equipment was rinsed three times with double distilled water before and after each fish dissection to minimise contamination. The fish were slowly defrosted until the flesh was in a workable, semi-frozen, condition. The mass and length of each individual was measured and body mass index (BMI) was calculated as $\text{length} \times \text{mass}^2$. Due to the frozen condition and small size of many of the fish, the sex was not determined. A fillet section (approximately three grams, but less with some smaller fish) without skin was carefully dissected, weighed, and stored in aluminium foil.

Great care was taken during preparation and dissections to minimise any contamination from dissection surfaces and other body parts. Two atmospheric procedural samples (clean 250 ml glass jars that were left open next to the sampling area) were analysed for each daily run of dissections. One sample was kept for backup if needed. At the end of the dissection, atmospheric procedural samples were closed off with aluminium foil and stored for later analysis.

1 M sodium hydroxide (NaOH), 0.5% sodium lauryl sulphate (SDS) and 1 M hydrochloric acid (HCl) were prepared for digestion (all solutions were filtered through a 25-micron sieve before use). Each sample of muscle tissue was placed into a rinsed flask with 50 ml NaOH and 25 ml of SDS. The sample in the flask was then capped with aluminium foil and placed in a heating mantle at no more than 60°C for 24 hours. After 24 hours, 50 ml HCl was added to each sample. The sample was then filtered through purpose-built 25-micron stainless steel filters and left to dry in clean, rinsed petri dishes until examination under the microscope. Atmospheric samples underwent the same digestion and filtrations steps.

2.3 Identification and measurement of microplastics

Dried extracts were inspected with a Nikon AZ 100 multifunctional microscope for MPs. Each MP was counted, measured (longest dimension), and characterised by colour and type (fibre, film, or fragment). Fibres are long filaments of plastic commonly found in clothing and textiles. Film is described as thin flat pieces of plastic, such as clingwrap or plastic packaging. Fragments are pieces of irregular plastic or spheres (GESAMP, 2019). Photographs were taken of some of the MPs for illustration in this article and as proof that the particles were microplastic and not natural composites. The MPs we found were too small to identify with the available (Attenuated Total Reflectance) ATR instrumentation.

The MP counts of each atmospheric sample for the daily runs were respectively subtracted per MP category. For example, if there was one 500 µm blue fibre in the atmospheric sample, then any blue fibre within a 250 µm size range (between 250 to 750 µm) was subtracted from the dataset as suspect contamination. If there was a distinct or unique colour present in the atmospheric sample, such as a blue-and-white fibre of a characteristic or notable pattern, then all such fibres, regardless of length, were ruled out as contamination. Counts were converted to MPs per gram (n/g) in wet mass.

2.4 Statistical analyses

We used GraphPad Prism Ver 9.4 for statistical analyses (GraphPad Software, www.graphpad.com) for summary statistics, comparisons, and linear regressions for positive data. Data were not normally distributed and therefore log-transformed. Mann-Whitney t-tests were used for comparisons between oceans. To compare proportional colours of the fibres and fragments between oceans, and between fibres and fragments within fish from the same ocean, we performed Chi-square and Fisher's exact tests of counts.

3. Results

3.1 Fish characteristics

The mean mass of the South Atlantic and Indian oceans anchovy was 6.5 and 7.2 g (Table 1), respectively, and the mean lengths were 10 and 11 cm, respectively (Table 1) (see Table S2 for more metrics). There was no significant difference for the mean mass of the anchovies between the two oceans (Figure 3b), but this was marginal, at $p < 0.0545$. A significant difference was found ($p = 0.0050$) for mean lengths of anchovies (Figure 3a). The mean body mass index difference (BMI) was not significant ($p = 0.9881$).

Table 1: Mass, length, and body mass index of anchovy collected off the South Atlantic and Indian oceans of South Africa.

	Fish mass (g)		Fish length (cm)		BMI	
	Atl	Ind	Atl	Ind	Atl	Ind
N	58	45	58	45	58	45
Min	3.3	3.2	7.5	8	0.77	0.85
Max	12	10	14	13	1.3	1.3
Median	5.3	7.6	10	11	0.99	1.0
Mean	6.5	7.2	10	11	1.0	1.0
Geometric mean	6.1	6.9	10	11	1.0	1.0
SD	2.5	1.8	1.7	1.1	0.12	0.071
CV%	39	25	17	10	12	7.1

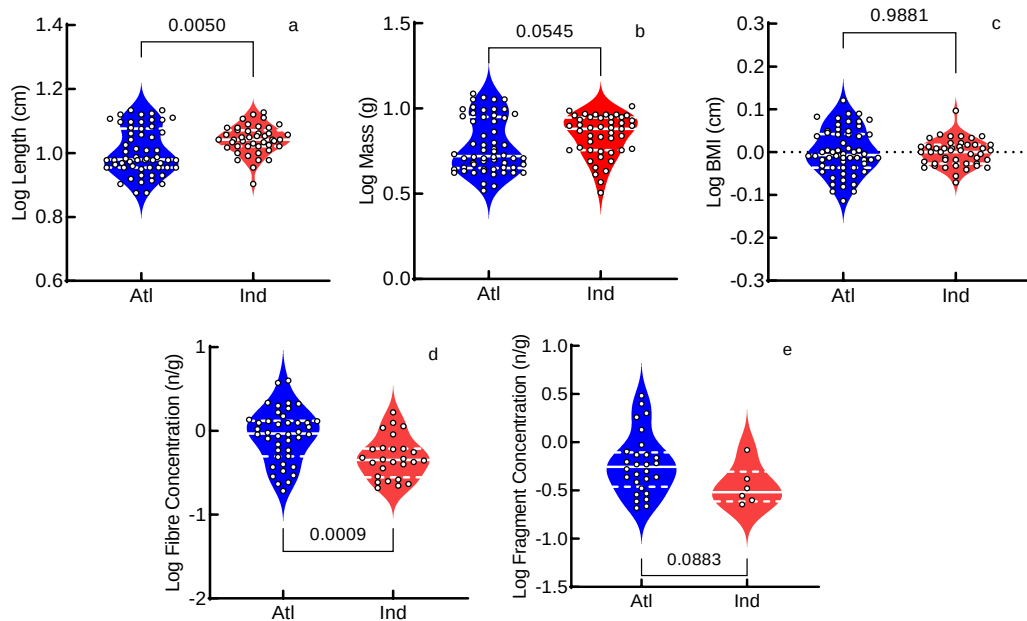


Figure 2. Violin plots and results of comparisons (Mann-Whitney) between fish length (a), mass (b), body mass index (c), fibre concentrations (d), and fragments concentrations (e) (wet mass) of South Atlantic and Indian Oceans anchovy. Coloured areas represent frequency distributions.

3.2 Microplastic concentrations

Fibres and fragments were detected (Table 2; Figure 3). Only one film fragment and one bead were characterised in the South Atlantic Ocean fish, which was ignored in further statistics. Of the 103 fish, 25 did not have any identifiable MPs (Table 2). Significantly higher concentrations ($p = 0.0009$) of fibres were found in South Atlantic anchovy muscle tissue, compared with Indian Ocean anchovy muscle tissue (Figure 2d). No significant differences were found for fragment concentrations in anchovy muscle tissue between the oceans (Figure 2e), but the lack of significance was marginal ($p = 0.0883$). Based on the differences in fragment and fibre concentrations, all further MP comparisons will assume that the two populations differ.

The South Atlantic Ocean fish had a lower proportion of fibres as part of the total MPs in their muscle tissue than their Indian Ocean counterparts (66% vs. 87%, respectively), and inversely for fragments (34% vs. 13%, respectively). The Chi-square test had a p -value of <0.0001 , a significant difference in fibre/fragment proportions between the fish of the two oceans. Therefore, the lighter and shorter fish from the South Atlantic Ocean had proportionally more fragments than fibres, compared with their Indian Ocean counterparts (Table 2).

Table 2. Concentrations (number per gram) of microplastics (fibres, and fragments) in muscle tissue of anchovy collected off the South Atlantic and Indian Ocean shores of South Africa.

Total (fibre + fragments)		Fibre		Fragment	
Atlantic	Indian	Atlantic	Indian	Atlantic	Indian

Number of samples	58	45	58	45	58	45
Number of positives	49	29	42	26	28	6
Percentage positive	84	64	72	58	48	13
Minimum	0.19	0.21	0.19	0.21	0.21	0.23
Maximum	4.3	25	40	17	30	8.3
Median	11	0.44	0.93	0.45	0.56	0.31
Mean	1.4	0.59	1.1	0.56	0.79	0.39
Geometric mean	1.0	0.49	0.87	0.48	0.59	0.35
Standard deviation	1.1	0.46	0.83	0.36	0.72	0.23
CV%	77	78	74	64	91	58



Figure 3: Photograph of blue and red fibres on 25µm sieve found in muscle tissue of anchovy

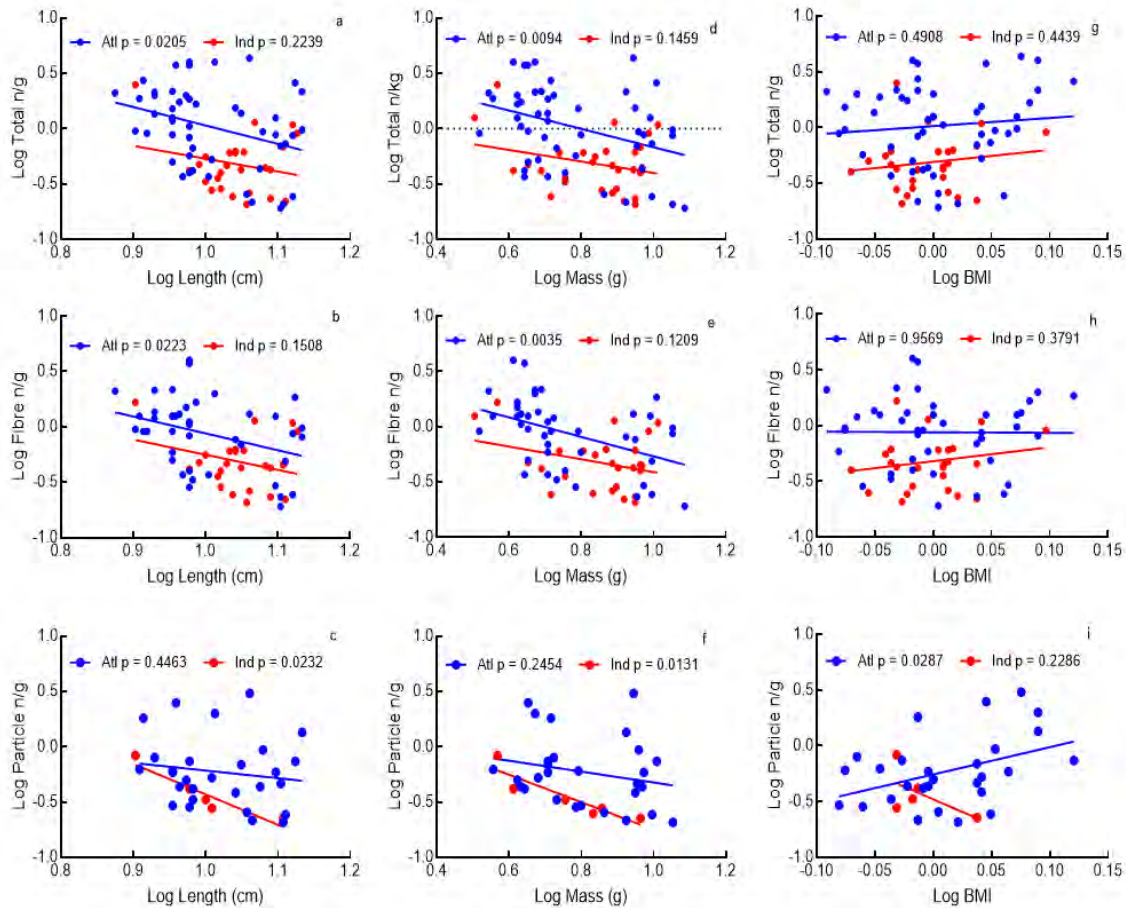


Figure 4: Linear regressions of log-transformed microplastics (all MPs, fibres, and fragments) concentrations (n/g) with body length (cm) (a, b, c), mass (g) (d, e, f), and body mass index (g, h, i) of anchovy collected off the South Atlantic and Indian Ocean shores of South Africa. P-values of the significance of the regressions are included on the graphs.

Figure 4 shows the linear regressions of biometric data with MP concentrations. Significant negative regressions were found for MPs and fibres regressed against both length (Figures 4 a and b) and length (Figures 4 d and e) for Atlantic Ocean anchovies, and with MPs against length and mass in the Indian Ocean fish. Regressions against BMI (Figures 5 g-i) were not significant, except for a positive regression of fragments in fish from the South Atlantic Ocean.

3.3 Microplastic characteristics

Fibre lengths were not significantly shorter in fish from the South Atlantic Ocean, compared with the Indian Ocean fish, and marginally so for fragments (Table 3, and Figures 5a and b). However, when the two largest fragments from the Atlantic Ocean collection was removed, the probability dropped to 0.0323. We therefore consider fragment sizes to be significantly larger in the Indian Ocean fish.

Table 3: Fibre and fragment dimensions (μm) extracted from muscle tissue of anchovy collected off the South Atlantic and Indian ocean shores of South Africa.

	Fibre		Fragment	
	Atlantic	Indian	Atlantic	Indian
N	126	59	47	7

Min	85	71	2.39	59.4
Max	3885	3942	1198	276.8
Range	3799	3871	1196	217.4
Median	500	601	74	105
Mean	739	837	122	122
Geometric mean	494	608	79	110
SD	695	732	191	71
CV%	94	87	157	58

Blue, red, and black fibres dominated in anchovy muscle tissue from both oceans (Figure 5c). When the proportions of the fragment and fibre colours (Table 4) were tested with Chi-square, there were no differences between the fibre and fragment colour proportions between the Indian Ocean and South Atlantic Ocean fish ($p = 0.1910$, and $p = 0.3559$, respectively). There was no significant difference between the colour proportions of fibres and fragments for the Indian Ocean fish (Chi square; $p = 0.2331$). However, there was a significant difference in fragment colour proportions of fibres and fragments in the South Atlantic Ocean fish ($p < 0.0001$). Notably, not all colours were found in fish from both oceans (Table 2).

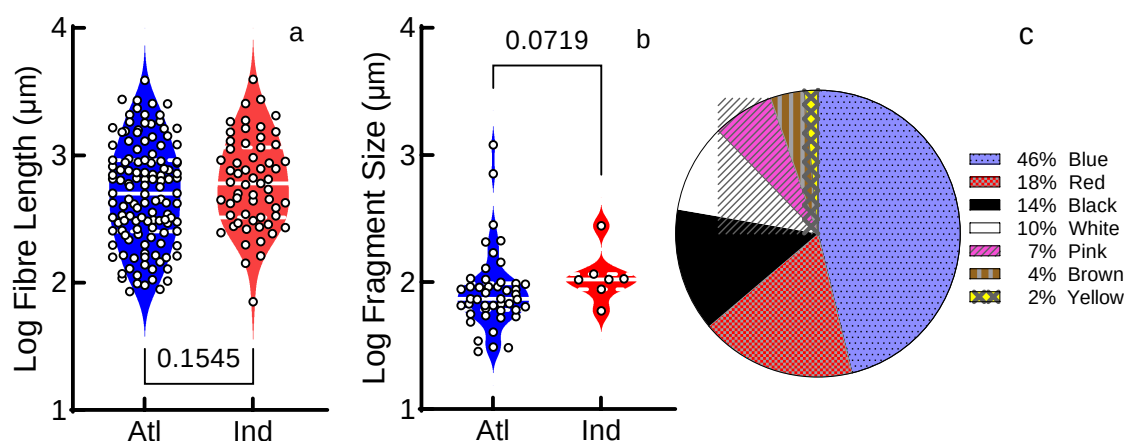


Figure 5. Microplastic characteristics: Violin plots and results of comparisons (Mann-Whitney) of fibre lengths (μm) (a), and maximum fragment dimension (b) found in anchovy muscle tissue from the two different oceans. Exclusion of the two large fragments (in 6b) resulted in a significant difference ($p = 0.0371$). Coloured areas are frequency distributions. (c) Proportional composition of fibre colours for both oceans combined.

Table 4. Number and percentages of colours of fibres and fragments in anchovy muscle tissue, per ocean.

Fibres	Blue	Black	Red	White	Yellow	Pink	Brown	Other
Atlantic	56	18	18	9	2	2	2	65
Indian	29	5	15	1	0	0	0	31
Atlantic %	33	10	10	5.2	1.2	1.2	1.2	38
Indian %	36	6.2	19	1.2	0.0	0.0	0.0	38

Fragment s	Blue	Black	Red	White	Yellow	Purple	Green	Other
Atlantic	27	1	10	0	6	2	4	40
Indian	6	0	0	0	0	0	0	6
Atlantic %	30	1.1	11	0.0	6.7	2.2	4.4	44
Indian %	50	0.0	0.0	0.0	0.0	0.0	0.0	50

4. Discussion

4.1 Fish characteristics

The South Atlantic Ocean fish were significantly shorter and lighter than those from the Indian Ocean, and there was no difference in BMI (Table 1, Figure 2). This difference reflects the life history of *E. encrasicolus* in the southern Benguela ecosystem, where spawning occurs primarily along the South Atlantic coasts, then moving to the Indian Ocean as they grow larger (van der Lingen and Huggett, 2003). Hence, anchovy from the Indian Ocean are typically larger than those from the Atlantic Ocean (Barange et al., 1999). Due to these differences, we assumed that, although the fish are the same stock (and have the same BMI), fish from the two oceans cannot be considered equivalent as there are also age differences. Any differences and correspondences in MP data should therefore be viewed in this context.

4.2 Microplastic concentrations

Plastic fibres and fragments larger than 25 µm occur in muscle tissue of anchovy from the South Atlantic and Indian Oceans off the South African coast (Table 2; Figures 4 a and b). The small tissue size used in the experiment was likely not the cause for non-detects, as we analysed approximately 35% of the mass of each fish. Therefore, we are confident that this represents actual presence and composition. Microplastic presence in tissue, however, was heterogeneous, as 25% of fish had no detectable fibres or fragments (Table 2). Indian Ocean fish that were heavier, longer, and presumably older (Table 1) had lower proportions of quantifiable MPs. Despite the differences in mass and length, this is in contrast with observations that MPs in anchovy GIT increased from west to east (Bakir et al., 2020), suggesting an increase in MP concentrations in sea water from west to east. Our data, however, suggest that the increase in anchovy GIT may be due to larger fish having larger GIT volumes than the smaller fish, as Bakir et al. (2020) measured per fish and we per tissue mass, as was found by McIlwraith et al. (2021).

Most of the MPs in anchovy muscle tissue were fibres, 64% overall, based on geometric means (from Table 2). Our findings regarding the predominance of fibres over fragments are supported by findings that fibres are the larger component of MPs in sea water (Barboza et al., 2020a; Frias et al., 2020; Jabeen et al., 2017; Mao et al., 2020; Nel et al., 2017). With fibres being more prevalent than fragments in marine environments, as was in the case of anchovy muscle tissue, our findings suggest that fragments and fibres are translocated to muscle tissue in approximate proportion (more fibres than fragments) to their presence in the environment at the MP size range we measured. Cellulosic fibres with brightly coloured dyes could be mistaken for MPs and thus skew results (Suaria et al., 2020). It is unlikely that the fibres we identified were cellulosic due to the sample digestion process, although this should still be considered, as ATR analysis was not possible for this study.

4.3 Uptake and translocation

Micro - and nano-plastics can be taken up into the body via ingestion, inhalation, and skin, where they can translocate to various organs (Dong et al., 2023; Gong et al., 2021). Ingestion is the most common route of exposure for larger particles.

The anchovy from the South Atlantic Ocean (West coast) are younger and feed on phytoplankton using filter feeding (James, 1987). As the anchovy grow larger and mature while moving to the Indian Ocean, they more readily feed on zooplankton (copepods and euphausiids) using selective feeding or 'biting' (James, 1987). Some of the zooplankton will also have ingested MPs and potentially transfer them to the anchovy (Desforages et al., 2015). The more selective 'biting' might result in less ingestion of MPs (and consequently uptake) compared with filter feeding. Northern anchovy (*Engraulis mordax*) had more MPs in juveniles than adults (Chavarry et al., 2022). The smaller gills rakers of juvenile anchovy may also increase MP uptake (Chavarry et al., 2022; Collard et al., 2017). Concentrations of MPs in muscle tissue decreased as the fish length and mass increased (Figure 5); this phenomenon may be due to growth dilution, as observed in other organisms. Growth dilution is commonly seen with contaminants such as metals and POPs, although studies on growth dilution of MPs are limited (Larsson et al., 2000; Li et al., 2018; McIlwraith et al., 2021; Uren et al., 2020). Growth dilution was present in both oceans for both fibres and fragments, even though fewer fragments than fibres were found. As discussed above, the transition to selective feeding could be a cause of the decrease in MPs seen in the fish. Rapid growth experienced by fish in its earlier life (Waldron et al., 1989) potentially assimilated MPs faster in muscle tissue, compared with slower rates of growth when more mature. The process of translocation of MPs from the GIT to the muscles could also slow down as the fish reach maturity. A study on growth dilution in freshwater fish found that MP concentration in the liver and muscle tissue decreased with size, whilst MPs in the GIT increased, possibly because the volume of the GIT is greater in larger fish (McIlwraith et al., 2021). Based on our findings, MP content in fish muscle is determined by a combination of feeding habits, age, size, habitat and translocation processes.

4.4 Possible different sources of microplastic between two oceans

Microplastics are carried to rivers via agriculture (Chen et al., 2020), stormwater runoff (Rathbone et al., 1998), wastewater treatment plants (Murphy et al., 2016), and informal settlements (de Villiers, 2018). Rivers and storm drain outlets thus contribute to the MP load of marine environments (GESAMP, 2015). The two large estuaries in Durban Bay and Richards Bay are sources of MPs to the Indian Ocean and the Agulhas Current (Nel et al., 2017). The constant influx of MPs to the Indian Ocean from these and other river mouths would increase the MP load of coastal Indian Ocean waters. Indian Ocean waters have more MPs than the South Atlantic Ocean (2.2 ± 0.65 L) in coastal South Atlantic Ocean waters, vs. 2.9 ± 0.54 L) in Coastal Indian Ocean waters (Rahman, 2019). The data format did not allow for significance testing. Indian Ocean waters had two-to-three times higher MP concentrations than the South Atlantic Ocean (Isobe et al., 2021; Tanhua et al., 2020). Increases in the MP load of the water would increase the encounter rate of MPs and therefore also MPs in the GIT of organisms in the Indian Ocean, as illustrated by Bakir. (2020). The sampled area of the Benguela current on the west coast of South Africa does not have large estuaries, but has notable upwelling zones (Lima et al., 2021).

Upwelling zones increase the uptake of other pollutants in organisms such as metals (Rejomon et al., 2009) and may cause a similar effect for MPs, but this requires further

research. Blue and black fibres were the dominant colours found in both oceans (Figure 6c); other studies have found the same (Campos da Rocha et al., 2022; Li et al., 2022a).

4.5 Comparisons with other data

Table 5: Comparative data of mean microplastics per gram (n/g) of muscle tissue and standard deviations ($\pm$) where available.

Species	Concentration (n/g)	Dominant shape	Dominant colour	Location	Author
<i>Mullus surmuletus</i> <i>Sardina pilchardus</i> <i>Solea solea</i> <i>Sparus Aurata</i> (Wild) <i>Sparus Aurata</i> (Farmed)	9.04E+04 $\pm$ 5.09E+04 8.86 E+04 $\pm$ 4.05E+04 9.38E+04 $\pm$ 2.85E+04 9.50E+04 $\pm$ 6.64E+04 8.66E+04 $\pm$ 2.43E+04	particle < 3 μ m	N/A	Tunisian coast/ Mediterranean Sea	Ferrante et al., 2022
<i>Dicentrarchus labrax</i> <i>Dicentrarchus labrax</i> <i>Scomber colias</i>	0.4 $\pm$ 0.7 0.7 $\pm$ 1.3 0.6 $\pm$ 0.8	Fibs 54% Frgs 45%	Blue	Portuguese coastal waters/ Northeast Atlantic Ocean	Barboza et al., 2020b
<i>Penaeus semisulcatus</i> <i>Epinephelus coioides</i>	0.36 0.16	Fibs 78%	N/A	Persian Gulf	Akhbarizadeh et al., 2019
<i>Alosa immaculata</i> <i>Mullus barbatus</i>	1.2 $\pm$ 0.10 1.1 $\pm$ 0.05	Fibs 51%	Black	Western coasts of Black Sea	Atamanalp et al., 2021
<i>Portunus plagicus</i>	1.0 $\pm$ 0.24	Fibs 100%	Black	Persian Gulf	Ahmadi et al., 2022
<i>Sillago sihama</i>	1.9 $\pm$ 0.46	N/A	Black/Grey	Persian Gulf	Abbasi et al., 2018
<i>Coryphaena hippurus</i>	0.0029 $\pm$ 0.005	Fibs 90%	White	Eastern Pacific Ocean	Li et al., 2022b
<i>Engraulis encrasicolus</i>	2.7	Fibs 99%	N/A	South Africa South Atlantic and Indian oceans	Ross, 2017
<i>Engraulis encrasicolus</i>	1.4 $\pm$ 1.1 0.58 $\pm$ 0.46	Fibs 73% Fibs 89%	Blue	South Africa South Atlantic Ocean Indian Ocean	This study

High MP concentrations found by Ferrante et al. (2022) (Line 1) is due to having measured very small particles, smaller than we were able to measure – and therefore is outside the scope of this comparison. For larger MP sizes, the concentrations were within the same two orders of magnitude, suggesting a fairly homogenous condition where measured, irrespective of species and locations. The low MPs measure by Li et al. (2022b) is likely due to different methods used, or simply that the fish had less MPs per gram of muscle. Anchovy from the South Atlantic Ocean in our study had MP concentrations in the higher range reported by others (Table 5), the anchovy from the Indian Ocean was at the lower range. Regarding the proportions of fibres and fragments, there were major differences, due either to real proportions in water, differences between species, and differences in extraction and identification methods used. Blue, black, and black-grey occurred the most often as colours although there is no standard way of reporting.

4.6 Estimated total microplastic in anchovy and transport thereof

The estimated stock of anchovy is between one and six million tons (DFFE, 2020). Assuming an average of one particle of plastic per gram of anchovy muscle and that 33% of the fish is muscle, South African anchovy has incorporated between 3.3×10^{11} and 2×10^{12} MPs (for 1 million tonnes and 6 million tonnes stock, respectively). Anchovy migrating from the South Atlantic Ocean therefore transport a large number of MPs to the Indian Ocean every year. This biologically-facilitated, inter-ocean transport of MPs by fish, has, to our knowledge, not been reported before. All accumulated pollutants in the MPs are also hitching a ride.

4.7 Estimated human intake

Anchovy are not commonly consumed by South Africans due to the fish being small, having a high lipid content, and has an unpleasant smell to some (Backteman, 2010). However, as the cost of food increases, anchovies may well provide a future additional and sustainable source of protein, as in Peru (Backteman, 2010).

Using the results of this study, it is possible to estimate the average human intake if anchovies were to be consumed. The South African average yearly intake of fish was approximately six to eight kilograms per person, per year in 2018 (South African Government, 2022). This would result in 8500 – 11000 MPs ingested from anchovies caught in the South Atlantic Ocean, and 3510 ± 2800 – 4700 MPs ingested from the Indian Ocean. Anchovy is however, also often used as fish meal and oil for livestock feed (Avila & Balloun, 1974; DFFE, 2020). Via trophic transfer, the plastics may end up in humans. Ingestion of MPs from marine organisms could be detrimental to human health (Bakir et al., 2020) although the risks of MP inhalation, and ingestion from food and fluids are probably far greater. An estimated 68 000 MPs per person per year via atmospheric deposition on meals while eating has been calculated (Catarino et al., 2018), orders of magnitude greater than the MPs from anchovy. Based on current knowledge, this level of human exposure to MPs via anchovy is negligible (GESAMP, 2020; Henry et al., 2019). Because only muscle tissue was analysed, we cannot calculate ingestion of whole organisms by predators.

5. Synthesis and conclusions

The anchovy from the South Atlantic Ocean is younger due to spawning occurring along the South Atlantic coasts from where they move to the Indian Ocean. They are from the same stock as the anchovy from the Indian Ocean with no significant difference in BMI, despite being shorter and lighter. However, Indian Ocean anchovy had less MPs in their muscle tissue, despite being larger, heavier, and collected from an area with higher concentrations of MPs (Bakir et al., 2020; Isobe et al., 2021; Tanhua et al., 2020). Higher MP content of the immediate surrounding sea water is therefore not the only factor affecting MP loads in muscle tissue of anchovy. Our findings suggest that the west-to-east movement during growth and changing food and feeding techniques of the anchovy are reasons for the significant difference in MP muscle concentrations. The younger fish use filter feeding on phytoplankton whilst the more mature fish use particulate feeding on zooplankton, the latter being more selective and less likely to cause accidental ingestion of MPs. Gill rakers of juvenile anchovy are also smaller and more likely to result in MP uptake (Chavarry et al., 2022; Collard et al., 2017). Translocation of MPs to muscle tissue may also slow down with age as fish grow less rapidly, and dilution by growth seems also to be involved.

We suggest that MPs translocate to muscle more readily earlier in life. Despite finding higher MP concentrations and a higher proportion of MP fragments in younger South Atlantic Ocean fish compared with Indian Ocean fish, there were no differences in fibre lengths, fragment dimension, or colour proportions. Fourier-transform infrared spectroscopy (FTIR) analysis was not possible for this study, thus visual inspection and photographs were used to

distinguish MP fragments and fibres. Fibres (particularly blue and black) were the most commonly found MP type in the anchovy muscle tissue from both oceans, an observation supported by other studies. Ingestion of MPs from anchovy by humans could be detrimental, however, MP uptake from other sources will be much larger.

Higher MP content in the Agulhas Current is likely due to large estuaries draining into it; the Benguela Current lacks large estuaries close to the area sampled. To the best of our knowledge, there are no studies comparing MP concentrations in fish muscle of same species from different oceans; thus comparing our findings to others was challenging. Future studies should include more conspecifics and their predators from more than one body of marine waters to compare MP background and MP content of marine organisms.

The transport of MPs from one ocean to the other in muscle tissue of anchovy may increase the MP concentration in the Indian Ocean. Billions of MPs (with associated pollutants such as POPs and mercury) are transported to the Indian Ocean in this manner and the implications thereof are currently scarcely understood.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that would influence the work reported in this paper.

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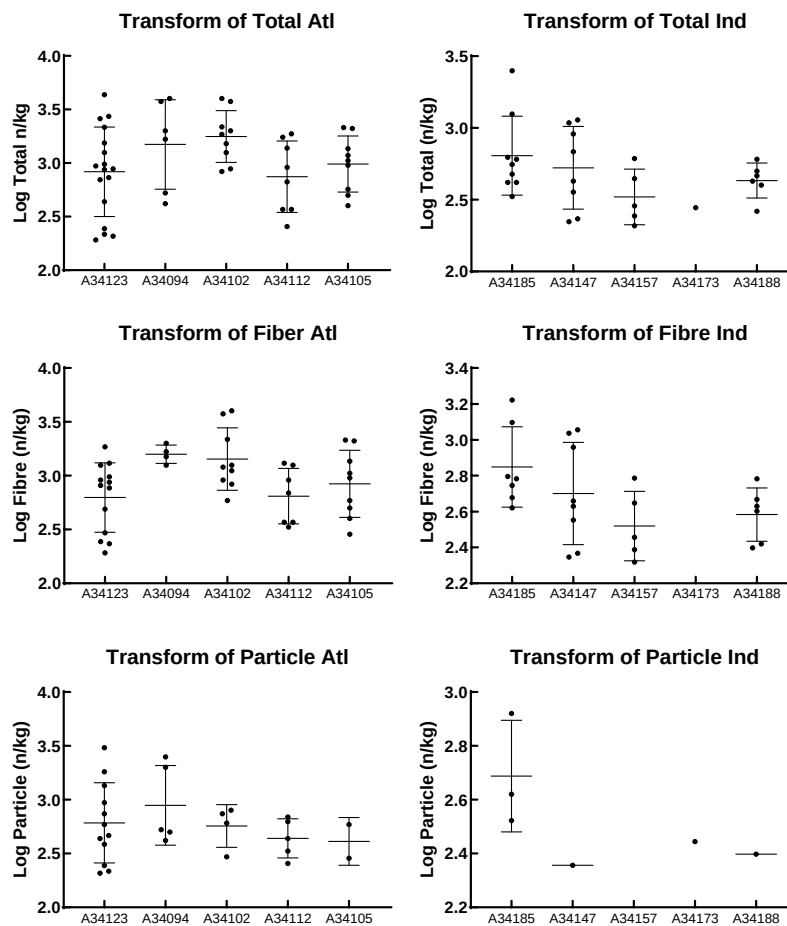
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Supplementary

Table S1 coordinates

Table S2

	Fish mass (g)		Fish length (cm)	
	Atl	Ind	Atl	Ind
n	58.0	45.0	58	44
Min	3.3	3.2	7.5	8
Max	12	10	14	13
Median	5.3	7.6	10	11
Mean	6.5	7.2	10	11
Geometric mean	6.1	6.9	10	11
SD	2.5	1.8	1.7	1.1
CV%	39	25	17	10



Chapter 3

Microplastics in the Indian and South Atlantic oceans translocate to the gills, digestive gland, and muscle of the chokka squid *Loligo reynaudii*

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Abstract

Microplastic research comparing data between oceans is relatively scarce, especially microplastic data associated with cephalopods. In this study, the gills, digestive gland, and mantle of the cephalopod *Loligo reynaudii* (chokka squid) sampled from the South Atlantic and Indian oceans off the coast of South Africa were analysed for microplastics (MPs). The chokka squid from the South Atlantic Oceans were significantly lighter due to slower growth in the colder Benguela Current but were otherwise comparable. The Indian Ocean contains approximately three times more MPs than the South Atlantic Ocean, but this was not reflected in organ concentrations. South Atlantic Ocean chokka squid had significantly higher mean concentrations of MPs but shorter fibres than Indian Ocean chokka squid. On the other hand, fibre length in mantle tissue of chokka squid from both oceans increased with mantle length. Dilution by growth is the likely reason for the lower MP concentrations in the Indian Ocean chokka squid (as they were heavier) but this does not explain why the fibres were longer. We argued three possible explanations, but none were satisfactory. Chokka squid from South Africa had more MPs when compared with other studies. The gills had the highest mean MP concentration followed by the digestive gland, than the mantle. Fibres were the dominant type of MP with blue as the dominant colour. Ingestion of MPs by humans from chokka squid was shown to be negligible compared with other routes of MP uptake. Squid may not be the best indicator of marine MPs, however, our findings can be used to track stocks and migrations of chokka squid in future research.

Keywords: microplastics; translocation: Indian Ocean: South Atlantic Ocean; squid

1. Introduction

Modern society causes environmental plastic pollution due to inadequate waste management and the ever-increasing production of plastic (Geyer et al., 2017; Sparks & Immelman, 2020; Verster & Bouwman 2020). Plastic ends up in oceans via rivers, stormwater outlets or by other means (GESAMP, 2015; Rathbone et al., 1998). Once the plastic enters a marine environment and is not removed, it will persist due to its durability (Vilakati et al., 2020). Plastic does, however, become weathered and smaller over time, becoming a potentially greater threat to ecosystems (GESAMP, 2015; Group of Experts on the Scientific Aspects of Marine Environmental Protection). Plastic shorter than 5 mm in the longest dimension is commonly considered as microplastic (MP) (Barnes et al., 2009; Eriksen et al., 2014). Microplastics are characterised according to how they are formed; if produced as a small particle, it is a primary MP, while those broken down via UV radiation, wave action, friction, and abrasion are known as secondary MPs (GESAMP, 2019). An estimated 92% of the plastic in the ocean is MP (Eriksen et al., 2014).

Microplastics can be transferred from one trophic level to another through a process known as trophic transfer (Abdolahpur Monikh et al., 2022; Athey et al., 2020; Chae et al., 2018; Farrell & Nelson, 2013; Hussain, 2001; Nelms et al., 2018; Santana et al., 2017). The trophic transfer is, however, not the only method in which larger organisms can be contaminated with MPs. Adherence to the skin of other organisms (del Carmen Alejo-Plata et al., 2019), adherence to gills (GESAMP, 2015; Gong et al., 2021), intake via lungs (Blackburn & Green, 2021; Jenner et al., 2022; Valavanidis et al., 2013), accidental ingestion by mistaking MPs as food (Compa et al., 2018), and accidental intake, in the case of filter feeders (GESAMP, 2020).

Microplastics have various effects on organisms. However, there are no standard methods to determine or measure this (Cowger et al., 2020; GESAMP, 2019; Goswami et al., 2020). The most commonly found effects across studies on MPs include but are not limited to, false satiation, oxidative stress, and decreased growth and survival (Batel et al., 2016; Cole et al., 2015; Mallik et al., 2021; Naidoo & Glassom, 2019; Sussarellu et al., 2016; Welden & Cowie, 2016). Decreases in growth and chances of survival are most likely a result of false satiation, as the organism has less energy available for growth and reproduction, which may result in smaller populations (Sussarellu et al., 2016; Welden & Cowie, 2016).

Microplastics in the environment are, however, not pristine polymers. MPs may already have potentially harmful chemicals incorporated as additives during production (Hermabessiere et al., 2017). Additives can leach from MPs into the environment (Henkel et al., 2022) and organisms after ingestion (Chua et al., 2014; Hermabessiere et al., 2017; Gaylor et al., 2012; Jang et al., 2016; Kühn et al., 2020 Tanaka et al., 2015 Wardrop et al., 2016). Metals and organic compounds such as persistent organic pollutants (POPs) and pharmaceuticals are often found in or on MPs in the environment (Imran et al., 2019; Joo et al., 2021; Koelmans et al., 2016; O'Donovan et al., 2018; Teuten et al., 2009; Velzeboer et al., 2014; Wang et al., 2020) and may transfer to organisms. However, knowledge on the transfer of pollutants and additives to organisms from MPs is limited (Bakir et al., 2016; GESAMP, 2015; Herzke et al., 2016; Lohmann, 2017; Rochman et al., 2013). Certain factors such as gut surfactants may facilitate the transfer of additives and pollutants to organisms (Bakir et al., 2014) though it may be negligible compared with other routes of uptake (GESAMP, 2015; Herzke et al., 2016; Lohmann, 2017). The adverse effects of MPs in nature are therefore more than the MPs themselves.

Studies of microplastics have been done on a large variety of marine organisms (Cliff et al., 2002; Fossi et al., 2018; Li et al., 2021; Maes et al., 2020; Naidoo & Glassom, 2019), but scarce on cephalopods (Gong et al., 2021). *Loligo reynaudii* (Chokka squid, also known as Cape Hope squid) is a coastal cephalopod/squid species in the South Atlantic and Indian

oceans off South Africa. They are important as both predator and prey to many other species (SANBI, 2022; Xie et al., 2021). Chokka squid feed on polychaetes, crustaceans, cephalopods, and teleost fish such as anchovy (Augustyn et al., 1994; Lipinski, 1992; Sauer & Lipinski, 1991). The young or smaller chokka squid tend to feed more on crustaceans, while the larger chokka squid feed on fish and other squid (Sauer & Lipiński, 1991; Uren et al., 2020b). Chokka squid is the most important commercially harvested cephalopod off the South African coasts, used for bait and consumed by humans (SANBI, 2022; Fries, 2010). Consuming chokka could therefore be a trophic pathway of MPs to marine predators and humans.

Chokka squid is present in both the cold Benguela Current (South Atlantic Ocean) and the warmer Agulhas Current (Indian Ocean) (Nel et al., 2017); some of the chokka squid also migrate between the two oceans (Lipiński et al., 2016). The population of chokka squid in the South Atlantic Ocean is far less than that of the Indian Ocean (DFFE, 2020). The Benguela Current is also associated with upwelling (Lima et al., 2021). Upwelling is the process by which strong winds displace surface water, causing upwelling of colder, deeper nutrient laden water to the surface (Garrison & Ellis, 2015; Stewart, 2005).

The aim of the current study was to measure and compare MP concentrations and characteristics incorporated within three organs (mantle muscle, gills, and digestive gland) of one squid species (Chokka) distributed over two oceans, each ocean with unique current systems (Figure 1). We predict that the differences in MP concentrations of the oceans will be reflected in MP concentrations in chokka squid tissues. The Indian Ocean has higher MP concentration than the South Atlantic Ocean (GESAMP, 2015; Isobe et al., 2021; Tanhua et al., 2020; Rahman et al., 2019). We also predict that gills will have higher MP concentrations than the other organs, as gills are in direct contact with the ambient water. Finally, we expect that MP concentrations in chokka squid organs will decrease with the increase in squid size as has been found with metal concentrations in chokka squid (Uren et al., 2020a). Calculations of human and predatory MP intake via chokka squid will be made.

2. Materials and methods

Chokka squid was caught by the Department of Environment, Forestry and Fisheries using mid-water trawls during the 2019 Pelagic Recruit Survey between June and July that covered the inshore shelf off the South African west and south coasts. The chokka squid samples were sent intact to the NWU Potchefstroom laboratories and frozen in clear low-density polyethylene bags stored in freezers. Twenty-eight chokka squid from the South Atlantic Ocean, and forty-two chokka squid from the Indian Ocean were used.

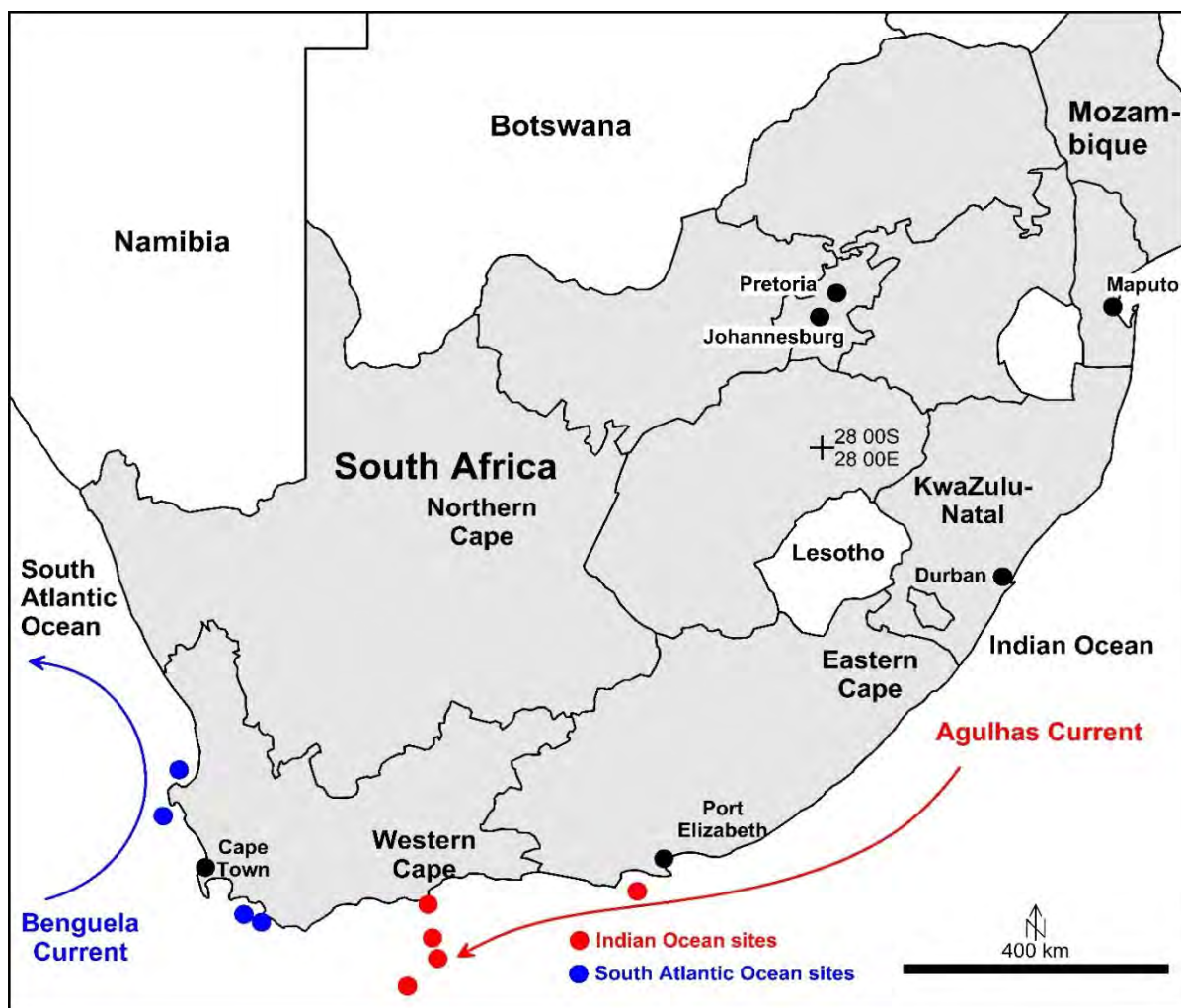


Figure 1: Map and locations of all used squid collection sites, including the two major currents.

All the equipment used for dissection was first rinsed three times with double distilled water before and after each sample was dissected. To quantify possible atmospheric MP contamination, two atmospheric samples were taken with each day's dissection, using clean open glass jars placed next to the dissection surface. The chokka squid were defrosted until workable, and their mass, mantle length, and gladius length were noted before dissection. The gills and digestive gland were removed whole, and a subsample of the mantle without skin (3-5 g) dissected. All the organs were wrapped separately in aluminium foil and frozen again to be used later during chemical digestion.

For chemical digestion, the mantle, gills, and digestive gland samples were weighed, and individually digested in clean, round-bottomed flasks. The sample mass never exceeded 5 g to ensure full digestion. The chemicals used were 1 mol of NaOH and 0.5% sodium lauryl sulphate (2:1). The volume added was calculated through the sample weight by adding the equivalent of 10 ml of NaOH per gram of sample similar to the method used by Ferreira et al., (2022). The sample was left for 24 hours to fully digest on a heating mantle at no more than 60°C. HCl (1 mol) was added to complete the digestion process.

The fully digested sample was filtered through a 25-micron stainless steel sieve which was left to dry in a sealed Petri dish. Once the samples were dry, they were inspected under a Nikon AZ 100 M microscope to find, categorise, count and measure each piece of plastic and to note the colour. The plastics were identified as either fibre, fragment, or film. Fibres are filaments of plastic commonly found in textiles. Film has the characteristics of being thin and flat, such as clingwrap or polyethylene packaging. Fragments are particles that are irregularly

shaped and are commonly thought to have broken off from a larger piece of plastic (GESAMP, 2019). For data analyses, the counts of the three most commonly found colours (blue, black, and red) were used — all other colours were classified as ‘other’.

The atmospheric sample underwent the same process as the chokka squid samples. Atmospheric contamination was subtracted. This was done by noting the colour and size of the atmospheric plastics and excluding similarly shaped and coloured plastics from the samples. Concentrations were expressed as numbers per gram (n/g).

For summary statistics, comparisons, and linear regressions GraphPad Prism (version 9.4) was used for positive data. Mann-Whitney t-tests were used for comparisons between oceans. Kruskal-Wallis with Brown-Forsythe and Welch Anova post-tests was used to compare between organs. Multiple unpaired t-tests were used to compare log-transformed MP concentrations and dimensions between oceans. Individual variances were assumed per data set. The false discovery rate applied was the two-stage step-up method of Benjamini, Krieger, and Yekutieli (GraphPad Software, www.graphpad.com) with the desired Q set at 1%. Chi-square and Fisher’s exact tests compared counts of fibre and fragment proportions between chokka squid from the same ocean, and between colours of fibres and fragments between oceans.

3 Results

3.1 Chokka squid characteristics

Indian Ocean chokka squid weighed significantly more than the South Atlantic Ocean chokka squid (109 g and 44 g, respectively; $p = 0.0064$; Table 1; Figure 2a). No significant differences were found for mean mantle ($p = 0.0961$) or mean gladius lengths ($p = 0.1218$; Figures 2b and c). Both South Atlantic Ocean and Indian Ocean chokka squid show dilution by growth, though not significantly so (Figures 2 c and d).

Table 1: Morphometrics of the chokka squid from the South Atlantic and Indian oceans including wet mass, mantle length and gladius length

	Wet mass (g)		Mantle length (cm)		Gladius length (cm)	
	Atlantic	Indian	Atlantic	Indian	Atlantic	Indian
N	28	38	28	38	28	38
Minimum	13	14	7.5	5.6	9.2	7.5
Median	40	87	12	14	13	15
Maximum	83	314	15	25	16	27
Mean	44	109	12	14	13	15
SD	19	88	1.9	5.1	1.8	5.5
Geo Mean	39	71	11	13	12	14
%CV	43	81	16	37	15	37

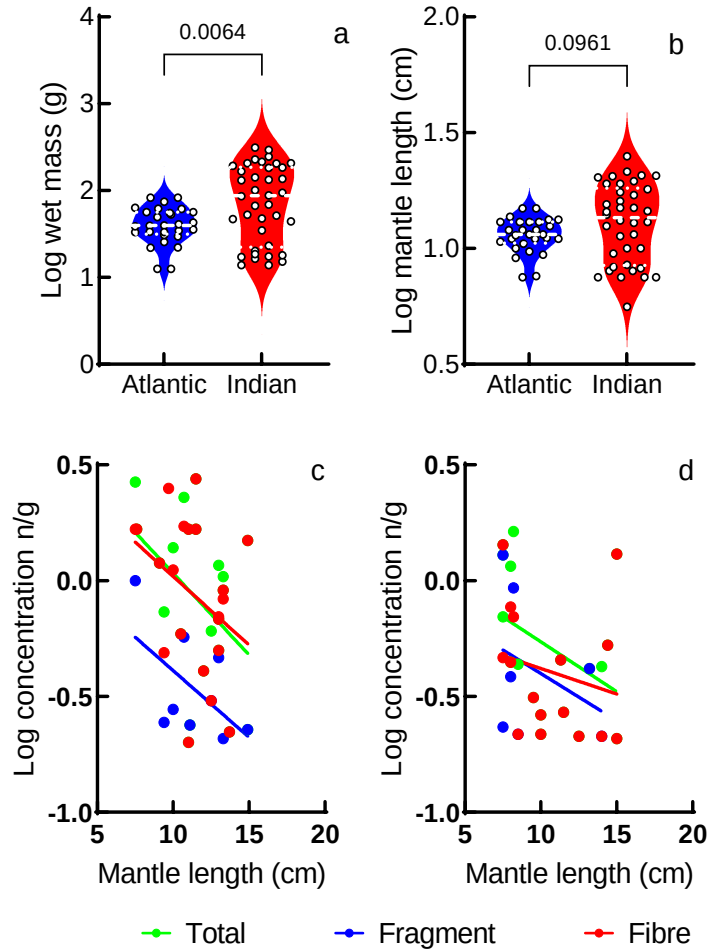


Figure 2: Violin plots and results comparisons (Mann-Whitney) between (a) mass and (b) mantle length. Coloured areas are frequency distributions. Linear regressions of log-transformed microplastics (all MPs, fibres, and fragments) concentrations (n/g) with mantle length (cm) of chokka squid collected off the South Atlantic (c) and Indian Ocean (d) South African shores.

3.2 Microplastic comparison between chokka squid from different oceans

Because fragments were found in low numbers (Table 2), most of our interpretations and discussion will concentrate on fibres.

The mantle was the only organ to show significant differences between the South Atlantic and the Indian Ocean chokka squid in terms of mean fibrous MP concentration $p < 0.001$ (Figure 3a). More fibres were found in the South Atlantic chokka squid organs, though not all significantly more (Table 2). Mean fibre length showed a significant difference in the digestive gland of chokka squid from the South Atlantic and Indian Ocean $p = 0.002$ (Figure 3c). No significant differences were seen in the fragments in regard to both mean concentration and length between the two oceans (Figures 3 b and d). No significant differences were observed in the colour of fibres between Indian and South Atlantic samples (Figure 3e). All three of the most commonly found colours (blue, black and, red) were

observed in greater mean quantities in the South Atlantic Ocean chokka squid, except for the variable colours (Figure 3e). Fibres that had more than one colour and colours as such as but not limited to yellow and purple, were placed in this category (Figure 3e). Fibres were more commonly found in both South Atlantic Ocean and Indian Ocean samples (Figure 3f). Significantly more fibres to fragments were found in the South Atlantic Ocean samples (Figure 3f). Significantly longer fibres were found in the larger chokka squid caught in the Indian Ocean. The mean length of the fibre increased in tandem with body length, gladius length, and wet mass (Figures 5 a and b; Table 3).

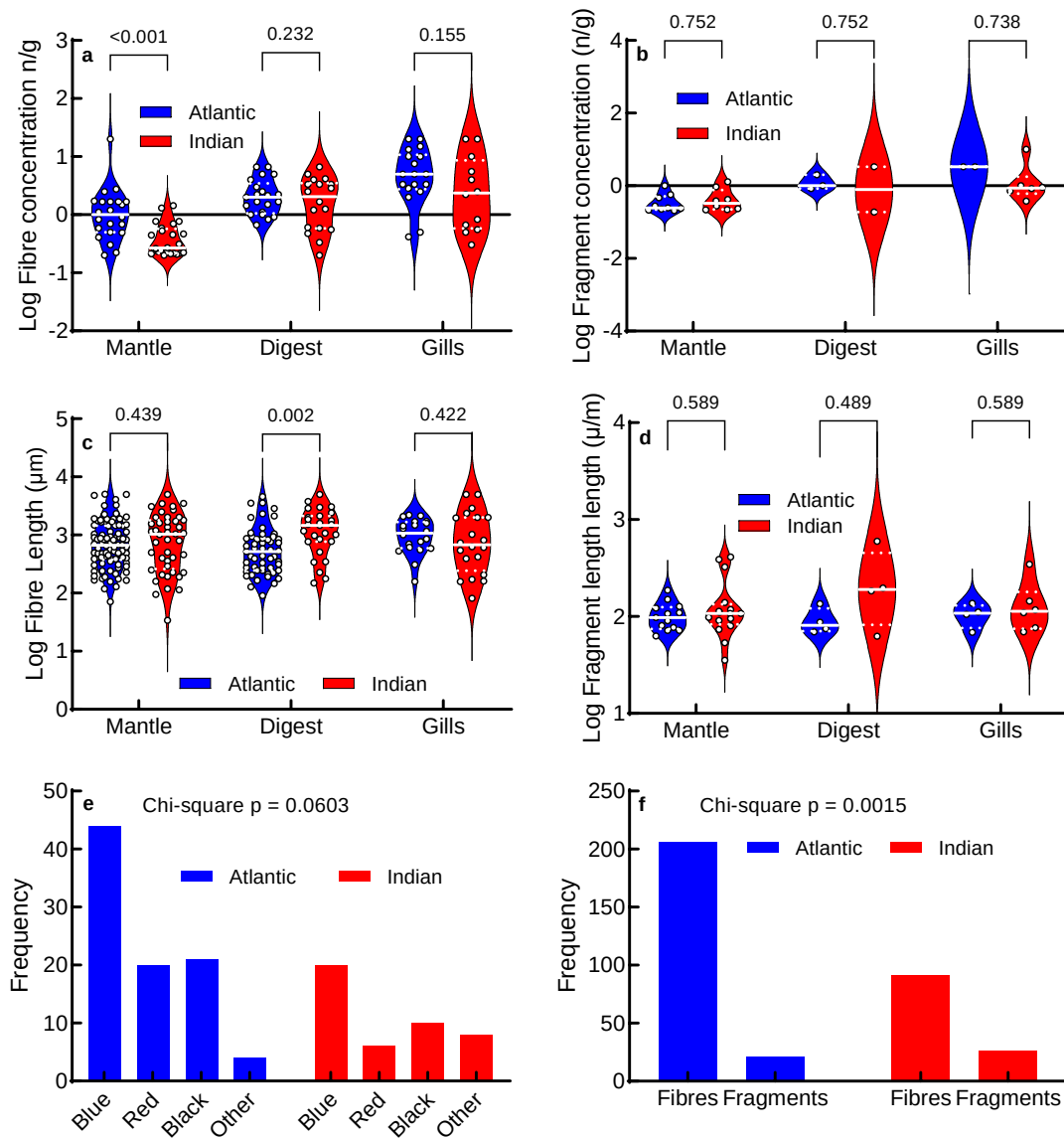


Figure 3: Violin plots of fibre and fragment concentrations (a and b) and dimensions (c and d) compared between the Indian and South Atlantic oceans using multiple t-tests. Chi-square test comparisons of colours from MP particles found in the chokka squid from the South Atlantic and Indian oceans (e). Chi-square test of fibres and fragments found in the South Atlantic and Indian oceans (f).

3.3 Microplastics in organs

Only one film particle was found which we excluded from further analyses. Fibres and fragments were present in all organs. The chokka squid from the South Atlantic Ocean showed significant differences in terms of mean MP fibre concentration in all three organs when compared with each other (Figure 4 and Table 2). The mantle had significantly lower mean MP concentrations than the digestive gland ($p = 0.0325$) and the gills ($p = 0.0002$). The digestive gland had significantly less MP fibres than the gills ($p = 0.0300$) (Figure 4a). The South Atlantic Ocean chokka squid mantle also had significantly less fragments than the digestive gland ($p = 0.0064$) and gills ($p = 0.0021$) (Figure 4b). The Indian Ocean chokka squid mantle showed significantly less MP fibres than the digestive gland ($p < 0.0001$) and the gills ($p = 0.0032$) (Figure 4c). There were no significant differences in the fragments mean concentrations between the organs of Indian Ocean chokka squid (Figure 4d). Neither fibre mean length nor fragment length showed significant differences between organs in both oceans (Figures 4 e to h; Table 3).

Table 2: Concentrations of MP fibres and fragments in three organs from chokka squid caught in the South Atlantic and Indian oceans

	Fibre concentration (n/g)					
	Atlantic			Indian		
	Mantle	Digest	Gills	Mantle	Digest	Gills
N	22	21	18	24	18	12
Minimum	0.20	0.67	0.42	0.20	0.20	0.30
Median	1.0	2.0	5.0	0.27	2.1	2.4
Maximum	20	6.7	20	1.4	6.7	20
Arithmetic Mean	2.0	2.5	7.2	0.4	2.3	5.6
SD	1.0	2.0	4.6	0.34	1.9	7.3
Geometric Mean	4.1	1.9	6.2	0.37	1.5	2.3
%CV	208	73	87	76	80	130
	Fragment concentration (n/g)					
	Atlantic			Indian		
	Mantle	Digest	Gills	Mantle	Digest	Gills
N	10	4	4	8	2	6
Minimum	0.2	0.8	2.5	0.22	0.19	0.36
Median	0.3	1.5	3.3	0.33	1.8	0.87
Maximum	1.0	2.0	10	1.3	3.3	10
Arithmetic Mean	0.37	1.5	4.8	0.50	1.8	2.3
SD	0.25	0.63	3.5	0.40	2.2	3.8
Geometric Mean	0.32	1.4	4.1	0.40	0.79	1.1
%CV	67	43	73	80	126	164

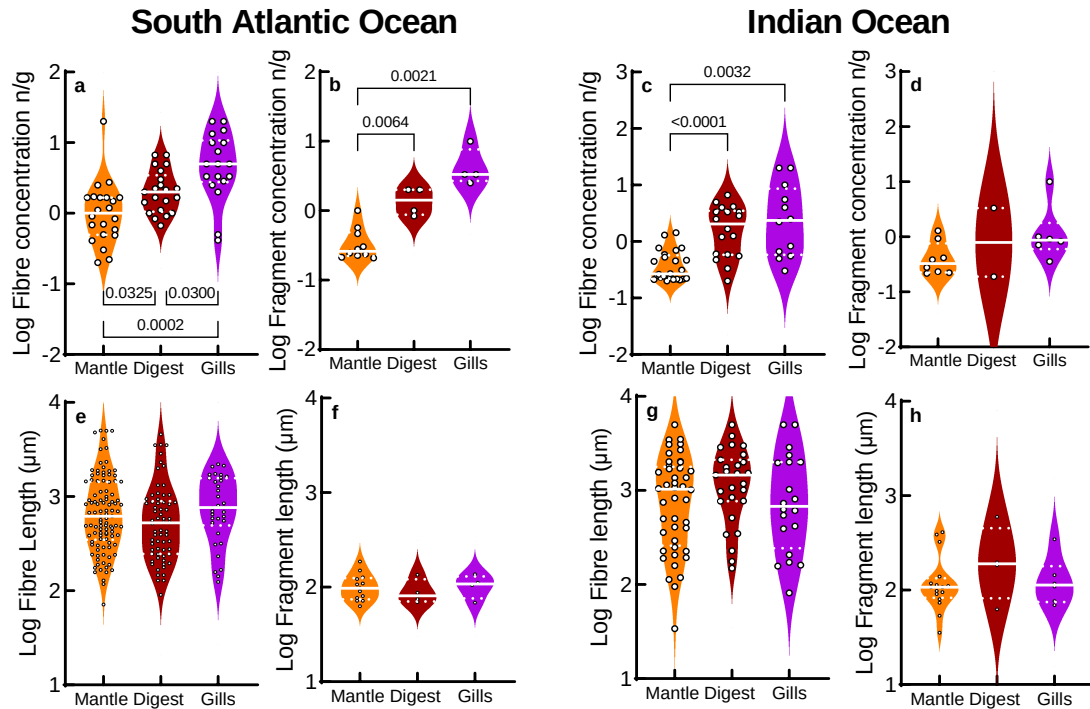


Figure 4: Violin plots of microplastic concentrations and dimensions between South Atlantic and Indian Ocean Chokka squid in three organs. Comparisons were done using Kruskal-Wallis with Brown-Forsythe and Welch ANOVA post-tests. Coloured areas are frequency distributions.

Table 3. Lengths of fibres and fragments in mantle, gills, and digestive glands of chokka squid from the South Atlantic and Indian oceans.

	Fibre length (µm)					
	Atlantic			Indian		
	Mantle	Digest	Gills	Mantle	Digest	Gills
n	106	66	34	44	27	20
Minimum	71	90	124	34	149	81
Median	620	525	765	1032	1457	680
Maximum	5016	4589	2205	5000	5000	5000
Arithmetic Mean	994	821	961	1232	1569	1357
SD	1066	938	632	1140	1190	1498
Geometric Mean	107	114	66	93	76	110
%CV	649	524	729	729	1118	734
	Fragment dimension (µm)					
	Mantle	Digest	Gills	Mantle	Digest	Gills
n	13	4	4	16	4	6
Minimum	63	69	68	35	63	69
Median	97	81	108	108	190	113
Maximum	186	135	137	410	598	345
Arithmetic Mean	104	92	105	147	260	144
SD	35	30	28	117	233	103
Geometric Mean	99	88	102	117	192	123
%CV	34	33	27	80	90	71

Figure 5 shows linear regressions of fibre length with mantle length and wet mass (Figures 5 a and b, respectively). Chokka squid from both oceans showed significant positive associations between body parameters and length of fibres.

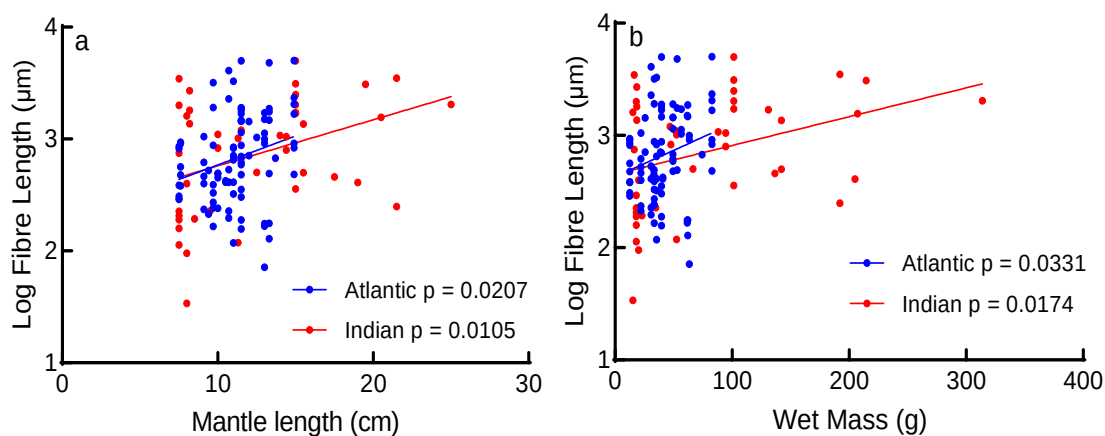


Figure 5: Linear regressions of mantle length to fibre length (a), and wet mass to fibre length (b)



Figure 6: Photograph of blue and white fibres on 25 μm sieve, found in squid mantle

4. Discussion

4.1 Chokka squid characteristics

In our samples, chokka squid from the South Atlantic Ocean were significantly lighter than those from the Indian Ocean (Figure 2a). However, there were no significant differences in their mantle lengths (Figures 2 b and c) indicating they were all adults. To the best of available knowledge, the chokka squid from both oceans are genetically the same species but morphologically different, resulting in lower body mass (Lipiński et al., 2016). The difference in mass could be due to lower temperatures which affect cephalopod growth (Forsythe et al., 1994; Forsythe, 2004; Hathfield, 2000; Jackson & Moltschaniwskyj, 2001; Olyott et al., 2007). For the purposes of this study, we consider the chokka squid from the two oceans comparable, as they are generally the same age, have been exposed for similar lengths of time to MPs, and are not genetically different.

4.2 Difference between the two oceans

Judging from their graphs, Tanhua et al. (2020) found approximately 0.05 n/l in Indian Ocean water, and 0.02 n/L in the South Atlantic Ocean. Isobe et al. (2021), based on their graphs, reported 0.01 n/l, and 0.06 n/l for the South Atlantic and Indian Ocean, respectively. Rahman et al. (2019) reported values of 2.9 ± 0.5 n/L and 2.2 ± 0.64 n/L, for the Indian and South Atlantic oceans, respectively. The different results between the three studies are likely due to sampling and filtration methods, but all three showed that Indian Ocean waters contained appreciably more MPs than the South Atlantic Ocean. Estimated relative MP distribution models based on Lebreton et al. (2012) found that the Indian Ocean water had higher MP concentrations than the South Atlantic Ocean water (GESAMP, 2015).

South Atlantic Ocean chokka squid, however, had significantly higher concentrations of fibres in their mantle despite being smaller (Table 1; Figures 2 a to c; Figure 3a). The other organs from the South Atlantic Ocean chokka squid also had higher fibre concentrations, however, the differences were not significant (Figure 3; Table 2).

Whilst it is uncertain why South Atlantic Ocean chokka squid have more MP fibres in their mantle, there are a few possible explanations. Chokka squid from the South Atlantic Ocean are suspected to occur in deeper waters than the Indian Ocean chokka (SANBI, 2022). Upwelling has also been associated with an increased heavy metal pollution in other organisms (Rejomon et al., 2009). However, water of upwelling regions had no higher concentrations of MPs than waters elsewhere (Kanhai et al., 2017). Without more information on the characteristics of the MPs of both oceans, the influence of this factor cannot be conclusively ascertained.

Chokka squid from the Indian Ocean also had higher concentrations of POPs than the chokka squid from the South Atlantic (Wu et al., 2019). One reason for the higher MP and POP concentrations of the Indian Ocean chokka squid could be due to the numerous large estuaries and industrial areas on the Indian Ocean coast, polluting the Agulhas Current flowing southwest (Nel et al., 2017; Kosek et al., 2019a; Kosek et al., 2019b). The Benguela Current flowing north up the east coast has one large estuary, the Orange River (Roux & Shannon, 2004) its plume pushing northwards and away from the sampled areas by the Benguela Current (Garrison & Ellis, 2015; Figure 1).

Both MPs and POPs are anthropogenic. The higher MP concentrations in the mantle of the South Atlantic Ocean chokka squid is therefore difficult to explain. On the one hand, MP concentrations in the Indian Ocean are higher than in the South Atlantic (first paragraph of this section) raising the expectation that chokka squid from the Indian Ocean would have higher concentrations, which was not the case. On the other hand, we found strong indications of dilution by growth in both oceans (Figures 2 c and d). Two mechanisms might be in play here.

Lower water temperatures cause cephalopods to grow slower (Forsythe, 2004; Hathfield, 2000; Jackson & Moltschaniwskyj, 2001) and have corresponding slower metabolism (Seibel et al., 1997; Seibel et al., 2000; Seibel & Carlini, 2001; Seibel & Drazen, 2007). Slower metabolism may also slow the removal of MPs from muscle tissue while chokka squid in warmer waters would have an accelerated removal. This may explain the higher MP concentrations in the chokka squid from the colder South Atlantic Ocean. However, we know of no such process.

On the other hand, Lipiński et al. (2016) suggest that juvenile chokka migrate from the south coast of South Africa (Agulhas Bank) westwards to the South Atlantic, while others stay or migrate a short distance eastward to the warmer Indian Ocean. If we assume that the juveniles start out with similar MP concentrations, those remaining or migrating eastward would grow faster, attaining more mass and length, as was the case for the chokka squid from this study (Figures 2 a and b). By growing faster, a higher rate of dilution by growth may explain the lower concentrations in the larger chokka squid in the Indian Ocean. This would imply that MP uptake would occur mainly during the juvenile stages — when growing larger, translocation would be less. We also found such indications for anchovy (Bothma et al., In prep).

There may be other mechanisms that we are unaware of, but our findings invite further investigations. However, as discussed in the next section, other findings confound the two explanations offered here.

4.3 Difference between organs

Uptake of MPs to the body via gills has been suggested or found by others (Gong et al., 2021; Kolandhasamy et al., 2018; Watts et al., 2014; Zhang et al., 2019). In both oceans, concentrations of fibres and fragments were higher in the gills and digestive tracts than in the mantle, although not always significantly so (Figures 4 a to d, Table 2). Fibre and fragment lengths, however, were essentially the same between organs in chokka squid from the same ocean (Figures 4 e to h). The higher fibre concentrations in the mantle from the South Atlantic chokka squid consisted of 20% shorter fibres than in the chokka squid from the Indian Ocean (means of 994 μm and 1232 μm , respectively), but the difference was not significant ($p = 0.6521$ and Table 3).

It may be that shorter fibres translocate easier to muscle tissue, resulting in higher concentrations in the South Atlantic Ocean chokka squid that also contained shorter fibres. However, there would be no reason not to find equally short fibres in the Indian Ocean squid, nor does it explain why the fibres were longer in the Indian Ocean (although the difference was not significant). The fibres in the digestive glands were double the length in the Indian Ocean (821 μm and 1569 μm ; Table 3). The two explanations argued in the previous section taken together with the argument that shorter fibres may translocate to muscle and the digestive gland are contradictory and poses a conundrum. The assumptions about movement of the chokka squid between the two oceans might not be helpful, and there may be processes we are not aware of.

4.4 Microplastic characterisation

Fibres in the digestive glands of chokka squid from the Indian Ocean was almost twice as long as in chokka squid from the South Atlantic Ocean (means of 1569 μm , and 821 μm , respectively; $p = 0.002$; Figure 3c, Table 3). Anchovy from the Indian Ocean also contained longer fibres than those from the South Atlantic (Bothma et al., In prep) although this difference was not significant. Having found dilution by growth for both oceans, implies that fibres become less and longer simultaneously. This is inexplicable, as we know of no mechanism that can make fibres longer. We are unable to expound on this dichotomy.

Fibres being the dominant type for MPs in tissue is supported by other studies on different organisms (Barboza et al., 2020; Frias et al., 2020; Jabeen et al., 2017; Mao et al., 2020; Nel et al., 2017) and two other studies done on cephalopods (Gong et al., 2021; Oliveira et al., 2020). Blue was the most dominant colour present in both the South Atlantic and Indian oceans, followed by black, then red (Figure 3e; Figure 6). The predominance of blue and black fibres were also reported by other scholars (Campos da Rocha et al., 2021; Li et al., 2022).

4.5 Estimated human intake

Almost all of the chokka squid is exported to Mediterranean countries such as Italy and Spain (Claasen, 2022). Chokka squid is therefore a likely pathway of MPs to humans. In 2017, Europeans consumed 0.67 kg of squid per capita (CBI, 2022) allowing estimations on human intake of MPs. Since the consumption of chokka squid is mainly muscle and excludes other organs, we based our calculations on mantle concentrations. Consuming chokka squid from the South Atlantic and Indian oceans results in an annual consumption of 1 340 and 268 MPs, respectively. Compared with an estimated annual intake of 68 000 MPs via atmospheric deposition on food consumed (Catarino et al., 2018), the relative contribution and potential threats posed by MPs in chokka squid is negligible.

Marine predators will typically consume whole squid, thus our data is not amenable to calculate predator intake.

4.6 Comparisons with other studies

There are some studies available for comparisons (Table 4) but it should be taken into account that methods vary between studies (Fossi et al., 2018; Cole et al., 2011). Only one study utilised the mantle / edible tissue for analysis (Table 4, Row 1; Daniel et al., 2021). We found substantially higher MP concentrations than in *Uroteuthis duvaucelii*. Only one study analysed gills but found far less MPs (Table 4, Row 2; Gong et al., 2021). The digestive gland MP concentrations from our study was similar to those found in wild *Sepia officinalis* (Table 4, Row 3; Oliveira et al., 2020). However, no other studies found concentrations (not per organism) of MPs as high as the present study. Only one study reported fibres and fragments with fibres predominating (Table 4, Row 2; Gong et al., 2021). While we found predominantly blue fibres, others reported mostly white or black/grey MPs. The methods used, localities, life histories, and prey of the different species may be the major reason for the large differences.

Table 4: Comparative studies on microplastics in cephalopods from different regions

	Species	Distribution	Organs used	Concentration	Dominant type	Dominant colour	Author
1	<i>Uroteuthis duvaucelii</i>	India Coastal waters of Kerala	Edible tissue	0.008 ± 0.02 items/g	Fraggs 69%	Transparent/white	Daniel et al., 2021
2	<i>Dosidicus gigas</i>	Peru Northern Humboldt Current	Gill Intestine Stomach	0.20 ± 0.24 items/g 0.74 ± 0.77 items/g 0.30 ± 0.24 items/g	Fibs 97% 93% 92%	Black-grey	Gong et al., 2021
3	<i>Sepia officinalis</i> (Wild)	Portugal Algarve coast	Stomach Intestine Digestive gland	0.14 ± 0.04 fibres/g mean total fibres/body weight 2.14 ± 1.10 fibres/g	N/A	Not analysed	Oliveira et al., 2020
4	<i>Sepia officinalis</i> (Farmed)	Portugal Ria Formosa lagoon system	Stomach Intestine Digestive gland	0.06 ± 0.02 fibres/g mean total fibre body weight 0.86 ± 0.34 fibres/g	N/A	Not analysed	Oliveira et al., 2020
5	<i>Vampyroteuthis infernalis</i>	Brazil Southwestern tropical Atlantic	Whole Organism	9.58 ± 8.25 particles per individual	Fraggs 89.0%	White	Ferreira et al., 2022
6	<i>Abralia veranyi</i>	Brazil Southwestern tropical Atlantic	Whole Organism	2.37 ± 2.13 particles per individual	Fraggs 62.0%	White	Ferreira et al., 2022
7	<i>Loligo reynaudii</i>	South Africa South Atlantic Ocean	Mantle Digestive gland Gills	2.0 fibres/g 2.5 fibres/g 7.2 fibres/g	Fibs 85% 93% 89%	Blue	This study
8	<i>Loligo reynaudii</i>	South Africa Indian Ocean	Mantle Digestive gland Gills	0.4 fibres/g 2.3 fibres/g 5.6 fibres/g	Fibs 73% 87% 77%	Blue	This study

5. Synthesis and conclusion

It was difficult to reconcile the results of concentrations, dimension, and colours of fibres and fragments in three different organs of chokka squid from two different oceans. This study was the first of its kind on *Loligo reynaudii*, a commercially important species of cephalopod. The size of the chokka squid differed significantly between oceans, likely due the slower growth rate in colder water, and not age. For the purposes of this study, we considered the chokka squid from the two oceans as comparable. Muscle tissues of the Indian Ocean chokka squid had significantly lower MP concentrations than muscle tissue from the South Atlantic Ocean chokka squid. However, Indian Ocean water had a higher MP concentration than the South Atlantic Ocean. This was contrary to our expectations.

Dilution by growth is a possible reason for the lower MP concentrations in the Indian Ocean chokka mantle tissue. However, the chokka squid from the Indian Ocean also had longer fibres in the mantle and digestive gland tissue. We argued three theories for this

dichotomy, but none explained all the findings satisfactorily. The gills had the highest mean MP concentration, followed by the digestive gland and then the mantle, as per our prediction, since gills are directly exposed to the ambient. In both oceans, fibres were the most dominant type of MP with blue the most dominant colour in both. Fibre lengths in chokka squid organs increased concomitant with increase in size.

There was a notable dearth of studies we could compare our results with, and even lesser used comparable methods and expression of concentrations. Despite this, the squid in our study had comparably higher mean MP concentrations and a higher proportion of fibres.

The chief shortcoming in the present study was relying on relatively scarce data on MP concentrations in the waters around the coasts of South Africa. Although all four sources agreed that MP concentration in the Indian Oceans was higher than the South Atlantic Ocean, there was no data on sizes, types, and colours. Given the topical nature of MPs in marine waters, this should be addressed as a matter of urgency as it will inform future studies and policy. We recommend that future studies also consider species from multiple trophic levels to better reflect true MP concentrations in the oceans. Our study also showed that chokka squid is a negligible pathway of MPs to humans. Taken together, it appears that chokka squid would not be a good indicator of MPs in oceans. However, the differences we did observe may be used to better track stocks and/or movement of the chokka squid.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that would influence the work reported in this paper.

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Chapter 4

Analysis, synthesis, and conclusions

1. Introduction

Microplastics (MP) are ubiquitous in marine environments (Cole et al., 2011) with many marine species found with MP contamination (Azevedo-Santos et al., 2019; Li et al., 2021; Markic et al., 2019). However, there are notable lacunae in information pertaining to the MP contamination differences in marine species from different oceans. For this study, two commercially important species were used, the chokka squid (*Loligo reynaudii*), and the anchovy (*Engraulis encrasicolus*) (DFFE, 2020). Both species occur off the South Atlantic and Indian Ocean coasts (DFFE, 2020; Department of Forestry, Fisheries, and the Environment). The two oceans adjoin, making it an ideal sampling area for this experiment. The anchovy is a major prey species of chokka squid (Augustyn et al., 1994; Lipinski, 1992; Sauer & Lipinski, 1991), thus transfer of MPs from anchovy to chokka squid is possible. Biomonitoring via these species may reflect MP concentrations in their ambient and consequent exposure to them. Both species are consumed by humans (Backteman, 2010; DFFE, 2020), the chokka squid more so, and could therefore be a route of MP exposure to humans. Microplastics have a variety of adverse effects on organisms that ingest or inhale these (Cowger et al., 2020; Foley et al., 2018; Gall & Thompson, 2015; GESAMP, 2019). No adverse effects have been reported in humans, though increased levels of exposure or pre-existing ailments may cause MPs to affect humans (GESAMP, 2020; Henry et al., 2019). It is therefore crucial that MP exposure to these two economically important species be tested, to determine how environmental differences may affect MP uptake.

The data available on MP concentrations and characteristics in muscle tissue from Chapters 2 and 3 allows comparisons between species from the same oceans. With the assumption of trophic transfer, we expect that chokka squid from each ocean will have higher MP concentrations in their tissues. We also expect that the fibre lengths and colour proportions will be the same.

Materials and methods

The log-transformed data from Chapters 2 and 3 were used, and compared with unpaired, two-way t-tests.

2. Results

Chokka squid and anchovy were analysed for MPs from the South Atlantic and Indian oceans. The differences between the MP content were discussed in Chapters 2 and 3 (Bothma et al., a and b, In prep). In this closing Chapter, the MP contents of the two species will be compared with each other and between the oceans.

2.1 Microplastic concentrations

Microplastic concentrations did not show significant differences between anchovy and squid from either ocean, although the difference was near-significant at $p = 0.0677$ for the South Atlantic (Figures 1 a and b), with the anchovy approximately having a 50% higher MP concentration. There was no significant difference in the MP concentration between anchovy and chokka squid muscle tissue from the Indian Ocean ($p = 0.9553$).

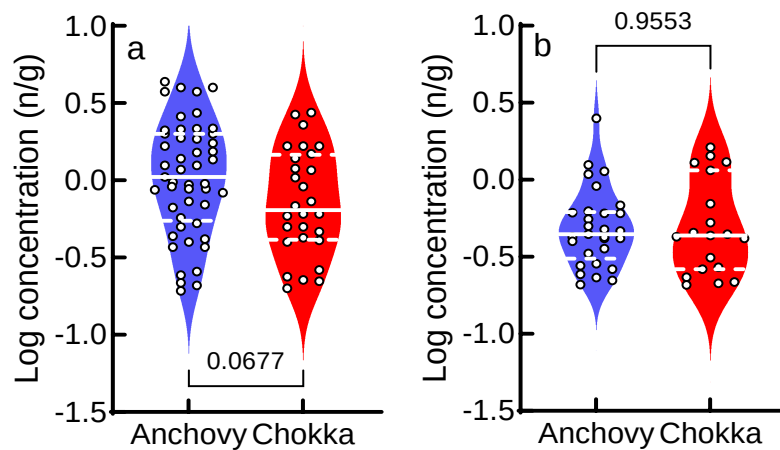


Figure 1: Violin plots of log MP concentration comparisons (unpaired, two-way, t-tests of log-transformed data) of anchovy and chokka squid from the South Atlantic Ocean (a) and the Indian Ocean (b).

2.2 Microplastic characterizations

Fibre lengths differed significantly between anchovy and squid from the South Atlantic Ocean ($p = 0.0172$) (Figure 2a) with anchovy fibres approximately 30% shorter. No significant differences in fibre length between anchovy and squid from the Indian Ocean was found (Figure 2b).

There was no significant difference in fibre colour proportions between anchovy and squid from the South Atlantic Ocean ($p = 0.1200$; Figure 3b). However, there was a significant difference in colour proportions of fibres in muscle tissue of the two species in the Indian Ocean ($p = 0.0040$; Figure 3a), with especially blue and red fibres occurring at half the frequency in chokka squid compared with anchovy.

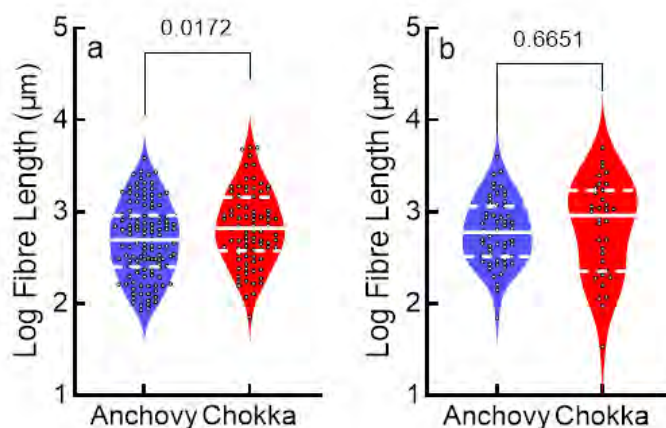


Figure 2: Violin plots of fibre lengths comparisons (unpaired, two-way, t-tests of log-transformed data) between muscle tissue of anchovy and chokka squid from the South Atlantic Ocean (a) and Indian Ocean (b).

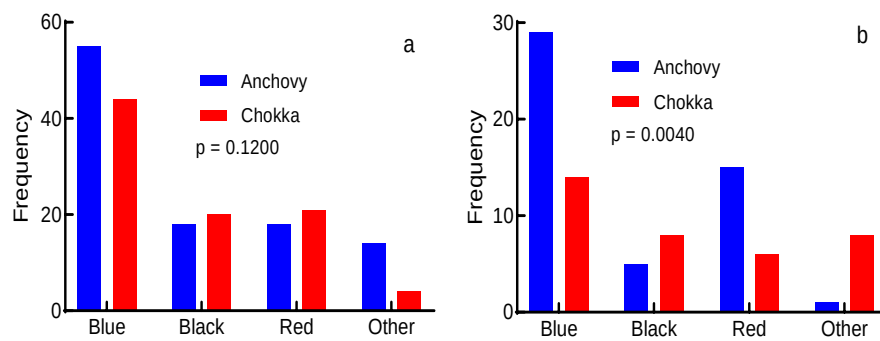


Figure 3: Bargraphs of comparisons (Chi-square) of proportional fibre colour frequencies in muscle tissues of anchovy and chokka squid from the South Atlantic Ocean (a) and Indian Ocean (b).

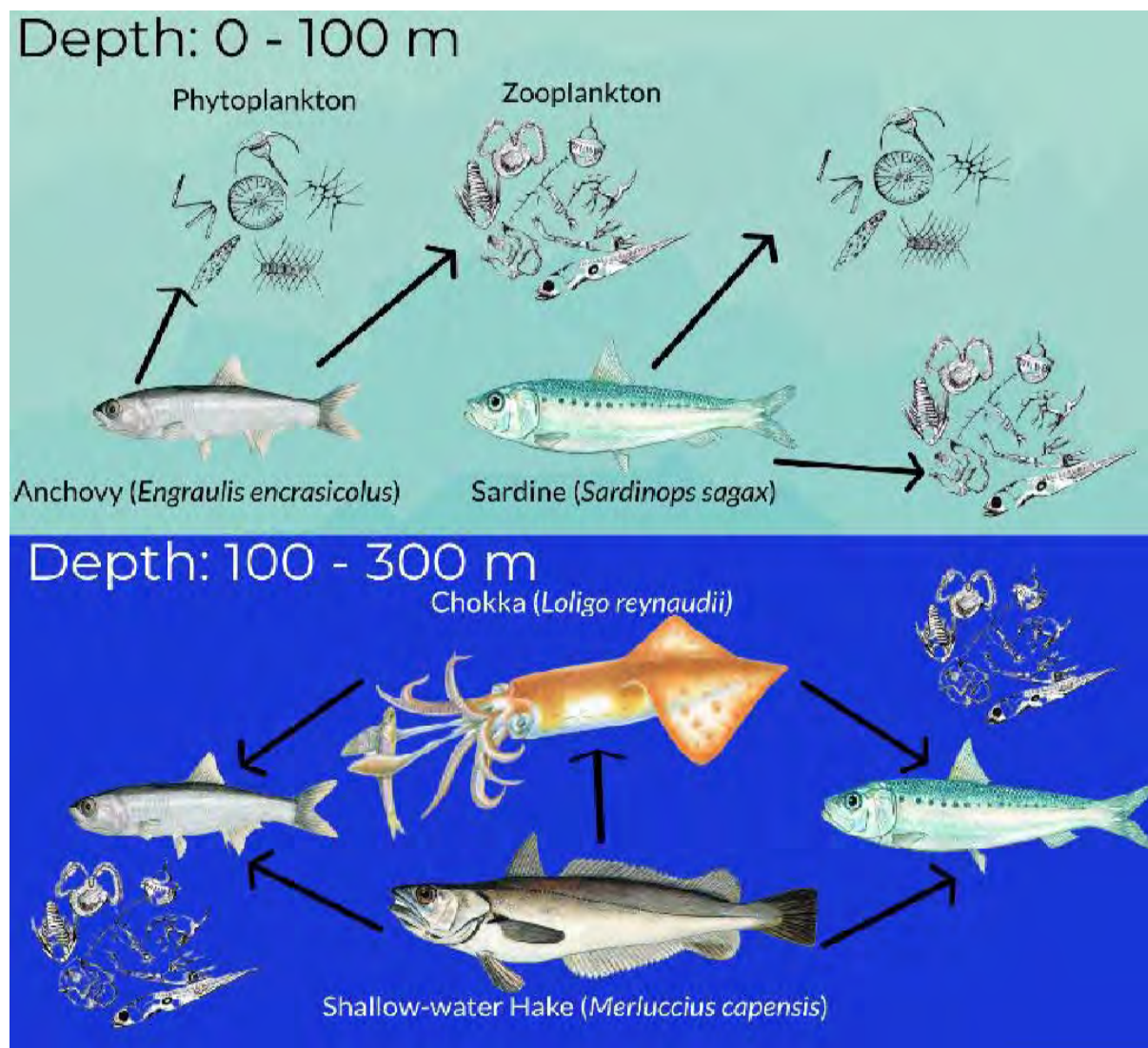


Figure 4: Simplified diagram of the food web and depth stratification of sardine, anchovy, hake and chokka squid in the South Atlantic and Indian oceans (Adapted from SAIAB, 2019; The South African Institute for Aquatic Biodiversity).

3. Discussion

3.1 Anchovy and chokka squid

The South Atlantic Ocean and the Indian Ocean are two adjoining oceans that make up the coasts of South Africa. Available concentrations of water show that the Indian Ocean has two-to-three times higher MP concentrations than the South Atlantic Ocean. The European anchovy (*Engraulis encrasicolus*) from both the South Atlantic and Indian Ocean contained MPs in muscle tissues with differing concentrations between the oceans. In contrast to the available water data, the South Atlantic anchovy had significantly higher mean concentrations of MPs in muscle tissues than the Indian Ocean anchovy (1.4 ± 1.1 n/g and 0.58 ± 0.46 n/g respectively). Similar to the anchovy, chokka squid (*Loligo reynaudii*) from the South Atlantic Ocean also had significantly higher MP concentrations in muscle tissues ($p < 0.001$). The three organs analysed from the squid (mantle, digestive gland, and gills) all showed higher concentrations of MPs in chokka squid from the South Atlantic Ocean, though only significantly so for the mantle. The chokka squid from the South Atlantic Ocean were significantly lighter than the Indian Ocean squid, due to the colder water in that region. Growth dilution of MPs was observed in both species.

3.2 Microplastic concentrations

Microplastic concentrations differed between anchovy and squid from the South Atlantic Ocean, the anchovy having 50% higher MP concentrations in its muscle tissue ($p = 0.0667$) (Figure 1a). Differences in trophic level may explain this variance (Walkinshaw et al., 2020) however, the Indian Ocean anchovy and chokka squid had no significant difference in MP concentration ($p = 0.9553$) (Figure 1b). There is currently no plausible explanation for this dichotomy. Chapter 3 argued similar problems in regards to contradicting data from the different oceans.

3.3 Microplastic characteristics

The dominant type of MP found in both species muscle tissue were fibres, fragments being scarce in chokka squid muscle tissue, therefore fibres were used for MP characteristic analysis. The South Atlantic Ocean chokka squid contained significantly longer fibres than the anchovy from the same ocean ($p = 0.0172$) (Figure 2a). The length of fibres in muscle tissue of chokka squid from both oceans increased in tandem with the size of the squid as discussed in Chapter 3, but I have no explanation for this phenomenon.

The significant difference in length of fibres from the South Atlantic Ocean anchovy and chokka squid may also be as a result of the different depths at which the organisms are usually found, as illustrated in Figure 4. This would, however, not explain why there was no significant difference in fibre length in the anchovy and squid from the Indian Ocean (Figure 2b). The dominant colour of MPs in both oceans and species was blue, although there were significant differences in the frequencies of colours in the Indian Ocean anchovy and chokka squid (Figures 3 a and b). No significant difference in the colours of the South Atlantic Ocean anchovy and chokka squid were found, despite differences in lengths and concentration (Figures 1a, 2a, and, 3a). The dominance of blue MPs in both oceans and species could indicate that the species selectively ingest blue MPs over others, or that blue MPs are most abundant.

3.4 Trophic transfer

Significant differences in colour may indicate that anchovy and chokka squid have different sources of MP exposure in the Indian Ocean (Figure 3b). Similar concentrations and lengths of MPs are potential indicators of MP trophic transfer from anchovy to chokka squid. However,

the previous finding in differing colours contradict this observation (Figures 1, 2, and 3). The South Atlantic Ocean anchovy and chokka had significant differences in length and concentration, but no significant difference in colour, indicating that there had been no trophic transfer.

3.5 Final observations

Dilution by growth of MP concentrations were observed in anchovy and chokka squid. This finding is supported by other studies that also found dilution by growth in fish muscle and liver tissues (McIlwraith et al., 2021). Squid and anchovy are poor bioindicators for microplastic pollution in seawater. The results of this study can rather be used as a comparative baseline for future studies. The significant differences between oceans in terms of MP concentrations in muscle tissue can be utilised to track stocks and migration patterns in fish and chokka squid.

4. Recommendations for further studies

I recommend that future studies focus on the following:

- More studies should be done on the translocation of MPs to tissues
- More than one trophic level should be studied
- As far as possible, methods should be standardised
- More studies should focus on species shared between adjacent oceans and seas
- Multiple organs should be sampled and analysed
- The uptake/trophic transfer of MPs to organisms should be thoroughly researched as there are underlying mechanisms currently unclear
- The bio-facilitated transfer of MPs between different oceanic regions should be investigated
- MPs in organs can be used as tracers to track movements of marine organism stocks and migrations
- The effect of upwelling on the uptake of marine organisms requires in-depth scrutiny
- Co-contaminants of MPs and their effect on uptake and translocation should be researched
- FTIR and/or ATR work should be implemented in MP studies

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