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Abstract

Synthetic microdebris (particles of <5 mm) are a worldwide concern because they can affect the community structure of the aquatic ecosystems, organism, and even food webs. For the biomonitoring of synthetic microdebris (especially nicroplastics, MPs), mainly benthic invertebrates are used, but crabs have been less cludied in the literature. We studied the synthetic microdebris contamination in wate: seaments, and three representative intertidal crabs (Neohelice granulata, Cyrtograp, s angulatus and Leptuca uruguavensis) with different lifestyles from the Bahía Blance estuary, Argentina. The results obtained show the presence of cotton-polyamide (P'1), polyethylene (PE), and polyethylene terephthalate (PET) in surface waters. In sediman, we identified cellulose modified (CE), polyester (PES), polyethylene (PE), and *elic*, tresin, while in crabs, cotton-PA and CE were the predominant ones. The MPs abundance ranged from 8 to 68 items L⁻¹ in surface water, from 971 to 2840 items Kg^{-1} in sediments, and from 0 to 2.58 items g^{-1} ww for the three species of crabs. Besides, paint sheets ranged from 0 to 17 in the total samples, with Cr, Mo, Ti, Pb, Cu, Al, S, Ba and Fe on their surface. There were significant differences between the microdebris abundances in the abiotic matrices but not among crabs species. The ecological traits of the different crabs helped to understand the accumulation of synthetic microdebris, an important characteristic when determining the choice of a good biomonitor.

Keywords: Microdebris; Microplastics; Paint sheets; Estuarine crabs; Metal ions; Chemical analytical techniques; Bahía Blanca estuary

Polymer abbreviations: CE, cellophane; PA, polyamide; PES, polyester; PE, polyethylene; TFE, tetrafluoroethylene

1. Introduction

In the last 5 years, studies on the contamination by synthetic microcontaminants (particles of <5 mm) in aquatic ecosystems have increased considerably, and it is currently the most addressed topic in scientific publications related to aquatic contamination (Imhof et al. 2016; Horton et al., 2017; Torres & De La Torre, 2021; Turner, 2021). Among synthetic microcontaminants, the most common are microplastics (MPs), tire wear particles and paints derived from marine structures and land traffic, that can be found in water, soil and air and even in organisms (Turner, 2021; Philipp et al., 2021; Forero Lopez et al., 2022). Primary MPs were initially produced as microsized beads and pellets for industrial, personal-care and medical (as vectors for drugs) uses (Barnes et al., 2009). Secondary MPs are the final product of different degradation mechanisms of larger plastics wastes, such as mechanical abrasion, photochemical oxidation by UV-B radiation, biologic and other processes (Barnes et al.,

2009; GESAMP, 2015). After these materials are used, these particles are discharged into the aquatic environment or enter wastewater treatment plants (WWTP), reaching marine systems as final disposal through currents and tides (Zhao et al., 2020; Xu et al., 2020).

An overlooked component of synthetic microdebris o microcontaminants are antifouling paint particles (APPs) that consist of a resin (polymer) combined with one or more additives and bear compositional similarities with MPs (Turner, 2021). Paint sheets enter marine environments through land-based activities, but their inputs are significantly enhanced more directly by the disturbance, erosion and weathering of coatings on coastal structures, boats and ships (Torres & De La Torre, 2021; Turner, 2021). Besides, they often have more additives used in marine antifouling than blas ics, leading to a shift in the surface characteristics and the release of harmful metalines ions and organometallics as Tributyltin (TBT) to the environment (Turner, 2021). Even when TBT-based antifouling products were banned because of their endocrine-disrupting effects causing imposex in marine gastropods and larval mortality in crabs and museu's (Amara et al., 2018), they are still available on the global market (Uc-Peraza et al., 2021). Therefore, APPs as part of the MPs pool is a promising area for investigations (Turner et al., 2022).

Once the part, les are in these ecosystems, they can affect community structure, organisms and even food webs. For example, they can serve as support and transport of microbes, bacteria, and other toxic organisms (Li et al., 2021); accumulate and carry toxic substances like metals and other xenobiotics (Tien et al., 2020; Forero López et al., 2021a) or be ingested by organisms and accumulated in their tissues (Anderson et al., 2016) to name a few. The latter has been well documented in filter-feeding organisms such as bivalves, especially those marketed that could threaten human health (Fernandez- Severini et al., 2019,

Mercogliano et al., 2020; Truchet et al., 2021a) that are also used as biomonitors of plastic particles in the environment.

In terms of selecting a proper benthic biomonitor of MPs, crabs have been less studied in the literature (Farrell and Nelson, 2013; Villagran et al., 2020, Yin et al., 2021; D'Costa, 2022), though they are an indispensable component of the benthic communities and represent a type of seafood that is easily obtained and frequently eaten by humans (Walkinshaw et al., 2020; Zhang et al., 2021a). Moreover, some research sugges s that crabs can hold MPs in their bodies for weeks and accumulate them in different orgons and tissues through diet and the respiratory system (Farrell and Nelson, 2013; Wat's et al., 2014; Zhang et al., 2021b; D'Costa, 2022). Thus, gills could represent the MPs taken from the water, while the digestive tract, the ones from food items and sediments (Villagran et al., 2020). Research on crabs as biomonitors of MPs is emerging, but some curters found that MPs can cause various adverse effects, including physical damage, such as internal abrasion, oxidative damage, inflammation, liver stress and growt's content (Yin et al., 2021; D'Costa, 2022). Also, plastics lodged in gills can decrease their ability to breathe and develop normal metabolic processes (Watts et al., 2016), while ingestion of plastics can block the digestive tract, producing false satiation and translocatir g in p other vital tissues and organs (Farrell and Nelson, 2013).

In South America, studies regarding MPs' presence in marine environments are developing. Still, some research has demonstrated a higher presence of plastic particles in sediments and water of coastal systems of Ecuador, Perú, Brazil, Uruguay and Argentina due to the high industrial development rates of these countries (Fernández Severini et al., 2019; De La Torre et al., 2020; Rodríguez et al., 2020; Capparelli et al., 2021; Díaz Jaramillo et al., 2021; Forero López et al., 2021a; Pazos et al., 2021; Truchet et al., 2021a). Notably, for Argentina, Forero López et al. (2021b) found significant densities of particles that reached

more than 33,000 items m⁻³ with toxic metals attached in the outlet of a malfunctioning wastewater plant in the Bahía Blanca estuary. This estuary is highly anthropized since it has urban settlements with untreated sewage waters, the largest deep port in Argentina and the largest petrochemical complex in South America that also produces polymers. Besides, the area has constant moves of cargo and naval ships and small-scale artisanal fishers.

However, there is little research on estuarine animals of this urbanized estuary, and they have advocated some crustacean and bivalves (Fernánde Severini et al., 2019, 2020), except for one study by Villagran et al. (2020) that analyzed MP in males of the burrowing crab *Neohelice granulata*. Furthermore, ecological trait 1.5 is a state of MPs, as has been recently observed by Truchet et al. (2021a) for mussels on Argentinian coasts, and therefore, it is essential to consider the approach in the study of plastic particles. For this reason, the objectives of this study were a) to assess MPs and other synthetic debris in water and sediments of different sites which in the Bahía Blanca estuary; b) to evaluate the possible bioaccumulation of these that is in different eggs, tissues and organs (gut, gills, carapace) of males and femal s of three representative intertidal crabs, *N. granulata*, *Cyrtograpsus angulatus* and *teptuca uruguayensis*; and c) to understand whether their abundance and composit on aried among tissues, *taxa*, gender and sites.

2. Material and methods

2.1 Study area and crabs' species

The Bahía Blanca estuary (BBe) (38°45′–39°25′ S and 61°45′–62°30′ W) is located in the SW Atlantic Ocean in the southern Pampas of Argentina and it constitutes the second-largest estuary of the country after La Plata, with almost 2,300 km². It is characterized by

large salt marshes and mudflats, extensive islands interconnected by tidal channels. From a chemical approach, the distribution of the parameters within the system is stable in terms of temperature, turbidity and water, while salinity varies in the inner area according to the season (Freije et al., 2008). Also, this estuary serves as a pool of nutrients that support primary and secondary production of the area and El Rincón, one of the first maritime protected areas of the world (Ferronato et al., 2021). Even when the BBe supports several ecosystem services and fisheries (Speake et al., 2020), it is heavily impacted by anthropogenic activities, like agriculture and cattle, industries Fore, and towns.

In addition, the estuary receives wastewater distroots from the Bahía Blanca city, the main urban settlement that supports ~300,000 inhabit. Its and additional freshwater inputs from other sewage plants and the industrial nuclear, including petrochemical refineries which discharge ~ 106,000 m³ day⁻¹ (Marcovecchipler al., 2021). The cities and industries enclosing the BBe are in continuous expansion and development: by 2002, the industrial area surrounding the petrochemical center bold only nine industries, while in 2012, it included more than 135. Also, the harbor area modifies the coastal environment through continuous dredging activities and the intill from the dredged areas, modifying the coastlines (Marcovecchio et al., 20 1).

Within the study area, two critical ports were selected: Puerto Cuatreros -PC-(38°44'50" S; 62°23'5" W) and Puerto Rosales -PR- (38°55' S; 62°03' W) (**Figure 1**). PC is located in the inner zone, and it has been used as a scientific station for the last 40 years. It is widely used for artisanal and recreational fisheries, with urbanized borders and rural lands used for agriculture and cattle. Besides, the area receives the freshwater inputs of the Sauce Chico river that flows through rural areas carrying heavy metals, pesticides and plastic particles (Girones et al., 2019; Villagran et al., 2019; Forero López et al., 2021a, b) and is

close to a wastewater plant ("Tercera Cuenca"). PR is located in the middle zone. Since it belongs to the General Port Consortium of Bahía Blanca, the area constantly moves fishing and army boats and medium and high altitude ships. Also close to PR, it is located the outlet of the wastewater plant of Punt Alta city that discharges ~ 19,000 m³ day⁻¹ (Marcovecchio et al., 2021).

In this estuary, the burrowing crab *N. granulata* and the genus of the rocky crabs *Cyrtograpsus* are the dominant macrobenthic species of the intertidal. The burrowing crab, *N. granulata*, inhabits the waters of the coastline, where they build their gregarious caves, called "cangrejales", in silty-clay sediments on tidal flats and salt charshes (Carcedo et al., 2021). The rocky crab, *C. angulatus*, inhabits the intertidation of upper sub-littoral zone, inhabiting rocky, sandy, and muddy bottoms. In contrast the fiddle crab, *L. uruguayensis*, was first reported in the estuary in 2019, representing a small population restricted to areas with sandy/silty substrates in the upper intertidation of the astrony. The three species of crabs are principally depresenting for *N. granulata* and *C. angulatus*, which have a broad feeding spectrum (**Table 1**).

2.2 Field sampling

Three replicates of surface water (1 L each) and intertidal sediments (100 g each) were taken from each sampling site in December 2020 and January 2021 (summer seasons), with glass bottles (for water) and a stainless steel shove (for sediments) previously conditioned following the methodology described by Forero López et al. (2021b). All materials employed during the sample and the MPs extraction were washed three times with filtered deionized water (0.22 μ m, pore size), conditioned with ethanol (70%), and then deionized water before covering them with aluminium foil. The materials were oven-dried at

70 °C. Water was taken at high tide, while the sediments from the mudflats inhabited by crabs, at low tide. The physicochemical parameters of the water column were measured *in situ* with a HANNA HI 9828 multi-sensor probe.

N. granulata and *L. uruguayensis* were handpicked from the intertidal at low tide in the summer seasons (December 2020 and January 2021), while *C. angulatus* was collected with a bottom trawling gear at high tide due to the different lifestyles of this species (**Table 1**). At PC, the most abundant crabs were *N. granulata* and *C. grgulatus*. Thus, 30 organisms of each sex and species were collected, except for females c^{f} the second species with lower densities that only 10 could be sampled. The same sampling v/as performed for *N. granulata* in PR, but in this case, as *C. angulatus* was not present in high densities, *L. uruguayensis* was collected from the upper intertidal. The sluggi h crabs or those lacking one or more appendixes were discarded.

2.3 Laboratory analyses

We used cotton clothes to avoid cross-contamination with clothes and samples in the laboratory. In the case of cracs, we measured the total weight (g) and width of the carapace (mm) of each organism In digestive tract and gills were dissected and pooled by species and gender and were weighed (g). For *N. granulata*, we obtained 3 pools per sex (10 organisms each), for *C. angulatus*, 3 pools were held for males and 1 for females (10 organisms each), and in the case of *L. uruguayensis*, we obtained 3 pools for males and 2 for females (10 organisms). In addition, three carapaces were randomly separated for each species. Eggs were also obtained from ovigerous females, but at the time, only *C. angulatus* and *N. granulata* were in this condition.

2.4 Isolation of MPs and other synthetic debris

Each replica of sediments was weighed and placed in aluminium boxes, covered with aluminium foil and dried to constant weight (65 \pm 5 °C for 72 h). We employed the methodology proposed by Masura et al. (2015) and Truchet et al. (2021a) for synthetic debris extraction. Due to the high content of organic matter in the sediments of BBe, aliquots of 10 g of dry sediments from each of the replicates (3 per site) were taken, placed in glass beakers (800 mL), added 30 mL of Fe (II) solution (0.05 M) and 30 mL of H₂O₂ 30%. Then, the samples were covered with aluminium foil and stirred for 10 minutes manually. To ensure the absence of synthetic particles from reagents, all chemicals em_r^{-1} oy d to make solutions were reagent grade, distilled water, and laboratory solutions were reinter through a 0.22 µm poresize filter.

Both types of samples, water and sediments, were digested at 70 °C until no organic matter was observed and sonicated for 20 minutes. In the case of sediments, a triple extraction with NaCl was conducted to extract MPs from them and sonicated for 20 minutes in each extraction. The supernatant (recent the extraction of each sample was filtered (0.45 μ m pore-size nitrocellulose), and the finers were placed in conditioned Petri glass dishes and dried at room temperature. In particular, NaCl supersaturated solution used to extract MPs from sediment samples (annot be efficient in removing APPs since they are denser than MPs of equivalent dimensions (Turner, 2021) due to their chemical composition. For this reason, the precipitate from the density separation was placed in conditioned Petri glass for manual observation of paint sheets.

In the case of organisms, between 10 and 15 mL of KOH 10% solution previously filtered were added to all pool samples and covered with a watch glass and aluminium (Thiele et al., 2019; Colombo et al., 2022). Then, filtered citric acid ($C_6H_8O_7$ 1.5 M) solution was added to each digested pool sample until its neutralization at pH 7 (Colombo et al.,

2022). All the digested samples were vacuum filtered through a nitrocellulose filter (0.45 μ m pore-sizes) and placed in conditioned Petri dishes. In addition, a total of 3 control blanks were employed to assess the possible contamination by MPs and other synthetic debris during their isolation, and no type of contamination was observed (Forero López et al., 2021a). Finally, all the samples were dried (55 ± 5 °C for 72 h) before sorting synthetic debris.

2.5 Microdebris characterization by instrument analysis

The morphological and physical identification of synthetic microdebris was made with a stereomicroscope Leica S8APO, classifying them according to their colors, shape, and sizes. The synthetic microdebris were classified into hiers, fragments, films, paint sheets, foams, microbeads and rubbers in terms of shape (*i.e.* Gago et al., 2019; Carretero et al., 2022). The particles were classified according to their sizes in three groups: < 0.5 mm, between 0.5 and 1 mm, and 1-5 mm

We used a micro Attenuater'. Toul Reflectance–Infrared (μ -ATR) to determine the plastic polymers and paint shee s using a Nicolet iN10 MX Ultrafast spectrometer with a SlideOn MicroTip Ge-ATR style. Also, a Scanning Electron Microscope (SEM) coupled with an energy dispersive λ ray analyzer (EDX). Finally, X-ray diffraction (XRD) was used to determine the crystal s ructure of some paint sheets following the methodology described by De La Torre et al. (2022a). The equipment was operated at a voltage of 45 kV and a current of 40 mA.

2.6 Data analyses

The condition index (CI) of organisms was calculated as shown in Equation 1:

$$CI = \frac{Carapace \ width \ (mm)}{Total \ weight \ (g)} x \ 100\% \ (Equation \ 1)$$

The data were analyzed for normality and equal variance tests. The Kruskal Wallis test was applied to compare the abundance of synthetic microdebris among different species, water and sediment samples. Because of the unbalanced pools in crabs, a general linear model (GLM) was used to assess possible differences between gender and tissues with species as a random factor. A Spearman correlation was employed to observe correlations between the total synthetic microdebris and the CI. All analyzes were performed with the free software R Core Team (2020) and Infostat Student Free Version (Universidad Nacional de Córdoba, Argentina).

3. Results

3.1 Synthetic microdebris in water c... sediments

The total synthetic debris , as 109 items in the sediment samples and 65 items in the water samples. In the case of vater, there were significant differences between both sites, with a higher abunda. $e^{in T}C$ (p< 0.05, mean: 55 ± 14 items L⁻¹ PC, and 13 ± 6 items L⁻¹ for PR) (**Figure 2a**). Contrariwise, in the sediments' samples, PR (2450 ± 378 items Kg⁻¹) exhibited a higher significant abundance of synthetic micro-debris than PC (1085 ± 182 items Kg⁻¹) (**Figure 2b**). For both abiotic compartments, the typical shape of MPs were fibers, while the dominant colors were transparent and blue (**Figure 3a-d**). Smaller debris predominated in the water samples with sizes < 0.5 mm (53%), while in the sediments, the most frequent size was 1-5 mm (47%) (**Figure 3e, f**), including paint sheets as another type (**Figure 3a**) that was undetected in water. In particular, green and black paint sheets were identified in sediments, and these microparticles were opaque. In both environmental

matrices predominated the secondary synthetic microparticles, and only 3% were microbeads (primary MPs).

3.2 Morphological metrics in crabs

The CI was higher in *N. granulata* since the species exhibited a higher weight and carapace width (**Table 1**). Lower CI followed in *C. angulatus* and *L. uruguayensis*. Besides, the CI between genders of the same species did not exhibit si_1 nit ant differences (p > 0.25).

3.3 MPs and other synthetic debris in crabs species

A total of 55 debris were found in the total c. ab samples (21 pools), and the detection rate was 86%—the total abundance (items z^{-1} ww) in crabs is shown in **Figure 2c**. No significant differences were detected in z^{-1} s total abundance among the different species of crabs (p > 0.25) (**Table 2**), but *L. uruguzvensis* had the highest abundance with 1.36 ± 1.16 items g⁻¹ ww, while *N. granulat* and the lowest abundance with 0.18 ± 0.16 items g⁻¹ ww, both species belonging to PK. In particular, the abundances did not show significant differences among tissues is the three species, but they exhibited a higher abundance in Gs than DT (**Figure 2d**) (**1. ble 2**). No statistical differences were observed between genders at each species in the particle abundance than males in all species, with 0.58 ± 0.8 and 0.43 ± 0.6 items g⁻¹ ww, respectively. According to the Spearman coefficient, in each species there was no significant correlation between the CI and the total abundance of items (*C. angulatus*: r = 0,69, p = 0,39; *L. uruguayensis*: r = -0.51, p = 0.38; *N. granulata* from PC: r = 0.35, p = 0.5; *N. granulata* from PR: r = -0.02, p = 0.97).

According to the types of items, fibers were the specific item in all the samples, representing 90% of the total synthetic microdebris. In particular, paint particles were found in both study sites, and in *N. granulata* and *C. angulatus*, with a higher prevalence in Gs tissues, with a mean of 0.1 ± 0.05 items g⁻¹ ww, while in DT the mean was 0.06 ± 0.03 items of paint sheets g⁻¹ (**Figure 3g, h**). For colors, black and transparent were dominant (**Figure 3i, j**), with 18% and 12%, respectively. Whereas, in terms of sizes, < 0.5 mm debris accounted for 32% of the total and predominated in Gs and DT (**Figure 3k, l**).

The detection of MPs in carapaces was 83%, with MFs being found in 10 carapaces of the 12 analyzed. The different species did not show significant differences in the abundance of items per carapace (p > 0.25). The abundance of NPs (items g^{-1} ww) for *N. granulata* from PR and PC, for *C. angulatus* and *L. uruguayen us* were in the following order: 0.14 ± 0.06 , 0.07 ± 0.06 , 0.67 ± 0.52 , 1.5 ± 1.7 , respectively. The fibers accounted for 94% of the total, with the predominant sizes of < 0.5 and 1-5 mm, while blue and black were the most common colors. Finally, in the case or eggs, MPs were found only in *N. granulata* from PR and PC. The averages were 2 items g^{-1} and 5 items g^{-1} for eggs, respectively. In general, 67% were fibers, and the rest were fragments. The predominant sizes were particles < 0.5 mm (67%), and the rest were particles between 0.5 and 1 mm, while blue was the dominant color.

3.4 Types of polymers

A subset of 30 synthetic debris were selected and analyzed using μ -ATR-FTIR and compared with reference spectra. Based on the μ -ATR-FTIR spectra, Cotton-PA, PE, and TFE-copolymer were the most common plastics in the water samples. In brief, μ -FTIR spectra displayed bands typically representative of Cotton-PA (**Figure 4a**), exhibiting N-H structural units around 3300 cm⁻¹ and strong amide I-amide II signals at 1650 and 1543 cm⁻¹.

Another strong peak at 1710 cm⁻¹ is associated with C=O symmetric stretching of Carbonyl functional groups.

PE was another type of synthetic identified microparticle (**Figure 4b**), exhibiting signals C-H stretching at 2850 cm⁻¹ and 2916 cm⁻¹, CH₂ stretching at 1450 cm⁻¹ and the TFE-copolymer presenting the characteristic absorption peaks C-F₂ halogen groups at 1147 cm⁻¹ and 1,209 cm⁻¹ (**Figure 4c**). On the other hand, the most common particles in sediment samples were PE, cellulose, PET, and alkyd resin inside the \triangle PPs. About 25% of synthetic microdebris found in sediments were green APPs. Cellu ose spectrum exhibited peaks between 3340 and 3390 cm⁻¹ (-O-H stretching), at 2905 cm⁻¹ (-C-H stretching) and an important peak at 1,061 cm⁻¹ assigned to the -C O- group (**Figure 4d**). PET spectrum presents at 1730 cm⁻¹ stretching of C=O of carb *as flic* acid group, belonging to the ester, the C-H stretching band of the aromatic ring case, at 3055 cm⁻¹, the peaks between 1340 and 1370 cm⁻¹ to the methylen *z g*, oup and vibrations of the ester C-O bond (**Figure 4e**). Finally, the predominant polymers in crabs samples were cotton-PA and CE, which were already described for abiotic matrices.

Following the characterization of synthetic microdebris, **Figure 5** showed the photography of an APP and SEM micrographs and EDX analysis on the surface of this debris. SEM micrographs show deep cracks and the development of a network of microcracks, fractures, and pin-holes. It can also be appreciated that multiple layers make up the paint sheet (**Figure 5a, b**). EDX spectrum on three different surface sites of the paint shows a strong carbon (C) peak, followed by oxygen (O), titanium (Ti) and iron (Fe), and minor peaks of calcium (Ca), potassium (K), silicon (Si), chromium (Cr), copper (Cu), and aluminium (Al) (**Figure 5c**). In particular, sulfur (S) peaks were identified in spectrums 1 and

3, while barium (Ba) peaks only were exhibited in spectrum 3, and magnesium (Mg) and molybdenum (Mo) peaks were observed in spectrum 2.

All the EDX spectra showed Cr and Cu characteristic peaks, which may indicate the presence of these metals as a pigment (Learner, 2004) and could explain the green color in the APP found in sediments and crabs. However, these elements are also used, like corrosion inhibitors (metallic oxides) and antifouling compounds (Yebra and Weinell, 2009). A SEM-EDX elemental mapping exhibited the presence of Ti, Ca, Fe, S, Al, Si, and Al in the paint sheet, indicating that these elements may be present as filler. and extenders (Learner, 2004). For example, TiO₂, CaCO₃, and BaSO₄ are commonly u. ad as extenders because they add bulk, texture, or rheological properties to paint. However, the change in the atomic percentages (Ca, Ti, Cu, and Cr) or absence of Leaks such as S, Ba, Pb, and Mo in some punctual EDX spectrums could indicate the lenching of some alkaline earth elements from aged micropaint (Luo et al., 2020; Simon et al., 2021) or due to their heterogeneous dispersion in their grain composition within the matrix (Turner, 2010). Finally, the XRD pattern of the APP (Figure 5d) nexhibited any sharp diffraction peaks except for the broad peaks characteristics of their prophous character nature of alkyd resin (Caramitu et al., 2018). This broad peak ove laps with the characteristics signals of oxide metallics, which were identified through S EM/EDX on the APP surface.

On the other hand, **Figure 6** shows SEM micrographs of the fiber found in surface waters. The fiber exhibited a high proportion of organic matter (diatom frustules), bacteria, and clay minerals adhered to the smooth surface of the microfiber. Also, various mechanical abrasions such as scale, pits, flakes, and fringe were observed on some surface regions (**Figure 6a**). EDX analysis on two different surface sites of the microfiber is presented in **Figure 6b**. Both sites exhibit a strong C peak with an atomic percentage of about 75 % Wt in

their composition, indicating their source of organic origin. Other small peaks of Al, Mg, P, Fe, Si, and Ca were also detected. In particular, spectrum 7 showed Ca and Mo, which signals that microfiber transport these elements present in the suspended particulate matrix.

4. Discussion

4.1 Water and sediments

In worldwide estuarine environments, the presence of MPs in the water column has been well addressed (**Table 3**). Previous works have reported higher concentrations in the BBe than in the present study (Forero López et al., 2021b). However, these authors used different sampling techniques, such as plankton nets. Although these nets are the most common method because they allow sampling a large volume of water, the amount and sizes of the MPs depend strictly on the mesh pizes (Gorokhova, 2015; Karlsson et al., 2020; De La Torre et al., 2022b). In this study, we used glass bottles because it has been suggested as an alternative method for shallow waters to allow different particles and less crosscontamination of the samples (Barrows et al., 2017). Despite this, the main disadvantages of using various techniques in MPs sampling without a global consensus are different concentration units that make it more challenging to establish further comparisons. If we compare concentrations according to the same methodologies, the BBe presents more significant amounts of MPs than other environments (**Table S1**), currently one of the most contaminated environments by plastics.

The higher abundance of MPs in PC water samples could be explained by the contributions of the Sauce Chico stream that flows through cattle and agricultural lands that were reported as a crucial source of MPs to aquatic environments in Argentina (Lajmanovich

et al., 2022). At the same time, the proximity to WWTP constitutes an essential input for MPs since the efficiency of these plants in removing MPs i s highly questionable (Forero López et al., 2022) since most of them are primary WWTP. In general, the synthetic fibers of PE, cellulose, nylon and cotton-PA are the predominant used in clothing, being the residues of household washing machines (Ross et al., 2021). Therefore, considering the composition of the main polymers found in the abiotic samples at both sites, most of the MPs in the BBe may be originated from the mechanical degradation of clothing textiles and hygiene products that enter the aquatic environment through sewage, avoiding water meatment processes (Forero López et al., 2021a, 2022).

The hydrodynamics of PC could also explain the higher amount of particles in surface waters since Angeletti et al. (2018) established the in PC the time of residence of suspended particles in the water is higher than in the cute: areas of the estuary. As a result, factors like wind directions can affect the distribution and accumulation of particles at different times and seasons. Nonetheless, the complexity of the circulation, currents' dynamics and the time of residence of the water in the BE make it challenging to establish the dynamics and time of residence of the anthropogenic particles introduced into the estuary.

Denser materials hill fibers of cellulose, PA, PE, and PES were found in the sediment samples, while APPs (alkyd resins) were most frequently in PR due to the port's constant ship movements. PE and PES were previously reported by Díaz Jaramillo et al. (2021) for sediments of the same estuary, while PE is the most frequent type of polymer in sediments of global studies (**Table S1**). Also, the sedimentological characteristics may be an important cause of the high amount of particles found in sediments of PR as this area has extensive tidal flats that favor depositional processes (Buzzi et al., 2021). Thus, synthetic particles can have

the same fate as sediment particles because they share hydraulically equivalent physical properties (Kane and Clare, 2019; Harris, 2020).

Even when APPs are more complex materials than plastics, some authors included them in the MPs pool (Turner, 2021). Furthermore, they have been scarcely studied in the literature because they have been undetected, overlooked, or evaded from the classification in the pool of microdebris (Turner, 2021). Since they contain a higher proportion of inorganic additives (Turner, 2021) (mainly metallic) than plastics and, consequently, are more toxic (Torres & De La Torre, 2021), they should not be considered as MPs, but as another type of synthetic contaminant. In particular, according to the roscute of SEM/EDX, MPs and APPs exhibited metals, cracks, pits and fissures, and some microorganisms adhered to the surface layer, indicating the degree of weathering of these microparticles (i.e., Forero López et al., 2021a, b; Truchet et al., 2021, among othclass, supporting the idea of synthetic microdebris as vectors of chemical and biological contaminants (Turner at al., 2022).

In the case of polymers' char c'erization, XRD is a technique used to determine the crystallinity degree of the change in crystallinity of polymers by weathering processes, chemistry treatment, or modifications, among others (Chen et al., 2021; Forero-López et al. 2022; Pizarro-Ortega ctai., 2022; De La Torre et al., 2022a). Moreover, this technique is also employed to quantify the crystalline phases (polymorphism) of polymers according to the distribution of their substituent in the chain (Pizarro-Ortega et al., 2022; Chen et al., 2021). It is well known that crystallinity influences to optical, thermal, and mechanical properties of polymers (Julienne et al., 2019). During the weathering processes of MPs, the crystallinity degree may be increased or decreased, affecting the sorption mechanisms between MPs and organic contaminants and metals present in the water column (De la Torre et al. 2022a; Fu et al. 2021). Finally, and since a considerable amount of these fibers are

cotton and cellulose-based in both abiotic matrices, most of the MPs in the BBe may originate from the mechanical degradation of clothing textiles and hygiene products that avoid water treatment processes (Forero López et al., 2021a, 2022).

4.2 Crabs

Decapods are an interesting group to study the accumulation of emerging contaminants since they are exposed to both abiotic comparements, and their different biological features play a role in MPs retention and to ficity (D'Costa, 2022). Since MP abundance might vary with taxa, feeding mode and habitat, it is recommended to include different taxonomic groups in any ecological assessment of the impact of synthetic microdebris, but few studies have achieved usis goal (Piarulli et al., 2020; Xu et al., 2020) and in Argentina, it was only studied in mussels (Truchet et al., 2021a) with promising results.

In terms of abundance the fiddler crab *L. uruguayensis* exhibited the highest values of items g-¹ ww of the species analyzed, with values even ranging to similar ones registered in other larger crabs (**Table 51**). In contrast, *N. granulata* and *C. angulatus* presented lower values even when they doubled the fiddler crab's size. Some research (Messinetti et al., 2018) exposed that strict filter-feeding organisms are more prone to MPs ingestion. Even when the three species analyzed share this same feeding pattern, *C. angulatus* and *N. granulata* have a broader range of eating patterns (**Table 1**), which might be an appropriate explanation of why *L. uruguayensis* exhibited a higher abundance of items. Meanwhile, no differences were observed according to genders, so the accumulation of MPs resulted in constant for these species.

Another possibility is the higher presence of MPs in the upper intertidal. Although we did not measure the abundance of particles in sediments of different intertidal zones, a previous study in the BBe by Díaz Jaramillo et al. (2021) explained that MPs abundance is higher in the upper intertidal in relation to the lower zone. Since *L. uruguayensis* inhabits this particular area (**Table 1**), this crab may bioaccumulate MPs from the upper layer of sediments, representing another hypothesis to explain the higher accumulation of these particles in this species.

Even when we registered differences in the CI between the three species, these differences were not attributed to the accumulation of microdebris, since there was no correlation between these measures. And thus, no conclusions could be reached for CI and synthetic microdebris accumulation, as it was *else* observed in the case of several bivalves' species, whose CI was unmodified by different size particles of MPs (i.e., Urban-Malinga et al., 2021; Truchet et al., 2021a).

Although *N. granulata* was the common crab between the two sampling sites, the organisms did not exhibit significant differences between sites. Villagran et al. (2020) observed the same situation for the same species and sites. This fact probably implies that some crabs' species have accumulation rates independent of the environmental concentrations of items or ingest them at constant rates (Remy et al., 2015; Cole et al., 2020). However, more research is needed to assess this possible idea by contrasting environmental concentrations with laboratory exposures.

N. granulata and *L. uruguayensis*, are crabs with a burrowing activity that exhibited a higher abundance of MPs in Gs than the DT. The same pattern was observed by Villagran et al. (2020) for males of *N. granulata* and, according to Kolandhasamy et al. (2018), adherence in some organs like Gs was reported to be a novel way for animals to uptake MPs beyond

ingestion. The higher predominance of MPs in Gs could be explained by the burrowing behavior of these two species since they remove sediments, organic matter and contaminants (Menone et al., 2006; Truchet et al., 2021b), possibly also including plastic particles that could be adhered to the Gs (Villagran et al., 2020). For Watts et al. (2016), crabs can take up MPs by ventilation into the gill chambers, where may remain for up to 22 days, which could be risky since in some species, like *C. maenas*, are found adhered to the posterior Gs, the major site for ion regulation. Consequently, the inspiration of plastic particles through the ventilatory mechanism can also reduce the fitness of crabe (viatts et al., 2014, 2016). Contrariwise, *C. angulatus* exhibited a different trend, where the DT presented a higher abundance than the gills. Even if the differences were not significant, this trend could be explained by this species' errant lifestyle in the esturity subtidal levels (**Table 1**), unlike the other two species whose Gs could be more p on the MPs inked to benthopelagic exchange pathways.

About the similar sizes of Mrc in Gs, it could be explained because they can take up plastic particles through inspiration across the Gs due to adherence to the hairlike setae on the external surface of Gs following aqueous exposure (Watts et al., 2014). In the case of the sizes found in the DT, even when we found similar sizes of MPs in water and DT, the smaller sizes of fibers found in the DT of crabs might be due to the breakdown of larger sizes in the gastric mill digestive processes, and those smaller plastic particles could return to the environment through the feces (Watts et al., 2015). Furthermore, the same authors found that crabs that ingested food containing MPs fibers showed reduced food consumption and a significant reduction in energy available for growth (Watts et al., 2015). In this way, it is essential to keep monitoring these contaminants since they might affect the overall food consumption and metabolism, especially in the species analyzed that ingest sediments with high MPs abundance.

Regarding MPs found in crabs' carapaces, their lifestyle might also be related to their abundance. The size of carapaces does not seem to directly influence the number of MPs adhered to them since no significant statistical differences were detected. In the case of *N. granulata*, the bioturbator role could lead to the adherence of particles in its carapace. *L uruguayensis* shares the same way of exposure due to similar habits, but the bioturbation caused by this species is relatively lower due to its small patchy distribution on the BBe compared to *N. granulata* (Truchet et al., 2019). On the other hand, it is possible that in *C. angulatus* while the tide rises, MPs can adhere to the carapace because of depressions, ridges and channels in the carapace.

Egg masses were registered in females of *N. aran data*. Still, *L. uruguayensis* was not gravid when field sampling was performed on the latest days of January and thus, this might have affected the proportion of ovigerous courbern females (Colpo & López Grecco, 2017; Truchet et al., 2019). None of the studie, on crabs found in the literature analyzed the presence of MPs in egg masses. MFs could have been adhered to eggs from the surrounding environment or transferred from the development stage, eggs could reach up to 0.5 mm in *N. granulata*, and during the reproductive season, when the gonad is growing, the reserves of the hepatopancreas decrease because they provide vitellogenin to the ovary. Therefore, it is possible that MPs of 0.5 mm could be translocated to the eggs, while the larger particles could have accidentally adhered from the environment, but more research is needed on this topic.

Cellulose, PA and CE were the most common MPs in crabs and in other worldwide species (**Table S1**), indicating that they might take particles from water and sediments and have a particular preference for the type of plastic particles, which still has not been reported.

As a result, the ecological features might not play a role in the type of bioaccumulated polymer. The cellulose-based polymers have been reported as one of the most common semisynthetic fibers in marine crustaceans (Remy et al., 2015) (**Table S1**), and in the present study, it might be associated with the textile products from primary WWTP. Even when cellulose might be unconsidered toxic for organisms, most cellulose fibers have additives or are colored by industrial dyes, resulting in toxicity for some marine organisms (Remy et el., 2015). And this is why it is necessary to keep on investigating the composition of the particles with SEM/EDX, Raman, XRD, and FTIR spectroscory: a nong others.

Nonetheless, the possible toxic effects of the epolymers are still not yet fully understood. As an example, CE can affect the digestive system and inhibit the growth rates of organisms (Kim et al., 2021a, 2021b), and adsorboxic metals like Cd onto MPs surface (Zhu et al., 2020). Whereas PA was not registered as an ordinary MP in the literature, its toxicity has been less studied in marine invertebrate, with unlikely adverse health effects in oxidative stress (Cole et al., 2020). Still, there are ovidence that nano PA are more toxic than MPs, and the passage of larger items through the intestinal tract and the gastric mill could physically damage MPs resulting in the formation of nanoplastics with immune responses (Watts et al., 2015; Cole et al., 2020).

In this study, we registered APPs (alkyd resin) with a higher prevalence in Gs of *N*. *granulata* and *C. angulatus*, coinciding with the findings of these particles in sediments and thus, crabs might be uptaken from this compartment instead of the water column. In addition to antifouling Cu paintings as probably the leading APP, TBTs were detected in multiple frameworks in the BBe, for different abiotic compartments and mussels (Quintas et al., 2017; 2021). Therefore, APPs might serve as a current and historical source of biocides (Turner et al., 2022), with probable adverse effects due to their chemical additives that need to be

further monitored. In PR, metals like Zn and Cu have been detected in the sediments and *N. granulata*, probably from APPs disposals (Truchet et al., 2021b), which could adversely affect the health of benthic organisms at environmentally relevant concentrations (Muller-Karanassos et al., 2021).

5. Conclusions

Synthetic microdebris corresponds to the so-called energing concern contaminants, a group of contaminants whose effects on the biota are still in completely studied. This work represents the first approach in selecting a suitable biomonitor species of these contaminants by studying the accumulation patterns of this debris in three widespread SW Atlantic benthic crab species with different ecological traits. We did not find statistical differences in the total amount of microdebris, while only *C. engulatus* and *N. granulata* accumulated APPs, possibly from different routes, like we and sediments, respectively and according to their lifestyles. Even when *L. uruguar ensis* is smaller than the other species, it exhibited a higher abundance –yet not significant- of microdebris, possibly taken from the upper-intertidal sediments inhabited maining of this species and where more particles are deposited. Cellulose, PA and CE and APPs we are the most common types of debris in crabs, indicating a possible selective accumulation rate of these contaminants, with still unknown effects on the biota that should be further studied, especially in the case of complex contaminants like APPs.

We also concluded that the ecological traits helped us understand the accumulation patterns of synthetic microdebris. Therefore, it is essential to address these approaches when selecting a good benthic biomonitor species for these contaminants. However, we accomplished that *N. granulata* was the most suitable biomonitor species for the BBe since it is widely distributed throughout the estuary in all seasons and sites, it is easy to sample, has a

broad feeding spectrum, is exposed to the aquatic and sedimentary phases and it has accumulated all the types of microdebris.

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Figure captions

Figure 1. Map of the study are . I. the Bahía Blanca estuary (BBe). The points indicate the sampling sites: Puerto Cuatreros (PC) and Puerto Rosales (PR).

Figure 2. Abundance (nea) \pm SD) of MPs and other synthetic micro-debris in **a**) water (items L⁻¹), **b**) sedimente (items Kg⁻¹) of PC and PR, **c**) crabs' species of PC and PR, and **d**) different tissues of crabs' species (items g⁻¹ ww).

Figure 3. Types, colors and sizes of MPs and other synthetic micro-debris in **a-f**) water and sediments; **g**⁻¹) gills and digestive tract of crabs.

Figure 4. μ-ATR-FTIR spectra of microplastics found in different samples: **a**) Cottonpolyamide, **b**) Polyethylene, **c**) Polytetrafluorethylene, **d**) Cellulose, **e**) Polyethylene terephthalate.

Figure 5. Photography and SEM micrograph obtained at different magnification: a) 250x andb) 5000x; c) punctuals EDX spectra of paint sheet found in sediment; d) XRD spectra of paint sheet found in sediment of BBe.

Figure 6. a) SEM micrograph obtained at 5000x and **b)** punctuals EDX spectra of a microfiber found in surface waters of the BBe.

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Figure 1



5





Figure 3









Figure 5

Figure 6



Crabs characteristics	Neohelice granulata	Cyrtograpsus angulatus	Leptuca uruguayensis	
Eating patterns	Omnivorous, deposit	nnivorous, deposit Omnivorous, deposit		
	feeder, scavenger feeder, scavenger		Deposit leedel	
	Burrower. Middle and higher levels of the intertidalErrant. Subtidal and lower levels of the intertidal.		Durrower Upper lovale	
Lifestyle			of the intertidel	
Weight (g)	11 ± 5.4	4.3 ± 2.5	0.98 ± 0.32	
Carapace width (mm)	25.3 ± 3.6	20 ± 3	13 ± 1.5	
Condition index (%)	41.5 ± 15.2	20.7 ± 7.8	7.48 ± 1.96	
Items in gills	0.17 ± 0.14	0.11 ± 0.07	1 ± 1	
Items in gut	0.06 ± 0.07	0.19 ± 0.11	0.36 ± 0.25	
Items in carapace	0.11 ± 0.07	0.67 = 0.52	1.5 ± 1.7	
Items in eggs	4 ± 2	ND	ND	
Total items	1.08 ± 1	0.25 ± 0.3	0.70 ± 0.6	

Table 1: Main characteristics of the crabs' species sample from the BBe. Data is represented in average \pm SD and synthetic particles are represented as item g⁻¹ ww

Abbreviation: ND, not detected

	numDF	denDF	<i>F-value</i>	p-value
Tissues	1	36	3.55	0.0678
Genders	1	36	0.58	0.4527

Table 2. Results of a general linear model (GLM) with species as a random factor.

Random factor sd: 0.70

Residual sd: 0.37

The random factor sd with a high value vs low residual sd, shows the efficiency of using a GLM with species as a random factor.



CRediT authorship contribution statement

D.M. Truchet: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Visualization; Validation; Supervision; Writing - original draft; Writing - review & editing

M.D. Ardusso: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Visualization; Writing - original draft; Writing - review & editing

A.D. Forero López: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Software; Visualization; Supervision; Writing - original draft; Writing - review & editing

G.N. Rimondino: Formal analysis; Investigation; Methodolo₅y, Resources; Writing - review & editing

Natalia S. Buzzi: Visualization; Resources; Funding ac uisition; Writing - review & editing F.E. Malanca: Visualization; Resources; Funding ac uisition

C.V. Spetter: Visualization; Funding acquisition

M.D. Fernández Severini: Investigation[.] ' e^{o} ources; Funding acquisition; Project administration; Supervision; Validation; V_{e} n ing review & editing

Graphical abstract



Highlights

- First multi-compartment approach of MPs and other synthetic microdebris in the BBe
- Cr, Mo, Ti, Pb, Cu, Ba, and Fe were detected on the surface of paint sheets.
- Cellulose and cotton-PA were the most common synthetic microdebris.
- Bioaccumulation in crabs might be dependent on their lifestyle and feeding spectrum.