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The Plastic Signature: Microplastic Ingestion and Phthalate Exposure in *Parapenaeus longirostris* from Three Tyrrhenian Sites (Mediterranean Sea)

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Academic Editor: Nicolas Kalogerakis

Received: 25 July 2025
Revised: 3 September 2025
Accepted: 12 September 2025
Published: 30 September 2025

Citation: Ciaralli, L.; Vencato, S.; de Lucia, G.A.; Valente, T.; Monfardini, E.; Libralato, G.; Manfra, L.; Radicioli, M.; Silvestri, C.; Dattilo, S.; et al. The Plastic Signature: Microplastic Ingestion and Phthalate Exposure in *Parapenaeus longirostris* from Three Tyrrhenian Sites (Mediterranean Sea). *Microplastics* **2025**, *4*, 67. https://doi.org/10.3390/microplastics4040067

Abstract

Microplastic pollution is pervasive in marine ecosystems and poses a growing threat to marine organisms and human health. This study simultaneously investigates microplastic ingestion and phthalate exposure in *Parapenaeus longirostris*, a commercially valuable and ecologically relevant Mediterranean crustacean occupying an intermediate trophic position. Specimens were collected from three coastal areas in the central Tyrrhenian Sea (Western Mediterranean): near the Tiber River mouth, one of the most polluted rivers in Italy, and two additional sites to the north and south. The frequency of individuals with ingested microplastics varied among locations: 78% near the Tiber River, 64% at site S, and 38% at site N, reflecting anthropogenic pressure gradients. Analyses confirmed the lower occurrence at site N, indicating higher ingestion near land-based pollution sources. Ingested microplastic polymer types varied among sites, reflecting location-specific contamination. Phthalates were present in shrimp muscle at all sites (5–1122 ng/g w.w.) with the highest average concentration (68.26 ± 55.74 ng/g) at the site with the highest microplastic ingestion. Although no statistical correlation was found, the similar spatial distribution of microplastics and phthalates suggests a potential link influenced by local pollution and individual variability. These findings provide novel evidence of microplastic and phthalate contamination in *P. longirostris*, highlighting its role as a trophic connector mediating contaminant transfer through the food web. While current levels suggest no

potential risk to human health, continued monitoring and further studies on exposure along trophic pathways are recommended.

Keywords: MP uptake; crustacean species; seafood; plastic pollution; plasticizers

1. Introduction

Plastic materials are deeply embedded in the infrastructure of modern society [1], being used across multiple sectors, including healthcare [2], construction [3], transportation, and communication [4]. Today, their unique combination of versatility and low cost makes them virtually indispensable [5,6]. The uncontrolled rise in global plastic production, estimated at 400 million tonnes in 2021 [7], combined with human activities on both land and at sea, has resulted in the continuous release of plastic into the oceans, with approximately 12.7 million tonnes entering the marine environment in a single year [8].

Microplastics (MPs) are synthetic particles smaller than 5 mm, typically resulting from the breakdown of larger plastic debris through physical, chemical, and biological processes [9]. Their small size and widespread presence have made MPs a pervasive emerging pollutant [10], whose distribution is influenced by a complex interplay of factors, including ocean currents, weather conditions, seasonal variability [11,12], and the physical properties of the particles, such as polymer type, density, and shape [13,14]. Research interest has increased in recent years to better understand their impacts on marine environments and organisms, as MPs often fall within the prey-size range of many marine species and may be ingested accidentally or mistaken for food [15–17]. The ingestion of MPs can cause physical damage such as occlusions and lacerations, which can lead to severe inflammation, oxidative stress, false satiety, and reduced overall fitness [18]. In addition to these effects, MPs can translocate into the circulatory system and disrupt normal physiological functions, causing genotoxicity and cytotoxicity in immune cells, increasing haemolysis, and promoting endothelial adhesion [19–21].

Concern about MP pollution extends beyond physical debris to associated chemicals, including persistent organic pollutants (POPs), additives, and adsorbed contaminants, for which MPs can act as vectors [22,23]. Among them, phthalates (PAEs) are synthetic chemicals widely used as plasticizers in PVC products, building materials, textiles, personal-care items, pharmaceuticals, and food packaging [24,25]. While they are not classified as highly persistent or bioaccumulative, their extensive use, continuous production, and release render them 'pseudo-persistent', resulting in ongoing environmental and human exposure [26–28]. Notably, nine PAEs are listed as Substances of Very High Concern (SVHCs) under the EU REACH Regulation [29].

In aquatic ecosystems, even low concentrations of PAEs can lead to oxidative stress, endocrine disruption, immunosuppression, metabolic disorders, genotoxicity, and apoptosis, ultimately impairing growth and development [24,30,31]. Correspondingly, human health may also be affected: in adult males, infertility and sperm damage have been reported [32], while in children, certain PAEs have been linked to hormonal disruption and reduced thyroid hormone levels [33].

PAEs can enter organisms through direct absorption from environmental matrices or dietary intake [34]. However, environmental plastics are considered the main source of PAEs in organisms [26,35], although the precise relationship between these contaminants remains unclear [36]. Their accumulation depends on properties such as $\log K_{ow}$ and water solubility, though they do not biomagnify in aquatic food webs [37].

Trophic transfer in the marine food web contributes to MP uptake [17,34], posing considerable risks to marine wildlife across multiple trophic levels [38–40] and to food security and human health [41–43]. Humans may ingest substantial amounts of MPs through seafood [44,45]; however, the actual health effects remain poorly understood [46,47]. In this context, species that are both ecologically important and widely consumed by humans represent promising candidates for assessing the potential transfer of MPs along the food chain.

Parapenaeus longirostris, the target species of this study, exemplifies this connection, holding both ecological and economic importance [48]. It is one of the most valuable species targeted by Mediterranean trawl fisheries, representing 23% of total crustacean landings between 2000 and 2008 [49,50], with catches rising from 7000 tonnes in 1970 to 22,700 tonnes in 2021 [48].

P. longirostris exhibits both active hunting and digging behaviour, preying on a diverse array of bathypelagic, benthic, and endobenthic organisms, including molluscs, other crustaceans, fish, echinoderms, and polychaetes [51]. Moreover, it plays a key trophic role in the Mediterranean food web, serving as an important prey for several predators, such as *Merluccius merluccius* [52,53], *Trachurus trachurus* [54], *Trachurus mediterraneus* [55], *Galeus melastomus* [56], *Etmopterus spinax* [57,58], and *Scyliorhinus canicula*.

Research on MP ingestion in decapods remains less extensive than for other marine taxa [59,60]; however, evidence of MP ingestion by *P. longirostris* has been documented in various parts of the Mediterranean Sea [61,62].

The dual role of *P. longirostris* as both a key trophic connector and a species frequently ingesting MPs [63–66] makes it a potentially valuable organism for assessing MP transfer to higher trophic levels through predation [17,38,67,68], as well as for exploring the risk of potential human health implications.

Among the growing body of literature on MP ingestion in marine organisms [69,70], few studies have adopted an integrated approach addressing both physical and chemical exposure (e.g., [31,71]). In particular, research on PAEs in marine wildlife is still limited, and their link to MP ingestion remains unclear [71,72]. Addressing this gap is crucial, as MPs provide only a partial view of contamination. Despite their recognized toxic effects [24], PAEs remain understudied, leaving uncertainties about the actual risks of plastic pollution.

This study addresses this gap by investigating *P. longirostris*, a widespread and commercially important crustacean that acts as an intermediate trophic connector within the food web, through an integrative approach combining physical and chemical endpoints to inform both environmental hazard assessment and seafood safety evaluation.

To assess the role of *P. longirostris* as a potential vector of MP-related risks through the food web under various environmental conditions, this study aims to:

(i) Investigate the abundance and the diversity of MPs ingested by *P. longirostris*;
(ii) Estimate the influence of the distance from the mouth of the Tiber River, as the main contamination source, on MP ingestion in *P. longirostris*;
(iii) Investigate the presence of PAEs in the target species;
(iv) Assess the potential relationship between ingested MPs and PAE concentrations in *P. longirostris* muscle tissue;
(v) Integrate the results on ingested MP abundance, muscle tissue PAEs, and spatial variability to provide a comprehensive assessment of contamination patterns and potential contamination risk.

2. Materials and Methods

2.1. Study Area

The Mediterranean Sea is a semi-enclosed basin recognised as the second largest hotspot for biodiversity globally [73,74], which faces intense anthropogenic pressures, including significant plastic pollution [75–77]. Rivers play a crucial role in transporting inadequately managed waste to marine systems, serving as major conduits for plastics entering the ocean [78–80]. The study area is situated in the central Tyrrhenian Sea (Figure 1) off the coast of Rome, where the Tiber River stands out as a primary pollution source [81], with significant contributions from plastic waste [61,82,83]. The Tiber River, which extends for approximately 405 km, with a hydrographic basin area of 17,375 km^2, is the largest river in Central Italy. Ranking third in length in Italy, and the most important river that flows into the central Tyrrhenian Sea, it is also one of the most polluted rivers in the country [81,83]. Within the study area, three different sampling sites were chosen at different distances from the mouth of the Tiber River. This study area was chosen not only because the Tiber River represents a major source of plastic pollution to the central Tyrrhenian Sea [81], but also because *P. longirostris* is commercially harvested both near the river mouth and in the adjacent northern and southern areas, located at approximately the same distance from the river mouth (33 and 29 miles, respectively). This spatial distribution allows investigation of how distance from the river mouth in both directions may influence MP ingestion patterns in the species. Comparing sites at varying distances and orientations from the main riverine source can reveal meaningful spatial patterns of contaminant distribution. A multi-site approach, including sites at different distances from impact sources, is commonly applied in the literature and has previously been used to investigate the distribution of MPs and PAEs [26,84–86]. Sample collection was conducted along three different transects at varying depths, from 130 to 550 m, at sampling sites off the localities of Civitavecchia (Northern Site; sampling site N; 41°52′35.01″ N, 11°39′01.16″ E; depth: 550 m; 33 miles from the Tiber River mouth), Fiumicino (Tiber River mouth; sampling site T; 41°41′13.51″ N, 12°06′13.39″ E; depth: 130 m; 7 miles away from Tiber River mouth), and Anzio (Southern Site; sampling site S; 41°18′51.83″ N, 12°03′28.92″ E; depth: 300 m; 29 miles away from Tiber River mouth), respectively. In particular, the Southern Site is located on the Albano Seamount, which has a peak depth of 250–280 m and a base depth of 580–590 m. Seamounts can influence local water circulation and particle retention, potentially affecting the accumulation of MPs [87,88], making the study of this site particularly interesting.

For each sampling location, an anthropisation score was calculated following the approach proposed by Liubartseva et al. [78], using a cities-to-rivers-to-shipping lanes ratio of 50:30:10% [89,90]. The sampling sites were ranked based on various coastal pressures [89], such as coastal population density, distance from major rivers, and the presence of shipping lanes. The distance from the shoreline was not considered, as all sites were located more than 3 km from it [78]. The final index provides a cumulative score for each site, offering an overview of the overall anthropogenic impact at each location.

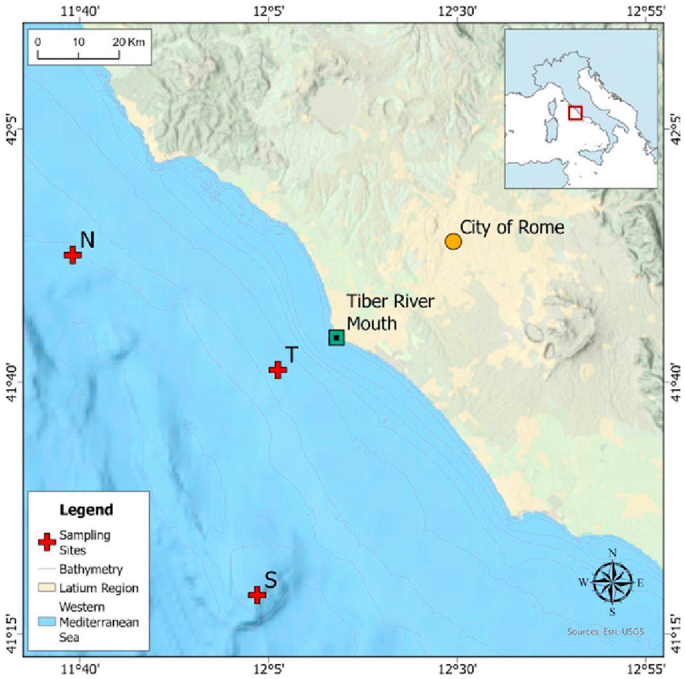

Figure 1. Sampling sites. The map displays the sampling sites (red crosses; N = Northern Site—Civitavecchia, T = Tiber mouth Site—Fiumicino, S = Southern Site—Anzio) selected for collecting specimens of *Parapenaeus longirostris*, during summer 2023, with a spatial reference point in the bottom right, the legend in the bottom left, and the scale in the top left. The mouth of the Tiber River is indicated with a green square mark.

2.2. Sample Collection and Analytical Methods

Between 17 July and 25 August 2023, *P. longirostris* individuals were collected by a professional fishing vessel in three sampling surveys, each corresponding to a different sampling site. Specimens were immediately wrapped in aluminium foil and preserved on ice directly on board to prevent secondary contamination. Following the protocol included in the Guidance on the Monitoring of Marine Litter in European Seas [62], a total of 150 individuals (50 per sampling site) were selected for investigation. Upon arrival at the laboratory, specimens were frozen at −20 °C for subsequent analysis of ingested MPs and PAEs.

Before dissection, total weight (Tw, g), total length (tL, cm), carapace length (cL, cm), stomach weight (sW, g), and intestine weight (iW, g) were recorded using a manual calliper and a precision scale (both accurate to 1 mm). Carapace length was measured from the right orbital edge to the midpoint of the posterior margin of the carapace. Mean carapace length and standard deviation were calculated to confirm that individuals were of similar size, minimising the influence of ontogenetic development stages on feeding behaviour and, consequently, on MP ingestion [91]. Finally, a subset of 20 individuals per site was analysed for PAEs in muscle tissue. The subsample was selected retrospectively, choosing 10 individuals with the highest number of ingested MPs and 10 individuals with none for each sampling location, resulting in a total of 60 samples.

2.2.1. Microplastics Analysis

The analysis of ingested MPs was conducted following the protocol for microlitter ingestion in fish as outlined in the aforementioned Guidance on the Monitoring of Marine Litter in European Seas [62], with specific modifications adapted for this case study [68], which recommends limiting the exposure of samples to 15% H_2O_2 at 40 °C for no longer than 5 days in order to minimise potential degradation of plastic particles. The selected

protocol is widely recognised and adopted as the most suitable compromise between tissue digestion efficiency and MP integrity. After dissection, the gastrointestinal tracts were divided into stomachs and intestines, each individually weighed and placed in 100 mL glass beakers. To remove all organic matter, the stomach and intestine contents were chemically digested with 15% H_2O_2 (Sigma-Aldrich®, St. Louis, MO, USA). After 5 days at 40 °C, the digestate was filtered through glass microfibre filter membranes (Whatman GF/B™; pore size: 1.0 µm) using a vacuum pump system (Cole-Parmer™ Air Cadet Vacuum/Pressure Pump, Vernon Hills, IL, USA). To prevent bias, any individuals with completely empty stomachs and intestines were excluded from the analysis. The stomach walls were separated from their contents, rinsed within the glass beaker with distilled water, and transferred to labelled glass Petri dishes for individual examination under a stereomicroscope. This step was crucial for both the effective digestion of the stomach content in H_2O_2, and for ensuring no particles remained adhered to the walls. Microparticles were then counted and photographed with a camera-equipped dissecting microscope (ZEISS Stemi 2000-C with Axiocam 208 colour, Carl Zeiss Microscopy GmbH, Jena, Germany). All microparticles were categorised by shape (fibre, filament, film, fragment, foam, granule, pellet, and bundle), colour (black, blue, green, grey, red, and white), and size (Size Class 1: 1 mm to 5 mm; Size Class 2: 330 µm to 1 mm; Size Class 3: 100 µm to 330 µm), with the lower limit set at 100 µm [91]. All of the items found were individually analysed using µFourier transform infrared (µFT-IR) spectroscopy (Nicolet iN5 FT-IR Microscope, Thermo Fisher Scientific, Madison, WI, USA) with OMNIC™ Series Software (version 9.13.1256) and the Aldrich™ Polymers FT-IR Spectral Library (Sigma-Aldrich, St. Louis, MO, USA). As this study exclusively focuses on MPs, this step was necessary to identify the precise chemical composition of each microparticle, and those with non-polymeric compositions were fully excluded from the investigation.

2.2.2. Phthalate Analysis

The presence of the most dominant phthalate congeners in aquatic environments (BBP: butylbenzyl phthalate; DBP: dibutyl phthalate; DEHP: di-2-ethylhexyl phthalate; DEP: diethyl phthalate; DMP: dimethyl phthalate) and one metabolite (MEHP: mono(2-ethylhexyl) phthalate, primary metabolite of DEHP) was investigated using solid-phase microextraction (SPME), followed by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS). The methodology, adapted from Saliu et al. [92], was modified for *P. longirostris* samples, as described below.

Each sample (approximately 0.2 g, wet weight) was pre-treated with 500 µL of acetone and 4 µL of deuterated internal standard, mixed with a metal spatula to break up the organic matrix and left for 30 min on an orbital shaker at room temperature (25 °C). After activation in 1 mL isopropanol (HPLC grade) for 20 min and a 10 s rinse with ultrapure Milli-Q water, C18 SPME fibres were inserted into the prepared mixtures—one fibre per muscle tissue sample—for 40 min at room temperature (25 °C) to allow analyte extraction. Fibres were then removed, rinsed for 10 s in Milli-Q water, and placed into glass vials containing 80 µL methanol (HPLC grade) for 25 min of desorption. The final extract was analysed by HPLC-MS/MS.

HPLC-MS/MS measurements were performed using a Thermo TSQ Fortis Tandem Mass Spectrometer (MS/MS) coupled with a Vanquish Ultra-High-Pressure Liquid Chromatograph (UHPLC). Chromatographic separation was achieved using a C18 Kinetex (Phenomenex, Torrance, CA, USA) 100 × 4.6 mm 100 A 1.7 µm column. Chromatographic separation was performed with a binary system consisting of water with 0.1% formic acid and methanol. The elution program was run at a constant flow rate of 0.5 mL/min. The start of the elution involved an initial flow rate of methanol that increased from 60% to

98% in 5 min and was maintained for 5 min. Electrospray ionization (ESI) was used as the ionization source. Initially, MS/MS (selected reaction monitoring) parameters were optimized to ensure accurate detection of the target contaminants. Next, conditions that provided sufficiently high signal intensities were identified, followed by appropriate peak integration of the selected transition for the quantitative analysis using the response factors assayed from the matrix-matched calibration (corrected with the labelled internal standard). The matrix-matched calibration curves for each analyte were drawn with 6 calibration points (50–75–125–250–500–1000 ng/g; diluting a mixed standard solution 500 ppm in methanol), with a correlation coefficient (R2) > 0.99 (Table 1). The limit of detection (LOD) and limit of quantification (LOQ) for BBP (butylbenzyl phthalate), DBP (dibutyl phthalate), DEHP (di-2-Ethylhexyl phthalate), DEP (diethyl phthalate), DMP (dimethyl phthalate), and MEHP (monoethylexyl phthalate, monoester of DEHP) were determined based on the standard deviation of the y-intercept of each regression line corresponding to each phthalate (Table 1). Three in-house quality control samples were created and tested to assess precision and recoveries that were <10% and 85–95%, respectively. Table S1 (Supplementary Materials) reports extended analytical data and compound-specific characteristics.

Table 1. Detection and quantification performance of phthalate ester compounds. * indicates values calculated based on the standard deviation of the y-intercept. LOD = limit of detection; LOQ = limit of quantification.

Phthalic Compound	ID Phthalic Compound	CAS Number	LODs (ng/g) *	LOQs (ng/g) *	R2
Butylbenzyl phthalate	BBP	85-68-7	8.51	25.78	0.9999
Dibutyl phthalate	DBP	84-74-2	9.18	27.81	0.9999
Di-2-ethylhexyl phthalate	DEHP	117-81-7	8.01	24.27	0.9999
Diethyl phthalate	DEP	84-66-2	6.27	19	0.9999
Dimethyl phthalate	DMP	131-11-3	9.84	29.81	0.9998
Mono(2-ethylhexyl) phthalate	MEHP	4376-20-9	10.3	31.23	0.9998
Bis(2-ethylhexyl)phthalate-3,4,5,6-d4	DEHP-3,4,5,6-d4	93951-87-2	-	-	-

2.2.3. Quality Assurance and Quality Control for Microplastics

To minimise secondary contamination, all analyses were performed in a clean laboratory. During the analyses, lab access was restricted to a maximum of two operators, wearing only 100% cotton clothing and lab coats. Additionally, three Dyson Purifier Cool™ air purifiers, equipped with H13 HEPA filters that capture particles as small as 0.3 µm, operated overnight and during the activities to ensure the air in the lab was purified. All dissecting tools were cleaned with ethanol and distilled water. To monitor secondary contamination, a blank sample was processed for every five samples (totalling 60 blanks). Any MPs matching the blank particles in shape, colour, polymer type, and size were subtracted from the results of the corresponding batch.

2.2.4. Quality Assurance and Quality Control for Phthalates

To minimise the risk of PAE background contamination, all processing and analyses were performed in a restricted-access room. Laboratory surfaces and equipment involved in sample processing were thoroughly cleaned using 99.9% HPLC-grade acetone or methanol, followed by rinsing with ultrapure, filtered Milli-Q water and baked at 300 °C before use [93]. After each cleaning step, laboratory materials were wrapped in aluminium foil until their next use. Plastic materials were strictly avoided during sample collection and handling.

2.3. Statistical Analysis

All analyses conducted in this study were performed with R (R studio Version 4.3.2, 2023), using the "base R", "stats", "MASS", "car", "lme4", and "vegan" packages for statistical analysis, and the "ggplot2" package for graphical outputs. For all analyses, a significance level of 0.05 (p-value < 0.05) was chosen.

The occurrence of MPs was recorded as a binary factor of presence–absence (0–1), and the abundance of MPs as the number of MPs found in each gastrointestinal tract. The frequency of occurrence (FO%) was computed by calculating the percentage of individuals with ingested MPs relative to the total number of individuals analysed. The relationship between FO% and sampling sites was investigated with a logistic regression model. A quasi-Poisson regression model was used to investigate the relationship between MP abundance, sampling sites, and gastrointestinal weight. Chi-square tests were performed to evaluate differences in MP characteristics (shape, colour, size class, and polymer) across the three sampling sites.

Similarly, the FO% was also calculated for PAEs, based on the proportion of individuals with detectable concentrations relative to the total number analysed. A two-way univariate permutational analysis of variance (PERMANOVA) on square-root-transformed data was performed to assess differences in PAE concentrations according to (i) sites/pollution conditions (3 levels: N, T, S), and (ii) the occurrence of plastic items found in organisms. Each term was analysed using 9999 random permutations and associated with a Monte Carlo test [94]. A Spearman rank correlation test was conducted to investigate the relationship between PAE load in each specimen and the number of plastic items detected in *P. longirostris*' gastrointestinal tracts. PAE concentrations below the detection threshold were classified as below detection limit (BDL), and a value equal to half the BDL was used for statistical analyses.

3. Results

3.1. Microplastic Ingestion

A total of 243 ingested MPs were identified in the gastrointestinal tract of *P. longirostris* individuals, and their distribution across sites is shown in Figure 2. MP ingestion was observed at all sampling sites, with an overall FO% of 60%, ranging from 38% at sampling site N, to 78% at sampling site T, and 64% at sampling site S. Each sampling site is influenced by various anthropogenic activities and pressures. Table 2 illustrates the anthropisation scores obtained for the three sampling sites, along with the FO% values and the mean abundance of items, including the standard error per site. The degree of anthropisation reaches its maximum value at the sampling site T, the site closest to the mouth of the Tiber River.

The FO% was significantly lower at sampling site N compared to sites T and S (logistic regression model, p-value < 0.001). This suggests that both T and S are associated with a higher probability of occurrence relative to site N. Moreover, the results indicate that the gastrointestinal weight is strongly positively correlated with the number of items collected (quasi-Poisson regression model, p-value < 0.001), suggesting a significant relationship between the gastrointestinal weight and the response variable. Conversely, the sampling sites T and S did not show significant effects on the number of items relative to sampling site N, as indicated by their non-significant coefficients.

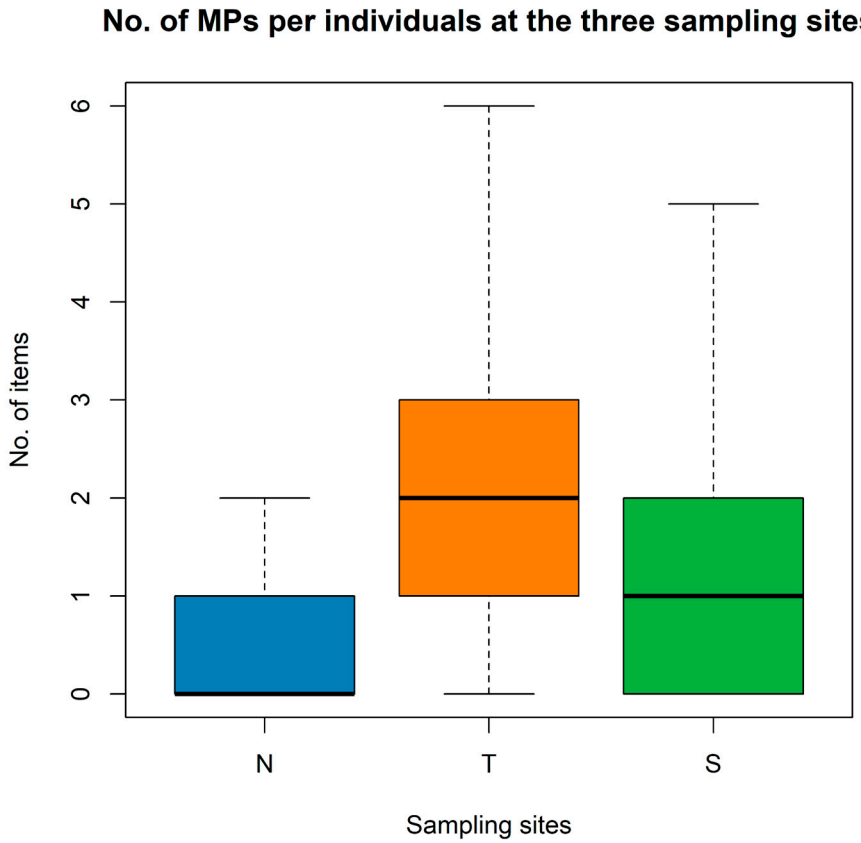

Figure 2. MP ingestion within the three sampling sites. Boxplots based on the analysis conducted on the gastrointestinal tracts of 150 individuals (50 per sampling site) of *Parapenaeus longirostris* caught off the Rome coast during July–August 2023. Each box represents the number of ingested MPs per individual at the sampling site (N = Northern Site, T = Tiber Mouth Site, and S = Southern Site).

Table 2. Ranking of the sampling sites along the central Tyrrhenian coast based on anthropogenic pressures, including coastal human population, river input, and shipping lanes. A cities-to-rivers-to-shipping-lanes ratio of 50:30:10% was applied. The degree of anthropisation is expressed as a cumulative score (● = 1; ○ = 0). The table also reports the frequency of occurrence (FO%) and microplastic (MP) abundance (mean ± SE) for each site.

Sampling Site	Area	Human Population	Distance Major River	Shipping Lane	Score	FO%	MPs Abundance
Northern Site (N)	Civitavecchia	●●●	●●	●	6	38%	1.44 ± 0.62
Tiber mouth Site (T)	Fiumicino	●●●●●	●●●	○	8	78%	1.96 ± 0.27
Southern Site (S)	Anzio	●●●●	●●	○	6	64%	1.44 ± 0.22

The most abundant type of MPs ingested at sampling site N was white polyethylene terephthalate (PET) fibre from Size Class 1 (1 mm to 5 mm), accounting for 36.1% of the total MPs at that site. In contrast, the most abundant MP type at sampling sites T and S was black resin-based fragments, measuring between 100 µm and 330 µm (Size Class 3) and accounting for 16.2% and 20.8% of the total, respectively. These differences are further confirmed by Chi-square tests, which show significant differences in the types of MPs ingested at the three sampling sites considering shape (X-squared = 29.147, df = 8, p-value < 0.001), colour (X-squared = 79.489, df = 12, p-value < 0.001), size class (X-squared = 34.195, df = 4, p-value < 0.001), and polymer (X-squared = 90.043, df = 16, p-value < 0.001).

The polymer composition percentages of the MPs ingested by *P. longirostris* at the different sampling sites are shown in Figure 3. PET is the most abundant polymer at sampling site N, representing 59.7% of the total. Resinous compounds (RES) are the dominant polymer at both sampling sites T and S, accounting for 42.4% and 61.1%, respectively. Additionally, polyacrylonitrile (PAN) exhibits notable abundance across all sites, with percentages of 20.8% at site N, 24.2% at site T, and 18.1% at site S (Figure 3).

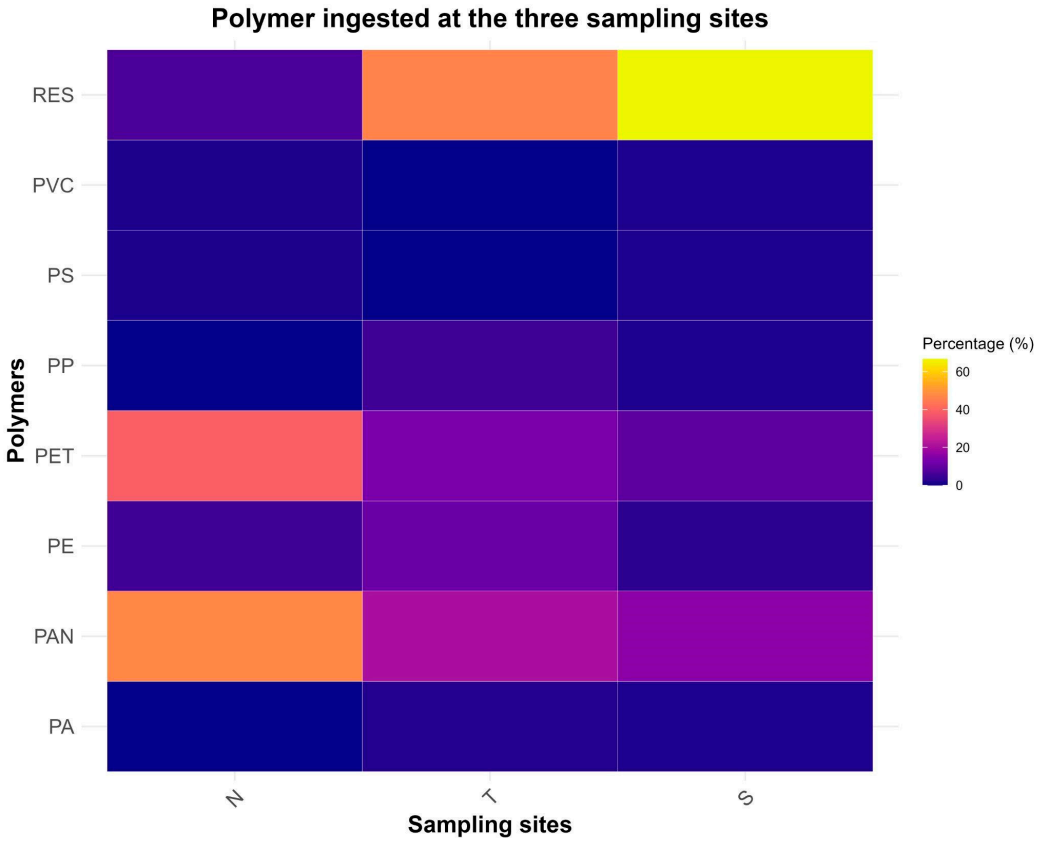

Figure 3. Ingested MP polymer composition. Heat map representing the results of FT-IR spectroscopy analysis conducted on all MPs recovered from the gastrointestinal tracts of *Parapenaeus longirostris* caught off the Rome coast during July–August 2023, at the three sampling sites (N = Northern Site, T = Tiber Mouth Site, and S = Southern Site), each represented by a single bar of the plot. (RES = resinous compounds; PVC = polyvinyl chloride; PS = polystyrene; PP = polypropylene; PET = polyethylene terephthalate; PE = polyethylene; PAN = polyacrylonitrile; PA = polyamide).

3.2. Phthalate Quantification

Among the five PAE congeners investigated (DMP, DEP, DBP, BBzP, DEHP) and the metabolite component (MEHP), only DEHP was detected and quantified in *P. longirostris* individuals. PAEs were detected at all the sampled sites and in 32% of the 60 individuals considered in the present analysis. More specifically, PAEs were detected in 26% of the individuals collected at site N, 40% at site T, and 25% at site S. The mean PAE concentration was 8.96 ± 1.69 ng/g (w.w.), 68.26 ± 55.74 ng/g (w.w.), and 18.75 ± 11.31 ng/g (w.w) for sites N, T, and S, respectively (Figure 4). For all three sites considered, PAE concentrations showed a median value of 5 ng/g. The highest concentration, 1122.28 ng/g (w.w.), was found at site T, while maximum values at N and S were 32.31 ng/g (w.w.) and 232.31 ng/g (w.w.).

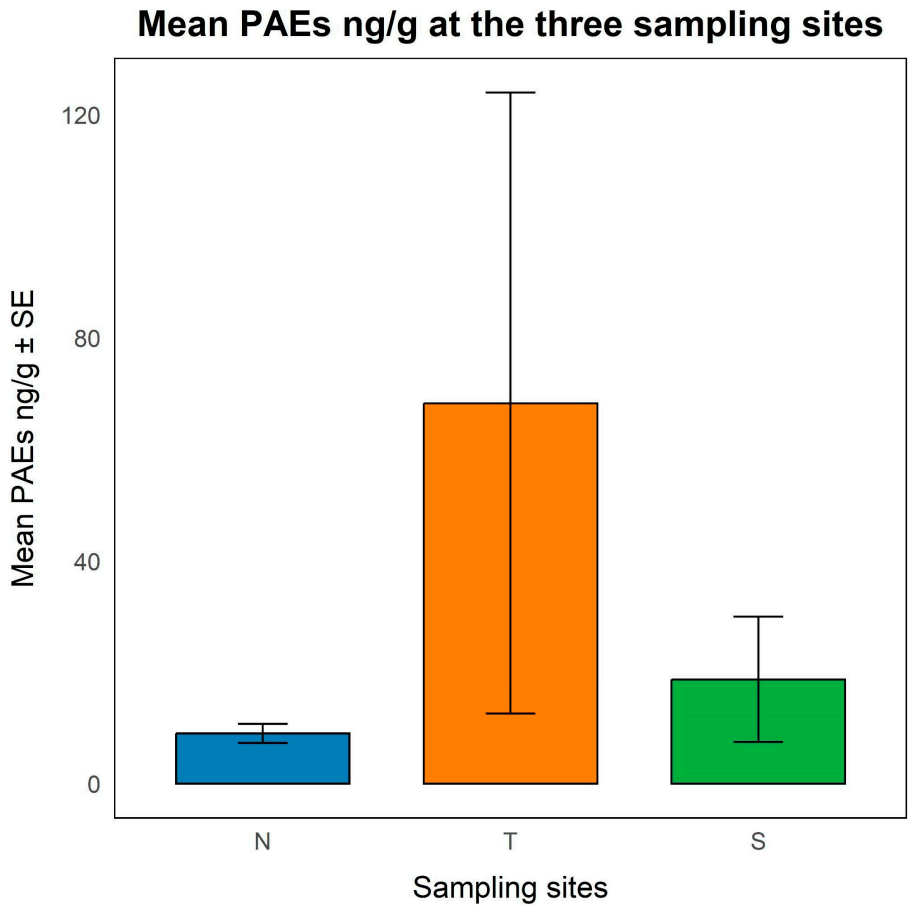

Figure 4. Bar plots of the mean PAE concentrations (ng/g, w.w.) in *Parapenaeus longirostris* according to different sites and contamination conditions (N = Northern Site, T = Tiber Mouth Site, and S = Southern Site).

PERMANOVA revealed no significant differences in PAE concentrations across the different sampling sites or in relation to the presence of plastic items found in organisms (Table 3). Although PAE levels were higher at the site with a higher FO% of MPs (Tiber mouth Site), no significant correlation was found between the PAE concentrations and the number of ingested MPs (Spearman's rho = 0.076, *p*-value = 0.564).

Table 3. Results of two-way PERMANOVA testing for differences in PAE concentrations in *Parapenaeus longirostris* in relation to sampling sites and the presence of ingested plastic items in the gastrointestinal tract.

Source of Variation	df	SS	MS	Pseudo-F	P(MC)
Site	2	33.095	16.547	0.81146	0.4517
MP Occurrence	1	0.70192	0.70192	4.57×10^{-2}	0.8529
Si × Oc	2	30.734	15.367	0.75357	0.4716
Res	54	1101.2	20.392	-	-
Total	59	1165.7	-	-	-

4. Discussion

This study provides new insights into MP ingestion by *P. longirostris* across three coastal sites with varying levels of anthropogenic impact, while also investigating the presence of PAEs in its muscle tissue. Deepening these aspects in trophic connector species

such as *P. longirostris* contributes to elucidating the transfer of MPs within marine food webs, possibly reaching higher trophic levels, including humans [42,68].

MP ingestion by *P. longirostris* was detected at all sampling sites, with variations in both the percentage and types of MPs ingested. The FO% differed significantly among sites, and all measured parameters (FO%, number of items, PAEs) followed a decreasing trend from the T site to S and N (T > S > N). The elevated values observed at site T were expected, given its high anthropogenic impact (Table 1), supporting the hypothesis that river mouths act as primary sources of marine pollution [81,95]. Unexpectedly, despite showing similar levels of anthropogenic pressure, as indicated by the anthropisation scores of the three sampling areas, sites S and N exhibited different frequencies of occurrence of MPs ingestion. This discrepancy may be influenced by physical factors, such as sea currents and topography, which are known to affect the transport and accumulation of marine litter, including MPs [78,96,97]. Investigating local hydrodynamic conditions could therefore help clarify the differences observed between the two sites. Although our study does not aim to provide a comprehensive assessment of the local hydrodynamic regime, a tentative explanation for the difference in MP FO% between sites N and S can be proposed based on coastal circulation patterns reported in the literature. Inghilesi et al. [81] provided valuable insights into the dynamics of Tiber River discharge using a three-dimensional, primitive-equation oceanographic model (ISPRA Coastal and Estuarine Princeton Ocean Model, ICE-POM) coupled with a Lagrangian particle dispersion model. They compared numerical results with remote sensing observations of the diffuse light attenuation coefficient at 490 nm (K490), a proxy for turbid riverine waters. Their simulations of a summer event showed that, under specific meteorological conditions, the Tiber plume can detach from the coast and propagate southward. This suggests that during our sampling campaign (July–August 2023), site S may have been reached by the plume. This hypothesis is further supported by the high occurrence of black resin fragments in samples from sites S and T, in contrast with the white PET fibres predominantly ingested at site N. These findings support the hypothesis that an accumulation pattern of MPs may occur near sampling site S, likely influenced by Tiber River discharge and seasonal plume propagation under specific meteorological conditions. However, confirming this hypothesis will require higher-resolution in situ and remote sensing measurements, combined with appropriate numerical modelling, an objective for future investigations. A detailed analysis is particularly crucial to understand the local dynamics at site S, which is located on the Albano Seamount, an area characterised by steep bathymetric gradients.

Species-specific traits such as prey-searching strategies, feeding depths, diel vertical migrations, and general foraging behaviour may influence the type and amount of MPs encountered by organisms, ultimately shaping the extent of their intake within marine food webs [98,99]. The feeding behaviour of the benthopelagic species *P. longirostris* likely drives the ingestion of MPs, which appears closely associated with local environmental pollution. The predominance of white PET fibres (from 1 mm to 5 mm) at site N aligns with previous findings, which identified fibres of the same size class as the most commonly ingested MPs by *P. longirostris* under similar environmental conditions [66,100,101]. This consistency may reflect the widespread environmental availability of synthetic fibres for *P. longirostris*, likely associated with textile degradation and domestic wastewater discharges originating from the river [102,103]. Although *P. longirostris* has previously been reported to ingest fragments [101], their overall occurrence was lower than that of fibres. The higher proportion of small black resin-based fragments (from 100 µm to 330 µm) ingested by specimens from sites T and S resembles the ingestion patterns observed in *Aristaeomorpha foliacea* by Ciaralli et al. [68] in the same area. Although MP ingestion is influenced by species-specific feeding strategies [98], local environmental conditions also modulate the types of MPs available

for ingestion. This finding highlights the interplay between intrinsic biological traits and site-specific exposure, supporting the hypothesis that the MP ingestion profile is driven by both ecological and environmental factors. Notably, the tendency of smaller MPs to sink faster has been demonstrated even for particles as small as 100 µm, further reinforcing the role of physical dynamics in shaping local MP availability [104]. In this context, our results point to the potential role of *P. longirostris* as a vector of such contaminants within the marine trophic web, where it acts as both prey and predator [105]. This suggests a contribution to MP transfer through the food web, a pathway whose confirmation is crucial to obtain an actual picture of the ecological consequences of plastic pollution. Consistently, Ciaralli et al. [68] also support the role of decapods as trophic intermediaries. Together, these findings highlight the need to investigate MPs in commercially exploited crustaceans and their role in the spread of plastic contamination.

Further insights into MP transfer can be gained by considering predators of *P. longirostris.* These include both non-commercial species (e.g., the elasmobranchs *G. melastomus, E. spinax, S. canicula*) and commercially important fishes such as *M. merluccius, T. trachurus*, and *T. mediterraneus*. All are widely distributed in the Mediterranean and feed actively on decapods [52,53,55,58,106,107], with some studies identifying *P. longirostris* among their preferred prey [53,56]. MP ingestion has been documented in several of these predators within the Mediterranean basin [56,108], and their co-occurrence in both shrimp and predator stomachs suggests a possible route of secondary ingestion through trophic interactions, although a direct link between the two observations cannot be established. Particularly, for *G. melastomus*, a significant correlation between stomach content weight and the number of ingested plastic particles has been observed, suggesting that MP bioaccumulation can occur via prey consumption [109]. This evidence, together with other reports on elasmobranchs [56,108], suggests that both non-commercial and commercial predators of crustaceans may play a pivotal role in transferring MPs to higher trophic levels; however, ingestion by economically important species raises an additional concern, as it highlights a potential pathway for these contaminants to enter seafood intended for human consumption.

Apart from the direct damage that MPs may cause to individuals, their ingestion by *P. longirostris* raises further concerns regarding the co-transfer of associated substances, which may accumulate in its edible tissues [110,111] and subsequently be ingested by predators.

Among the PAEs investigated in this study, only DEHP was detected in the samples, yet it is one of the most commonly reported hazardous compounds in the marine environment [26,27,112,113]. The absence of other target PAEs may be related to the specific types of MPs ingested by these shrimps. Indeed, PAEs are mainly used as plasticizers in PVC, which was almost absent among the plastics ingested in this study, whereas PET, PAN, and resins predominated. While PAEs are absent from most resins, they are widely used in synthetic polyester and acrylic fibres, which may explain their association with PET and PAN detected here.

The spatial distribution of both MPs and PAEs showed notable similarities, with higher DEHP concentrations and MP ingestion in *P. longirostris* at the Tiber mouth, closely resembling the anthropisation scores previously calculated for the study sites. This may point to common underlying drivers. Although these concordant trends do not establish a causal relationship, they suggest that site-specific anthropogenic pressure may simultaneously influence MP ingestion and PAE contamination, highlighting a potential link that warrants further investigation.

While the use of PAEs as indicators of MP pollution is still debated, several studies suggest a correlation between MP abundance and PAEs, since MPs themselves are identified as a PAE source [36]. In this work, no significant correlation was found between the PAE

concentrations and the number of ingested MPs by *P. longirostris*. However, to understand if PAEs could be considered reliable tracers of the presence and extent of plastic pollution in ecosystems, an accurate interpretation of these results requires an integrated understanding of exposure pathways [36,71]. Previous studies [33] indicate that the dietary uptake of high molecular weight PAEs, such as DEHP, is primarily driven by bioaccumulation from food rather than by direct absorption from water in marine organisms. In the context of our study, this evidence, together with the detected presence of DEHP in muscle tissues, supports the notion that ingested MPs represent a major source of PAEs in *P. longirostris*. This provides insight into the potential release of toxic compounds from ingested MPs in this species and contributes to the existing literature, which highlights that fully assessing the ecological consequences of plastic pollution requires further investigation of the link between MP ingestion and PAE accumulation. Such studies are particularly relevant for species like *P. longirostris*, given its potential role as a trophic connector for contaminants across different levels of the food web, potentially including humans.

To the best of our knowledge, contamination of *P. longirostris* by PAEs has only been previously investigated in the Sea of Marmara [114], where DEP, DEHP, and DBP were not detected. However, the PAE levels detected in *P. longirostris* individuals in this study are lower than or comparable to those found in other marine invertebrates from the Mediterranean basin, such as the molluscs *Ostrea edulis* (50 ng/g) and *Mytilus galloprovincialis* (27 ng/g) [115], and the sea anemones *Actinia equina* (26–90 ng/g) and *Anemonia viridis* (23–117 ng/g) [116]. These levels are also consistent with those observed in other Mediterranean crustaceans (15–300 ng/g) [117,118]. Other prawn and shrimp species monitored for PAE contamination show concentration ranges similar to those observed in this study. For instance, coastal mud shrimp from the East China Sea showed concentrations between 1.58 and 212 ng/g [119]. Similarly, DEHP levels detected in the African river prawns *Macrobrachium vollenhovenii* [120] and the red prawns *Aristeus antennatus* [31,121] ranged from <50 to 140 ng/g and <100 to 482 ng/g [113], respectively, in line with the DEHP levels found in *P. longirostris* in this study.

Among the already discussed predators of *P. longirostris*, *Merluccius merluccius* and *Mullus barbatus* have been reported with DEHP in the central Adriatic and Ligurian Seas (*M. merluccius*: 107 ± 74.8 and 93.1 ± 39.1 ng/g w.w.; *M. barbatus*: 36.6 ± 40.5 and 78.3 ± 91.9 ng/g w.w.) [112]. The occurrence of DEHP in these species suggests that trophic transfer from lower to higher levels may represent a relevant pathway not only for MPs but for associated contaminants as well, with *P. longirostris*, as both trophic connector and prey, potentially contributing to this process.

Although we did not perform a full quantitative risk assessment, the DEHP concentrations detected in *P. longirostris* (8.96 ± 1.69, 68.26 ± 55.74, and 18.75 ± 11.31 ng/g w.w. for sites N, T, and S, respectively) are consistent with those reported in other edible invertebrates and lower than or comparable to levels observed in edible fish species from Dettoto et al. [112], which are considered safe for human consumption. Taking into account typical daily seafood consumption rates (55.5 g/day for adults and 52.5 g/day for children [122]) and the Tolerable Daily Intake (TDI; 5×10^4 ng/kg bw/day) and Reference Dose (RfD; 2×10^4 ng/kg bw/day) from the literature [123,124], these concentrations suggest that DEHP intake through *P. longirostris* would likely pose a low potential risk to human health. Nevertheless, further targeted exposure assessments would be valuable to confirm this conclusion. In particular, DEHP, as one of the most harmful PAEs, should be closely monitored in organisms along the food web, as consuming fish and seafood can still result in human exposure to PAEs [125].

5. Conclusions

The ingestion of MPs by marine organisms may have cascading effects on marine ecosystems and human health through seafood consumption, underscoring the need for deeper investigation into their sources, pathways, and impacts.

This study provides the first evidence of the simultaneous occurrence of MPs and PAEs (DEHP) in *P. longirostris* from the central Tyrrhenian Sea, with MPs detected in the gastrointestinal tract of 60% of individuals and DEHP found in the muscle tissue of *P. longirostris* at all sampled sites in 32% of individuals. The simultaneous presence of MPs and PAEs in *P. longirostris* highlights the widespread nature of environmental contamination and the species' potential role in the trophic transfer of these pollutants. In this context, *P. longirostris* emerges as an ecologically and commercially important species suitable for evaluating both physical and chemical exposure in natural conditions, reflecting the threats present in its habitats and potentially acting as a trophic connector of contaminants across food web levels.

Although a full quantitative risk assessment was not conducted, the DEHP concentrations detected are comparable to or lower than those reported in other edible invertebrates and fish species considered safe for human consumption, suggesting only a potential, rather than immediate, risk to seafood consumers. Nevertheless, continued monitoring of DEHP and other PAEs in seafood species is recommended to confirm whether PAE levels in seafood species might represent a concern for consumers.

Within this framework, the critical need for effective management strategies to mitigate plastic pollution is evident. Preventive measures, such as stricter regulations on plastic production and use, and improved waste management, remain essential to reduce inputs at the source. At the same time, innovative remediation approaches, including recently developed plastic removal and filtration technologies, could provide complementary tools to limit the spread of MPs and associated contaminants. Implementing these measures within an integrated monitoring and management framework would contribute to reducing ecological risks while supporting the sustainable use of marine resources.

Future research should therefore focus on unravelling the complex interactions between MPs, associated contaminants, and marine organisms, while considering all potential exposure pathways to refine human health risk assessments.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/microplastics4040067/s1: Table S1: Table summarizing the HPLC-MS/MS analytical parameters and key characteristics of target compounds employed for the quantification of phthalates in *Parapenaeus longirostris* samples.

Author Contributions: Conceptualization, M.M., G.A.d.L., L.C., and S.V.; methodology, S.D., P.M.R., M.M., and T.V.; software, L.C., S.V., and M.R.; validation, G.L. and L.M.; formal analysis, L.C., S.V., M.R., P.M.R., and S.D.; investigation, L.C., S.V., and T.V.; resources, M.M., G.A.d.L., and S.D.; data curation, L.C., S.V., and P.M.R.; writing—original draft preparation, L.C. and S.V.; writing—review and editing, T.V., E.M., L.M., M.R., C.S., and V.L.; visualization, G.L., L.M., D.B., V.L., G.G., C.S., and M.C.; supervision, M.M., G.A.d.L., L.M., and G.L.; project administration, M.M., L.M., and M.M.; funding acquisition, M.M., G.A.d.L., L.M., and S.D. All authors have read and agreed to the published version of the manuscript.

Funding: This work was carried out in the framework of the BIOPLAST4SAFE project (code: PREV-B 2022-12377008: Biomonitoring of biodegradable micro- and nanoplastics: from the environment to humans in a One Health perspective—Investimento E.1—SALUTE AMBIENTE—BIODIVERSITA'—CLIMA—Missione 6—linea di Investimento 1.4—CUP: I55I22000510001) with the technical and economic support of the Italian Ministry of Health–PNC funds. Laura Ciaralli was supported by a PhD fellowship grant (PhD in Biology, University of Naples Federico II) funded by ISPRA (Rome, Italy).

Institutional Review Board Statement: Ethical review and approval were not required for this study in accordance with Italian legislation (D.L. 04/04/14 N.26 art1 a.1), which specifies that ethical approval is not necessary for experiments conducted on invertebrates.

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw data supporting the conclusions of this article will be made available by the authors on request, due to reasons related to the privacy policy of the project.

Acknowledgments: The authors wish to acknowledge the BIOPLAST4SAFE project (CUP: I55I22000510001) for providing the financial and logistical support that made this study possible. Furthermore, the authors wish to thank the National Research Council of Italy, Institute of Polymers, Composites and Biomaterials (CNR-IPCB), Institute of Catania, for their hospitality in facilitating the research, Raffaella Piermarini, Viviana Belvisi, and Melissa di Mauro for their support and contribution. Additionally, the authors would like to express their sincere gratitude to the fishers for their invaluable assistance in data collection and for their continued support throughout the study. We thank the anonymous reviewers for their constructive comments, which helped improve the clarity and quality of this manuscript.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

MPs	microplastics
PAEs	phthalate acid esters
μFT-IR	μFourier transform infrared

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