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Microplastics in commercial seafood: *Pleoticus muelleri* as a case study in an estuarine environment highly affected by human pressure (Southwestern Atlantic).

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Abstract

Plastic pollution in seafood has become a worldwide safety concern due to its possible harm to humans. This is the first study which has investigated the length-weight relationship, growth patterns and condition factor, together with the concentrations of microplastics (MPs) and mesoplastics (MesoPs) in Pleoticus muelleri from the Bahia Blanca Estuary (BBE), Argentina. Forty-nine individuals were collected from three sampling stations in the BBE, and each abdominal muscle with the gastrointestinal tract was analyzed. P. muelleri showed an isometric growth pattern (b = 3.0054) with values of K similar among the individuals collected (ranged between 0.80 and 0.91), considering them in good condition compared to other crustacean species around the world. 96% of shrimp presented transparent or black synthetic fibers as prevalent types, with an abundance average of (3.0 ± 2.90) MPs/g w.w. and (0.053 ± 0.16) MesoPs/g w.w., as well as a dominant size range of 0.5-1.5 mm, in accordance with recent studies in the same area. The linear regression analysis showed that K was independent of the concentration of MPs ingested by P. muelleri, with R^2 ranging between 0.024 and 0.194 indicating that MPs contamination does not affect the nutritional condition of shrimp. SEM/EDX detected the presence of elements like C, O, K, and Mg, tissue residues and fractures on the surface of the analyzed fibers. FTIR confirmed different types of polymers in shrimp related to textile fabrics probably from untreated sewage discharges from nearby cities. The results of this research provide useful information for a better understanding of MPs contamination in seafood, suggesting P. muelleri as a suitable species for monitoring MPs in estuarine ecosystems. Likewise, more research is required to know the effects of MPs on food safety in humans.

Keywords: Microplastics, Emerging contaminants, shrimp; Condition Factor; Cellulose; Food web.

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Graphical abstract



1. Introduction

Plastics, are one of the most used materials worldwide due to their low cost, malleability, high strength-to-weight ratio, and thermal and electrical insulating properties (Ardusso et al., 2021), which makes them practically impossible to replace, although they are negative for the environment (Wang et al., 2015; Barnes et al., 2009; Andrady, 2003). Aquatic and terrestrial ecosystems are threatened by the presence of the so-called "plastic pollution". Microplastics (MPs, plastic particles <5 mm) are part of the plastic pool, and can be found in most marine environments. Their presence is mainly originated by the fragmentation of bigger plastic items by mechanical, physical, or chemical action. Thus MPs, as emerging pollutants, have become an important global environmental problem because of their wide distribution and their long residence time in the environment (Andrady, 2011). Furthermore, they can be unintentionally assimilated by organisms in different ways, like air-water inhalation, indirectly through web chains, or by direct ingestion (Anderson et al., 2016). Crustaceans such as shrimp ingest everything in their path including MPs from water and sediments, as well as copepods, fish larvae, and other item-prey which may also incorporate MPs from the aquatic environment (Curren et al., 2020). Thereby, shrimp pose a threat when consumed by fish and top predators like whales, or by humans. Although the cephalothorax of shrimp is not consumed by humans, the abdominal muscle is, together with the gastrointestinal tract where MPs accumulate, posing a great risk to food safety (Curren et al., 2020; Fernández-Severini et al., 2020). Hence MPs can be detected at several trophic levels and can be extensively transferred throughout the food chain (Savoca et al., 2019). Nowadays, this transfer is a major concern, as fisheries and aquaculture provide a critical proportion of the world's food supply. Seafood safety

associated with MPs as a stressor is a key issue to consider therefore, studies focused on the presence of plastics, their characteristics, and seafood sanitary conditions are of utmost importance.

The length-weight relationship and condition factor in crustaceans have been welldocumented in different marine species worldwide (Wong et al., 2015; Uddin et al., 2016; Sousa et al., 2019). This relationship is an important tool in fisheries and aquaculture management as it provides information on growth patterns and on the general condition of the organism, reflecting fluctuations in the uptake and allocation of energy (Guino-o, 2012). Thus, these parameters are influenced by many factors such as the reproductive cycle (Chu et al., 1995), water temperature, nutrient supply, and by pollutants (Castilho et al., 2007). Another important parameter for the management of culture systems is Fulton's condition factor (K), used to quantify an animal's physical wellbeing, and a useful complement to growth estimates of crustaceans (Piratheepa et al., 2013). It is important to point out that there are no available studies in the literature, concerning the length-weight relationship or condition factor K of *Pleoticus muelleri*, making the present study the first one to use this tool. It is necessary to strengthen the knowledge about different aspects of its biology to maintain an effective conservation plan.

This study is focused on *Pleoticus muelleri* Argentine red shrimp, a highly consumed seafood species, commonly available in local markets and also exported to many countries for human consumption. Argentina, at the moment, is the second most important exporter of shrimp in Latin America (179,000 tons), increasing year by year. Lately, shrimp have been considered as one of the most valuable fishery resources in Bahia Blanca Estuary (BBE) (FAO, 2016; Secretaria de Agricultura, Ganadería y Pesca, 2021). Since MPs may

affect the health of commercially exploited organisms, food security, and human health, the analysis of MPs abundance, distribution, and composition as well as the sanitary state of the red shrimp is of significant relevance. To better understand the biological aspect and its implication for fishery management, we investigated the length-weight relationship, growth patterns, and condition factors of this species, together with the possible interaction with ingested MPs. We hypothesize that, in *P. muelleri*, the presence of MPs is high and may represent a risk to food safety.

2. Materials and Methods

2.1. Research area

The Bahía Blanca Estuary (BBE) is located on the southwest Atlantic coast ($38^{\circ} 45' - 39^{\circ} 25'$ S and $61^{\circ} 45' - 62^{\circ} 30'$ W), with an extension of about 2.300 km². This estuary is a mesotidal temperate system and has an irregular triangular shape, divided into inner, middle, and external zones. The area comprises three main channels (Principal, Embudo, and Bermejo), two bays (Falsa and Green) and Brightman cove. There are NE-SW oriented islands which are not covered by the tides, while there are wetlands covered by the sea at least twice a day. Furthermore, this estuary is strongly influenced by the wind and presents low fluvial input, which is only significant on rainy seasons (Piccolo & Perillo, 1990).

BBE represents one of the most important coastal environments of South America, from an economic and ecological perspective, with heavy maritime traffic. In the inner zone are the cities of Bahía Blanca (~ 300.000 inhab.), Punta Alta, and General Daniel Cerri, along with one of the most important petrochemical and industrial parks from Argentina, formed by petroleum, chemicals, and plastic industries producing polyvinyl

chloride and different types of polyethylene which could be a potential source of primary microplastics. Furthermore, most of these industries, as well as the neighboring cities discharge their effluents in the estuary with partial or no treatment (Fernández-Severini et al., 2019). The Principal channel, with a length of 67 km, gives access to the most important deep-water port system in the Argentine Atlantic which includes the ports of Ingeniero White, Galván, Rosales, Cuatreros and Belgrano, being the last one the largest Argentine naval base (Biancalana et al., 2018). Also, the "Natural Maritime Reserve (Reserve of Multiple Uses Bahía Blanca, Bahía Falsa, and Bahía Verde)" (Ley 12.101/98) is located in the middle and external zones of the estuary. This reserve protects and preserves numerous islands such as Bermejo, Green, Embudo, among others, as well as an important number of streams and channels until they reach the open sea. Hence, these sites, which are a highly productive and eutrophic ecosystem, represent an important economic area for many artisanal fisheries, being P. muelleri one of the most commercialized species. However, it receives sediments from the dredging of the Principal channel and Galván port, which could carry pollutants to the area (Truchet et al., 2019).



Figure 1. Map of the study area, the Bahía Blanca Estuary (BBE). The dots show the sampling stations: VC, Vieja channel; EC, Embudo channel; GI, Green Island.

2.2. Field sampling

P. muelleri were collected during spring/summer 2019, by artisanal fisherman, from three sampling stations in the BBE (**Figure 1**): Vieja channel (VC) ($38^{\circ} 50' 01.1"$ S - $62^{\circ} 12' 34.7"$ W), Embudo channel (EC) ($38^{\circ} 55' 59.99"$ S - $62^{\circ} 9' 0"$ W), and Green Island (GI) ($39^{\circ} 15' 23.5"$ S - $62^{\circ} 11' 40.3"$ W). VC is located in the middle zone of the estuary and represents the area where the cities of Bahía Blanca, Punta Alta, and Ingeniero White discharge their untreated sewage waters, which have proved to be toxic for the zooplanktonic fraction and to modify environmental quality parameters (Tombesi et al.,

2000; Biancalana et al., 2012; Dutto et al., 2012; Berasategui et al., 2018). EC and GI, are placed in the external zone, and make up the "Natural Maritime Reserve". A total of 49 organisms were randomly selected, specifically 20 shrimp came from EC, 19 from GI, and 10 from VC. The organisms were collected by anchoring shrimp fishing nets and immediately frozen in the same boat, so they were not subjected to intestinal purification. Shrimp were immediately transported in iceboxes to the laboratory at -20° C for preservation until further analysis. Each shrimp was washed with distilled water (previously filtered through 0.22 µm - pore size membrane filter) and biometric data (total wet weight (g) and length (cm)) were determined according to the methodology described by Fernandez-Severini et al. (2020). Then, each organism was dissected by discarding the cefalotorax and keeping the abdominal muscle with the gastrointestinal tract, and placed individually in a previously conditioned glass beaker.

2.3. Cleaning procedure

In order to prevent airborne MPs contamination, the samples were processed in a laboratory with restricted access and all the materials used during the analyses were conditioned according to the methodology described by Fernandez-Severini et al. (2020) and Forero-López et al. (2021a). Cotton lab coats, facemasks, and nitrile gloves were worn during laboratory work. Moreover, reactive solutions and distilled water used to prepare these solutions were filtered through a 0.22 μ m-pore size membrane filter before their use to ensure the elimination of MPs from reagents. Control blanks (n = 3) were utilized to assess MPs contamination levels during sample processing analysis. MPs found in the blanks (mean: 4 items ± 4.35) were subtracted from the number of items found in the samples.

2.4. Laboratory analysis

MPs extraction from shrimp abdominal muscles containing the gastrointestinal tract was based on the methodology proposed by Thiele et al. (2019). The tissues of each individual were placed in a glass beaker within 10 to 15 mL of 10% KOH solution and quickly covered with a watch glass and aluminum foil to prevent airborne particle contamination. Periodic manual agitation at 60°C for 48 hours was carried out until the tissue was completely digested, that is, without visual appreciation of it. Additional KOH solution was added as it evaporated during digestion. Once the samples were cooled, they were neutralized with 1.5 M citric acid until reaching a pH 7, verified by indicator strips. Each sample was vacuum filtered through a glass fiber filter (0.70 μ m-pore size) and placed in conditioned glass Petri dishes. A stereomicroscope (Leica S8 APO) and an optical microscope (Nikon Eclipse LV100) were used to identify, record, photograph, and classify plastic debris based on their size as MPs (< 5 mm) or MesoPs (> 5mm), colour, and shape (fragment, pellet, fiber, or films). Items grouped as MPs were also classified in five size ranges <0.5 mm, 0.5–1.5 mm, 1.5–2.5 mm, 2.5–3.5 mm, 3.5–5 mm.

2.5. Plastic characterization

In order to delve into the chemical composition, characterization of the surface morphology and atomic composition of MPs, fibers larger than 500 µm were manually separated with a hypodermic needle or dissection tweezers and resuspended in distilled water, with subsequent vacuum filtering through a nitrocellulose filter (0.45 µm pore size, Millipore). MPs were analyzed by Nicolet iN10 FTIR infrared microscope and by a dual-

stage ISI DS 130 SEM with an EDAX 9600 quantitative energy dispersive X-ray analyzer according to the methodology described by Forero-López et al. (2021c).

2.6. Length-weight and condition factor measurements

The length-weight relationship of shrimp was expressed in allometric form (Li et al., 2016):

$$W = a L^b$$

where W is the weight (g), L is the total length (cm), *a* is the constant showing the initial growth index, and *b* is the slope showing the growth coefficient. If shrimp retains the same shape, it grows isometrically (b = 3). When weight increases more than length (b > 3), it shows positive allometric however, when the length increases more than weight (b < 3), it indicates negative allometric (Li et al., 2016). The proper fit of the growth model was given by the determination coefficient (R^2). The correlation coefficient (r) was calculated between the weight and total length of shrimp.

To evaluate the nutritional condition of shrimp, condition factor (K) by Fulton (1904) was calculated for each individual (VC, EC and GI) and adapted for decapod crustacean (Lalrinsanga et al., 2012):

$$K = ((W / L^3) \times 100)$$

where W is the total weight (g) and L the total length (cm) of the organism.

2.7. Statistical analysis

Statistical analyses of the concentration of MPs found in shrimp were performed using the free software R Core Team (2017). Also, an analysis of variances (ANOVA-one way) was performed to evaluate the statistical differences between the concentrations MPs found in abdominal muscle with the gastrointestinal tract of the shrimps from each sample station, previous verification of the homoscedasticity of the variances, and the normal distribution of the residues.

On the other hand, a linear regression analysis was carried out for each sampled station, considering condition factor as the dependent variable and the abundance of MPs as the independent variable, in order to observe if a dependence relationship existed between them.

3. Results and discussion

All the collected *P. muelleri* individuals presented a good correlation regarding their body weight and their body length. In general, the body weight varied between 1.34 and 35.4 g, whereas the samples of the abdominal muscle weighed between 0.56 and 14.58 g. The body length of shrimp varied between 5.5 and 16.2 cm. The values of length and corresponding weight of shrimp were plotted in **Figure 2**. The estimated (*b*) value given in the allometric equation was 3.0054 (W = 0.008 L^{3.0054}), with R^2 of 0.95. Thereby, the slope (*b*) of regression was 3, corresponding with an isometric growth of *P. muelleri* at BBE (*t* = 0.0518, *p* > 0.95), meaning the shrimp retained the shape while growing. The correlation coefficient (*r*) between total length and body weight of the shrimp was 0.94 (*r* > 0.5), showing that the length-weight relationship was positively correlated. Since this is the first

study analyzing the length-weight relationship in *P. muelleri*, there is no data to compare. However, similar results were seen in other species like *Penaeus monodon*, which recorded a (*b*) value of 3.2183 in pooled shrimp (W = 0.0039 L^{3.2183}) and a coefficient of determination (R^2) of 0.97, corresponding to an isometric growth (Piratheepa et al., 2013). In the case of *Macrobrachium felicinium* pooled shrimp there was an isometric growth with a (*b*) slope of 3.003 (W = 0.0016 L^{3.003}) and an R^2 of 0.99 (Okayi & Iorkyaa, 2004).



Figure 2. Length-weight relationship of *P. muelleri*.

As can be seen in **Table 1**, condition factor K was similar for the individuals collected from VC, EC, and GI at BBE, with an average of (0.80 ± 0.1) , (0.85 ± 0.1) , and (0.91 ± 0.2) , respectively. The lack of information related to K factor for other shrimp species does not allow intercomparisons, but it places this study as a potential starting point for future research. Nevertheless, the values found here are similar to those obtained with other crustacean species all around the world, where the authors argued that they were in

good condition. Recently, Rossiter et al. (2021) studied different species of *Macrobrachium sp* giant prawn, and reported a condition factor of (1.12 ± 0.23) . Another study in the oriental *Macrobrachium nipponense* river prawn showed K ranged between 0.78 to 1.34 (Aminisarteshnizi, 2021). Also, Kaka et al. (2019) studied different penaeid shrimp species in Kenya and found K values between (0.76 ± 0.084) and (0.45 ± 0.02) , being these results similar to those obtained in this study.

Of the 49 shrimp collected at the different sampling sites, 96% presented MPs; however, shrimp from EC showed higher total content of MPs in the abdominal muscle than organisms from VC and GI. A total of 295 plastic particles were found in shrimp samples with an average of (3.0 ± 2.90) MPs per gram of wet weight (items/g w.w.) and (0.053 ± 0.16) MesoPs/g w.w.. A previous study carried out by our group reported a mean concentration of 1.31 MPs/g w.w. for P. muelleri individuals collected from the inner zone of the estuary (Fernández-Severini et al., 2020), using a different digestion method. Some authors have reported that the technique employed to digest the tissue from organisms can affect the visual identification of plastic particles due to incomplete digestion or physical damage of plastic items (Fernandez-Severini et al., 2020; Hara et al., 2020). The mean MPs concentration found in the abdominal muscle of P. muelleri in the present study was similar, or comparatively lower, than those reported for other shrimp species worldwide. Hossain et al. (2020) reported an average of (3.40 ± 1.23) MPs/g w.w. in *Penaeus monodon* and (3.87 ± 1.05) MPs/g w.w. in *Metapenaeus monocerous* from the Bengal Bay, Bangladesh. Recently, a study on *Metapenaeus affinis* white shrimp, one of the most important fish resources of the Persian Gulf, showed an average abundance of (4.31 ± 1.7) MPs/g w.w. (Keshavarzifard et al., 2021). Also, Gurjar et al. (2021), studied three different shrimp species in the northeastern part of the Arabian Sea, and estimated the highest average value of (70.32 ± 34.67) MPs/g w.w. in pooled shrimp.

Table 1. Morphometric results, condition factor (K), microplastic (MPs) and mesoplastics (MesoPs) content in *P. muelleri* in each sample site.

Sampling sites	FC(K)	Body weight (g)	Body length (cm)	Abdominal muscle	Plastic particles		
				weight (g)			
					Total	Abundance	
					plastic		
				0	particle	s	
VC	0.80	2.88 - 9.20	7.2-10.2	1.07 – 3.57	65	$3.69\pm2.02~MPs/g.w.w$	
		(5.52 ± 1.96)	(8.74±0.92)	(2.15 ± 0.73)	4	$0.40 \pm 0.12 \ MesoPs/g.w.w$	
EC	0.85	1.41 - 35.4	5.5-16.2	0.63 - 14.58	158	3.54 ± 3.52 MPs/g.w.w	
		(9.82 ± 8.93)	(9.96 ± 3.08)	(3.98 ± 3.60)	1	$0.029 \pm 0.13 \text{ MesoPs/g.w.w}$	
GI	0.91	1.34 - 13.05	6-11.2	0.52- 5.15	67	$2.15\pm2.43~MPs/g.w.w$	
		(6.53 ± 2.58)	(8.81 ± 1.25)	(2.23 ± 1.0)			

In particular, MPs content in shrimp from VC (middle zone) ranged from 2.68 to 6.80 MPs/g w.w., with a mean value of (3.69 ± 2.02) MPs/g w.w.. The external zones EC and GI presented ranges from 0 to 9.62 MPs/g w.w. (mean = (3.54 ± 3.52) MPs/g w.w.) and 0 to 11.22 MPs/g w.w. (mean = (2.15 ± 2.43) items/g w.w.), respectively (**Table 1**). The analysis of variances (ANOVA-one way) showed no statistical differences between sample sites according to MPs content in *P. muelleri* (F = 1.54, *p* = 0.225). The linear regression analysis showed that condition factor K was independent of MPs concentration found in *P. muelleri* on each sample site, with a coefficient of determination (R^2) of 0.170 in VC, 0.024 in EC, and 0.194 in GI. Therefore, K seemed to be independent of the content of MPs ingested by the animal. Our results agree with those reported by Devriese et al.

(2015), where no correlation was observed between K of *Crangon crangon* brown shrimp and the ingestion level of MPs, indicating that MPs contamination does not affect the nutritional condition of shrimp. In the same way, and analyzing other species, Foekema et al. (2013) found no significant association between K of seven fish species of the North Sea and the presence of ingested plastic particles.

Fibers were the most common morphotype found and varied from 0.059 to 7.44 mm in size while plastic particles between 0.5 and 1.5 mm were the most common, representing 44.75% of the total MPs detected at all sampling stations. Size ranges from 1.5 to 2.5 mm, <0.5 mm, and 2.5 - 3.5 mm represented percentages of 22.71%, 22.03%, and 3.74%, respectively. Size range from 1.5 to 2.5 mm was predominant at VC and GI, while the range <0.5 mm was predominant at EC (see Figure 3). These results are in accordance with those described by Forero-López et al. (2021c), who reported the presence of plastic particles with size ranges from 0.047 to 13.5 mm in the water column during the same sampling period. Otherwise, MesoPs (> 5 mm) were also found, representing 1.69% of total plastic particles (Figure. 3a), with mean value of (0.40 ± 0.12) MesoPs/g w.w. at VC, while at EC was (0.029 ± 0.13) MesoPs/g w.w.. Villagran et al. (2020) and Fernández-Severini et al. (2020) reported the same predominant size ranges of MPs and the presence of MesoPs in equivalent tissues of regional decapod organisms such as Neohelice granulata burrowing crab and *P. muelleri* shrimp. Furthermore, this size range pattern was in accordance with the results shown in recent studies in other shrimp species in the world (Li et al., 2021; Pradit et al., 2021; Yan et al., 2021; Daniel et al., 2020; Hossain et al., 2020). Regarding other types of locally-consumed seafood, Amarilladesma mactroides and Brachidontes rodriguezii mussels, presented an abundance of (0.33 ± 0.1) MPs/g w.w. and

 (0.17 ± 0.07) MPs/g w.w., respectively, (Truchet et al., 2021b) which were lower than those presented in this study for *P. muelleri*.

Different plastic colours were recorded as can be seen in **Figure 3b**. Transparent MPs were the most abundant fibers found in shrimp samples from VC (52.16%) and EC (37.71%), while black and blue fibers were most abundant at GI with percentages of 26.87% and 26.37%, respectively, while other colours such as red, yellow, grey, and green were also observed (**Figure 3b**). The predominance of blue, black, and transparent plastic particles in the water column, bivalves, and crustacean from the BBE and Claromecó beaches, was previously reported (Villagran et al., 2020; Fernandez-Severini et al., 2020; Forero-López et al., 2021a and c; Truchet et al., 2021b). A study in southeastern Brazil showed the same coloured pattern in the Atlantic ghost crab (*Ocypode quadrata*), where most of the microfibers were black (39%), blue (38%), and transparent (7%) (Lopes Costa et al., 2019). Another study, in the Persian Gulf, reported similar results in tissues of *M. affinis* samples, in which white/transparent MPs (43%) were the most abundant followed by blue (21%) and black (18%) (Keshavarzifard et al., 2021).



Figure 3. (a) Percentage of range sizes and (b) colours of plastic particles found in the abdominal muscle of *P. muelleri* from each sampling site at BBE.



3.1. Chemical composition of MPs

Figure 4. SEM micrograph of the fibers found in the digestive tract of *P. muelleri* obtained at different magnifications: (a) 176x, (b) 519x, (c) 964x, and (d) 875x.

SEM/EDX microphotographs showed the presence of tissue residues, fractures, and pits on the surface of the analyzed fibers (**Figure 4**). Mechanical degradation of textiles caused by washing processes has been assessed as the main source of MPs in watercourses and oceans (De Falco et al., 2019; Henry et al., 2019). The appearance of irregular, acicular crystals of different sizes, whose nucleation and growth result from the evaporation of potassium citrate-containing water residues (formed by the neutralization of KOH with

citric acid) during the drying period, was also observed (McGinty et al., 2020). An EDX analysis of these crystals revealed the presence of C, O, K, and Mg elements (**Figure 5a**, spectrum 21) in their composition. On the other hand, **Figure 5b** shows a SEM/EDX analysis on two different surface sites of multicolour synthetic fiber. All the EDX spectrums exhibit a strong C peak, followed by O, and a peak of K (**Figure 5b**). In particular, peaks of P and S also were detected on the fiber surface.

Experimental C	EDX ANALYSIS		
	Element	Spectrum	Spectrum
	analysis	21	23
	Mg	8.81%	6.04%
	0	29.78%	24.69%
Spectrum 23	Si	0.36%	0.19%
+ Spectrum 20	K	9.54%	4.1 %
	С	50.06%	64.3%
	Cl	1.45%	0.67%

Spectrum 10	Spectrum 10			EDX ANALYSIS		
		Element analysis	Spectrum 9	Spectrum 10		
		Mg	0.14%	0.35%		
40-E	1 9 33 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0	20.05%	28.22%		
		Al	0.05%	0.09%		
		Si	0.12%	0.16%		
	No. 1997	к	7.41%	7.3%		
Spectrum 9		Ca		0.43%		
		Na	0.09%	0.08%		
1 mil		Р	0.2%	0.23%		
	Bertune and Link A.	С	71.48%	62.92%		
40-		s	0.18%			
		Cl	0.28%	0.22%		

Figure 5. SEM/EDX micrograph of the fibers found in the abdominal muscle of *P*. *muelleri* obtained at different magnifications: (a) 176x, (b) 519x, (c) 964x, and (d) 875x.

The spectroscopic analysis was performed on 25 randomly selected fibers larger than 0.5 mm (500 µm) in order to be manipulated with precision tweezers and transferred to the reflectance mirror of the spectrometer. Practically all the fibers analyzed corresponded to textile in which the main polymer was the cellulose-based matrix, followed by polyester (polyethylene terephthalate) and two-component mixed materials. 62.5% of the analyzed fibers were cellulose-based materials presenting the typical pattern of a glycoside (1427 and 1314 cm⁻¹ corresponding to C-H and in-plane O-H bending, respectively; 1160, 1108, and 1053 cm⁻¹ corresponding to different stretching modes of C-O bond; and 899 cm⁻¹ assigned to the β -glycosidic C-O-C stretching) (Abderrahim et al., 2015; Comnea-Stancu et al., 2017). In Figure 6a, the spectrum of a representative cellulosic fiber is compared with the spectrum of pure microcrystalline cellulose. Some of these fibers presented this characteristic pattern, but with the appearance of new signals at 1733, and 1577 and 1538 cm⁻¹ (marked with arrows in Figure 6a). Those peaks were assigned to the oxidized functional groups which appeared as result of the glycosidic ring degradation (Zghari et al., 2018). 12.5% of the fibers showed good correlation with polyester or polyethylene terephthalate patterns (aromatic overtones at 1964 cm⁻¹, C=O stretching at 1715 cm⁻¹, aromatic C=C stretching at 1508 cm⁻¹, and C-CH₂ rocking at 720 cm⁻¹) (Figure 6b). Finally, 20.8% of fibers correspond to cotton-polyamide blend with the appearance of glycosidic signals, as well as new and intense bands such as Amide I and Amide II at 1635 and 1535 cm⁻¹ in the carbonyl region, confirming the presence of this functional group (Peets et al., 2017). In Figure 6c the spectrum of a representative fiber of this kind is compared with the spectrum of 30% polyamide - 70% cotton fabric. All these materials are widely used as textile fibers, thus, are expected to be found in places affected by domestic discharges. As was previously reported, BBE receives poorly treated

wastewater effluents. Forero-López et al. (2021c) found a mean value of 6162 MPs/m^3 in the water column of BBE, where the highest concentrations were detected in the middle zone of the estuary, a site that receives untreated sewage effluents from the Bahía Blanca city. Previous works reported the presence of semi-synthetic cellulosic materials, polypropylene, and polyethylene fibers in shrimp from the VC sampling site. Cellulose and polyamide were observed in brown shrimp (*M. monocerous*) and tiger shrimp (*P. monodon*) from offshore waters of the Northern Bay of Bengal, Bangladesh (Hossain et al., 2020). Likewise, cellulose-based materials were identified as the dominant plastic type in *P. australiensis* freshwater glass shrimp from Victoria, Australia (Nan et al., 2020).

From the analyzed fibers, just one item (4.2%) was not properly identified. The spectrum exhibits a pronounced signal at the carbonyl group region (1704 cm⁻¹). No match was achieved with the references used (personal spectra library recorded at the same experimental conditions) or Omnic 8 software library. However, a certain correspondence with poly (acrylic acid) could be made, indicating the presence of this monomer as a component of a blend, or the presence of some acrylate as a constituent of the polymer.



Figure 6. FT-IR spectra of microplastics particles found in the abdominal muscle of *P*. *muelleri* at BBE: (a) cellulose, (b) poly ethylene terephthalate, and (c) cotton-polyamide (70:30).

Natural or man-made cellulosic materials are widely used in various fields (e.g.: textiles, paper, engineering, medical, or construction) due to their high mechanical strength, large surface area, flexibility, and biodegradability (Pengiran et al., 2021), which is why they were reported as the main constituent of oceanic fibers all over the world (Suaria et al., 2020). The presence of additives such as dyes, flame retardants, or fabric softeners, can modify both physicochemical properties and surface charge, and thus their biodegradability, resulting in distinct toxic effects on organisms (Ventura et al., 2020; Kim et al., 2019). There are limited studies on the ecotoxicity of natural microfibers on aquatic organisms. Kim et al., 2021 reported that natural microfibers (lyocell) were more toxic to *Daphnia magna* than synthetic microfibers such as polypropylene or polyethylene terephthalate. On the other hand, Wang et al. (2020) informed that cellulose nanofibrils were key factors to induce oxidative stress in *Scenedesmus obliquus*, *Daphnia magna*, and *Danio rerio*. However, in South America, especially in BBE, there are no studies on the effects of cellulosic fibers and their additives in local organisms.

4. Conclusions

In the present study, a significant number of MPs, mainly semi-synthetic textile fibers, were detected in *P. muelleri* from the BBE. In turn, the widespread presence of these microplastic fibers could be related to the untreated sewage water discharge of domestic effluents from the cities of Bahía Blanca, Punta Alta, and Ingeniero White. As condition factor K, showed that shrimp were in good condition, not being affected by the concentrations of MPs in the BBE. However, the collateral damage induced by the ingestion of MPs, which may contain chemicals or bacteria, was not investigated in this study and could describe a possible health problem. Since *P. muelleri* is a seafood species widely consumed by humans, more research on MPs levels and their effects is required to assess comprehensive risk factors that could affect food safety.

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Declaration of interests

xThe 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.

□The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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