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Assessing microplastic abundances in freshwater fishes in a subtropical African reservoir

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Abstract
Microplastics are emerging pollutants of global concern, and their presence in the aquatic environment poses a serious risk for aquatic biota. While many studies have been conducted on the presence of microplastics in marine habitats, little research has been done in freshwater African reservoirs on microplastic pollution and their impacts on fish within the subtropical regions. To bridge this gap, the current study investigated microplastic abundances and distribution in freshwater fish within Nandoni reservoir, South Africa, across two seasons (i.e., hot–wet, cool–dry). Fish were randomly sampled using seine and gill nets from seven sites within the reservoir. In the laboratory, fish were then sorted according to taxa before dissecting them to remove the gills and the gastrointestinal tract (GIT). The organs were digested using hot hydrochloric acid and hydrogen peroxide, and the microplastics were classified according to their colours and shapes. Among the 94 fishes (i.e., 8 species) examined, microplastics were detected in 86.6% of the eight species caught. Microplastics were dominant in the gills and GIT during the cool–dry and hot–wet seasons, respectively. High microplastic abundances were found in the gills of *Micropterus salmoides* and the GIT of *Coptodon rendalli*, where fibres and the transparent colour were the most dominant. The results further showed high microplastic abundances in benthopelagic feeders highlighting that habitat influences fish consumption of microplastics whether directly or indirectly. Significant differences were observed in the feeding zone and season for all microplastic types. Microplastic sources in the reservoirs could be due to anthropogenic activities such as illegal dumping, fishing, and agriculture. Thus, there is a need for further investigation into the relation of fish weight, fish sex and body in relation to microplastic pollution. The highlighted ecological factors should be taken into consideration for future research and management actions aimed at mitigating and protecting the negative impacts of microplastic pollution on environmental and human health.

Keywords Microplastics · Benthopelagic · Gastrointestinal tract · Gills · Seasonal variation · Microplastic ingestion

1 Introduction

Plastic products are notable for their lightness, durability, versatility, and low cost of production [1, 2]. Nonetheless, global concern has been expressed over the widespread distribution and the impacts of plastic on the environment [1], with plastic pollution in our natural waters increasing every day due to human activities [3]. Plastic debris is accumulating in aquatic environments such as rivers and reservoirs at an alarming rate, thereby posing significant threats to aquatic

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ecosystems [4]. As a result, plastic pollution in aquatic environments is one of the emerging concerns around the world. Removal of plastics is difficult as they are the hardest materials to remove once they break into smaller particles, i.e., micro– or nanoplastics [5]. Cole et al. [6] highlighted that up to 10% of plastic pieces end up in aquatic ecosystems due to the rising manufacturing of plastic products, broad use, and poor waste management. Geyer et al. [7] estimates suggest more than 80% of plastic waste is in the natural environment and landfills. Thus, these plastic litter when they enter the aquatic and terrestrial environment, they can be broken down by wave action and ultraviolet light, forming small particles called microplastics [8). Microplastics are small particles with a diameter of less than 5 mm [9].

Environmental issues related to microplastic contamination have become a global problem due to their enormous influence on human health, the environment, and food safety [10, 11]. Their presence in the natural environment may result in ingestion by a variety of aquatic animals, such as crustacean taxa and commercially significant fish species, leading to negative effects on fish species [12–14]. Emerging environmental concerns involve accidental microplastic consumption by freshwater fishes [15]. Ingestion of microplastics by fish can hinder food passage and may obstruct feeding appendages [16–19]. Microplastic, when consumed by fish, leads to negative effects such as reduced growth rate, reduced productive ability, and increased death rate, which will have detrimental effects on the fitness of the fish population [9, 20, 21]. For example, European perch *Perca fluviatilis* in early developmental stages has reduced survival rates when exposed to high microplastic abundance within the natural environment [22]. Microplastics have also been discovered in studying the gastrointestinal contents of Rio de la Plata Estuary littoral freshwater fish, with all the fish caught containing microplastics [3]. Additionally, when mammals consume fish containing microplastics, they can bioaccumulate within the kidneys, intestines, and liver, leading to oxidative stress, growth, and developmental inhibition of their tissues ([10]. Many fish have been found to contain microplastics across various systems around the world and can cause microplastics to be passed along the food chain leading to biomagnification [10, 23–25]. According to Singh et al. [26], microplastic are readily ingested by fish accidentally due to their tiny size and similarity to phytoplankton [3, 13, 14, 20]. These ingested microplastics can also decrease the swimming ability of fish species, which impacts fish resistance time when swimming against high water flows [26]. Furthermore, after exposure to microplastics, the predatory performance and efficiency of a young common goby *Pomatoschistus microps* is significantly reduced [20]. Certain microplastic polymeric polymers have been found to suppress acetyl cholinesterase (AChE) activity in the fish's brain [10, 27].

However, the information available on plastic abundance and impacts in reservoirs is scarce and the sources that result in their pollution [10]. The aquatic ecosystems can be polluted by many factors such as pollution from industries, agricultural activities, mining, and urban development, which have resulted in increased production of plastics for commercial and domestic use [4, 28]. Freshwater environments have been identified as hotspots of microplastics, which are attributed to various sources, including illegal discharge of untreated wastewater from blocked pipes, effluent from wastewater treatment plants, and human littering [30]. Over 600 taxa have been documented to ingest plastic particulates [13, 31–34] with fishes being amongst the most affected taxa [35].

According to Khan et al. [3], studies have shown that most microplastics are found near effluent discharge points. A study by Dalu et al. [13] highlighted that all fish caught contained microplastics in their gastrointestinal tract, and more microplastics were found downstream of the wastewater effluent. Furthermore, Jabeen et al. [36] found microplastics in 95.7% of freshwater fish, and the abundance of microplastics in fish intestines was much higher than in the fish stomach. Microplastics ingested by fish may vary based on fish size and habitat [10]. As the majority of microplastic particles, with the exception of polyethylene and polypropylene, are likely to sink to the bottom, demersal fish species are the most vulnerable to microplastics [32]. Jabeen et al. [36] highlighted that demersal species had substantially higher microplastic abundances than pelagic fishes and that the feeding habit of freshwater fish played a crucial role in microplastic consumption. However, Rummel et al. [37] highlighted that pelagic fish species had more microplastics in their stomachs than demersal fish species. Dalu et al. [13] found that pelagic feeders had higher microplastic abundances than benthopelagic and demersal. Moreover, studies [e.g., 5, 13, 37] have reported that microplastic consumption by fish increases with size. However, Carbery et al. [38] highlighted that microplastics consumed by fish are primarily not related to other factors such as environmental conditions but related to the plastic abundances in the environment [39, 40].

The effects of seasonality have been found to significantly impact both freshwater fish populations [e.g., 41–43] and the distribution of plastics [14, 44, 45] in aquatic ecosystems. Temperature changes, rainfall patterns, and water flow variations influence fish behaviour, reproduction, and migration, often concentrating them in specific areas during certain seasons [46, 47]. These changes also affect the movement and accumulation of microplastics [48, 49]. During heavy rainfall or snowmelt, microplastics from urban runoff and agricultural areas are more likely to enter water bodies, leading to seasonal increases in contamination [50–52]. Therefore, it can be postulated that warm seasons (i.e., hot-wet,

hot-dry) may enhance the breakdown of plastics into microplastic particles, increasing their bioavailability and potential ingestion by fish, thus disrupting food webs and ecosystems [53].

While many studies have been conducted on the presence of microplastics in marine habitats, little research has been done in freshwater reservoirs on microplastic pollution and their impacts on fish within the subtropical regions. To bridge this gap, the study investigated the presence and abundance of microplastics in freshwater fishes within a subtropical reservoir in South Africa across two different seasons (i.e., hot-wet, cool-dry). We quantified the diversity, types and abundances of microplastics in various freshwater fish species within the reservoir. We hypothesized that (i) during the hot–wet season, freshwater fishes are most likely to consume more microplastics than in cool–dry season, as the reservoir receives more water from the Luvuvhu River, which is highly polluted from the catchment, and (ii) transparent fibres would be the most dominant type of microplastic in benthic fish feeders as they are not visible in water columns and are most likely to accumulate in sediments thereby being more likely to be directly or indirectly consumed due to their high frequency of occurrence.

2 Materials and methods

2.1 Study area

The Nandoni reservoir (22°59′11′′S, 30°36′16.19′′E) is situated near Ha–Mutoti, Ha–Budeli and Ha–Mphego villages in Thulamela Municipality, Vhembe District, Limpopo province, South Africa (Fig. 1) [54]. The reservoir lies along the Luvuvhu River with a catchment area of 1 380 km^2 and a total water capacity of 3785.4 m^3. The mean annual rainfall for the catchment ranges from 610 to 800 mm, with an average annual discharge of 519 million m^3 [55]. The typical summer and winter temperatures are 23 °C and 17 °C, respectively [55]. The predominant wind direction throughout summer and winter is from the east to southeast. The terrain of the reservoir region belongs to the Soutpansberg group, consisting of low–lying, undulating terrain that is underlain by a series of gneiss [55].

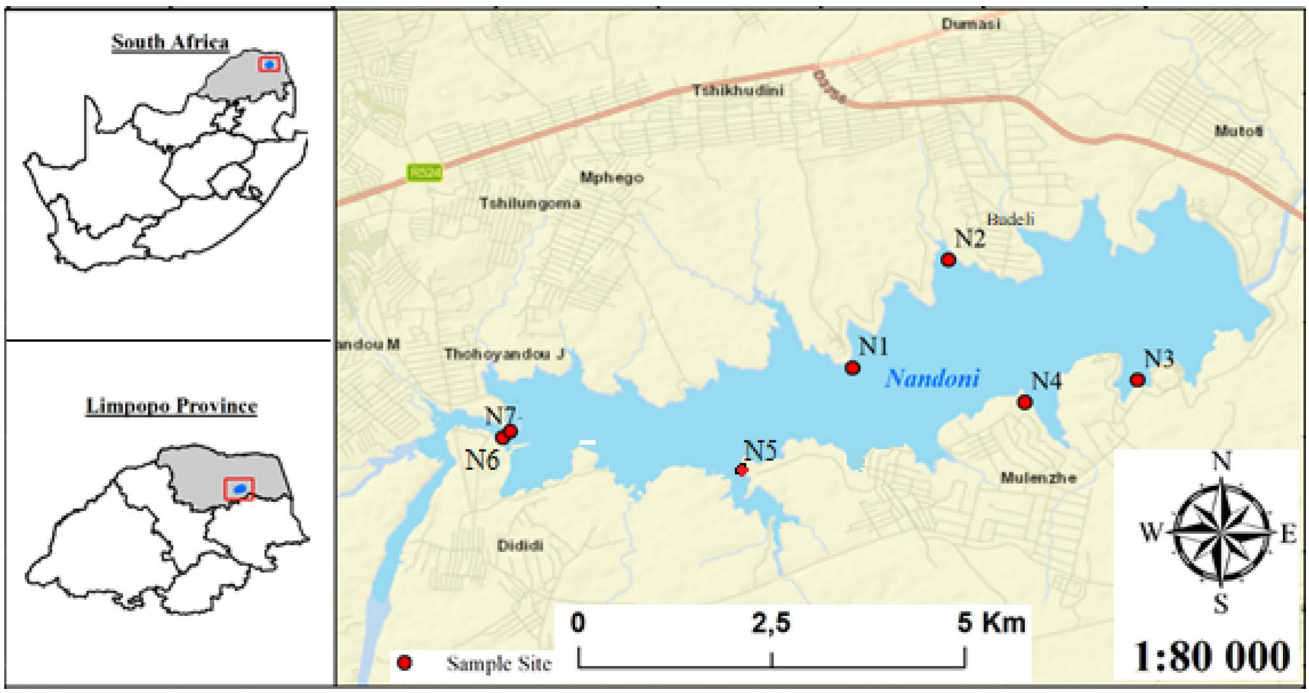

Fig. 1 The map of Nandoni reservoir in the Limpopo Province of South Africa highlighting the study sites

2.2 Fish collection

Two sampling methods, gill net (35 m × 2.75 m long), with mesh sizes of 35, 45, 57, 73, 93, 118 and 150 mm (i.e., approximately 5 m per mesh size) and seine net (i.e., 5 mm mesh size; 20 m (long) × 2 m (deep) with purse) were used for fish collection. Upon collection, the fish were immediately ethically euthanised through an overdose of 40 mg L^{-1} clove oil added to the collection buckets with the sampled fish and reservoir water [56] to prevent the fish from regurgitating anything that they have consumed because of stress or being confined in a small area. Once euthanised, the fish were properly preserved in buckets containing 70% ethanol and transported to the university laboratory for further processing. In the laboratory, the fish were separated according to their taxa using keys by Skelton [57] and the standard length to the nearest mm was measured.

2.3 Extraction and enumeration of freshwater fish microplastics

The collected fish were dissected to remove the gastrointestinal tract and gills before being placed in separate labelled glass test tubes. The glass test tubes were covered with aluminium foil to prevent external contaminants. All equipment was thoroughly cleaned using milliQ distilled water, and extreme care was taken to prevent contamination (i.e., wearing gloves and lab coats). The 55% nitric acid was added to each glass test tube until the gastrointestinal tract or gills were fully submerged or covered before being allowed to react for 6 h. After that, the remaining samples with most of the organics digested were heated to 100 °C to break down any remaining tissue for 1 h, following methods by Claessens et al. [58]. The sample was then vacuum filtered onto a Millipore membrane filter (diameter 47 mm, pore size 2 µm), which was then placed into a clean, covered petri dish waiting for further laboratory analysis. Each sample filter was thoroughly analysed under a compound light microscope (Olympus) at × 100 magnification, where all microplastic particles were visually identified and enumerated using colour and morphology [59]. The microplastics identified were enumerated as number of microplastic particles per fish. The total counts from each filter were multiplied by appropriate microscope conversion (optical factor F) and sample volume to provide the absolute abundances. Furthermore, particle size was measured after calibrating the microscope for a selected number of particles ($n = 1\,574$) based on the following four size classes: < 25 µm, 25–200 µm, 200–360 µm, and > 360 µm.

2.4 Freshwater fish microplastic quantification

We ensured that we prevented contamination during microplastic quantification in the laboratory as it is one of the important steps by using clean, non-plastic tools and glassware, wearing cotton laboratory coats, and working in a dust-free laboratory where the air-conditioner was turned off. The samples were handled in controlled conditions, cover with aluminium foil and regularly checked for contamination by running blanks [24].

The microplastics were classified into commonly used morphological shapes such as fibres, fragments, foam and film, according to Free et al. [60]. The colours were further categorised as blue, black, red, white, green, black, transparent, and other colours representing commonly occurring microplastic colours in aquatic research work [61]. Presumed microplastic particles were considered microplastics if they possessed unnatural shapes (e.g., perfectly spherical and sharp edges) and colouration (e.g., multi-coloured and bright colouration) [8, 59]. Since visual inspection alone has been deemed not sufficient to characterise and quantify microplastics, hence further physical form analysis was conducted based on Mintenig et al. [62]. We used Nile Red dye (CAS 7385–67–3, HYD0718–500 mg, Hycultec, Beutelsbach) to stain all the suspected microplastics [63]. Once the potential microplastics were stained, a blue light fluorescein was used to fluoresce and further identify the suspected microplastics to confirm microplastics because visual analysis alone is not adequate to quantify them [64]. A lipophilic dye like Nile Red helps identify microplastics as it colours the hydrophobic properties of plastic by staining them and illuminating them under a blue fluorescein light, reducing the risk for underestimation or overestimation of microplastics.

2.5 Data analysis

A simple *t-test* was used to analyse the fish species richness and standard length per season (i.e., hot–wet and cool–dry). Before multivariate analysis, the microplastic abundance data was log($x+1$) transformed to reduce variation and heteroscedasticity. We further tested for the homogeneity of variance and normality. Hence, the data was found to violet several parametric test assumptions and semi–parametric tests were utilised. Thus, a Kruskal–Wallis ANOVA was used to investigate differences in microplastic types and colour across locations (i.e., GIT, gut), fish habitats (i.e., benthopelagic, demersal), and seasons (i.e., hot–wet and cool–dry) using STATISTICA version 12. Species-level microplastic transformed abundance data was utilised based on Bray–Curtis similarity for the non–metric multidimensional scaling (*n*–MDS) ordination test analysis to help visualise the microplastic data as 2–2-dimension ordination figures for microplastic location, fish habitat and seasons in PRIMER version 6 [65, 66]. Multiple regression tests were conducted to assess the relationships between fish standard length with microplastic colour and types in STATISTICA version 12.

3 Results

3.1 Fish community structure

During the two study seasons, 94 individual fishes belonging to 4 families, namely, Centrarchidae, Cichlidae, Cyprinidae, and Mochokidae, were collected from the Nandoni reservoir, dominated by *Micropterus punctulatus* (Table 1). Significant differences (*t-test*, $p=0.015$) were observed in fish species richness across seasons. Furthermore, the cool–dry season had high fish abundances ($n=52$) caught with a high number of juvenile fish, while only while only 42 fish were caught during the hot–wet season. A total of 7 fish species across 4 families were collected, *Micropterus salmoides* (standard length (SL) range 24.5–31.5 cm, $n=3$), *Coptodon rendalli* (SL range 6.9–22.3 cm, $n=16$), *M. punctulatus* (SL range 16.4–18.9 cm, $n=4$), *Tilapia sparrmanii* (SL range 3.4–8.9 cm, $n=4$), *Chiloglanis paratus* (SL range 4.4–7 cm, $n=5$), *Labeo cylindricus* (SL range 6.7–19.6 cm, $n=5$) and *Labeobarbus marequensis* (SL range 6.5–8.2 cm, $n=13$) (Table 1). The fish standard length was significantly different (*t-test*, $p=0.001$) across seasons. The catch per unit effort (CPUE) was 5.2 for cool–dry season and the CPUE was 6.0 for the hot–wet season. The hot–wet season was mostly dominated by juveniles of spotted bass *Micropterus punctulatus* (standard length (SL) range 10.2–21.3 cm, $n=34$) and Mozambique tilapia *Oreochromis mossambicus* (SL range 7.4–12.4 cm, $n=8$).

3.2 Microplastic ingestion by fish

A total of 94 fish caught were examined for microplastics in GIT and on the gills. In total, 2 905 microplastic particles were detected, with abundances ranging from 0 to 78 particles per fish and the cool–dry season had high microplastic abundances compared to the hot–wet season. For the hot–wet season, 91.6% of microplastic particles were found in the gills, and 81.6% of microplastic particles were found in the GIT. All the species found in the cool–dry season had microplastics in their gills and GIT (Table 1). Fibres were the most common microplastic type found during the cool–dry season, with foam being the least dominant. Similarly, during the hot–wet season fibres were the most common microplastic type, with foam and film being the least dominant.

3.3 Fish species–variation in microplastic ingestion

Using Kruskal–Wallis analysis, foam ($H=1.982$, $df=1$, $p=0.371$) and fragments ($H=2.942$, $df=1$, $p=0.230$) were found to show no significant differences across locations, with all other microplastic types being significantly different ($p<0.05$) (Table 2). The feeding zone and season had significant differences ($p<0.05$) observed for all microplastic types (Table 2). Generally, the cool–dry season had high microplastic abundances (particle abundance range 0–78), compared to the hot–wet season (particle abundance range 0–39). Generally, *M. punctulatus* species had more microplastics in their gills (94.1% of the fishes) compared to the GIT (85. 2% of the fishes). Similarly, *O. mossambicus* had more microplastic in their gills (89.0%) than in the GIT (78.0%). Fibres were the most dominant microplastic type found in the gills of *M. punctulatus* (particle range 4.5 ± 4.0) and *O. mossambicus* (particle abundance mean 4.6 ± 4.9),

Table 1 Microplastic abundances observed in freshwater fishes in Nandoni reservoir across study seasons (hot–wet, cool–dry)

Fish species	Location	Season	*n*	Mean	SL range	MP range	Foam	Beads	Fragment	Fibre	Film
Micropterus punctulatus	Gills	Hot–wet	34	9.9 ± 8.3	10.2–21.3	0.0–32.0	0.2 ± 0.8	4.0 ± 6.5	0.5 ± 1.4	4.5 ± 4.0	0.8 ± 1.5
Oreochromis mossambicus	Gills	Hot–wet	8	11.3 ± 6.5	7.4–12.4	4.0–23.0	2.1 ± 3.4	2.5 ± 2.8	1.4 ± 1.8	4.6 ± 4.9	0.6 ± 1.1
Micropterus punctulatus	GIT	Hot–wet	34	5.6 ± 7.4	10.2–21.3	0.0–25	0.5 ± 1.0	2.2 ± 5.2	0.6 ± 1.3	1.7 ± 2.0	0.6 ± 1.8
Oreochromis mossambicus	GIT	Hot–wet	8	12.3 ± 13.4	7.4–12.4	0.0–39	0.9 ± 2.7	2.8 ± 7.0	1.0 ± 1.5	3.4 ± 3.5	4.2 ± 7.0
Coptodon rendalli	Gills	Cool–dry	16	29.8 ± 20.3	6.9–22.3	4.0–71.0	2.3 ± 5.4	9.3 ± 10.0	2.6 ± 3.2	10.7 ± 9.7	4.9 ± 4.5
Micropterus salmoides	Gills	Cool–dry	3	46 ± 29.1	24.5–31.5	21.0–78.0	3.0 ± 5.2	16.0 ± 7.9	1.0 ± 1.7	18.7 ± 17.6	7.3 ± 2.1
Micropterus punctulatus	Gills	Cool–dry	4	28.8 ± 18.0	16.4–18.9	6.0–46.0	5.3 ± 9.8	3.3 ± 5.3	5.3 ± 6.8	9.0 ± 12.8	6.0 ± 6.7
Tilapia sparrmanii	Gills	Cool–dry	4	16.0 ± 11.6	3.4–8.9	5.0–27	2.0 ± 4.0	5.8 ± 7.1	0.8 ± 1.0	6.8 ± 4.3	0.8 ± 1.0
Chiloglanis paratus	Gills	Cool–dry	6	28.7 ± 8.5	4.4–7	20.0–40.0	4.0 ± 4.4	6.7 ± 5.9	3.7 ± 3.7	11.2 ± 8.2	3.2 ± 2.9
Labeo cylindricus	Gills	Cool–dry	5	30.0 ± 7.6	6.7–19.6	21.0–40.0	1.0 ± 1.2	7.0 ± 6.1	3.0 ± 3.3	13.6 ± 5.9	5.4 ± 4.4
Coptodon rendalli	GIT	Cool–dry	16	11.4 ± 18.4	6.9–22.3	1.0–68.0	1.8 ± 3.2	7.7 ± 7.8	1.8 ± 2.1	8.1 ± 8.2	4.8 ± 3.7
Micropterus salmoides	GIT	Cool–dry	3	29.3 ± 9.7	24.5–31.5	11.0–40.0		12.3 ± 3.8	3.3 ± 4.2	9.7 ± 6.0	4.0 ± 3.6
Micropterus punctulatus	GIT	Cool–dry	4	13.0 ± 8.1	16.4–18.9	5.0–23.0		0.8 ± 1.5	2.3 ± 2.9	7.8 ± 3.2	2.3 ± 2.6
Tilapia sparrmanii	GIT	Cool–dry	4	5.3 ± 9.2	3.4–8.9	0.0–19.0		0.3 ± 0.5	0.3 ± 0.5	2.8 ± 4.3	2.0 ± 4.0
Chiloglanis paratus	GIT	Cool–dry	6	0.5 ± 1.2	4.4–7	0.0–3.0				0.5 ± 1.2	
Labeo cylindricus	GIT	Cool–dry	5	25.4 ± 20.6	6.7–19.6	2.0–47.0	2.4 ± 3.3	7.8 ± 8.3	4.2 ± 3.9	6.6 ± 7.0	4.4 ± 4.1
Labeo cylindricus	Both	Cool–dry	2	27.6 ± 12.6	3.0–5.7	8.0–54.0	1.6 ± 2.5	4.8 ± 4.9	3.5 ± 4.4	11.5 ± 8.1	6.1 ± 4.4
Labeobarbus marequensis	Both	Cool–dry	13	9.0 ± 2.0	6.5–8.2	7.0–11.0	0.5 ± 0.7			3.5 ± 3.5	5.0 ± 7.1

n taxon fish abundances, MP microplastic, SL standard length, GIT gastrointestinal tract

Table 2 The Kruskal–Wallis analysis results for freshwater fish microplastic abundances found in the Nandoni reservoir among locations, feeding zones and seasons

	Location			Feeding zone			Season		
	H	***Df***	***p***	**H**	***Df***	***P***	**H**	***Df***	***P***
Colour									
Red	**18.502**	**2**	**< 0.001**	**27.319**	**1**	**< 0.001**	**61.207**	**1**	**< 0.001**
Black	**20.643**	**2**	**< 0.001**	**5.692**	**1**	**0.017**	**16.806**	**1**	**< 0.001**
Blue	2.237	2	0.327	**4.128**	**1**	**0.042**	**9.893**	**1**	**0.002**
Green	**25.101**	**2**	**< 0.001**	**8.767**	**1**	**0.003**	**23.598**	**1**	**< 0.001**
White	**17.672**	**2**	**< 0.001**	1.429	1	0.232	**4.393**	**1**	**0.036**
Yellow	**10.218**	**2**	**0.006**	0.148	1	0.700	0.192	1	0.661
Other	0.136	2	0.934	0.647	1	0.421	0.002	1	0.967
Transparent	**14.742**	**2**	**0.006**	**5.411**	**1**	**0.020**	**9.785**	**1**	**0.002**
Type									
Foam	1.982	2	0.371	**7.776**	**1**	**0.005**	**5.677**	**1**	**0.017**
Beads	**8.414**	**2**	**0.015**	**5.397**	**1**	**0.020**	**13.612**	**1**	**< 0.001**
Fragments	2.942	2	0.230	**10.818**	**1**	**0.001**	**17.724**	**1**	**< 0.001**
Fibre	**22.877**	**2**	**< 0.001**	**11.212**	**1**	**< 0.001**	**27.036**	**1**	**< 0.001**
Film	**12.266**	**2**	**0.002**	**20.724**	**1**	**< 0.001**	**40.22**	**1**	**< 0.001**
Total	**20.779**	**2**	**< 0.001**	**18.792**	**1**	**< 0.001**	**40.141**	**1**	**< 0.001**

Significant values ($p < 0.05$) are indicated in bold

with foam (particle abundance mean 0.2 ± 0.8) and film (particle abundance mean 0.6 ± 1.1) being the least dominant microplastic for two species, respectively. Beads (particle abundance mean 2.2 ± 5.2) and film (particle abundance mean 4.2 ± 7.0) were the most dominant microplastics found in the GIT of *M. punctulatus* and *O. mossambicus*, respectively. The transparent colour was common in both fish species in the gills and GIT.

All the species found in the cool–dry season had microplastics in their gills and GIT (Table 1). Generally, *M. salmoides* had high microplastic abundances in the gills (particle abundance range 21–78), with *C. rendalli* (particle abundance range 4–71) having the least dominant microplastic abundance in the gills. Fibres were the most dominant microplastic type found in the gills and GIT of all fish species (Table 1). Film (particle abundance mean 0.8 ± 1.0) and foam (particle abundance mean 1.0 ± 1.2) were the least dominant microplastic type found in the gills of *T. sparrmanii*, and *L. cylindricus*, respectively. Beads (particle abundance mean 0.3 ± 0.5) and fragments (particle abundance mean 0.3 ± 0.5) were the least dominant microplastic type found in the GIT of *T. sparrmanii*. The transparent colour was the most dominant colour in the GIT and gills, whereas blue and green were the least dominant colour in all the fish species. Based on Kruskal–Wallis analysis, microplastic colours were found to be significantly different ($p < 0.05$) (Table 2), with the exception of colour blue ($H = 2.237$, $df = 2$, $p = 0.327$) and other ($H = 0.136$, $df = 2$, $p = 0.934$) across the different locations (i.e., gills, GIT). Across the different feeding zones, microplastic colour white ($H = 1.429$, $df = 1$, $p = 0.232$), yellow ($H = 0.148$, $df = 1$, $p = 0.700$) and other ($H = 0.647$, $df = 1$, $p = 0.421$) were the only colours not significant, whereas across seasons, microplastic colour yellow ($H = 0.192$, $df = 1$, $p = 0.661$) and other ($H = 0.002$, $df = 1$, $p = 0.967$) where the two colours not significant (Table 2).

During the cool–dry season, *Labeo cylindricus* had high microplastic abundance (range 8–54) compared to *L. marequensis* (range 7.0–11.0) (Table 1). Fibres were the common microplastic type found in *L. cylindricus* (mean 11.5 ± 8.1) and *L. marequensis* (mean 3.5 ± 3.5). Generally, foam was the least common microplastic type in *L. cylindricus* (particle abundance mean 1.6 ± 2.5) and *L. marequensis* (particle abundance mean 0.5 ± 0.7). Red was the most dominant colour found in *L. cylindricus*, with blue being the least dominant colour. Transparent was the most dominant colour found in *L. marequensis*, with red and yellow being the least dominant colour found in the fish species.

Generally, within the gills most of the microplastic particles were larger than 360 µm, with the small particles being found in most of the fish species. *Oreochromis mossambicus* showed a slightly different pattern, with 25–200 µm particle size dominating (Fig. 2). Within the gastrointestinal tract elevated quantities of the < 25 µm particle size was observed compared to the gills, with no clear patterns for most fishes (Fig. 2). Based on the Kruskal–Wallis test, no significant differences ($p > 0.05$) were observed habitats (i.e., benthopelagic, demersal), whereas for the location (i.e., gills, GIT), significant differences ($p = 0.026$) were only observed for < 25 µm particle size which significantly high in the GIT and rest of the size fractions were not significant ($p > 0.05$).

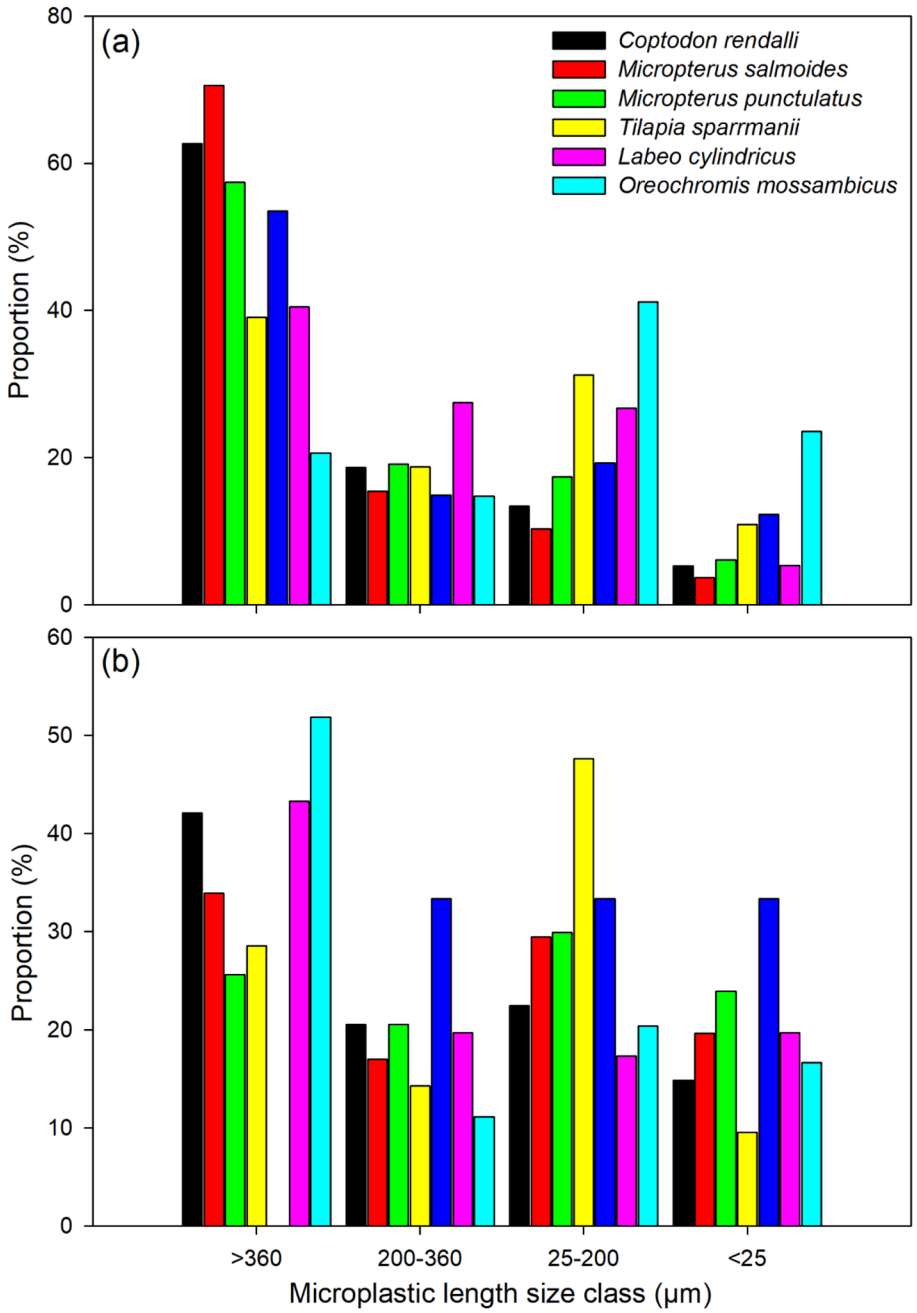

Fig. 2 Variation in microplastic sizes across selected fish species and number from the Nandoni reservoir, South Africa: **a** fish gills and **b** gastrointestinal tract

Based on the *n*–MDS analysis, the different fish parts which were examined (i.e., GIT, gills) showed no major differences in microplastics, with the gills having a large microplastic abundances compared to the GIT (Fig. 3a). Similar patterns were observed for seasons, with the hot–wet season having a large spatial range of microplastics within the fishes (Fig. 3b). Furthermore, the feeding zone also had similar patterns with demersal fish species having varied microplastic fishes compared to benthopelagic fishes (Fig. 3c).

3.4 Relationship among freshwater fish standard length with microplastic type, colour and total abundance

The multiple regression analysis highlighted an $r = 0.44$ ($r^2 = 0.19$, adjusted $r^2 = 0.12$), which was significant at $F_{14,162} = 2.795$, $p < 0.001$ (standard error estimate 0.20). Furthermore, regression analysis results highlighted no significant ($p > 0.05$) relationships for the fish's standard length with selected colours (i.e., red, black, green, white) and type (i.e., foam, beads, fragments, fibre, film) abundances (Table 3). The fish standard length had a positive and significant relationship with

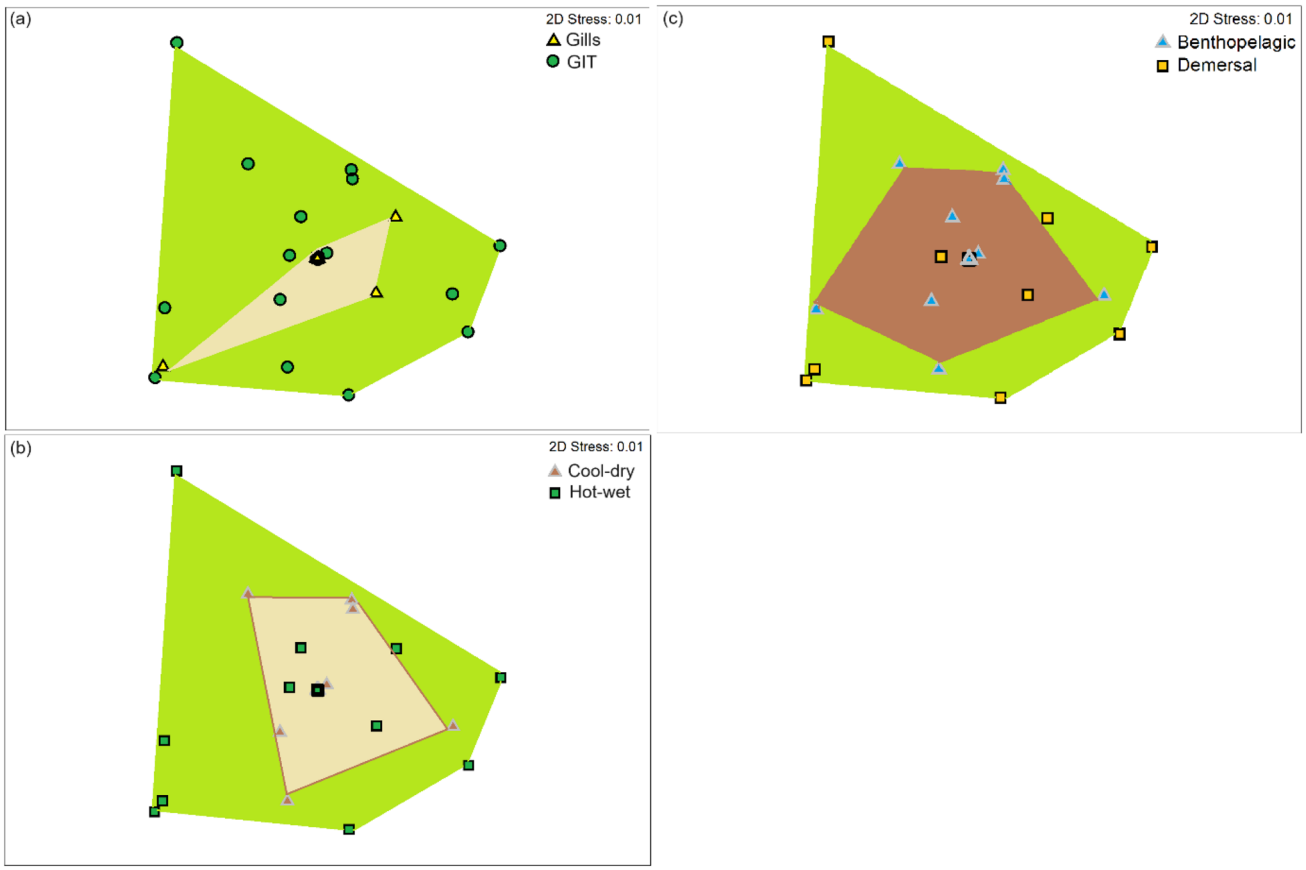

Fig. 3 Non–metric multidimensional plots of **a** fish location (i.e., gastrointestinal tract (GIT), gills), **b** seasons and feeding zone within water for fish microplastics within Nandoni reservoir, South Africa

Table 3 Multiple regression analysis results for freshwater fish collected in the Nandoni reservoir highlighting the relationship among the fish standard length with microplastic colour and type

	B	*b**	*T*	*P*
(Constant)	**1.14 ± 0.04**		**29.431**	**< 0.001**
Microplastic colour				
Red	0.001 ± 0.08	0.02 ± 0.15	0.106	0.916
Black	0.07 ± 0.07	0.13 ± 0.13	0.945	0.346
Blue	−0.001 ± 0.12	−0.001 ± 0.08	−0.008	0.993
Green	−0.10 ± 0.09	−0.11 ± 0.09	−1.185	0.238
White	0.08 ± 0.06	0.14 ± 0.11	1.281	0.202
Yellow	**0.16 ± 0.06**	**0.27 ± 0.11**	**2.579**	**0.011**
Other	**0.24 ± 0.09**	**0.20 ± 0.08**	**2.593**	**0.010**
Transparent	**0.20 ± 0.08**	**0.42 ± 0.16**	**2.706**	**0.008**
Microplastic type				
Foam	−0.02 ± 0.07	−0.03 ± 0.10	−0.318	0.751
Beads	0.07 ± 0.07	0.16 ± 0.17	0.915	0.361
Fragments	−0.03 ± 0.06	−0.04 ± 0.09	−0.474	0.636
Fibres	0.06 ± 0.08	0.12 ± 0.17	0.694	0.489
Film	−0.03 ± 0.07	−0.05 ± 0.13	−0.413	0.680
Total abundances	**−0.30 ± 0.11**	**−0.73 ± 0.27**	**−2.670**	**0.008**

For significant values ($p < 0.05$), the numbers are indicated in bold

microplastic colour yellow ($t_{162} = 2.57$, $p = 0.011$), other ($t_{162} = 2.59$, $p = 0.010$) and transparent ($t_{162} = 2.71$, $p = 0.008$) which highlighted that with increases in fish size, there was an increase in the consumption of microplastic coloured yellow, other and transparent. The fish standard length further highlighted a negative and significant relationship with total microplastic abundances ($t_{162} = -2.67$, $p = 0.008$), which indicates that with an increase in fish size, there was a decrease in the consumption of microplastics (Table 3).

4 Discussion

We hypothesized that in the hot–wet season, fish are more likely to consume more microplastics than in the cool–dry season, as the reservoir receives more water from the Luvuvhu River, which is highly polluted by various activities [13]. The results showed that in the cool–dry season, fish consumed more microplastics compared to the hot–wet season, thereby rejecting my initial hypothesis. This could be due to reduced water flows entering the reservoir, which led to increased concentrations of microplastic to be suspended or to sink which were then ingested by fish. The reservoir is surrounded by lodges, agricultural activities, and villages, and these could be the main sources of contamination, including littering of plastics around the reservoir shoreline. Microplastics, when exposed to the aquatic environment, particles can either float or sink; thus, fishes mistake them for prey [67].

The study assessed microplastic abundances in freshwater fishes in the Nandoni reservoir, South Africa. The results showed that 86.6% of fish species were contaminated with microplastic and this was consistent with Parvin et al. [10], who reported 73.3% of fish caught having microplastic in their digestive tract. The findings were in contrast with Rummel et al. [37], who reported that 5.5% of fish had microplastic in their stomach. However, the presence of microplastics in fish species in the present study is low compared to Dalu et al. [13], who reported that all fishes caught had microplastics in the Luvuvhu and Crocodile River systems. Similarly, Park et al. [68] and Hossain et al. [5] also reported that all fish had microplastic in their digestive tract. Microplastic in the fishes could be due to numerous factors such as the littering, disposal of domestic waste near the reservoir, partially treated raw sewage discharge, burst sewage pipes, and increased urbanisation.

High microplastic ingestion by fish could be as a result of preferred habitat [13]. Based on the feeding zone and preferred habitat all fish collected *T. sparrmanii*, *C. paratus, L. cylindricus*, *C. rendalli*, *L. marequensis*, and *O. mossambicus* were classified as benthopelagic, whereas *M. salmoides* and *M. punctulatus* were classified as demersal, with high microplastic abundances being observed in *C. rendalli* and *M. salmoides*. The high microplastic abundance in *C. rendalli* a microphyte feeder may be due to the accidental consumption of microplastics as food, and *M. salmoides* a predator may be due to consuming a prey that is contaminated by microplastics which is then passed on to higher trophic level [63]. Benthopelagic fish reside and forage within waters just above the bottom, feeding mostly on zooplankton and benthos [69]. This habitat provides benthopelagic fish with a large feeding ground and a variety of dietary sources, which increases the chance of microplastic ingestion. This observation was supported by Bessa et al. [70] who reported high microplastic abundances in the benthopelagic fishes compared to demersal fishes within the Mondego Estuary, Portugal. However, the results were in contrast with Rummel et al. [37], Park et al. [68] and Dalu et al. [13] which reported high microplastic abundances in pelagic fish than demersal and benthopelagic fishes. However, Tien et al. [71] and Parvin et al. [10] highlighted those demersal fish had higher microplastic abundances than benthopelagic and pelagic fishes.

Microplastic ingestion by fish is highly dependent on particle size [72, 73]; the smaller the microplastic size, the more likely the particles are to be ingested or transmitted into the digestive system of a fish [6, 16]. The presence of small-size particles (i.e., < 25 µm) presents an opportunity for these microplastics to be absorbed and assimilated by fish species and is more likely to cause serious complications. Although we observed no differences in microplastic particle size ingestion among habitats in the current study, Corami et al. [74] observed that pelagic fish species tended to ingest more large particles than those ingested by the benthic fish species. Large microplastics may be retained in the GITs [73] and may result in disturbance of ingestion, physical obstruction, death, and malnutrition [75, 76]. Furthermore, fish species may ingest these microplastic particles through their prey, which they may be feeding on [74]. The present results showed high microplastic abundances found in the gills compared to the GITs for all fish species. These results agreed with Yona et al. [77], who reported high microplastic abundances in the gills compared to the GITs of coral reef fish in three outer islands of Papua, Indonesia. Furthermore, the results were also similar with Barboza et al. [78] who reported microplastics in high abundance in the gills (60.2–85.1%) than in the GITs (49.0–78.9%). In contrast, Hurt et al. [79] found high microplastic abundances in the gills *Dorosoma cepedianum* from Evergreen and Bloomington Lakes, Illinois, USA. During a fish's interaction with its environment, the large microplastic particles stay trapped in the gills, while the tiny

ones may pass through and accumulate in the GITs [78]. In this present study, this could explain the high microplastic abundances found in the gills compared to the GITs.

Five microplastic types (i.e., form, fibres, beads, film, fragments) were observed in the fish GIT and gills. The observed colours were red, yellow, green, blue, transparent, white, black, and others. The dominant microplastic type found in both gills and GIT were fibres. The results agrees with our hypothesis that transparent fibres will be the most dominant type of microplastic in benthic fish feeders as they readily sink and are mainly likely to accumulate in sediments [10]. Previous studies [10, 13, 80–82] found that fibres were the dominant microplastic type in fish species. Similarly, Neves et al. [83] reported the dominance of fibre microplastics in commercial fish of the Mendoza River and Portuguese Coast, Argentina. This could be attributed to the high abundance of microplastic fibres in the reservoir from sewage (i.e., discharge of semi–treated sewage and/or burst sewage pipes). The fish might have mistakenly identified these particles as food and ingested unintentionally while feeding, breathing, moving around and filtering water [81, 84–86], or ingested a contaminated prey [13, 16, 20, 78].

In this present study, transparent was the most dominant colour and the abundance of transparent plastic fibres and fragment in the fish gills and GIT were reported in numerous studies [5, 10, 36]. Around the Nandoni reservoir, the surrounding communities practice fishing using monofilament gill nets, littering of shopping and packaging bags, these plastic particles are transparent, and while these microplastics are in water column, they break down into smaller particles which might be ingested by fishes. However, in contrast with our study, Barboza et al. [78] and Neves et al. [83] reported blue as the most dominant colour preferred by fish, with blue microplastics being more likely to be consumed by fish and their prey than microplastics of other colours [78]. In contrast, de Sa et al. [20] reported that white microplastics were the most preferred plastic type by common goby *Pomatoschistus microps* juveniles from the Lima and Minho rivers, northwest Iberian Peninsula due to its resemblance to brine shrimp *Artemia nauplii*, which is typically pallid in colour and serves as an essential food source for the fish.

The fish standard length showed a positive and significant relationship with microplastic colour yellow, other and transparent which indicates that with an increase in standard length, there was an increase in the uptake of microplastic coloured yellow, other and transparent by the fishes. The fish standard length further showed a negative significant relationship with total microplastic abundances, suggesting that an increase in standard length can result in a decrease in the uptake of microplastic by the fishes. This was consistent with previous research by Dalu et al. [13], who reported a positive correlation between standard length and high microplastic uptake by fish. The results of the present study suggest that bigger fish are more likely to consume microplastics. However, this result contradicts the findings by Chen et al. [87], who observed no correlation between microplastic abundance and fish standard length.

5 Conclusions

Microplastics were detected in 86.6% of freshwater fishes, with fibres being the most abundant microplastic type and transparent being the most dominant colour. Microplastic were high during the cool–dry season compared to the hot–wet season, and the results suggested that microplastic contamination in the Nandoni reservoir was more severe in the cool–dry season. This could be attributed to anthropogenic activities such as littering, agriculture and fishing. However, more studies are required to understand these dynamics. The results further revealed a high prevalence of microplastics in benthopelagic consumers, suggesting that feeding habits and fish habitat can affect the consumption of microplastics by fish. The high abundances of microplastics were observed in the gills of *M. salmoides* and the GIT of *C. rendalli*. This study's findings can be used as baseline data for the subtropical regions for the status of microplastics and further set the way for future investigations into microplastic pollution in aquatic environments. The highlighted ecological factors should be consideration for future research and management actions aimed at mitigating and protecting the negative impacts of microplastic pollution on environmental and human health.

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Author contributions TD: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Funding, Supervision, Writing—original draft, review and editing; STT: Methodology, Data curation, Formal analysis, Investigation, Writing—original draft, review and editing; FD: Visualization, Methodology, Investigation, Writing—original draft, review and editing;; NW: Visualization, Methodology, Investigation, Funding, Writing—original draft, review and editing; LFM: Visualization, Methodology, Investigation, Writing—original draft, review and editing.

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Data availability All data has been presented in the manuscript.

Declarations

Ethics approval and consent to participate The study has been ethical approved by University of Mpumalanga School of Biology and Environmental Sciences Research Ethics Committee number: UMP/Themba/BScHons/2023 and University of Mpumalanga Animal Sciences Research Ethics Committee number: AS/TDalu 01–150322, and permission to carry out the study was granted by Limpopo Economic Development, Environment and Tourism: CPM01753.

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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