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Microplastics in Commercially Important Small Pelagic Fish Species From South Africa

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This study documented the levels of microplastics in three commercially important small pelagic fish species in South African waters, namely European anchovy (*Engraulis encrasicolus*), West Coast round herring (*Etrumeus whiteheadi*) and South African sardine (*Sardinops sagax*). Data suggested variation between species with a higher concentration of microplastics for *S. sagax* (mean of 1.58 items individual^{-1}) compared to *Et. whiteheadi* (1.38 items individual^{-1}) and *En. encrasicolus* (1.13 items individual^{-1}). The occurrence of microplastics was also higher for *S. sagax* (72%) and *Et. whiteheadi* (72%) compared to *En. encrasicolus* (57%). Microfibers accounted for 80% of ingested microplastics (the remainder were plastic fragments) with the main ingested polymers being poly(ethylene:propylene:diene) (33% occurrence), polyethylene (20%), polyamide (20%), polyester (20%), and polypropylene (7%). The abundance of ingested items was not significantly correlated with fish caudal length or body weight, and spatial investigation indicated an increase in the abundance of ingested items from the West to the South coast. *Etrumeus whiteheadi* is proposed as a bio-indicator for microplastics for South Africa.

Keywords: commercial small pelagic fish species, *Engraulis encrasicolus* (European anchovy), *Etrumeus whiteheadi* (West Coast round herring), *Sardinops sagax* (South African sardine), marine litter, microplastics

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INTRODUCTION

Plastics are valuable resources with numerous societal benefits. Global plastics production was almost 360 million tonnes in 2018, of which 7% was attributed to the Middle East and Africa (PlasticsEurope, 2019). In South Africa in 2015, most plastic consumption was attributed to product packaging (53%), followed by building and construction (13%), and agriculture (9%) (Plastics SA, 2019).

Reduction of marine plastic litter through plastic waste management is the current focus of international efforts (Babayemi et al., 2019). Impacts of marine plastic litter are varied and include ingestion by biota (macro, meso, and microplastics including microfibers) or entanglement/collision [e.g., ghost fishing caused by Abandoned Lost or otherwise Discarded Fishing Gear (ALDFG)]. Plastics can also act as substrate for a wide variety of species increasing their potential for long-range transport (GESAMP, 2020). Microplastics are widespread in the

environment with some known environmental and ecological impacts. Field and laboratory studies have demonstrated the ingestion of microplastics by a large range of marine organisms representing various trophic levels including seabirds, marine mammals, fish and invertebrates (GESAMP, 2015) and detrimental physical effects of microplastics have been reported following ingestion (Wright et al., 2013), including mortality (Maes et al., 2020). There is also evidence that microplastics can act as carriers for harmful sorbed co-contaminants (i.e., hydrophobic organic compounds, additives, pathogens) with the potential for transfer to biota following ingestion (Rochman et al., 2013; Tanaka et al., 2013; Bakir et al., 2014). However, the transfer of sorbed co-contaminants from microplastics to biota may be negligible compared to other routes of exposure (Bakir et al., 2016; Herzke et al., 2016; Koelmans et al., 2016; Lohmann, 2017).

Plastic litter in biota within and off South Africa has been studied on a macro scale in sharks (Cliff et al., 2002), turtles (Ryan et al., 2016a), albatrosses and Southern Ocean fur seals (Ryan et al., 2016b), on a micro scale in fish (Naidoo et al., 2015, 2020b; Ross, 2017; Naidoo and Glassom, 2019) and inland waterbirds (Reynolds and Ryan, 2018). Data on the abundance of microplastics in commercially important fish species for South Africa is, however, limited and there is a need to address this knowledge gap. Small pelagic fish are of particular importance as they occupy a vital place in marine food webs and the fishery for these species is South Africa's largest and second-most valuable (DFFE, 2020). Whilst most of the catch is processed into agri- and aqua-feeds, small pelagic fish are an important source of food with canned sardine (pilchard) being one of the most important basic food items in the diets of South Africans (Isaacs, 2016). Investigation in microplastic contamination in small pelagic fish is therefore required to assess some related ecological impacts as well as understanding the potential for direct uptake via the human diet.

The aim of this study was to investigate the abundance of microplastics in three commercially important South African small pelagic fish species, namely European anchovy (*Engraulis encrasicolus*), West Coast round herring (*Etrumeus whiteheadi*) and South African sardine (*Sardinops sagax*). The main objectives were to (i) apply a proposed approach for the extraction and quantification of microplastics in small pelagic fish, (ii) to investigate interspecific differences in microplastic ingestion, (iii) to identify the main plastic and polymer types ingested by biota, (iv) to investigate spatial variations with the identification of "accumulation zones" of microplastics contamination, and (v) to identify and propose a suitable bio-indicator species for the monitoring of microplastics in South African waters.

MATERIALS AND METHODS

Biota Sampling

Samples of the three small pelagic fish species were collected during the 2019 Pelagic Recruit Survey that covered the inshore shelf along the South African coastline from the Orange River mouth (West Coast) to Mossel Bay (South Coast) in seven geographical areas or strata (A–G; **Figure 1**, Shabangu et al., 2019). Echosounders were used to identify biomass hotspots along survey transects and midwater trawls (nylon net) were used to catch pelagic fish. Trawl composition varied from one to several species, with all three small pelagic species often being caught in a single trawl. Collected biota samples (i.e., intact individuals) were stored intact and frozen (−20°C) in labeled (date and location of capture) plastic bags until ready for dissection.

Chemicals

The chemicals used in this study are listed in **Table 1**.

Quantification of Contamination and Quality Control

Contamination control procedures were followed to reduce contamination of samples. Cotton lab coats were worn to avoid contamination from clothing items. Prior to use, all glassware and dissection kits were cleaned using a laboratory detergent and rinsed using reverse osmosis (RO) water and covered with RO rinsed foil until ready for use. Gastrointestinal tracts (GITs) were removed under a fume cupboard and quickly transferred to RO rinsed 120 mL glass specimen jars covered with RO rinsed foil. Jars were then transferred to a PCR workstation with laminar flow for the addition of chemical solutions. All chemical solutions used in this study were previously filtered using a 47 mm diameter, 0.2 μm regenerated cellulose membrane filter. Contamination monitoring within the laboratory was carried out by using negative and positive controls. Negative controls consisted of blank filters processed in the same way as environmental samples for each batch of samples processed. Positive controls consisted on the spiking of some filters and checking for particle recovery.

Microplastics in Biota

A total of 593 individual fish were processed during this study comprising of 178 *En. encrasicolus*, 188 *Et. whiteheadi* and 227 *S. sagax*, collected from seven stations per species split across the survey strata as shown in **Figure 1**. For strata with a homogeneous population size distribution, the caudal length (equivalent to standard length) and wet body weight were recorded for five individuals per species, per sampling station (thus representing each stratum), and averaged values were used for calculations. Individual fish caudal length (0.1 cm) and wet body weight (0.1 g) were recorded prior to removal of the GITs under a pre-cleaned fume cupboard to avoid ambient contamination. Stomach weight was collected for all individuals. To determine potential health effects from microplastics on sardines, the lengths and weights of all individual fish were measured in strata E and F. The wet weight of the tissues was recorded and each GIT was transferred to a 120 mL glass jar, pre-cleaned with RO water. To compensate for ambient contamination, an empty glass jar was left open inside the fume cupboard, during the time required to dissect one individual as a blank. The number of items in the blank were deducted from the total number of items in fish for the corresponding batch. As the size of *En. encrasicolus* varied greatly across strata, for sites where individuals were too small for dissection

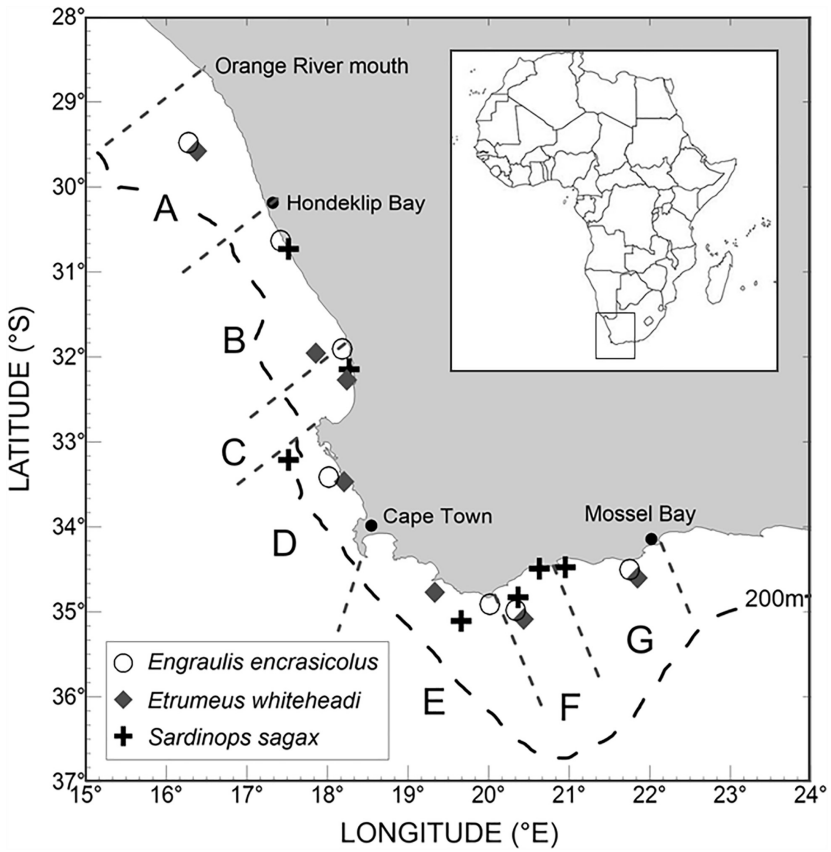

FIGURE 1 | Locations of midwater trawls where biota (small pelagic fishes: white circles for *Engraulis encrasicolus*; gray diamonds for *Etrumeus whiteheadi*; black crosses for *Sardinops sagax*) samples were collected during the 2019 Pelagic Recruit Survey and processed for microplastics. The 200 m depth contour is indicated by the black dashed line and the borders of survey strata A to G by gray dashed lines. Note that sardines were collected from two trawls in stratum F in order to obtain sufficient fish for processing.

TABLE 1 | List of chemicals, manufacturers, and suppliers.

Chemicals	Molecular formula	Manufacturer/supplier	Purity (%)
Potassium hydroxide	KOH	Lasec, South Africa	Analytical Reagent
Sodium hypochlorite	NaClO	Lasec, South Africa	13% active chlorine
Ethanol	C_2H_6O	VWR	95% purity
Nile Red	$C_{20}H_{18}N_2O_2$	VWR	99% purity

(∼5 cm), the outside of the individuals were rinsed with MilliQ water (18.2 M′Ωcm and TOC < 10 ppb) and the head and tail removed. The remaining body was then placed in a glass jar as previously described. A fixed volume of 40 mL of a 30% KOH:NaClO solution was added to each pot in an PCR workstation with laminar flow to avoid particle contamination and each sample was sonicated using a VWR ultrasonic cleaning bath for 5 min (Enders et al., 2017). Each sample was then incubated at 40°C for 24 h before filtration using a pre-rinsed Whatman GF/D filter (2.7 μm porosity). Identification of the extracted microplastics was carried out using the fluorescence tagging of polymers using Nile Red coupled with digital imaging and an automated particle counting method developed at Cefas based on ArcGIS (Maes et al., 2017). As a validation step, each filter was examined under a microscope (VWR Stereo microscope, VisiScope SZT360-6) to remove any false positives from the fluorescence of biological items and to differentiate suspected anthropogenic-origin items into fibers and fragments based on their morphology. For additional quality assessment, a GF/D filter was spiked with a known number of plastic particles to investigate recovery rates using both a visual (digital imaging and microscopy) and an automated particle counting method. Data were corrected from the procedural control (i.e., negative control) to compensate for ambient contamination. A summary of the methods is shown in **Supplementary Figure S1**.

Quality Control and Polymer Identification Using Fourier-Transform Infrared Spectroscopy

Polymer identification of particles was carried out using attenuated total reflection Fourier transform infrared spectroscopy (ATR-FT-IR) with a Thermo Fisher Scientific Nicolet iS5 ATR-FTIR with OMNIC software (version 9.9.473) and by comparison of their IR spectra to a polymer library. ATR-FT-IR has been shown to be a fast and effective tool for the identification of polymers of plastic marine debris, including those ingested by marine organisms (Jung et al., 2018). Due to size limitation using ATR-FT-IR, only particles above ∼250 μm in size were analyzed. In total, 2.3% of particles were selected for polymer identification. Spectra were collected in absorbance mode in the range 4000–400 cm^{-1} at a resolution of 4 cm^{-1}. Polymer identification was verified based on the% match (>70%) against polymer libraries (HR Nicolet Sampler Library, HR Spectra IR Demo and Hummel Polymer Sample Library). Quality control was carried out with the analysis of a polystyrene (PS) and polyethylene (PE) reference material before each batch. Categorization of the extracted particles for quality control is shown in the supporting information section (**Supplementary Figure S3**).

Statistical Analysis

A Poisson log-linear model (**Supplementary Figure S2**) was applied to model the relationships between the number of items $individual^{-1}$ in fish against species, geographic range (strata), and stomach weights using Eq. 1.

$$\log(\mu) = fn(Species,\ Strata,\ Stomach\ weight) \quad (1)$$

where μ is the Poisson mean and *fn (Species, Strata, Stomach weight)* is a linear function of the three potential explanatory variables. We use the link function log (μ) to constrain μ to be positive. Statistical analysis was carried out using R Core Team (2019). Once all combinations of the explanatory variables were fitted into the model, the Akaike Information Criterion (AIC) was used to judge the suitability of the models.

The effect of microplastics on fish health and fitness was investigated using the caudal length to body weight ratio. Symmetric distribution of the ratios suggested Normality and the following linear model was applied using (Eq. 2).

$$Ratio = f(Count,\ Strata) + error \quad (2)$$

where $f(Count,\ Strata)$s a linear *function* of Count and Strata and the error is assumed to be Normally distributed with mean 0 and constant variance.

With further consideration of fish health, a linear regression model was applied to investigate the potential relationship between mean microplastic count, in each species, against the mean weight and mean caudal length of fish, within each geographical location. Finally, a modified bootstrap analysis was applied on results obtained on microplastics abundance to evaluate the optimum sampling sizes for each species for future monitoring (Manly, 2006).

RESULTS

Interspecific Variation

This study confirmed the presence of microplastics in GITs of commercially important small pelagic fish species in South African waters, specifically *En. encrasicolus* (n = 178), *Et. whiteheadi* (n = 188), and *S. sagax* (n = 227). A total of 813 suspected microplastics were detected in 406 fish across all the species out of 593 individuals, representing a 68% occurrence overall. The corresponding total mean concentration of microplastics for biota across all the strata was 1.36 items $individual^{-1}$. The mean occurrence of microplastics was substantially lower for *En. encrasicolus* (57%) as compared to *Et. whiteheadi* and *S. sagax* (each 72%) (**Table 2**).

Mean number of microplastics (mean number of items $individual^{-1}$) ranged from 1.13 items $individual^{-1}$ for *En. encrasicolus* followed by *Et. whiteheadi* and *S. sagax* with 1.38 and 1.58 items $individual^{-1}$, respectively (**Table 2**), and varied between strata (**Figure 2**). The results from the Poisson log-linear model (AIC values) suggested that species, strata and their interaction influence the abundance of microplastics in fish species. This makes inter-comparisons and identification of global trends difficult (**Table 3**). The mean number of items $individual^{-1}$ per species and per stratum is shown in **Figure 2**, along with 95% confidence intervals derived from the fitted log-linear model with the interaction between species and strata. The overall trend, irrespective of species, indicated a slight decrease from stratum A to B followed by a distinct increase in abundance of ingested items to stratum C. The mean number of items $individual^{-1}$ was then generally stable until further increasing in stratum G. For *En. encrasicolus*, the abundance of microplastics was generally constant and linear across strata. This trend was however different for *Et. whiteheadi* and *S. sagax* which showed a gradual increase in the abundance of ingested items from the strata B to G (**Table 2** and **Figure 2**). This interspecific difference was particularly clear for stratum G in which *Et. whitehead* and *S. sagax* ingested substantially higher numbers of microplastic items (means of 1.80 and 2.38 items $individual^{-1}$, respectively) as compared to *En. encrasicolus* (mean of 0.92 items $individual^{-1}$) (**Figure 2**).

The mean number of items per individual was also plotted against mean fish body weight (**Figures 3**, **4**) and mean fish caudal length (**Figures 3**, **5**) for all strata per species to assess whether larger organisms showed a higher ingestion of particles. Linear regression models were applied to derive levels of significance, but the mean number of items $individual^{-1}$ was not significantly linearly related ($p > 0.05$) to fish body weight or to body caudal length for all species under investigation.

Main Microplastic Types and Sources

Fibers and fragments were the most common types of microplastics found for all the species under investigation. Overall, for all the species, fibers represented 80% of the analyzed particles and fragments 20%. This was consistent across species with fibers representing 82, 81, and 76% of the particles analyzed for *En. encrasicolus, Et. whitehead,* and *S. sagax*,

TABLE 2 | Number of individuals studied, number of individuals with micro plastics, % occurrence per strata, and mean number of items individual^{-1} per species [range in ()] for anchovy (*Engraulis encrasicolus*), West Coast round herring (*Etrumeus whiteheadi),* and sardines (*Sardinops sagax*).

Strata	Species	Number of individuals	Number of individuals with microplastics	% occurrence	Mean number of items individual^{-1} (range of number of items in individual)		Fibers vs. fragments (%) per species		Fibers vs. fragments (%) per stratum	
							Fibers	Fragments	Fibers	Fragments
A	***Engraulis encrasicolus***	36	24	67	1.22 (0–4)	1.19	93	7	85	16
	Etrumeus whiteheadi	25	15	60	1.16 (0–6)		76	24		
	Sardinops sagax	0	0	NA	NA			NA		
B	***Engraulis encrasicolus***	25	17	68	1.24 (0–6)	1.04	85	15	73	27
	Etrumeus whiteheadi	24	12	50	0.75 (0–2)		79	21		
	Sardinops sagax	25	14	56	1.12 (0–6)		54	46		
C	***Engraulis encrasicolus***	10	5	50	1.10 (0–5)	1.53	60	40	82	18
	Etrumeus whiteheadi	33	29	88	2.18 (0–7)		96	4		
	Sardinops sagax	50	37	74	1.30 (0–4)		91	9		
D	***Engraulis encrasicolus***	10	6	60	1.50 (0–7)	1.37	92	8	79	21
	Etrumeus whiteheadi	10	5	50	1.10 (0–4)		50	50		
	Sardinops sagax	50	35	70	1.50 (0–7)		96	4		

(Continued)

TABLE 2 | Continued

Strata	Species	Number of individuals	Number of individuals with microplastics	% occurrence	Mean number of items individual^{-1} (range of number of items in individual)		Fibers vs. fragments (%) per species		Fibers vs. fragments (%) per stratum	
							Fibers	Fragments	Fibers	Fragments
E	***Engraulis encrasicolus***	25	16	64	0.96 (0–3)	1.35	71	29	66	34
	Etrumeus whiteheadi	25	18	72	1.20		81	19		
	Sardinops sagax	24	21	88	1.88 (0–4)		47	53		
F	***Engraulis encrasicolus***	47	27	57	0.94 (0–4)	1.26	95	5	86	14
	Etrumeus whiteheadi	46	37	80	1.50 (0–5) (0–6)		93	7		
	Sardinops sagax	49	30	61	1.33 (0–9)		70	30		
G	***Engraulis encrasicolus***	25	12	48	0.92 (0–3)	1.70	77	23	89	11
	Etrumeus whiteheadi	25	20	80	1.80 (0–7)		89	11		
	Sardinops sagax	29	26	90	2.38 (0–7)		100	0		
Total	***Engraulis encrasicolus***	**178**	**102**	**57**	**1.13 (0–7)**	**1.36**	**82**	**18**	**80**	**20**
	Etrumeus whiteheadi	**188**	**136**	**72**	**1.38 (0–7)**		**81**	**19**		
	Sardinops sagax	**227**	**163**	**72**	**1.58 (0–9)**		**76**	**24**		

Bold values were used to highlight total values.

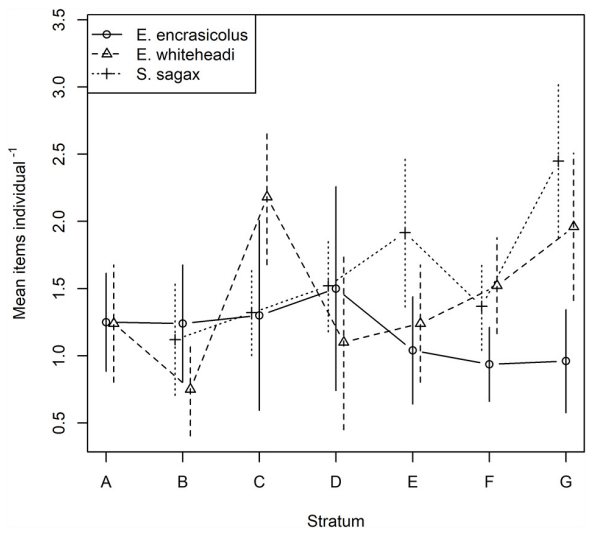

FIGURE 2 | Mean number of items individual^{-1} by stratum for *Engraulis encrasicolus* (n = 178), *Etrumeus whiteheadi* (n = 188), and *Sardinops sagax* (n = 227). Also shown are 95% confidence intervals from the interaction Poisson log-linear model. *S. sagax* was not collected from Stratum A.

TABLE 3 | Summary of AIC values for the Poisson log-linear models in decreasing order of model quality (low values of AIC are best).

Variables in model	AIC
Strata + Species + Strata.Species	1919.9
Strata + Species	1931.2
Strata + Species + Stomach weight	1932.2
Species	1938.2
Species + Stomach weight	1940.2
Strata	1940.8
Stomach weight	1952.6
None	1950.8

respectively (**Table 2**). The most commonly found polymers were poly(ethylene:propylene:diene) (EPDM, 33%), polyethylene (PE, 20%), polyamide (PA, 20%), polyester (PET, 20%), and polypropylene (PP, 7%) (**Supplementary Figure S3**).

DISCUSSION

Uncertainties and Evaluation of Method

The Nile red screening method for microplastics was applied for a fast and cost-effective assessment of the occurrence of microplastics in biota collected in South Africa. This method has recently been used for large-scale mapping of microplastics (Wang et al., 2018). Due to the nature of the project, only small, portable items were used during this study. This included the use of a portable ATR-FTIR for plastic particle validation and polymer identification with a validation step restricted to particles down to about 250 μm in size. No micro-FTIR or Raman spectroscopy was accessible on site and are commonly used for the identification of smaller size particles (Kniggendorf et al., 2019). The presence of false positives has been previously identified as a source of error when applying the Nile red screening method (Maes et al., 2017; Kukkola et al., 2020). Additional steps were therefore required for plastic confirmation and removal of false positives, including visual observation using digital imaging and microscopy.

Interspecific Variation

This study documented and compared levels of microplastics in three small pelagic fish species found off the coast of South Africa, namely *En. encrasicolus, Et. whiteheadi,* and *S. sagax.* All three species are planktivorous but show resource partitioning and feed primarily on different components of the plankton. *Sardinops sagax* are able to retain small particles so phytoplankton (diatoms and dinoflagellates) is on occasion relatively important, but the majority of the dietary intake of this species is via filter-feeding on smaller zooplankton such as poecilostomatoid and small calanoid copepods as well as fish eggs (Van Der Lingen, 2002). Phytoplankton is of less importance to *En. encrasicolus* which primarily particulate-feeds on larger zooplankton such as calanoid copepods and euphausiids (James, 1987). The diet of *Et. whiteheadi* has been reported to consist entirely of zooplankton (euphausiids, large copepods and decapods; Wallace-Fincham, 1987) but it is likely they also eat larval and early juveniles stages of fish (van der Lingen and Miller, 2011).

Given their planktivorous nature and the overlap in size between their natural prey and microplastics, the ingestion of

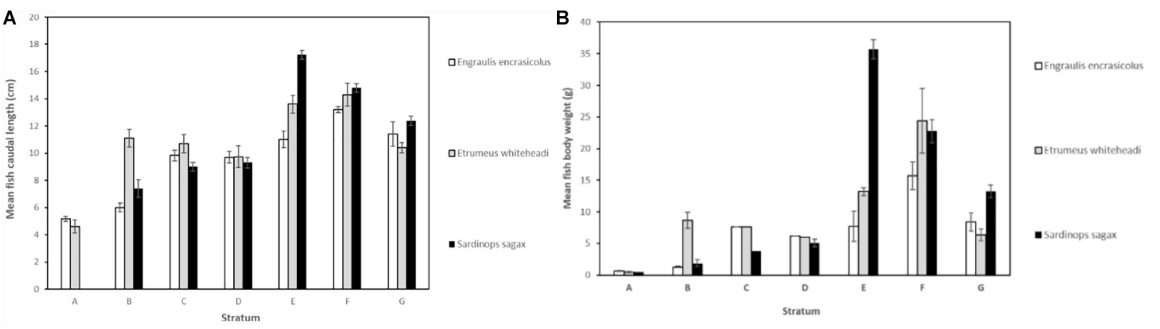

FIGURE 3 | Mean **(A)** fish caudal length (in cm) and **(B)** mean fish body weight (g) (mean ± 95% CI) for *Engraulis encrasicolus* (n = 178), *Etrumeus whiteheadi* (n = 188), and *Sardinops sagax* (n = 227).

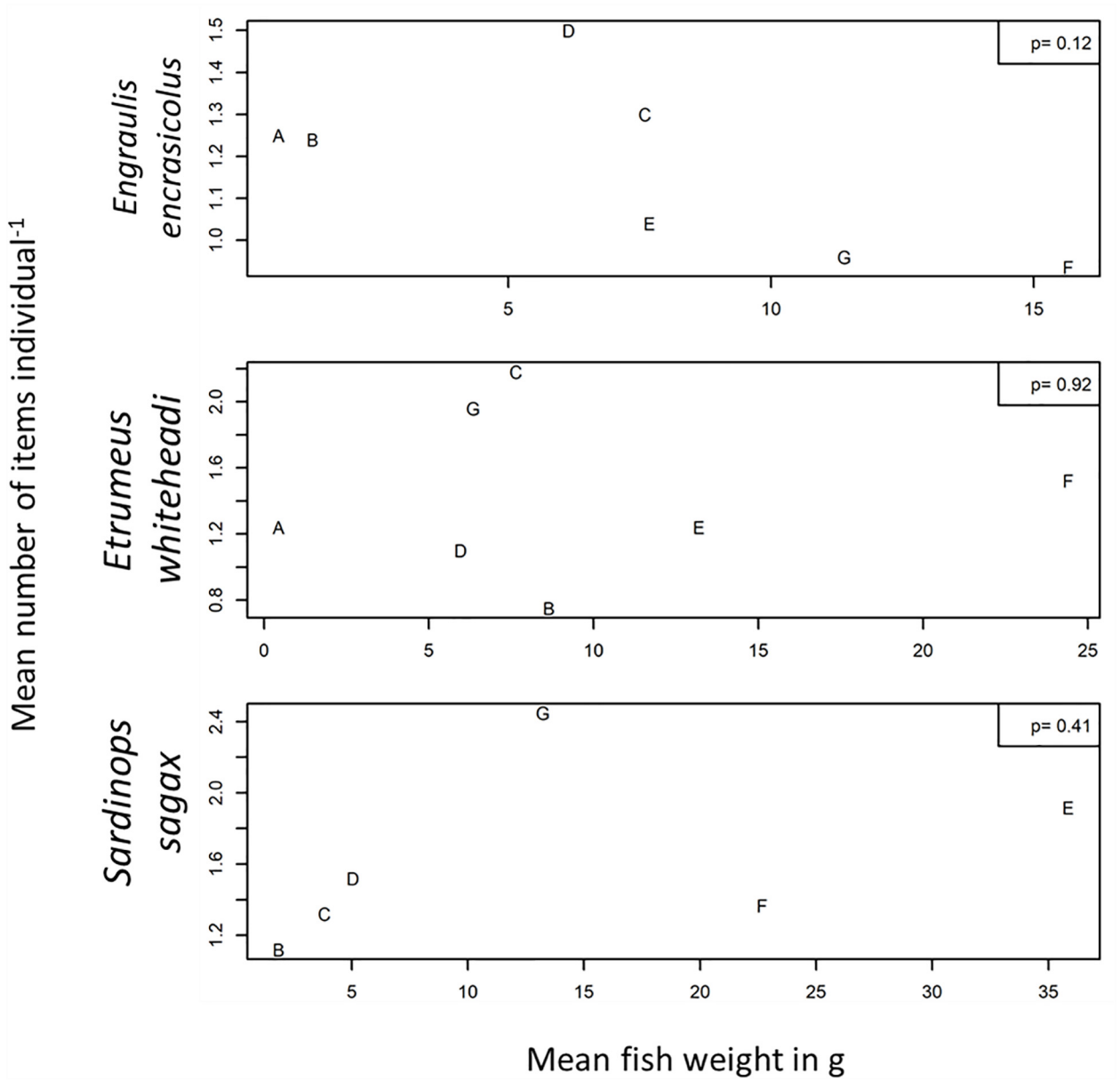

FIGURE 4 | Mean number of items individual^{-1} plotted against mean weight (g^{-1}) for *Engraulis encrasicolus, Etrumeus whiteheadi* and *Sardinops sagax* for each stratum. Also shown are *p*-values for a linear regression model.

microplastics by these small pelagic fish species is predictable. Detailed comparative analysis of diet, feeding morphology and behavior and energetics of sardine and anchovy (van der Lingen et al., 2006), as well as a comparison of nitrogen stable isotope ratios that reflect the trophic position of all three species (van der Lingen and Miller, 2011; Van Der Lingen and Miller, 2014), have demonstrated clear differences in their feeding ecologies in South African waters, suggesting that microplastic ingestion levels may vary between them. Such interspecific differences were indeed observed, with the Poisson log-linear model indicating that species, location (stratum) and their interaction all had significant effects on microplastic abundance in these small pelagic fish species. Overall, *En. encrasicolus* had both the lowest occurrence (57%) and lowest mean abundance (1.13 items individual^{-1}) of microplastics, whereas *Et. whiteheadi* and *S. sagax* had the same occurrence (72%), the mean abundance of microplastics was higher in sardine (1.58 items individual^{-1}) than in round herring (1.38 items individual^{-1}).

The interspecific difference reported here supports the hypothesis of Collard et al. (2017) that planktivorous fish species with the most efficient filtration apparatus will be more likely to ingest microplastics. Those authors determined filtration areas and particle retention thresholds of *En. encrasicolus*, *Clupea harengus,* and *Sardina pilchardus* in European waters

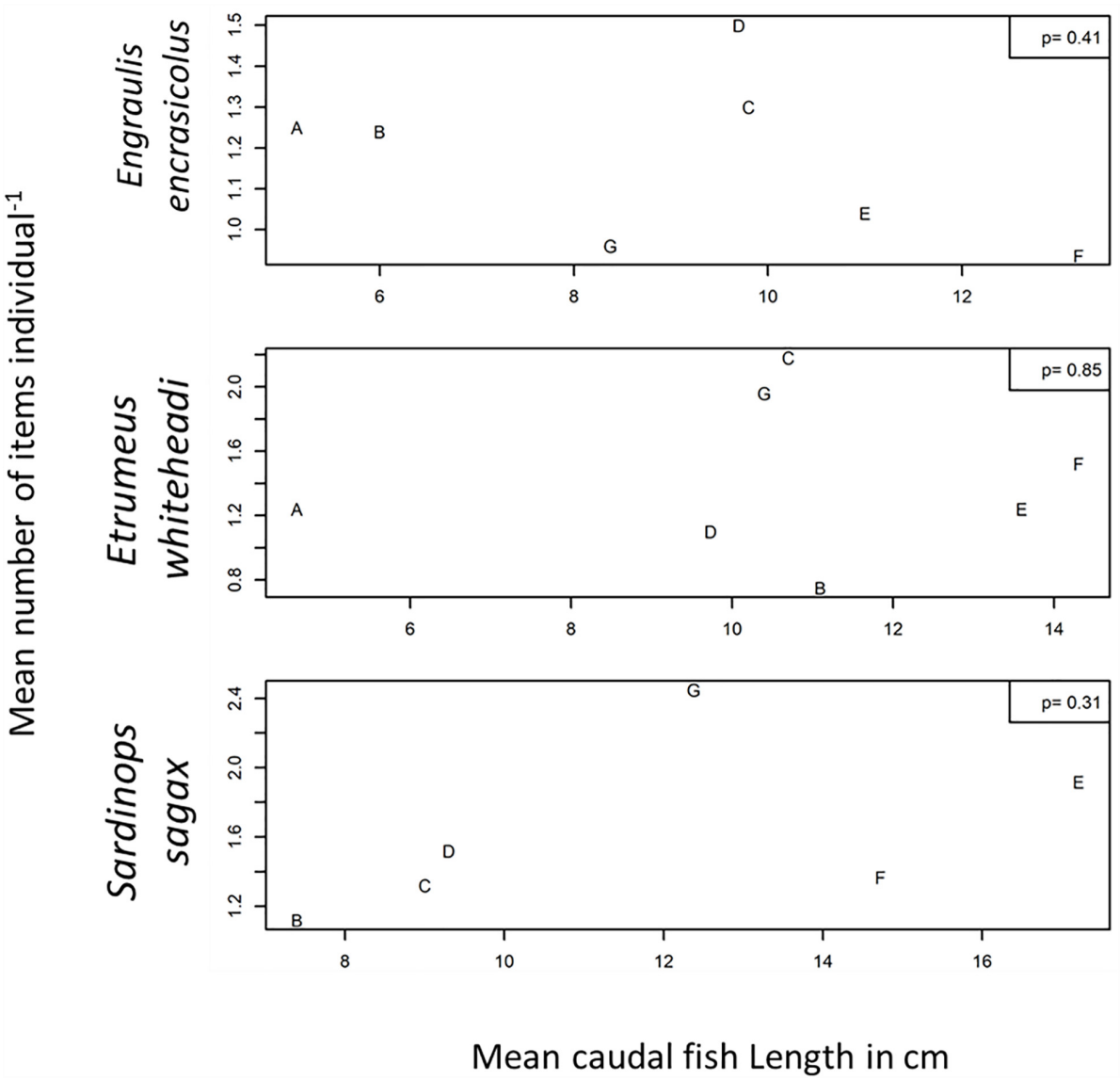

FIGURE 5 | Mean number of items individual^{-1} plotted against mean caudal length (cm) for *Engraulis encrasicolus*, *Etrumeus whiteheadi* and *Sardinops sagax* for each stratum. Also shown are *p*-values for a linear regression model.

and compared these with their anthropogenic particle (including microplastics) levels, and reported that sardine, which had the largest area and closest gill raker spacing, ingested more fibers and smaller fragments than anchovy or herring. *S. sagax* has a markedly smaller gill raker gap than either *En. encrasicolus* or *Et. whiteheadi* in southern African waters (Vorsatz et al., 2015), which provides a plausible mechanism to explain the higher occurrence and abundance of microplastics in sardine compared to the other two species reported here. However, the hypothesis that retention of smaller particles results in increased microplastics ingestion is in contrast to findings by Lopes et al. (2020), who reported that planktivorous species from Alanto-Iberian waters that fed on larger zooplankton prey (*Trachurus trachurus*, *Scomber scombrus,* and *En. encrasicolus*) accumulated more microplastics than those feeding on smaller prey size (*S. pilchardus*, *Sc. colias,* and *Boops boops*), and recommended *T. trachurus* as a suitable bio-indicator species for monitoring microplastics in that ecosystem. While studies on bioaccumulation of microplastics are limited, there is indication that plastic particles can be ingested and transferred in the planktonic food web (Setälä et al., 2014), representing another potential route of uptake by biota.

Data on the occurrence and abundance of microplastics in sardines, anchovies, and round herrings from various studies of the same or similar species, including from South African waters, are listed in **Table 4**. It must be noted that methods used in those studies are not all standardized and that sample sizes vary substantially between them, hence inferences made from

comparing those data are questionable. Nonetheless, we feel that they provide a useful contextualization of our results. Ross (2017) assessed ingestion of microplastics by the same three epipelagic species from the a similar geographical area investigated in this study and reported an overall microplastics occurrence of 67%, which was consistent with the overall 68% occurrence observed in this study using markedly larger sample sizes. In contrast to our findings, however, Ross (2017) found that *En. encrasicolus* had the highest (80%) and *Et. whiteheadi* the lowest (44%) occurrence of microplastics, whereas observations from the two studies for sardine were similar (76% from Ross, 2017 and 72% from this study; **Table 4**). Despite these differences, estimates from both studies for *En. encrasicolus* off South Africa are higher than the median occurrence of 30% calculated from available data for anchovies in the literature (**Table 4**). More strikingly, estimates of microplastic occurrence in *S. sagax* off South Africa are the highest yet reported for this species compared to a median value of 25% from the eight values reported in **Table 4**. We could find no other reports of microplastic occurrence in *Etrumeus* spp. but the median value from Ross's (2017) and this study is 58%.

Our estimate of mean microplastics abundance for *En. encrasicolus* is substantially (< 50%) lower than that estimated by Ross (2017) but is above the median value of 0.48 items individual^{-1} for anchovies listed in **Table 4**, where mean abundance ranges from 0.0 to 4.0 items individual^{-1} across six species from three genera. Similarly, Ross (2017) reported a higher mean microplastics abundance and substantially larger range for *S. sagax* than observed in this study, and abundance values from both studies are the highest yet reported for this species and compare to a median of 0.35 (range 0.0–2.8 items individual^{-1}) across the four species in four genera of sardines reported in **Table 4**. We could find no microplastic abundance data for any other *Etrumeus* species but Sparks and Immelman (2020) reported that *Et. whiteheadi* from a single sample collected in shelf-edge waters off South Africa's South Coast (in Stratum G of this analysis) had a mean abundance of 3.3 (± 0.5 SE) items individual^{-1}, which is somewhat but not substantially higher than the value of 1.80 (± 0.55 95% CI) 2.5 items individual^{-1} estimated for this species in Stratum G in this study.

Main Plastic Types and Sources

Fibers were the main type of ingested items in biota, representing 80% of the extracted items in this study. Microfibers are known to represent a large fraction (>85%) of microplastic debris found along shorelines globally (Carr, 2017). This was in agreement with Ross (2017) who reported that microfibers represented 99% of the items extracted from *En. encrasicolus*, *S. sagax*, *Et. whiteheadi*, *Lampanyctodes hectoris,* and *Maurolicus walvisensis* from the West and South coasts of South Africa. Similarly, Naidoo et al. (2016) reported that plastic fibers were ingested most commonly by *Mugil cephalus* in the Port of Durban off South Africa's East Coast. Naidoo et al. (2020b) also reported that fibers represented 68% of ingested items by juvenile *Oreochromis mossambicus*, *Terapon jarbua*, *Ambassis dussumieri* and *Mugil* spp., within four mangroves also along the East Coast of South Africa, and Sparks and Immelman (2020) that filaments (i.e., fibers) comprised 67% of microplastics ingested by three species of intertidal mussel around Cape Town.

Microfibers can enter the environment from both primary sources (fibers < 5 mm in size) or resulting from the fragmentation of larger items (Henry et al., 2019). Whereas wastewater treatment plants have been previously thought to be a primary source of microfibers in the environment, recent studies have shown that direct input from shedding of fibers, from common fabric and textiles, could represent a much important source (Carr, 2017; Conley et al., 2019). De Villiers (2019) investigated the occurrence of microfibers in river sediments adjacent to South Africa's coastline and reported a significant positive relationship between river sediment, microfiber levels, and the percentage of households in the catchment area that do not have access to piped water. That study also suggested that rivers represent direct vectors for the transport of fibers to the marine environment from rural communities, for whom rivers are the only source of accessible water, from clothes-washing activities. It is however worth noting that natural fibers (cellulose and of animal origin) represent a greater proportion of oceanic fibers (91.8%) as compared to synthetic fibers (8.2%) despite synthetic polymers currently accounting for two-thirds of global fiber production (Suaria et al., 2020).

The main commonly found polymers in biota in our study were poly(ethylene:propylene:diene) (EPDM, 33% occurrence), polyethylene (PE, 20%), polyamide (PA, 20%), polyester (PET, 20%), and polypropylene (PP, 7%) (**Supplementary Figure S3**). While data on the type of polymer found in biota for South Africa is limited these results are consistent with a meta-analysis by Erni-Cassola et al. (2019) that indicated the prevalence of PE, followed by PA, PET, and PP in aquatic environments globally.

Spatial Variation

Significant variation in the occurrence and abundance of microplastics in biota across the different strata were also observed in this study. The ingestion of particles throughout the inshore shelf suggested the widespread occurrence of microplastics in the pelagic environment along the South African coastline, between the Orange River mouth and Mossel Bay, with a significant increase in the number of items per individual in biota from the West to the South coast observed. This study was carried out in the southern Benguela coastal upwelling ecosystem (west of Cape Agulhas; Strata A–E) and the Agulhas Bank temperate shelf ecosystem (east of Cape Agulhas; Strata F and G), and prevailing oceanographic conditions differ between the two (Hutchings et al., 2009). The southern Benguela is characterized by seasonal upwelling that brings cool waters from the depths of the Central South Atlantic to the surface inshore, and warm Agulhas Current water offshore. The Agulhas Bank displays characteristics of both upwelling and temperate shelf ecosystems, and its dynamics are markedly impacted by the Agulhas Current that transports warm, salty water from the Indian Ocean along South Africa's East and South coasts. Because of the differing origins and transport routes of source waters of these two systems it might be expected that microplastics load off the West Coast are lower than those off the South Coast, as suggested by the results of this study.

TABLE 4 | Occurrence and number of items individual^{-1} for anchovies, sardine and round herring reported in the literature from several locations (number of individuals examined indicated where possible; ns: not specified; nd: not determined).

Fish/species		Location	Number of individuals examined	Occurrence (%)	Mean number of items per individual	References
Anchovies	*Coilia ectenes*	Yangtze estuary, China	18	nd	4.0 ± 1.8	Jabeen et al., 2017
	Ctengraulis mysticetus	Pacific Ocean, Columbia	30	3	0.03 ± 0.03 SE	Ory et al., 2018
	Ctengraulis mysticetus	Pacific Ocean, Panama	10	0	0	Ory et al., 2018
	Engraulis encrasicolus	Mediterranean Sea, Spain	ns	nd	1.18 ± 0.40	Ferrer et al., 2016
	Engraulis encrasicolus	Atlantic and Indian oceans, South Africa	25	80	2.68 (1–7)	Ross, 2017
	Engraulis encrasicolus	Atlantic Ocean and Mediterranean Sea (United Kingdom, France, Spain)	20	40	0.85	Collard et al., 2017
	Engraulis encrasicolus	Mediterranean Sea, Spain	>15	14.28	0.48	Compa et al., 2018
	Engraulis encrasicolus	Southern Tyrrhenian Sea	19	nd	0.26	Savoca et al., 2020
	Engraulis encrasicolus	Atlantic Ocean, Portugal	131	79	0.48 median (0.10–0.90 interquartile range)	Lopes et al., 2020
	Engraulis encrasicolus	**Atlantic and Indian oceans, South Africa**	**178**	**57**	**1.13 (0–7)**	**This study**
Sardines	*Engraulis japonicus*	Tokyo Bay	64	nd	2.3 ± 2.5	Tanaka and Takada, 2016
	Engraulis mordax	Pacific Ocean, United States (California)	10	30	0.3 ± 0.5 SD	Rochman et al., 2015
	Engraulis ringens	Pacific Ocean, Chile	76	1.3	0.01 ± 0.01 SE	Ory et al., 2018
	Engraulis ringens	Pacific Ocean, Peru (40)	40	0	0.0	Ory et al., 2018
	Opisthonema libertate	Pacific Ocean, Columbia	40	0	0.0	Ory et al., 2018
	Opisthonema libertate	Pacific Ocean, Ecuador	40	5	0.05 ± 0.04 SE	Ory et al., 2018
	Sardina pilchardus	Atlantic Ocean and Mediterranean Sea (United Kingdom, France, Spain)	20	45	0.55	Collard et al., 2017
	Sardina pilchardus	Mediterranean Sea, Spain	>15	15.24	1.43 ± 0.79	Compa et al., 2018
	Sardina pilchardus	Southern Tyrrhenian Sea	27	nd	0.53	Savoca et al., 2020
	Sardina pilchardus	Atlantic Ocean, Portugal	76	58	0.16 median (0.00–0.53 interquartile range)	Lopes et al., 2020
	Sardinops sagax	Atlantic and Indian oceans, South Africa	25	76	2.8 (1–16)	Ross, 2017
	Sardinops sagax	Pacific Ocean, Chile	7	0	0.0	Ory et al., 2018
	Sardinops sagax	**Atlantic and Indian oceans, South Africa**	**227**	**72**	**1.58 (0–9)**	**This study**
	Strangomera bentincki	Pacific Ocean, Chile	10	0	0.0	Ory et al., 2018
Round herring	*Etrumeus whiteheadi*	West and South coasts, South Africa	25	44	**0.8 (1–3)**	Ross, 2017
	Etrumeus whiteheadi	Indian Ocean, South Africa	15	nd	3.3 ± 0.5	Sparks and Immelman, 2020
	Etrumeus whiteheadi	**West and South coasts, South Africa**	**188**	**69**	**1.38 (0–7)**	**This study**

Bold values were used to highlight data coming from this study as compared to other reported studies from the literature.

TABLE 5 | Recommended criteria for the selection of a bioindicator species for the monitoring of microplastics in biota for SA coastal waters.

Criteria	Biota		
	Engraulis encrasicolus	***Etrumeus whiteheadi***	***Sardinops sagax***
Wide geographical distribution	✓	✓	✓
Representative of a specific monitoring area	✓	✓	✓
Species that are not protected or endangered	✓	✓	✓
Suitable particle retention time within organisms (hours)	4–158[1–3]	4–158[1–3]	4–158[1–3]
Already used as bioindicator/biomonitoring species	✓	×	✓
Ability to ingest and retain small to larger sized particles	<5 mm[1–3]	<5 mm[1–3]	<5 mm[1–3]
Species that can be kept in cages for easy field deployment and collection	×	×	×
Invertebrate species, which require less staff training (cost-effective) for handling than vertebrate species	×	×	×
Perform sampling in a cost-effective manner by synergies with pre-existing programs	✓	✓	✓
Commercially important species with public health implications	✓	✓	✓
Ease of sample preparation and validated analytical protocol using Nile Red polymer fluorescence tagging	✓	✓	✓

[1]*(Ward and Kach, 2009; Catarino et al., 2017).* [2]*(Brett and Grooves, 1979).* [3]*(Nelms et al., 2018).*

Along the South African coastline, macro litter has been observed in higher abundance close to urban centers (Ryan et al., 2009), whereas, micro litter on beach sediments has not been observed to be driven by population demographics, but rather by large scale ocean currents (Nel et al., 2017). Microplastic concentrations are higher in environmental matrices near or in highly populated areas, close to large coastal wastewater treatment plant discharge points, in rivers and harbors, and are affected by seasonal changes in river runoff (Naidoo et al., 2015; Nel et al., 2017; de Villiers, 2018). De Villiers (2019) noted higher microplastic levels in river sediments in KwaZulu-Natal and the Wild Coast (both along the South African East Coast) which are comparable to levels in the sediments of rivers along the Cape South Coast (ca. 20–26°E).

We also found that microplastics abundance was not significantly linearly related with individuals' caudal length or weight, which is in agreement with other studies that reported no correlation between total number of particles per fish and fish mass (Naidoo et al., 2020b) or fish size (Vendel et al., 2017).

Selection and Proposal of a Bioindicator for Microplastics for South African Waters

One of the objectives of this study was to identify and propose a suitable and sustainable indicator species for the monitoring of microplastics in biota for South African waters, with fish considered to be appropriate bio-indicators of larger microplastics (Ryan et al., 2020). A list of criteria for the selection of a suitable bio-indicator for microplastics has been modified from GESAMP (2019) and is presented in **Table 5**. The species used in this study, *En. encrasicolus*, *Et. whiteheadi* and *S. sagax,* fulfilled most of these criteria; in particular, they are widely distributed in shelf waters along the South African coastline (**Supplementary Figure S4**) and are already being collected as part of annual research surveys, indicating that a broad-scale assessment of microplastics could be cost-efficiently integrated into an existing monitoring program.

In consideration of a microplastics bio-indicator and the development of a potential monitoring plan, a bootstrap (Manly, 2006) analysis to generate confidence intervals was carried out to estimate optimum sample size for each species (**Figure 6**). Output from the analysis suggested a sample size of 150 individuals across

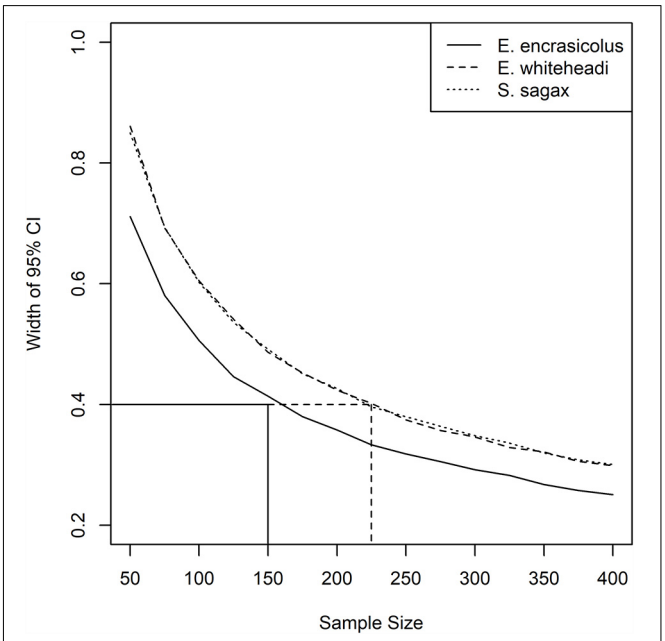

FIGURE 6 | Widths of 95% bootstrapped confidence intervals for sample sizes between 50 and 400, for *Engraulis encrasicolus*, *Etrumeus whiteheadi,* and *Sardinops sagax*. Note that the lines for *Etrumeus whiteheadi* and *Sardinops sagax* are virtually identical.

all strata (22 individuals per stratum) sampled in the 2019 Pelagic Recruit survey for *En. encrasicolus,* and 225 individuals for both *Et. whiteheadi* and *S. sagax* across all strata (32 individuals per stratum), to obtain a precision of 0.4 items of MP per individual, as measured by a 95% confidence interval. Plots for *Et. whiteheadi* and *S. sagax* were similar due to their nearly identical standard deviations of 1.532 and 1.537, respectively. The CI widths for *En. encrasicolus* were also lower due to a lower standard deviation of 1.29 in this study. As mentioned previously, the Poisson log-linear model suggested that the effect of fish species was not similar across strata and the interaction between MP items in the fish species and strata was an important factor to consider (**Supplementary Table S3**). In other words, the pattern of MP abundance across strata was different, depending on the species. Given that the fish in this study were caught together, rather than caught per species, the interaction relationship is unlikely to have been influenced by the sampling technique.

Although fewer *En. encrasicolus* would be required for microplastics assessment to achieve the desired precision, it was clear that both *Et. whiteheadi* and *S. sagax* showed spatial trends in microplastics abundance whereas anchovy did not, suggesting that the former two species might represent more suitable candidates for monitoring programs. *Sardina pilchardus* has already been proposed as a bio-indicator for microplastics for the Mediterranean Sea (Fossi et al., 2018) and the selection of *S. sagax* would allow for global comparisons. However, its suitability in a South African context is under question as the local *S. sagax* population is presently in a depleted state (DFFE, 2020), which could negatively impact sample collection until it recovers. Given this, the present large population size of *Et. whiteheadi*, and the recommendation by Lopes et al. (2020) that planktivorous species that feed on larger zooplankton prey (which this species does) be used as bio-indicator species for monitoring microplastics, we suggest the latter as a suitable regional microplastics bio-indicator specific for South African waters. This despite the lack of knowledge of plastic ingestion by this genus elsewhere which will make global comparisons difficult. The estimate of 225 fish as a required sample size applies to the area covered in the 2019 Pelagic Recruit Survey and would need to be adjusted for other surveys that cover a larger spatial extent.

Potential Impacts on Human Health

The occurrence of microplastics in biota has caused several concerns, ranging from its effects on biodiversity and populations to potential risks to food safety and human health. As fish consumption in South Africa grew by more than 26% between 1994 and 2009, there is potential for the transfer of microplastics from fish to humans following consumption (Naidoo et al., 2020a). It has been suggested by the Food and Agriculture Organization (FAO) that the transfer of sorbed co-contaminants and additives from the ingestion of plastic particles would be negligible due to the low dietary exposure to such contaminants (Lusher et al., 2017). However, the transfer of plastics along the food chain to humans and impacts on human health has been identified as a gap in the understanding of marine plastic pollution in a South African perspective and data on levels of transferral of microplastics from edible aquatic species to humans are currently lacking (Godfrey, 2020; Naidoo et al., 2020a). This gap must be filled in order to make predictive decisions in regard to safety for consumption (Naidoo et al., 2020a).

CONCLUSION

The data presented in this study confirmed the occurrence of microplastics, principally microfibers, in the commercially important small pelagic fishes *En. encrasicolus*, *Et. whiteheadi,* and *S. sagax* in South Africa, with an overall occurrence of 68%. The abundance of microplastics in these fish was impacted by both species and location, as well as the interaction of these two factors, but the overall pattern indicated an increase from the West to the South coast. Microplastic abundance levels in South African anchovy and sardine are above median values for similar species elsewhere but were not correlated with fish biological characteristics in any of the three species examined here. We propose that *Et. whiteheadi* be used as a bio-indicator for microplastics in South African waters and that the collection and processing of this species be included in regular survey programs. Finally, an increase in fish consumption in South Africa suggests potential for the transfer of microplastics to human following consumption with unknown related effects on human health. However, the impacts of microplastics and associated co-contaminants on human health have been suggested to be minor by both the World Health Organization and FAO due to the low dietary exposure to such contaminants.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: 10.14466/CefasDataHub.100.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the fish were collected as part of an already existing fisheries survey conducted by the South African Government. Research was conducted on dead fish.

AUTHOR CONTRIBUTIONS

FP-W, TM, CL, and AdB designed and coordinated the study. CL, AsB, and YG collected the samples. AdB, YM, GM, DD, FT, and RH carried out the laboratory work. JB carried out the statistical analysis. AdB and CL wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2020.574663/full#supplementary-material

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