Marine Pollution Bulletin 202 (2024) 116371

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

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

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ARTICLE INFO

Keywords:
Microplastics
Fibre length
Tissue translocation
Indian Ocean
South Atlantic Ocean

ABSTRACT

Comparative microplastic (MP) data for cephalopods between oceans is scarce. Our aim was to quantify, characterise, and compare MPs in gills, digestive gland, and mantle of chokka squid from the South Atlantic Ocean (SAO) and Indian Ocean (IO) off the coast of South Africa. South African squid had more MPs compared with other studies (means = 2.0 and 0.4 in SAO and IO squid mantle, respectively). Blue fibres were dominant. Identifiable MPs were polyethylene. Despite IO water having higher MP concentrations than the SAO, SAO squid had higher MP concentrations. Dilution by growth is the likely reason for the lower MP concentrations. Fibres were shorter in SAO than IO squid. However, we could not explain why fibre and mantle lengths from both oceans were positively correlated. Squid may not be the best indicator of marine MPs. The characteristics of MPs in squid can be used to track stocks and migrations.

1. Introduction

Modern society generate environmental plastic pollution due to inadequate waste management and the ever-increasing production of plastic (Geyer et al., 2017; Sparks and Immelman, 2020; Verster and Bouwman, 2020). Plastic ends up in oceans via rivers, stormwater outlets or other means (GESAMP, 2015; Rathbone et al., 1998). Once the plastic enters a marine environment it will persist due to its durability (Vilakati et al., 2020). Plastic does, however, become smaller over time and a potentially greater threat to ecosystems (GESAMP, 2015). 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 by how they are formed; if produced as small particles, it is a primary MP, while those broken down via UV radiation, wave action, biological action, friction, and abrasion are known as secondary MPs (GESAMP, 2019; Sambolino et al., 2023). 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 (Abdolahpur Monikh et al., 2022; Athey et al., 2020; Chae et al., 2018; Farrell and 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), 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 aquatic 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 of 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 and Glassom, 2019; Sussarellu et al., 2016; Welden and Cowie, 2016). Pertinent to this study, experimental exposure of the octopus *Amphioctopus fangsiao* to polystyrene MPs caused histopathological damage, inflammation of the intestine, oxidative stress, metabolic disorders, gene expression, changes in the gut microbiome, and a range of changes in biomarkers (Zheng et al., 2022).

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.,

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https://doi.org/10.1016/j.marpolbul.2024.116371
Received 20 October 2023; Received in revised form 5 April 2024; Accepted 9 April 2024

2020; Naidoo and Glassom, 2019), but scarce on cephalopods (Gong et al., 2021; Sambolino et al., 2023). *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 a short-lived species with a mean life span of between 323 and 316 days for males and females respectively (Lipiński et al., 2020). They are important as both predator of and prey for 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; Lipiński, 1992; Sauer and Lipiński, 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 and 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). Cephalopods interact with marine plastic debris in many ways, including as shelter and as substrate to lay eggs on (Freitas et al., 2023). Ingestion is another interaction, both of larger pieces of polyethylene terephthalate (PET), polyethylene (PE), and polyvinylchloride (PVC), and as MPs through direct and indirect ingestion (Freitas et al., 2023). Consuming chokka could therefore be a trophic pathway of MPs to humans and marine predators such as dolphins, sharks, and penguins (Aznar-Alemany et al., 2019; Gong et al., 2023; Recabarren-Villalón et al., 2023).

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 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 and 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 (*Loligo reynaudii*) distributed over two oceans, each ocean with unique current systems (Fig. 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, 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. Twenty-eight samples from the South Atlantic Ocean (four sites) and thirty-eight samples from the Indian Ocean (five sites) were used for this study. At least five squid were used from each site, with no site >10. 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.

All the equipment used for dissection was rinsed thrice 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

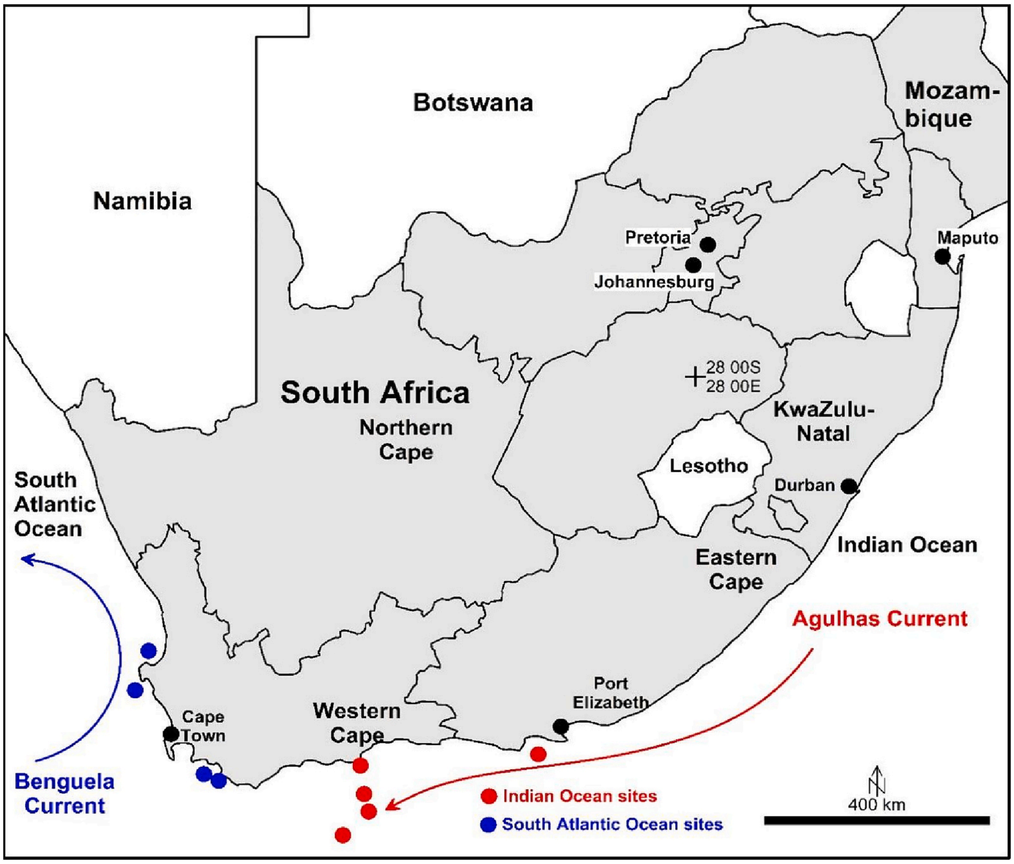

Fig. 1. Map and locations of all used squid collection sites, including the two major currents.

to the dissection surface. The chokka squid were defrosted until workable, and their wet mass, mantle length, and gladius length noted before dissection. The gills and digestive glands 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, glass flasks. To ensure complete digestion, the wet mass of samples never exceeded 5 g. 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 h to fully digest on a heating mantle at no >60 °C. HCl (1 mol) was added to complete the digestion process.

The fully digested sample was filtered through a 25-μm 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 categorised 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).

An aliquot of the sample was deposited on a zinc selenide (ZnSe) transmissive window for subsequent analysis. Polymer analyses was done using FTIR according to Maurizi et al. (2023) at the Department of the Built Environment, Aalborg University, Denmark. The analyses were executed using a Focal Plane Array (FPA) – μFTIR. The Cary 620 FTIR microscope, integrated with a Cary 670 IR spectroscope (Agilent Technologies, USA), was used for scanning the ZnSe transmission windows' active area. This microscope, fitted with a 15 × Cassegrain objective, delivered a 5.5 μm pixel resolution on its 128 × 128 MCT FPA detector. Scanning was performed in transmission\n mode, covering a spectral range of 3750–850 cm^{-1} at 8 cm^{-1} resolution, with 30 co-added scans. A separate background tile, collected before each sample and involving 120 co-added scans, ensured accurate background correction. The result was a chemical image of the sample area, where each pixel held a corrected IR spectrum. These images were analysed using the siMPle software Primpke et al. (2020) v. 1.3.1β, which detects particles, quantifies their morphology, and estimates their volume and wet mass automatically (Simon et al., 2018).

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 were 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 %. Linear regression slopes were compared by calculating a two-tailed *p*-value whether the slopes differ from each other, i.e. whether they are parallel or not.

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; Fig. 2a). No significant differences were found for mean mantle ($p = 0.0961$) or mean gladius lengths ($p = 0.1218$; Fig. 2b and c). There was a significant difference ($p = 0.0119$) between the mass/mantle length ratios of squid from the two oceans (Fig. 2d).

3.2. Microplastic comparison between chokka squid from different oceans

We extracted and characterised 490 MPs. 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 $p < 0.001$ (Fig. 3a). The linear regression of total MP concentrations with mantle length of squid from the SAO was significantly negative ($p = 0.0384$). All other regressions for fragments, fibres, and total concentrations were all negative, but not significant in both oceans (all $p > 0.1$). However, the regression slopes for fibres, fragments, and total MPs in the mantle of squid from each ocean were not significantly different from each other ($p > 0.8$). Neither were there any differences in between the slopes of all six regressions at once (in effect overlaying Fig. 2e and f), nor pairwise between oceans for fibres, fragments, and total MPs ($p > 0.6$).

More fibres were found in the South Atlantic chokka squid organs, though not all were significantly higher (Table 2). Mean fibre length showed a significant difference in the digestive gland of chokka squid from the South Atlantic and Indian Ocean $p = 0.002$ (Fig. 3c). No significant differences were seen in the fragments in regard to both mean concentration and length between the two oceans (Fig. 3b and d).

No significant differences were observed in the colour of fibres between Indian and South Atlantic samples (Fig. 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 (Fig. 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 (Fig. 3e). Fibres were the dominant morphotype in both South Atlantic Ocean and Indian Ocean samples (Fig. 3f). Significantly more fibres to fragments were found in the South Atlantic Ocean samples (Fig. 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 (Fig. 5a and b; Table 3).

3.3. Microplastics in organs

Only one film particle was found which we excluded from further

Table 1
Morphometrics of the chokka squid from the South Atlantic and Indian oceans including wet mass, mantle length and gladius length. n = number of samples; SD = standard deviation of the arithmetic mean; %CV = coefficient of variation.

	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

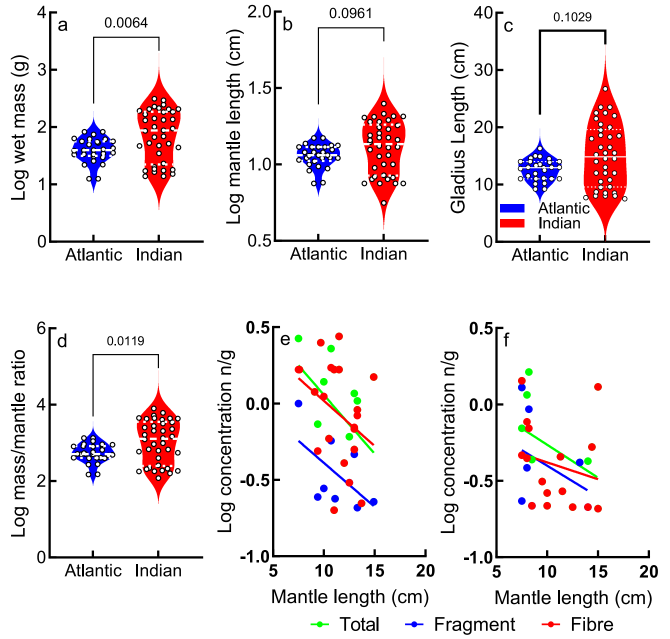

Fig. 2. Violin plots and results comparisons (Mann-Whitney) between (a) wet mass, (b) mantle length, (c) gladius length, and (d) mass/mantle length ratio. Coloured areas are frequency distributions. Linear regressions of log-transformed microplastics (all MPs, fibres, and fragments) concentrations (n/g)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

Table 2

Concentrations of MP fibres and fragments in three organs from chokka squid caught in the South Atlantic and Indian oceans. n = number of samples; SD = standard deviation of the arithmetic mean; %CV = coefficient of variation.

	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

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 (Fig. 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) (Fig. 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) (Fig. 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) (Fig. 4c). There were no significant differences in the fragments mean concentrations between the organs of Indian Ocean chokka squid (Fig. 4d). Neither fibre mean length nor fragment length showed significant differences between organs in both oceans (Fig. 4e to h; Table 3).

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

Due to sample loss during transport, only a limited number of MPs could be characterised with μFTIR (Table 4). All identified MPs were polyethylene. The largest fibre measured 0.27 mm long, with a mass of 1 μg.

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 (Table 1; Fig. 2a). However, there were no significant differences in their mantle and gladius lengths (Table 1; Fig. 2b and c). The mass/mantle length ratios of squid from the two oceans were, however, significantly different (Fig. 2d). 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 sea water temperatures in the SAO compared with the IO which would affect cephalopod growth (Forsythe et al., 1994; Forsythe, 2004; Hatfield, 2000; Jackson and 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 (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) also 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 mantles despite being smaller (Table 1; Fig. 3a). The other organs from the South Atlantic Ocean chokka squid also had higher fibre concentrations, however, the differences were not significant (Fig. 3; Table 2).

Whilst it is uncertain why South Atlantic Ocean chokka squid had 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) and increased transport of MPs (Wang and Chen, 2023). 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

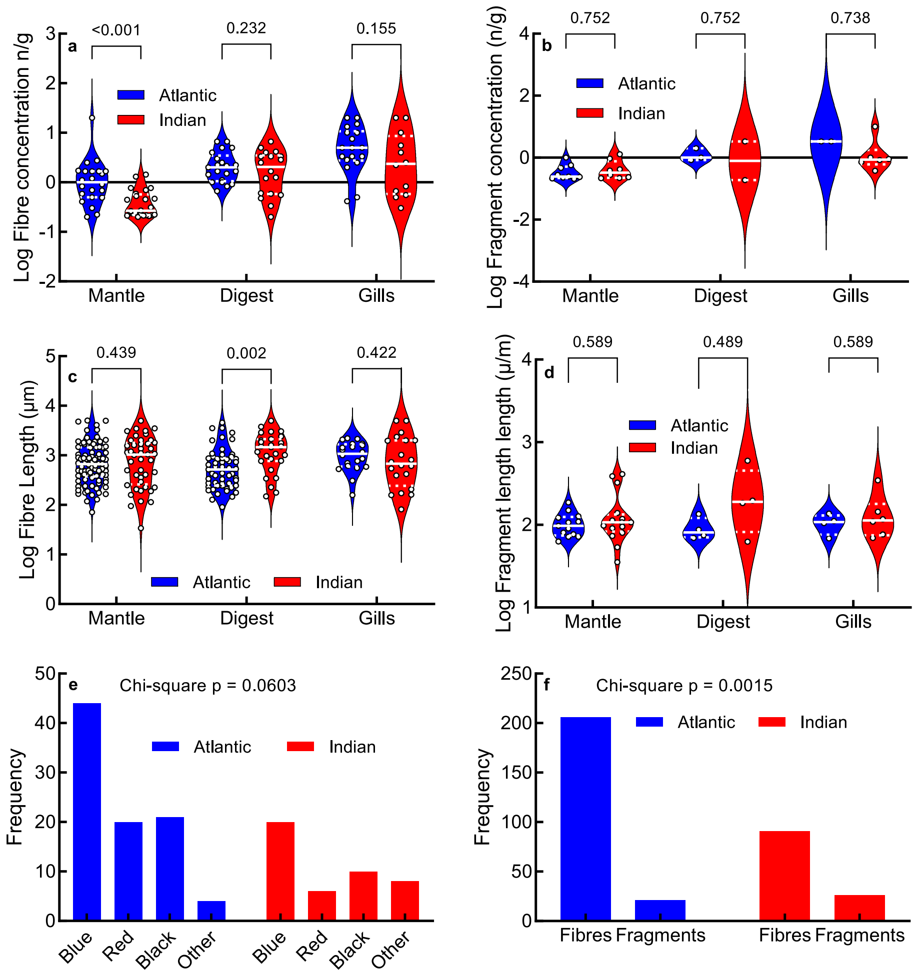

Fig. 3. Violin plots of fibre and fragment concentrations (a and b) and dimensions (c and d) compared between the Indian and South Atlantic oceans using multiple *t*-tests. Coloured areas are frequency distributions. Chi-square test comparisons of colours from MP particles found in the chokka squid from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

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 and Shannon, 2004) its plume pushing northwards and away from the sampled areas of the Benguela Current (Garrison and Ellis, 2015; Fig. 1).

Since 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 (Fig. 2e and f). There were also no significant differences ($p > 0.8$) between slopes for fibres, fragments, and total MPs within and between the two oceans, independent of concentrations suggesting that somatic growth and MP concentration dynamics are the same between the oceans, further supporting growth by dilution. Two mechanisms might be in play here.

1) Lower water temperatures cause cephalopods to grow slower (Forsythe, 2004; Hatfield, 2000; Jackson and Moltschaniwskyj, 2001) and have corresponding slower metabolism (Seibel et al., 1997; Seibel et al., 2000; Seibel and Carlini, 2001; Seibel and 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.

2) 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 greater mass and length, as was the case for the chokka squid from this study (Fig. 2a 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. While growing larger, translocation to the organs would be less. We also found similar indications for anchovy (Bothma et al., In prep).

Differences in digestion mechanisms between juvenile and adult squid might be in play as well. In octopus, the juvenile stages typically have internal digestion while adults begin external digestion before ingestion (Freitas et al., 2023). However, this is not the case with squid and cuttlefish that ingest their prey whole or bitten in pieces (rejecting the digestive tract, presumably with the MPs inside the prey's digestive

Table 3

Lengths of fibres and fragments in mantle, gills, and digestive glands of chokka squid from the South Atlantic and Indian oceans. n = number of samples; SD = standard deviation of the arithmetic mean; %CV = percentage coefficient of variation.

	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)					
	Atlantic			Indian		
	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

tract as well) and digested internally (Bidder, 1950). Squid has a very efficient digestive mechanism for managing food particles in the caecum. Endocytosis occurs in the digestive gland (Semmens, 2002), but how much this plays a role in capturing and translocating MPs from food to tissues is not known. The squid's efficient digestive system allows for their predatory lifestyle and perpetual swimming (Bidder, 1950).

There may be other mechanisms that we are not aware 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 of squid 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 (Fig. 4a to d, Table 2). Fibre and fragment lengths, however, were essentially the same in the present study between organs in chokka squid from the same ocean (Fig. 4e 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 half the length in the Indian Ocean squid compared with South Atlantic squid (821 μm and 1569 μm, respectively; 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 were almost twice as long as in chokka squid from the South Atlantic Ocean (means of 1569 μm, and 821 μm, respectively; $p = 0.002$; Fig. 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. 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

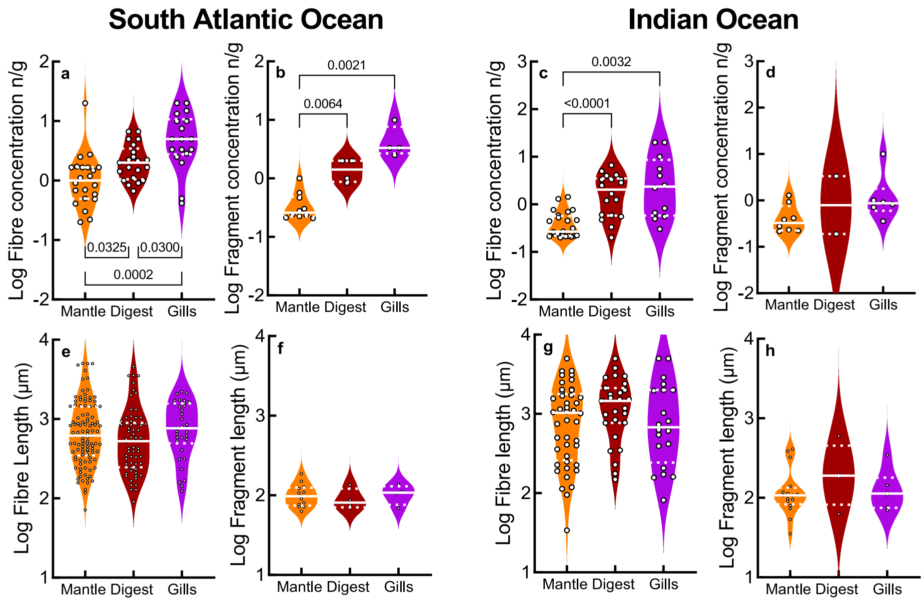

Fig. 4. Violin plots of microplastic concentrations and dimensions between South Atlantic and Indian Ocean Chokka squid in three organs. Coloured areas are frequency distributions. Comparisons were done using Kruskal-Wallis with Brown-Forsythe and Welch ANOVA post-tests. Only *p*-values of significant pair-wise comparisons are shown on the graphs. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

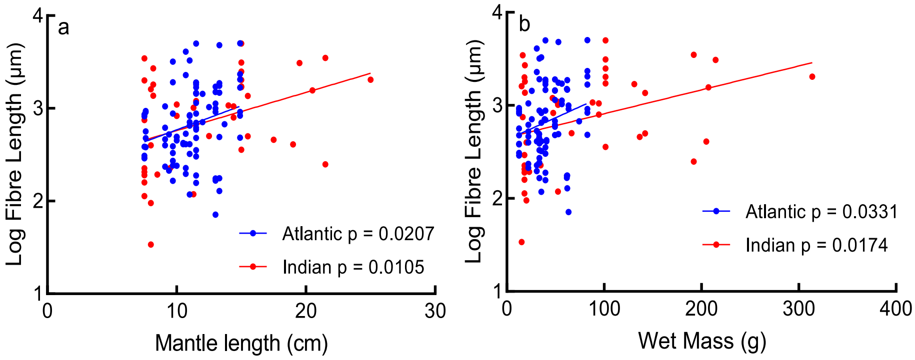

Fig. 5. Linear, least square, regressions of mantle length with fibre length (a), and wet mass with fibre length (b). The p-values of the slopes are provided.

Table 4
Characteristics of microplastics found in mantle tissue of chokka squid. Dim = dimension.

	Polymer	Area μm^2	Longest dim μm	Narrowest dim μm	Volume μm^3	Mass ng
MP1	Polyethylene	3200	90	45	57,000	55
MP2	Polyethylene	360	29	16	2300	2.2
MP3	Polyethylene	24,000	270	110	1,080,000	1000
MP4	Polyethylene	570	38	20	4500	4.3

other studies on different organisms (Barboza et al., 2020; Burger et al., 2024; Frias et al., 2020; Jabeen et al., 2017; Mao et al., 2020; Nel et al., 2017; Sambolino et al., 2023) and three studies done on cephalopods (Gong et al., 2021; Oliveira et al., 2020; Wang and Chen, 2023). Daniel et al. (2021) found mainly fragments in edible squid tissues from the Arabian Sea. In our study, blue was the most dominant colour in both the South Atlantic and Indian oceans, followed by black, then red (Figs. 3e; 6). The predominance of blue and black fibres was also reported by others (Campos da Rocha et al., 2021; Li et al., 2022; Sambolino et al., 2023).

The qualitative FTIR analyses available to us identified all as polyethylene (Table 5). This corresponds somewhat with the results of others who also found polystyrene and polypropylene (Table 5). The only other comparable study from the southern hemisphere also predominantly found polyethylene (Table 5, Ferreira et al., 2022) supporting our finding.

4.5. *Comparisons with other studies*

There are some studies available for comparisons (Table 5) 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 5, 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 5, 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 5, 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

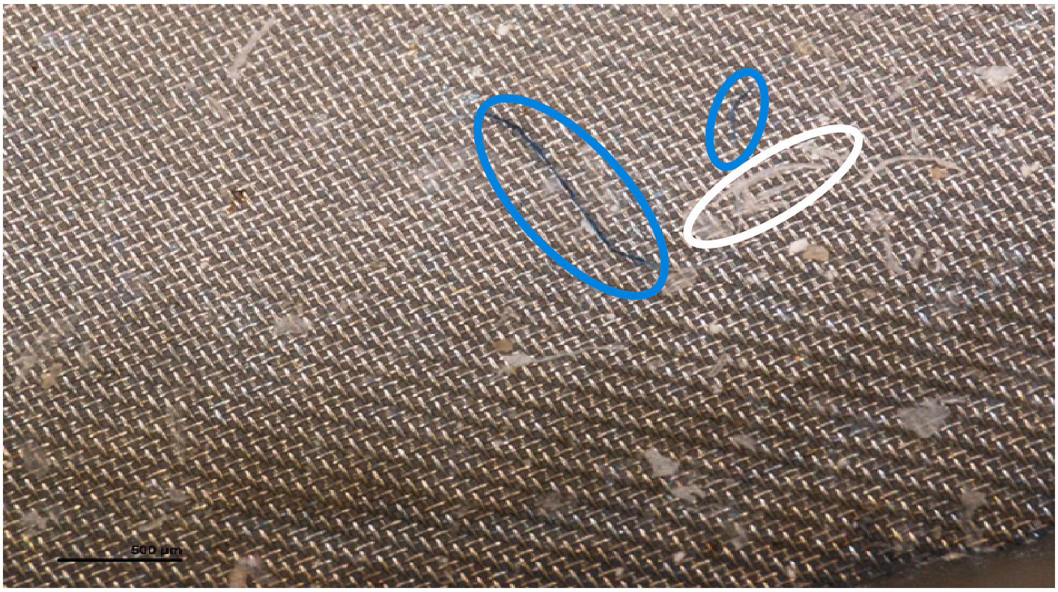

Fig. 6. Photograph of blue (blue ovals) and white (white oval) fibres on 25 μm sieve, from digested squid mantle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Comparative studies on microplastics in cephalopods from different regions.

Species	Locality	Organ	Concentration n/g	Dominant type	Dominant polymer	Dominant colour	Reference
Uroteuthis duvaucelii	India, Arabian Sea	Edible tissue	0.008	Frags	PP (40 %)	Transparent	Daniel et al., 2021
Dosidicus gigas	Peru	Gill	0.2	Fibs 97 %	Cellophane (79 %)	Black-grey	Gong et al., 2021
		Intestine	0.74	Fibs 93 %			
		Stomach	0.3	Fibs 92 %			
Sepia officinalis (Wild)	Portugal	GIT	0.12 fibres/g	Fibs 90 %	LDPE	Not analysed	Oliveira et al., 2020
Sepia officinalis (Farmed)	Portugal	GIT	0.06 fibres/g	Fibs 90 %	LDPE	Not analysed	Oliveira et al., 2020
Vampyroteuthis infernalis	Brazil	Whole organism	9.58	Frags 89 %	PE (34 %)	White	Ferreira et al., 2022
Dosidicus gigas	South Africa	Stomach	0.24	Frags 54.55 %	PET (32 %)	Blue	Wang and Chen, 2023
Sepsia officinalis	Italy	GIT	0.035	frags	N/A	Black	Armellini et al., 2023
Abralia veranyi	Brazil	Whole organism	2.37	Frags 62 %	PE (34 %)	White	Ferreira et al., 2022
Loligo reynaudii	South Africa SAO	Mantle	2.0 fibres/g	Fibs 85 %	PE	Blue	This study
		Digestive gland	2.5 fibres/g	Fibs 93 %			
		Gills	7.2 fibres/g	Fibs 89 %			
Loligo reynaudii	South Africa IO	Mantle	0.4 fibres/g	Fibs 73 %		Blue	This study
		Digestive gland	2.3 fibres/g	Fibs 87 %			
		Gills	5.6 fibres/g	Fibs 77 %			

for the large differences.

5. Synthesis and conclusions

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 less 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. It should be noted, however, that MPs do have negative effects on cephalopods (Zheng et al., 2022).

The shortcoming of 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 and trophic transfer. Our study also showed that MPs might have negative effects on squids from both oceans. 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.

CRediT authorship contribution statement

Francois Bothma: Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Ryan Christian Uren:** Conceptualization, Data curation, Investigation, Writing – review & editing. **Lucian Iordachescu:** Data curation, Formal analysis, Software. **Carl D. van der Lingen:** Data curation, Funding acquisition, Resources, Writing – review & editing. **Hindrik Bouwman:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Software, Supervision, Visualization, Writing – review & editing.

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.

Data availability

Data and detailed results from the statistical tests will be made available on request.

Acknowledgements

We thank the Department of Forestry and Fisheries and the Environment for providing the samples used in this study. We would also like to extend thanks to Willie Landman from the NWU for his assistance with microscopy work and the use of the microscope. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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