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Sediment grain size determines microplastic exposure landscapes for sandy beach macroinfauna☆

Peter Vermeiren[a,b,*], Diego Lercari[c], Cynthia C. Muñoz[a,b], Kou Ikejima[b], Eleonora Celentano[c], Gabriela Jorge-Romero[c], Omar Defeo[c]

[a] Department of Environmental Science, Faculty of Science, Radboud University, Heyendaalseweg 135, 6525 AJ, Nijmegen, the Netherlands
[b] Laboratory for Coastal Ecology and Conservation, Faculty of Agriculture and Marine Science, Kochi University, 200 Otsu, Monobe, Nankoku City, Kochi, 783-8502, Japan
[c] UNDECIMAR, Departamento de Ecología y Evolución, Facultad de Ciencias, Universidad de la República, Iguá 4225, Montevideo, 11400, Uruguay

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ABSTRACT

Despite the global occurrence of microplastic contamination on sandy beaches, evidence of microplastic distribution within beaches remains contradictory. When conflicting evidence is used to inform sampling surveys, it increases uncertainty in resulting data. Moreover, it hampers spatially explicit risk characterization of microplastic pollution to intertidal fauna. We aimed to guide sampling designs for microplastic monitoring on beaches, and to quantify macroinfauna exposure to microplastics. Microplastic abundance, quantified between 5 mm–66 µm, lacked a significant zonation across the top sediment layer of sub-terrestrial, upper and lower midlittoral, and swash zones at two sites with varying anthropogenic influence on a microtidal dissipative beach in Uruguay. Microplastic abundance decreased exponentially with increasing grain size, as revealed by Bayesian Poisson regression, although the decrease was less steep compared to prior knowledge regarding sediment – plastic interactions obtained for large (millimeter-sized) industrial pellets. Significant differences in microplastic contamination between the two sites with varying anthropogenic influence likely related to their proximity to a freshwater canal. Corresponding field measurements of body burdens of fibers and irregular particles were significantly lower for the polychaete *Euzonus (Thoracophelia) furcifera*, despite its preference for finer sediments with higher microplastic loads, compared to the isopods *Excirolana braziliensis* and *Excirolana armata*. Results provide critical insights toward representative sampling of microplastics within beach sites. Specifically, we caution against sampling limited to the drift line, and instead recommend: 1) reporting beach morphodynamic characteristics; 2) using clearly defined, ecologically-informed zonation schemes; and 3) accounting for sediment grain size as a covariate to normalize among reported contamination levels. The results contribute valuable baseline data toward realistic exposure landscapes relative to the sediment grain size preferences of macroinfauna, needed to inform laboratory experiments.

1. Introduction

Plastic materials are used in a diverse range of products, with their success, in part, due to characteristics such as longevity, strength, ease of manipulation, and low production costs (Andrady and Neal, 2009). Nonetheless, the large amount of plastics currently being produced has created environmental contamination on a global scale (Borrelle et al., 2020). This contamination is augmented because many plastics have a long lifespan, yet are only single or short-term use, and appropriate waste management is generally lacking (Borrelle et al., 2020). Microplastics are most commonly defined as plastic particles with their longest axis <5 mm (Hidalgo-Ruz et al., 2012). The small size of microplastics makes them available to organisms throughout aquatic food webs (Au et al., 2017). A wide range of physical and chemical effects of microplastics on individual organisms has been documented (Haegerbaeumer et al., 2019), resulting from direct interactions with

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* Corresponding author. Department of Environmental Science, Faculty of Science, Radboud University, Heyendaalseweg 135, 6525, AJ, Nijmegen, the Netherlands.

E-mail address: p.vermeiren@science.ru.nl (P. Vermeiren).

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microplastics or indirectly through interactions with hazardous chemicals and microbial communities for which microplastics provide a substratum (Laist, 1997; Gregory, 2009; Gall and Thompson, 2015). The impacts of microplastics on individuals can eventually affect the structure and functioning of faunal communities (Lin et al., 2020).

Sandy beaches are one of the marine compartments most exposed to microplastics due to their high accumulation potential (Everaert et al., 2018). Several studies have shown differences in microplastic contamination among beaches concerning a suite of natural and anthropogenic factors (Browne et al., 2010; Vermeiren et al., 2016). Among them, human population density has consistently been identified as a key driver of microplastic contamination (Corcoran et al., 2020; Vetrimurugan et al., 2020). Despite the widespread detection of microplastic contamination among beaches, however, the distribution of microplastics within a beach, including across-shore zonation, is poorly understood. This gap limits our understanding of microplastic zonation within beaches and subsequently the potential exposure of fauna to microplastics.

Sampling near the high tide line, outlined by the drift line where deposition of natural debris occurs (McLachlan and Defeo, 2018), has been supported by observations of microplastics accumulation at this location (Bravo et al., 2009; Heo et al., 2013; Lee et al., 2015; Moreira et al., 2016; Karkanorachaki et al., 2018). Nevertheless, several studies failed to detect clear zonation in microplastic contamination across a beach (e.g. Hidalgo-Ruz and Thiel, 2013; Mathalon and Hill, 2014; Besley et al., 2017), or found contrasting zonation patterns (Turra et al., 2014). The absence of clear across-shore zonation could result from high variability in microplastic concentrations at small scales (Turra et al., 2014; Kim et al., 2015; Fisner et al., 2017; Bancin et al., 2019).

High variability in microplastic concentrations at small scales could be driven by variation in environmental factors within a beach. Experimental observations demonstrated increased retention of fine microplastic particles (>100 μm) within columns filled with glass beads resembling coarse silt and fine sand when compared to coarser substrates (Waldschläger and Schüttrumpf, 2020). Nonetheless, evidence for a relation between sediment grain size and microplastic deposition within sandy beaches is poor. The absence of a clear relation could be attributed to a general focus of field surveys on patterns among beaches (Browne et al., 2010, 2011; Mathalon and Hill, 2014; Urban-Malinga et al., 2020) rather than across-shore zonation within beach sites where patchiness in sediment grain size can occur. Nonetheless, a recent study did observe a higher abundance of industrial microplastic pellets on beaches with very fine, fine, and medium sand compared to coarser sediments in the Laurentian Great Lakes of North America (Corcoran et al., 2020). These results argue for increased attention to the role of sediment grain size in microplastic deposition patterns.

Knowledge of relations between microplastic deposition and environmental factors within beaches could be used to identify areas of high exposure risk for beach macroinfauna to microplastics. Isopods and polychaetes are numerically dominant macroinfauna on sandy beaches (Lercari and Defeo, 2006) and play a fundamental role in the ecosystem (Bergamino et al., 2011). Isopods and polychaetes have been used as bioindicators of environmental change in sandy beaches (Omena et al., 2012; Bessa et al., 2014; Machado et al., 2017) and could be useful indicators of microplastic impacts on sandy beaches. Reported effects of microplastics on marine isopods and polychaetes include increased mortality, reduced energy reserves, reduced regeneration rates, and altered enzyme activities (e.g. Wright et al., 2013; Leung and Chan, 2018; Korez et al., 2019), with others observing no distinct effects (Hamer et al., 2014). Little is known about variations in contamination levels in organisms' home ranges and the level of microplastic ingestion in their natural environment. Nonetheless, knowledge of exposure and ingestion is fundamental to quantifying the impacts of microplastic contamination and informing relevant experimental designs on microplastic effects (Horton et al., 2017).

We aimed to quantify the exposure of sandy beach macroinfauna to microplastic pollution and to guide sampling designs for monitoring microplastics across sandy beach zones. We hypothesized that: 1) microplastics display highest abundance in the upper midlittoral zone that is delimited at the top by the drift line; 2) microplastic abundance is higher at beach sites with higher levels of anthropogenic influence after accounting for confounding environmental factors; 3) microplastic abundance decreases toward coarser sediment grain sizes; and 4) ingestion of microplastics is highest for macroinfauna species whose habitat preferences overlap with areas of high microplastic contamination. To test these hypotheses, we modelled variations in microplastic abundance at two beach sites with different levels of anthropogenic influence, while accounting for variation in environmental factors, particularly sediment grain size. Subsequently, we assessed the exposure to microplastics among three sandy beach macroinfauna species: the isopods *Excirolana braziliensis* and *Excirolana armata*, and the polychaete *Euzonus (Thoracophelia) furcifera*, by measuring body burdens of microplastics.

2. Material and methods

2.1. Study area and sampling design

Sampling was conducted at two sites with varying degrees of anthropogenic influence along a 22-km long beach between Barra del Chuy and La Coronilla, eastern Uruguay (Fig. 1). Exposed to the Atlantic Ocean, this microtidal dissipative beach is characterized by fine to very fine well-sorted sands, a gentle slope, heavy wave action, and a wide surf zone (Lercari and Defeo, 2006, 2015). The beach supports the highest richness, diversity, abundance, and biomass of macroinfauna among Uruguayan beaches (Lercari and Defeo, 2006, 2015). A natural stream at the northeastern limit, Chuy Stream, and an artificial stream at the southwestern limit, Andreoni Canal, delimit the beach.

Sampling was conducted at two sites. One site was adjacent to the town of La Coronilla and located 1 km northeast from the mouth of the Andreoni Canal. The Andreoni Canal is 68 km in length and drains a wide basin of around 270,000 ha with predominantly agriculture and cattle rearing. The canal's discharge into the ocean flows towards the northeast and affects the quality of the beach around La Coronilla (Lercari et al., 2002; Jorge-Romero et al., 2019). Microplastics originating from the canal's discharge and human population in La Coronilla are hypothesized to result in microplastic contamination at this site. Therefore, this site will be referred to as the "high impact" site. For comparison, a "low impact" site was selected 13 km from the mouth of the Andreoni Canal and roughly in the middle between Barra del Chuy and La Coronilla. The low impact site is comparable in morphology to the high impact site (Appendix 1) but is bordered by a well-developed dune system, while the dunes at the high impact site are eroding. Population density at the high impact site, with a mean (±SE) of 100 ± 7 ind km^{-2} in a 1 km radius, is 100x higher than at the low impact site (Appendix 1). Sampling was conducted in austral winter during clear weather on 27 June and August 15, 2019 in the low and high impact sites, respectively. The flow of the Andreoni Canal is highest during austral winter (up to 89 m^3/s), and onshore winds dominate during this season (Gianelli et al., 2019).

Surface sediment at each site was collected for analysis of microplastic contamination and sediment grain size distribution following a stratified random sampling design (Defeo and Rueda, 2002). A 60 m stretch along the beach at each site was divided into four parallel zones based on the influence of wave action on dominant physical variables such as slope, moisture content, organic matter content, and grain size (Table 2), and on Dahl's description of the intertidal distribution of characteristic crustaceans in relation to those physical variables (McLachlan and Defeo, 2018), specifically: sub-terrestrial, upper midlittoral, lower midlittoral and swash zones (Fig. 1C). Five sediment samples, each at least 5 m apart, were randomly collected per zone. Each sample consisted of two duplicates (adjacent to each other): one

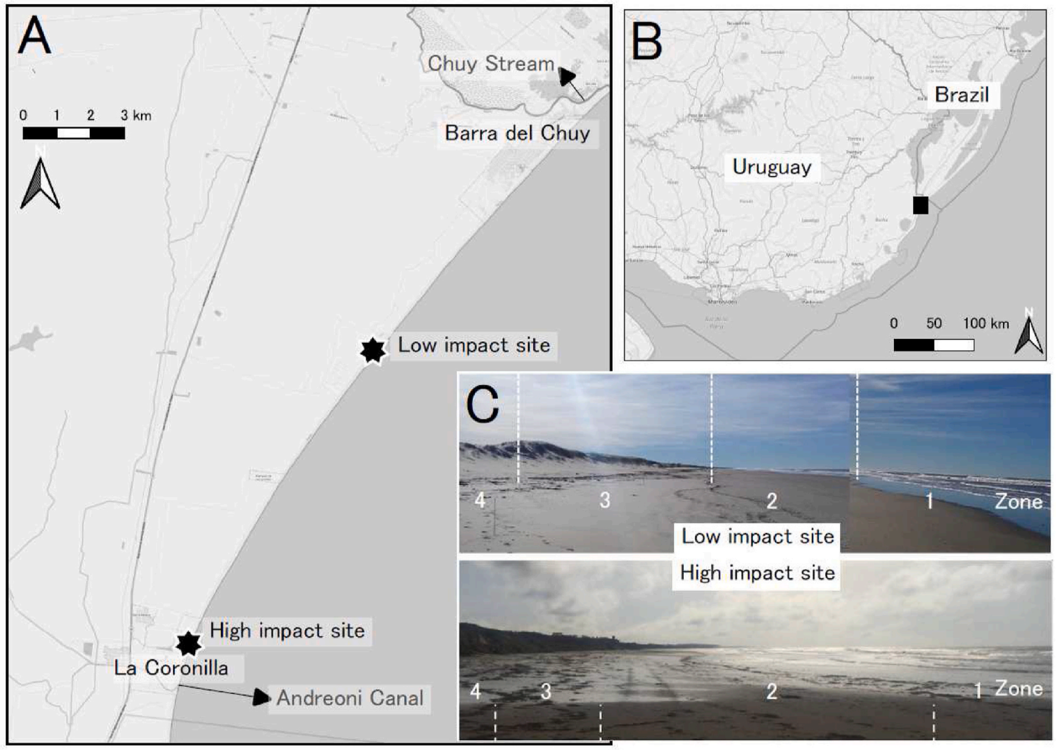

Fig. 1. A) Low and high impact sites along the beach between La Coronilla and Barra del Chuy, Uruguay; B) overview map with a black square indicating the study area; C) photographs of the beach sites with indication of the four parallel zones (1) swash, 2) lower midlittoral, 3) upper midlittoral, 4) sub-terrestrial), as identified by the influence of wave action and Dahl's description of the intertidal distribution of characteristic crustaceans (McLachlan and Defeo, 2018).

reserved for microplastic and one for grain size, moisture content, and organic matter content analysis. Samples were taken using glass Petri dishes of 100 mm diameter to a depth of 15 mm (volume: 118 mL), allowing a sample of about 100 g dry sediment to be collected. The depth of the samples was chosen in line with the overall research aim of characterizing microplastic exposure for sandy beach macroinfauna, whose main feeding activities are centered on the top, surface layer of the beach sediment (Defeo et al., 1997; Otegui et al., 2012). Petri dishes were pressed upside down into the sediment, dug out by hand, and flipped over. Any excess sediment was scooped off and the Petri dish closed. All samples were stored individually in zip lock bags at room temperature until laboratory analysis.

Macroinfauna sampling focused on the isopods *Excirolana braziliensis* and *Excirolana armata*, and the polychaete *Euzonus (Thoracophelia) furcifera*. Core samples were taken in each zone and sieved through 500 μm mesh. Macroinfauna were handpicked and stored in glass vials (−20 °C) until analysis of ingested microplastics.

2.2. Sediment and microplastic analysis

Sediment grain size distribution was established with laser diffraction to 1 μm accuracy. Therefore, 2 g wet sediment was dried overnight at 50 °C after which organic matter was removed using 1 mL of 30% H_2O_2 overnight. Then, the sediment was analyzed using a Malvern Mastersizer 2000 laser diffraction particle sizer coupled with a hydro200 S dispersion unit. Sodium hexametaphosphate, $(NaPO_3)_6$, 5.5 g/L was used as a dispersant. Results were averaged across five repeated measurements per sample. To characterize each zone at each site, the moisture content (%, established on 2 g sediment dried at 80 °C for 24 h, and measured to 1 mg accuracy), organic matter content (%, by loss on ignition of 2 g dried sediment at 500 °C for 5 h, measured to 1 mg accuracy), the zone width (m), and the slope per zone (cm/m, 1 mm accuracy) were recorded.

Microplastic quantification and identification were conducted following the protocol of Vermeiren et al. (2020), in three phases: preparation, separation, and identification (see Appendix 2 for a detailed description and flow chart). During phase 1, macroinfauna was removed from the sediment by gently sieving through a 500 μm sieve to avoid chitinous materials that could be mistaken for microplastics during fluorescence dying in phase 3 of the protocol. Any other materials were added back to the sample. Organic matter content in the samples was then reduced using 40 mL of Fenton's reagent. In phase 2, microplastics were separated from relatively heavier sediments in an overflow density separation column with top overflow (OC-T), using $ZnCl_2$ with a density of 1.5 g/cm^3 as separation fluid. The mixture in the OC-T was stirred for 3–4 min with a glass stick to obtain a homogeneous solution. After 24 h rest, the top layer containing the microplastics was overflown until 200 mL was collected into a glass beaker. Subsequently, another 50 mL was carefully decanted from the OC-T into the beaker. The sediment was then stirred again until completely homogenized and left to rest for another 24 h, before repeating the overflow. The collected liquid from both overflows was then vacuum filtered using 47 mm glass fiber filters (GFF) with 0.7 μm pore size. The filters were dried at 55 °C for 48 h and stored in glass Petri dishes.

In the 3rd phase, microplastic concentrations on the filters were established using Nile Red dye assisted automated counting following Vermeiren et al. (2020, Appendix 2). Nile Red dye is solvatochromic based on the polarity of its environment and fluoresces when sorbed to plastics (Shim et al., 2016; Maes et al., 2017). Stained filters were then photographed, in a dark room, under a green light using a binocular microscope fitted with an orange filter. The filter cuts out wavelengths below 600 nm, thereby allowing visualization of the fluorescence emitted by Nile Red dye at 637 nm. Images of filters were analyzed with ImageJ (Schneider et al., 2012) for abundance and size distributions (length of the longest axis of individual particles) of microplastics using a color threshold to separate plastics from non-plastics using parameters for Hue (0–43), Saturation (0–255), and Brightness (160–255) on a scale of 0–255 (Vermeiren et al., 2020). The lower size limit of particles detected was set to 33 μm which corresponds to a particle that is at least 3 image pixels large. As a precautionary measure, the detection limit was doubled to yield a quantification limit of 66 μm and only microplastics larger than the quantification limit were considered in further statistical analyses, hence leading to a definition of microplastics in the current study as particles with their longest axis 5 mm–66 μm. The longest axis was considered to capture the maximum dimension of microplastics defined as < 5 mm (Hidalgo-Ruz et al., 2012).

Nile Red assisted automatic quantification was combined with the identification of 70 randomly selected particles using micro Fourier-transform infrared (μFT-IR) spectroscopy in transmission mode on a JASCO FT/IR-6600 interfaced with an IRT-7200 microscope taking readings between 400 and 6000 cm^{-1} at a resolution of 4.0 cm^{-1}. The combination of Nile Red assisted quantification with μFT-IR validation provides a cost-effective solution for large-scale monitoring of microplastics in sediment samples (Vermeiren et al., 2020) such as the large number of samples in the current study.

2.3. Analysis of microplastic ingestion by macroinfauna

Macroinfauna collected and euthanized in the field were sorted in the laboratory under a binocular microscope into the three species: *E. braziliensis*, *E. armata,* and *E. furcifera*. For each species, adult individuals from the same beach zone were selected for analysis of microplastic body burdens. Individuals were rinsed with distilled water to remove any materials from the outside of the body. After blotting with tissue paper, individual wet weight (accuracy: 0.1 mg) was established. Individuals were placed in separate glass test tubes with Fenton's reagent, consisting of 0.5 mL aqueous 0.05 M Fe (II) solution and 0.5 mL of 30% hydrogen peroxide, for 48 h. Individuals were then gently squashed with a blunt glass rod and contents of the vial concentrated unto 25 mm GF/C filters with 1.2 μm pore size.

Filters were stained with Nile Red dye, dried overnight at 55 °C, and inspected at 40x magnification on a NIKON FXA epi-fluorescence microscope using a 365 nm UV light and a 495 nm blue excitation filter. Filters were systematically traversed following a grid along which fluorescent particles were counted and categorized as irregular particles or fibers following Hartmann et al. (2019). Counting was performed by two independent observers and results averaged. The length of the longest axis of a subset of 16 irregular particles and 12 fibers was established as a preliminary assessment of the size distribution of ingested microplastics.

2.4. Quality control

Glass materials were used as much as possible to avoid contamination in the field (e.g. Petri dishes for sample collection) and the lab (beakers, vials, and OC-T columns). All materials were washed with tap water and subsequently rinsed with distilled water. Cotton clothes were worn to avoid accidental contamination. Samples were processed in a small closed laboratory space. Samples were covered throughout the process with aluminum foil to avoid contamination from the external environment. Sediment and macroinfauna samples were corrected against laboratory blanks that were analyzed at the middle and end of the sample processing.

2.5. Statistical analysis

Analyses were conducted on quantifiable microplastics (longest axis: 5 mm–66 μm) expressed as the abundance of microplastics per Petri dish (volume: 118 mL). To identify differences in microplastic abundance (nr. Plastics/118 mL) across beach sites and zones, a two-way ANOVA was conducted including zone (the 4 zones, Fig. 1C) and beach site (high vs. low impact site) as factors. Microplastic abundance was log-transformed to meet assumptions of homogeneity and normally distributed residuals.

To assess differences in microplastic abundance at two beach sites with different levels of anthropogenic influence, a Bayesian regression model was constructed that accounted for the influence of sediment grain size (including prior knowledge on the magnitude of this influence). Specifically, an exponential model with a Poisson error distribution was selected, suitable to modelling microplastic abundance (nr. Plastics/118 mL) as count data skewed toward zero. The exponential model included an intercept, α, a parameter, β_{site}, which captures the influence of the fixed categorical predictor of either high or low impact site, and a parameter, β_{grain}, which captures the influence of the continuous predictor of mean sediment grain size. The likelihood model for the observations of microplastic abundance, y_i, within each sample can then be described following eqns. (1) and (2),

$$y_i \sim poisson(z_i) \tag{1}$$

$$\log(z_i) = \alpha + \beta_{site}x_{site} + \beta_{grain}x_{grain} \tag{2}$$

where x_{site} and x_{grain} are the input data regarding beach site and mean sediment grain size (μm), respectively.

Prior knowledge regarding model parameters was included in the model as follows: a wide lognormal prior distribution (log mean = 1, log SD = 1) for α accommodated the positive nature of count data while allowing inference mainly based on the data. We hypothesized a positive effect of anthropogenic impacts on microplastic abundance. Yet, a wide normal prior distribution centered at mean = 0 (SD = 25) was chosen to avoid forcing a positive value in case of conflicting evidence in the data. A normal prior distribution (mean = −1.004, SD = 1) for β_{grain} was based on previous relations between sediment grain size and industrial pellets (i.e. large-sized microplastics) on beaches across the Laurentian Great Lakes (Corcoran et al., 2020, Appendix 3). This allowed us to explore whether the microplastics (including much finer particles than studied by Corcoran et al., 2020) sampled on oceanic sandy beaches in the current study followed a similar pattern.

The likelihood model and prior distributions were combined during Bayesian inference to obtain posterior distributions for each of the parameters. Numerical simulations were conducted using four Markov Chain Monte Carlo sampling chains of 10,000 iterations and a burn-in of 2000 iterations, using the Gibbs sampler implemented in the rJAGS package (version 4.10, Plummer, 2019) in the R statistical software (version 3.6.3, R Core Team, 2020).

Body burdens of ingested microplastics (nr. irregular particles or fibers/mg body weight) were compared among species and microplastic categories (fibers vs. irregular particles) with a two-way ANOVA. Body burdens were log-transformed to meet assumptions of homogeneity and normally distributed residuals.

3. Results

3.1. Microplastic characteristics

Polyolefins, plastics with densities lower than seawater, made up about half (52.4%) of validated particles. This was followed by polyesters (12.7%) and polyvinyls (11.1%), common polymers with densities heavier than seawater. Other polymers, including polystyrene, polyurethane, polyacrylonitrile, and polysiloxane, contributed less than 10% (Appendix 4). Non-plastics contributed 7.9%.

No macroplastics (i.e. plastics ≥5 mm) were detected. The number of microplastics (<5 mm) extracted on both beach sites increased toward the smaller sizes (Fig. 2). The probability density distribution peaked for microplastics with sizes smaller than the quantification limit (LOQ, 66 μm) and then decreased for microplastics with sizes closer to the detection limit (LOD, 33 μm, Fig. 2). Microplastic size distributions were comparable across zones at the low impact site. By contrast, at the high impact site, coarser microplastics were more prevalent in the lower midlittoral and swash zones, whereas the finest microplastics were more

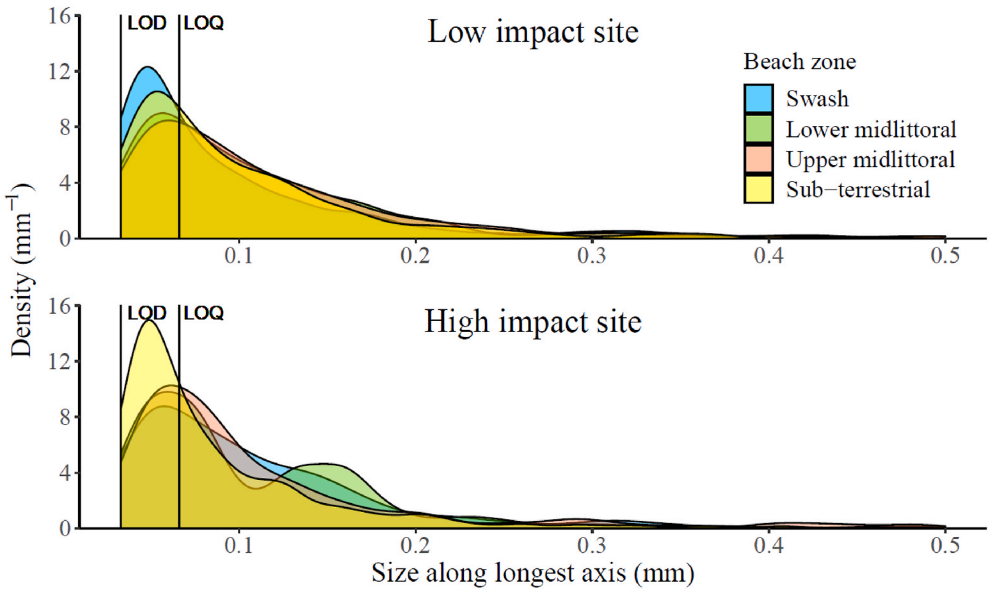

Fig. 2. Probability density distribution of microplastic across their size range (0.5 mm–33 μm) across beach zones in the low and high impact sites. LOD: limit of detection (33 μm), LOQ: limit of quantification (66 μm).

prevalent in the sub-terrestrial zone.

3.2. Anthropogenic and environmental influences on microplastic abundance

The low impact site, with 60 m, was slightly wider than the high impact site, with 47 m (Table 2). Beach slope at both sites was gentle and highest at the top of the beach. The sediment grain size was coarsest at the top of the beach at the low impact site, closely followed by the drift zone at the high impact site. Average microplastic abundance was significantly higher at the high impact site than at the low impact site (Fig. 3, Table 1). Beach zones and the interaction between beach zones and sites did not influence microplastic abundance (Fig. 3, Table 1).

The sediment grain size was strongly related with microplastic abundance within beach sites, with abundance about twice as high at the high compared to the low impact site (Fig. 4) and the 95% probability interval of the posterior of β_{site} not including zero (Table 3). The posterior effect of sediment grain size on microplastic abundance, parameterized as β_{grain}, was smaller than prior expectations, yet, also had a substantial effect with the 95% probability interval of the posterior not including zero (Table 3).

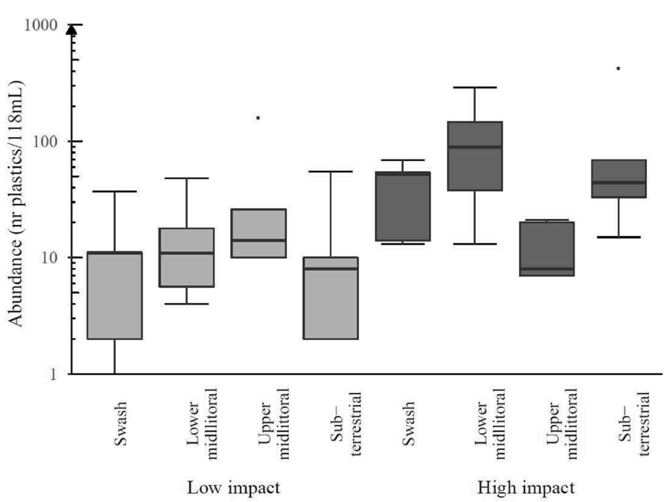

Fig. 3. Microplastic abundance (nr. plastics 5 mm–66 μm/118 mL), measured in the top (15 mm) sediment layer, on a log scale across beach zones at the high and low impact sites. Boxplots encompass the median, 75th, and 25th percentile with whiskers extending 1.5 times the interquartile range.

Table 1
Two-way ANOVA on: A) microplastic abundance (log-transformed) considering beach site, zone (levels: the 4 parallel zones), and their interaction; and B) microplastic body burdens (plastics/mg, log-transformed) considering species (levels: 3 species), type (levels: fiber or irregular particle), and their interaction. Microplastic abundance expressed as nr. plastics 5 mm–66 μm/118 mL. df: degrees of freedom. *Significant effects with $p < 0.05$.

	Factors	df	F statistic	p-value
A	Beach site*	1	5.5	0.025
	Zone	3	1.3	0.29
	Interaction	3	1.5	0.23
B	Species*	2	13	<0.001
	Plastic type	1	0.031	0.86
	Interaction	2	0.15	0.86

3.3. Microplastic exposure and ingestion by macroinfauna

The mean size (±SD) of microplastics ingested was 35 ± 30 μm for irregular particles and 484 ± 234 μm for fibers. Body burdens did not differ substantially among fibers or irregular particles but did vary significantly among species (Table 1), with a Tukey post hoc comparison test identifying significantly lower body burdens for *E. furcifera* compared to both isopod species (Fig. 5).

4. Discussion

4.1. Spatial variation in microplastic abundance

Microplastic abundance in the top sediment layer did not differ among zones (Table 1, Fig. 3). The deposition of microplastics in drift lines was suggested to relate to the magnitude and strength of wave action during different tidal cycles (Hinata et al., 2017) and was supported by observations of microplastic accumulation at the high tide line (e.g. Lee et al., 2015; Moreira et al., 2016; Karkanorachaki et al., 2018). Many studies comparing multiple beaches focus their sampling at the

Table 2
Main physical characteristics of the four zones analyzed at each site.

Beach	Zone	Slope of beach (cm/m)	Zone width (m)	Median grain size (µm)	Mode grain size (µm)	Moisture content (%)	Organic matter content (%)
Low impact	Swash	4.2	16	225	225	14.9	0.2
	Lower midlittoral	5.5	12	230	231	11.6	0.1
	Upper midlittoral	3.5	24	225	225	7.5	0.1
	Sub-terrestrial	10	8	263	261	1.4	0.2
High impact	Swash	4.7	8	216	217	15.8	0.1
	Lower midlittoral	3.9	7	223	224	15.4	0.2
	Upper midlittoral	4.8	24	259	260	8.5	0.1
	Sub-terrestrial	8.2	8	239	240	2.4	0.1

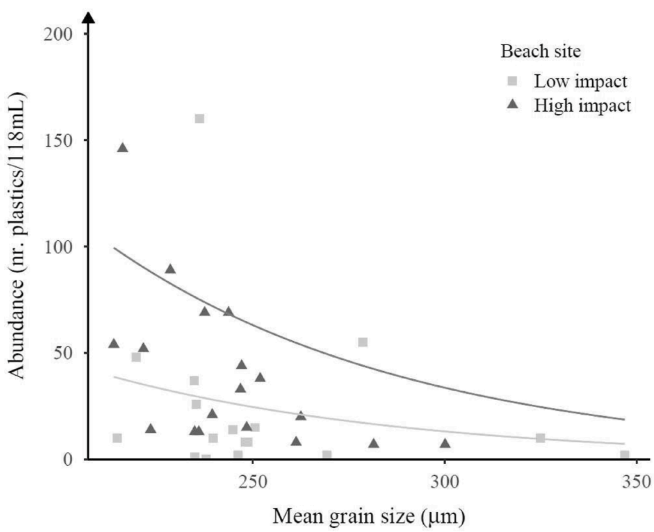

Fig. 4. Bayesian Poisson regression of microplastic abundance (nr. plastics 5 mm–66 µm/118 mL), measured in the top (15 mm) sediment layer, relative to the mean sediment grain size and the factor beach site (Table 3). Note that two samples with outlying abundance fall outside the y-axis range.

Table 3
Prior and posterior parameter distributions (median and 95% probability interval) for the Bayesian Poisson regression with a log link function of microplastic abundance at two sandy beach sites in Uruguay. Priors were derived from Corcoran et al., (2020) (Appendix 3).

Parameter	Prior	Posterior
Intercept α	2.81 (0.39–18.81)	6.34 (5.75–6.97)
Effect of beach site β_{site}	−0.44 (−49.6 – 49.6)	0.94 (0.84–1.05)
Effect of sediment grain size β_{grain}	−1.00 (−2.90 – 0.95)	−0.013 (−0.015––0.010)

high tide line (Besley et al., 2017) following this presumed deposition pattern. Our results, however, did not support the concept of microplastic accumulation at the drift line, in agreement with previous observations of no or contrasting patterns in microplastic abundance across the intertidal (e.g. Hidalgo-Ruz and Thiel, 2013; Mathalon and Hill, 2014; Turra et al., 2014; Besley et al., 2017). These results suggest that sampling designs targeting only the drift line might not be appropriate for each beach type and that more attention needs to be given to the type of beach and its physical characteristics.

The contradictory evidence in microplastic deposition patterns among studies could originate from the use of different classification schemes of across-shore zones, and to a difference in morphodynamics

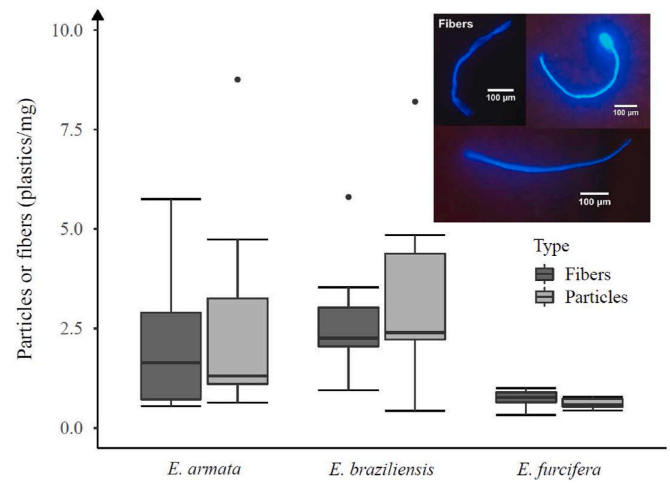

Fig. 5. Body burdens of microplastic irregular particles and fibers (nr. plastics/mg body weight) across three sandy beach species. Inset displays example fibers observed.

among beaches. We distinguished four zones based on the influence of wave action and Dahl's description of the intertidal distribution of characteristic macroinfauna (McLachlan and Defeo, 2018). Other sampling designs rely on levels determined by tides (Mathalon and Hill, 2014; Besley et al., 2017) or use poorly documented zonation schemes and vague terminologies such as backshore or high strandline (Turra et al., 2014; Kim et al., 2015). We investigated a microtidal dissipative beach, characterized by fine grain sizes, a gentle slope, a wide surf zone, and a reduced astronomic tide (<0.5 m), with water levels determined by wind direction and speed (Lercari and Defeo, 2006, 2015, Table 2). Because of these morphodynamic characteristics, the study sites did not have one distinct drift line, but rather natural debris was spread across a broader zone (Fig. 1C). A clear reporting of the morphodynamics of the beaches investigated, and appropriate zonation schemes for such beaches, would improve interstudy comparability. Given the ultimate aim of assessing the risk of microplastics to fauna, we argue for the use of ecologically-based zonation schemes, as the one used in the current study.

It should be noted that we focused our sampling on the top 15 mm of sediment, in line with the research aim of establishing exposure landscapes for macroinfauna feeding at the sediment surface. Nonetheless, microplastics can accumulate in deeper sediment layers (Turra et al., 2014; Waldschläger and Schüttrumpf, 2020). The relation between microplastics at the sediment surface and their accumulation into deeper sediment layers deserves further attention to complement our knowledge on microplastics pollution within sandy beaches.

4.2. Anthropogenic and environmental influences on microplastic abundance

Microplastic abundance, measured in the top sediment layer, decreased exponentially with increasing grain size in both sites (Fig. 4). These results support experimental observations of microplastic (>100 μm) retention near the surface in fine sediments, such as silt and fine sand, compared to coarser sediments (Waldschläger and Schüttrumpf, 2020). The exponential decrease in microplastic abundance with increasing grain size in our study (Fig. 4) was less steep than the decrease observed previously in larger (retained on a 2.5 × 3.0 mm sieve) industrial microplastic pellets among beaches in the Laurentian Great Lakes of North America (Corcoran et al., 2020, Table 3). However, a comparable decrease was observed in microplastic (≥500 μm) abundance in relation to the fine (<63 μm) sediment grain size fractions among estuarine sites (Enders et al., 2019). The mechanisms governing microplastic deposition, retention, and resuspension are complex and still poorly understood (Alimi et al., 2018; Chubarenko et al., 2020). Under experimental conditions, smaller microplastics infiltrated deeper into the substratum than larger microplastics (Waldschläger and Schüttrumpf, 2020). Beach sediments are known traps for pollution that retain particles deposited by wind or wave action (McLachlan and Defeo, 2018). Potentially, smaller microplastics might be more easily retained and integrated into the sediment matrix, even in coarser sediments, as compared to larger sized microplastics. This hypothesis could explain the difference between our results and the prior knowledge derived from Corcoran et al. (2020). Additionally, these results argue for accounting for sediment granulometry when comparing among sites, similar to normalization procedures typically employed in sediment contamination assessment (Kersten and Smedes, 2002). Since sandy beaches are dynamic over time, accounting for features such as morphodynamics and grain size can also offer insights into temporal dynamics. A well-defined theoretical framework based on beach morphodynamics and microplastic deposition regimes is urgently needed to synthesize current results regarding microplastic abundance and understand their spatial and temporal dynamics.

Average microplastic abundance was significantly higher at the high compared to the low impact site (Table 1). Human populations are a source of plastic debris to the environment, and human population density correlates strongly with microplastic contamination (Corcoran et al., 2020; Vetrimurugan et al., 2020). The population density within a 1 km radius was 100x larger at the high compared to the low impact site (Appendix 1). Nevertheless, the town of La Coronilla, close to the high impact site, had a population of only 1184 individuals (INE, 2011). Another main difference between the study sites was their proximity to the Andreoni Canal mouth (1 and 13 km from the high and low impact site, respectively). Proximity to rivers increases concentrations of debris (Acha et al., 2003) including microplastics (Frère et al., 2017; Bancin et al., 2019). Additionally, the mixing of fresh and saltwater at the mouth of rivers increases microplastic deposition (Vermeiren et al., 2016). Likely, the large catchment drained by the Andreoni Canal could contribute to explaining differences in microplastic contamination between both sites, warranting further research into the capacity of river outflows to influence contamination levels on remote beaches.

4.3. Microplastic exposure and ingestion by macroinfauna

Differences in microplastic exposure, associated with preference in sediment grain size among beach macroinfauna, were not reflected in body burdens. The isopods *Excirolana armata* and *Excirolana braziliensis* actively scavenge on the sediment surface. *Excirolana armata* generally occurs on finer substrata (mean grain size around 200 μm) than *E. braziliensis*, although the latter is known to also occur on fine sediments in Uruguay (Defeo et al., 1997). Meanwhile, the polychaete *Euzonus (Thoracophelia) furcifera* is a non-selective deposit-feeder that burrows in fine to very fine substrates (Otegui et al., 2012). The preference of *E. furcifera* for the finest grain size among the three species investigated, coupled with the increasing microplastic abundances at finer sediment grain sizes (Fig. 4), leads to the highest microplastic exposure for this polychaete when compared to the two isopods. Nevertheless, the microplastic body burden of *E. furcifera* was the lowest among the three species (Fig. 5). This difference is even more pronounced if we consider body burdens/g soft tissue (the exoskeleton of the isopods was included in the current body mass measurements). Experimental evidence suggests that microplastics are ingested non-selectively by the marine isopod, *Idotea emarginata*, and can be eliminated without clogging its digestive system (Hamer et al., 2014). Likewise, observations of comparable microplastic burdens in sediment and fecal casts of polychaetes inhabiting the intertidal of Halifax Harbor, Canada, suggest that ingestion and elimination are in equilibrium (Mathalon and Hill, 2014). Our results illustrate that body burdens do not simply reflect exposure levels in the environment. Consequently, detailed data for the currently studied species, including ingestion, elimination, and transit rates, are needed to improve assessments of the exposure and risk of macroinfauna to microplastics.

Increasing laboratory research focuses on the adverse effects of microplastic on macroinvertebrates. Yet, few studies consider in situ ingestion (Pinheiro et al., 2020). Moreover, the use of unrealistically high exposure concentrations in laboratory studies has been criticized (Browne et al., 2015). Our study provides critical baseline information on exposure levels relative to the sediment grain size within habitats of macroinfauna (Fig. 4). Microplastics ingested by species at the base of food webs, such as Polychaeta and Isopoda, can be transferred to higher trophic levels (Farrell and Nelson, 2013; Pinheiro et al., 2020), ultimately affecting population and community structure, and ecosystem functioning (Ma et al., 2020).

4.4. Data overview and method evaluation

The small size of microplastics encountered on the sandy beach sites in the current study (Fig. 2) poses methodological constraints to their quantification. Similar to our observed increase in abundance with decreasing microplastic size, Eo et al. (2018) found increasing numbers of microplastics with decreasing sizes until a peak at 100–150 μm. The drop in microplastic abundance at even smaller sizes could be attributed to selective removal of such small particles, resulting from e.g. increased infiltration in the sediment, selective ingestion by organisms, or rapid fragmentation into nanoplastics. Macroinfauna in the current study ingested microplastics of on average 35 μm. However, fibers with a larger mean size of 484 μm were also ingested. Alternatively, methodological constraints limit the quantification of small microplastics with lower recovery rates and higher omission during microscopic quantification with decreasing particle size (Eo et al., 2018). The photographs in the current study were taken at a higher magnification than those of Vermeiren et al. (2020), allowing us to lower the quantification limit (LOQ) from 125 μm in Vermeiren et al. (2020) to 66 μm in the current study (Fig. 2). The drop off in microplastic abundance occurred for microplastic sizes below our set LOQ (Fig. 2).

The small size of microplastics in the current study can limit polymer identification with μFT-IR. Microplastics were randomly handpicked under a microscope and pressed between two mini-KBr plates to allow measurement using transmission μFT-IR. The 7.9% of particles identified as non-plastics after μFT-IR validation could be cases where sediment attached to the microplastic or the tweezers used to pick up the microplastics might have been measured rather than the particle itself. These results indicate the limitation of hand-picking particles for μFT-IR validation when the particles become very small. In the current study, the majority of particles were smaller than 250 μm (Fig. 2). Eo et al. (2018), sampling microplastics down to 20 μm, identified 29% non-plastics and 1% not identified particles after spectroscopic validation. Vermeiren et al. (2020), utilizing the same protocol as in the current study, identified 8.3% non-plastics after μFT-IR validation.

Solutions to improve false positive and negative identifications such as automated μFT-IR imaging arrays are, at present, expensive and time-consuming, thereby preventing their routine use in large-scale monitoring at the current time (Vermeiren et al., 2020). The low LOQ, combined with the comparable performance of μFT-IR validation across studies, indicates the suitability of the cost-effective protocol, developed and validated for estuarine sediments (Vermeiren et al., 2020), for use on sandy beach ecosystems.

5. Conclusions

Increasing reports regarding microplastic abundance on sandy beaches worldwide highlight the urgency to address theese emerging contaminats. Nonetheless, synthesis of results among studies remains a major obstacle. Our study related microplastic abundance, quantified down to 66 μm, to sediment grain sizes across beach zones. Results provide critical insights toward representative sampling of microplastics in sandy beaches. Specifically, we caution against sampling limited to the drift line, and instead recommend: 1) reporting beach morphodynamic characteristics; 2) using clearly defined, ecologically-informed zonation schemes; and 3) accounting for sediment grain size as a co-variate to normalize among reported contamination levels. Despite the increased effort needed to sample, extract, and identify small microplastics that cannot be identified visually, we urge increased attention to those smaller microplastics as they are most relevant to ingestion by macroinfauna. We linked sediment concentrations to parallel field measurements of body burdens for three dominant sandy beach species, highlighting a species-specific discrepancy between sediment concentrations and body burdens. These results contribute valuable baseline data toward realistic exposure landscapes relative to the sediment grain size preferences of macroinfauna, needed to inform laboratory experiments.

Author statement

PV, OD, CM and DL designed the study, with funding acquisition by OD, DL, PV and KI. Methodology was designed and validated by PV, CM and DL, with formal analysis, visualization of results, and writing by PV and CM. All authors contributed to data collection and processing, and manuscript review and editing.

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

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