

# Investigating microplastic contamination and biomagnification in a remote area of South Australia

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## ABSTRACT

**Context.** Microplastics are widespread in aquatic ecosystems and are commonly recorded in water, sediment and a broad spectrum of marine biota. Yet, the extent to which organisms ingest microplastics directly or indirectly by trophic transfer is largely unknown. **Aims.** This study characterises microplastic abundance across intertidal water, sediment, and marine biota species of different trophic levels, and investigates whether biomagnification occurs. **Methods.** Water, sediment, molluscs, crustaceans and fish were sampled from a single area in southern Australia. **Key results.** Microplastics were recorded in 35% of water, 45% of sediment and 39% of biota samples. Plastic load was  $0.36 \pm 0.08$  microplastics  $\text{g}^{-1}$  DW for sediment,  $0.50 \pm 0.17$  microplastics  $\text{L}^{-1}$  for water, and  $0.70 \pm 0.25$  microplastics individual $^{-1}$  for biota. Biomagnification was not found, although similarities in plastic characteristics across biota may imply trophic transfer. Most of the microplastics were fibres (97.5%) of blue, black and transparent colour. Spectral analysis ( $\mu$ -FTIR) indicated that polyester (50%) and polyethylene (42.3%) dominated the polymer compositions. **Conclusions.** There were no significant differences in microplastic contamination among biota species, with no biomagnification identified. **Implications.** We provide information on biomagnification of microplastics alongside a still uncommon characterisation of contamination in water, sediment and biota.

**Keywords:** biomagnification, biota, contamination, marine debris, microplastic, plastic pollution, southern hemisphere, trophic transfer.

## Introduction

Plastic pollution has become a pressing global issue in recent decades, with the United Nations labelling it as one of our most significant environmental challenges (United Nations Environment Programme 2014, 2021). Plastics have become an integral part of the modern world because of their durability, persistence, low cost and versatility (Hamilton et al. 2021). Over time, if not properly managed, plastic waste can be transported to the marine environment where it breaks down into different-sized plastics from macro-, meso- to micro- (<5 mm in size) and even nanoplastics, under the influence of ultra-violet radiation, photo-oxidative reactions and mechanical forces (Worm et al. 2017). Microplastics of different size, shape and density have been identified in biota of all trophic levels, from filter feeders to deposit feeders, and from small primary consumers to top predators (Cole et al. 2013; Lusher 2015; Nelms et al. 2018; Miller et al. 2020; Sarker et al. 2022). Once ingested, microplastics may induce ecotoxicological risks and physical damage, as well as potentially desorbing a cocktail of associated chemicals to biological tissues (Wright et al. 2013; Huang et al. 2021). Despite this, knowledge of the risks that microplastics pose to biota is still limited.

Many studies focus on the ingestion and abundance of microplastics in individual species (Soo et al. 2021) or relative to the environment (e.g. bioconcentration). However, microplastics can also be ingested indirectly as a result of trophic transfer and biomagnification, whereby contaminated prey items are consumed by predators, and contamination is higher than that of the prey (Farrell and Nelson 2013). Few studies have evaluated the occurrence

of biomagnification of microplastics across broad food webs and trophic levels in the wild (but see e.g. Covernton et al. 2022), with most undertaken in experimental laboratory conditions (Farrell and Nelson 2013; Nelms et al. 2018; Costa et al. 2020). These studies have shown mixed results of trophic transfer and biomagnification, likely because exposure conditions can be unrealistic compared with what occurs naturally in the wild, research questions vary, and few studies quantify multiple trophic levels and organisms (Miller et al. 2020). Furthermore, the extent of microplastic biomagnification in predators from ingested prey is unresolved because of unknown ingestion, retention, egestion or depuration rates (Chagnon et al. 2018; Wang et al. 2021; Covernton et al. 2022). Ingestion is a major pathway of microplastic uptake in aquatic biota; therefore, a broad understanding of the contamination of the surrounding environment (e.g. water, sediment), together with information across food webs, will be key to determining whether feeding habits, species-specific traits, or trophic levels pose different microplastic exposure risks. There is still a lack of understanding as to the fate and cycling of microplastics within complex and interconnected food webs and ecosystems (Nelms et al. 2018).

Microplastic ingestion has been reported globally, including in wild bivalve molluscs collected along South Australia's coastline (Klein et al. 2022), and in South Australian seafood species sold for human consumption (Wootton et al. 2021a; Ogunola et al. 2022). However, there is still limited research worldwide characterising multiple marine matrices (e.g. water, sediment and biota) within the same location (but e.g. Kazour et al. 2019; Miller et al. 2023). In addition, there are no microplastic data recorded for the coastal marine areas of the Yorke Peninsula region and the western area of Gulf Saint Vincent (South Australia). We aimed, first, to analyse microplastic contamination across water, sediment and marine biota from different trophic levels, and, second, to investigate whether biomagnification of microplastics (using trophic magnification factors) was occurring in these coastal environments. Namely, we sampled invertebrates such as gastropods, bivalves and decapod crustaceans, as well as fish. Overall, we characterised microplastic contamination in three environmental matrices, in an important, yet understudied, region of the southern hemisphere, and increased our knowledge of biomagnification potential in wild conditions, across a wide number of species and trophic levels.

## Materials and methods

### Study area

The study was conducted at Black Point, a coastal site on the Yorke Peninsula, (Fig. 1) located in the south-east of South Australia. Black Point is in a remote, rural area, 175 km from the state capital city of Adelaide, and is characterised by a

protected bay and long sandy beach with a low human population (<100 inhabitants, in 2016 Australian census), although population numbers increase during the summer months.

### Sample collection

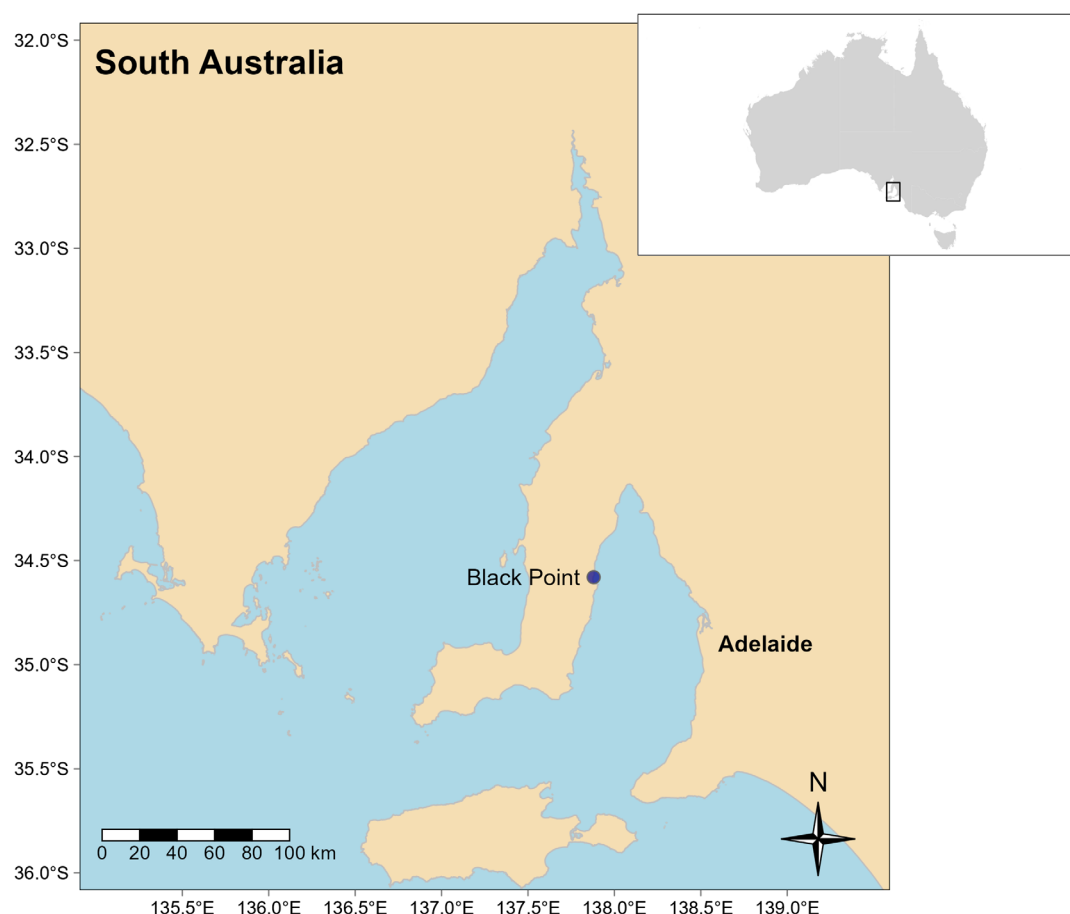
In March 2022, surface-water samples ( $n = 20$ ) were collected from the intertidal zone by using 1 L of pre-washed polypropylene plastic containers. Surface sediment ( $n = 40$ ) was collected from the top layer (~5 cm) of the benthic intertidal environment at a water depth of <20 cm, into 70-mL polypropylene sampling containers (i.e. the top layer of the sediment was displaced towards the container with added caution). Water and sediment samples were collected randomly in the intertidal environment (>50 m from the high tide line) across a 100-m radius. All samples were transported on ice and stored frozen ( $-5^{\circ}\text{C}$ ) until further analysis.

At the same time and location where the sediment and water were collected, a total of 145 marine macro-benthic invertebrate and vertebrate biota was sampled. Yellowfin whiting (*Sillago schomburgkii*,  $n = 18$ ) and blue swimmer crab (*Portunus armatus*,  $n = 18$ ) were collected with seine net and rod and line. Other fauna, including razorfish (*Pinna bicolor*,  $n = 49$ ), mussel (*Brachidontes* spp.,  $n = 51$ ) and black nerite snail (*Nerita atramentosa*,  $n = 9$ ) were collected by hand. Fish were euthanased using the Ike Jime method (ethics approval, S-2020-090 from The University of Adelaide and Ministerial exemption ME9903203) and all biota were individually wrapped in foil and placed immediately on ice. In the laboratory, total weight and total length (including shell or carapace) were measured and recorded (Table 1). All biota samples were then rinsed with ultrapure water (Milli-Q Advantage A10, filter 22  $\mu\text{m}$ ). Whole soft tissue contents of nerite snail, mussel and razorfish and gastrointestinal tracts of blue swimmer crab and yellowfin whiting were dissected and extracted with forceps, weighed, wrapped with aluminium foil and stored frozen ( $20^{\circ}\text{C}$ ) for subsequent microplastic analyses (Karlsson et al. 2017).

### Microplastic extraction

Each replicate water sample was filtered onto 25- $\mu\text{m}$  stainless steel Hollander woven mesh filters in a filtration connected to a vacuum pump (Williams et al. 2020; Cutroneo et al. 2021). All filters were transferred to pre-cleaned glass jars to dry at room temperature ( $\sim 21^{\circ}\text{C}$ ) and were examined under a dissecting stereo-microscope (Leica M80 with integrated IC90E camera, magnification 7.5 $\times$  to 60 $\times$ ).

A subsample of 2 g was weighed from each replicate sediment sample and freeze-dried in glass centrifuge tubes. To remove organic matter, Fenton's reagent (10 parts ferrous heptahydrate,  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ; 10 parts 30% hydrogen peroxide,  $\text{H}_2\text{O}_2$ ; and 1 part hydrochloric acid, HCl) was added to the dried sediment samples. The samples were left overnight



**Fig. 1.** Map of study location (Black Point) in South Australia, Australia.

**Table 1.** Biota examined for microplastic analysis for biomagnification.

Species	Animal type	Trophic level, trophic position	Sample size (n)	Body weight (g)	Shell, carapace or body length (cm)	Tissue or gut weight (g)	MP items (individual <sup>-1</sup> )	MP items (g <sup>-1</sup> WW)
Nerite snail	Gastropod	2, primary consumer <sup>A</sup>	9	29.91 ± 15.02	3.83 ± 0.88	5.49 ± 2.75	1.33 ± 0.58	0.48 ± 0.31
Mussel	Bivalve mollusc	2, primary consumer <sup>B,C</sup>	51	0.78 ± 0.43	1.53 ± 0.08	0.13 ± 0.02	0.39 ± 0.12	3.71 ± 1.22
Razorfish	Bivalve mollusc	2, primary consumer <sup>B,C</sup>	49	132.55 ± 5.72	21.16 ± 0.38	19.18 ± 0.92	0.57 ± 0.11	0.03 ± 0.01
Blue swimmer crab	Decapod crustacean	2.97, secondary consumer <sup>B,D</sup>	18	195.59 ± 11.84	6.66 ± 0.07	14.50 ± 1.03	0.56 ± 0.20	0.04 ± 0.02
Yellowfin whiting	Fish	3.2, tertiary consumer <sup>E</sup>	18	181.22 ± 9.89	35.51 ± 0.58	15.24 ± 1.42	0.67 ± 0.25	0.05 ± 0.02

Morphometrics and microplastic loads are means ± s.e. MP, microplastic. Trophic level was obtained from the literature.

<sup>A</sup>Zhang *et al.* (2017).

<sup>B</sup>Duarte *et al.* (2009).

<sup>C</sup>Wijsman *et al.* (2019).

<sup>D</sup>Goldsworthy *et al.* (2017).

<sup>E</sup>FishBase, R. Froese, and D. Pauly, see <http://www.fishbase.org>.

(12 h), and then 10 mL of zinc chloride (ZnCl<sub>2</sub>) was added to each sample to stop the reaction. Reacted sediment samples

were centrifuged for 20 min (15°C, 500g) and filtered (as per water samples). The 25-µm stainless steel Hollander woven

mesh filters were similarly pretreated and examined under the dissecting microscope (Williams et al. 2020; Cutroneo et al. 2021; Reineccius et al. 2021).

For biota samples, soft tissue (razorfish, mussels, nerite snails), or gastro-intestinal tract (blue-swimmer crabs, yellowfin whiting) of each individual was transferred to a large glass beaker and digested with 10% potassium hydroxide solution (KOH) (Rochman et al. 2015). The added solution was at least three times the volume of biological material, and the glass beakers were covered with aluminium foil to prevent any air contamination. Aluminium foil never came in contact with KOH or digested solution. The samples were heated for 8–12 h at 60°C in the oven, as recommended by Dehaut et al. (2016) and Lusher et al. (2017a). The use of 10% KOH at 60°C is an efficient and effective method to digest biota for microplastic analysis (Dehaut et al. 2016). The recovery rate of using KOH at this temperature is suitable when using short digestion times (Karami et al. 2017; Prata et al. 2019), with the added benefit of facilitating global comparisons to studies that use this methodological approach (Lusher et al. 2017a; Hartmann et al. 2019). The resultant solution was filtered through stainless steel sieves of 1 mm and 38 µm. Microplastics retained on each of the sieves were examined under the dissecting stereo-microscope (Ogunola et al. 2022). Overall, for all sample types, microplastics in the filters or sieves were visually examined, manually scanning the whole sample, with potential microplastics identified, counted and information on shape (fibre, film or fragment) and colour was recorded, following methods and criteria in Hidalgo-Ruz et al. (2012).

### Spectroscopic analyses and identification

Polymer identification was conducted with attenuated total reflectance Fourier transform infrared spectroscopy (µ-FTIR; Bruker Hyperion). A subset of 20% of the suspected microplastics (including 20% of the portion of microplastics from each of the sediment, water and biota samples) was randomly selected for µ-FTIR analysis following the recommendations for spectral analysis (Lusher et al. 2017b; Wootton et al. 2021a). This step was undertaken primarily to confirm and validate that samples collected as putative microplastics were plastic. The FTIR spectrum of each potential microplastic piece was recorded using an average of 64 scans in the range of 3900–650 cm<sup>-1</sup> with the atmospheric water CO<sub>2</sub> region between 2500 and 1900 cm<sup>-1</sup> excluded when compared with the BRUKER Hyperion ATR spectral library for polymers. A threshold score of at least 70% (hit quality) was used to ensure the reliability of the identification of microplastics (Wootton et al. 2022; Zhang et al. 2022).

### Quality control and assurance

Quality control procedures were undertaken to limit the risk of external contamination during field sampling and laboratory analysis. All sampling and storage containers were

cleaned, triple rinsed with ultrapure water and dried in a laminar flow before use. During field sampling, containers were fully submerged beneath the surface prior to being opened, so as to limit their exposure to open air. In the laboratory, all dissecting tools and bench surfaces were wiped with 70% ethanol and lint-free wipes (Kimtech Science Kimwipes) between sessions. Dissection tools were triple rinsed with ultrapure water and dried in a laminar flow and covered with aluminium foil before use, as was all glassware, such as beakers. All chemicals and solvents were filtered before use with 25-µm Stainless steel Hollander mesh filter. Sample processing was conducted in a laminar flow to exclude external contamination. Field and laboratory procedural blanks and airborne contamination controls (i.e. open vials with water and no tissue) were used to check for any background contamination and were processed, sieved and examined under the microscope in the same manner as other samples (Wootton et al. 2021a). Although polypropylene containers were used to collect sediment and water samples, they were thoroughly cleaned and rinsed prior to use (see above), and blank controls were run to check for contamination. Additionally, polypropylene was found only in biota samples, where no polypropylene was utilised. Throughout the sample preparation, natural clothing and bright pink polyester laboratory coats were worn, as well as blue nitrile gloves, to control for potential cross contamination from the operator's garments. No contamination occurred in any of the dissection, digestion, or microscopy control samples.

### Data analysis

Microplastic load is reported in water as the number of microplastic items per litre of water filtered and in sediments as microplastic items per gram of dry sediment (MPs g<sup>-1</sup> DW). The frequency of occurrence of microplastic was also estimated (i.e. percentage of samples with at least one piece of plastic). In biota, data are presented as the frequency of occurrence of microplastic, and as the average number of microplastic items both per individual and per gram wet weight (MPs g<sup>-1</sup> WW) to facilitate comparisons across the literature. Single-factor permutational multivariate analysis of variance (PERMANOVA) was used to compare plastic loads in biota.

The biomagnification potential of microplastics was determined using trophic magnification factors (TMF) testing the relationship between trophic levels (TL) and microplastic abundance (MP). TMF evaluates the change in contaminant concentrations per trophic level, assuming that the diet is a major route of exposure to microplastics, and trophic level a main driver of the accumulation of microplastics in organisms and food webs. Trophic magnification factor (TMF) was calculated using an approach similar to that used in previous studies (Borgå et al. 2012; Diepens and Koelmans 2018; Alava 2020). For broader comparison purposes, we use microplastics per organism and microplastics per weight approaches, with



the latter being better suited for comparisons among differently sized organisms. First, a linear regression of microplastic abundance and species trophic levels was expressed by the following equation:

$$MP = a + bTL$$

where TL represents the trophic level of the organisms and the intercept ( $a$ ) represents the baseline of microplastic abundance in the environment. The trophic magnification factor (TMF) along the food web, was then estimated as the antilog of the slope ( $b$ ) as follows:

$$TMF = 10^b \text{ or } e^b$$

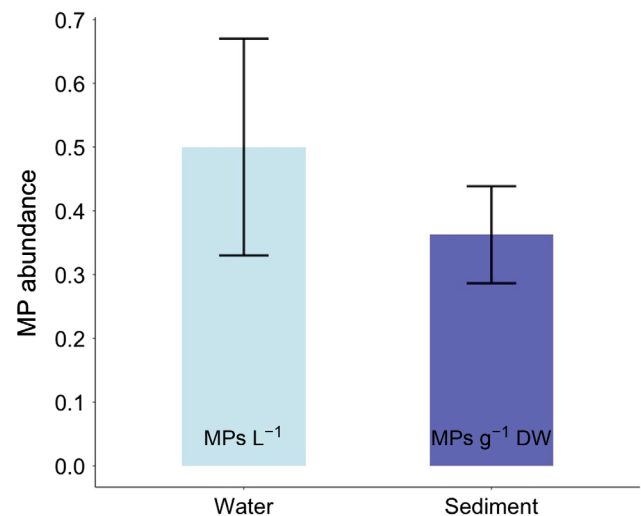
whereby a positive slope results in a TMF value of  $>1$  and indicates that microplastics are biomagnifying, whereas a zero or a negative slope indicates that microplastics are not biomagnifying (Borgå *et al.* 2012).

Species trophic levels were derived from values obtained from FishBase (see <http://www.fishbase.org>) and previous studies using stable nitrogen or carbon isotope ratios of diet/tissue analysis (Borgå *et al.* 2012; Zhang *et al.* 2022). Data analysis was performed in R (ver. 4.1.1, R Foundation for Statistical Computing, Vienna, Austria, see <https://www.r-project.org/>) with 'car' (ver. 3.1-2, see <https://CRAN.R-project.org/package=car>; Fox and Weisberg 2019), 'Rmisc' (ver. 1.5.1, R. M. Hope, see <https://cran.r-project.org/package=Rmisc>), 'ggplot2' (ver. 3.4.2, see <https://CRAN.R-project.org/package=ggplot2>; Wickham 2016), 'ggpubr' (ver. 0.1, A. Kassambara and M. A. Kassambara, see <https://cran.r-project.org/package=ggpubr>), 'dplyr' (ver. 1.1.2, H. Wickham, see <https://github.com/tidyverse/dplyr>), 'tidyverse' (ver. 2.0.0, see <https://cran.r-project.org/package=tidyverse>; Wickham *et al.* 2019), 'raster' (ver. 3.6-20, R. J. Hijmans, J. Van Etten, J. Cheng, M. Mattiuzzi, M. Sumner, J. A. Greenberg, O. P. Lamigueiro, A. Bevan, E. B. Racine and A. Shortridge, see <https://cran.r-project.org/package=raster>) and 'sf' (ver. 1.0-12, see <https://cran.r-project.org/package=sf>; Pebesma 2018) packages. PERMANOVA analyses were calculated using PRIMER software (ver. 7, see <https://www.primers-e.com/>).

## Results

### Microplastics in water and sediment

Microplastics in surface water and sediment at Black Point averaged  $0.50 \pm 0.17$  microplastics  $L^{-1}$  of water and  $0.36 \pm 0.08$  microplastics  $g^{-1}$  DW of sediment (mean  $\pm$  s.e.) (Fig. 2). In total, 45% of the sediment samples (18 of 40 samples) had microplastics present, whereas only 35% of the water samples (7 of 20) were contaminated.

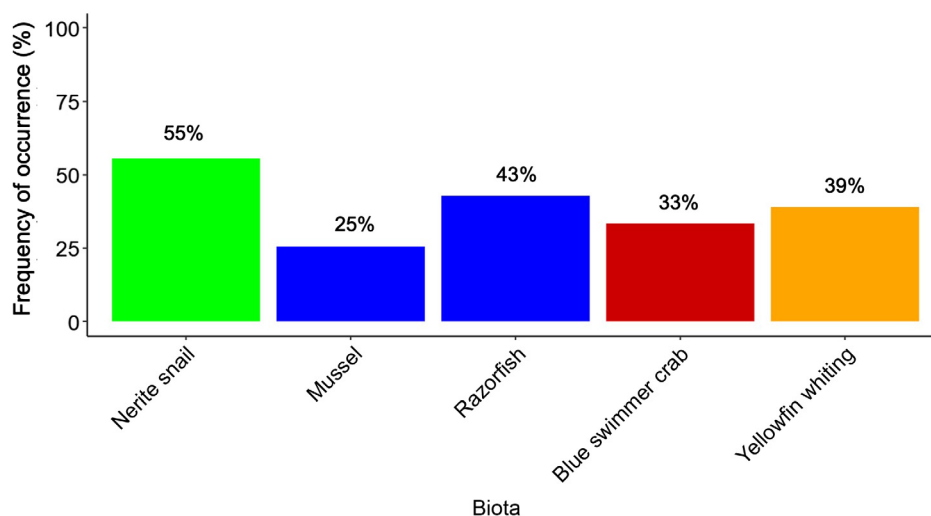


**Fig. 2.** Average abundance ( $\pm$ s.e.) of microplastics (MP) in water and sediment samples. Note that water is measured as microplastics per litre, and sediment as microplastics per gram of dry weight.

### Microplastics in biota

Microplastics were found in all biota species. The frequency of occurrence was 35.8% (52 of 145 samples) across all species, although there were variations among species. Nerite snails (55%) and mussels (25%) had the highest and lowest frequency of occurrence of microplastics respectively. The frequencies of razorfish, yellowfin whiting, and blue swimmer crab varied between 33 and 43% (Fig. 3). Similarly, the average number of items of microplastic per individual was highest in nerite snail (1.33 microplastics, range 0–5), which was more than double the amount found in yellowfin whiting, blue swimmer crab, and razorfish, with 0.67 (range, 0–4), 0.57 (range, 0–2) and 0.56 (range, 0–3) microplastics per individual respectively. Mussels had the lowest microplastic abundance, with 0.39 (range 0–5) microplastics per individual (Table 1, Supplementary Fig. S1). No significant differences were observed between species for microplastic abundance (PERMANOVA,  $F_{4,140} = 1.97$ ,  $P = 0.08$ , Supplementary Table S1). Consequently, no biomagnification was evident for microplastic abundance per individual (slope = 0.01,  $R^2 < 0.01$ , TMF = 1.0).

When weight was considered, mussels had the highest average level of microplastic (3.71 microplastics  $g^{-1}$  WW), followed by nerite snails (0.48 microplastics  $g^{-1}$  WW). This is likely driven by the low bodyweight of the mussels and the few specimens that had up to five microplastic pieces per individual (Table 1, Fig. S1). Significant differences were recorded among species (PERMANOVA,  $F_{4,140} = 4.04$ ,  $P = 0.01$ , Table S1). Although mussels had the highest average level of microplastics per gram wet weight, they were only significantly higher than razorfish (Table 1). This high contamination per wet weight was driven by a few individuals having concentrations of up to five microplastics

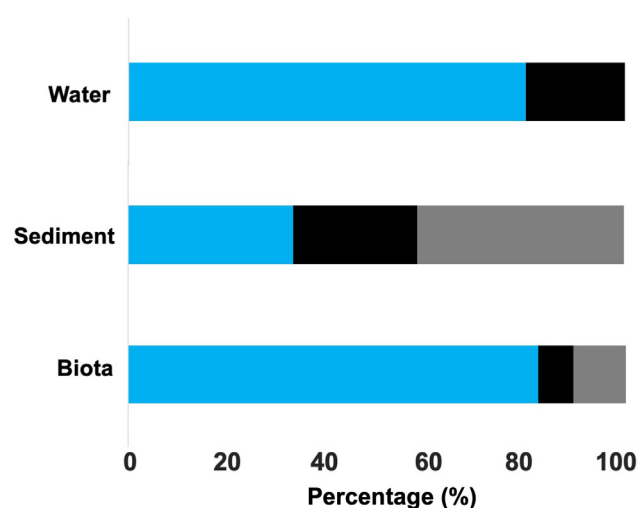


**Fig. 3.** Frequency of occurrence of microplastic ingestion for biota species: gastropods (green); bivalve mollusc (blue); decapod crustacean (red); fish (orange).

per specimen as well as low individual size or weight (Fig. S1). Additionally, nerite snail had significantly higher numbers of microplastics per gram wet weight than did razorfish, blue swimmer crab and yellowfin whiting (Table S1). When considering all species, the slope was negative and thus the TMF suggested dilution (slope =  $-0.12$ ,  $R^2 = 0.02$ , TMF = 0.76). However, this value was strongly driven by mussels, and in particular the few individual mussels that had high values (Fig. S1b). When mussels were removed, the TMF value was close to one (slope =  $-0.01$ ,  $R^2 = 0.01$ , TMF = 0.98), suggesting no biomagnification or dilution occurred.

### Characteristics of microplastics across environmental and biota samples

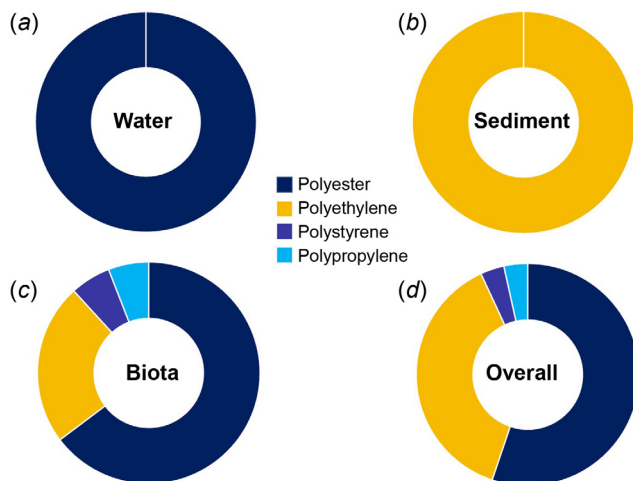
The shapes of the microplastics in this study were dominated by fibres (97.5%), with the remaining pieces being fragments, with the latter found only in higher trophic-level organisms (two fragments in yellowfin whiting and one fragment in a blue swimmer crab). There were three colours of microplastic fibres, namely, blue, black and transparent. There were differences in the colour of microplastics found in the different environmental matrices. Microplastics in water and biota were mostly blue (80 and 82.4% respectively), whereas in sediments 41.7% of pieces were transparent, followed by 33.3% blue and 25% black (Fig. 4). Comparing across individual species, blue microplastics dominated with ratios (75–90%) closely matching those of blue microplastics in the water (Fig. S2). We found transparent microplastics (fibres), matching those found in the sediment, only in three species (i.e. nerite snails 8.3%, razorfish 10.7% and yellowfin whiting 16.7%). Microplastic pieces were all  $>300\ \mu\text{m}$ , with only five pieces (i.e. 4% of total) larger than 1 mm



**Fig. 4.** Percentage of the microplastic colours found in water, sediment and biota ( $n = 7, 18$  and  $52$  respectively). The colours shown in the figure represent the colours of the microplastic (blue, black and transparent).

and found in water and biota samples (mussel, nerite snail and razorfish).

When further identifying the microplastics under the  $\mu$ -FTIR, four polymer types were identified, with clear differences among environmental matrices. All microplastics tested were verified as plastic. All the microplastics tested from water were identified as polyester, whereas the pieces tested from sediment were all polyethylene (Fig. 5). Biota had a large portion of polyester (64.7%), followed by polyethylene (23.5%). One piece of polypropylene and one of polystyrene were found in blue swimmer crabs and yellowfin whiting respectively, with polyester being dominant across species (Fig. 5, S3).



**Fig. 5.** Polymer composition of microplastic identified using the  $\mu$ -FTIR in (a) water, (b) sediment, (c) biota, and (d) combined polymer composition of all matrices. Number of microplastics tested; water = 4, sediment = 7, biota = 17.

## Discussion

This study has reported the occurrence of microplastics in surface water, sediment, and biota from three different trophic levels in an area with limited urban impact. The results showed that contamination was generally low, and although fibres dominated, polyester was most prevalent in water, polyethylene in sediment, and biota had a mix of polymers mostly reflective of their immediate environment. The shape of microplastic pieces across biota was dominated by fibres, also matching findings in both the sediment and water. The same three colours were found across biota, water and sediment, but blue dominated in water and biota, implying a likely route for uptake. Microplastics were found in all the sampled species, including gastropods, bivalve molluscs and fish, spread across different trophic levels and feeding strategies. Although there were variations in the frequencies of occurrence and abundances of microplastics, we found no indication of trophic magnification of microplastics across this food web. Species from the lower trophic level had both the highest and lowest microplastic loads per individual, namely, the nerite snail and mussels respectively. Yet, both nerite snails and mussels had microplastic loads (microplastics  $\text{g}^{-1}$  WW) several times higher than in the other sampled species, with size, ecological and species-specific traits likely playing a role (Miller *et al.* 2020; Covernton *et al.* 2022).

Microplastic abundance in the water at Black Point (0.50 microplastics  $\text{L}^{-1}$ ) was lower than contamination found in intertidal water across other coastal regions of South Australia, and which ranged from 2.9 to 16.3 microplastics  $\text{L}^{-1}$  (Klein *et al.* 2022). However, levels of microplastic in sediment (average 0.36 microplastics  $\text{g}^{-1}$  DW)

were higher than in coastal areas on the eastern coast of Australia, where microplastics ranged from 0.08 to 0.35 microplastics  $\text{g}^{-1}$  DW (Jahan *et al.* 2019). The only other study sampling South Australian marine sediments with comparable sampling approaches and units to our study focused on deep offshore oceanic sediment where between 0 and 13.6 microplastics  $\text{g}^{-1}$  DW were recorded (Barrett *et al.* 2020). When comparing the contamination of biota from Black Point to other regions, the frequency of occurrence was expectedly low and matched values observed both in Australia and worldwide, namely in regions with lower human presence. For example, 35.5% of fish (Wootton *et al.* 2021a) and 48% of crustaceans (Ogunola *et al.* 2022) across Australian marine waters had microplastic present; however, up to 92% of mussels (Klein *et al.* 2022) and 100% of oysters (Jahan *et al.* 2019) sampled from coastal urban areas contained microplastic. Likewise, these trends match global microplastic contamination, where frequency of occurrence and plastic load is generally higher in more populated and urban regions than in isolated areas (Jang *et al.* 2014; Gola *et al.* 2021). In addition to human activity, other factors such as currents, winds and hydrodynamics will also contribute to the spatial variations across regional areas in South Australia (Reisser *et al.* 2013; Klein *et al.* 2022; Ogunola *et al.* 2022; Leterme *et al.* 2023). Nonetheless, accurate comparison of microplastic loads across studies is still difficult because of a wide variety of sampling, laboratory, and quality-control methods (Provencher *et al.* 2020a; Wootton *et al.* 2021b). Multiple factors including variations in collection methods, sieve size, sample size, use of chemical digestion, units of measurement, contamination control and validation of polymer identification hamper robust comparisons (Provencher *et al.* 2020a; Omeyer *et al.* 2022). This is a major issue across microplastic research. It is important to work towards harmonised and reproducible approaches for sampling and analysis of microplastics to increase repeatable and comparable data.

The majority of the microplastics observed were fibres (98.8%), which is consistent with the prevalence of fibres in coastal and marine environments globally (Barrows *et al.* 2018; Ferreira *et al.* 2020; Hamilton *et al.* 2021). Laundry of textile clothes or fabrics in washing machines has been recognised as a major source of contamination of fibres (e.g. polyester) to aquatic environments in effluent or wastewater (Napper and Thompson 2016; Hernandez *et al.* 2017; De Falco *et al.* 2019). Although the level per litre of contamination of treated wastewater is generally low (e.g.  $\sim 1$  microplastics  $\text{L}^{-1}$ ) (Browne *et al.* 2011; Ziajahromi *et al.* 2021), because of the large volumes of discharge, wastewater plants can be an important pathway to environmental contamination. Estimates of effluent loads from treated wastewater in Australia can reach up to 133 million microplastics ( $>25 \mu\text{m}$  in size) per day (Ziajahromi *et al.* 2021). Despite the low values of microplastic found in this study, communities such as Black Point have seasonal increases in human population over the summer months,

and it is worth investigating whether these fluctuations in population density are reflected in the local environment (Klein *et al.* 2022), together with a potential increase in recreational fishing, considering fishing gear may also be a source of microplastic fibres such as polyethylene (Andrady 2011; Silva-Cavalcanti *et al.* 2017).

All individuals were collected from Black Point and were likely to have been exposed to similar environmental conditions. Although there were variations in microplastic load (per individual or per gram WW) and uptake among species, there was no evidence for biomagnification. Similarities in microplastic shape and colour across biota species indicated that trophic transfer could potentially still be occurring, but not being magnified up trophic levels. Likewise, regarding polymers, although noting that we tested only a portion of all microplastics for polymer type. Evaluations of trophic transfer and biomagnification of microplastics across food webs and marine ecosystems are limited and results remain inconsistent, with magnification not demonstrated (Akhbarizadeh *et al.* 2019; Covernton *et al.* 2022; Miller *et al.* 2023). Overall, meta-analyses have not supported biomagnification across marine food webs in wild settings (Miller *et al.* 2020), although there are reports implying trophic transfer among different groups of species (Sarker *et al.* 2022; Miller *et al.* 2023) or within a group of species (e.g. fish; Zhang *et al.* 2022). Whereas variations are likely to be due to regional factors, different microplastic contamination, as well as species ecological traits and interconnections within a food web, other key points to consider are differences in organisms' egestion rates and plastic sizes in the environment (Ward *et al.* 2019; Xiong *et al.* 2019). Modelling approaches (Alava 2020) and links between microplastic contamination and stomach fullness suggest egestion rates play a major role, at least for larger microplastics (Covernton *et al.* 2022), with laboratory studies also showing that trophic dilution occurs, including at high contamination loads (Kim *et al.* 2018; Elizalde-Velázquez *et al.* 2020). To further resolve these inconsistencies regarding biomagnification, we are likely to need a combination of laboratory or mesocosm-based studies with realistic levels of microplastic contamination. Replicating assessments on the same or similar species across regions with different environmental contamination loads may also be beneficial (including across an intertidal–subtidal gradient).

Differences in polymer type across matrices are likely to be a result of the density and fate of different plastic types, with higher-density plastics often sinking and low-density plastics staying afloat. Here, the most abundant types of polymers were polyester (density  $\sim 1.38 \text{ g cm}^{-3}$ ) in the water column and polyethylene (density  $\sim 1.0 \text{ g cm}^{-3}$ ) in the sediment. Biofouling and weathering (such as in the case of polyethylene), together with site- and environment-specific conditions (e.g. wave action and water turbidity, in the case of polyester) are likely to contribute to these findings. Similar results have been found in South Australia's coastline,

with high prevalence and numbers of polyester fibres in water samples (Leterme *et al.* 2023), and polyethylene in sediment (Hayes *et al.* 2021). Although we acknowledge that we analysed only a limited number of samples, polymer characteristics reflect the connectivity between biota and the environmental matrix, with more polyethylene being found in mussels, and polyester in razorfish and yellow-fin whiting, implying that organisms that forage in intertidal and subtidal areas of Black Point are susceptible to microplastics from the sediment and water column respectively. Nonetheless, we also found polystyrene in fish but not in the local environment at this sampling time, which could be linked to variations in environmental contaminations or broader habitat use of these organisms. Moreover, the prevalence of blue fibres in biota was similar across species (trophic levels) and is likely to confirm ingestion from the water, where blue fibres dominated, but results also reflect uptake from sediment by the uptake of transparent microplastics. Overall, the occurrence of blue, black and transparent fibres is common (Ogunola *et al.* 2022) and is likely to result from both intentional or unintentional ingestion (Roch *et al.* 2020; Li *et al.* 2021).

Microplastic ingestion is pervasive in marine biota across all trophic levels and feeding strategies including gastropods, bivalve molluscs, decapod crustaceans and fish, which are important fishery resources (Karlsson *et al.* 2017; Hamilton *et al.* 2021; Zaki *et al.* 2021). Yet, we still need to further understand the risk of harm, and physical and chemical effects of different levels and types of microplastics in biota (Lusher *et al.* 2017b; Provencher *et al.* 2020b). Our study is one of few studies to sample across three environmental matrices (water, sediment and biota), and different trophic levels, providing evidence of low levels of microplastic contamination (but see e.g. Kazour *et al.* 2019; Miller *et al.* 2023). Overall, characterising microplastic contamination across environmental matrices is critical for a comprehensive understanding of the occurrence (i.e. how much and where) and the potential fate of microplastics in coastal ecosystems. Although low environmental contamination may restrict trophic magnification, results are in line with previous findings. It is recommended that future studies select more species, and replicate assessments across regions with different environmental contamination to help unfold how species-specific traits and environmental factors may influence vulnerability to microplastics in coastal environments. Ultimately, understanding the fate of plastic pollution in marine food webs, particularly the uptake, transport through diverse trophic level species, and retention time, would further help unravel the effects of microplastics at individual, population and ecosystem levels.

## Supplementary material

Supplementary material is available [online](#).



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**Data availability.** The data that support this study are available in FigShare (doi:10.6084/m9.figshare.23071187).

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