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Microplastic accumulation and ecological impacts on benthic invertebrates: Insights from a microcosm experiment

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ABSTRACT

Microplastic (MP) pollution poses a global concern, especially for benthic invertebrates. This one-month study investigated the accumulation of small MP polymers (polypropylene and polyester resin, 3–500 μm, 250 μg L^{-1}) in benthic invertebrates and on one alga species. Results revealed species-specific preferences for MP size and type, driven by ingestion, adhesion, or avoidance behaviours. Polyester resin accumulated in *Mytilus galloprovincialis*, *Chamelea gallina*, *Hexaplex trunculus*, and *Paranemonia cinerea*, while polypropylene accumulated on *Ulva rigida*. Over time, MP accumulation decreased in count but not size, averaging 6.2 ± 5.0 particles per individual after a month. MP were mainly found inside of the organisms, especially in the gut, gills, and gonads and externally adherent MP ranged from 11 to 35 % of the total. Biochemical energy assessments after two weeks of MP exposure indicated energy gains for water column species but energy loss for sediment-associated species, highlighting the susceptibility of infaunal benthic communities to MP contamination.

1. Introduction

The global concern for microplastics (MP) contamination in both biotic and abiotic samples underlie a serious lack of management in the value chain from the producer to the consumer of plastic materials (Roland et al., 2022) that ultimately get discarded into the environment. Plastic, in its many polymers, shapes and processes is an extremely versatile and cheap material, ubiquitously present in many aspects of daily life since the beginning of mass production in the 1950s (Kane and Clare, 2019). It was estimated that 367 million tons of plastic materials were manufactured in 2020 (Plastics Europe, 2021) and about 8 million metric tons of plastic waste are expected to enter annually in the world's ocean (NASEM, 2022). Unlike the native primary MP that enter the environment through various channels, secondary MP are generated by progressive fragmentation and degradation of larger particles to fine microparticles by means of physical, chemical and biological processes that lead to the reduction of structural integrity of these plastics (Andrady, 2011; Eriksen et al., 2014; Imhof et al., 2012). The resulting fragments can have different colours, shapes and sizes ranging from few centimetres to few microns, therefore MP were categorized into 5 main classes in function of their size: macroplastics (<200 mm); mesoplastics (200–5 mm); large MP (5–1 mm); small MP (1 mm–1 μm) and nanoplastics (<1 μm) (Andrady, 2017; Koelmans et al., 2015). The MP size influence not only the mobility of the polymers (and therefore their dispersion) but also their potential ingestion which is a function of the organisms size and of their buccal apparatus and digestive system, as smaller organisms can ingest only smaller particles. Polymers are numerous and varied in composition. Following the level of plastic production and release, six main categories of polymers (Dehaut et al., 2016) emerge among the others, these include: polypropylene (PP), high and low density polyethylene (HDPE and LDPE), polyvinyl chloride (PVC), polyurethane (PUR), polyethylene terephthalate (PET) and polystyrene (PS). Recent reports have assessed the ubiquitous presence of MP in various environmental matrices from remote sparsely populated regions of the globe such as the poles to remote lakes and deserts (Munari et al., 2017; Fang et al., 2018; Peng et al., 2018; Zhang et al., 2019; Sfriso et al., 2020; Abbasi et al., 2021). Following processes of fragmentation, fouling and flocculation, ingestion and egestion by biota,

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MP eventually became negatively buoyant, settling on the seabed (Porter et al., 2018). Marine fouling on plastics was reported to increase sinking velocity for large plastic pieces, but the development of a microbial biofilm alone was insufficient to induce sinking of common low density MP such as polyethylene and polystyrene (Kaiser et al., 2017). Therefore, the phenomena of ingestion and expulsion by organisms (Cole et al., 2016) and the flocculation in shallow coastal areas by capture in mucopolysaccharides and transparent exopolymer particles (Summers et al., 2018) should represent significant sinking pathways toward the bottom. Eventually the seabed should be considered as an important sink for MP (Claessens et al., 2013; Van Cauwenberghe et al., 2013; Woodall et al., 2014), especially in transitional water systems and coastal areas where the mixture of fresh and salt-waters favours the precipitation of colloids and primary productivity in the water column. The organisms of the benthic communities associated with the seabed are constantly in contact with MP (Coppock et al., 2021) facilitating the burial of the particles on coastal areas, especially smaller MP (<1 mm) that are missing in surface waters and tend to accumulate in sediments (DeSmit et al., 2021), macroalgae (Sfriso et al., 2021) and benthic organisms (Sfriso et al., 2020).

An extensive literature of laboratory experiments exists describing the effects of MP on aquatic organisms and many papers deals with marine environmental contamination. However a severe lack of realism was highlighted by many authors for laboratory experiments (Weis, 2020; Weis and Palmquist, 2018). This seems to especially pertain the environmental contamination levels which are often lower of orders of magnitude in comparison with laboratory tests (Burns and Boxall, 2018). Additionally, criticisms were raised regarding the short duration of the experiments (h/days) and the size of the particles (Weis and Palmquist, 2018) which do not necessarily reflect the preferential size range of ingestion and accumulation in the different aquatic organisms. Additionally, many authors in toxicological or ingestion studies of MP focus their attention on low-density polymers such as PS, PP and PE, and were largely neglecting high-density polymers. In a wide review by Haegerbaeumer et al. (2019) out of 32 articles that examined the effects of MP on benthic marine invertebrates, only 5 investigated the role that high density polymers can play such as: poly(methyl methacrylate) (PMMA), polyamide (PA), polylactic acid (PLA), PET and PVC. These prompts were considered for the development of this study in order to reveal the temporal dynamics of accumulation in microcosm for a coastal macrobenthic community of the Northern Adriatic Sea. MP fragments of irregular shape, for six dimensional classes, from two polymer types, representative for low- and high-density MP (respectively with density lower or higher than seawater = [1.02 g cm^{-3}]) were implemented at environmentally relevant concentrations aiming to: 1) evaluate accumulation patterns for density, amount and size of MP over time for selected benthic organisms; 2) evaluate MP accumulation in target organs of the investigated benthic species; and 3) evaluate biochemical energy allocation (loss and gain) for different benthic species as an indicator of the state of well-being of the organisms.

2. Material and methods

2.1. Sampling area and organisms

All the organisms were collected from the Venice lagoon (Italy) in proximity of the sea inlets. The collected organisms represent the dominant benthic species in the reference study area. The animals used in the experiments included: *Paranemonia cinerea* Contarini (1844) – (deposit feeder, predator – macrophyte surface/mid water); *Mytilus galloprovincialis* Lamarck (1819) – (filter feeder – mid water); *Chamelea gallina* L. (1758) – (filter feeder – sediment infaunal); *Palaemon serratus* Pennant (1777) – (grazer/scravenger/omnivore – all water column); *Tritia nitida* Jeffreys (1867) – (scravenger – sediment infaunal/epifaunal) and *Hexaplex trunculus* L. (1758) (scravenger/predator – sediment infaunal/epifaunal). The macroalga *Ulva rigida* C. Agardh (1823) was used in the experiment as representative of primary producers, the base of the tropic food chain. Specialist predators were excluded from the study. The densities of the organisms were not chosen by a typical density pattern that would occur in the wild but for the experimental need to have enough individuals for all the analysis.

2.2. Quality assurance and quality control

In order to minimize MP contamination during handling, and analysis of the samples all the analytical procedures were performed on a clean dedicated bench, and the operators wore cotton clothing and lab coats during analyses performance. All the glassware and the aquariums were carefully pre-cleaned by Contrad™ 70 liquid detergent and tap water and then rinsed at least two times with filtered Milli-Q water. The samples and the glassware were capped with aluminium foil and glass caps during the analyses to minimize airborne contamination. One analytical blank was performed at every cycle of analyses, and was considered acceptable, with <3 particles per filter. The Whatman® glass microfiber filters, Grade GF/F (0.7 μm) used during the analyses were calcinated at 550 °C for 2 h before use to remove traces of MP contaminants. All used reagents were filtered with GF/F filters before use.

2.3. Microplastic production

The MP were produced by fragmentation in a top-down approach. Two fluorescent polymers were selected for microplastic production, representing low- and high-density polymers: red polypropylene (d ≈ 0.90–0.92 g cm^{-3}) and polyester resin (d ≈ 1.30–1.35 g cm^{-3}). Red polypropylene (PP) MP were produced utilizing the plastic body of a red fluorescent marker (tratto VIDEO, F.I.L.A. S.p.a.). Green fluorescent polyester resin (PER) was made by dissolution of few milligrams of fluorescein and polymerization of 50 mL of polyester resin (Poly Top, 3C Commerciale Chimica Colori S.r.l.) with a commercial catalyst. Different techniques were used for polymers fragmentation: scraping with a scalpel, grinding in an electric spice grinder, gently grating on a fine cheese grinder, and eventually grating with an abrasive sponge (grit 100). The particles obtained from all these techniques were pooled together and sieved on a Vibratory Sieve Shaker (RETSCH model AS200control) to separate different dimensional ranges: (<30 μm)–(30–60 μm)–(60–125 μm)–(125–250 μm)–(250–500 μm)–(500–1000 μm). The MP produced were then pooled again in equal weight per each dimensional range. Pharmaceutical grade diatomaceous Earth (Lochside Natural Products Ltd) was used to mix, dilute and partition MP into equal parts, and a mixture of approx. 100 mg of diatomaceous earth/MP mix was used to introduce the same MP amount in the aquariums to get a final MP concentration of 250 μg L^{-1} [PP + PER]. Details about the number of particles per dimensional range introduced in the experiment are provided in supplementary material (Fig. S2).

2.4. Experimental design

Two sets of microcosm experiments were prepared in glass aquariums (Fig. 1). The first experimental setup was carried out to investigate the fate and accumulation of prepared fluorescent MP (final concentration of 250 μg L^{-1} [PP + PER]) over time periods (3, 7, 14 and 30 days). The second experimental setup was conducted in the same conditions of the first experiment for 14 days to explore the MP accumulation in different target organs of the tested organisms and measure the macronutrient constituents (proteins, carbohydrates, lipids) in the soft tissues. The total macronutrients were converted into energy to evaluate energy gain or losses in different species due to MP accumulation in comparison with control conditions. The following conversion factors were used (FAO, 2003): carbohydrates 16 J mg^{-1}; proteins 17 J mg^{-1}; lipids 37 J mg^{-1}.

The first experimental setup consisted of 3 aquariums each filled with 25 L of seawater (filtered by fiberglass filters GF-F to eliminate MP

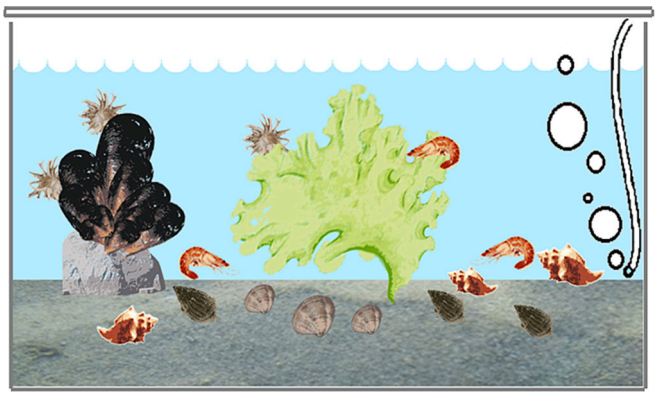

Fig. 1. Schematic representation of aquariums setup.

and suspended particles). A bottom sediment layer (5 cm) of calcinated sand (i.e. 550 °C for 2 h to eliminate MP contaminants) was placed in each aquarium. The oxygenation and water circulation were ensured by means of small air pumps with free bubbling fixed midwater. Two 9 W led lights were used as light source with a day/night cycle of 12 h. Each aquarium was covered by a Plexiglas lid to prevent airborne MP contamination. The test organisms were sampled and depurated for 2 weeks and three water changes were performed with filtered seawater during depuration in order minimize environmental MP contaminants, the organisms were then transferred to the experimental aquariums. For each aquarium, an amount of 30 g of prewashed macroalga (*U. rigida*) was added. Filter feeders and deposit-feeders were fed every 24 h with 50 mg per aquarium of pre-dissolved microalgae dry powder (*Nannochloropsis gaditana* by My Superfoods Ltd). A minimum of 12 individuals for each species was placed in each aquarium and a mixture of MP was added. At every sampling time (3, 7, 14 and 30 days) two individuals for each species were sampled together with 5 g of *U. rigida* and about 60 g of sediment (a pool from three sediment cores per aquarium). All samples were packed in aluminium foil and frozen for analysis.

The second experimental setup was composed of two aquariums (25 L) with addition of fluorescent MP and two aquariums set as negative control (without MP addition). All the organisms were collected after 2 weeks and pooled as control aquariums and as exposed ones. Half of the organisms from the MP exposed aquariums were utilized for the macronutrient analyses. The second half of the exposed samples was used within 24 h to separate (when possible) the internal organs: hepatopancreas, gonads, gut, gills, and "other tissues" (which represent the remaining internal organs thereafter pooled together). The soft tissues of the animals were washed with NaOH 1 % solution with intense shaking for 1 min to remove loosely attached external MP particles, then they were quickly rinsed with a $NaHCO_3$ solution, placed on a Petri dish and the organs were carefully separated.

2.5. Digestion, MP purification and analysis

The organisms, macroalgae and the sediments were freeze-dried completely and stored in the dark. The dried organisms from each aquarium pool were counted, separated into shells and soft tissues, weighted, and processed together. The lyophilized soft tissues from each organism were washed with NaOH 1 % solution with intense shaking for 1 min in a glass bottle in order to remove loosely attached external MP particles. For each type of organism, these wash solutions and any discarded shells were pooled (by mixing the three-aquarium wash replicates together in one), recording the respective counts and weights of the involved organisms. The washed organisms were transferred into a 100 mL flask and digested with NaOH 1 % solution at 40 °C for 24 h (Sfriso et al., 2020) with a biomass/solution ratio of 20 mg dw/mL. The wash pool solutions were digested by the same method.

MP were extracted from macroalgae following the protocol of Sfriso et al. (2021) by shaking of 1 g dw of *U. rigida* in 100 mL extracting solution (NaCl 30 g L^{-1}, Na_2EDTA 100 mM) for 30 min. The extraction protocol was repeated (2 times) with additional 50 mL of extraction solution.

For sediment, MP were extracted by density floating in $CaCl_2$ solution with of d ≈ 1.4 g cm^{-3}. The extraction was performed in 50 mL flasks from 2 g of dried sediment. The $CaCl_2$ solution was added reaching the edge of the flask and the sediment was mixed with a magnetic stirrer bar (coated by aluminium foil) for 5 min, then the sediment was left to settle. Subsequently, MP were recovered by overflowing the solution from the flask collecting the supernatant. The extraction procedure was repeated 3 times for full recovery.

The purification of MP from all digested extracts was carried out following the oil-extraction protocol (Crichton et al., 2017) with some minor modifications. Briefly, an aliquot of the digests was transferred to separation funnel and was diluted with filtered tap water followed by addition of 10 mL of filtered sunflower oil. The mixture was shaken for 1 min then settled for 5 min. Aqueous fraction was discarded, and the restored oil fraction was re-purified with additional filtered water to recover the MP. The oil fraction was diluted with hexane and filtered with Whatman® glass microfiber filters, Grade GF/F. The filters were washed with 10 mL of ethanol:methanol:isopropanol solution (90:5:5 v/v) to remove organic contaminants and residual oil.

The qualitative/quantitative analysis of MP was performed using epifluorescence microscopy (Olympus BX51 Microscope) with UV excitation by scanning the whole filter (or half or one quarter in function of the MP abundance) in a 10× magnification and capturing the red (PP) and green (PER) fluorescent particles by a digital camera. The images were processed by ImageJ software to measure the Feret diameter hereinafter used to describe the size of the particles. The size detection limit for the smallest particles was 3 μm.

2.6. Macronutrients analyses

Prior to analyses, shells were removed and the organisms were dried, then ground to fine powder and kept in dry place. The total protein analysis was performed by digestion of ground organisms in 0.5 M NaOH for 15 min at 105 °C. Total protein concentration was measured spectrophotometrically using Bradford protein assay (Bradford, 1976). Bovine serum albumin was used for the calibration curve.

The total carbohydrates analysis was performed by digestion in H_2SO_4 72 % at 30 °C for 1 h and measurement by phenol-sulphuric acid assay (Dubois et al., 1956). Glucose was used for the calibration curve. The total lipid analysis was performed by ultrasound assisted extraction for 30 min (Metherel et al., 2009) in a hexane/isopropanol (3/2, v/v) solution (Hara and Radin, 1978) followed by gravimetric determination in aluminium capsules after drying.

2.7. Statistical analysis

Non-parametric Wilcoxon rank sum tests were performed in order to assess significant differences between MP contents accumulated over a time period (3, 7, 14 and 30 days) for each organism. Spearman Rank-Order correlations were performed to assess significant correlations between MP contents in each species. Furthermore, linear regressions were applied to MP accumulated in all the organisms and the relative allocated energy. Regression coefficients, relative *p*-value, R-squared and all statistical analyses were performed using R software, version 3.5.3.

3. Results

3.1. Sediment and macroalgae MP accumulation

The microcosm experiment follow over time the sedimentation of MP by means of accumulation as item g^{-1} dw in macroalgae and sediments.

Macroalgae (*U. rigida*) were by weight the main accumulators of MP after 3 days with 140 item g^{-1} dw (Fig. 2) with dominance of PP (PP 85 %: PER 15 %). The MP content reduced in *U. rigida* over time retaining a higher content of large-size low-density particles after 30 days. Overall, macroalgae proved to be non-specific accumulators in terms of size of MP fragments. In fact, the histogram of Fig. 3a illustrates that the distributions of dimensional frequencies spread over a wide size range.

The sediment, unlike macroalgae, showed a slower MP accumulation trend over time which almost never exceeded 19 ± 8.8 item g^{-1} dw, except at 14 days with a peak accumulation of 60 ± 77 item g^{-1} dw for a median size of 18 μm (Table. 1). The size range frequency distribution for sediment MP (Fig. 3b) displayed an equalized distribution in terms of PP (52 %) and PER (48 %) following the distribution of the MP mixture introduced into the aquariums. Eventually the first (3 days) and the last time point (30 days) displayed the same median size and similar interquartile ranges.

3.2. Benthic organisms MP accumulation

The entity and the dynamics of MP bioaccumulation in the tested organisms were measured by the normalization to items per individual in order to compare the organisms with highly variable sizes, weights and feeding habits.

After 3 days of MP exposure, the filter feeder *M. galloprovincialis* displayed the highest MP accumulation (19 ± 17 item $individual^{-1}$) whereas a maximum accumulation of 5.8 ± 4.0 item $individual^{-1}$ was recorded for all other organisms (Fig. 2a). Unlikely, very small amounts of externally adherent MP were recorded on all organisms in comparison with internally accumulated ones. The mussels (*M. galloprovincialis)* occupying the water column were the first organisms interacting with sinking particles. Although the average MP contamination in the mussel was high, MP (especially low-density ones) were rapidly expelled as early as one week (Fig. 2b) and remained within 5.2 ± 8.9 item $individual^{-1}$ without significant changes in size (median size 11 μm — Table.1) up to 30 days. The second filter feeder used in the experiment, the infaunal clam *C. gallina*, in spite of the similar MP size and

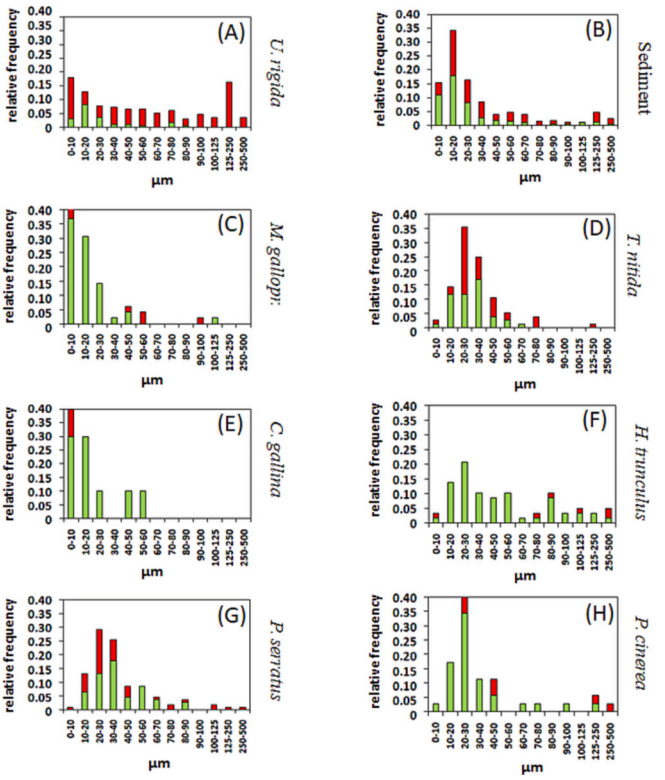

Fig. 3. Relative size frequencies of microplastics in (A) *U. rigida*, (B) sediment, (C) *M. galloprovincialis*, (D) *T. nitida*, (E) *C. gallina*, (F) *H. trunculus*, (G) *P. serratus*, (H) *P. cinerea*. The size is represented by the Feret diameter of the particles. Red: PP; green: PER. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

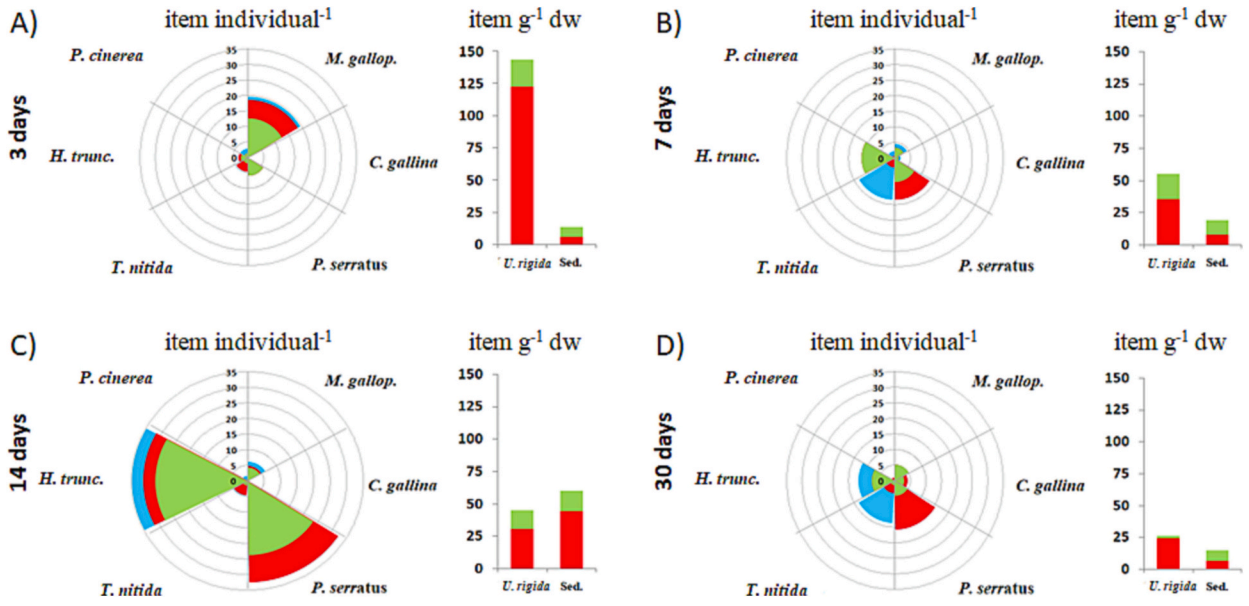

Fig. 2. Rose graph of microplastic content per individual (item $individual^{-1}$) at 3 days (A) 7 days (B) 14 days (C) and 30 days (D) in *P. cinerea*, *M. galloprovincialis* (*M. gallop.*), *C. gallina*, *P. serratus*, *T. nitida* and *H. trunculus* (*H. trunc.*). Side histograms represent the respective microplastic contamination in macroalgae (*U. rigida*) and sediment (Sed.) as item g^{-1} dw to track changes in the environmental microplastic contamination over time. Average standard deviation of total MP content (PER + PP) between aquariums was in: *M. galloprovincialis* 9 item $individual^{-1}$, *C. gallina* 1 item $individual^{-1}$, *P. serratus* 10 item $individual^{-1}$, *T. nitida* 3 item $individual^{-1}$, *H. trunculus* 7 item $individual^{-1}$, *P. cinerea* 1 item $individual^{-1}$, *U. rigida* 26 item g^{-1}, sediment 25 item g^{-1}. Green (*PER*-polyester resin); Red (PP-polypropylene); Blue (external loosely associated microplastics PP + PER). The reported values for PER and PP are the mean of 3 replicates. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Median size (μm — measured as Feret diameter of the particles) and interquartile range at 72 h and at 1–2–4 weeks in all organisms and in the sediment. Legend: nd — not determined; Global — median value of all time points.

Sample	3 days	7 days	14 days	30 days	Global
	μm				
Sediment	21(23)	15 (18)*72h	18 (16)	21 (25)	18 (20)
U. rigida	33 (60)	29 (47)	35 (64)	74 (66)*all	41 (66)
M. galloprovincialis	13 (9)	23 (9)	9 (12)	9 (4)*1w	11 (12)
C. gallina	0 (nd)	19 (6)	9 (10)	14 (5)	11 (10)
P. serrarus	39 (21)	36 (13)	29 (22)	28 (9)	30 (14)
T. nitida	26 (15)	35 (13)	25 (12)	28 (8)	27 (12)
H. trunculus	44 (56)	38 (29)	30 (43)	44 (51)	39 (48)
P. cinerea	29 (10)	20 (4)	21 (nd)	27 (19)	26 (10)

* Wilcoxon rank sum test significant difference over time for $p < 0.05$.

distribution frequencies to *M. galloprovincialis* (Fig. 3c and e), showed a disparate bioaccumulation behaviour over time. MP content was the lowest in *C. gallina* (<1.4 ± 0.4 item individual^{-1}) for the first two weeks and the peak accumulation was recorded at 30 days with 4.2 ± 2.5 item individual^{-1}. This suggested a much slower accumulation process of MP in *C. gallina* and probably a greater selectivity for MP during feeding. Despite that, the final accumulation after 30 days for both filter feeders were quite similar in terms of MP accumulation. The sea anemone *P. cinerea* thrived in the water column, especially attached to macroalgae and mussels. These organisms were expected to be highly contaminated by MP, in proportion to the high concentrations accumulated by the substrate organisms (i.e., *U. rigida/M. galloprovincialis*). Indeed, a significant correlation (Spearman correlation $R^2 = 0.90$, $p < 0.05$; Table S1) was calculated between the MP content in *P. cinerea* and that in *M. galloprovincialis*. However, the average MP accumulation of *P. cinerea* was the lowest with a peak at 3 days of 1.5 ± 1.3 item individual^{-1} (Fig. 2) for a median particle size of 26 μm. This deposit feeder accumulated mainly PER and traces of PP (appeared only after 7 days).

The shrimp (*P. serratus*) displayed the highest MP accumulation (avg. 17 ± 11 item individual^{-1}) with the lowest values recorded after 3 days (5.8 ± 4.0 item individual^{-1}) and the highest after 14 days (32 ± 16 item individual $^{-1}$). The median particles size decreased over time from 39 μm to 28 μm indicating a progressive loss of larger MP in favour of the retention of smaller ones; moreover, no preference was recorded regarding polymer type.

The shrimps moved only occasionally in the water column or on macroalgae and spent most of the time grazing on surface sediments. This epibenthic position and the omnivore diet could produce a niche overlap which may explain the correlation found between the MP content in *P. serratus* and *H. trunculus* (Spearman correlation $R^2 = 0.90$, $p < 0.05$; Table S1). Interestingly, even though *H. trunculus* accumulation patterns mirrored those of *P. serratus*, the size distribution of the accumulated particles was very different (Fig. 3f, g). *H. trunculus* selectively accumulated high-density PER (90 % of the particles) with a median particle size of 39 μm and the accumulated particles were spread over a much wider dimensional range than all other animals. The gastropod *H. trunculus*, after the *T. nitida*, showed the highest concentrations of external adhering MP, especially after 14 days. Surprisingly, the mud snail *T. nitida* showed a constant MP concentration (average 4 ± 0.8 item individual^{-1}) with a marked preference for PP and an average concentration of external adhering particles at 7 and 30 days which was 4 times higher than the internally accumulated MP. Overall, the mean MP accumulation into the organisms was 7.4 ± 6.5 item individual^{-1}. The MP accumulated internally reduced as counts but not as size and persisted into the organisms up to 1 month with 6.2 item individual^{-1}. If we consider only the average accumulated particles (excluding those attached externally) at all sampling times, *P. serratus* and *H. trunculus* displayed the highest accumulation (13–17 item individual^{-1}), conversely *P. cinerea* and *C. gallina* displayed the lowest one (0.9–1.5 item individual^{-1}). Surprisingly the external adhering MP were a minority fraction of the total, ranging averagely (as all organisms) between 11 % and 35 % of the total MP from 3 to 30 days with few exceptions as previously stated for *H. trunculus* and *T. nitida*.

3.3. Target tissue accumulation

The second experiment was carried out in order to verify the fate of ingested MP inside the benthic organisms. The experiment took place under the same conditions of the first one and terminated after 14 days [the point of highest average MP accumulation for many organisms recorded from the first set of experiments]. Regarding the separated organs from the animals, the hepatopancreas was distinguishable only in *H. trunculus*; gonads were not distinguishable in *P. cinerea* and *P. serratus* and no gills exist in *P. cinerea*. In particular, the MP with more transitory nature are those into the gut as they can be expelled when they are not absorbed into the walls of the intestinal lumen. Indeed, considerable fractions of MP were accumulated into the gut of *P. cinerea*, *P. serratus* and *M. galloprovincialis* and these exceeded 50 % of the total accumulated MP (Fig. 4).

Moreover, important fractions of the accumulated MP (36–69 %) were found strongly attached to the gills of filter feeders (*M. galloprovincialis*, *C. gallina*). In "other tissues" MP were identified from all the organisms in the range from 8 % to 68 % of the total MP (Fig. 4). Among all samples with distinguishable gonad, the highest gonad MP accumulations were recorded in *T. nitida* and *H. trunculus* (23 % and 55 % of the total accumulated MP, respectively).

Generally, by considering only the MP accumulated in internal organs and tissues (i.e., ΣMP in gonad-other tissues-hepatopancreas,), filter feeders displayed the lowest percentages of bioaccumulated MP ranging 14–19 % of the total accumulated MP. Whereas *H. trunculus* and *T. nitida* displayed the highest percentages of accumulated MP in internal tissues from 79 to 91 % of the total MP. This suggest that that a significant fraction of MP was actually accumulated and it was not transient in the digestive tract of the organisms.

3.4. Energy storage and dynamics

In order to estimate gains or losses in terms of energy (i.e., identify metabolic disruptions at 14 days) the organisms utilized in the second experiment were analyzed for protein, carbohydrates and lipids and the respective composition was converted to energy. Among the organisms exposed to MP, the organisms living in the water column (*P. cinerea* and *M. galloprovincialis*) displayed positive energy gains in a ratio with control (Table 2). These organisms were not negatively affected by exposure to MP. In fact, MP exposure seemed to induce a slight energy gain. The shrimp *P. serratus* showed no changes in energy content and

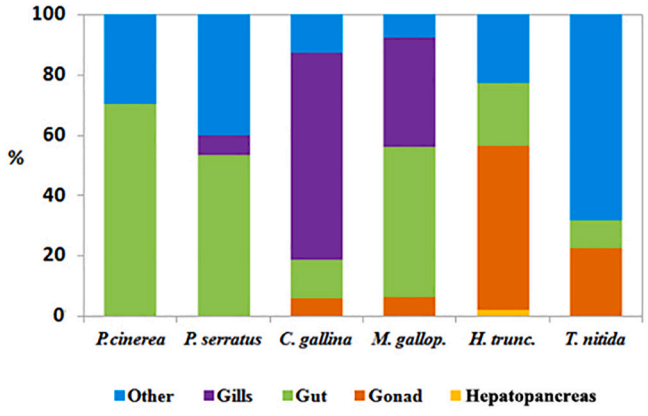

Fig. 4. Microplastics relative abundance for *P. cinerea*, *P. serratus*, *C. gallina*, *M. galloprovincialis*, *H. trunculus* and *T. nitida* in different organ tissues (hepatopancreas, gonad, gut, gills and other tissues) at 14 days.

Table 2
Biochemical composition of the organisms at 14 days in control and exposed conditions. The data were converted in J g^{-1} dw and expressed as biochemical energy gain/loss (ratio + MP:Ctrl) in presence of MP as estimate of the organism wellbeing.

%dw	*P. cinerea*		*C. gallina*		*P. serratus*		*H. trunculus*		*M. galloprovincialis*		*T. nitida*	
	Ctrl	+MP	Ctrl	+MP	Ctrl	+MP	Ctrl	+MP	Ctrl	+MP	Ctrl	+MP
Carbohydrates	3.9	5.2	15	15	2.6	2.4	10	5.7	7.7	9.4	9.0	8.8
±sd	0.2	0.3	0.2	0.4	0.2	0.2	0.3	0.3	0.3	0.5	0.6	0.4
Lipids	9.0	10	6.2	6.6	5	5.58	8.3	5.7	5.1	7.3	5.5	9.3
±sd	0.6	0.0	1.5	0.3	0.3	0.22	2.0	0.2	0.1	0.4	0.3	1.0
Proteins	31	31	33	30	38	36	39	34	34	35	43	33
±sd	0.0	0.1	0.3	0.3	0.2	0.4	0.1	0.6	0.6	0.0	0.3	0.4
kJ g^{-1} dw	9.2	9.8	10.3	9.9	8.7	8.7	11.3	8.8	8.9	10.1	10.8	10.4
±sd	0.3	0.1	0.6	0.2	0.2	0.2	0.8	0.2	0.2	0.2	0.3	0.5

	P. cinerea	*C. gallina*	*P. serratus*	*H. trunculus*	*M. galloprovincialis*	*T. nitida*
Energy ratio	5.8 %	−4.3 %	−0.7 %	−21 %	13 %	−3.5 %

was apparently unaffected by MP exposure. In contrast, the organisms *C. gallina*, *T. nitida* and *H. trunculus* (most closely associated with the sediments) displayed an energy loss with a peak in *H. trunculus* (−21 % in comparison with control). No significant statistical correlations were found between the total content of accumulated MP and energy allocation. However, considering only MP accumulated in internal organs and tissues (i.e., ΣMP in gonad-other tissues-hepatopancreas), a significant inverse correlation emerged between accumulated MP in all the organisms and the allocated energy (Adjusted $R^2 = 0.63$; $p = 0.036$; regression details are provided in supplementary material). In accordance with these results, non-externalizable MP have a more transitory nature and are expected to produce fewer effects over time.

4. Discussion

Microplastics are pervasive contaminants, detected across many environments including the atmosphere, mountain lakes, freshwater bodies, terrestrial lands, deep oceans, and polar regions (Munari et al., 2017; Fang et al., 2018; Free et al., 2014; Gasperi et al., 2015; Horton et al., 2017; Van Cauwenberghe et al., 2013). Small MP often resemble small food particles and interact with organisms often leading to ingestion by a wide array of aquatic fauna (Wang et al., 2019) and recent field studies focusing on invertebrate benthic trophic chains suggested that biomagnification and accumulation of MP are unlikely to occur toward predators (Bour et al., 2018; Setälä et al., 2016; Sfriso et al., 2020), conversely, filter feeders and grazers, at the lowest trophic levels, exhibited the highest concentrations of MP per individual. The marine benthic invertebrates that populate the coastal waters are potentially strongly exposed to contamination by MP especially in areas with high water residence time which is the case for the site of origin of the organisms tested in this study: the Venice lagoon, which display adjacent tributary rivers, elevate plastic debris flows (Liubartseva et al., 2016) and residence times on the seaward side of the lagoon ranging 20–40 days (Sfriso et al., 2021). Taking into consideration the contamination reported for the Venice lagoon, near the collection point of the studied organisms, high concentrations of small microplastics (S-MP) were reported in water (Corami et al., 2021) in the range 4900–16,800 items L^{-1} for an estimated mass of approx. 45–83 μg L^{-1}. The concentrations reported by the authors for the sediments were between 170,000 and 200,000 items kg^{-1} fw for an estimated mass of approx. 81–490 μg kg^{-1} fw. These concentrations are comparable in terms of mass with the amount of MP implemented in this study (250 μg L^{-1}). Moreover, the amount of contamination of the sediments recorded for field samples by Corami et al. (2021) exceeded by 3 times the maximum quantity of MP recorded in the aquarium sediments (60,000 items kg^{-1}). Therefore, the data obtained under the experimental conditions should represent credible small MP accumulation patterns in highly contaminated environments. The results highlight how MP are especially represented in the smaller dimensional range under 30–50 μm, whose identification is often very demanding and commonly neglected (Setälä et al., 2016).

The experiment displayed a preferential accumulation in terms of low-density MP (PP) on macroalgae probably due to the longer sinking time and retention in the water column in comparison with high density MP (PER), which quickly sank when introduced into the aquariums. The mucopolysaccharides present onto the macroalga *U. rigida* work as a glue capturing MP from the water column (Sfriso et al., 2021), as low density particles stay longer in the water phase it is reasonable to assume more low density particles should stick to macroalgae. In the coastal areas where high-density polymers (Rayon, Nylon, PET, polyester) represent significant fractions of the contamination, this behaviour not always seem to reflect in the polymer composition of MP from macroalgae (Feng et al., 2020; Zhang et al., 2022). Interestingly, it must be emphasized that the vast majority of the recorded high-density polymers (from field samples) often correspond to fibres which were not considered in this experiment. Additionally, fibres are reported to more easily entangle on the laminar or tubular mucilaginous structures of the macroalgae in comparison with fragments (Ng et al., 2022).

In the reconstructed microcosm environment all invertebrates accumulated MP but surprisingly the actinia *P. cinerea* generally proved to be extremely effective in maintaining low levels of MP accumulation at all time periods. This selectivity could be confirmed by the MP accumulation recorded for the anemones from the Spain coast (Janssens and Garcia-Vazquez, 2021) which in a comparison with the gastropods *Phorcus lineatus* and *Steromphala umbilicaris* presented always markedly lower concentrations. This low MP accumulation found in anemones was justified by Janssens and Garcia-Vazquez (2021) as a consequence of the feeding strategy bound to the catch of food particles with tentacles before ingestion, a selective process in which organisms are able to effectively discriminate food from inert debris. Accordingly, the lowest accumulation was also recorded for the anemones in a study on the benthic communities of Terra Nova Bay (Sfriso et al., 2020). This seems to reflect in the energy ratio of *P. cinerea*, slightly positive, showing not to be negatively affected by MP contamination. The heavily contaminated shrimp *P. serratus* seemed almost unaffected by accumulated particles. Conversely the infaunal clam *C. gallina* (always displaying low MP accumulation) and the sea snail *H. trunculus* displayed an energy loss when exposed to MP. Even considering only the two filter feeders used in the experiment in a comparison, *C. gallina* and *M. galloprovincialis* accumulated both similar small high density MP in the size range reported by Defossez and Hawkins (1997) for the feeding habits of *M. edulis* and *Ruditapes* spp. (7.5–15 μm). However the mussel was not affected by the introduction of MP, it displayed an energy gain instead at 14 days. Though mussels are usually recognized as sensitive biological indicators of pollution in monitoring programs (Viarengo and Canesi,

1991) no negative effects on their energy status were recorded. Wright et al. (2013) detected a significant decrease in the energy budget of lugworms exposed to uPVC (unplasticized polyvinylchloride) MP, and similar results were recorded by Ribeiro et al. (2017) with the depletion of energy reserves in the clam *Scrobicularia plana* exposed to polystyrene MP at 1 mg L^{-1}. However, no adverse effects of MP were recorded for mussels both by Van Cauwenberghe et al. (2015) and Revel et al. (2019) confirming our results and pointing out how the effects of MP were probably species-specific.

The expected trophic relationships between organisms do not appear to be reflected in the accumulation patterns. No relationships have been experimentally detected between macroalgae and *P. serratus*, that usually graze on *U. rigida*, nor between *H. trunculus* and the prey *M. galloprovincialis*. This seems to be in agreement with the observations of Bour et al. (2018) on the occurrence of MPs in analyzed biota, not influenced by organism habitat nor trophic level. The only significant relationships found in MP accumulation patterns in our tests were between *H. trunculus* and *P. serratus* and between *P. cinerea* and *M. galloprovincialis*, however these accumulation patterns over time did not match for type and size of accumulated MP. The authors Bour et al. (2018) and Porter et al. (2023) reported a lack of relationship between individual MP body burden and: environmental position within the sediment (demersal, epifaunal and infaunal); feeding mode; mobility, habit or organism weight. This may be the case for the accumulation relationship found between *H. trunculus* and *P. serratus* which both predominantly played the role of deposit-feeders on surface sediments and the overlap of niche and feeding strategies may have influenced the similar accumulation trends in these organisms. However, it does not explain the relationship found between *P. cinerea* and *M. galloprovincialis* which instead seemed linked to positioning in the aquarium and certainly not to the feeding mode since part of the anemones were positioned on the mussels. Feeding mode alone is not a sufficient parameter to infer on MP accumulation as in our results organisms with similar feeding strategies have not always shown the same accumulation pattern of MP over time. The authors (Bour et al., 2018; Porter et al., 2023) suggested that more subtle processes may be involved in defining MP body burden such as: food availability and biofilm formation. The possibility cannot be ruled out that food availability during laboratory exposure experiments could introduce confounding factors by impacting on the physiological condition of the tested organisms (Chae and An, 2020) or promoting formation of hetero-aggregations of MP and organic material (Li et al., 2021) that settle on the bottom foraging epibenthic organisms. Additionally, Gutow et al. (2019) described the role of gastropod pedal mucus as a vector for the transfer of MP to other organisms that forage on it, potentially influencing MP accumulation in shrimps.

From the results, despite the differences in composition and size of the MP accumulated in the different species, it is clear how the organisms closely associated with the sediment suffered a reduction of the energy at two weeks, (*C. gallina*, *H. trunculus* and *T. nitida*), conversely the organisms in the water column (*P. cinerea*, *P. serratus*, *M. galloprovincialis*) were weakly or positively affected. The investigation into the distribution of MP between different tissues reveals how organisms in the water column showed on average a higher MP content in the gut. This fraction is mostly considered transitory. In depuration experiments carried out by Saborowski et al. (2019) and Chae and An (2020) on the Atlantic ditch shrimp *Palaemon varians* and the mussel *M. galloprovincialis*, clearance of the gut was recorded already at 24 h suggesting that MP in shrimp and mussels are probably more transient and reflect a short-term contamination. As regards the clam *C. gallina* (and to a lesser extent *M. galloprovincialis*), however, a high accumulation of MP was found to be associated with the gills, as reported for the wedge *clam Donax trunculus* for which the gills were reported as the main target for MP accumulation (Tlili et al., 2020). Small MP can enter the body not only from ingestion but also through the body surface (Kolandhasamy et al., 2018), especially gills for which internalization was recorded in mussels (Ikuta et al., 2022). Previous reports highlighted MP translocation from the gut to the hemolymph in *Carcinus maenas* and *in Mytilus edulis* (Browne et al., 2008; Farrell and Nelson, 2013) or the adsorption from external tissues (Kolandhasamy et al., 2018) reaching the internal organs. In addition to this, a study carried out on *M. galloprovincialis* reported MP accumulation reaching the gonadial tissue (Von Hellfeld et al., 2022). Both filter feeders showed a slight contamination at the level of the gonads, but the organisms that showed the highest levels of accumulation in the gonads were the gastropods *H. trunculus* and *T. nitida*. There is little evidence in the literature to make comparisons, however it is reasonable to assume that with the spawning of the gametes part of the MP accumulated at the level of the gonads can be expelled. This consideration (still lacking in evidence) however implies the fact that outside of the reproductive period the accumulated MP at the level of the gonads remain within the organism and are therefore less transitory in comparison with the gut; furthermore, the role that these may have on gastropods at the reproductive level is not known yet.

Ultimately, we evaluated the goodness of the tested organisms as biomonitors for the accumulation of MP. Ideal biomonitors for synthetic contaminants should be: 1) sedentary, 2) easy to identify, 3) abundant, 4) long lived, 5) available for sampling all year round, 6) large enough to provide enough sample for analysis, 7) tolerant to a wide range of physico-chemical parameters, and 8) accumulators for the contaminant with a simple correlation between the tissue content and the surrounding environment (Storelli et al., 2001). In compliance with these parameters, the macroalga *U. rigida* and the gastropods *H. trunculus* and *T. nitida* represent the best organisms to be considered as biomonitors. Indeed, the proposal to use gastropods as biomonitors for MP contamination is not new, in fact it had already been advanced by some authors (Akindele et al., 2019; Curren et al., 2024; Zaki et al., 2021). Similarly, we already suggested the use of macroalgae as candidate biomonitors for MP in transitional water systems (Sfriso et al., 2021) and a similar proposal was put forward by Feng et al. (2020). However, according to the parameters already mentioned by Storelli et al. (2001) it would be necessary to add especially in the case of MP that: "the organism should not present any preferential accumulation in terms of 9) size of MP or 10) MP polymer type". Under these circumstances, in fact, only *U. rigida* and *H. trunculus* showed a homogeneous distribution for all the dimensional ranges. We therefore suggest a combined use of macroalgae and gastropods since they should behave as complementary biomonitors in coastal areas.

5. Conclusions

In conclusion, this study sheds light on the concerning issue of MP contamination and its accumulation dynamics within benthic invertebrates. The results indicate that, high percentages of MP were accumulated in the internal tissues of the studied organisms and impacted more significantly on infaunal species, confirming that benthic species are especially at risk of deleterious effect by MP. The assessment of the energy balance of various benthic species after two weeks of MP exposure revealed different impacts on their well-being. Organisms inhabiting the water column exhibited positive energy gains compared to the control group, while those closely associated with sediments experienced energy loss, with the highest loss observed in *H. trunculus*. Collectively, these findings underscore the greater susceptibility of infaunal benthic communities to MP contamination.

High-density MP, represented by polyester resin, tended to be preferentially accumulated in several species, while low-density MP, represented by polypropylene, accumulated externally mainly on the macroalga *U. rigida*. Different species of benthic invertebrates exhibited species-specific preferences for MP size ranges and polymer types, with varying accumulation behaviours, including ingestion (as function of the feeding strategies), adhesion, or avoidance. Over the course of one month, MP accumulation within the organisms showed a decrease in

counts but not in particle size. Notably, a significant portion of MP was found to accumulate internally, with 11 to 35 % of MP adhering externally to the organisms. The study also identified specific organs, such as the gut, gills, and gonads, where MP accumulation occurred, indicating potential pathways for MP transfer within these species. Definitely, this microcosm study portrays only a small, simplified window of the complexity of coastal ecosystems. To date, no comprehensive studies evaluate the whole ecological quality in response to MP contamination and future efforts should be directed to fill this critical knowledge gap.

The supporting information provide additional material on the statistical analyses performed and some images of the microplastics identified by epifluorescence microscopy. Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2024.116231.

CRediT authorship contribution statement

Andrea Augusto Sfriso: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. **Abdul-Salam Juhmani:** Conceptualization, Investigation, Writing – original draft. **Yari Tomio:** Formal analysis. **Adriano Sfriso:** Supervision. **Flavio Rizzolio:** Resources. **Muhammed Adeel:** Investigation. **Mohammad Wahsha:** Resources. **Cristina Munari:** Resources. **Michele Mistri:** Funding acquisition, Supervision.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used CHAT-GPT in order to rephrase few sentences of the text. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

References

Abbasi, S., Turner, A., Hoseini, M., Amiri, H., 2021. Microplastics in the Lut and Kavir Deserts, Iran. Environ. Sci. Technol. 55, 5993–6000. https://doi.org/10.1021/acs.est.1c00615.

Akindele, E.O., Ehlers, S.M., Koop, J.H.E., 2019. First empirical study of freshwater microplastics in West Africa using gastropods from Nigeria as bioindicators. Limnologica 78, 125708. https://doi.org/10.1016/j.limno.2019.125708.

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. https://doi.org/10.1016/j.marpolbul.2011.05.030.

Andrady, A.L., 2017. The plastic in microplastics: a review. Mar. Pollut. Bull. 119, 12–22.

Bour, A., Avio, C.G., Gorbi, S., Regoli, F., Hylland, K., 2018. Presence of microplastics in benthic and epibenthic organisms: influence of habitat, feeding mode and trophic level. Environ. Pollut. 243, 1217–1225. https://doi.org/10.1016/j.envpol.2018.09.115.

Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3.

Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). Environ. Sci. Technol. 42, 5026–5031. https://doi.org/10.1021/es800249a.

Burns, E.E., Boxall, A.B.A., 2018. Microplastics in the aquatic environment: evidence for or against adverse impacts and major knowledge gaps. Environ. Toxicol. Chem. 37, 2776–2796. https://doi.org/10.1002/etc.4268.

Chae, Y., An, Y.-J., 2020. Effects of food presence on microplastic ingestion and egestion in *Mytilus galloprovincialis*. Chemosphere 240, 124855. https://doi.org/10.1016/j.chemosphere.2019.124855.

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Mar. Pollut. Bull. 70, 227–233. https://doi.org/10.1016/j.marpolbul.2013.03.009.

Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C., Tamara, S.G., 2016. Microplastics alter the properties and sinking rates of zooplankton faecal pellets. Environ. Sci. Technol. 50, 3239–3246. https://doi.org/10.1021/acs.est.5b05905.

Coppock, R.L., Lindeque, P.K., Cole, M., Galloway, T.S., Näkki, P., Birgani, H., Richards, S., Queirós, A.M., 2021. Benthic fauna contribute to microplastic sequestration in coastal sediments. J. Hazard. Mater. 415, 125583 https://doi.org/10.1016/j.jhazmat.2021.125583.

Corami, F., Rosso, B., Morabito, E., Rensi, V., Gambaro, A., Barbante, C., 2021. Small microplastics (<100 μm), plasticizers and additives in seawater and sediments: oleo-extraction, purification, quantification, and polymer characterization using micro-FTIR. Sci. Total Environ. 797, 148937 https://doi.org/10.1016/j.scitotenv.2021.148937.

Crichton, E.M., Noël, M., Gies, E.A., Ross, P.S., 2017. A novel, density-independent and FTIR-compatible approach for the rapid extraction of microplastics from aquatic sediments. Anal. Methods 9, 1419–1428. https://doi.org/10.1039/c6ay02733d.

Curren, E., Yu, D.C.Y., Leong, S.C.Y., 2024. From the seafloor to the surface: a global review of gastropods as bioindicators of marine microplastics. Water Air Soil Pollut. 235, 45. https://doi.org/10.1007/s11270-023-06823-6.

Defossez, J.-M., Hawkins, A.J.S., 1997. Selective feeding in shellfish: size-dependent rejection of large particles within pseudofaeces from *Mytilus edulis*, *Ruditapes philippinarum* and *Tapes decussatus*. Mar. Biol. 129, 139–147. https://doi.org/10.1007/s002270050154.

Dehaut, A., Cassone, A.-L., Frère, L., Ludovic, H., Charlotte, H., Emmanuel, R., Gilles, R., Christophe, L., Philippe, S., Arnaud, H., Guillaume, D., Ika, P.-P., 2016. Microplastics in seafood: benchmark protocol for their extraction and characterization. Environ. Pollut. 215, 223–233. https://doi.org/10.1016/j.envpol.2016.05.018.

DeSmit, J.C., Anton, A., Martin, C., Rossbach, S., Bouma, T.J., Duarte, C.M., 2021. Habitat-forming species trap microplastics into coastal sediment sinks. Sci. Total Environ. 772, 145520 https://doi.org/10.1016/j.scitotenv.2021.145520.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for the determination of sugars and related substances. Anal. Chem. 28, 350–356.

Eriksen, M., Lebreton, L.C., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan, P.G., Reisser, J., 2014. Plastic pollution in the world's oceans: more than 5 trillion plastic pieces weighing over 250,000 tons afloat at sea. PloS One 9 (12), e111913. https://doi.org/10.1371/journal.pone.0111913.

Fang, C., Zheng, R., Zhang, Y., Hong, F., Mu, J., Chen, M., Song, P., Lin, L., Lin, H., Le, F., Bo, J., 2018. Microplastic contamination in benthic organisms from the Arctic and sub-Arctic regions. Chemosphere 209, 298–306. https://doi.org/10.1016/j.chemosphere.2018.06.101.

FAO, 2003. Food and Nutrition Paper 77 – Food Energy – Methods of Analysis and Conversion Factors. FAO — Food and Agriculture Organization of the United Nations, Rome, Italy. https://www.fao.org/3/y5022e/y5022e04.htm#bm4 (accessed 2023-04-01).

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3. https://doi.org/10.1016/j.envpol.2013.01.046.

Feng, Z., Zhang, T., Wang, J., Huang, W., Wang, R., Xu, J., Fu, G., Gao, G., 2020. Spatio-temporal features of microplastics pollution in macroalgae growing in an important mariculture area, China. Sci. Total Environ. 719, 137490 https://doi.org/10.1016/j.scitotenv.2020.137490.

Free, C.M., Jensen, O.P., Mason, S.A., Eriksen, M., Williamson, N.J., Boldgiv, B., 2014. High-levels of microplastic pollution in a large, remote, mountain lake. Mar. Pollut. Bull. 85, 156–163. https://doi.org/10.1016/j.marpolbul.2014.06.001.

Gasperi, J., Dris, R., Mirande-Bret, C., Mandin, C., Langlois, V., Tassin, B., 2015. First overview of microplastics in indoor and outdoor air. In: Proceedings of the 15th EuCheMS International Conference on Chemistry and the Environment, Leipzig, Germany, 20–25 September 2015, pp. 1–3.

Gutow, L., Bartl, K., Saborowski, R., Beermann, J., 2019. Gastropod pedal mucus retains microplastics and promotes the uptake of particles by marine periwinkles. Environ. Pollut. 246, 688–696.

Haegerbaeumer, A., Mueller, M.T., Fueser, H., Traunspurger, W., 2019. Impacts of micro- and nano-sized plastic particles on benthic invertebrates: a literature review and gap analysis. Front. Environ. Sci. 7 https://doi.org/10.3389/fenvs.2019.00017.

Hara, A., Radin, N.S., 1978. Lipid extraction of tissues with a low-toxicity solvent. Anal. Biochem. 90, 420–426. https://doi.org/10.1016/0003-2697(78)90046-5.

Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E., Svendsen, C., 2017. Microplastics in freshwater and terrestrial environments: evaluating the current understanding to identify the knowledge gaps and future research priorities. Sci. Total Environ. 586, 127–141. https://doi.org/10.1016/j.scitotenv.2017.01.190.

Ikuta, T., Tame, A., Takahashi, T., Nomaki, H., Nakajima, R., 2022. Microplastic particles are phagocytosed in gill cells of deep-sea and coastal mussels. Front. Mar. Sci. 9, 1034950. https://doi.org/10.3389/fmars.2022.1034950.

Imhof, H.K., Schmid, J., Niessner, R., Ivleva, N.P., Laforsch, C., 2012. A novel, highly efficient method for the separation and quantification of plastic particles in sediments of aquatic environments. Limnol. Oceanogr. Meth. 10, 524–537. https://doi.org/10.4319/lom.2012.10.524.

Janssens, L., Garcia-Vazquez, E., 2021. Dangerous microplastics in topshells and anemones along the north coast of Spain. Mar. Pollut. Bull. 173, 112945 https://doi.org/10.1016/j.marpolbul.2021.112945.

Kaiser, D., Kowalski, N., Waniek, J.J., 2017. Effects of biofouling on the sinking behavior of microplastics. Environ. Res. Lett. 12, 124003 https://doi.org/10.1088/1748-9326/aa8e8b.

Kane, I.A., Clare, M.A., 2019. Dispersion, accumulation, and the ultimate fate of microplastics in deep-marine environments: a review and future directions. Front. Earth Sci. 7 https://doi.org/10.3389/feart.2019.00080.

Koelmans, A.A., Besseling, E., Shim, W.J., 2015. Marine Anthropogenic Litter, 1st ed. Springer Cham, p. 447.

Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., Shi, H., 2018. Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. Sci. Total Environ. 611, 635–640. https://doi.org/10.1016/j.scitotenv.2017.08.053.

Li, J., Wang, Z., Rotchell, J.M., Shen, X., Li, Q., Zhu, J., 2021. Where are we? Towards an understanding of the selective accumulation of microplastics in mussels. Environ. Pollut. 286, 117543 https://doi.org/10.1016/j.envpol.2021.117543.

Liubartseva, S., Coppini, G., Lecci, R., Creti, S., 2016. Regional approach to modeling the transport of floating plastic debris in the Adriatic Sea. Mar. Pollut. Bull. 103, 115–127. https://doi.org/10.1016/j.marpolbul.2015.12.031.

Metherel, A.H., Taha, A.Y., Izadi, H., Stark, K.D., 2009. The application of ultrasound energy to increase lipid extraction throughput of solid matrix samples (flaxseed). Prostaglandins Leukot. Essent. Fatty Acids 81, 417–423. https://doi.org/10.1016/j.plefa.2009.07.003.

Munari, C., Infantini, V., Scoponi, M., Rastelli, E., Corinaldesi, C., Mistri, M., 2017. Microplastics in the sediments of Terra Nova Bay (Ross Sea, Antarctica). Mar. Pollut. Bull. 122, 161–165. https://doi.org/10.1016/j.marpolbul.2017.06.039.

NASEM — The National Academies of Sciences Engineering and Medicine, 2022. Reckoning with the U.S. Role in Global Ocean Plastic Waste. The National Academies Press, Washington, DC. https://doi.org/10.1007/978-3-319-16510-3.

Ng, K.L., Suk, K.F., Cheung, K.W., Shek, R.H.T., Chan, S.M.N., Tam, N.F.Y., Cheung, S.G., Fang, J.K.-H., Lo, H.S., 2022. Macroalgal morphology mediates microplastic accumulation on thallus and in sediments. Sci. Total Environ. 825, 153987 https://doi.org/10.1016/j.scitotenv.2022.153987.

Peng, X., Chen, M., Chen, S., Dasgupta, S., Xu, H., Ta, K., Du, M., Li, J., Guo, Z., Bai, S., 2018. Microplastics contaminate the deepest part of the world's ocean. Geochem. Perspect. Lett. 9, 1–5. https://doi.org/10.7185/geochemlet.1829.

Plastics Europe — Group Market Research, 2021. Plastics — The Facts 2021.

Porter, A., Lyons, B.P., Galloway, T.S., Lewis, C., 2018. Role of marine snows in microplastic fate and bioavailability. Environ. Sci. Technol. 52, 7111–7119. https://doi.org/10.1021/acs.est.8b01000.

Porter, A., Godbold, J.A., Lewis, C.N., Savage, G., Solan, M., Galloway, T.S., 2023. Microplastic burden in marine benthic invertebrates depends on species traits and feeding ecology within biogeographical provinces. Nat. Commun. 14 (1), 8023. https://doi.org/10.1038/s41467-023-43788-w.

Revel, M., Lagarde, F., Perrein-Ettajani, H., Mélanie, B., Farida, A., Rossana, S., Julien, R., Katherine, C., Priscilla, D., Bruno, C., Amélie, C., Catherine, M., 2019. Tissue-specific biomarker responses in the blue mussel *Mytilus* spp. exposed to a mixture of microplastics at environmentally relevant concentrations. Front. Environ. Sci. 7, 33. https://doi.org/10.3389/fenvs.2019.00033.

Ribeiro, F., Garcia, A.R., Pereira, B.P., Fonseca, M., Mestre, N.C., Fonseca, T.G., Ilharco, L.M., Bebianno, M.J., 2017. Microplastics effects in *Scrobicularia plana*. Mar. Pollut. Bull. 122, 379–391. https://doi.org/10.1016/j.marpolbul.2017.06.078.

Roland, G., Jenna, R.J., Lavender, L.K., 2022. Production, use, and fate of all plastics ever made. Sci. Adv. 3, e1700782 https://doi.org/10.1126/sciadv.1700782.

Saborowski, R., Paulischkis, E., Gutow, L., 2019. How to get rid of ingested microplastic fibers? A straightforward approach of the Atlantic ditch shrimp *Palaemon varians*. Environ. Pollut. 254, 113068.

Setälä, O., Norkko, J., Lehtiniemi, M., 2016. Feeding type affects microplastic ingestion in a coastal invertebrate community. Mar. Pollut. Bull. 102, 95–101. https://doi.org/10.1016/j.marpolbul.2015.11.053.

Sfriso, A.A., Tomio, Y., Rosso, B., Gambaro, A., Sfriso, A., Corami, F., Rastelli, E., Corinaldesi, C., Mistri, M., Munari, C., 2020. Microplastic accumulation in benthic invertebrates in Terra Nova Bay (Ross Sea, Antarctica). Environ. Int. 137, 105587 https://doi.org/10.1016/j.envint.2020.105587.

Sfriso, A.A., Tomio, Y., Juhmani, A.-S., Sfriso, A., Munari, C., Mistri, M., 2021. Macrophytes: a temporary sink for microplastics in transitional water systems. Water 13, 3032. https://doi.org/10.3390/w13213032.

Storelli, M.M., Storelli, A., Marcotrigiano, G.O., 2001. Heavy metals in the aquatic environment of the Southern Adriatic Sea, Italy: macroalgae, sediments and benthic species. Environ. Int. 26, 505–509. https://doi.org/10.1016/S0160-4120(01)00034-4.

Summers, S., Henry, T., Gutirrez, T., 2018. Agglomeration of nano- and microplastic particles in seawater by autochthonous and de novo-produced sources of exopolymeric substances. Mar. Pollut. Bull. 130, 258–267. https://doi.org/10.1016/j.marpolbul.2018.03.039.

Van Cauwenberghe, L., Vanreusel, A., Mees, J., Janssen, C.R., 2013. Microplastic pollution in deep-sea sediments. Environ. Pollut. 182, 495–499. https://doi.org/10.1016/j.envpol.2013.08.013.

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environ. Pollut. 199, 10–17. https://doi.org/10.1016/j.envpol.2015.01.008.

Viarengo, A., Canesi, L., 1991. Mussels as biological indicators of pollution. Aquaculture 94, 225–243. https://doi.org/10.1016/0044-8486(91)90120-V.

Von Hellfeld, R., Zarzuelo, M., Zaldibar, B., Cajaraville, M.P., Orbea, A., 2022. Accumulation, depuration, and biological effects of polystyrene microplastic spheres and adsorbed cadmium and benzo(a)pyrene on the mussel *Mytilus galloprovincialis*. Toxics 10, 18. https://doi.org/10.3390/toxics10010018.

Wang, W., Gao, H., Jin, S., Li, R., Na, G., 2019. The ecotoxicological effects of microplastics on aquatic food web, from primary producer to human: a review. Ecotoxicol. Environ. Saf. 173, 110–117.

Weis, S.J., 2020. Aquatic microplastic research — a critique and suggestions for the future. Water 12, 1475. https://doi.org/10.3390/w12051475.

Weis, S.J., Palmquist, K.H., 2018. Reality check: experimental studies on microplastics lack realism. Appl. Sci. 11, 8529. https://doi.org/10.3390/app11188529.

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1, 140317 https://doi.org/10.1098/rsos.140317.

Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol. 23, R1031–R1033. https://doi.org/10.1016/j.cub.2013.10.068.

Zaki, M.R.M., Zaid, S.H.M., Zainuddin, A.H., Aris, A.Z., 2021. Microplastic pollution in tropical estuary gastropods: abundance, distribution and potential sources of Klang River estuary, Malaysia. Mar. Pollut. Bull. 162, 111866 https://doi.org/10.1016/j.marpolbul.2020.111866.

Zhang, T., Wang, J., Liu, D., Sun, Z., Tang, R., Ma, X., Feng, Z., 2022. Loading of microplastics by two related macroalgae in a sea area where gold and green tides occur simultaneously. Sci. Total Environ. 814, 152809 https://doi.org/10.1016/j.scitotenv.2021.152809.

Zhang, Y., Gao, T., Kang, S., Sillanpää, M., 2019. Importance of atmospheric transport for microplastics deposited in remote areas. Environ. Pollut. 254, 112953 https://doi.org/10.1016/j.envpol.2019.07.121.