communications earth & environment

Article

https://doi.org/10.1038/s43247-024-01300-2

Microplastics and nanoplastics size distribution in farmed mussel tissues

Silvia Fraissinet[1] ✉, Giuseppe E. De Benedetto[2], Cosimino Malitesta[1], Rupert Holzinger[3] & Dusan Materić[3,4] ✉

Microplastics and nanoplastics are hazardous to ecosystems, wildlife, and through seafood, also for human health. Due to biological, chemical, and physical characteristics, nanoplastics can slip through cell membranes, being even more toxicologically important than microplastics. Thermal Desorption - Proton Transfer Reaction - Mass Spectrometry was used to analyze mussels from the Apulian region, Italy. All the analyzed organisms have plastics (values ranging from 10 to 187 ng of microplastics and nanoplastics per dry weight mg). The highest mass of plastics was detected in the size groups >2.2 μm (218 ng per dry weight mg) and 20–200 nm (187 ng per dry weight mg). Upscaling data we estimated that people in Europe could ingest more than 2 mg of nanoplastics per year through seafood consumption. The detected presence of nanoplastics in farmed mussels here presented contributes to establishing a baseline for monitoring these pollutants.

Plastic manufacturing started at the beginning of the 20th century and, after World War II became a cheap and useful alternative to more expensive products[1]. The earnings of plastics were evident, lightweight, strong, inexpensive, and durable material, moreover able to be transformed and modified to fit consumer demands[2].

In 2021 production of plastics touched 390 million tonnes worldwide. In Europe, 15% of the production is for packaging materials, and even if more the one-third is sent to recycling facilities, over 23% is still sent to landfills[3]. Improper waste management or accidental discharge from activities like construction, farming, aquaculture, and mariculture are the principal sources of plastic that end up in the marine environment[4]. Plastic can reach the sea directly as micro-or nano - range items intentionally produced for humans (primary microplastics (MPs); primary nanoplastics (NPs) or otherwise, as big pieces that, in the environment, undergo a decomposition process that can break down their bonds forming smaller sizes particles known as secondary MPs and eventually secondary NPs[4–8]. Up to date, MPs are being detected in organisms' tissues and different ecosystems worldwide[9–18] while NPs, even if their detection is still challenging, were found in every environmental compartment (air, water, soil)[19–21] even in the most remote places of the world[22,23], but still hardly in bivalves[24,25].

Investigating nanoplastics is challenging due to instrument limitations, their low concentrations in the environment, and their conjugation with interfering substances. Some authors suggested the combination of different analytical techniques to reach the final goal of quantification, identification, and characterization of NPs[26]. One of the most promising approaches is the Thermal Desorption - Proton Transfer Reaction - Mass Spectrometry (TD-PTR-MS) technique, this recently proposed method couples high sensitivity and high mass resolution providing data on plastics presence in the samples with a limit of detection (LOD) of <10 ng[27,28]. Usually, the preferred way to express data about the presence of MNPs detected in environmental samples is number of particles/g dry weight or wet weight or number of particles/individual[5,29] due to limit of quantification (LOQ) and limit of detection (LOD) constrains posed by instruments. Some authors proposed harmonization of size and shape information with mass data because particle size has a considerable influence on mass information: a 0.7–1 μg reduced to one single particle can correspond to a size between 50 and 200 μm[30].

Nevertheless, the interference of the surrounding matrix is still a concern while using this technique[24,28]. In particular, with biota samples removing the organic matrix sufficiently without damaging the polymers is an essential step. The optimization of a digestion protocol effective on the matrix preserving the plastic integrity is a crucial point to proceed in the quantification and identification with sophisticated and highly sensitive approaches such as TD-PTR-MS.

Microplastics and nanoplastics (MNPs) can negatively impact all biological organization levels from ecosystem to subcellular[31]. MNPs can

[1]Department of Biological and Environmental Science and Technology, University of Salento, Lecce, Italy. [2]Laboratory of Isotopic and Analytic Mass Spectrometry, Department of Cultural Heritage, University of Salento, Lecce, Italy. [3]Institute for Marine and Atmospheric Research Utrecht, Utrecht University, Princetonplein, Utrecht, the Netherlands. [4]Helmholtz Centre for Environmental Research – UFZ Department of Analytical Chemistry, Permoserstraße 15, 04318 Leipzig, Germany. ✉e-mail: silvia.fraissinet@unisalento.it; dusan.materic@ufz.de

also offer a suitable surface for the settlement of microorganisms or invasive species, enabling their migration to distinct locations[32]. Moreover, has been proved that decreasing the size increases the toxicity, thus NPs having a high surface-volume ratio can severely interact with toxic organic compounds[31]. MPs and NPs can be ingested by aquatic organisms and bioaccumulate along the trophic chain[33]. Their nanoscopic dimension allows their passage through biological membranes entering cells and tissues[34]. These conditions made nanoplastics an even more severe threat to all organisms than MPs, raising serious concerns about human exposition[35].

Bivalves are largely consumed by humans, and as sessile benthic filter feeders, organisms are highly affected by MPs pollution. These organisms are widely distributed all over the globe and can provide a useful and interesting assessment of NPs' presence in the surrounding environment acting as bioindicators[36]. Within the Bivalvia class, mussels are well-known studied animals, used as model organisms for ecotoxicological studies, as well as for sentinel organisms for marine pollution for several years[36,37] and were proposed as a target group to study the accumulation and toxicity of NPs as well[38]. Nevertheless, up to date, a little information about the presence of NPs in marine organisms has been reported[25] Most of the performed studies, are focused on the polystyrene NPs' toxic effects[38–40] along with feeding-controlled experiments.

To fulfill this lack of data, nanoplastics presence in organisms was investigated, and to provide a comprehensive size distribution of MNPs in mussels, the TD-PTR-MS technique was combined with a cascade filtration, and five mussels were analyzed. In the present work, the micro and nanoplastics size distribution in farmed mussel tissues is provided, quantifying plastics in the nanogram range and chemically identifying them. The measured values were then upscaled to estimate the nanoplastics uptake by Europeans in a year by mussels consumption.

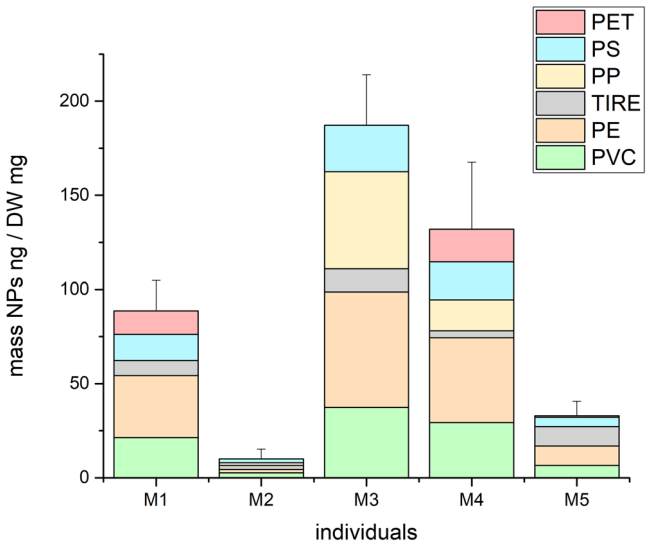

Fig. 1 | Nanoplastic presence (particles size 20–200 nm) in all the analyzed mussels. The panel shows the amount of nanoplastics detected and quantified in nanogram in all mussel tissues related to the mussels' dry weight. The error bars represent the global standard deviation.

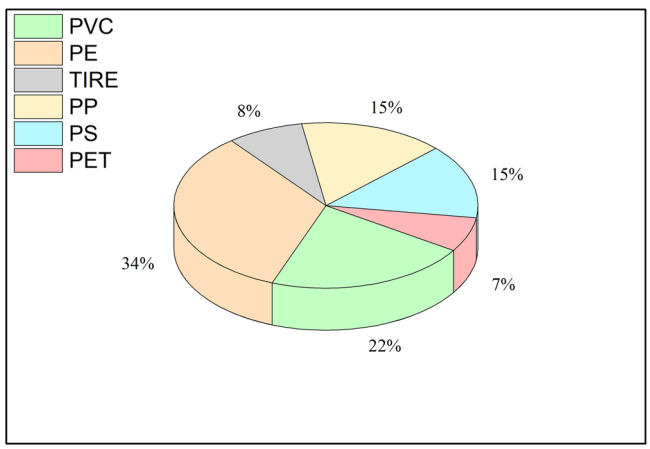

Fig. 2 | Abundance of polymers found in the analyesd tissues.Relative distribution of nanoplastics polymers in all the analyzed mussles (size 20–200 nm).

Results

Nanoplastics in all mussels tested

Mussels used in this study were bought in a local fish market in the Apulian region (Italy). The size ranges 20–200 nm were analyzed in all five mussels. These five specimens have similar sizes (details reported in Supplementary Table 2). The mussel M4 is the smallest one followed by M5 and M2. A high quantity of nanoplastics was registered in each organism (details in Supplementary Table S4). The highest amount of plastic was found in M3, with 187 ± 27 ng/mg DW, followed by M4 (132 ± 36 ng NPs/ mg DW) and M1 (89 ± 16 ng NPs/ mg DW). Plastics were chemically identified, and a high diversity of nanoplastics was registered in each mussel. PE, PVC, and PS were detected in all the analyzed individuals. PP was present in M2, M3, M4, while PET in M1, M4 and M5 (0.7 ng MNPs/mg DW) (Fig. 1). The most frequent and abundant polymer is PE, followed by PVC and PP. The less-represented polymers are PET (7%) and tire particles (8%) (Fig. 2).

Micro- and nanoplastics size distribution

The organism with the highest amount of MNPs was selected for a more complete study of the MNP size distribution, and the relevant results are summarized in Fig. 3. Some of the most common polymers found in the marine environment (Polystyrene PS; Polypropylene PP; Tire; Polyethylene PE; Polyvinyl-chloride PVC) were detected in each size class. Most of the plastics were detected in the highest size group >2.2 μm (218 ng/mg DW), which was the first step of the cascade filtration, and in the lowest size group 20–200 nm (187 ng/mg DW). The size group with less amount of plastic was 700–1400 nm (25 ng/mg DW).

The most frequent polymers are PP, PS, PVC and PE. PP and PS are present in all the size classes while PVC and PE were absent in few size ranges. However, when present, PE is the most abundant polymer detected,

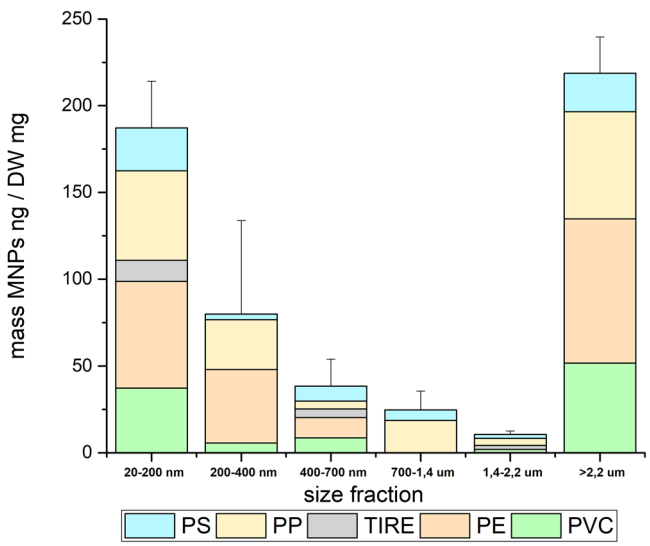

Fig. 3 | Micro- and nanoplastic size distribution in mussel (M3) tissues. Different colors represent different polymers (PS polystyrene, PP polypropylene; Tire; PE polyethylene, PVC Polyvinyl chloride). The error bars represent the standard deviation of triplicates.

always followed by PP, except for the 400–700 nm size class in which PVC and PS are equally present constituting the second most abundant polymers (22%). The lowest plastics diversity was measured in the 700–1400 nm size class, with the dominance of PP (75%) and the presence of PS (25%), PP was the dominant polymer (38%) also in the 1400–2200 nm size group. It is interesting to notice that PE is widely present in all the size classes except for the above ones, indicating that in the analyzed organisms were not present PE particles of those dimension and in absence of PE, PP is the most abundant polymer detected. In both, the smallest 20–200 nm and the biggest size class >2200 nm there are small differences between PE and PP contribution, representing the most abundant plastics found (Fig. 4).

Comparison between MPs and NPs

To compare MPs and NPs quantity in M3 the microplastics size threshold was set at the size class 700–1400 nm, considering NPs below 700 nm then the data were summed up. The mass of NPs is similar or higher (PE and Tire) to the mass of MPs for all the polymers. PE and Tire are more abundant in the nano range of our data (Supplementary Fig. 1).

Figure 5 shows the polymer contribution of both MPs and NPs, which is quite similar. It is not surprising that PE and PP are the most represented polymers, with a higher contribution of PE in the nanometer range (37%) followed by PP (27%), while in MPs the presence of PE and PP is analogous (about 30%). PE (including LDPE and HDPE) and PP are the two most produced plastics in 2021[3], and the most abundant and frequently found polymers in the Mediterranean Sea and consequently in organisms[25,41,42]. The third produced polymer in 2021 was PVC which is also abundant in both MPs and NPs but its contribution in higher in the micro range[3]. While the presence of PS percentage is almost the same in the micro and nanometric range, there is a big difference in the quantity of tire found in NPs and MPs, in the nano range this polymer type contributes by 5% to the total of polymer found while in micro range its percentage is 0.94%.

Discussion

Mussels are commonly used to assess toxicity and bioaccumulation of environmental pollutants[37,43,44]. Laboratory studies have been performed feeding mussels with MPs and NPs (especially PS) to investigate the possible physiological responses[39,45–49]. Some authors report the mussel's ability to excrete foreign particles through pseudofeces[50], but there is evidence that small particles can cross the gastro-intestinal barrier reaching the tissues (2–4 µm) while particles larger than 10–20 µm are not able to cross the barrier, remaining in the intestinal tract and being expelled subsequently[51]. NPs can cause different responses in mussels, but there is no information about their excretion. Wang et al. [42] evaluated the effects of 70 nm PS particles and reported their accumulation in the digestive tract of mussels[42]. Some authors, suggested different uptake routes in mussel hemocytes depending on size, with a higher internalization of smaller (50 nm) particles

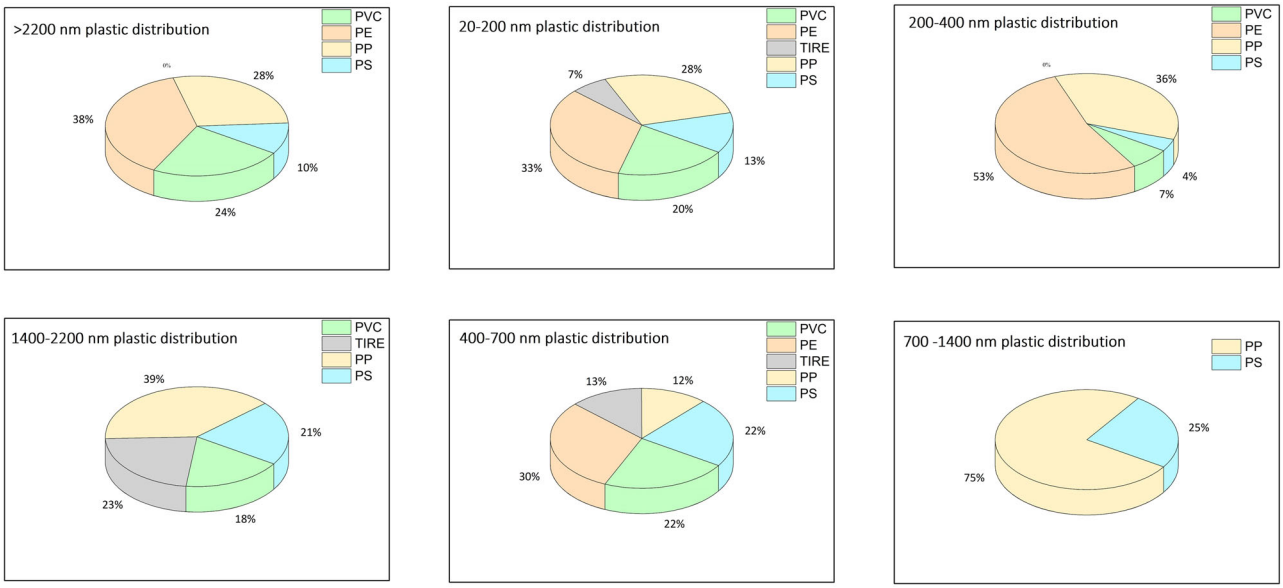

Fig. 4 | Plastic polymer distribution in each size fraction. Each panel corresponds to a different size fraction.

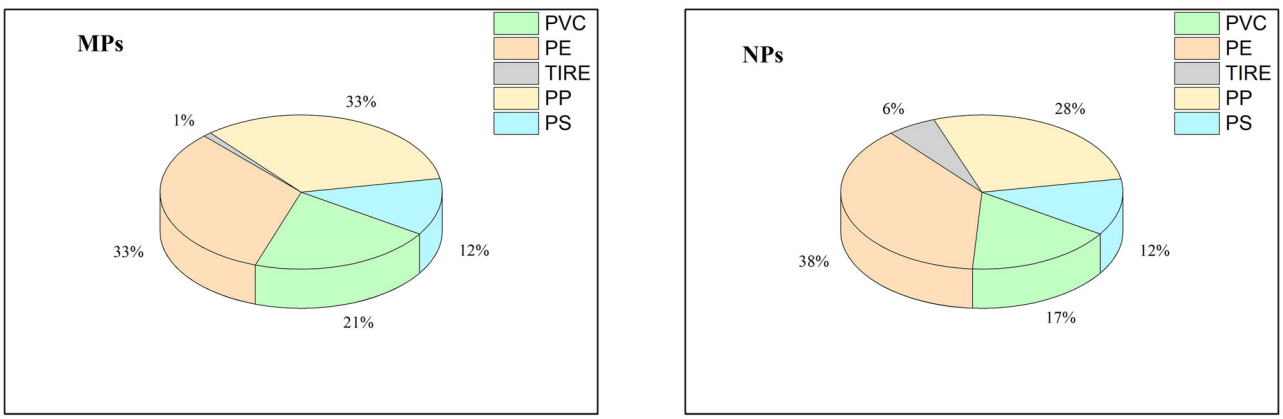

Fig. 5 | Microplastics and nanoplastics comparison. The two panels represent the plastic polymer percentage contribution in the micro and nano size range.

while the distribution in tissues was independent of size[47]. NPs at the laboratory concentration can cause severe stress to the bivalve immune system compromising also larval development and fertility[52,53].

Although several studies have been carried out to assess the toxicity of NPs particles, often together with MPs, in laboratory experiments, very little information is available on their presence in organisms in environmental conditions. This lack of data makes it impossible to build a baseline of NPs concentration in the marine environment Indeed, the NPs in the marine environment are smaller compared to the quantities tested in lab experiments[20,23], also our data confirm the actual amount of MNPs found in mussels is lower than the particles' concentration used in laboratory experiments. We measured distinct levels of NPs contamination in the five tested mussels, ranging from tens to hundreds of ng NPs/mg DW. Our data may reflect the variability of the real contamination; however, more measurements are needed before robust conclusions can be made.

Recently the concentration of micro and nanoplastics in deployed and non-deployed oysters has been assessed[25]. Reporting data as μg/g ww (wet weight), the authors detected most of the plastic in the deployed oysters (respect to the non-deployed ones) showing the highest values in the 1–22 μm fraction. The median polymers concentration in the 1–22 μm fraction were 1611 μg/g ww (PE), 25 μg/g ww (PS), 121 μg/g ww (PVC), 169 μg/g ww PP, and 21 μg/g ww PMMA (poly(methyl methacrylate)). While in the fraction below 1 μm the polymers median concentrations were 3 μg/g ww (PS), 5206 μg/g ww (PE), 82 μg/g ww (PVC) and 2749 μg/g ww (PP). Despite the differences in methodology applied and the size ranges selected, our data are comparable to these findings both in terms of concentration and polymer composition. Considering that both oysters and mussels are widely consumed seafood the reported data rises concerns.

Mussels are not able to discriminate between natural particles or artificial particulate contaminants, ingesting both regardless of their chemical composition[54]. However, it is possible that mussels in natural conditions can manage the MNPs uptake by modulating their filtration when exposed to increasing external stressors in the marine environment[55]. Recently the mass content of MPs in historical series of mussel samples from the North and the Baltic seas has been assessd[56]. Summing values of different pooled organisms reported masses between 2−12 μg/ g DW indicating the most represented polymers as PET and PVC. We measured much higher content of plastic in our samples and a different polymer composition. This difference can be explained by local pollution which can vary several orders of magnitude at regional scale[56], for example the measured microplastics polymer composition and abundance along the North Sea is different within the North Sea itself, and from the Mediterranean Sea, known as one of the most plastic polluted sites on the world[57–59]. Mussels feeding strategy is filtration, and the plastic content within their tissues is directly influenced by the surrounding environment, indeed the abundance of MPs in seawater and the abundance of MPs in bivalves have a reported positive correlation[42,56,60].

Bivalve mollusks are highly exposed to plastics during farming. In aquaculture and mariculture, most floating structures are made of plastics (often Expanded polystyrene (EPS) or plastic buoys (PVC) and are stabilized by ropes and lines (usually made of PE and PP). In mussel farming, plastics are ubiquitous, especially PP and PE: hybrid ropes built with natural materials and mixed with synthetic filaments (PE and PP) are used to attract more larvae during the recruitment period, as well as anti-predator nets to protect shellfish are mostly made of PP (followed by PE)[61].

Mussels are one of the most widely consumed foods worldwide and are of serious concern as a potential source of NPs for humans. Although there are still some aspects that need to be investigated in detail, our results show a concerning situation that needs to be addressed. To ensure a safe seafood product for consumers, mussels must be grown in clean water and undergo a depuration process according to HACCP recommendations[62]. To date, micro- and nanoplastics have not been included in the list of potential human hazards associated with mussel consumption. The depuration time is several days, but our results underline that this process needs to be regulated differently or improved to "clean" these organisms from MNPs before they reach the consumer. There are several works dealing with MPs in mussels from the fish market[63–67]. Recently, has been reported that, on average, a consumer on the Asturian coast could ingest up to 109 MPs particles per serving of mussels[67], while an American adult male consumed approx. 142 MPs particles daily, mainly from seafood[68]. In UK consumers eat approx. 70 MPs particles in a 100 g portion of processed mussels[44]. These animals are farmed to fit human demands being the most farmed product in aquaculture facilities after finfishes, in 2020 the global production touched 1108.3 Kilotons LWE (live weight). In Europe, mussels are the second main farmed product consumed, principally supplied by Spain (which provides 80% of EU production). Recently (2018–2019), the consumption of mussels slightly exceeded 1.20 kg LWE per capita in Europe[69]. Upscaling our data we estimated the presence of approx. 2 tons of NPs (DW) (2114 Kg DW) in the total world mussel production and, that a European consumes slightly more than 2 mg of NPs (DW) (2.2×10^{-6} Kg) per year. In absence of a consistent dataset regarding NPs presence in farmed bivalves it is impossible to understand possible contamination pathways through the trophic chain, and knowing the actual extend of the problem.

Comparing micro and nanoplastics in our sample there are no differences in polymer composition. Even if these results are referred to just one mussel, they are still quite interesting. We found PP, PE, and PVC as the most abundant and frequent polymers in both micro- and nano-range, and a possible correlation between a source of contaminants and their presence in the organisms tested. As also observed by other authors these three polymers are the dominating polymers also in the analysis of oysters 'tissues both in the micro- and nanorange, with a slight prevalence at the microscale, same of out data except for PE, that in our case is more abundant at the nanoscale[25]. Generally, bivalves contain comparable amount of small and large microplastics, but the smallest plastic size found in bivalve organisms is 3.6 μm[70,71]. Despite the global attention posed to these contaminants their presence in the environment is progressively increased, suggesting a gap of knowledge and consequently in pragmatical actions, making more difficult the proposal of mitigation measures especially for nanoplastics[72].

To conclude, there is an urgent need to establish viable approaches that can provide an assessment of the actual MNPs contamination of the marine environment, especially since MNPs have already been found in human blood, proving that we are in close contact with these pollutants by breathing, drinking and eating[73,74]. Bivalves have a high tolerance to environmental stressors and have already been shown to ingest and accumulate NPs in laboratory experiments. Mussels are sessile powerful filter-feeders, widespread and abundantly farmed in diverse aquatic environments, investigate MNPs' presence in these animals can help to better understand eventually transferring to humans as for other contaminants or MPs[75–78]. Our findings show important levels of contamination reflecting the actual concern for NPs' interaction with fauna and perhaps humans and pose a new benchmark for comprehension of the problem extension. This is critical information needed to assess the ecological risks of NPs and the potential impacts on marine wildlife. Further investigations are needed to deepen its potential ecological implications. An interdisciplinary approach needs to be pursued not only to assess and monitor the nanoplastic presence but also to find reliable solutions to mitigate this problem.

Materials and methods

Chemicals and materials

KOH pellets, H_2O_2 (30%) solution, and NH_4OH (25%) solution were purchased from Sigma-Aldrich (Milano, Italy). All the solutions were prepared with ultrapure water. The digestion solution was freshly prepared every day. Glass fiber filters, Quartz fiber filters and, Anodisc filters were supplied by Sigma-Aldrich. PS standard spheres of 1 μm in diameter (PS-ST-1.0, Microparticles GmbH, Berlin, Germany).

Sample preparation

Several organisms were bought in a local market and stored at −20 °C. Five specimens of similar sizes were (average shell length 5.56 cm ± 0.52) selected and digested. Information about the mussel's length and dry weight (DW)

are provided in Supplementary Table 1. One organism within five was randomly chosen and all filters corresponding to the 6 different particle's size fractions were analyzed covering the entire size range from >2.2 µm to 20–200 nm. Then for all the digested mussels, the lower size fraction (20–200 nm) was analyzed providing information about the presence of nanoplastics in seafood from the fish market.

Tissues were defrosted and the whole animal was dried in an oven at 60 °C for 48 h, then grounded to a powder and weighed, the powder was placed in glass bottles.

Samples were treated slightly changing the digestion protocol optimized in a previous work[65]. A digestion solution prepared with KOH 2.5% and H_2O_2 5% was added to the bottles that were then placed in a water bath at 60 °C for 3 h. After 3 h bottles were left cooling and the solution was centrifugated for 5 min at 3000 rpm. The supernatant was neutralized with 10% citric acid added dropwise, while the precipitate was treated using an acidic solution with 5 mL of 25% of formic acid and 10% of sodium citrate solution. After 30 min at room temperature, also the acid solution was neutralized using 2.5 mL of 25% NH_4OH. Both supernatant and precipitate were filtered on the same membranes using a water jet (vacuum) pump (KNF- LABOPORT® N 86 KT.18).

Six steps of filtration were performed using decreasing pore size filters starting from 2.2 µm (Quartz filter) −1.4 µm (Glass fiber filter) −0.7 µm (Glass fiber filter) −0.4 µm (Glass fiber filter) −0.2 µm (Anodisc filter); 0.02 µm (Anodisc filter). Each bottle was rinsed three times with prefiltered ultra-pure water and the rinsing water was filtered on the same membrane. After each filtration, the filter was washed with prefiltered MilliQ water, and placed in a glass petri dish. Note that cascade filtration could induce such artifact that some smaller particles could stack in the bigger size filter matrix.

Each filter was analyzed in triplicates. For pore sizes from 2.2 µm to 700 nm replicas were obtained punching the filters using a metal tool having 1 mm perfect circular pieces, while for the 400 nm pore size filters a 2 mm puncher was used. While Anodisc filters (with 200 nm and 20 nm pore size) were cracked using a metal needle, each piece was photographed and stored in a glass vial. From the collected pictures, each Anodisc filter piece area was measured using ImageJ software[79].

Knowing the dry weight of the sample and the filter's area is possible to estimate the amount of sample on each filter piece for each size fraction (Supplementary Table 2). The same approach was used to calculate the mass content of plastic in all five mussels at 20 nm, values reported in Supplementary Table 3. Then, we used the mass content of plastics to upscaling the data to the global production and the global consumption of mussels, trying to estimate a possible contamination at worldwide scale. The former data are expressed in live weight which means the weight of the animal alive, while our data are referred to dry weight of the animal without the shell. Knowing by literature that in approx. 1 kg of mussels the 36.5% of weight is represented by shell[80] and the mussel water content is up to 80%[81], the live weight was converted in dry weight, then this value was used to estimate the NPs content in produced and consumed mussels.

TD-PTR-MS analysis

The analysis was performed using TD-PTR-MS 1000, IONICON Analytik, Austria, where the setting was: ramping from 35 to 360 °C at 40 degrees/min, E/N ~ 105 Td.

Data were processed using PTRwid.13 custom-made software[82] The TD-PTR-MS signal was integrated over 7 min starting when the TD temperature reached 200 °C, to include in the analysis the thermal degradation products of each polymer (which are different at different temperatures).

Each plastic polymer shows a specific ion signal at certain temperatures. TD-PTR-MS can monitor these signals at high resolution quantifying each organic ion produced by the thermal desorption process (an example of mass spectrum and thermogram of detected polymers types related to a mussel sample are provided in Supplementary Figs. 2 and 3). This approach has been exploited to study environmental pollutants in different environmental matrices[22,23,83–88]. TD-PTR-MS measurements and fingerpinting analysis were performed as described in previous works[22,23,28,89]. For the tire standard, we sampled around 1 cm^3 of tire material from three commonly used car tire brands in the Netherlands. We made small particles using the ultrafine saw, a process we adopted from our previous work[28]. We loaded a small amount of tire powder with a surgical needle and generated the library mass spectra. The average mass spectrum had been added to the library, available in our previous work[89](see the data availability section) and used for fingerprinting. Note that our reported values are not compensated for ionization losses, so they are semiquantitative and represent the minimum (low threshold) of the plastics present in the samples. Effectively, based on PS standard, we can assume the realistic values to be at least 3-fold higher. Even if this technique seems to be one of the most promising, there are some limitations: 1) sample matrices interference: the sensitivity of the analyzer, which can detect and measure even small impurities; 2) the lack of information about the morphology and dimension of particles, and 3) the presence of humidity in the sample, which can cause the formation of clusters that can clog the system[86,90]. Knowing these issues, we tried to minimize the organic matrix on the filters, treating mussels using a digestion protocol with an efficiency of 98%. The size fractions obtained information about the size, even if the morphology of the particles remains unknown. To avoid cluster formation, all the filter pieces after the punching were placed in an oven to dry at 50 °C for 24 h.

QC/QA

Working in a laboratory is almost impossible to avoid plastic materials, so we tried to minimize the contamination as much as possible. During the distinct phases of this work, particular attention to preserving samples was paid: 1) Sample preparation: The procedures were performed under a laminar flow cabinet, which was carefully cleaned before any activity using paper and prefiltered water. Operative staff wore cotton coats (or clothes) and nitrile gloves. Glass materials were baked before use. All the containers were covered with aluminum foil to reduce exposure to air. Along with the sample preparation, a procedural blank (referred to in the supplement as "biota blank BB") was performed; 2) TD-PTR-MS analyses were carried out in triplicates, both for samples and blanks. Three blanks per experiment were included: a) procedural blanks to assess the contamination during the laboratory operations; b) material blanks to assess the possible contamination due to the filter material; c) system blanks to assess the contamination of vials and instruments; 3) Data analysis and fingerprinting: all the mass spectra were corrected for blank signal, and masses below the 3σ detection limit were not considered for the analysis. The fingerprinting was then performed by four algorithms using a high number of ions (40) with $m/z > 120$, following[28].

Procedural blanks (biota blank BB) were analyzed along with mussel samples. In blank samples, all the size ranges tested showed plastic presence (fingerprinting output). Nonetheless, often the plastic present in the blanks showed a fingerprinting weak match (e.g. in the size range 1400–2200 nm) or their mass were quite lower than the quantity registered in mussels (e.g. in the size range 20–200 nm). Just for the size range 400–700 nm due to technical problems during the analysis blanks were not available.

Moreover, to assess the possibility of false negative responses, on the 700–1400 nm size fraction, a spiking experiment was performed using 1 µm PS standard spheres. A water solution (concentration 20 ng/µl) was prepared to have a final load of 100 ng on samples. Three sample and blank replicas were spiked with 100 ng of PS. In mussels' samples, PS was detected in both spiked and not spiked filters, due to the PS contamination of organisms tissues as we showed in the results section, while the blank filters were completely devoid of PS. However, in this test experiment a positive increment of PS quantity in spiked filters (recovery/ionization efficiency 30%) was detected (Supplementary Fig. 4). These levels are well in the range of tests previously reported – e.g. 15%[28]. The reason for such a low "recovery" figure is mainly instrumental[23]. In short, the ionization (proton transfer reaction PTR) happens on volatilized organic molecules, but some thermolysis s products (such as CO_2, CO, CH_4) do not get ionized, so as the neutral fragments. These plastic vapor parts are not visible in our system. In addition, attempting plastic identification is very challenging, especially when plastics are embedded in a matrix with high organic content like organisms.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

All data needed to evaluate the conclusions in the paper including raw files (measures related to nanoplastics) and processing stages of all the data analysis are available via: https://doi.org/10.5281/zenodo.10629512. All the data measures related to microplastic size fraction are available via https://doi.org/10.5281/zenodo.10634968. The threshold between micro and nano fractions is set at 700 nm as described in the manuscript.

Code availability

Fingerprint codes are already published and available via https://doi.org/10.24416/UU01-HKNCGC.

Received: 25 May 2023; Accepted: 4 March 2024;
Published online: 15 March 2024

References

1. Thompson, R. C., Moore, C. J., Vom Saal, F. S. & Swan, S. H. Plastics, the environment and human health: current consensus and future trends. *Phil. Trans. R. Soc. B* **364**, 2153–2166 (2009).
2. Napper, I. E. & Thompson, R. C. Plastic Debris in the Marine Environment: History and Future Challenges. *Global Challenges* **4**, 1900081 (2020).
3. Plastics – the fast Facts 2023 • Plastics Europe. Plastics Europe, https://plasticseurope.org/knowledge-hub/plastics-the-fast-facts-2023/ (2023).
4. Peng, L. et al. Micro- and nano-plastics in marine environment: Source, distribution and threats — A review. *Sci. Total Environ.* **698**, 134254 (2020).
5. Vitali, C., Peters, R. J. B., Janssen, H.-G., Nielen, M. W. F. & Ruggeri, F. S. Microplastics and nanoplastics in food, water, and beverages, part II. Methods. *TrAC Trends Anal. Chem.* **157**, 116819 (2022).
6. Thompson, R. C. Microplastics in the Marine Environment: Sources, Consequences and Solutions. In *Marine Anthropogenic Litter* (eds. Bergmann, M., Gutow, L. & Klages, M.) 185–200 (Springer International Publishing, Cham, 2015). https://doi.org/10.1007/978-3-319-16510-3_7.
7. González-Pleiter, M. et al. Secondary nanoplastics released from a biodegradable microplastic severely impact freshwater environments. *Environ. Sci. Nano* **6**, 1382–1392 (2019).
8. Gaylarde, C. C., Baptista Neto, J. A. & Da Fonseca, E. M. Nanoplastics in aquatic systems - are they more hazardous than microplastics? *Environ. Pollut.* **272**, 115950 (2021).
9. Akdogan, Z. & Guven, B. Microplastics in the environment: A critical review of current understanding and identification of future research needs. *Environ. Pollut.* **254**, 113011 (2019).
10. Piazzolla, D. et al. Preliminary Investigation of Microlitter Pollution in Low-Energy Hydrodynamic Basins Using Sabella spallanzanii (Polychaeta: Sabellidae) Tubes. *Bull. Environ. Contam. Toxicol.* **104**, 345–350 (2020).
11. Kumar, R. et al. Impacts of Plastic Pollution on Ecosystem Services, Sustainable Development Goals, and Need to Focus on Circular Economy and Policy Interventions. *Sustainability* **13**, 9963 (2021).
12. Koutnik, V. S. et al. Distribution of microplastics in soil and freshwater environments: Global analysis and framework for transport modeling. *Environ. Pollut.* **274**, 116552 (2021).
13. Kane, I. A. et al. Seafloor microplastic hotspots controlled by deep-sea circulation. *Science* **368**, 1140–1145 (2020).
14. Gasperi, J. et al. Microplastics in air: Are we breathing it in? *Curr. Opin. Environ. Sci. Health* **1**, 1–5 (2018).
15. Girard, E. B. et al. Sponges as bioindicators for microparticulate pollutants? *Environ. Pollut.* **268**, 115851 (2021).
16. Zhang, F., Man, Y. B., Mo, W. Y., Man, K. Y. & Wong, M. H. Direct and indirect effects of microplastics on bivalves, with a focus on edible species: A mini-review. *Crit. Rev. Environ. Sci. Technol.* **50**, 2109–2143 (2020).
17. Moore, R. C. et al. Microplastics in beluga whale (Delphinapterus leucas) prey: An exploratory assessment of trophic transfer in the Beaufort Sea. *Sci. Total Environ.* **806**, 150201 (2022).
18. Panebianco, A., Nalbone, L., Giarratana, F. & Ziino, G. First discoveries of microplastics in terrestrial snails. *Food Control* **106**, 106722 (2019).
19. Xu, S., Ma, J., Ji, R., Pan, K. & Miao, A.-J. Microplastics in aquatic environments: Occurrence, accumulation, and biological effects. *Sci. Total Environ.* **703**, 134699 (2020).
20. Ter Halle, A. et al. To what extent are microplastics from the open ocean weathered? *Environ. Pollut.* **227**, 167–174 (2017).
21. Wahl, A. et al. Nanoplastic occurrence in a soil amended with plastic debris. *Chemosphere* **262**, 127784 (2021).
22. Materić, D., Ludewig, E., Brunner, D., Röckmann, T. & Holzinger, R. Nanoplastics transport to the remote, high-altitude Alps. *Environ. Pollut.* **288**, 117697 (2021).
23. Materić, D. et al. Nanoplastics measurements in Northern and Southern polar ice. *Environ. Res.* **208**, 112741 (2022).
24. Zhou, A. et al. Microplastics and their potential effects on the aquaculture systems: a critical review. *Rev. Aquaculture* **13**, 719–733 (2021).
25. Ribeiro, F. et al. Out of sight but not out of mind: Size fractionation of plastics bioaccumulated by field deployed oysters. *J. Hazardous Materi. Lett.* **2**, 100021 (2021).
26. Zhang, W., Wang, Q. & Chen, H. Challenges in characterization of nanoplastics in the environment. *Front. Environ. Sci. Eng.* **16**, 11 (2022).
27. Ivleva, N. P. Chemical Analysis of Microplastics and Nanoplastics: Challenges, Advanced Methods, and Perspectives. *Chem. Rev.* **121**, 11886–11936 (2021).
28. Materić, D. et al. Micro- and Nanoplastics in Alpine Snow: A New Method for Chemical Identification and (Semi)Quantification in the Nanogram Range. *Environ. Sci. Technol.* **54**, 2353–2359 (2020).
29. Dellisanti, W. et al. A short review on the recent method development for extraction and identification of microplastics in mussels and fish, two major groups of seafood. *Marine Pollut. Bull.* **186**, 114221 (2023).
30. Primpke, S., Fischer, M., Lorenz, C., Gerdts, G. & Scholz-Böttcher, B. M. Comparison of pyrolysis gas chromatography/mass spectrometry and hyperspectral FTIR imaging spectroscopy for the analysis of microplastics. *Anal. Bioanal. Chem.* **412**, 8283–8298 (2020).
31. Galloway, T. S., Cole, M. & Lewis, C. Interactions of microplastic debris throughout the marine ecosystem. *Nat. Ecol. Evol.* **1**, 1–8 (2017).
32. Zettler, E. R., Mincer, T. J. & Amaral-Zettler, L. A. Life in the "Plastisphere": Microbial Communities on Plastic Marine Debris. *Environ. Sci. Technol.* **47**, 7137–7146 (2013).
33. Sana, S. S., Dogiparthi, L. K., Gangadhar, L., Chakravorty, A. & Abhishek, N. Effects of microplastics and nanoplastics on marine environment and human health. *Environ. Sci. Pollut. Res.* **27**, 44743–44756 (2020).
34. Hollóczki, O. & Gehrke, S. Can Nanoplastics Alter Cell Membranes? *ChemPhysChem* **21**, 9–12 (2020).
35. Yee, M. S.-L. et al. Impact of Microplastics and Nanoplastics on Human Health. *Nanomaterials* **11**, 496 (2021).
36. Gonçalves, J. M. & Bebianno, M. J. Nanoplastics impact on marine biota: A review. *Environ. Pollut.* **273**, 116426 (2021).
37. Goldberg, E. D. The Mussel Watch concept. *Environ. Monit. Assess.* **7**, 91–103 (1986).
38. Sendra, M., Sparaventi, E., Novoa, B. & Figueras, A. An overview of the internalization and effects of microplastics and nanoplastics as

pollutants of emerging concern in bivalves. *Sci. Total Environ.* **753**, 142024 (2021).
39. Gonçalves, J. M., Sousa, V. S., Teixeira, M. R. & Bebianno, M. J. Chronic toxicity of polystyrene nanoparticles in the marine mussel Mytilus galloprovincialis. *Chemosphere* **287**, 132356 (2022).
40. Merzel, R. L. et al. Uptake and Retention of Nanoplastics in Quagga Mussels. *Global Challenges* **4**, 1800104 (2020).
41. Sharma, S., Sharma, V. & Chatterjee, S. Microplastics in the Mediterranean Sea: Sources, Pollution Intensity, Sea Health, and Regulatory Policies. *Front. Mar. Sci.* **8**, 634934 (2021).
42. Wang, D. et al. Quantitative and qualitative determination of microplastics in oyster, seawater and sediment from the coastal areas in Zhuhai, China. *Marine Pollut. Bull.* **164**, 112000 (2021).
43. Franzellitti, S., Canesi, L., Auguste, M., Wathsala, R. H. G. R. & Fabbri, E. Microplastic exposure and effects in aquatic organisms: A physiological perspective. *Environ. Toxicol. Pharmacol.* **68**, 37–51 (2019).
44. Li, J., Liu, H. & Paul Chen, J. Microplastics in freshwater systems: A review on occurrence, environmental effects, and methods for microplastics detection. *Water Res.* **137**, 362–374 (2018).
45. Cappello, T. et al. Time-dependent metabolic disorders induced by short-term exposure to polystyrene microplastics in the Mediterranean mussel Mytilus galloprovincialis. *Ecotoxicol. Environm. Saf.* **209**, 111780 (2021).
46. Green, D. S., Colgan, T. J., Thompson, R. C. & Carolan, J. C. Exposure to microplastics reduces attachment strength and alters the haemolymph proteome of blue mussels (Mytilus edulis). *Environ. Pollut.* **246**, 423–434 (2019).
47. Sendra, M. et al. Nanoplastics: From tissue accumulation to cell translocation into Mytilus galloprovincialis hemocytes. resilience of immune cells exposed to nanoplastics and nanoplastics plus Vibrio splendidus combination. *J. Hazardous Mater.* **388**, 121788 (2020).
48. Rist, S., Baun, A., Almeda, R. & Hartmann, N. B. Ingestion and effects of micro- and nanoplastics in blue mussel (Mytilus edulis) larvae. *Marine Pollut. Bull.* **140**, 423–430 (2019).
49. Auguste, M. et al. Impact of nanoplastics on hemolymph immune parameters and microbiota composition in Mytilus galloprovincialis. *Marine Environ. Res.* **159**, 105017 (2020).
50. Wagner, M. et al. Microplastics in freshwater ecosystems: what we know and what we need to know. *Environ. Sci. Eur.* **26**, 12 (2014).
51. Ribeiro, F., O'Brien, J. W., Galloway, T. & Thomas, K. V. Accumulation and fate of nano- and micro-plastics and associated contaminants in organisms. *TrAC Trends Anal. Chem.* **111**, 139–147 (2019).
52. González-Fernández, C. et al. Cellular responses of Pacific oyster (Crassostrea gigas) gametes exposed in vitro to polystyrene nanoparticles. *Chemosphere* **208**, 764–772 (2018).
53. Cole, M. et al. Microplastics, microfibres and nanoplastics cause variable sub-lethal responses in mussels (Mytilus spp.). *Marine Poll. Bull.* **160**, 111552 (2020).
54. Brehm, J. et al. In-depth characterization revealed polymer type and chemical content specific effects of microplastic on Dreissena bugensis. *J. Hazardous Mater.* **437**, 129351 (2022).
55. Capolupo, M., Valbonesi, P. & Fabbri, E. A Comparative Assessment of the Chronic Effects of Micro- and Nano-Plastics on the Physiology of the Mediterranean Mussel Mytilus galloprovincialis. *Nanomaterials* **11**, 649 (2021).
56. Halbach, M. et al. 30 years trends of microplastic pollution: Mass-quantitative analysis of archived mussel samples from the North and Baltic Seas. *Sci. Total Environ.* **826**, 154179 (2022).
57. Suaria, G. et al. The Mediterranean Plastic Soup: synthetic polymers in Mediterranean surface waters. *Sci. Rep.* **6**, 37551 (2016).
58. Alessi, E., Di Carlo, G., Campogianni, S., Tangerine, B. & Pietrobelli, E. *Out of the plastic trap: saving the Mediterranean from plastic pollution*. 1–28 (WWF Mediterranean Marine Initiative, 2018).
59. Lorenz, C. et al. Spatial distribution of microplastics in sediments and surface waters of the southern North Sea. *Environ. Pollut.* **252**, 1719–1729 (2019).
60. Ward, J. E. et al. Selective Ingestion and Egestion of Plastic Particles by the Blue Mussel (Mytilus edulis) and Eastern Oyster (Crassostrea virginica): Implications for Using Bivalves as Bioindicators of Microplastic Pollution. *Environ. Sci. Technol.* **53**, 8776–8784 (2019).
61. Lusher, A., Hollman, P. C. H. & Mendoza-Hill, J. *Microplastics in Fisheries and Aquaculture: Status of Knowledge on Their Occurrence and Implications for Aquatic Organisms and Food Safety*. (Food and Agriculture Organization of the United Nations, Rome, 2017).
62. FAO, W. Code of practice for fish and fishery products. *WHO*, Geneva, Switzerland 1–268 (2020).
63. Cho, Y., Shim, W. J., Jang, M., Han, G. M. & Hong, S. H. Abundance and characteristics of microplastics in market bivalves from South Korea. *Environ. Pollut.* **245**, 1107–1116 (2019).
64. Nalbone, L., Cincotta, F., Giarratana, F., Ziino, G. & Panebianco, A. Microplastics in fresh and processed mussels sampled from fish shops and large retail chains in Italy. *Food Control* **125**, 108003 (2021).
65. Fraissinet, S., Pennetta, A., Rossi, S., De Benedetto, G. E. & Malitesta, C. Optimization of a new multi-reagent procedure for quantitative mussel digestion in microplastic analysis. *Marine Pollut. Bull.* **173**, 112931 (2021).
66. Bom, F. C. & Sá, F. Are bivalves a source of microplastics for humans? A case study in the Brazilian markets. *Marine Pollut. Bull.* **181**, 113823 (2022).
67. Masiá, P., Ardura, A. & Garcia-Vazquez, E. Microplastics in seafood: Relative input of Mytilus galloprovincialis and table salt in mussel dishes. *Food Res. Int.* **153**, 110973 (2022).
68. Cox, K. D. et al. Human Consumption of Microplastics. *Environ. Sci. Technol.* **53**, 7068–7074 (2019).
69. European Commission. Directorate General for Maritime Affairs and Fisheries. *The EU Fish Market: 2021 Edition*. (Publications Office, LU, 2021).
70. Vital, S. A. et al. Do microplastic contaminated seafood consumption pose a potential risk to human health? *Marine Pollut. Bull.* **171**, 112769 (2021).
71. Ding, J. et al. Microplastics in global bivalve mollusks: A call for protocol standardization. *J. Hazardous Mater.* **438**, 129490 (2022).
72. Sarkar, B. et al. Challenges and opportunities in sustainable management of microplastics and nanoplastics in the environment. *Environ. Res.* **207**, 112179 (2022).
73. Leslie, H. A. et al. Discovery and quantification of plastic particle pollution in human blood. *Environ. Int.* **163**, 107199 (2022).
74. Sangkham, S. et al. A review on microplastics and nanoplastics in the environment: Their occurrence, exposure routes, toxic studies, and potential effects on human health. *Marine Pollut. Bull.* **181**, 113832 (2022).
75. Jović, M., Onjia, A. & Stanković, S. Toxic metal health risk by mussel consumption. *Environ. Chem. Lett.* **10**, 69–77 (2012).
76. Carbery, M., O'Connor, W. & Palanisami, T. Trophic transfer of microplastics and mixed contaminants in the marine food web and implications for human health. *Environ. Int.* **115**, 400–409 (2018).
77. Smaal, A. C. European mussel cultivation along the Atlantic coast: production status, problems and perspectives. In *Sustainable Increase of Marine Harvesting: Fundamental Mechanisms and New Concepts* (eds. Vadstein, O. & Olsen, Y.) 89–98 (Springer Netherlands, Dordrecht, 2002). https://doi.org/10.1007/978-94-017-3190-4_8.
78. Beyer, J. et al. Blue mussels (Mytilus edulis spp.) as sentinel organisms in coastal pollution monitoring: A review. *Marine Environ. Res.* **130**, 338–365 (2017).
79. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* **9**, 671–675 (2012).
80. Moore, D., Heilweck, M. & Petros, P. Saving the planet with appropriate biotechnology: 2. Cultivate shellfish to remediate the atmosphere. *Mexican J. Biotechnol.* **6**, 31–91 (2021).

81. Prato, E. et al. Proximate, fatty acids and metals in edible marine bivalves from Italian market: Beneficial and risk for consumers health. *Sci. Total Environ.* **648**, 153–163 (2019).
82. Holzinger, R. PTRwid: A new widget tool for processing PTR-TOF-MS data. *Atmos. Measur. Tech.* **8**, 3903–3922 (2015).
83. Oyama, B. S. et al. Chemical characterization of organic particulate matter from on-road traffic in São Paulo, Brazil. *Atmos Chem Phys* **16**, 14397–14408 (2016).
84. Salvador, C. M. et al. Measurements of submicron organonitrate particles: Implications for the impacts of NOx pollution in a subtropical forest. *Atmos. Res.* **245**, 105080 (2020).
85. Allen, S. et al. An early comparison of nano to microplastic mass in a remote catchment's atmospheric deposition. *J. Hazardous Mater. Adv.* **7**, 100104 (2022).
86. Velimirovic, M., Tirez, K., Voorspoels, S. & Vanhaecke, F. Recent developments in mass spectrometry for the characterization of micro- and nanoscale plastic debris in the environment. *Anal. Bioanal. Chem.* **413**, 7–15 (2021).
87. Kirchsteiger, B., Materić, D., Happenhofer, F., Holzinger, R. & Kasper-Giebl, A. Fine micro- and nanoplastics particles (PM2.5) in urban air and their relation to polycyclic aromatic hydrocarbons. *Atmos. Environ.* **301**, 119670 (2023).
88. Hale, R. C., Seeley, M. E., King, A. E. & Yu, L. H. Analytical Chemistry of Plastic Debris: Sampling, Methods, and Instrumentation. In *Microplastic in the Environment: Pattern and Process* (ed. Bank, M. S.) 17–67 (Springer International Publishing, Cham, 2022).
89. Materić, D., Holzinger, R. & Niemann, H. Nanoplastics and ultrafine microplastic in the Dutch Wadden Sea – The hidden plastics debris? *Sci. Total Environ.* **846**, 157371 (2022b).
90. Ellis, A. M. & Mayhew, C. A. *Proton Transfer Reaction Mass Spectrometry: Principles and Applications*. (John Wiley & Sons, 2013).

Acknowledgements

This study is part of the project "I-Plastics: Dispersion and impacts of micro- and nano-plastics in the tropical and temperate oceans: from regional land-ocean interface to open ocean". The authors would like to acknowledge the funding from the JPI Oceans International Consortium. Dutch Research Council (Nederlandse Organisatie voor Wetenschappelijk Onderzoek – NWO) project "Nanoplastics: hormone- mimicking and inflammatory responses?" (grant number OCENW.XS2.078), and project "Size distribution of nanoplastics in indoor, urban and rural air" (OCENW.XS21.2.042).

Author contributions

S.F. prepared samples, performed TD-PTR-MS experiments, analyzed the raw data, produced figures and graphs, wrote the manuscript, and incorporated edits and comments all other authors had during internal revisions. G.E.D.B and C.M. proposed the initial concept, provided comments and edits, and revised the manuscript. R.H. contributed to the conceptualization and planning of the work, provided comments and revisions to the manuscript. D.M. improved the initial concept, supervised the TD-PTR-MS analysis, performed the fingerprinting, supervised the data analysis, and contributed to the manuscript writing, editing, and revising.

Funding

Open Access funding enabled and organized by Projekt DEAL.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s43247-024-01300-2.

Correspondence and requests for materials should be addressed to Silvia Fraissinet or Dusan Materić.

Peer review information *Communications Earth & Environment* thanks Elvis Genbo Xu and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editors: Ilka Peeken, Clare Davis and Aliénor Lavergne. A peer review file is available.