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Microplastics contamination of fish from the Creeks along the Kenya coast, western Indian Ocean (WIO)

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ABSTRACT

Microplastics (MPs) are a global threat to marine life, but information available on the extent of pollution along the Kenya Indian ocean coast is absent. Ingestion of MPs by five common pelagic fish from the region was investigated in this study. Fish tissues were digested in 10 % potassium Hydroxide (KOH) at 60 °C for 14 hours and the MPs were separated by floatation method using supersaturated Sodium Chloride (NaCl) solution. The benthic fish studied (*Acanthopagrus berda* had a mean of 0.48 ± 0.06 MPs per gram (g^{-1}) tissue, *Gerres oyena* 0.20 ± 0.02, *Terapon jarbua*0.20 ± 0.06) had higher MPs contamination compared to the pelagic (*Rastrelligerkanagurta*0.054 ± 0.011) and reef fish (*Leptoscarus vaigiensis*0.038 ± 0.009) reflecting the influence of habits and habitat on MPs contamination in fish. Fish caught in the second sampling had higher MPs contamination compared to the first and fish from Mida Creek had higher MPs contamination compared to those from Tudor and Port-Reitz probably because they were mainly benthic fish. Gut and gills of all species contained significantly higher concentrations of microplastics than the flesh. Thus, we recommend that fish be gutted and thoroughly clean the gills before cooking to reduce MPs contamination. There is need for further research to evaluate the risk posed to humans by the consumption of marine water fish that ingested microplastics

Keywords Microplastic contamination; fish; Creeks; Western Indian Ocean Kenya Coast.

INTRODUCTION

Pollution of the oceans with microplastics and their potential impact along marine food web through consumption is of increasing concern (Cole *et al*., 2013; Eerkes- Medrand*et al.,* 2015; Romeo *et al*., 2015; Beer *et al*., 2018). Barnes *et al*. (2009) defined microplastics as synthetic particles measuring < 5mm in diameter. They can be primary in nature if they are particles manufactured for product production in which case they are referred to as nurdles or they can be secondary if the particles are degraded from microplastic debris (Lusher *et al*., 2013; Free *et al*., 2014). Plastic degradation occurs through physical, microbial and ultra-violet radiation processes (Moore, 2008; Andrady, 2011; Rummel, 2014) although very slowly. Microplastics are further classified as fibers, fragments, films, beads or foams based on shape (Cole *et al*., 2013; Claessens *et al*.,

2013; Mathalon and Hill, 2014; Hartline *et al*., 2016). Fibers originate mainly from plastic bags, fishing nets and clothing (Claessens *et al*., 2013; Hartline *et al*., 2016), while fragments often originate from plastics that do not unfurl into filamentous threads such as molten plastics or plastic films, filters, and geo textiles (Cole *et al*., 2011; Claessens *et al*., 2013).

Due to their large surface area and hydrophobic nature, microplastics absorb a lot of persistent organic particles (POPs) and because of their minute size (1µm-5mm), they can be easily swallowed by a diversity of organisms since they mimic prey particles and sediment grains (Hong *et al*., 2018). Research has shown that marine invertebrates and vertebrates ingest microplastics (Neves *et al*., 2015; Van Cauwenberghe *et al*., 2015; Devriese *et al*., 2015; Li *et al*., 2016; Nelms *et al*., 2018; Awour, 2020) with some such as crabs taking them in through the gills (Wright *et al*., 2013; Setela *et al*., 2014; Cole *et al*., 2015; Weiden and Cowie, 2016; Karlsson *et al*., 2017). Microplastics transport POPs into marine organisms, as well as plastic additives, such as Bisphenol-A and nonylphenol, which leach out into the organisms (Koelmans *et al*., 2014). Bioaccumulation and biomagnification of microplastics to higher trophic levels has also been reported (Farrell and Nelson, 2013; Setala *et al*., 2014).

Microplastics are therefore harmful to organisms along the food webs as well as the environment. For instance, styrene in polystyrene is an endocrine disrupter, while polyester contains hazardous level of monomers associated with respiratory irritation, cell mutation, and is toxic to aquatic environments (Lithner *et al*, 2011). Polyethylene and polyamides (nylon) although thought to be benign, may absorb POPs from the environment (Rochman *et al*., 2013) such as pesticides and polychlorinated biphenyls (PCB's), known to disrupt immunity and cell division (Lauby-Secretan *et al*., 2013; Hable and Nguyen, 2013). Microplastics toxins in low density polyethylene (LDPE) cause liver stress including: single cell glycogen depletion, necrosis, and fatty vacuolation (Rochman *et al*., 2013). Microplastics have been known to cause inimical physiological effects, leading to a decrease in feeding ability, energy accumulation, and reproduction for small-size organisms at lower Atrophic levels (Cole *et al*., 2013; Sussarellu *et al*., 2016). However, information on contamination of fish by microplastics is not well documented (Romeo *et al*., 2015) creating a knowledge gap, more so, no study has been done on contamination of fish by microplastics along the Kenya Coast.

Owing to the toxic effects of microplastic contamination to organisms along the food webs and the ever-increasing release of plastics into the ocean, it is important to understand the extent of the problem, to effectively mitigate it. Therefore, the main objectives of this study were to; a) assess the presence and abundance of microplastics in the gut, gills and muscles of five of the most common marine fish species from the creeks along the Kenya coast in WIO. b)characterize the microplastics by shape and colour. Considering the importance of the marine trophic web, as prey for big fish and food to humans, this study makes an important contribution to knowledge of microplastics occurrence in fishes in Kenyan inshore waters.

MATERIALS AND METHODS

1. Sampling sites

The study was carried out in two creeks in Mombasa County (Tudor, Port-Reitz) and one creek in Kilifi County (Mida) along the Kenya Coast (Fig. 1). The creeks

are enclosed and surrounded by informal settlements, highly populated villages, and manufacturing industries (Okuku *et al*., 2011, 2019; Maritim *et al*., 2016), hence may be prone to plastic pollution.

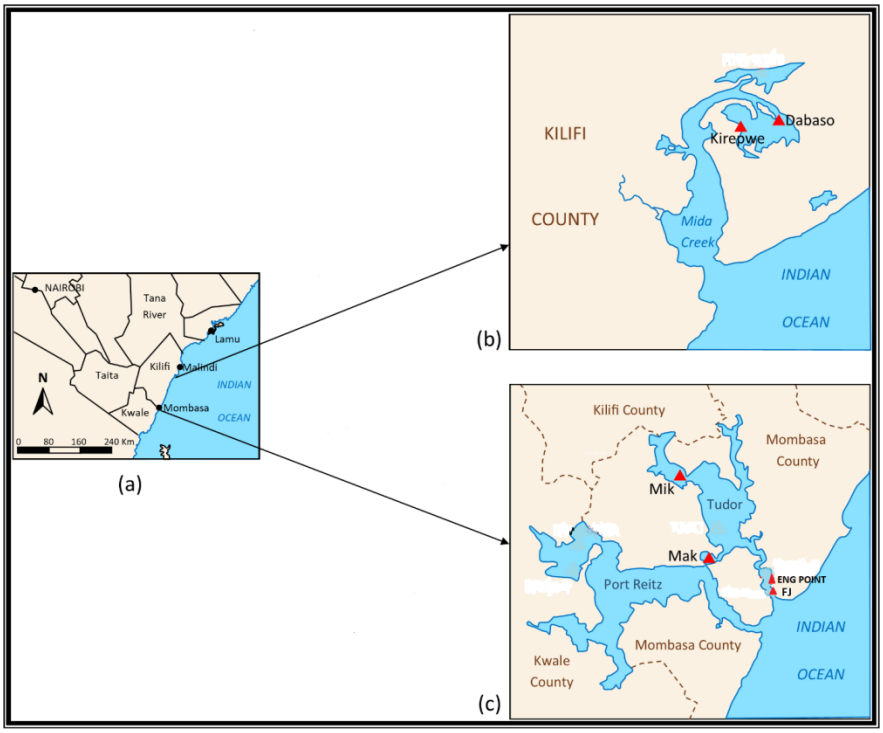

Fig. 1. Map of Kenya showing the study sites a) Kenya Coastal region; b) Mida Creek (Dabaso and Kirepwe); c) Mombasa Island with Tudor (Fort Jesus-FJ, English Point-Eng Point and Mikindani-Mik) and Port-Reitz (Makupa-Mak) Creeks.

The Kenya Coastal region experiences two rainy seasons with two maxima in May and October and an average mean annual rainfall of 1204 mm (Obiero and Onyando, 2013). The region experiences fairly high average temperatures ranging between 26 and 32°C, a small diurnal range of between 7 and 9°C (Obeiro and Onyando, 2013). Port-Reitz creek receives freshwater from rivers Mwache, Cha Shimba, and Mwambone, while Tudor creek is fed by two main seasonal rivers; Kombeni and Tsalu which arise from around Mariakani town, 32 km Northwest of Mombasa (Kitheka *et al*., 1999) (Fig. 1a). Tudor creek passes under Nyali Bridge and is bordered by Makupacauseway which dissects it into Tudor creek to the East and Port-Reitz to the West (Kitheka *et al*., 1999) (Figure 1c). In port-Reitz Creek, fish samples were analyzed only from Makupa station due to limited availability of fish. Midacreek within Watamu Marine National Park is a semi-pristine environment and was considered as a control (Fig. 1b). In Mida Creek two stations were sampled; that is, Dabaso and Kirepwe. In Tudor fish were sampled from 3 stations; Fort Jesus, English Point and Mikindani while from Port Reitz only Makupa was sampled.

2. Sampling strategy

All institutional and national guidelines for the care and use of laboratory

animals were followed. Sampling was done in January/February2018 (Jan 2018) during the dry period and in September 2018 (Sept 2018) during the short rainy season to collect fish samples for microplastic extraction and analysis. Fish samples were bought from the local fishers encountered at the sampling stations or the landing sites (Tudor, Port-Reitz and Mida Creeks) and the number and species depended on availability in the catch. At each station, GPS coordinates were recorded (Table 1) using a handheld GPS (version; Mitac mio168).

Table 1.Longitude and latitudes of Sampling stations of the Creeks along the Kenya coast, western Indian Ocean.

Site	Station	Latitude (South)	Longitude (East)
	Mikindani (Mik)	4° 41´´ 51´	39° 21´´ 12´
	English Point (Eng point)	4° 1´´34.7´	39° 38´´47.5´
Tudor	Fort Jesus (FJ)	4° 1´´29´	39° 67´´ 96´
Port-Reitz	Makupa (Mak)	4° 2´´16.5´	39° 38´' 50.1´
	Kirepwe	3° 3´´ 23.5´	39° 48´´ 47´
Mida	Dabaso	3° 20´´39.8´	39° 59.1´´2.8´

The fish were sorted according to species and placed into ziploc bags that were labeled and then placed in cooler boxes with ice for transportation to the laboratory. In the laboratory, the fish were washed with distilled water, and rinsed in 70 % ethanol to get rid of any particle affixed to the body surface. The fish samples were subdivided into three replicate groups of equal numbers based on species and location.Fish lengths (cm) and weights (g) were measured to the nearest 0.1 mm and 0.1g, respectively (Karami *et al*., 2017). The samples were wrapped in aluminum foil to avoid external contamination, placed in ziplocs and stored at -40°C until further analyses.

3. Processing and analysis of fish samples

Sample processing and analysis were done at the Kenya Marine and Fisheries Research Institute (KMFRI) and the University of Nairobi (UON) Laboratories. The fins were chopped off and discarded. The fish were dissected by making a cut just below the throat and extending the cut down the ventral side to the anal pore (Gupta and Mullins, 2010). The gut, gills and the rest of the fish were separated into different samples, weighed, chopped into smaller pieces and digested using 10 % KOH (1g: 5 ml) (Foekema *et al*, 2013; Eriksen *et al.*, 2013; Rochman *et al*., 2015; Dehaut *et al*., 2016; Kuhn *et al*., 2017; Thiele *et al*., 2019) at 60°C for 14 hours (modified protocol). Fins and bones did not digest completely and some organic matter was evident on samples of *Rastrelliger kanagurta* and *Leptoscarus vaigiensis*. Such samples were digested in 55 % Nitric acid (HNO_3) solution (10mL/g) for a further five minutes to remove any organic material (Collard *et al*., 2015). Acid digestion was done in a fume cupboard, in glass jars covered with watch glasses. The digestates were diluted by adding 100 mL of distilled water to protect the filtration equipment and ease floatation (Collard *et al*., 2015). The microplastic particles were density separated by adding filtered supersaturated Sodium Chloride (NaCl) solution (1.35g cm^{-3}), in the ratio of 1: 3 (sample: salt solution), and left to settle overnight (12 hours) (Rochman *et al*., 2015; Kuhn *et al*., 2017 modified protocol). The supernatant was filtered by vacuum pump filtration over 0.8 µm membrane filters. The filters with particles

were placed in covered glass petri-dishes, and then dried at 40°C for 12 hours before being examined under a dissecting microscope at X40 magnification (Claessens *et al*, 2013; Lusher *et al*., 2013). Suspected microplastics were confirmed using the hot needle test (Witte *et al*, 2014; Devries *et al*, 2015), and further characterization done. The microplastics were characterized by shape and categorized as fiber, fragment or film and their color noted.

4. Quality control

Owing to the very light weight and mobility of Microplastics (MPs), caution was observed while analyzing samples toguarantee no contamination of samples by particles from the air. Sample processing and analyses were done in a clean room with no air flow (windows and doors shut) and limited human traffic. Samples were covered with aluminum foil and glass covers whenever not in use, while glassware and metal equipment were used. Equipments were rinsed with deionized water prior to use. Working surfaces were thoroughly cleaned using 70 % ethanol three times and allowed to dry before use (Hidalgo-Ruz*et al.*, 2012). Hand gloves were used and cotton laboratory coats were worn throughout. Long term blanks were measured (1 blank per sample analysis). A moistened filter paper (30mm diameter, Whatman No. 1) (Lusher *et al.*, 2017) per sample was placed in a petri dish and left exposed during the processing and analysis period. A series of blanks set during the analyses process were examined for contamination.

5. Data analysis

Shapiro-Wilk's test was used to test data normality and all data was found to be normally distributed after log transformation. Species abundance and the mean concentrations of microplastics in species during the different seasons were compared using one- way ANOVA and the Turkey's post hoc test separated the means. One- way ANOVA was also used to compare the concentration of microplastics and the weight of tissues followed by a Turkey's test. A spearman's correlation was done to determine the relationship between the mean microplastic concentration and mean lengths and weights of the organisms. Species pairwise comparisons were done using Turkey's test. Fish data from the two seasons was combined for von Bertalanffy growth curves.

The assessment of MPs in the guts, gills and muscles was done in accordance with procedure and ethical guidelines for animal experiments in the University of Nairobi and KMFRI

RESULTS

1 Fish distribution and size

A total of 225 specimens from five different species were obtained, most of which are benthic (Nelson, 1994: Sheaves, 2006: Fischer *et al*., 1990: Lieske and Myers, 2004; Froese and Pauly, 2020), and included *Geres oyena* (Forsskal, 1775*), Acanthopagrus berda* (Forsskal, 1775) and *jarbua* (Forsskal, 1775). *Gerres oyena* lives in coastal waters and is a carnivore (Cyrus and Blaber, 1982), *Acanthopagrus berda* (Forsskal, 1775) is predominantly marine (Nelson, 1994: Sheaves, 2006) with some living in euryhaline estuarine environments (Leu and Chou, 1996), and is an omnivore (Nasir, 2000: Shelta *et al*., 2018), *Leptoscarus vaigiensis* (Quay & Galmard, 1824) is reef associated grazing fish (Locham *et al*., 2015) while, *Rastrellige rkanagurta* (Cuvier 1816) is pelagic and omnivore (Collette, 2001).

During the first sampling all five species were while in the second sampling only four species were encountered (due to absence of *L. vaigiensis* that had been

encountered at English point and was not encountered again) and all species were represented by fewer individuals. Mida and Tudor Creeks had a higher variety of fish species compared to Port-Reitz. In Mida this was mainly because of the high diversity encountered in Dabaso station while in Tudor all three stations sampled had different species. *Gerres oyena* was the most predominant species (91 individuals representing 44 %) and occurred in all stations except Fort Jesus and English Point (Table 2). Data on species diversity from the two sampling campaigns did not vary significantly (ANOVA: F = 0.77, df =1, P = 0.790). Although *G. oyena* was dominant, it did not occur in Port-Reitz Creek during the second sampling campaign.

The average (± SE) weights and lengths of the different fish species showed wide ranges (Table 3) with the greatest mean weight range being observed in *G. oyena* perhaps because of its occurrence in different stations and seasons. The heaviest *G. oyena* individuals were encountered in Makupa followed by those from Dabaso while Mikindani had the smallest individuals. *L. vaigiensis* individuals were as heavy as the heaviest *G. oyena* individuals from Makupa while *A. berda* had the smallest individuals.

Table 2. Occurrence of different fish species in different sites and stationsof the Creeks along the Kenya coast, western Indian Ocean.

Site	Station	Species	Jan 2018	Sept 2018
Mida	Dabaso	*G. Oyena*	-	16
		A. Berda	31	9
		T. Jarbua	37	6
	Kirepwe	*G. Oyena*	15	-
Port-Reitz	Makupa	*G. Oyena*	11	-
Tudor	Mikindani	*G. Oyena*	31	18
	Fort Jesus	*R. Kanagurta*	35	6
	English Point	*L. Vaigiensis*	10	-

Table 3. Mean ((± SE) lengths and weights of fish of different species per site and station of the Creeks along the Kenya coast, western Indian Ocean.

			Jan 2018		Sept 2018	
site	Station	Species	Av. Bw (g)	Av.TL (cm)	Av. Bw (g)	Av. TL (cm)
	Dabaso	*G. oyena*	-	-	79.9±0.1	15.6±0.02
	Dabaso	*A. berda*	11.9±0.1	9.7±0.03	74.3±0.01	9.8±0.01
Mida	Dabaso	*T. jarbua*	16.3±0.17	10.8±0.01	74.8±0.1	10.2±0.01
	Kirepwe	*G. oyena*	50.4±0.04	14.9±0.1	-	-
Port-Reitz	Makupa	*G. oyena*	143.8±1.4	28.2±0.06	-	-
	Mikindani	*G. oyena*	35.6±0.7	12.8±0.2	28.6±0.01	19.4±0.03
	Fort Jesus	*R. kanagurta*	118.6±0.15	22.3±0.08	32.5±0.06	10.3±0.1
Tudor	English Point	*L. vaigiensis*	143.1±0.74	20±0.04	-	-

2.Length-weight relationship and Fish growth

The estimated a and b constants (Table 4) for *G. oyena* varied widely from those obtained by Kanak Tachihara (2006) who calculated the relationship as $W= 0.0035L^{2.89}$ for fish from Okinawa Island

Japan, El Agamy (1988) $W= 0.00812L^{3.13}$ for fish from the Arabian Gulf, and Letourneur *et al*., (1998) $W= 0.012L^{3.232}$ for fish from New Caledonia. The mean b-value was low as all fish species except *A. berda* had negative allometry, hence did not obey the cubic law (Wootton, 2012). The growth of weight relative to length for *A. berda* was positively allometric showing that weight of fish increases lightly more than the cube of its length. The length-weight relationship for different investigated fish species was shown in Figure (2).

Table 4. Parameters of the Length-weight relationship for different fish species Creeks along the Kenya coast, western Indian Ocean.

Species	Station	a-value	b-value	R^2	n	Non-linear equation
G. oyena	Dabaso	3.1266	0.3675	0.4962	17	$W= 3.1266L^{0.3675}$
G. oyena	Kirepwe	4.2102	0.3275	0.8813	16	$W= 4.202L^{0.3275}$
G. oyena	Makupa	6.3678	0.3042	0.9796	12	$W=6.3678L^{0.3042}$
G. oyena	Mikindani	11.631	0.0964	0.0127	46	$W= 11.631L^{0.0964}$
R. kanagurta	Fort Jesus	1.5434	1.3915	0.393	41	$W= 1.5434L^{1.3915}$
A. berda	Dabaso	0.0107	3.054	0.9143	40	$W= 0.0107L^{3.054}$
T. jarbua	Dabaso	0.1226	2.0467	0.4172	43	$W= 0.1226L^{2.0467}$
L. vaigiensis	English Point	0.0273	2.8453	0.9137	10	$W= 0.0273L^{2.8453}$

Y-intercept (a-value), slope of the curve (b-value) and the coefficient of determination (R^2)

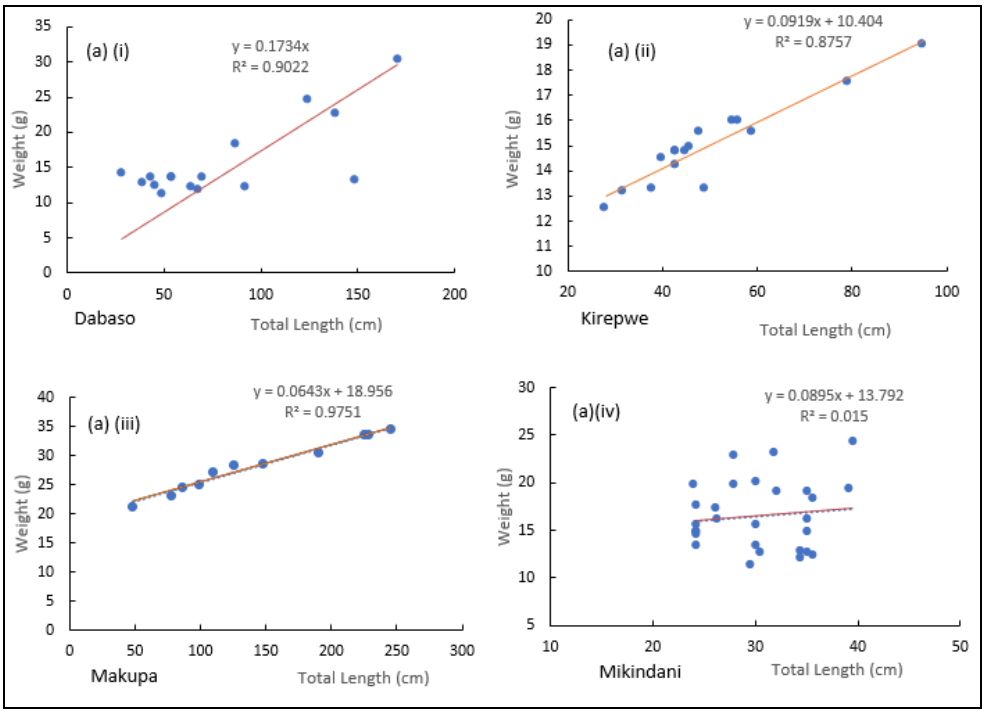

Fig. 2. The length-weight relationship for a) *Gerres oyena*,

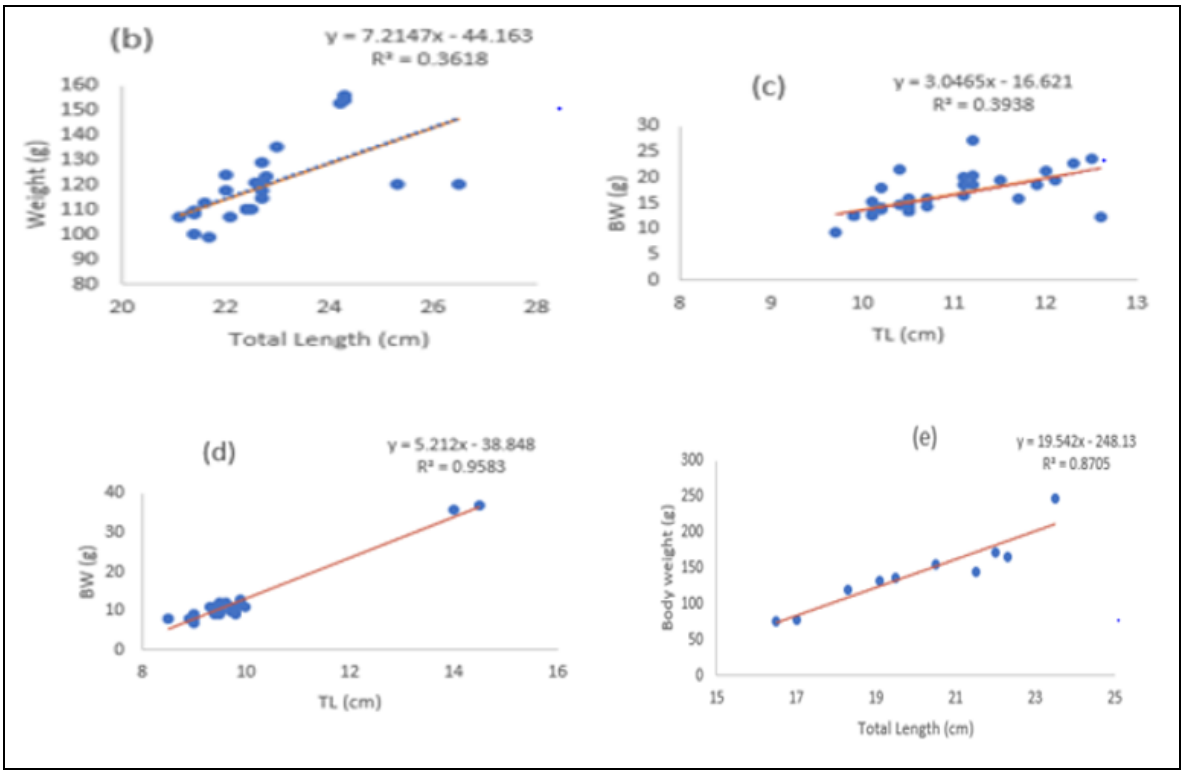

Fig. 2 (Cont.). The length-weight relationship b) *Rastrelliger kanagurta*, c)*Terapon jarbua*, d) *Acanthopagrus berda*, e) *Leptoscarusvaigiensis* from the creeks along the Kenya coast.

3. Overall mean concentration (g^{-1}) of microplastics in different fish species

The overall (±SE) mean microplastics concentration in different species was below 1 microplastic per gram of tissue (Table 5) and was significantly different *for R. kanagurta, T. jarbua*and *A. berda* ($p < 0.05$) between the first and second sampling periods.

Overall, the mean concentration of microplastics showed significant difference ($F = 12.69$, $df = 11$, $p < 0.01$) among species. *Acanthopagrus berda* from Mida had the highest mean concentration, while *G. oyena* from Mida and *L. vaigiensis* from, Tudor had the lowest mean concentration of microplastics per gram tissue.

Table 5. The concentration of MPs (±) in different fish species from different creeks and stations along the Kenya coast.

Site	Station	Species	Jan 2018	Sept 2018	Mean conc
Mida	Dabaso	*G. oyena*	-	0.18±0.041	
		A. berda	0.16±0.003	0.52±0.01	0.480 ± 0.058
		T. jarbua	0.15±0.008	0.31±0.01	0.240± 0.04
	Kirepwe	*G. oyena*	0.041±0.032	-	
Port-Reitz	Makupa	*G. oyena*	0.1±0.034	-	
Tudor	Mikindani	*G. oyena*	0.2±0.02	0.21±0.011	0.209 ± 0.051
	Fort Jesus	*R. kanagurta*	0.07±0.01	0.16±0.004	0.132 ± 0.011
	English Point	*L. vaigiensis*	0.04±0.001	-	

Apairwise comparison in MPs concentration between different stations but the same sampling period, and same species but different sampling seasons produced varied results (Table 6). Significant differences ($p < 0.05$) were observed in the concentration of MPs in *G. oyena* between different stations during the same sampling season (Table 6).There was no significant difference in the concentration of MPs in *G. oyena* from Mikindani during the different sampling seasons ($t = 0.45$, $df = 2$, $p = 0.07$). On the other hand, significant differences ($p < 0.05$) were observed in the concentration of MPs in all the other fish species between same species and different sampling seasons.

Table 6. Pairwise comparison of mean microplastic concentration (X± SE) in fish

Stations and seasons	Mean	Std. Err Mean	t- value	df	P
Same species (*Gerresoyena*)					
Jan - Sept Mik (same stn; diff sampling)	0.014	0.031	0.45	2	0.70
Mik - Mak Jan (diff stn; same sampling)	0.169	0.020	8.51	2	0.01
Mik - Kir Jan (diff stns; same sampling)	0.159	0.029	5.55	2	0.03
Mik - Dab Sept (diff stn; same sampling)	0.033	0.045	0.72	2	0.54
Kir Jan - Dab Sept (diff stns; diff sampling)	0.141	0.056	2.50	2	0.13
Other species					
R. kanagurta Jan -Sept (FJ) (diff sampling)	0.408	0.153	7.62	2	0.012
A. berda Jan – Sept (Dab) (diff sampling)	0.177	0.060	6.93	2	0.039
T.jarbua Jan – Sept (Dab) (diff sampling)	0.036	.012	5.98	2	0.040

4-Concentration of microplastics in different fish species per gram tissue

Microplastics were observed in all investigated fish samples (Fig.3) with the benthic fish like *A. berda* (0.480 ± 0.058) *G. oyena* and *T. jarbua* (0.240 ± 0.04) each having higher mean concentration compared to the pelagic,*R. kanagurta* (0.132 ± 0.011) and reef fish *L. vaigiensis* (0.04 ± 0.001),(Fig. 3) and the differences were significant ($Chisq_4 = 5504$, $p = < 0.01$).

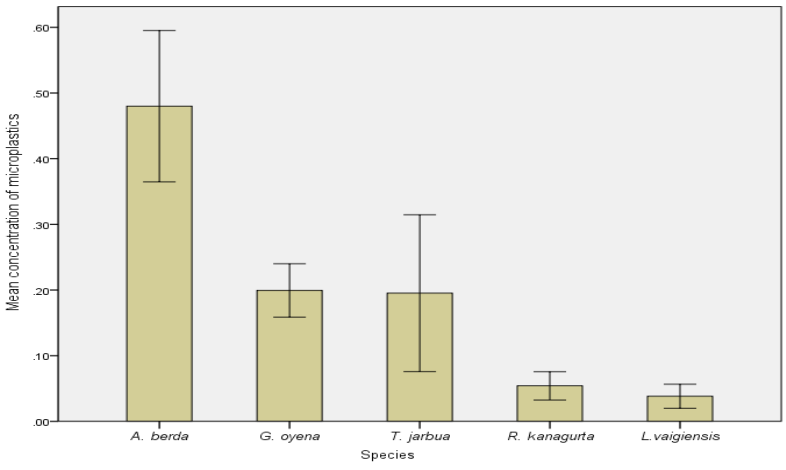

Fig. 3. Mean (± SE) microplastics concentrations for the different fish species with standard error bars (MPs per gram).

A correlation between MPs concentration and body length and weight showed that all fish species except *R. kanarguta* had increased MPs concentration with increase in body length (Fig. 4 a), while *G. oyena* and *L. vaigiensis* showed a decrease in MPs concentration with increase in body weight (Fig. 4 b).

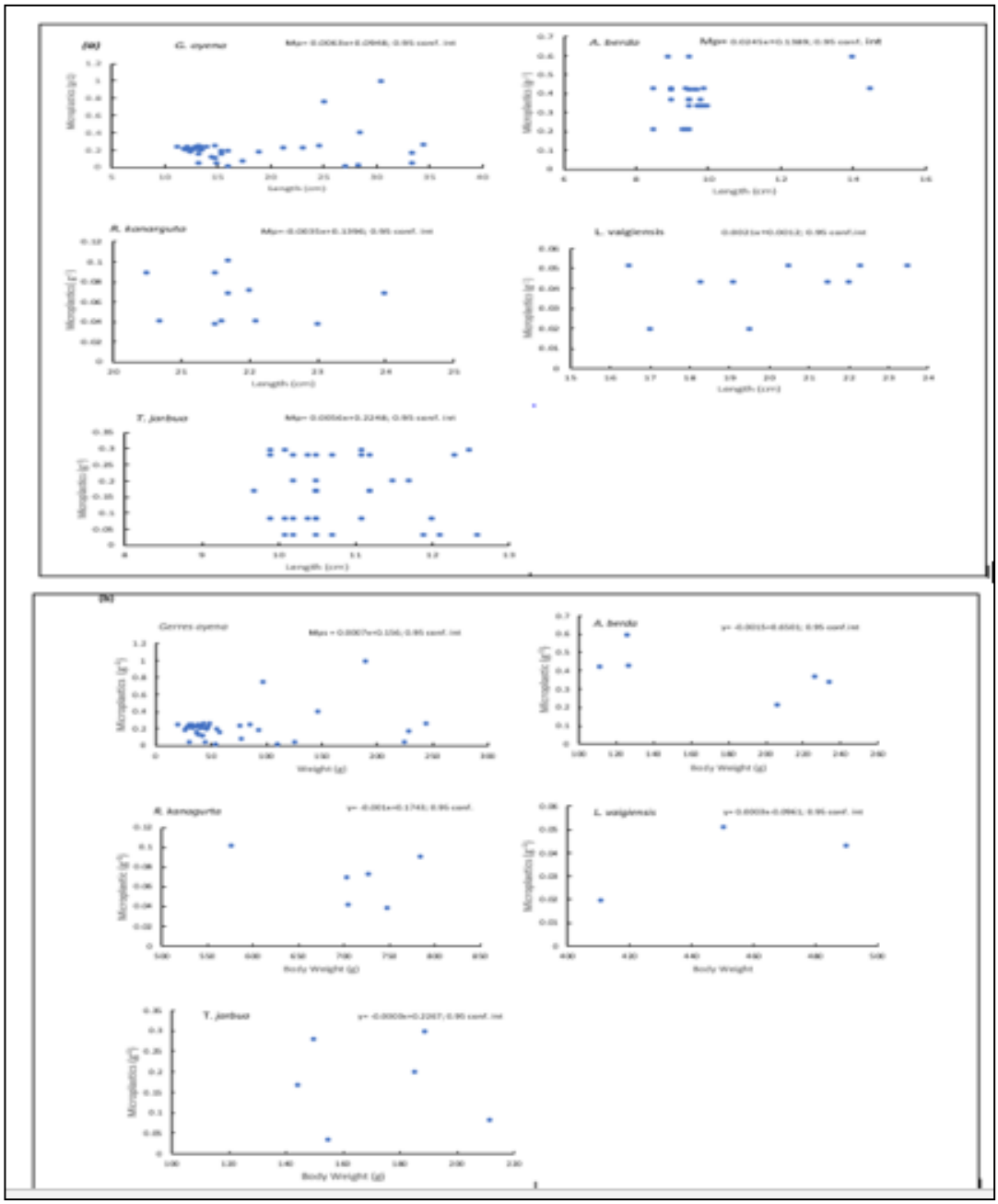

Fig. 4. Relationship between (a) MPs concentration and body length and (b) MPs concentration and body weight in the different species.

5 Mean (± SE) concentrations of microplastics in different organ tissues in different species

All the fish species contained microplastics in their guts, gills and body tissues (Table 7). There were significant differences (F = 22.725, df = 20, p = 0.002) in the mean microplastic concentration in the guts between species. Guts of *A. berda* (7.41±0.42) had significantly higher mean MPs concentration, compared to *G. oyena, R. Kanxagurta, T. jarbua* and *L. vaigiensis* but the latter three were not significantly different (F = 1.549, df = 20, P = 0.211) (Table 7. With respect to the gills, *A. berda*and *G. oyena* had significantly higher mean concentrations of MPs per gram tissue compared to other species but the two were not significantly different (p > 0.05). Generally, the mean concentrations of MPs in the rest of the fish body were lower than 0.1 MPs g^{-1} across all species and stations.

Based on station, the guts of *G. oyena* from Dabaso had a significantly higher (F = 12.692, df = 7, p < 0.05) mean concentration (g^{-1}) of microplastics compared to those from Kirepwe, Mikindani and Makupa but the latter three were not significantly different (p > 0.05). Similarly, the gills of *G. oyena* from Dabaso had a significantly higher mean concentration of MPs (g^{-1}) (F =13.142, df = 7, p = 0.001) compared to other stations (Table 7)**.**

Table 7 Mean (x± SE) concentration of microplastics in various organs per gram tissue of the different fish species

Site	Station	Species	MPs g^{-1}	Microplastics in organs g^{-1}		
				Guts	Gills	Body
Mida	**Dabaso**	***G. oyena***	0.181±0.041	*3.557±0.15	*2.599±o.23	0.042±0.001
Mida	**Kirepwe**	***G. oyena***	0.041±0.032	1.398±0.05	1.339±0.07	0.004±0.001
Tudor	**Mikindani**	***G. oyena***	0.209±0.051	1.43±0.02	0.172±0.05	0.015±0.001
Port-Reitz	**Makupa**	***G. oyena***	0.1±0.034	0.94±0.01	1.92±0.12	0.02±0.0
Tudor	**Fort Jesus**	***R. Kanagurta***	0.132±0.011	1.44±0.03	0.74±0.01	0.01±0.01
Tudor	**English Point**	***L. vaigiensis***	0.04±0.001	0.56±0.1	0.45±0.1	0.01±0.01
Mida	**Dabaso**	***A. berda***	0.48±0.058	*7.41±0.42	*2.82±0.08	0.081±0.01
Mida	**Dabaso**	***T. jarbua***	0.240±0.04	1.38±0.02	1.97±0.025	0.031±0.01

* indicates high concentration of microplastics

6- Microplastic types by shape and colour in the tissues of different fish species

Most of the MPs recovered from the fish were fibers (91.4 %) and a small percentage (8.6 %) were fragments (Fig. 4). Similarly, significantly higher (F=22.721, df = 20, P< 0.001) proportions of fibers were observed in fish gills, compared to fibers in guts, and in fish body (Fig 5). In addition, clear balls of fibers were observed in the guts of some fish of *R. kanagurta* and *G. oyena* species (Fig. 6).

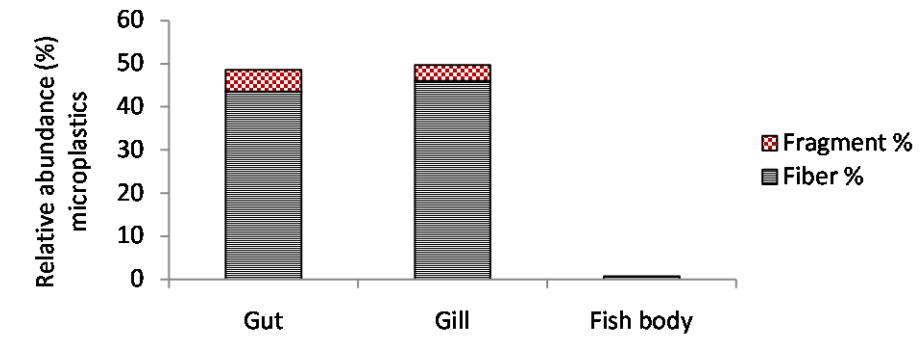

Fig. 5. Mean percentage concentration of microplastic shapes observed in organs of different fish species from the creeks along the Kenya coast.

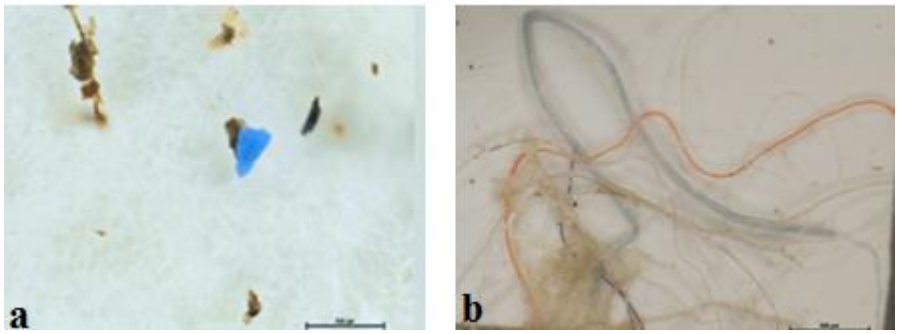

Fig. 6.Examples of microplastic types by shape recovered from fish tissues: (a) Blue fragment from the gut of *R. kanagurta* of Fort Jesus (b) Red, blue and black tangled fibers from the gills of *G. oyena* of Makupa

The majority of the microplastics were blue (36.4%) and black (34.2%) followed by white (18.4%), green (6.5%), red 3.3%), and purple (0.9%) (Fig.7).

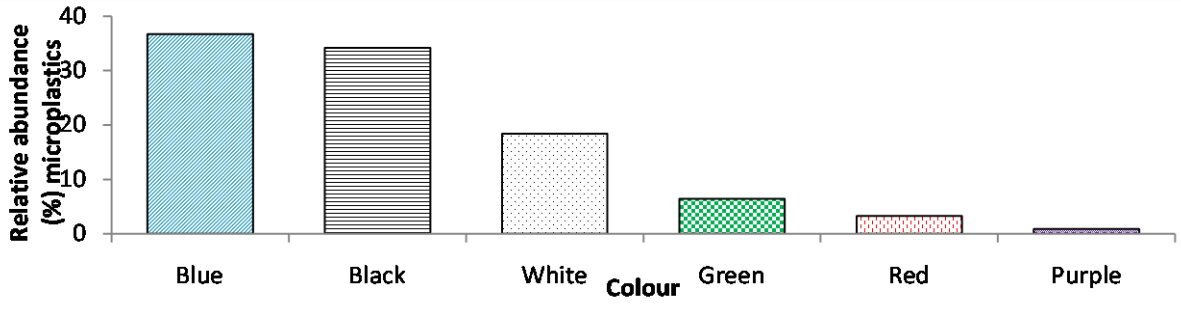

Fig. 7. Mean percentage concentration of microplastic colours observed in different fish species from the creeks along the Kenya coast

DISCUSSION

Kosore *et al*. (2018), Awour *et al*., (2020) and Kerubo *et al*. (2020; 2021) distinctly showed that microplastics were abundant in the Kenya's marine environments and are interacting with zooplankton and macro-invertebrates by way of ingestion. Fish are economically important as human food (Barboza, *et al*., 2018). Some of the risks associated with marine fish are the incorporation of microplastics and adsorbed chemicals into the food web through trophic transfer (Setälä *et al*., 2018). Ingestion of microplastics increases toxicity of plastic chemicals such as nonylphenols, bisphenol A and antioxidants in the organisms through leaching (Hermabessiere *et al.*, 2017). It is therefore imperative to assess the interaction of microplastics in marine fish, as a potential risk to humans. The present study focused on the sites around Mombasa due to the rapid increase in human population and high solid wastes from tourism and industrial sectors (Okuku, 2019). Microplastics are present everywhere including Dabaso within Watamu Marine National Park, a protected area expected to be free from microplastic contamination (Kerubo *et al*, 2020; 2021).

Growth in fish is isometric if body weight increases with increase in total body length (b = 3), positively allometric if the b-value is greater than 3 and negatively allometric if the b-value is far less than 3 (Ricker, 1975; Wootton, 2012). Based on Fish Base data, different fish species attain maturity at different body lengths;*G. oyena* attains maturity at an average total body length of 22 cm (Roux, 1986), *T. jarbua* 13 cm (Lieske and Myers, 1994), *A. berda* 20-22 cm (Smith and Smith, 1986), *R. kanagurta* 19.9 cm (Sommer *et al*., 1996) and *L. vaigiensis* at 16.5-18.4 cm total body length for fish in parks and reserves and attains a maximum body length of 35 cm (Randall, 1986). In this study, the collected samples of *G. oyena*, from Makupa could be considered mature, while the rest of the fish sampled were immature. The length-weight relationship gave a good fit to the length and weight of *A. berda* (James *et al*., 2003; Ontomwa *et al*., 2018), while data for the length-weight relationship for other fish species did not. The length-weight relationships for *A. berda*, indicates isometric allometry, an indication that the species had homogenous groups in their populations with body weights varying independently with the cube of the total length. The high coefficient of determination implied proportional increase in weight and length. These results affirm earlier research on *A. berda* from the North Coast of Kenya, ($W=0.0191L^{2.988}$) with a coefficient determination ($R^2 = 0.9676$) (Anam *et al*., 2019) and from Shimoni artisanal fishery, Kenya (Ontomwa *et al*., 2018). The length-weight relationship of *A. berda* could have influenced microplastic ingestion during feeding. Isometric growth could be attributed to the phenotype of the species, condition of the fish, the environment and food availability (Ontomwa *et al*., 2018; Anam *et al*., 2019).

The length-weight relationship for *G. oyena* and *L. vaingiesis* imply positive correlation and negative allometric growth, while the length-weight relationships for *R. kanagurta*, and *T. jarbua* indicate negative correlation and negative allometric growth patterns. Negative allometry indicates that the species had heterogenous groups with body weights varying differently with the cube of total length. Such growth could be attributed to feeding and spawning biological aspects which have much impact on the length- weight relationships. These results contrast previous research results for *G. oyena* from the Gulf of Suez, $W= 0.094L^{3.11}$ (Saber *et al*., 2020), and from

Caledonia (W= 0.0120 $L^{3.232}$) (Letourneur *et al*., 1998) but agree with results for *G. oyena* from Okinawa Island Southern Japan, W= $0.035L^{2.89}$ (Kanak and Tachihira, 2006), *T. jarbua* from Mindano, Philippines, W= $0.0006L^{2.8484}$(Fortaleza *et al*., 2019) and *L. vaigiensis* from Shimoni artisanal Fishery, Kenya, W = $0.0000129L^{2.3}$ (Ontomwa *et al*., 2018), showing that length of fish increased more than weight. The results also contrast results of *R. kanagurta* from Mangalore India, W= $0.0045L^{3.2234}$ (Hulkot *et al*., 2013), which indicate positive correlation and allometry. The negative allometric growth could be attributed to several factors including insufficient feeding, age, sex, health condition of the fish, poor food quality and availability, low salinities and poor habitat conditions (Sarre and Potter, 2000; Froese, 2006).

This study established that fish within the Creeks along the Kenya coast are contaminated with microplastics, including those from Mida Creek which was expected to be free of microplastic contamination. Significant variations in microplastic concentrations among species could be explained by differences in habitats and feeding behaviour that affect ingestion of microplastics. For example, *A. berda* is demersal and feeds on benthic invertebrates mainly barnacles, crabs and oysters, (Fischer *et al*., 1990) known to ingest and accumulate microplastics (Neves *et al*., 2015; Li *et al*., 2016; Nelms *et al*., 2018; Awour, 2020) hence the high microplastics in the species. *Geres oyena* was the most abundant and widespread species in the investigated sites. It is demersal species that inhabiting inshore areas and feeds on small organisms and benthic invertebrates living in sandy bottoms (Lieske and Myers, 2004; Froese and Pauly, 2020),while *T. jarbua* is demersal feeding mainly on white (*Paneaus indica*) and brown (*Paneaus monoceros*) shrimps and on small fishes which may accumulate MPs and pass them on to a higher trophic predator. *Rastrelliger kanagurta* is pelagic and omnivore, feeding on algal materials and small invertebrates (Collette, 2001), while *L. vaigiensis*, is pelagic, reef associated inhabiting seagrass areas and is herbivorous feeding on sea grasses and algae (Sommer, 1996; Locham *et al*., 2015; Froese and Pauly, 2017). MPs are likely to arrive into the coastal environment through rivers and may have high concentration in the surface waters (Kerubo *et al*., 2020), yet pelagic fish tend to accumulate much less compared to the demersal fish.

Small invertebrates accumulate microplastics passing them up trophic levels, thereby increasing microplastics in higher trophic levels (GESAMP, 2016) as was the case with *A.berda*and *G. oyena*. The high contamination with MPs of *A. berda* from Dabaso both in the gut and gills could not be explained as it was not the site with the highest MPs concentration in the surface water and the sediments (Kerubo*et al*, 2020; 2021) although Awuor *et al*, (2020) found that MPs concentrations in the invertebrates in Dabaso were comparable to other sites along the Kenya Coast. On the contrary, Mikindani had recorded relatively high MPs in the sediments (Kerubo *et al*., 2021) and *G. oyena* population from that site had not accumulated as high MPs in the gut and gills as was observed in the population from Dabaso. This suggests that several factors playing together influence MPs contamination in fish and not just the level of contamination of the environment.

Leptoscarus vaigiensis from English Point had the lowest microplastic concentrations in the gut and gills which could be as a result of the fish not spending much time in the creeks being reef associated species and only occasionally venturing into the creeks (Locham *et al*, 2015). This could imply that the reefs are

less contaminated with MPs compared to the creeks. The current results are consistent with earlier research where similar concentrations have been observed in the digestive tracts of the Mediterranean lantern fishes which are both shallow and deper-living but pelagic feeders, mainly feeding on mesopelagic fish (Romeo *et al*., 2016)and the South African catfish which is omnivore with carnivorous tendency (Silva-Canti *et al*., 2017), but were lower than those obtained in the gastrointestinal tracts of fish from other regions of the world, such as crevalle jack (*Caranix hippos*, Linnaeus, 1766) (Froese and Pauly, 2017). The relatively high microplastic concentrations in both juvenile and mature fish samples indicates that size or age does not influence microplastic ingestion in fish.

Microplastic concentrations were significantly lower in fish body tissues but significantly higher in the guts and gills of all the fish species. The low microplastic concentration in the fish body could be attributed to the total weight due to inclusion of bones and fins. The high concentration of microplastics in the guts and gills of *A. berda* and *G. oyena* from Dabaso could imply transfer of the contaminants to humans in high concentrations if the fish is consumed whole. For example, in this study, consuming 1 kg whole *A. berda* from Dabaso could transfer as much as 1031 ± 0.42 microplastics, while the same quantity of gutted fish with gills discarded would transfer about 81 ± 0.02 MPs particles only.

Microplastics were mainly fibers agreeing with earlier research by Nelms *et al*. (2018), reporting similar results in the Atlantic Mackerel with fibers (72 %) being higher than fragments (Nelms, 2018), as well as in the digestive tracts of the South African catfish (Silva-Canti*et al*., 2017). Fibers made up 88 % of the microplastics in five fish species in China (Jabeen *et al*. 2017), 96% of microplastics in fish from Texas (Fazey and Ryan, 2016) and were predominant in the stomachs of Mediterranean lantern fishes (Romeo *et al*., 2016) among others. The occurrence of clear balls of fibers in guts of some fish is consistent with earlier research reports of bunched balls of microplastics in the digestive tract of the *Lates niloticus* (Linnaeus, 1758) and the *Oreochromis niloticus* (Linnaeus, 1758) in Lake Victoria (Biginagwa *et al*, 2016).

The high percentage of fibers suggests waste water treatment, domestic waste water, fishing ropes and nets, degraded plastic bags, synthetic textiles and tourism activities could be the main sources of microplastics (Khan *et al*., 2018; Graca *et al*., 2017). The presence of film fragments suggests light weight plastics could be the source. It is worthwhile to note that the source of microplastics directly influences their concentration in water bodies and subsequently fishes (Free *et al*., 2014).

Most of the microplastic particles from fish in this study were blue and black. Earlier research reported red, blue, and white elongated fibers in the Gulf of Mexico with no proportions (Philips, 2015). Variations in microplastic particle colours implied multiple sources of the pollutants. Further investigation is required to establish the source of microplastics in the demersal and pelagic fishes of the creeks along the Kenya Coast and the subsequent impact on human health.

Conclusions

The study established that both demersal and pelagic fish ingest microplastics and body size or age did not influence their ingestion. Growth of the fish species in the study deviated from the norm in literature for the same species which could probably suggest that fish are affected by microplastics in the aquatic environment. Although all the fish species had

microplastics in their guts, gills and the rest of the body, fish body had significantly lower values than the gut and gills. But the fact that microplastics are found in the fish body tissue is alarming as it demonstrates gut tissue or gill tissue transfer of microplastics.

In retrospect, this study shows that common fish in the creeks along the Kenya coast ingesting microplastics could pose a risk to humans especially if they are consumed whole.

Microplastics of different shapes and colours were ingested most of which blue were indicating multiple sources of these pollutants. High microplastic concentrations in demersal and pelagic fish indicate that microplastics in the creeks along the Kenya coast accumulate in sediments and the water column and differences in feeding modes influence ingestion.

This study has implications for fishery and wildlife management. Understanding of the results could benefit the National and International Governments, environmental advocacy groups such as NEMA, and Intergovernmental organizations. Moreover, this study supports the February 2017 ban on production, and use of light weight plastics by the Kenya government as there were both fiber and film particles found in the study.

Recommendation

It is more safely to remove gut and gills from all fish regardless of their size before being processed or cooked for human consumption.Research is needed to determine the source of microplastics. It is necessary to compel more effective actions and mitigations for plastic waste management to reduce the microplastic numbers in the oceans.

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Microplastics contamination of fish from the Creeks along the Kenya coast, western Indian Ocean (WIO)

تلوث الأسماك باللدائن الدقيقة من الجداول على طول ساحل كينيا ، غرب المحيط الهندي(WIO)

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المستخلص

تشكل اللدائن الدقيقة (MPs) تهديدًا عالميًا للحياة البحرية ، لكن المعلومات المتاحة عن مدى التلوث على طول ساحل المحيط الهندي في كينيا غائبة. تناولت الدراسة خمسة أسماك بحرية شائعة من المنطقة . تم هضم أنسجة الأسماك في 10٪ هيدروكسيد البوتاسيوم (KOH) عند 60 درجة مئوية لمدة 14 ساعة وتم فصل جزيئات البلاستك الأنسجة من الاسماك بطريقة التعويم باستخدام محلول كلوريد الصوديوم مفرط التشبع .(NaCl) وكان متوسط تواجدها لكل جرام من الاسماك القاعية مساوى 0.48 جرام جزيئات بلاستك/جرام اسماك للنوع *Acanthopagrus berda* ، 0.2 جرام جزيئات بلاستك/جرام اسماك فى كل من *Terapon jarbua* ، *Gerres oyena* وكان لديها تلوث أعلى من جزيئات البلاستك مقارنةً بالاسماك السطحية (*Rastrelliger kanagurta*) 0.011 جرام جزيئات بلاستك/جرام اسماك وأسماك الشعاب المرجانية (*Leptoscarus vaigiensis*) جرام جزيئات بلاستك/جرام اسماك 0.038 مما يعكس تأثير العادات والموئل على تلوث الاسماك بهذه الجزيئات من البلاستك. كان للأسماك التي تم اصطيادها من Mida Creek في العينة الثانية تلوثًا أعلى بجزيئات البلاستك مقارنة بالأسماك الأولى بتلك الموجودة في تيودور و كذلك الموجودة فى بورت ريتز ربما لأنها كانت في الأساس أسماك قاعية. تحتوي الأمعاء والخياشيم من جميع الأنواع على تركيزات أعلى بكثير من اللدائن الدقيقة مقارنة باللحم. وبالتالي ، نوصي بإخراج الأحشاء وتنظيف الخياشيم جيدًا قبل الطهي. هناك حاجة إلى مزيد من البحث لتقييم المخاطر التي يتعرض لها الإنسان نتيجة استهلاك أسماك المياه البحرية التي يتناولها.