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Baseline

Abundance and characteristics of microplastics in retail mussels from Cape Town, South Africa

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ABSTRACT

With the increased occurrence of plastics in the marine environment, ingestion of microplastics (MPs) by marine invertebrates such as mussels is increasing globally. This study investigated the occurrence of microplastics in mussels sold at supermarkets and wholesalers in Cape Town, South Africa. Soft tissue was extracted from mussels, digested and identified by microscopy and FTIR-ATR. MP filaments (70%) and fragments (30%) were the only types of MPs identified and an average of 0.04 MPs/g soft tissue and 3.8 MPs/mussel recorded. Blue/green (44%) and black/grey (40%), smaller than 2000 μm were the most prominent MPs recorded and the main polymer type was filamentous polyethylene terephthalate (PET). Our results suggest that retail mussels in Cape Town do not contain as high concentrations of MPs when compared to other investigations and routine monitoring of seafood in the country is suggested.

Excess plastics in the marine environment may change the ecology of ecosystems and marine life as marine organisms either consume plastics or become entangled in substances made of plastic material (Barnes et al., 2009; Dehaut et al., 2016; Derraik, 2002). Most marine debris found in oceans are from land-based sources (Barnes et al., 2009) and according to Schwarz et al. (2019), 99% of plastic pollution is unaccounted for. As it is well known that plastics degrade into microplastics (MPs) via various chemical and mechanical means (Barnes et al., 2009; Beaumont et al., 2019; GESAMP, 2019), the prevalence of MPs in marine resources (seafood) is not well known (Verster et al., 2017).

MPs are characterised as polymer particles smaller than 5 mm (GESAMP, 2019) with recent studies indicating there is an increasing occurrence of microplastics in the marine environment (Bakir et al., 2020; Horton and Barnes, 2020; Ribeiro et al., 2020). Primary MPs are designed for various cosmetics (face rubs) or production of plastic materials (nurdles) whereas secondary MPs come from plastics that fragment and degrade into smaller pieces, including fibres (Karlsson et al., 2017). Whether of primary or secondary sources, MPs are detrimental to the health and wellbeing of the marine environment due to obstruction of digestive tracts and absorbed contaminants potentially being transferred into marine organisms (Karlsson et al., 2017). According to Guzzetti et al. (2018), the increase in the human population is directly proportional to the increase in MPs found in coastal environments. With marine environments already being exposed to MPs, there is a growing concern regarding human exposure to MP contamination when consuming marine resources such as mussels.

Mussels filter-feed on microorganisms and nutrients that float in its surrounding waters in the intertidal and subtidal zones, where many environmental parameters are reported on (De Witte et al., 2014). Environmental parameters include high variations in temperature, salinity, prolonged periods of desiccation, wind disturbance and exposure to anthropogenic contaminants (including MPs) (Yap et al., 2017). According to Barnes et al. (2009), mussels in rocky shores are exposed to plastics entering the coastal environment, including MPs that stem from plastic broken into smaller particles as a result of wave action.

Mussels provide an indication of what is present in the waters surrounding them as they are sessile and ubiquitous in many coastal areas (Bråte et al., 2018; Li et al., 2018a, 2018b, 2018c). According to Li et al. (2018a, 2018b, 2018c), mussels have the ability to filter water at 300 ml per minute at 100% oxygen saturation. As such, mussels are being considered as good bioindicators for many forms of pollutants due to their ability to withstand various environmental changes in their habitat, their distribution allows for easy monitoring and they have efficient filtration abilities (Bråte et al., 2018; Yap et al., 2017). Not only are the biological and ecological implications of MP consumption by mussels an issue, but as resources consumed by humans, the passing on of MPs in mussels poses human health risks (Seltenrich, 2015).

Mussels are a common source of food in coastal areas with studies

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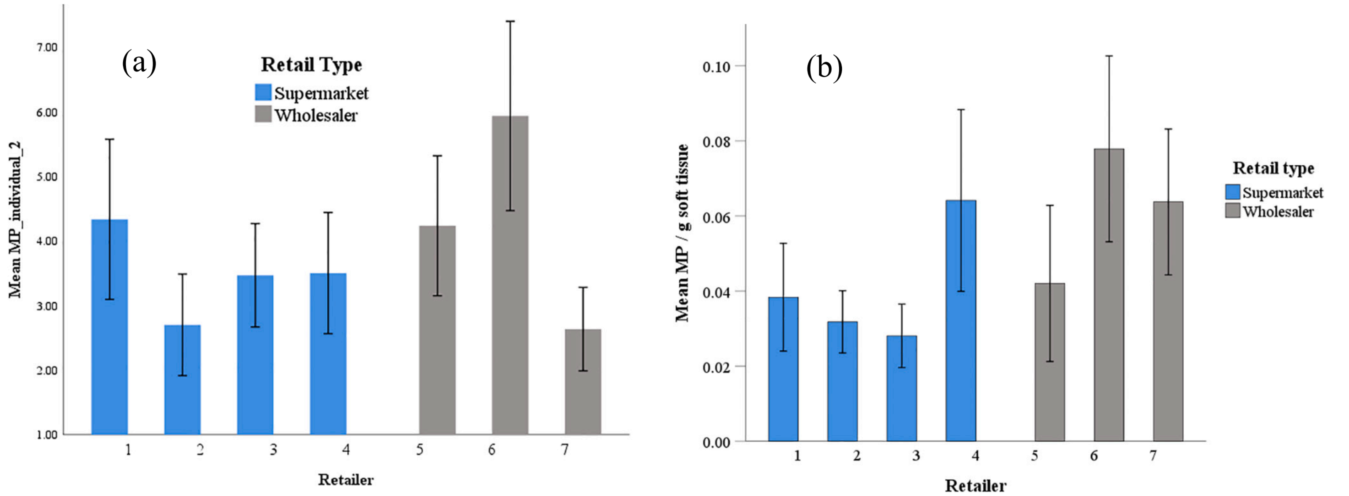

Fig. 1. Mean MP per individual (a) and per gram soft tissue wet weight (b) from seven retailers in Cape Town, South Africa. Retailers indicated in blue are supermarkets and grey are wholesalers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

indicating that 73.8 million metric tons of shellfish were produced in 2014 (Bürgener et al., 2014). According to Beaumont et al. (2019), 19% of the global population (estimated at 1.4 billion people) consume more than 20% of the ocean's food supply. This indicates how heavily reliant humans are on the ocean for sources of protein. The occurrence of plastics and MPs in the ocean could impact fisheries stocks when the consumption of plastics may result in poor animal health, starvation and ultimately death. Also, when seafood is consumed by humans, the chemicals sorbed onto plastics may potentially be passed along the food chain via biomagnification and ultimately result in contaminated seafood being consumed by humans (Frias et al., 2010).

According to Bürgener et al. (2014), the total seafood consumed from marine resources harvested in South Africa was estimated at 156,068 tons in 2015, with imported seafood consumption estimated at 312,753 tons in 2010. The occurrence of MPs in consumable mussels have been discovered in various countries including the United Kingdom, Germany and China (Li et al., 2018a, 2018b, 2018c; Li et al., 2015; Van Cauwenberghe and Janssen, 2014). With seafood consumption rates of 7.5 kg per capita, per year in South Africa (Britz and Venter, 2016), the potential concentrations of MPs in mussels consumed in South Africa is unknown. In this study, we sourced mussels from seven retailers (supermarkets and wholesalers) in Cape Town, South Africa with the aim of determining the abundance and characteristics of MPs.

Retail *Choromytilus meridionalis* (black mussels) */ Mytilus meridionalis* (blue mussels) were sourced between July and August 2019 and frozen until analyses were done. Fresh or frozen mussels were obtained at each of these sites, based on availability and coded based on whether from a supermarket (supermarket 1–4) or wholesaler (wholesaler 5–7). The mussel's origins were from various mussel farms in Saldanha Bay (140 km north of Cape Town), but some packaging did not report on the origins of the mussels.

Digestion of mussel tissue was based on the method by (Sparks, 2020). In brief, 30 mussels from respective retailers were processed individually. Petri dishes were rinsed three times with 10 μm filtered reverse osmosis (RO) water and mussels placed on these, allowed to defrost. Once the mussels had been defrosted, individual shell length (mm) and total and soft tissue weight (g) measured. The mussel soft tissue of individuals mussels were transferred to pre-cleaned glass jars (byssal threads were not included in sample analyses). A 10% KOH (w:v) acid solution was added and mussels placed in an oven for 48 h at 60 °C. Thereafter, the jars were removed from the oven, allowed to cool and the digestates filtered through a 20 μm nylon mesh using a vacuum pump. The mesh was stored in pre-cleaned petri dishes and left to dry before identification was done.

For microplastic identification and characterisation, each mesh was removed from the petri dish and MP identification conducted using a Stereo Microscope (Zeis) at magnification ranging from x10 to x20. MPs, per mesh, were counted and classified per type, size and colour according to criteria recommended by GESAMP (2019). MP shapes were categorised as filament and fragment as no other types were recorded (foam, fragment, film and pellet). Six categories were used for characterisation of MP size as follows: >5000, 5000–2000 μm, 2000 μm–1000 μm, 1000 μm–500 μm, 500 μm–125 μm and <125 μm. Colours were categorised as Blue/Green, Black/Grey, Red/Pink, White and Transparent.

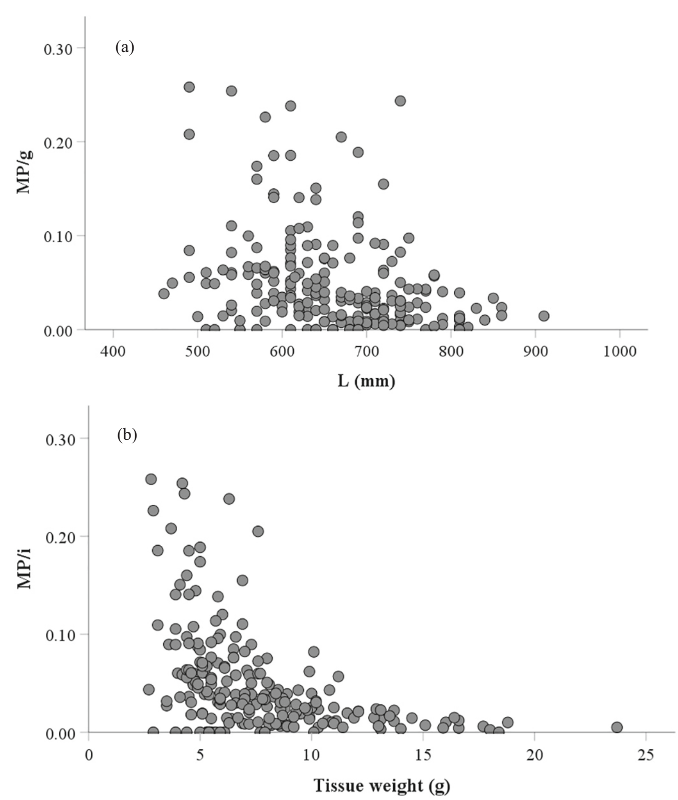

Fig. 2. Spearman Rank Correlation between microplastics per mussel tissue weight and tissue weight from the respective retailers (a) and fresh/frozen mussels (b).

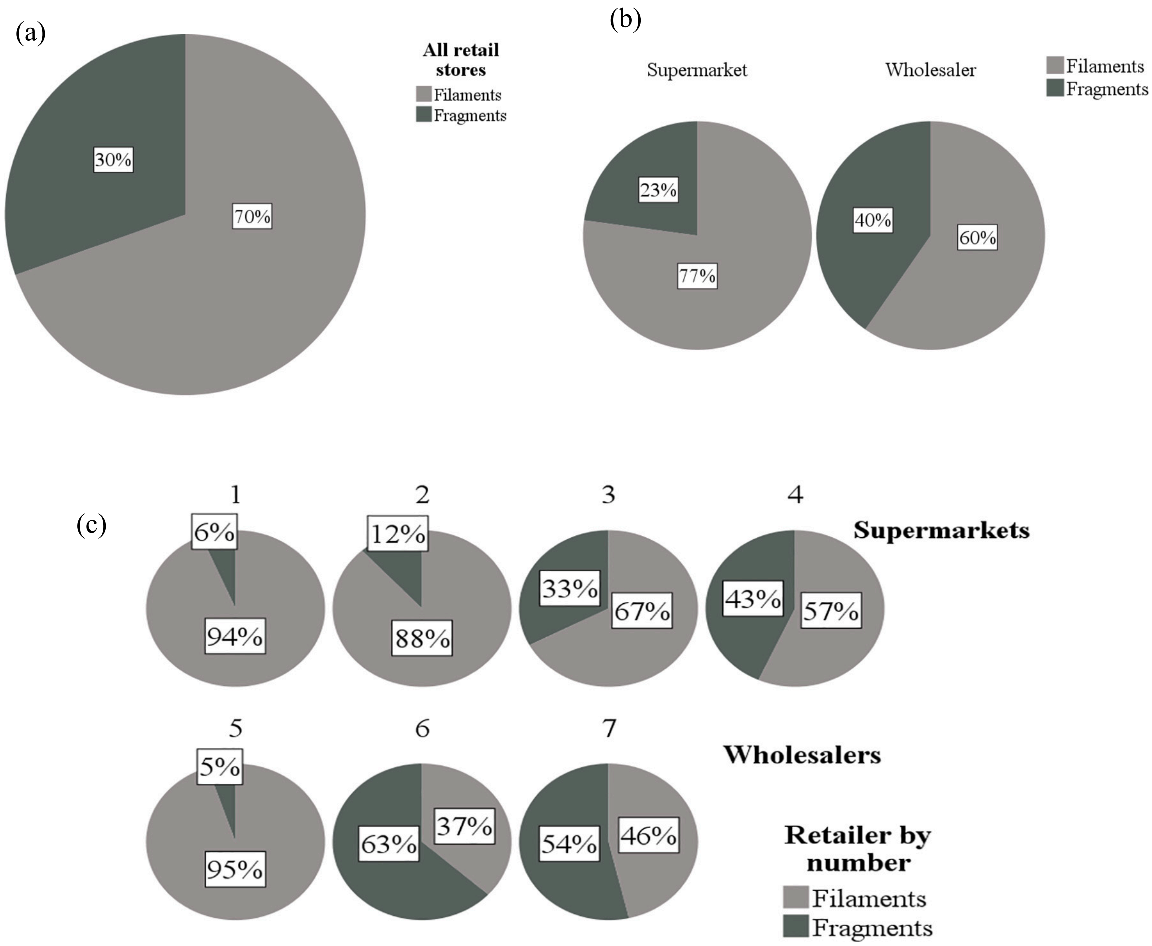

Fig. 3. Microplastics based on shape for all retailers combined (a), retail type (supermarket/wholesaler) (b) and respective retailers (c).

Polymer identification was done via spectroscopy using a Perkin Elmer Two FTIR-ATR Spectrometer. Spectra were recorded in the wavenumber ranging from 5000 to 450 cm^{-1} with a resolution set to 4 cm^{-1} and a data interval of 1 cm^{-1}. The ATR crystal was cleaned before use with 70% propynol and background scans done prior to each sample analysis. Filaments and fragments >500 μm were placed on the FTIR using a fine tweezers and compressed against the diamond head at a force of at least 80 N. The MPs scanned were identified by comparing scans with a polymer reference database (Open Specy) (Cowger et al., 2020) and sources from the literature (Asensio et al., 2009; Jung et al., 2018; Peets et al., 2017).

As the mussels were sourced from retailers, we cannot account for MP contamination from the mussel farms and processing of mussels. The laboratory in which sample processing was done, windows and air vents remained closed to decrease the risk of air-borne contamination. Empty petri dishes were placed next to workbenches and assessed for air-borne contamination daily (at most, three fibres were recorded on a given day and accounted for in the final MP calculations). When working in the laboratory, cotton lab coats were worn with the same clothes, where possible, for the duration for the processing of samples. Bench surfaces were cleaned prior to use with ethanol, for prevention of any contamination. RO water was filtered through a 10 μm mesh and used for all solutions of samples processed. The jars used for digestion as well as the petri dishes were rinsed three times with filtered RO water and blank control samples for jars taken. All containers were covered with foil whilst samples were being processed. Extraction efficiencies for fibres recorded recovery rates of 90–85% for MPs between 250 and 1000 μm in size.

Statistical analysis was conducted using SPSS (v26). Statistical tests for normality and equal variance was conducted, resulting in neither criterion being met to do parametric analyses. Non-parametric tests were subsequently done using the Mann-Whitney test to compare between two groups and the Kruskal-Wallis for multiple groups. Non-parametric Spearman Rank correlations (r_s) were used to assess relationship between groups. Results are reported as means (±SE) and significances set at $p < 0.05$.

A total of 804 microplastics (MPs) were recorded in 87% of mussels processed from seven retailers sampled in Cape Town, South Africa. Mean MPs for all retailers combined was 3.83 (±0.2 SE) MPs/individual (MPs/i) and 0.04 (±0.003 SE) MPs/g soft tissue wet weight (MP/g), respectively (Fig. 1). Mussels (MPs/i) sampled at wholesalers (N = 3; n = 30; mean = 4.26 ± 0.3 MPs/i) had significantly higher (U = 6530, p = 0.009) MPs/i than mussels from supermarkets (N = 4; n = 40; mean = 3.5 ± 0.2 MPs/i), with no significant differences recorded in average MPs/g between supermarkets (0.04 ± 0.03 MPs/g) and wholesalers (0.04 ± 0.06 MPs/g). The higher concentrations of MPs were significantly negatively correlated to mussel length (mm) when based on weight ($r_s = -0.34$, $p < 0.05$) (Fig. 2a). Mussel length vs MPs/i however was not significantly correlated ($r_s = 0.14$, $p > 0.05$) (graph not indicated). When based on tissue weight (MP/g), MP concentrations were significantly negatively correlated with MP/i ($r_s = -0.53$, $p < 0.05$) (Fig. 2b), but there were no significant correlations between mussel tissue weight and MP/g ($r_s = 0.16$, $p > 0.05$) (graph not indicated).

Analysis of MPs in retailers indicated that mussel MP concentrations were similar across supermarkets, ranging from 2.7 ± 0.39 MP/i at supermarket two to 4.33 ± 0.62 MPs/i at supermarket one (Table S1). The

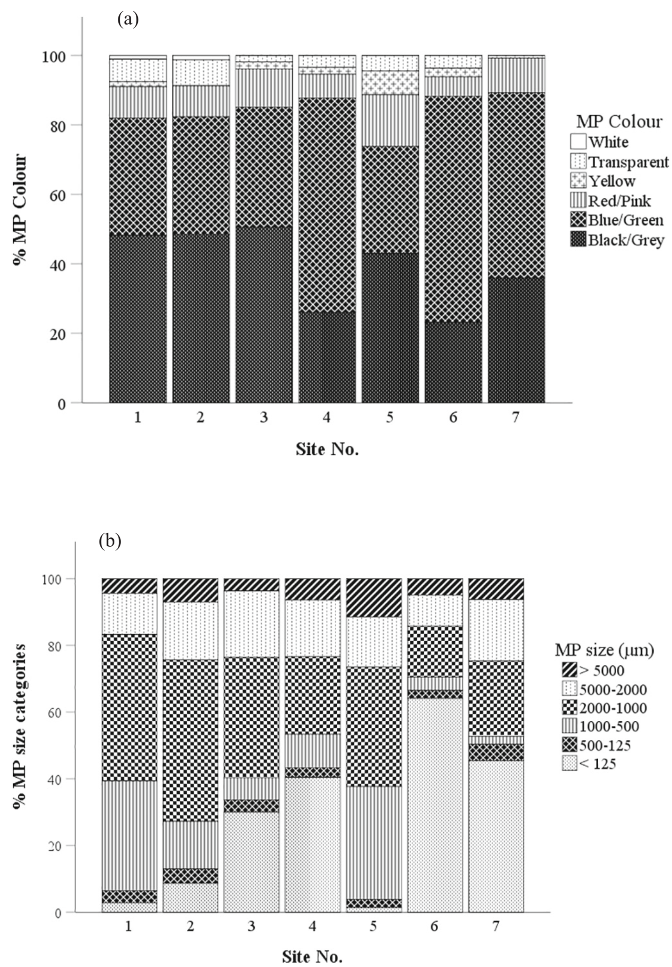

Fig. 4. Percentage colour (a) and size (b) distribution of mussel MPs for all retailers sampled in Cape Town, South Africa.

MP mean abundances at wholesalers ranged from 2.63 ± 0.32 MPs/i at wholesaler seven to 5.59 ± 0.73 MP/i at wholesaler six, the highest concentration of MPs for all retailers sampled. Mussels sampled at supermarket four and wholesaler five were fresh (as opposed to all the remainder mussels sampled being frozen) and there was no significant difference between MPs in fresh (n = 60) and frozen mussels (n = 150) (U = 4391, p = 0.78). The unequal sample size is however noted and the results interpreted with caution.

Of the five MP type categories proposed by GESAMP (2019) - fragments, filaments, foam, pellets and film, only filaments (30%) and fragments (70%) were recorded in mussels from all retailers (Fig. 3a). Based on retail type, supermarket mussels had more filaments (77%) than wholesalers (60%) (Fig. 3b). Supermarket one (94%) and two (88%), as well as wholesaler five (95%) mussels contained more than 80% filaments (Fig. 3c). Based on colour, most MPs in mussels were dark (44% blue/green and 40% black/grey). Analysis of MPs based on colour indicated that for all retailers combined, wholesalers contained more blue/green MPs (50%) than supermarkets (41%), with supermarkets (44%) having higher concentrations of black/grey MPs than wholesalers (34%) (Fig. 4a). There were however no evident differences in MPs colour between supermarkets and wholesalers. Analysis of MPs based on size indicated that most MPs in mussels from all retailers were smaller than 2000 µm. Supermarket one and two and wholesaler five had higher concentrations of smaller MPs (<1000 µm) (Fig. 4b).

Of the 804 MPs recorded, 72% (n = 579) was larger than 500 µm, which was the minimum size sampled for FTIR-ATR polymer type identification. A total of 31 (5%) MPs were scanned for polymer identification with 52% of samples being PET (Fig. 5a) and the remainder of MPs having similar ratios of polymer types. Based on MP type, fragments (n = 7) comprised 57% Latex, 29% PET and 14% PVC. Filaments (n = 24) was comprised of 58% PET, 13% polyester-cotton and cotton, respectively, and the remainder of polymers comprising 4% cellulose acetate, EVA, HDPE and nylon, respectively (Fig. 5b). FTIR scans of examples of polymers analysed are shown in Fig. 6.

To our knowledge, this is the first study in South Africa to investigate the occurrence of MPs in mussels sourced from local retailers (Cape Town). The results confirm the occurrence of MPs in mussels destined for human consumption with concentrations generally being lower than that reported elsewhere for farmed mussels (De Witte et al., 2014; Li et al., 2018a, 2018b, 2018c; Li et al., 2015; Renzi et al., 2018; Van Cauwenberghe and Janssen, 2014) (Table S2).

When comparing wild vs retail mussels, other studies reported higher concentrations of MPs in retail than wild mussels (De Witte et al., 2014; Li et al., 2018a; Renzi et al., 2018). Our results were different to these results as retailer mussels compared to wild mussels collected in Cape Town, South Africa (Sparks, 2020) reported an average of 4.27 MPs/i for wild mussels, compared to 3.83 MPs/i for retail mussels (this study). The differences were even more significant when compered per unit weight. Sparks (2020) reported 2.8 MPs/g in *Mytilus galloprovincialis* compared to 0.04 MPs/g sampled in this study. The differences in MPs between wild vs farmed mussels could be as a result of good farming and processing practices at the mariculture facilities in Saldanha Bay. However, it could also be that the cleaning of mussels at processing plants (away from the mussel farms) could be cleaning mussels of MPs.

Five of the seven retailers sampled indicated that the source of mussels was from Saldanha Bay (Table S1) and it is probable that the remaining two sources of mussels were also from that area. Saldanha Bay is the only site for mussel mariculture in South Africa, producing 3053 tons of mussels in 2019 (Clark et al., 2020). The bay is the only enclosed bay in South Africa and declared an economic development zone for aquaculture and other industrial activities, in particular, the exporting of metal ores (Clark et al., 2020). Of the 1700 tons of mussels produced in Saldanha Bay in 2015, only 102 tons were exported (DAFF, 2016), indicating that the major market for mussels farmed in Saldanha Bay is for the South African market.

Mussel farming in Saldanha Bay started in the mid-1980s and the farming practice takes place by raft culture, where mussels are attached to ropes hanging from rafts (Stenton-Dozey et al., 2001). Although in a controlled setting, mussels hanging from ropes are able to feed on particles in the natural environment. As filter-feeders, mussels feed on prey based on particles of particular sizes in the ambient environment. The ropes and rafts are potential sources of MPs, in particular filaments, as studies reporting on the occurrence of MPs in farmed mussels indicated that filaments were most abundant (De Witte et al., 2014; Li et al., 2015; Renzi et al., 2018), as reported in our study. A study by Li et al. (2018a) reported that PET was the most abundant MP present in supermarket mussels in the United Kingdom, similar to the polymer type reported in this study. Mathalon and Hill (2014) reported that cultured mussels farmed in Canada are processed on polyethylene ropes which are possible sources of MPs for farmed mussels. Polyethylene and other fibrous MPs used in the mussel farming sector, together with other environmental contaminants, should be further investigated to ascertain the sources of MP contamination in these aquaculture farms.

The MPs extracted from mussels were mainly dark in colour (black and blue), suggesting that the MPS were from similar sources. The high prevalence of dark MPs for all samples is similar to results (of predominant black and blue MPs) reported elsewhere (Abidli et al., 2019; Li et al., 2015). Furthermore, Sparks (2020), also recorded a high prevalence of blue and black MPs from mussels in Cape Town, even though these colours were prevalent at lower ratios. It can also be postulated that the uptake of darker MPs could relate to prey selectivity based on colour as it has been previously reported that mussels (filter-feeders) ingest MPs that reflect their prey preference (De Witte et al., 2014). Prey

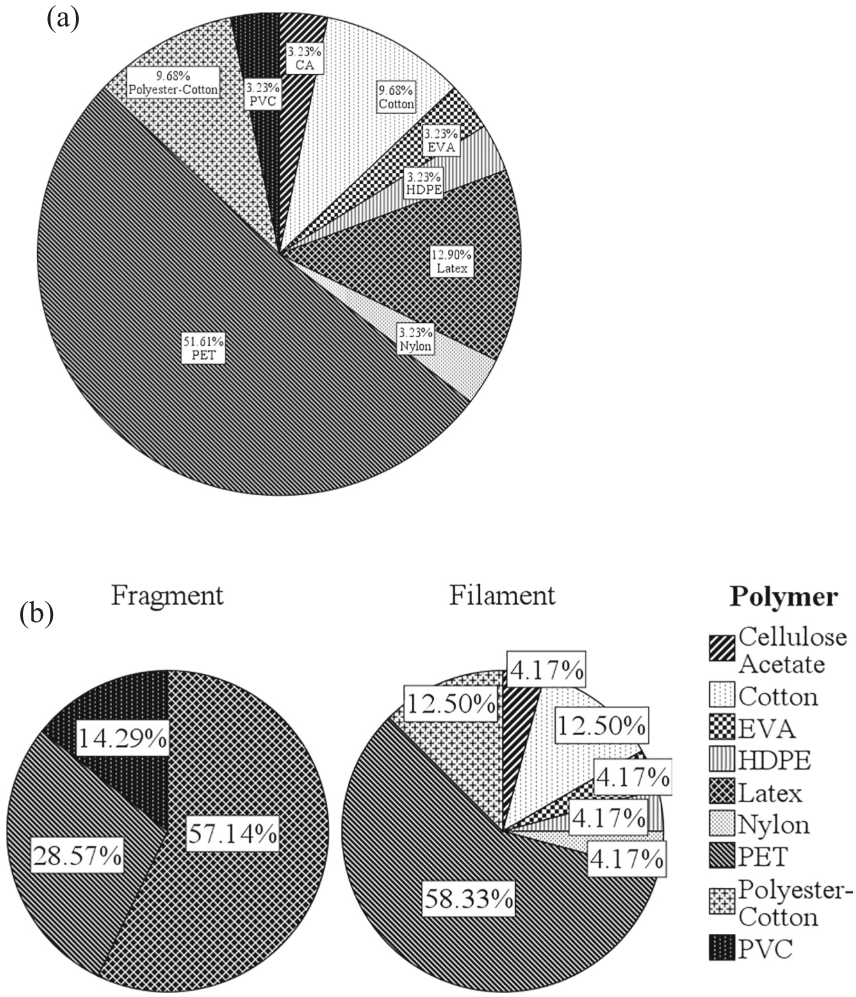

Fig. 5. Percentage polymer types confirmed using FTIR-ATR in retailer mussels sampled (a) and MP type (b) in Cape Town, South Africa.

preference based on colour associations with MPs is however controversial, as other authors have not found evidence to suggest that marine organisms actively take up MPs based on colour (Lusher et al., 2013; Schuyler et al., 2014).

The size of MPs recorded in retail mussels varied for the smaller sized classes and generally all mussels comprised 80% smaller MPs (<2000 μm). MP size category <125 μm was the most abundant size category recorded in mussels sampled from wholesalers (except at wholesaler five). The higher concentrations of smaller MPs (Fig. 4b) at wholesaler five coincides with a higher prevalence of filaments (Fig. 3c). Similar results were also reported by other studies on commercial/retail mussels (Li et al., 2018a, 2018b, 2018c; Li et al., 2015). The higher prevalence of smaller MPs recorded in the mussels coincides with the prey size preference of mussels (De Witte et al., 2014). The polymer type (51% PET) recorded in this study is similar to that recorded elsewhere (Digka et al., 2018; Naji et al., 2018) and indicates the prevalence of this type of plastic consumed by the mussels analysed, since the very low occurrence of contaminants during sample analysis did not influence the type of MP recorded. Of interest is the prevalence of latex (Fig. 5b and Fig. 6) suggesting that protective gear (eg. gloves) used during the handling (at mussel farms) and processing (at factories) of mussels could also be potential sources of MP contamination of retail mussel.

Once in the marine environment, MPs are colonized by microorganisms (including pathogens) that may provide sensory cues to attract MPs to be consumed by marine invertebrates (Harrison et al., 2018). MPs in mussels may then also be vectors of pathogens, antibiotics and other contaminants (such as metals and organic contaminants) that may pose a threat to human health (Naik et al., 2019). Since this analysis was not done in this study, leachate contamination of MPs requires further investigation for mussels consumed in South Africa.

According to Golden et al. (2016), 19% of the global population (estimated at 1.4 billion people) consume more than 20% of the ocean's food supply, indicating the reliance of humans on resources from the ocean. Van Cauwenberghe and Janssen (2014) estimated that when consuming an average portion of mussels (250 g wet weight), a person in Germany consumes approximately 90 MP particles. Based on estimates from our study (0.04 MPs/g), an average portion of mussels (250 g) consumed from South African retailers contains approximately 10 MP particles. With 179 t of mussels produced annually in South Africa (DAFF, 2016), this amounts to a total of 7.2 million potential MP particles (based on MP particles/g) that can be consumed when eating farmed mussels in South Africa. Whether the continuous consumption of MPs could have detrimental effects on human health requires further investigation (Li et al., 2015; Van Cauwenberghe and Janssen, 2014) as it is still unclear whether (or how) MPs and sorbed additives and contaminants are taken up (Bakir et al., 2020).

The study confirmed the occurrence of MPs in mussels sold at retailers in Cape Town, South Africa. Mussels, from the seven retailers were mainly filamentous and fragmented MPs, with filaments comprising 70% of all the MPs found. MP colour was mainly dark and smaller than 2000 μm. Most (if not all) of the mussels were cultured along the west coast of South Africa (Saldanha Bay). Although this study indicates the occurrence of MPs in retail mussels, the concentrations measured were lower than that reported elsewhere. Further

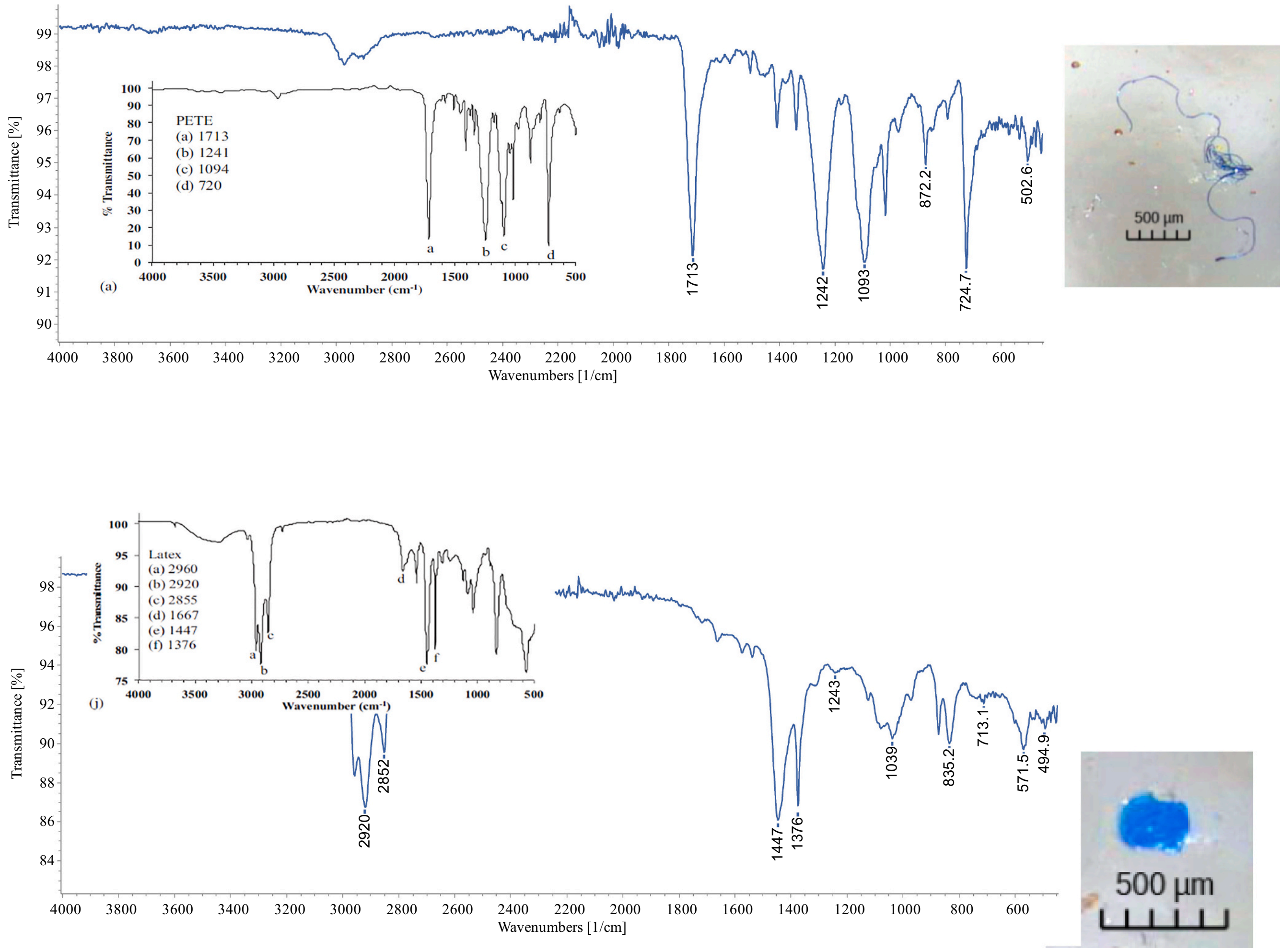

Fig. 6. Polymer identification of two polymers sampled in mussels: a) PET and b) Latex. Inserts of reference polymers from Jung et al. (2018).

investigations are needed to ascertain the effects of MP uptake in farmed mussels, as the occurrence of MPs in farmed mussels may affect growth rates, which in turn may affect mussel aquaculture productivity. Even though this may be the first study in South Africa to report on the occurrence of MPs in mussels destined for human consumption, it is recommended that routine monitoring of MPs takes place for farmed mussels and other seafood products in South Africa.

CRediT authorship contribution statement

Conrad Sparks: Conceptualization, Funding acquisition, Methodology, Investigation, Supervision, Writing – review & editing. **Adetunji Awe:** Methodology, Resources, Investigation. **Jade Maneveld:** Investigation, Writing – original draft.

Declaration of 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2021.112186.

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