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Uptake and absorption of fluoranthene from spiked microplastics into the digestive gland tissues of blue mussels, *Mytilus edulis* L.

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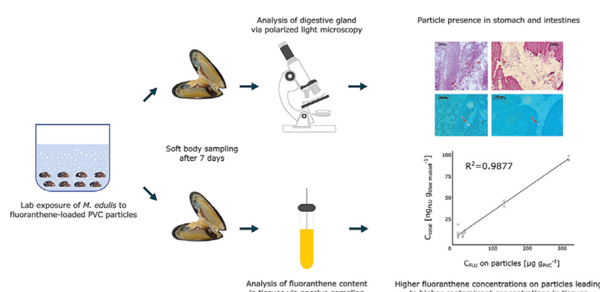
HIGHLIGHTS

- Internal fluoranthene concentration of mussel tissue determined by equilibrium passive sampling.
- Proof of contaminant transfer from plastics to organismal tissues.
- Microplastics identified as source of fluoranthene contamination.

GRAPHICAL ABSTRACT



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ABSTRACT

The present work intended to investigate the fate of contaminant-loaded microplastics if ingested by benthic filter feeder *Mytilus edulis* under laboratory conditions. In the course of a 7-day experiment the mussels were exposed to PVC microplastics in a size range ≤40 µm, in doses of 2000 particles L^{-1} (11.56 mg L^{-1}). Particles were either virgin or loaded with one of four different nominal concentrations of the polycyclic aromatic hydrocarbon (PAH) fluoranthene (500, 125, 31.25 and 7.8125 µg g^{-1}). Verification of fluoranthene concentrations on the particles provided evidence of the high absorptive capacity of PVC for this PAH, indicating that comparable particles may serve as considerable accumulation sites for high concentrations of hydrophobic contaminants in the aquatic environment. Analysis of digestive gland tissues via polarised light microscopy revealed the occurrence of particles and particle aggregates within stomach and intestines of all mussels treated with microplastics, thus making the xenobiotic bioavailable. Results of contaminant analysis in mussel tissues via equilibrium sampling point to a considerable capability of microplastics for the accumulation of hydrophobic contaminants from the environment and their potential to act as vehicles for the transport of theses contaminants into organismal tissues.
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1. Introduction

The ubiquity and abundance of plastics litter has become a problem of global extend. Considered as just a matter of aesthetic disturbance in the beginning, plastics pollution is now considered to pose a serious threat to organisms and ecosystems throughout

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the planet, eventually also affecting human health (Vethaak and Leslie, 2016; Wright and Kelly, 2017). A considerable amount of the litter finds its way into the oceans and onto the shores. Up to 95% of the waste on shorelines, the sea surface and sea floor are plastics (Galgani et al., 2015). By now, plastics are littering all spheres of the marine environment. They have been found in all oceans and marginal seas, from the surface via the water column down to the sea floor, they form extensive garbage patches where wind and currents accumulate and capture the floating litter (Moore et al., 2001). Plastics debris pollutes sediments in harbours as well as on coastlines and beaches (Claessens et al., 2011; Vianello et al., 2013; Nor and Obbard, 2014), and has even been identified in regions far away from any direct human influence, like remote islands (Barnes, 2005), the deep sea (Bergmann et al., 2017) and the polar regions (Barnes et al., 2010; Waller et al., 2017; Obbard, 2018).

At the end of their life cycle the great advantage of plastics – their durability – turns into their greatest drawback. Plastics are not biodegradable and thus may persist for decades or even centuries. In fact, via various environmental influence factors, such as photo-oxidation caused by UV radiation or mechanical abrasion by wind and wave action, larger items become brittle and undergo continuous fragmentation (Andrady, 2003). So, in the course of time the particle scale shifts from macro to micro.

The fact that plastics litter may be severely hazardous for marine organisms has been well documented within the scope of numerous studies. Due to different size classes, all levels of the food web are affected – from marine mammals (de Stephanis et al., 2013; Bravo Rebolledo et al., 2013), sea turtles and birds (Avery-Gomm et al., 2013; Provencher et al., 2014) to fish and invertebrates (Rummel et al., 2016; Abbasi et al., 2018), from large whales to microscopic planktonic organisms. Adverse effects result from entanglement in lost ropes or fishing gear and ingestion of debris and small particles, which are either intentionally taken up as a consequence of mistaking them for food or, as in the case of filter feeders, by chance together with their actual food. Another way of unintentional uptake is the feeding on prey which before has ingested (micro-) plastics.

However, plastics reveal another dimension of potential risk: a cocktail of chemicals associated with them. These are, on the one hand, added during the production process, as flame retardants, plasticisers, pigments and fillers (Rochman, 2015). On the other hand, due to its physical and chemical properties, the debris acts as a magnet to all kinds of hydrophobic contaminants and persistent organic pollutants, like e.g. polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) or organochlorines (e.g. DDT), and also metals present in the surrounding water or sediment. Mato et al. (2001) were among the first to detect the accumulation of PCBs on polypropylene pellets from the surrounding seawater on Japanese coasts. Antunes et al. (2013) sampled resin pellets from different Portuguese beaches and found PAHs, PCBs and DDT sorbed to them, concentrations ranging from 53 to 44 800 ng g^{-1} plastics for PAHs, from 2 to 233 ng g^{-1} for PCBs and from 0.41 to 42 ng g^{-1} for DDT. The chemical load attached to floating plastics debris may be transported over long distances to pristine areas. Biofouling or a density higher than that of seawater lead to contaminant transport to the sediment.

The relative contaminant load increases with decreasing size and thus a higher surface–volume ratio. Sorption capacity and rate may vary with respect to polymer as well as contaminant type (Teuten et al., 2007; Rochman, 2015). However, the life cycle of plastics in the marine environment is a very dynamic one with constant changes in size, surface and material characteristics, location and availability to marine organisms. In this context, it has been suggested that the longer a plastics particle remains in the marine environment the higher the contaminant concentrations it is likely to accumulate (Engler, 2012; Antunes et al., 2013). Teuten et al. (2009) tested PVC, PP and PE for their sorptive capacities under simulated UV radiation, indicating an increase compared to virgin pellets. Different studies have shown that concentrations on particles are orders of magnitude higher than those in the surrounding seawater (Ogata et al., 2009). Moreover, plastics appear to sorb higher amounts of contaminants in comparison with sediments. This could be demonstrated by Teuten et al. (2007) in an experiment with the PAH phenanthrene, different polymer types and sediment, in which the sorption to plastics was up to three orders of magnitude higher than to sediment.

Taking into account their vast number and high accumulation potential on the seafloor, contaminant-loaded microplastics may pose a serious risk especially to benthic invertebrates living at the sediment-water interface or within the sediment, like shellfish, crustaceans or polychaetes, many of which are important ecosystem engineers and keystone species.

A considerable number of experimental studies have examined the ingestion of microplastics by different invertebrate species under laboratory conditions in order to understand mechanisms of uptake, accumulation and depuration as well as the fate and effects of ingested particles within the organisms (Lusher, 2015). They provided proof that different organisms, like the lugworm *Arenicola marina* (Browne et al., 2013; van Cauwenberghe et al., 2015), mussels *Perna viridis* (Rist et al., 2016) and *M. edulis* (von Moos et al., 2012), the oyster *Crassostrea gigas* (Sussarellu et al., 2016) or various species of sea cucumbers (Graham and Thompson, 2009) actually take up particles of the tested polymer types nylon, PVC, HDPE and PS with diverse effects on the physiological, histological, cellular and humoral level.

von Moos et al. (2012) could show an uptake of HDPE particles < 80 μm into the stomach and digestive gland of *M. edulis* only after 3 h of exposure and an accumulation in the lysosomal system leading to inflammatory responses and histological changes like the formation of granulocytomas or sub-cellular organelle impairments such as lysosomal membrane destabilisation. Comparable results concerning regions of particle accumulation and biological responses were gained by Wegner et al. (2012), Wright et al. (2013a), Avio et al. (2015), Paul-Pont et al. (2016) and Pittura et al. (2018).

Based on the findings of high plastics loads in the marine environment, their capacity to accumulate contaminants in concentrations exceeding those in the surrounding water and sediment by orders of magnitude and substantial proof of particle uptake by marine organisms a growing concern has arisen as to the potential of microplastics to play a crucial role in the transfer of hazardous substances to these organisms. Once ingested, contaminants may desorb from the plastics into digestive fluid, tissues and cells (Teuten et al., 2009). Browne et al. (2013) could demonstrate that pollutants and additives from ingested PVC would desorb to the tissues of *Arenicola marina* in concentrations high enough to cause negative biological effects.

Recent studies with *Mytilus galloprovincialis* by Avio et al. (2015) and Pittura et al. (2018) point to a microplastics-mediated transfer of sorbed PAHs into mussel tissues, thus further strengthening the concept of a vector function. Responses on the molecular and cellular level point to toxicological challenges which may be only slight during short-term exposure but gain greater significance in the long run. Furthermore, results of the 28-day exposure by Pittura et al. (2018) point to a shift from mechanical to chemical toxicity in the course of time.

By an exposure experiment with the blue mussel *M. edulis* the present study aimed at answering the following questions: (1) Do the mussels as benthic filter feeders ingest microplastics particles in a size range comparable to that of their food spectrum? (2) If a

potential uptake can be verified, where can particles be detected? and (3) Is an uptake of the pollutant sorbed to the particles detectable in organismal tissues, and does it correlate with the pollutant concentration on the particle?

PVC was chosen as model plastics as it is among the most frequently produced polymer types and also due to its higher density in comparison with seawater, which makes it sink to the bottom and thus available to benthic organisms. The PAH used in the experiment was the fourring component fluoranthene, which is one of the most widespread PAHs in the marine environment and one of the 16 PAHs the US EPA has listed as priority pollutants. In 2019 it was added to the candidate list of substances of very high concern (SVHCs) by the European Chemicals Agency (ECHA) due to the carcinogenic, toxic to reproduction, persistent, bioaccumulative and toxic (PBT) and very persistent and very bioaccumulative (vPvB) properties (European Chemical Agency, 2019). It has already been detected on plastics fragments in the field (Rios et al., 2007).

2. Material and methods

2.1. Collection and pre-experimental maintenance of test organisms

Individuals of the species *M. edulis* were collected on December 6, 2018 at (55°01′3.2″N 8°26′4.30″E) on the island of Sylt at a water temperature of 7.4 °C and a salinity of 31.0. After being transferred to the lab at Bremerhaven they were placed in two 20-Litre aquaria filled with pre-cooled natural seawater (temperature: 8 °C, salinity: 31) and kept in a temperature controlled lab at 8 °C. Seawater originates from a reference site south of Helgoland (54°08′48″N/07°53′00″E). Via membrane pumps air was added to the aquaria in order to guarantee an optimal oxygen supply.

Acclimatisation of the mussels took place over a period of 6 days. Each day half of the water volume in the aquaria was replaced by artificial seawater, consisting of deionised tap water to which the appropriate amount of aquarium salt (Coral Pro Premium) was added to gain a salinity of 31 and which was cooled down to a temperature of 8 °C. Salinity and temperature were checked with a salinometer. Artificial seawater was stored in 75-Litre drums in the temperature controlled lab. The drums were covered in order to prevent any airborne particle contamination. The light regime followed a day-to-night-rhythm of 8 h–16 h. During acclimatisation time the mussels were not fed.

Two days prior to the experiment start the mussels were removed from the aquaria and thoroughly cleaned from any epibiota with a knife. After cleaning they were put back into the aquaria.

2.2. Preparation and loading of PVC particles

As a model plastics for this experiment PVC was chosen. Its density of 1.38 g cm^{-3} (Kuchling, 2014) makes it negatively buoyant and thus available for benthic organisms like *M. edulis*. The PVC particles (Werth Metall, Erfurt, Germany; purity: >99.7%) were kindly provided by the Fraunhofer Institute for Chemical Technology (ICT) in Pfinztal, Germany. The original particles in a size spectrum <63 μm were sieved in an analytical screening machine (Control AS; Retsch GmbH, Haan, Germany) in order to gain the required sizes spectra. Grain size of PVC particles was controlled by laser diffraction spectroscopy (LS 13 320 Multi-Wavelength Particle Size Analyzer, Beckman Coulter). A small amount of PVC particles was directly introduced in the Universal Liquid Module (Beckman Coulter) until an obscuration between 8 and 12% was reached. Since the particles agglomerate in water sonication was used during analyse (Sonication Control Unit, Beckman Coulter). Three subsamples were analysed according to Fraunhofer Theory. The results showed that over 99% of the particles ranged between 0.5 and 40 μm in diameter. To remove unwanted additives and contaminations, the particles were cleaned at Hamburg University of Applied Sciences (HAW). For that, between 4 and 5 g of the particles were transferred into glass vials. 60 mL of n-hexane were added to each vial, and the vials were put into an ultrasonic bath for 15 min. After the particles had settled at the bottom of the vials, the hexane was carefully decanted and fresh hexane was added for another cleansing step. The whole process was repeated three times.

The cleaned particles were subsequently loaded with four different concentrations of fluoranthene (Sigma-Aldrich, Darmstadt, Germany), which were nominally set at 500, 125, 31.25 and 7.8125 μg g^{-1} PVC. Thus, a rather broad spectrum of contamination was to be depicted. The fluoranthene powder was dissolved in hexane and the stock solution of 500 μg mL^{-1} was diluted by further addition of hexane to gain the required concentrations 125, 31.25 and 7.8125 μg mL^{-1}. The loading solutions were added to the particles in the glass vials. After an exposure time of 48 h the hexane was removed with the help of a nitrogen stream. The particles in one vial were left unloaded. From each vial an aliquot was taken to measure the actual fluoranthene concentration on the particles via high performance liquid chromatography with fluorescence detection (HPLC-FD) (High-Performance Liquid Chromatograph Chromaster system with 5310 column oven, 5160 pump and 5260 autosampler, equipped with a 5450 fluorescence detector; Hitachi High-Technologies Europe GmbH, Krefeld, Germany).

2.3. Determination of actual concentrations on the particles

Aliquots of the loaded PVC particles were transferred into ASE vials, 10 mL of methanol (99.9%) was added and the vials were placed in an ultrasonic bath for 45 min. This process was repeated three times. Subsequently 1 mL was extracted from each concentration sample, transferred into GC vials and fluoranthene contents measured via HPLC-FD.

2.4. Calculation of daily particle doses and weighing of particles

To ensure that the treatment aquaria contained a sufficient quantity of microplastics for an up-take by the mussels, a daily dose of 2000 particles mL^{-1} was chosen. For the calculation of the corresponding weight an ideal spherical shape of the particles and an average diameter of 20 μm were assumed. The equations used are listed below:

Thus, 0.1156 g of PVC particles were calculated as daily dose per aquarium per day. This makes 11.56 mg of microplastics per litre.

$$V_{particle} = \frac{4}{3}\pi \times r_{particle}^3 \tag{1}$$

$$m_{particle} = V_{particle} \times \rho_{particle} \tag{2}$$

$$m_{dose} = c \times m_{particle} \times V_{aquarium} \tag{3}$$

where: V = volume [cm^3], r = radius [cm], $m_{particle}$ = particle mass [g], ρ = particle density [g cm^{-3}], m_{dose} = dose per aquarium per day [g], c = concentration [particles mL^{-1}].

2.5. Test of initial contaminant status of mussels

In order to determine the initial status of the mussels, one day prior to the start of the experiment, six mussels were prepared for

contaminant analysis. After the determination of total weight and shell length, each mussel was carefully opened, drained and net weight was measured. Subsequently, the soft body was completely extracted from the shell, weighed and packed into a piece of previously annealed aluminium foil. The package was shock-frozen in liquid nitrogen and stored at −25 °C. Subsequently empty shells were weighed.

2.6. Experimental setup

The experimental setup encompassed a total of 18 15-L glass aquaria filled with a volume of 10 L each for the 6 different treatments: control (without either microplastics or fluoranthene), clean microplastics, microplastics loaded with fluoranthene at four different nominal concentrations (Fig. 1). Each treatment was carried out in triplicates.

The aquaria were placed in a temperature controlled lab at 8 °C and filled with artificial seawater with a salinity of 31 and a temperature of 8 °C. During the acclimatisation period the water was kept in the covered 75 L drums in the temperature controlled lab to acquire the necessary temperature. In order to avoid any cross contamination, the control aquaria were placed at some distance from the ones with microplastics. Each aquarium contained eight mussels. All aquaria were covered with acrylic glass plates and aerated using membrane pumps. Sufficient oxygen supply as well as water circulation was guaranteed by two Teflon tubes per aquarium. The tube positioned at the bottom was additionally weighted with a stainless steel screw nut, the second tube was positioned in the middle of the water column. The light-dark-regime was 10:14 h. During the 7-day experiment the test organisms were not fed.

Water exchange and re-dosing of the microplastics particles took place every 24 h. Prior to the start of water exchange a small amount of salinated MilliQ water (salinity: 31) was added to the vials with the daily particle doses. In order to homogenise the mixture and to dissolve lumps, the vials, covered with a piece of annealed aluminium foil, were dipped into an ultrasonic bath for five times.

Two sets of aquaria were used for the experiment so that after each water exchange the used set could be cleaned from any remains of microplastics and organic particles. Cleaning was carried out with a metal sponge and a detergent. After cleaning, the aquaria were rinsed with deionised tap water.

In the course of the water exchange the mussels were removed from the aquaria, their shells cleaned from plastics remains with salinated MilliQ water. After that they were returned to a clean aquarium. This procedure was carried out for the treatment mussels as well as for the control mussels. Having finished the water exchange for all three replicates of a treatment, the microplastics were added to the aquaria. Vials were thoroughly rinsed with salinated MilliQ water to get all particles out. Finally, the aquaria were covered again with the acrylic glass plates.

2.7. Mussel behaviour

Subsequently, the behaviour of mussels was observed. Therefore, aquaria were checked for spontaneous spawning activities and the opening status of the mussels was documented. Following the behavioural checks, the water in the aquaria was stirred with a metal spoon to re-suspend the microplastics particles, which meanwhile had settled on the bottom. Behavioural checking and subsequent stirring was carried out every hour, in total for eight times per day, and the opening status of the test organisms was checked every 2 h, in total for five times per day.

2.8. Post experiment sampling

After seven days, all mussels were removed from their aquaria and kept in the temperature controlled lab until dissection. The shell lengths were measured using a vernier caliper. Individuals were carefully opened with a knife by cutting the posterior adductor muscle. Thereafter mussels were rinsed with clean artificial seawater, before there were briefly drained to gain the total wet weight (ww) of each mussel. Subsequently, the mussel was completely opened. Tissue of the digestive gland was removed with scissors and tweezers, placed in cryo vials and immediately shock-

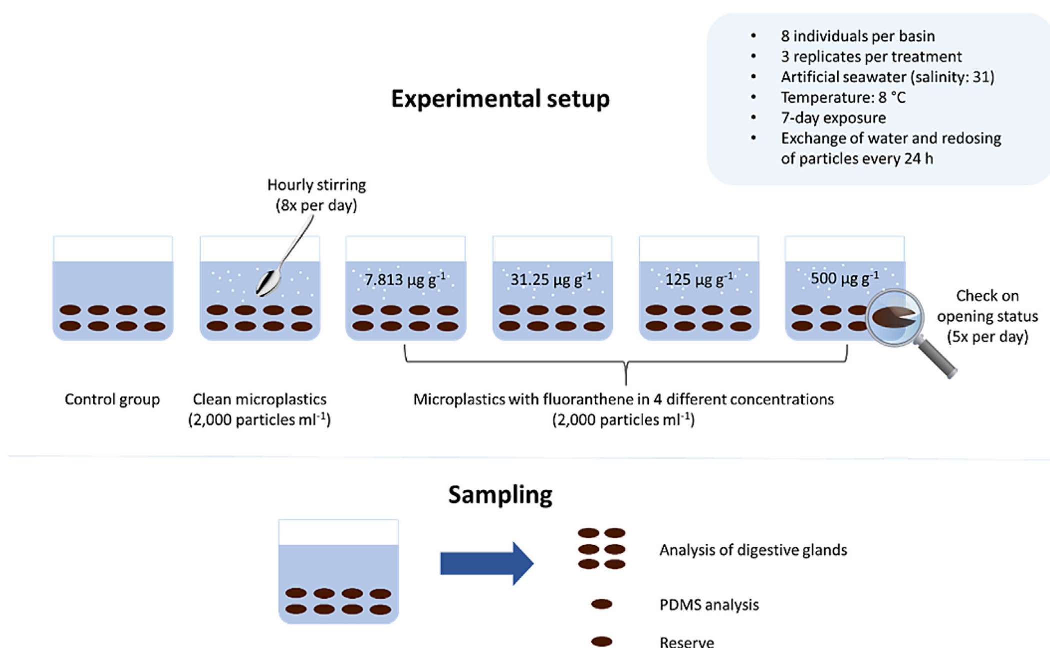

Fig. 1. Schematic representation of experimental setup and sampling of the mussels.

frozen in liquid nitrogen. The frozen samples were stored at −80 °C.

After dissection, the remaining tissue was removed and shell weight was determined. In total 6 individuals (18 individuals per treatment) from each aquarium were sampled for CI calculation and visual inspection for the presence of particles (digestive gland). Further, one individual from each aquarium (3 per treatment) was sampled for contaminant analysis (whole soft bodies). One individual was left as spare in case of potentially occurring lethality. Data of shell length, total wet weight (ww) and shell weight were used to calculate the condition index (CI) of mussels, using the following formula:

$$\frac{weight\ softbody\ [g]}{weight\ shell\ [g]} \times 100 \tag{4}$$

CI of mussel was used to compare mussel from the different treatments regarding their physiological status.

2.9. Tissue preparation and staining

Frozen soft body samples were fixed on pre-cooled aluminium chucks for cryostat-sectioning. Tissue sections of 10 mm were obtained using a cryotome (CryoSTAR NX70; Microm International GmbH, Neuss, Germany) with chamber/knife temperature of 23/25 °C. Sections were stored at −80 °C until staining.

A hematoxylin and eosin staining of the digestive gland was performed. The nuclei and basophilic cytoplasm were stained blue whereas muscles, connective tissue and acidophilic cytoplasm were stained pink. The slides were fixed in Baker's formalin for 15 min and rinsed in distilled water (Milli-Q, Millipore). Afterwards followed the incubation in Gills hematoxylin for 15 s. The slides were rinsed for 20 min under running deionised water and rinsed once more in distilled water for 10 s, before incubation in eosin-phloxine-solution for 30 s. Afterwards the samples were cleaned in 80% ethanol. The slides were dried at room temperature, provided with one drop of Euparal, capped and dried for three days.

2.10. Tissue analysis

The H&E stained digestive gland cryo sections were screened for the presence of micro plastic particles by using a microscope (Zeiss, Axioskop) at 100× magnification and polarised light.

2.11. Contaminant analysis in mussel tissue

Analysis of fluoranthene concentration in the mussel tissues was carried out via passive equilibrium sampling at the HAW Hamburg. This method has detection limits in the lower nanogram-per-litre range was successfully applied in the course of several studies for the determination of PAHs in sediments and pore water as well as in biota (Jahnke et al., 2009; Witt et al., 2010). Applying the technique of solid phase micro-extraction (SPME) (Witt et al., 2009), glass fibres with a 10 μm polydimethylsiloxane (PDMS) coating (GF 10; Fiberguide Industries, Stirling, USA) served as sampling phases for the analyte in the tissue matrix. The analyte diffused into the PDMS until an equilibrium between the two phases was reached, which follows a specific distribution coefficient.

Each frozen soft body was homogenised under the addition of some MilliQ water in an Ultra-Turrax Tube Drive (Ika-Werke GmbH, Staufen, Germany) for ca. 1 Min. During that process, the samples were regularly put on ice in order to keep them cooled. 10 mL of the homogenate were transferred into chromacol vials and closed with a PTFE-lined septum cap. The septum was pierced with a syringe cannula, via which two to three PDMS fibres, the length of which had been previously measured, were inserted into each vial.

The vials were placed in an end-over-end shaker and shaken for 24 h at room temperature until equilibrium was reached. Subsequently the fibres were removed, cleaned from particles with a lint-free cloth and some MilliQ water and stored in annealed aluminium foil at −20 °C until further processing. Measurements of contaminant load were carried out via thermodesorption in the GC/MS (gas chromatography/mass spectrometry; Agilent Technologies, Waldbronn, Germany).

In the programmed temperature vaporising injector (PTV) each fibre was heated up for the contaminant to detach and change into the gaseous phase. Fluoranthene was detected and analysed via mass spectrometry. Subsequently, the concentration on the fibres was calculated using the following equations:

$$\beta = \frac{response}{a}\ [pg] \tag{5}$$

$response$ = area of peaks measured GC/MS
a = gradient of the calibration line

The gradient of the calibration line was determined via a three-point calibration (10 pg μL^{-1}, 80 pg μL^{-1} and 160 pg μL^{-1}) using an external standard.

$$c_{fibre} = \frac{\beta}{length_{fibre}}\ [pg\ cm^{-1}] \tag{6}$$

c_{fibre} = fluoranthene concentration on PDMS fibre

$$c_{PDMS} = \frac{c_{fibre}}{V_{fibre}}\ [pg\ \mu L^{-1}] \tag{7}$$

V_{fibre} = fibre volume = 0.0877 $\mu L\ cm^{-1}$

A distribution coefficient $K_{PDMS/mussel}$ of 7.85 for fluoranthene has been determined in the course of a previous work (Vogel, 2015):

$$K_{PDMS/mussel} = \frac{c_{PDMS}}{c_{mussel}}\ [kg\ L^{-1}] \tag{8}$$

Using this distribution coefficient, the fluoranthene concentration in the mussel sample could be calculated as follows:

$$c_{mussel} = \frac{c_{PDMS}}{K_{PDMS/mussel}}\ [ng\ g^{-1}] \tag{9}$$

2.12. Statistical analysis

Data for calculating the CI were evaluated with a Kruskal-Wallis ANOVA followed by a Mann-Whitney pairwise test and a Dunn's post hoc test using the software PAST (version 3.22). The level of significance was set at $p < 0.05$. All other table, graphics and calculations were created using Microsoft Excel 2010.

3. Results

3.1. Loading of PVC particles

With the applied method of a 48-h incubation in a hexane-fluoranthene-solution, the PVC particles could be successfully loaded with different concentrations of the PAH. However, an exact loading according to the nominal and the calculated nominal

Table 1
Provides an overview of nominal and calculated nominal fluoranthene concentrations on the PVC.

Vial	Treatment	Nominal FLU concentration [μg g^{-1} PVC]	Calculated nominal FLU concentration [μg g^{-1} PVC]
1	MP	0	0
2	FLU 4	500	502.36
3	FLU 3	125	124.98
4	FLU 2	31.25	31.16
5	FLU 1	7.8125	7.91

Table 2
Calculated nominal fluoranthene concentrations and actual concentrations as measured via HPLC-FD.

Calculated nominal FLU concentration [μg g^{-1} PVC]	Actual FLU concentration [μg g^{-1} PVC]	Deviation from calculated nominal concentration [%]
0	n.n.	–
502.36	318.75	37
124.98	133.02	6
31.16	18.51	41
7.91	13.77	74

concentrations, respectively, was not achieved (Table 1) (Table 2).

3.2. Mussel survival, spontaneous spawning, shell opening and formation of filamentous excretions

None of the mussels died in the course of the experiment and there was no evidence for a spontaneous spawning incident during the whole experiment. According to the observations made during the daily routine (Table 3), valves were opened regularly with no significant differences between the treatments. When placed in the clean aquaria with artificial seawater having been renewed, almost all mussels opened their valves.

The check in the morning, 1 h prior to water exchange, revealed that at this time of day the majority of the individuals had closed valves. During the regular checks, long filamentous excretions were detected, which the mussels in all treatment aquaria (both clean and contaminated microplastics) produced in the course of the day and which were expelled via the exhalant siphon. This was not observed in the control aquaria.

3.3. Distribution of plastics particles

After the plastics-water suspension had been dosed into the respective aquarium, the water showed a certain turbidity indicating that the particles were dispersed in the water column. However, due to the high density of the PVC, they settled more or less quickly and also formed conglomerates with each other as well as with byssal threads and other organic particles originating from the mussels. Regular stirring with the metal spoon brought the particles/conglomerates in suspension before they sank to the bottom again. Aeration by the two tubes did not suffice to keep the particle conglomerates in constant suspension.

Table 3
Average numbers of mussels with open valves in the different treatments as observed during the whole day as well as shortly after water renewal and particle re-dosing.

	Total observation time	Shortly after water exchange and particle re-dosing
Control	20.3	23.9
Microplastics (MP)	19.6	23.3
FLU 1	20.6	23.6
FLU 2	19.2	23.0
FLU 3	21.0	23.7
FLU 4	21.1	23.1

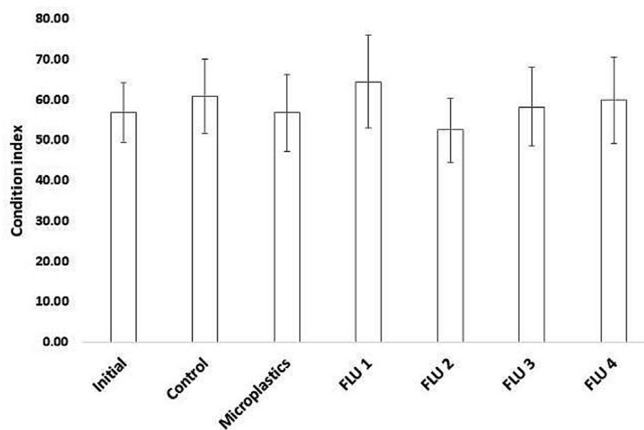

Fig. 2. Condition indices of the initial sampling group and the mussels of the different treatments. There were no significant differences between the groups.

3.4. Condition index

The calculated condition indices of the mussels are displayed in Fig. 2. No significant differences were observed between the different treatments as well as the individuals of the initial sampling (Kruskal-Wallis ANOVA; $p = 0.1041$, $n = 18$ per treatment) (Fig. 2).

3.5. Verification of microplastics uptake via polarised light microscopy

Particles and particle aggregates could be detected in the tissues of the digestive gland, specifically in the lumen of stomach, gut and tubuli of the mussels treated with PVC (Fig. 3). No particles were found penetrating into the tissues or cells of the inspected tissues.

The samples of the initial status individuals as well as those of the control did not show any of them. Each of the treatment mussels had ingested microplastics. No qualitative differences were observed between the different treatments. As only digestive glands were examined for the presence of microplastics, no statement can be made as to their occurrence in other tissues and organs, such as hemolymph, mantle or gills.

3.6. Analysis of fluoranthene in mussel tissues

The mussel soft bodies were analysed via passive sampling, and fluoranthene concentrations in organismal tissues were calculated from those measured on the PDMS fibres. Results for average concentrations of fluoranthene in mussel tissues are displayed in Table 4. Values indicate that with increasing fluoranthene concentrations on the particles also tissue concentrations in mussels increased ($R^2 = 0.9796$) (Fig. 4). Average concentrations in mussels from the fluoranthene treatments ranged from 4.53 ng g^{-1} wet weight for the lowest concentration on the PVC to 95.96 ng g^{-1} wet weight for the highest concentration. However, fluoranthene was also detected in the control and clean microplastics samples with concentrations of 3.71 and 2.71 ng g^{-1} wet weight, respectively.

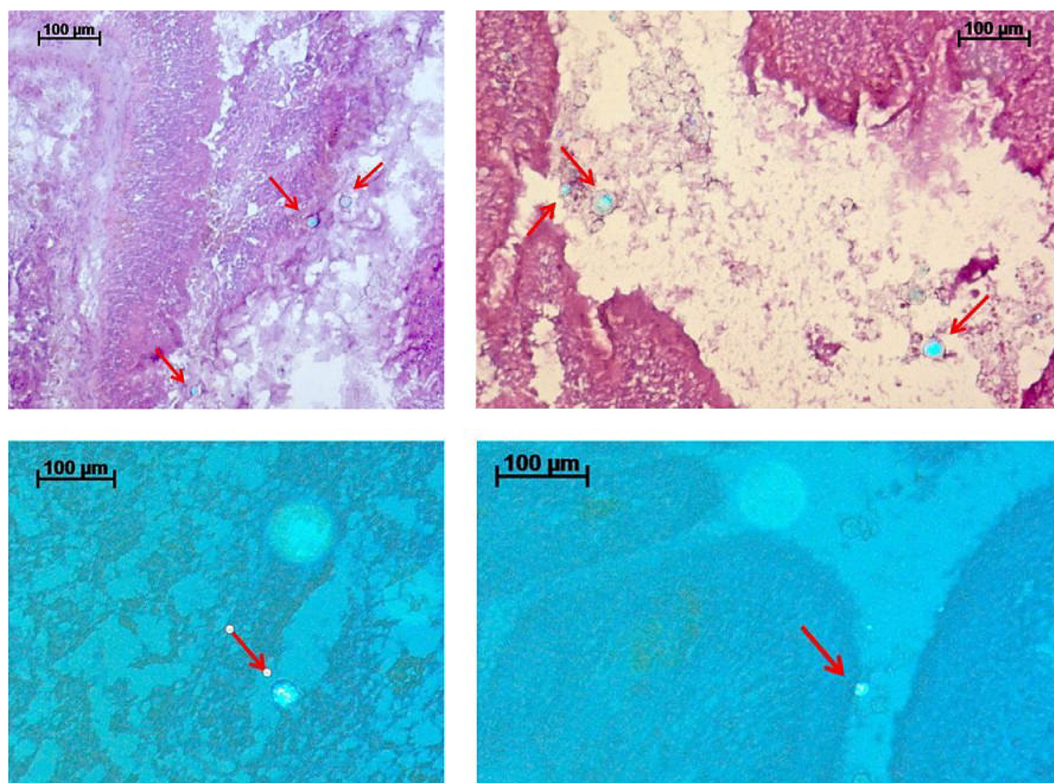

Fig. 3. Microplastics in the stomach and intestine of *M. edulis* after the 7-day exposure, visualised by polarised light microscopy.

Table 4
Average concentrations of fluoranthene in PDMS and mussel tissues.

Treatment	c_{FLU} on PVC particles [µg g^{-1}]	Average FLU concentration in mussel samples [ng g_{ww}^{-1}]
Control	0	3.71
MP	0	2.71
FLU 1	13.77	4.53
FLU 2	18.51	10.85
FLU 3	133.02	38.60
FLU 4	318.75	95.96

4. Discussion

Since their detection in the Sargasso Sea in the 1970s microplastics have been subject to a growing scientific interest. The results of numerous studies have indicated that microplastics have become ubiquitous in the marine environment and pose serious threats to organisms and ecosystems. The whole dimension of the pollution situation is still unknown due to the extreme vastness of the area of interest - oceans, coastlines, seafloors, sediments - and to weaknesses in sampling methodologies, excluding especially the very small particle fractions from consideration. However, with plastics production further increasing and the sector of one-way products continuing to grow, with insufficient concepts of recycling and the lack of proper disposal in various countries, these trends are very likely to be reflected in plastics abundances in the marine sphere in the future.

Recently, scientific interest has also turned towards the fact that microplastics have the potential to sorb hydrophobic contaminants like PAHs or PCBs from the surrounding sea water and to accumulate them in concentrations exceeding those in the water by orders of magnitude. In combination with diverse chemical substances added to the synthetic polymers during the production process, the particles may turn into accumulation hotspots and transporters of toxic cocktails in the marine environment.

Microplastics ingestion by marine organisms could be demonstrated in the field as well as under laboratory conditions for a large number of species, including benthic invertebrates like mussels. In this context, the question arose whether the particles may act as vectors for contaminant transport into organisms with desorption processes occurring preferably under gastric conditions as well as in lipid-rich tissues. Thus, besides acting as potential physical stressors microplastics could transfer elevated concentrations of chemical stressors to biota.

In the course of this work condition index data, calculated from wet meat weight and shell weight, were used to make sure that the individuals of the different treatments were in a comparable physiological status. As no significant differences were observed between the initial status and the control on the one hand and the treatment groups on the other hand, a comparability could be assumed.

Plastics-mediated uptake of fluoranthene into mussel tissues, even in case of the highest concentration on the PVC particles, did not induce lethal effects during the 7-day exposure, as the survival rate of 100% indicates. Moreover, the doses of neither the microplastics particles nor the PAH led to prolonged periods of valve closure, which could have been observable in the course of the regular checks during the day. This behavioural trait is a protective mechanism of mussels in cases of environmental disturbance, which may be the presence of predators, falling dry during low tide or also the presence of contaminants and potentially harmful particles in the surrounding water (Wright et al., 2013b; Rist et al., 2016).

Also low food concentrations have been observed to lead to periods of valve closure, thus reducing oxygen consumption rate

Fig. 4. Fluoranthene concentrations in blue mussel samples plotted against the actual fluoranthene concentrations in the aquaria. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and metabolism (Riisgård et al., 2003). During the experiment of the present work the mussels were not provided with algal suspension as a food source so that they were confronted with just nutritionally unusable and potentially harmful microplastics particles. While using a comparable particle size spectrum (≤50 μm) as has been applied for the present work, even the lowest particle concentration tested by Rist et al. (2016) was almost twice as high (21.6 mg L-1). This may indicate that probably the PVC concentration of the present work was still too low to induce valve closure as a protective mechanism against potential mechanical damage in *M. edulis*. Furthermore, even the highest fluoranthene concentration with which the animals were confronted via particle uptake did not result in valve closure as a possible reaction to prevent continuing intake of a chemically harmful substance.

The present work aimed at testing the ingestion of PVC particles, both virgin and contaminated with different concentrations of the PAH fluoranthene by *M. edulis* under laboratory conditions during a period of seven days. Uptake into the gastrointestinal system was verified by microscopic examination of digestive gland tissue sections. Via passive sampling a concentration-dependent uptake of fluoranthene in mussel tissues could be demonstrated.

The concentration of 11.56 mg of PVC particles L^{-1} used in this work was much lower than microplastics concentrations used in the studies of Browne et al. (2008) (0.5 g L^{-1}), von Moos et al. (2012) (2.5 g L^{-1}), Avio et al. (2015) (1.5 g L^{-1}), Rist et al. (2016) (up to 2.16 g L^{-1}) or Santana et al. (2018) (0.125 g L^{-1}) but several orders of magnitude higher than the ones Paul-Pont et al. (2016) and Sussarellu et al. (2016) applied in their experiments (0.032 mg L^{-1} and 0.023 mg L-1, respectively). A comparable concentration was used by Pittura et al. (2018) with 10 mg L^{-1}.

It has been criticised that most experiment concentrations by far exceed those detected in the field up to now, referring to both water column and sediments, and that they are thus not of environmental relevance (Koelmans et al., 2017). However, due to methodological restrictions, smaller size fractions often have remained unconsidered in many previous studies so that actual particle amounts may be assumed to be much higher. A refinement of sampling techniques and sample processing protocols combined with precise detection instruments such as FTIR or Raman spectroscopy as well as appropriate modelling approaches have recently been introduced (Enders et al., 2015; Erni-Cassola et al., 2017). The application of asymmetric field flow fractionation, dynamic light scattering and

pyrolysis gas chromatography coupled with mass spectrometry provides promising methods for particle identification on the nano-scale (Paul-Pont et al., 2018). All these may help to carry out more exact analyses and a more realistic estimation of plastics loads of the small and smallest size classes in the marine environment, which are expected to further increase in the future, especially at pollution hotspots like coastlines and in countries with insufficient or even no waste management for plastics litter. Taking this into consideration, at least the more moderate particle concentrations used in today's experimental studies might actually be representative for pollution situations in the world's highly contaminated environments.

The applied loading method using dissolved fluoranthene in a hexane solution led to comparably high concentrations of the PAH on the PVC particles in the used size spectrum, although the nominal concentrations could not be reached during the 48-h loading period. Nevertheless, the results of the loading process demonstrate a high capacity of the PVC particles in the chosen size spectrum for fluoranthene.

The final fluoranthene concentrations on the particles were much higher than contaminant concentrations which have been used so far by other studies. Pittura et al. (2018) chose a benzo(*a*) pyrene concentration of 15 μg g^{-1} low density polyethylene for their laboratory experiment with *Mytilus galloprovincialis*. Avio et al. (2015) applied polystyrene and polyethylene particles loaded with 200–260 ng g^{-1} of pyrene for their exposure experiment with the same organism. Rist et al. (2016), who also used PVC particles and fluoranthene in their laboratory study with *Perna viridis*, reached a maximum concentration of 20 ng g^{-1} after particle incubation in a fluoranthene-seawater solution for 24 days.

Environmental concentrations of fluoranthene specifically and PAHs in general detected in the field so far are mostly lower by orders of magnitude than the ones used in this work. Karapanagioti et al. (2011) collected plastics pellets at Greek beaches and found fluoranthene concentrations of up to 89 ng g^{-1} plastics, and total PAH concentrations of up to 500 ng g^{-1}. Resin pellets from the Portuguese coast sampled by Antunes et al. (2013) carried PAH loads up to 44.8 μg g^{-1}, which is actually a range comparable to the two lower concentrations on the experiment particles (13.77 and 18.51 μg g^{-1}, respectively), even if it encompasses a mixture of PAHs and not just fluoranthene.

Via polarised light microscopy PVC particles were successfully traced within the stomach and intestinal lumen of the mussels, verifying an uptake of microplastics in the size range ≤40 μm. Thus, the results of studies like Browne et al. (2008), von Moos et al. (2012), Avio et al. (2015) and Magni et al. (2018), which showed an uptake of polyethylene and polystyrene particles of different size classes in a general spectrum < 100 μm into the gastro-intestinal tract as well as other tissues of *M. edulis*, *Mytilus galloprovincialis* and *Dreissena polymorpha*, could be confirmed for the higher-density polymer type PVC.

The findings also provide proof that particle doses do not necessarily need to be exceedingly high, as has been the case in the named studies (see above), to lead to an organismal uptake, even during an exposure of only seven days. Particle uptake at even lower microplastics doses could also be shown by Sussarellu et al. (2016) for *Crassostrea gigas* during a 2-month experiment and by Paul-Pont et al. (2016) for *Mytilus* spp. during seven days of exposure and a subsequent 7-day depuration phase. Both studies detected particles only in stomach and intestine but not in digestive tubules or other tissues. Furthermore, the apparently unfavourable resuspension situation within the aquaria did not prevent particle uptake. Although a considerable amount of particles was observed to accumulate to larger conglomerates, which sank to the bottom and were thus assumed to be no longer available to the test organisms, there was obviously still enough material in suspension to result in an uptake which could later be seen under the microscope.

The filamentous excretions, which were observed in the treatment aquaria during the regular checks, may be an indicator of an increased elimination of the particles as a result of pre-as well as post-ingestive selection. Beside the production of pseudofaeces and faeces, which has already been demonstrated in the presence of microplastics by Wegner et al. (2012), Kesy et al. (2017), Santana et al. (2018) and Khan and Prezant (2018), this could be an additional way in dealing with indigestible foreign substances.

As only cryo sections could be analysed, a quantification of total microplastics loads within mussel tissues was not possible. Visual inspection, though, lead to the impression that all mussels in the microplastics treatments may have accumulated comparable loads of particles within their guts. Due to the fact that detected accumulations appeared to be of rather small dimensions compared to the relatively high doses of particles added daily to the aquaria, it may be assumed that a certain amount of ingested particles is actually excreted relatively quickly. Short retention times have also been proposed by Pittura et al. (2018), who did not find significant quantitative differences between the tissues of mussels exposed to HDPE particles for seven, 14 and 28 days, respectively. This phenomenon can be seen as a reflection of the feeding mode of the mussels, which is first of all an unspecific uptake of particles in the filtered water and subsequent sorting into food and unusable matter.

The chosen size fraction of PVC particles (≤40 μm) was to reflect particles fitting into the food spectrum of *M. edulis* in order to guarantee an uptake by the mussels. Furthermore, smaller size classes seem to be retained more readily within the mussel tissues. Browne et al. (2008) detected a higher abundance of smaller particles in comparison to larger ones in their experiment mussels, and van Cauwenberghe et al. (2015), based on the results of their laboratory exposure experiments, proposed a size limit of particle retention by *M. edulis* which ranges between 10 and 30 μm.

The passive sampling results of fluoranthene concentrations in mussel tissues point to a concentration-dependent uptake of fluoranthene in the tissues of *M. edulis* as a consequence of the daily exposure to contaminated microplastics. In relation to the concentration on the PVC particles, those in mussel tissues were much lower. Bioaccumulation under experimental conditions was described by Avio et al. (2015), who found pyrene concentrations in the digestive gland of *Mytilus galloprovincialis* being nearly twice as high as those on the contaminated particles used in the experiment. However, although PAH concentrations in their experiment (200–260 ng g^{-1}) were considerably lower than those applied in the present work, the particle concentrations, on the other hand, were exceedingly higher (1.5 g L^{-1}). Thus, comparably higher fluoranthene concentrations in mussel tissues may have been the result of higher particle availability.

After a 7-day exposure Pittura et al. (2018) could measure a significant increase in benzo (a)pyrene concentrations in the digestive gland of *Mytilus galloprovincialis* for treatments with the PAH alone and sorbed to LDPE particles, with no statistically relevant differences between the two treatments. Furthermore, they proposed high concentration levels in the tissues to be rather a consequence of actual desorption of the contaminant from the particles with subsequent accumulation than of disproportionately large particle amounts retained in the organism with the contaminant still sorbed to them.

Microplastics-mediated uptake and accumulation of PAHs in organisms seems to be of a more persistent nature than transfer via organic matter, as the results of Paul-Pont et al. (2016) propose. In their study they exposed *Mytilus* spp. to a mixture of microalgae

and either fluoranthene alone or the PAH sorbed to polystyrene spherules. Whereas directly after the 7-day exposure concentrations in the digestive gland of both groups did not show significant differences, the measurements after another seven days of depuration revealed the highest contaminant concentrations in mussels treated with the fluoranthene-loaded PS spherules. This may suggest that there is a possible direct impact of the microplastics particles on PAH-detoxification processes and should be subject to further research.

The findings of increasing fluoranthene concentrations in mussel tissues also indicate that enhanced desorption of contaminants from ingested microplastics may be facilitated by the special conditions in the gastrointestinal tract, as proposed by Sakai et al. (2000) and Bakir et al. (2014), who showed that gut surfactants as well as temperature and pH in the gut had a significant influence on leaching rates of sorbed contaminants. Furthermore, tissues with high lipid content are considered to provide favourable conditions for a pollutant transfer from plastics to biota (Koelmans et al., 2014). Generally it can be stated that a higher lipid content results in a greater desorption and bioaccumulation potential (Livingstone and Pipe, 1992). This implies that uptake and accumulation potential may vary considerably between different tissues as well as different biota and also the degree of hydrophobicity of a contaminant.

When identifying potential target locations of contaminant transfer as well as desorption times, it seems to be reasonable to also consider the question of particle size. Small microplastics and especially nanoplastics, having the potential of entering the circulatory system and other tissues, could transfer pollutants directly to these sites, as Browne et al. (2013) propose. Also retention times may be comparably longer for these particles as they are potentially too small to be quickly recognized as foreign matter by organismal structures and maybe also difficult to get rid of, once they have entered the tissues. Furthermore, desorption rates are estimated to be much faster with the smaller size fractions due to the higher surface-volume ratio (Koelmans et al., 2013; Bakir et al., 2016). When ingested particles are small enough to transcend biological membranes and thus confront the cells themselves with a combination of physical and chemical hazards, this may constitute a challenge to marine organisms of still unrateable extend.

The results of passive sampling, though, also revealed the presence of fluoranthene in the samples of control and clean microplastics groups. This may indicate a background contamination of the animals with the examined PAH resulting from exposure scenarios at the site from which they were sampled. Due to the fact that fluoranthene was also detected in the tissues of a mussel sampled prior to the experiment start (initial status), a contamination of the control and clean microplastics-treated mussels during the exposure may be excluded. The similar concentrations detected in mussels from control, clean microplastics and FLU 1 may indicate that the background contamination within the animals may have prevented further desorption from ingested PVC particles at this lowest applied concentration level due to the lack of a concentration gradient.

When examining uptake and bioaccumulation of contaminants and the role of microplastics as potential vectors in the course of laboratory experiments, the chosen concentrations of both particles and sorbed pollutants are commonly rather high, in most cases by far exceeding the respective concentrations measured so far in the field. Initial body burdens of experimental organisms taken from the field are much lower so that a gradient exists enabling contaminant transfer from plastics to organismal tissues. In studies intending to evaluate the accumulation of chemicals and its biological consequences for various marine species, this is due reasonable. However, it should be kept in mind that transfer may also take place in the other direction in cases where body burdens in organisms are actually higher than concentrations on the plastics particles they have ingested. Some studies could show that the addition of clean plastics to polluted systems, including organism, lead to a transfer of chemicals to these particles and a reduced bioaccumulation by the organisms (Teuten et al., 2007, 2009; Gouin et al., 2011; Koelmans et al., 2013). Nevertheless, there still remains the potentially adverse health effect associated to the particles themselves and the question whether these may be effectively excreted or rather retained and translocated from one tissue to another with also their contaminant load being kept within the body. Furthermore, as is also remarked by Teuten et al. (2009), the likelihood of plastics persisting in the environment being clean (As a result of production processes they already contain several chemical additives) and staying clean is very low.

5. Conclusion and outlook

In the present study it could be demonstrated that under laboratory conditions *M. edulis* ingests PVC microplastics in a size spectrum $\leq$40 μm. By applying a novel, fast and straightforward equilibrium sampling approach PAH levels achieved from contaminated microplastics were determined in the tissues of blue mussels. Like that, the vector function of the particles in transporting the sorbed polycyclic aromatic hydrocarbon fluoranthene into mussel tissues could be verified. Increasing contaminant concentrations on the particles resulted in increasing tissue concentrations. Even the highest fluoranthene concentration of 500 $\mu g\ g^{-1}$ PVC did not induce lethal effects during the 7-day exposure.

The higher sorptive capacity of microplastics in comparison with natural matter as well as their ability of sorbing a great variety of organic chemicals has been demonstrated by several studies (Teuten et al., 2007; Rochman, 2015; Paul-Pont et al., 2016; Ziccardi et al., 2016). First experimental results, including those gained in the course of the present work, indicate the potential of microplastics to transport their freight of sorbed contaminants into organismal tissues. Amounts of micro- and nanoplastics in the world's oceans, in sediments and on shores and coastlines are expected to further increase in the future, and thus they are to be considered as a serious threat to the health of organisms and the functioning of ecosystems.

Author credit statement

Nicole Stollberg, Writing – original draft, Investigation, Methodology, Validation. Silja Denise Kröger, Writing – original draft, Investigation, Validation. Mathias Reininghaus, Writing – original draft, Investigation, Validation. Jens Forberger, Validation, Preparation. Gesine Witt, Supervision, Writing – review & editing, Method development, Conceptualization. Matthias Brenner, Project administration, Supervision, Writing – review & editing, Resources, Methodology, Conceptualization.

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.

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References

Abbasi, S., Soltani, N., Keshavarzi, B., Moore, F., Turner, A., Hassanaghaei, M., 2018. Microplastics in different tissues of fish and prawn from the Musa Estuary, Persian Gulf. Chemosphere 205, 80–87.

Andrady, A.L. (Ed.), 2003. Plastics and the Environment. Wiley, Hoboken, NJ.

Antunes, J.C., Frias, J.G.L., Micaelo, A.C., Sobral, P., 2013. Resin pellets from beaches of the Portuguese coast and adsorbed persistent organic pollutants. Estuar. Coast Shelf Sci. 130, 62–69.

Avery-Gomm, S., Provencher, J.F., Morgan, K.H., Bertram, D.F., 2013. Plastic ingestion in marine-associated bird species from the eastern North Pacific. Mar. Pollut. Bull. 72 (1), 257–259.

Avio, C.G., Gorbi, S., Milan, M., Benedetti, M., Fattorini, D., d'Errico, G., Pauletto, M., Bargelloni, L., Regoli, F., 2015. Pollutants bioavailability and toxicological risk from microplastics to marine mussels. Environ. Pollut. 198, 211–222.

Bakir, A., Rowland, S.J., Thompson, R.C., 2014. Enhanced desorption of persistent organic pollutants from microplastics under simulated physiological conditions. Environ. Pollut. 185, 16–23.

Bakir, A., O'Connor, I.A., Rowland, S.J., Hendriks, A.J., Thompson, R.C., 2016. Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. Environ. Pollut. 219, 56–65.

Barnes, D.K.A., 2005. Remote islands reveal rapid rise of southern hemisphere, sea debris. Sci. World J. 5, 915–921.

Barnes, D.K.A., Walters, A., Gonçalves, L., 2010. Macroplastics at sea around Antarctica. Mar. Environ. Res. 70 (2), 250–252.

Bergmann, M., Wirzberger, V., Krumpen, T., Lorenz, C., Primpke, S., Tekman, M.B., Gerdts, G., 2017. High quantities of microplastic in arctic deep-sea sediments from the HAUSGARTEN observatory. Environ. Sci. Technol. 51 (19), 11000–11010.

Bravo Rebolledo, E.L., van Franeker, J.A., Jansen, O.E., Brasseur, S.M.J.M., 2013. Plastic ingestion by harbour seals (*Phoca vitulina*) in The Netherlands. Mar. Pollut. Bull. 67 (1–2), 200–202.

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 (13), 5026–5031.

Browne, M.A., Niven, S.J., Galloway, T.S., Rowland, S.J., Thompson, R.C., 2013. Microplastic moves pollutants and additives to worms, reducing functions linked to health and biodiversity. Curr. Biol.: CB 23 (23), 2388–2392.

Claessens, M., de Meester, S., van Landuyt, L., de Clerck, K., Janssen, C.R., 2011. Occurrence and distribution of microplastics in marine sediments along the Belgian coast. Mar. Pollut. Bull. 62 (10), 2199–2204.

de Stephanis, R., Giménez, J., Carpinelli, E., Gutierrez-Exposito, C., Cañadas, A., 2013. As main meal for sperm whales: plastics debris. Mar. Pollut. Bull. 69 (1–2), 206–214.

Enders, K., Lenz, R., Stedmon, C.A., Nielsen, T.G., 2015. Abundance, size and polymer composition of marine microplastics ≥10mm in the Atlantic Ocean and their modelled vertical distribution. Mar. Pollut. Bull. 100 (1), 70–81.

Engler, R.E., 2012. The complex interaction between marine debris and toxic chemicals in the ocean. Environ. Sci. Technol. 46 (22), 12302–12315.

Erni-Cassola, G., Gibson, M.I., Thompson, R.C., Christie-Oleza, J.A., 2017. Lost, but found with nile red: a novel method for detecting and quantifying small microplastics (1 mm to 20 mm) in environmental samples. Environ. Sci. Technol. 51 (23), 13641–13648.

European Chemical Agency, 2019. Accessed: 10.03.2019. https://echa.europa.eu/-/six-new-substances-added-to-the-candidate-list?utm_source= linkedin.com&utm_medium=social%20&utm_campaign=candidate_list.

Galgani, F., Hanke, G., Maes, T., 2015. Global distribution, composition and abundance of marine litter. Pages 29–56 of: Bergmann, Melanie, Gutow, Lars, & Klages, Michael. In: Marine Anthropogenic Litter. Springer International Publishing, Cham.

Gouin, T., Roche, N., Lohmann, R., Hodges, G., 2011. A thermodynamic approach for assessing the environmental exposure of chemicals absorbed to microplastic. Environ. Sci. Technol. 45 (4), 1466–1472.

Graham, E.R., Thompson, J.T., 2009. Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. J. Exp. Mar. Biol. Ecol. 368 (1), 22–29.

Jahnke, A., Mayer, P., Broman, D., McLachlan, M.S., 2009. Possibilities and limitations of equilibrium sampling using polydimethylsiloxane in fish tissue. Chemosphere 77 (6), 764–770.

Karapanagioti, H.K., Endo, S., Ogata, Y., Takada, H., 2011. Diffuse pollution by persistent organic pollutants as measured in plastic pellets sampled from various beaches in Greece. Mar. Pollut. Bull. 62 (2), 312–317.

Kesy, K., Hentzsch, A., Klaeger, F., Oberbeckmann, S., Mothes, S., Labrenz, M., 2017. Fate and stability of polyamide-associated bacterial assemblages after their passage through the digestive tract of the blue mussel *Mytilus edulis*. Mar. Pollut. Bull. 125 (1–2), 132–138.

Khan, M.B., Prezant, R.S., 2018. Microplastic abundances in a mussel bed and ingestion by the ribbed marsh mussel Geukensia demissa. Mar. Pollut. Bull. 130, 67–75.

Koelmans, A.A., Besseling, E., Wegner, A., Foekema, E.M., 2013. Plastic as a carrier of POPs to aquatic organisms: a model analysis. Environ. Sci. Technol. 47 (14), 7812–7820.

Koelmans, A.A., Besseling, E., Foekema, E.M., 2014. Leaching of plastic additives to marine organisms. Environ. Pollut. 187, 49–54.

Koelmans, A.A., Besseling, E., Foekema, E., Kooi, M., Mintenig, S., Os- sendorp, B.C., Redondo-Hasselerharm, P.E., Verschoor, A., van Wezel, A.P., Scheffer, M., 2017. Risks of plastic debris: unravelling fact, opinion, perception, and belief. Environ. Sci. Technol. 51 (20), 11513–11519.

Kuchling, H., 2014. Taschenbuch der Physik. Carl Hanser Verlag, München.

Livingstone, D.R., Pipe, R.K., 1992. Mussels and environmental contaminants: molecular and cellular aspects. In: Gosling, Elizabeth (Ed.), The Mussel Mytilus: Ecology, Physiology, Genetics and Culture. Development in Aquaculture and Fisheries Science. Elsevier, Amsterdam, London, New York, Tokyo, pp. 425–464.

Lusher, A.L., 2015. Microplastics in the marine environment: distribution, interactions and effects. Pages 245–307 of: Bergmann, Melanie, Gutow, Lars, & Klages. In: Michael (Ed.), Marine Anthropogenic Litter. Springer International Publishing, Cham.

Magni, S., Gagné, F., André, C., Della Torre, C., Auclair, J., Hanana, H., Parenti, C.C., Bonasoro, F., Binelli, A., 2018. Evaluation of uptake and chronic toxicity of virgin polystyrene microbeads in freshwater zebra mussel *Dreissena polymorpha* (Mollusca: Bivalvia). Sci. Total Environ. 631, 778–788.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C., Kaminuma, T., 2001. Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. Environ. Sci. Technol. 35 (2), 318–324.

Moore, C.J., Moore, S.L., Leecaster, M.K., Weisberg, S.B., 2001. A comparison of plastic and plankton in the north pacific central gyre. Mar. Pollut. Bull. 42 (12), 1297–1300.

Nor, N.H.M., Obbard, J.P., 2014. Microplastics in Singapore's coastal mangrove ecosystems. Mar. Pollut. Bull. 79 (1–2), 278–283.

Obbard, R.W., 2018. Microplastics in Polar Regions: the role of long range transport. Current Opinion in Environmental Science & Health 1, 24–29.

Ogata, Y., Takada, H., Mizukawa, K., Hirai, H., Iwasa, S., Endo, S., Mato, Y., Saha, M., Okuda, K., Nakashima, A., Murakami, M., Zurcher, N., Booyatumanondo, R., Zakaria, M.P., Le Dung, Q., Gordon, M., Miguez, C., Suzuki, S., Moore, C., Karapanagioti, H.K., Weerts, S., McClurg, T., Burres, E., Smith, W., van Velkenburg, M., Lang, J.S., Lang, Rich C., Laursen, D., Danner, B., Stewardson, N., Thompson, R.C., 2009. International Pellet Watch: global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. Mar. Pollut. Bull. 58 (10), 1437–1446.

Paul-Pont, I., Lacroix, C., González Fernández, C., Hégaret, H., Lambert, C., Le Goïc, N., Frère, L., Cassone, A.-L., Sussarellu, R., Fabioux, C., Guyomarch, J., Albentosa, M., Huvet, A., Soudant, P., 2016. Exposure of marine mussels *Mytilus spp*. to polystyrene microplastics: toxicity and influence on fluoranthene bioaccumulation. Environ. Pollut. 216, 724–737.

Paul-Pont, I., Tallec, K., Gonzalez-Fernandez, C., Lambert, C., Vincent, D., Mazurais, D., Zambonino-Infante, J.-L., Brotons, G., Lagarde, F., Fabioux, C., Soudant, P., Huvet, A., 2018. Constraints and priorities for conducting experimental exposures of marine organisms to microplastics. Frontiers in Marine Science 5, 1–22.

Pittura, L., Avio, C.G., Giuliani, M.E., d'Errico, G., Keiter, S.H., Cormier, B., Gorbi, S., Regoli, F., 2018. Microplastics as vehicles of environmental PAHs to marine organisms: combined chemical and physical hazards to the mediterranean mussels, Mytilus galloprovincialis. Frontiers in Marine Science 5, 207.

Provencher, J.F., Bond, A.L., Hedd, A., Montevecchi, W.A., Muzaffar, S.B., Courchesne, S.J., Gilchrist, H.G., Jamieson, S.E., Merkel, F.R., Falk, K., Durinck, J., Mallory, M.L., 2014. Prevalence of marine debris in marine birds from the North Atlantic. Mar. Pollut. Bull. 84 (1–2), 411–417.

Riisgård, H.U., Kittner, C., Seerup, D.F., 2003. Regulation of opening state and filtration rate in filter-feeding bivalves (Cardium edule, *Mytilus edulis*, Mya arenaria) in response to low algal concentration. J. Exp. Mar. Biol. Ecol. 284 (1–2), 105–127.

Rios, L.M., Moore, C., Jones, P.R., 2007. Persistent organic pollutants carried by synthetic polymers in the ocean environment. Mar. Pollut. Bull. 54 (8), 1230–1237.

Rist, S.E., Assidqi, K., Zamani, N.P., Appel, D., Perschke, M., Huhn, M., Lenz, M., 2016. Suspended micro-sized PVC particles impair the performance and decrease survival in the Asian green mussel *Perna viridis*. Mar. Pollut. Bull. 111 (1–2), 213–220.

Rochman, C.M., 2015. The complex mixture, fate and toxicity of chemicals associated with plastic debris in the marine environment. Pages 117–140 of: Bergmann, Melanie, Gutow, Lars, & Klages. In: Michael (Ed.), Marine Anthropogenic Litter. Springer Inter- national Publishing, Cham.

Rummel, C.D., Löder, M.G.J., Fricke, N.F., Lang, T., Griebeler, E.-M., Janke, M., Gerdts, G., 2016. Plastic ingestion by pelagic and demersal fish from the north sea and baltic sea. Mar. Pollut. Bull. 102 (1), 134–141.

Sakai, S., Urano, S., Takatsuki, H., 2000. Leaching behavior of PCBs and PCDDs/DFs from some waste materials. Waste Manag. 20 (2–3), 241–247.

Santana, M.F.M., Moreira, F.T., Pereira, C.D.S., Abessa, D.M.S., Turra, A., 2018. Continuous exposure to microplastics does not cause physiological effects in the cultivated mussel *Perna perna*. Arch. Environ. Contam. Toxicol. 74 (4), 594–604.

Sussarellu, R., Suquet, M., Thomas, Y., Lambert, C., Fabioux, C., Pernet, M.E.J., Le Goïc, N., Quillien, V., Mingant, C., Epelboin, Y., Corporeau, C., Guyomarch, J., Robbens, J., Paul-Pont, I., Soudant, P., Huvet, A., 2016. Oyster reproduction is affected by exposure to polystyrene microplastics. Proc. Natl. Acad. Sci. U. S. A 113 (9), 2430–2435.

Teuten, E.L., Rowland, S.J., Galloway, T.S., Thompson, R.C., 2007. Potential for plastics to transport hydrophobic contaminants. Environ. Sci. Technol. 41 (22), 7759–7764.
Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkhavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. Philosophical transac- tions of the Royal Society of London. Series B, Biological sciences 364 (1526), 2027–2045.
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.
Vethaak, A.D., Leslie, H.A., 2016. Plastic debris is a human health issue. Environ. Sci. Technol. 50 (13), 6825–6826.
Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A., Da Ros, L., 2013. Microplastic particles in sediments of Lagoon of Venice, Italy: first observations on occurrence, spatial patterns and identification. Estuarine. Coastal and Shelf Science 130, 54–61.
Vogel, Ulrike, 2015. Untersuchung von PCB und PAK in Miesmuscheln (Mytilus edulis) mitHilfe von passive sampling im Vergleich zur herkömmlichen Analyse mittels ASE. Bachelor's thesis. University of Applied Science, Hamburg.
von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46 (20), 11327–11335.
Waller, C.L., Griffiths, H.J., Waluda, C.M., Thorpe, S.E., Loaiza, I., Moreno, B., Pacherres, C.O., Hughes, K.A., 2017. Microplastics in the Antarctic marine system: an emerging area of research. Sci. Total Environ. 598, 220–227.
Wegner, A., Besseling, E., Foekema, E.M., Kamermans, P., Koelmans, A.A., 2012. Effects of nanopolystyrene on the feeding behavior of the blue mussel (*Mytilus edulis* L.). Environ. Toxicol. Chem. 31 (11), 2490–2497.
Witt, G., Liehr, G.A., Borck, D., Mayer, P., 2009. Matrix solid-phase mi- croextraction for measuring freely dissolved concentrations and chemical activities of PAHs in sediment cores from the western Baltic Sea. Chemosphere 74 (4), 522–529.
Witt, G., Bartsch, C., Liehr, G.A., Thiele, R., McLachlan, M.S., 2010. Using solid-phase microextraction to evaluate the role of different carbon matrices in the distribution of PAHs in sediment-porewater systems of the Baltic Sea. J. Soils Sediments 10 (7), 1388–1400.
Wright, S.L., Kelly, F.J., 2017. Plastic and human health: a micro issue? Environ. Sci. Technol. 6634–6647.
Wright, S.L., Rowe, D., Thompson, R.C., Galloway, T.S., 2013a. Microplastic ingestion decreases energy reserves in marine worms. Curr. Biol.: CB 23 (23), R1031–R1033.
Wright, S.L., Thompson, R.C., Galloway, T.S., 2013b. The physical impacts of microplastics on marine organisms: a review. Environ. Pollut. 178, 483–492.
Ziccardi, L.M., Edgington, A., Hentz, K., Kulacki, K.J., Kane Driscoll, S., 2016. Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: a state-of-the-science review. Environ. Toxicol. Chem. 35 (7), 1667–1676.