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Baseline

The effect of interspecific and intraspecific diversity on microplastic ingestion in two co-occurring mussel species in South Africa

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ABSTRACT

Interspecific and intraspecific diversity are essential components of biodiversity with far-reaching implications for ecosystem function and service provision. Importantly, genotypic and phenotypic variation within a species can affect responses to anthropogenic pressures more than interspecific diversity. We investigated the effects of interspecific and intraspecific diversity on microplastic ingestion by two coexisting mussel species in South Africa, *Mytilus galloprovincialis* and *Perna perna*, the latter occurring as two genetic lineages. We found significantly higher microplastic abundance in *M. galloprovincialis* (0.54 ± 0.56 MP items g^{-1} WW) than *P. perna* (0.16 ± 0.21 MP items g^{-1} WW), but no difference between *P. perna* lineages. Microbeads were the predominant microplastic (76 % in *P. perna*, 99 % in *M. galloprovincialis*) and polyethylene the prevalent polymer. Interspecific differences in microplastic abundance varied across locations, suggesting diverse sources of contamination. We suggest that microplastic ingestion can be species-specific even in organisms that coexist and play similar functional roles within ecosystems.

1. Introduction

Globally, microplastics (MPs; < 5 mm; Arthur et al., 2009) are of great concern due to their multiple negative ecological impacts (Chen et al., 2021; Khalid et al., 2021; Mao et al., 2022; Zhao et al., 2022) and the threats they pose to human health (Carbery et al., 2018; F. Zhang et al., 2020). MPs are the most abundant portion of plastic debris in the world's oceans (Eriksen et al., 2014) and because of their ubiquity, small size and different polymer compositions, are readily bioavailable to a wide range of aquatic organisms, particularly filter feeders (Law, 2017; Botterell et al., 2019; Thushari and Senevirathna, 2020; Wang et al., 2020). The responses of different species to MP ingestion include physical harm, impairment of the immune, reproductive, digestive and endocrine systems as well as toxicological effects such as neurotoxicity and genotoxicity related to the leaching of chemicals (Wright et al., 2013; De Sá et al., 2018; Gunaalan et al., 2020; Agboola and Benson, 2021; Raju et al., 2022).

Bivalves are among the most targeted species in plastic pollution research (Q. Zhang et al., 2020; Bom and Sá, 2021; Li et al., 2021; Ding et al., 2022) and are regarded as sentinel species because of their filter-feeding behaviour, wide distribution and sensitivity to contaminants (Fabbri et al., 2014; Amiard and Amiard-Triquet, 2015; Li et al., 2019). The detection of MPs has been extensively documented in mussels (De Witte et al., 2014; Li et al., 2016; Zhao et al., 2018; Sparks, 2020; Joyce and Falkenberg, 2023) and selective processes occurring during ingestion, translocation and ejection of MPs have been described (Ward et al., 2019; Li et al., 2021 for a review).

In coastal areas, mussels are key habitat engineers that enhance local biodiversity providing nurseries, refuge and feeding areas (Commito et al., 2005; Borthagaray and Carranza, 2007; Gestoso et al., 2013; Lefcheck et al., 2019). They also provide key ecosystem services such as water filtration (Newell, 2004; van Leeuwen et al., 2010), coastal protection (Folkard and Gascoigne, 2009; Borsje et al., 2011; Ysebaert et al., 2019) and food provision (Wijsman et al., 2019). Bivalves are a food

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source for many organisms (Dame and Kenneth, 2011) including humans (Danopoulos et al., 2020); consequently, the ingestion of MPs by mussels can result in bioaccumulation through the food web (Farrell and Nelson, 2013; Santana et al., 2017; Carbery et al., 2018; Santos et al., 2021) with potential repercussions for higher trophic levels.

While current research has primarily focused on the effects of MP ingestion at the species level, there is a growing recognition of the importance of intraspecific variation in understanding the ecological impacts of plastic pollution (Nicastro et al., 2023b). Intraspecific variation includes the genotypic and phenotypic diversity within and among populations of a species. Such diversity plays a crucial role in regulating ecological and evolutionary processes (Palkovacs and Post, 2009; Harmon et al., 2009; Schweitzer et al., 2011; Zardi et al., 2015; Des Roches et al., 2018). Critically, there is evidence that intraspecific variation can have even greater implications for ecosystem function and services than differences among species (i.e., Cardinale et al., 2012; Raffard et al., 2018; Nicastro et al., 2020; Cozzolino et al., 2022). Worldwide, intraspecific diversity is declining due to anthropogenic threats (Hughes et al., 1997; DiBattista, 2008; Willoughby et al., 2015; Leigh et al., 2019) including climate change (Pfenninger et al., 2012; Siefert, 2012; Nicastro et al., 2013; Saada et al., 2016), species introductions (Laikre et al., 2010) and pollution (Rocha-Olivares et al., 2004; Cozzolino et al., 2023 in press). Emerging findings indicate that intraspecific phenotypic diversity plays a role in influencing the degree of MP entrapment and ingestion within biogenic habitats and among reef fish (Nanninga et al., 2020; Cozzolino et al., 2022; Nicastro et al., 2022). Similarly, it has been reported that intraspecific genotypic variation can modulate the behavioural and physiological responses of mussels when exposed to MP leachates (Cozzolino et al., 2023 under review).

Here, we assess the composition and abundance of MPs ingested by the two dominant mussel species in South Africa, the invasive Mediterranean mussel *Mytilus galloprovincialis*, and the indigenous brown mussel *Perna perna*. The former invaded South Africa through a

northeast Atlantic-derived population in the 1970s (Grant and Cherry, 1985; Daguin and Borsa, 2000; Zardi et al., 2018) and rapidly extended its distributional range, now occurring over approximately 3700 km of the shoreline of southern African (Ma et al., 2021). *Perna perna*, is endemic to South Africa where it exhibits two distinct genetic entities generally described as the eastern and western lineages (Zardi et al., 2007a, 2011; Ntuli et al., 2020). The two lineages have different evolutionary histories (Cunha et al., 2014) and physiological adaptations (Nicastro et al., 2010; Zardi et al., 2011; Nicastro et al., 2012), as well as distinct geographical distributions (Zardi et al., 2015). The western lineage (here referred to as PP_{west}) inhabits the coast of Namibia down to the south-east coast of South Africa but is absent from the cold waters of the Benguela system, where it is probably excluded by subtle effects of low temperature on its capacity for competition, or larval survival (McQuaid et al., 2015). On the other hand, the eastern lineage (referred to as PP_{east}) extends from the south-east coast eastwards to the subtropical bioregion up to central Mozambique (Fig. 1). The phylogeographic break between the two genetic lineages lies in the transitional zone between the warm-temperate and subtropical bioregions and is maintained by both local adaptation and limited connectivity through larval dispersal (Zardi et al., 2007a, 2011).

The distributions of the two species (*M. galloprovincialis* and *P. perna*) and the two lineages (PP_{west} and PP_{east}), overlap on the southeast coast (Fig. 1; Zardi et al., 2007b; Barker, 2021) with *P. perna* from both genetic lineages dominating the lower mussel zone, *M. galloprovincialis* the upper, and mixed species beds on the mid shore (Bownes and McQuaid, 2006). The two species show different physiological parameters with *M. galloprovincialis* exhibiting faster feeding and metabolic rates than *P. perna* (Van Erkom Schurink and Griffiths, 1991). Similarly, at the intraspecific level, the eastern lineage of *P. perna* appears to have a higher filtration rate than the western lineage (Berry and Schleyer, 1983; Van Erkom Schurink and Griffiths, 1991). On this basis, we hypothesised that *M. galloprovincialis* would ingest more MPs than

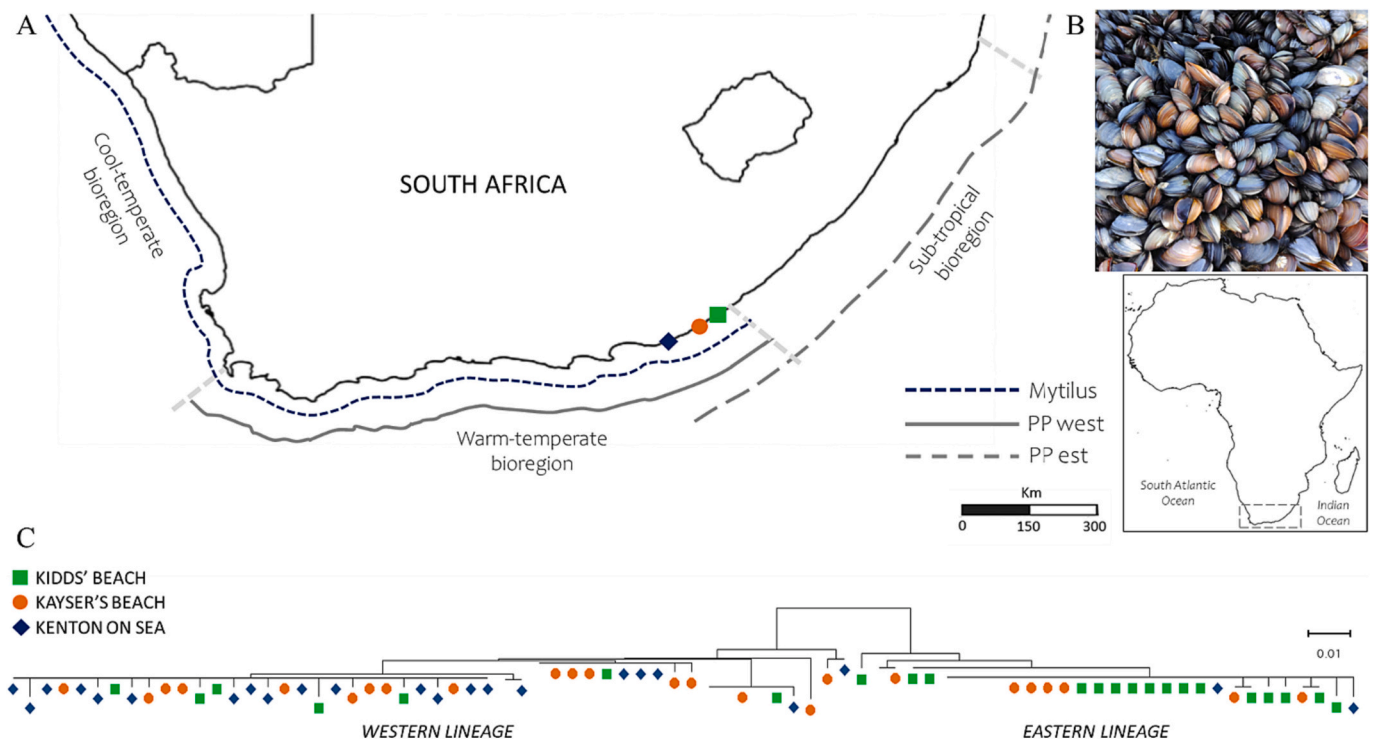


Fig. 1. (A) Study area and sampling locations in the overlapping zone of the distributional ranges of *P. perna* lineages and *M. galloprovincialis* along the South African coastline. Blue dashed line shows the distribution of *M. galloprovincialis*. Solid and dashed grey lines show the distribution of *P. perna* western (PP_{west}) and eastern lineage (PP_{east}) respectively. Dashed borders identify limits of biogeographic regions; (B) mixed bed of *P. perna* and *M. galloprovincialis* at Kayser's Beach; (C) geographic distribution pattern of the mtDNA lineages and neighbour-joining phylogram based on sequences from cytochrome oxidase I (COI). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

P. perna and that the eastern lineage of *P. perna* would ingest more MPs than the western lineage. We tested these hypotheses by comparing the ingestion of MPs (expressed as number of items per gram of wet weight biomass) between species (*M. galloprovincialis* vs *P. perna*) and between genetic lineages (PP_{west} vs PP_{east}).

2. Materials and methods

2.1. Study area and sampling

Samples of *Mytilus galloprovincialis* and *Perna perna* were collected at three locations along a 200 km section on the south-east coastline of South Africa were the two species and the two lineages co-exist (Zardi et al., 2007a). The three locations were Kenton-on-Sea (−33.693619, 26.670574), Kayser's Beach (−33.212563, 27.611679) and Kidd's Beach (−33.142848, 27.705601) and they were sampled during spring low tide in March 2022 (Fig. 1A). These are small coastal towns found at various distances from major urban and industrial areas. Kenton-on-Sea lies approximately halfway between the industrial centres of East London (180 km) and Port Elizabeth (130 km) and it is situated between the Bushmans and Kariega rivers (< 2 km). Kayser's Beach is located about 35 km from East London and next to the Chalumna River (< 2 km). Kidd's Beach is the closest to the urban centre of East London but also the least susceptible to river runoff. In general, the sewage infrastructure in the area is deficient and some wastewater treatment sites have inadequate conveyance and treatment capacity (i.e., Kenton-on-Sea and Kidd's Beach; Buffalo City Metropolitan Municipality Annual Report 2020/21; Green Drop Eastern Cape 2022).

In the field, synthetic clothing was avoided. At each location, mussels were haphazardly collected from the mid shore where both species coexisted intertidally in mixed mussel beds (Fig. 1B). Mussels were placed in sealed plastic bags and transported to the laboratory under dark cool conditions. In the laboratory, mussels were cleaned of sand and frozen at −20 °C intact in their shells to avoid external contamination of the soft tissues. Mussels were not subjected to depuration before analysis in order to measure the absolute MP abundance, including those particles recently ingested or potentially translocated to the tissues (Li et al., 2015).

2.2. Genetic analysis

Since the two lineages of *P. perna* are morphologically identical, all individuals were identified genetically. To do so, total genomic DNA was extracted from approximately 1 mm³ of mantle edge of each individual before analysis. Genetic analysis was carried out following a standard proteinase K and ammonium acetate extraction method adapted from Sambrook et al. (1989). Approximately 650 bp portion of the mitochondrial cytochrome oxidase subunit I (mtDNA COI) gene was PCR amplified and sequenced using the universal primers LCO 1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTG-3') and HCO 2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') from Vrijenhoek (1994). Amplifications were performed in a solution containing 1 µl (10 to 100 ng) of genomic DNA, 5 mM GoTaq Buffer (5×), 0.2 mM of dNTP (Promega, Madison, WI, USA), 1.5 mM MgCl₂, 0.2 mM of each primer and 1 U of GoTaq DNA polymerase (Promega). The PCR cycling profile comprised an initial denaturation step at 94 °C for 2 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 90 s, and a final extension at 72 °C for 5 min (adapted from Zardi et al., 2007a). PCR products were cleaned using purified ethanol/sodium acetate precipitation (Green and Sambrook, 2016) and directly sequenced with the corresponding PCR primers. Sequencing was performed on an Applied Biosystems 3130xl Genetic Analyser, using Sanger technology and the BigDye® Terminator v3.1 kit. MEGA software v6 (Tamura et al., 2013) was employed to construct a rooted neighbour-joining tree to identify the genetic origin of each individual using unique sequences of the eastern and western lineages retrieved from Zardi et al. (2007a;

Table S1).

2.3. Microplastic quantification

Individuals of each species were defrosted and rinsed with pre-filtered ultrapure water (purified by an Elix equipment and filtered through a GF/C Whatman 1.2 µm pore size) to remove potential external contaminants adhered to the shells. The maximum lengths of shells were measured using Vernier callipers (0.01 mm precision) and soft tissue was extracted and weighed (g WW) using a Sartorius microbalance (0.001 g precision), then rinsed again with pre-filtered ultrapure water. A variable number of individuals is often used in digestions to ensure comparable biomass among replicates (e.g., Li et al., 2015, 2016; Teng et al., 2019). Because *Mytilus galloprovincialis* were relatively small, four to six randomly selected individuals with shell lengths ranging between 2.1 and 5.1 cm and wet-tissue biomass ranging from 0.14 to 5.48 (g WW) were pooled to form a composite sample. There were five such samples (mean weight of 8.47 ± 0.21 g WW) per site. We analysed a total of *N* = 70 individuals of *M. galloprovincialis* across the three sites. Individuals of *P. perna*, ranging from 4 to 7 cm shell length and 3.26 to 10.91 g WW were analysed individually to allow subsequent intraspecific analysis.

Soft tissue digestions and MP extractions were conducted using the adapted protocol of Dehaut et al. (2016). Each composite sample of *M. galloprovincialis* was placed in a 250 mL flask and 1.8 M KOH solution was added to digest the organic matter. The solution was covered with aluminium foil and placed in the oven at 60 °C for 24 h. After incubation, the warm solution was filtered (vacuum <5 cm Hg) through a Whatman GF/C glass-fibre filter (diameter 47 mm, 1.2 µm pore size). The resulting filters were placed in glass Petri dishes with lids, dried in the oven at 40 °C for 24 h and thereafter examined for the presence of MPs under a stereomicroscope (BestScope 3044A) operated at 100× magnification. Filters were kept sealed when not in use to avoid airborne contamination. MPs were counted and classified according to their shape (e.g., pellet, fragment, fibre, film, foam, microbead; Frias et al., 2018).

The entire laboratory analysis for MP determination was conducted within a laminar flow cabinet. To limit post-sampling contamination, gloves and 100 % cotton laboratory coats were worn during the process. In addition, all equipment used was non-plastic (i.e., glass or metal), and was rinsed twice with pre-filtered ultrapure water between each sample extraction. To account for possible contamination, a procedural (blank) control (containing KOH solution only) was performed in parallel to each digestion batch, yielding no procedural contamination.

2.4. Polymer identification

To identify polymer types, Fourier-transform infrared spectroscopy (FTIR) analysis was performed on a haphazardly collected subsample (*n* = 15) for each species and lineage. FTIR was conducted in attenuated total reflectance (ATR) mode with air as the background spectrum. All polymers were identified using a Nicolet iN10 Fourier infrared micro spectroscope (Thermo Fisher Scientific Co., Waltham, Massachusetts, USA). All spectra were obtained from 4000 to 600 cm^{−1}. Recorded spectra (Fig. S1) were compared against commercial FTIR spectral libraries (Hummel Polymer and Additives Library and FBI fibre library and PerkinElmer). Identifications were validated when scores higher than 0.75 were obtained.

2.5. Statistical analyses

The statistical analyses comprised two distinct assays, an intraspecific comparison of ingested MPs between genetic lineages of *P. perna* (PP_{west} vs PP_{east}) and an interspecific one, between *M. galloprovincialis* and *P. perna*. Both analyses were performed using the count of MPs as the dependant variable. As the dataset for both *M. galloprovincialis* and *P. perna* was characterised by excessive zero-counts, we applied a generalized linear model (GLM) with a negative binomial law to deal

with overdispersion of the data (using `glm.nb` from the R package MASS, Venables and Ripley, 2002). Further, MP counts per wet weight was accounted for by using an offset term (log of the WW). The GLM used for the intraspecific analysis included lineage and location as fixed factors plus their interaction. The interspecific comparison was performed using a generalized linear model with negative binomial designed with species and location as fixed factors and accounting for their interaction. In both regressions, models with higher AIC values were selected to account for the interaction terms or not. Homogeneity of variances and dispersion were checked using the package Dharma (Hartig, 2022). Where appropriate, Tukey's post-hoc tests were used to perform pair-wise comparisons between factors' modalities using the R package "emmeans" (Lenth, 2023) and using the compact letter display from the R package "multcomp" (Hothorn et al., 2008). Data analyses and visualization were conducted using R Studio 2022.07.0 (R Core Team, 2022).

3. Results

3.1. Genetic analysis

All DNA sequences obtained matched with public records of COI genes in GenBank. High-quality chromatograms showing no variable nucleotide positions or double peaks were identified. The rooted neighbour-joining tree (Fig. 1C) revealed $n = 44$ individuals of *P. perna* belonging to the western lineage and $n = 25$ individuals belonging to the eastern lineage. The abundance of individuals of the western lineage decreased towards east and vice versa for individuals of the eastern lineage (Kenton on Sea: 21 PP_{west} and 2 PP_{east} ; Kayser's Beach: 16 PP_{west} and 7 PP_{east} ; Kidds' Beach: 7 PP_{west} and 16 PP_{east}).

3.2. Microplastic ingestion

Microplastics (MPs) were present in 52 % of eastern individuals and 43 % of western ones. The abundance of ingested MPs was similar across the distinct genetic lineages (Negative binomial glm, $P = 0.99$) and among distinct locations ($P > 0.05$; Fig. 2A; Table S2A). On average, we found values of 0.21 ± 0.25 MP items g^{-1} WW for the eastern lineage and 0.14 ± 0.19 items g^{-1} WW for the western lineage. The most common MPs found were microbeads which accounted for 73.2 % of total debris in PP_{west} and 80 % in PP_{east} . The remainder consisted of microfibrils. No fragments, plastic pellets or foams were detected in the individuals analysed.

To test for the effect of species on ingestion of MPs, all individuals of *P. perna* ($N = 70$) and *M. galloprovincialis* ($N = 70$ individuals pooled in $N = 5$ composite samples per location) were used. MPs were present in 46 % of the *P. perna* mussels analysed (0.16 ± 0.21 items g^{-1} WW in *P. perna*) and 87 % of *Mytilus galloprovincialis* (0.54 ± 0.56 MP items g^{-1} WW). There was a significant interaction term between species and locations, meaning that the species effect differed between locations, and this prevents us from interpreting the effects of species and locations separately. Such significant interaction resulted from the lower difference in MP items g^{-1} WW between *M. galloprovincialis* and *P. perna* at Kidds' Beach compared to Kayser's Beach (Negative binomial glm, $P = 0.00123$, Table S2B). No interaction occurred between species at Kayser's Beach and Kenton's Beach (Negative binomial glm, $P = 0.88$, Table S2B). This is better illustrated by pairwise comparisons (Table 1; Fig. 2). MP items g^{-1} WW were significantly higher in *M. galloprovincialis* than in *P. perna* at Kayser's Beach and Kenton-on-Sea (Tukey's post-hoc test, $P < 0.05$; the mean difference between the two species for these locations was 0.62 MP items g^{-1} WW) but not at Kidds' Beach ($P > 0.05$; Table 1; Fig. 2). Similarly, there were no significant differences in MP items g^{-1} WW between the three locations for *P. perna*

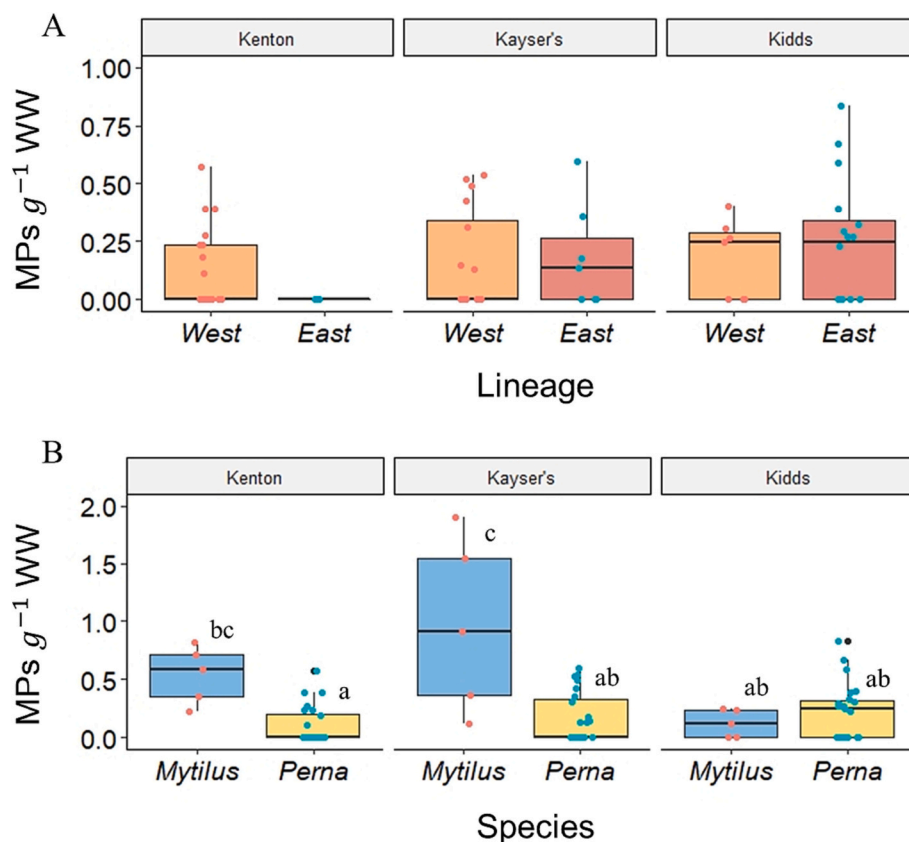


Fig. 2. Microplastic abundance per gram of wet weight biomass. (A) Intraspecific comparison between *P. perna* lineages (B) interspecific comparison between *P. perna* and *M. galloprovincialis* (Means of boxplots not sharing common superscript letters are significantly different, $P < 0.05$, Tukey's post-hoc test).

Table 1

MP abundance per gram wet weight biomass (MP x g WW; mean \pm SD), total number of individuals analysed, total MP items and type (microbead or fibre) found in each species (*Perna perna* and *Mytilus galloprovincialis*) and location ($n = 3$). Means of MP x g WW not sharing common superscript letters are significantly different ($P < 0.05$, Tukey's post-hoc test).

Location	MP x g WW	Total individuals	Total MPs	Microbead	Fibre
<i>Perna perna</i>					
Kenton on Sea	0.10 \pm 0.17 ^a	24	20	16	4
Kaysers' Beach	0.17 \pm 0.21 ^{ab}	23	23	16	7
Kidd's Beach	0.22 \pm 0.24 ^{ab}	23	23	18	5
<i>Mytilus galloprovincialis</i>					
Kenton on Sea	0.54 \pm 0.24 ^{bc}	20	23	23	0
Kaysers' Beach	0.97 \pm 0.75 ^c	20	41	40	1
Kidd's Beach	0.12 \pm 0.12 ^{ab}	30	5	5	0

($P > 0.05$) unlike *M. galloprovincialis* ($P < 0.05$).

Overall, we found $N = 66$ MPs in *P. perna* (76 % being microbeads and 24 % microfibre) and $N = 69$ MPs in *M. galloprovincialis* (99 % being microbeads and only 1 % microfibre). Fourier-transform infrared spectroscopy (FTIR) analysis revealed microbeads to be largely composed of polyethylene (40–53 %) and to a lesser degree, polypropylene (20–33 %) and polystyrene (13–20 %). Interestingly, *M. galloprovincialis* also contained relatively high quantities of alginate microbeads (26 %).

4. Discussion

Our study showed notable variation in microplastic (MP) ingestion between the two mussel species. The invasive *Mytilus galloprovincialis* exhibited higher ingestion rate than the indigenous *Perna perna*, although this effect was location dependent. Our results indicated no effect of intraspecific diversity on the rates of MP ingestion.

Variation in the filtration rate between *M. galloprovincialis* and *P. perna* likely accounts for the differences observed in the ingestion of MP. Previous studies have shown that *M. galloprovincialis* has faster feeding and metabolic rates than *P. perna* (Van Erkom Schurink and Griffiths, 1991, 1993). For instance, filtration rates of a 50 mm *M. galloprovincialis* are around 2,27 l/h compared to 1,32 l/h for a similarly sized *P. perna*. Likewise, filtration rates for a 1 g dry weight individual would be c. 4.1 l.h⁻¹ and 2.61 l.h⁻¹, respectively (Van Erkom Schurink and Griffiths, 1991). Our results for MP abundances in *P. perna* are in line with those described for Southern India (Patterson et al., 2021) but lower than those reported in studies conducted in Brazil (e.g., Santana et al., 2016; Birnstiel et al., 2019). By contrast, the values we report for *M. galloprovincialis* are higher than those found for this species in Europe with average of 0.08 \pm 0.09 MP items g⁻¹ WW in Portugal, 0.16 \pm 0.11 MP items g⁻¹ WW in Italy, 0.11 \pm 0.12 MP items g⁻¹ WW in Spain (Vandermeersch et al., 2015) and 0.23 MP items g⁻¹ WW along the Turkish coasts (Gedik and Eryaşar, 2020), although exceptions exist (e.g., 1.62 MP items g⁻¹ in Spain, Masía et al., 2022). Interestingly, the MP values presented for *M. galloprovincialis* during this investigation are lower than those reported from the Western Cape of South Africa where the species also occurs (2.8 MP items g⁻¹ WW, see Sparks, 2020). However, direct comparison between studies should be treated cautiously. Lack of standardized protocols and quantification units may account for discrepancies (Claessens et al., 2013; Dehaut et al., 2016; Phuong et al., 2016, 2018; Q. Zhang et al., 2020) and, in South Africa, disparity in the degree of urbanisation among South African bioregions can be considerable and may partially explain the different values.

The lack of significant differences in MP ingestion between the

different genetic lineages of *P. perna* was unexpected. From an evolutionary point of view, the two lineages have independent origins and a non-sister relationship (as shown by mitochondrial (COI) and nuclear (ITS) sequence data) across a wide geographic range (Cunha et al., 2014). Most importantly, they show different physiological traits. Laboratory and field experiments have shown that *P. perna* on the east coast (eastern lineage) are physiologically more tolerant of sand inundation and high temperatures than those on the south coast (western lineage) (Zardi et al., 2006b, 2011; Nicastro et al., 2023a). These studies also highlight marked behavioural differences consistent with the geographical and intertidal distributions of each lineage along sharp environmental clines, indicating their strong adaptive significance (Zardi et al., 2006a; Nicastro et al., 2010; Nicastro et al., 2012; Zardi et al., 2015; Barker et al., 2021). In the face of such divergences, our results do not support intraspecific differences in ingestion rates. Direct comparisons of the filtration rates of the different lineages of *P. perna* are currently lacking and the few existing studies conducted on the two different lineages are not comparable due to lack of standardized methodology (Berry, 1978; Berry and Schleyer, 1983; Bayne et al., 1984). Thus, future research efforts should directly compare the filtration rates of the two lineages to better understand the susceptibility of the distinct *P. perna* populations to MP ingestion.

Microplastic pollution is a growing environmental concern worldwide. In South Africa, MP assessments are now available for a number of aquatic organisms (e.g., Reynolds and Ryan, 2018; Nel and Froneman, 2018; Naidoo et al., 2020; Bakir et al., 2020; Sparks, 2020) and in various ecosystems, including estuaries (Naidoo et al., 2015), beach sediments (Ryan and Moloney, 1990; de Villiers, 2019) and sea-surface water (Ryan, 1988). High MP contamination has been reported in the study area along the south-eastern coastline (Nel and Froneman, 2015). In this study, we found differences in the abundance of MPs between species across sampling locations, possibly indicating different anthropogenic pressure and/or variable sources and environmental contamination among sites. Specifically, no significant differences in MP abundance between species were found at Kidd's Beach compared to the other two sites. Such differences are most likely related to the distance of the sampling sites from point sources such as river mouths (Suteja and Purwiyanto, 2022). Noticeably, the primary contributors to plastic pollution are land-based sources including domestic, industrial, and agricultural activities, with rivers, as the main pathway through which plastic debris enters marine ecosystems (Browne et al., 2007; Browne et al., 2011; Driedger et al., 2015; Sun et al., 2019). In our case, while Kidd's Beach is about 25 km away from the mouth of the Buffalo River, the other two sites (Kenton-on-Sea and Kayser's Beach) are next to river mouths (i.e., < 2 km; Bushman's River and Chalumba River respectively) and thus are potentially exposed to higher MP concentrations.

Microbeads and fibres were the only MP type we reported. No foam, fragments, or pellets were found during our experiment. This is only partially in agreement with previous MP assessments in South Africa suggesting fibres (Naidoo et al., 2015; Nel and Froneman, 2015; Nel et al., 2017; Reynolds and Ryan, 2018; Bakir et al., 2020; Sparks, 2020), fragments or pellets (Ryan and Moloney, 1990; Ryan et al., 2018) as the most common MP types (Pereao et al., 2020 for a review). There are no former records of microbeads along the south-eastern coast of South Africa. These are primary MPs commonly employed in personal care and cosmetic products (UNEP, 2015) and enter the aquatic environment through untreated effluents (Fendall and Sewell, 2009; Napper et al., 2015). The presence of rivers and wastewater treatment plants in our study area further suggest this pathway. We found polyethylene and polypropylene to be the most common polymers, which is in agreement with other global reports (Eriksen et al., 2013; Gouin et al., 2015; UNEP, 2015). To the best of our knowledge, this study is the first to report the ingestion of alginate bead by filter feeders. Alginate-based bioplastics exhibit low-toxicity and biodegradability, and find extensive applications in food, pharmaceuticals, and as bio-adsorbents for pollutants (Asghari et al., 2022; Zhang et al., 2022). In light of our results, further

research is needed to understand their persistence in the coastal habitat and their effect on coastal filter-feeders.

Despite considerable evidence of the ecological importance of intraspecific variation, we found no indication that MP ingestion differs between distinct genetic lineages of *P. perna* along the south-east coast of South Africa. We did, however, find significant differences in the relative abundance of MP between our two species, suggesting that MP ingestion is most likely species-specific even in organisms that coexist and play a similar functional role within ecosystems. The implications of our findings extend from the immediate effects of MP ingestion on mussels to the broader consequences at the ecosystem level. In coastal environments, mussels are key ecosystem engineers (e.g., Suchanek, 1985) that provide numerous ecosystem functions and services such as water filtration and nutrient cycling (Karatayev et al., 2002; Newell, 2004; van Leeuwen et al., 2010). MP ingestion in mussels can result in negative impacts such as physical damage and reduced fitness (Von Moos et al., 2012; Détrée and Gallardo-Escárate, 2018; Alnajjar et al., 2021) that could alter mussel filtration efficiency with cascading effects on water quality and nutrient cycling. The species-specific differences in MP ingestion observed during the current investigation may adversely affect the competitive interaction between the two coexisting species, ultimately contributing to shifts in mussel abundance and distribution and consequences on food and habitat availability for other organisms. Such cascading effects would potentially lead to changes in community structure that could affect the overall composition of the intertidal and subtidal habitats. In addition, mussels are the primary target species for artisanal fisherfolk on the South African coast and form an important component of the diet of coastal communities (Fielding, 1996). Although *P. perna* is the main target species and shows less MP contamination than *M. galloprovincialis*, our results raise the possibility of potential risk for human health. To strengthen and validate our field observations, future research should involve direct MP assessments in both species and lineages within a controlled laboratory environment.

CRedit authorship contribution statement

Lorenzo Cozzolino: Conceptualization, Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Katy R. Nicastro:** Conceptualization, Formal analysis, Resources, Writing – review & editing, Funding acquisition, Project administration, Supervision. **Sebastien Lefebvre:** Data curation, Formal analysis, Writing – review & editing. **Luana Corona:** Investigation. **Pierre William Froneman:** Resources, Writing – review & editing. **Christopher McQuaid:** Writing – review & editing, Supervision. **Gerardo I. Zardi:** Conceptualization, Formal analysis, Resources, Writing – review & editing, Funding acquisition, Supervision.

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.

Data availability

Data will be made available on request.

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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.2023.115649>.

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