Bull Environ Contam Toxicol (2017) 99:655–661
DOI 10.1007/s00128-017-2186-3

Ingestion and Egestion of Microplastics by the Cladoceran *Daphnia magna*: Effects of Regular and Irregular Shaped Plastic and Sorbed Phenanthrene

Camilla Krogh Frydkjær[1] · Niels Iversen[1] · Peter Roslev[1]

Received: 28 April 2017 / Accepted: 26 September 2017 / Published online: 13 October 2017

Abstract The presence of microplastics in aquatic ecosystems is of increasing global concern. This study investigated ingestion, egestion and acute effects of polyethylene microplastics in *Daphnia magna*. Fate of regular shaped microplastic beads (10–106 µm) were compared with irregular shaped microplastic fragments (10–75 µm). *Daphnia magna* ingested regular and irregular microplastic with uptake between 0.7 and 50 plastic particles/animal/day when exposed to microplastic concentrations of 0.0001–10 g/L. Egestion of irregular fragments was slower than that of microplastic beads. The EC50 for irregular microplastic was 0.065 g/L whereas microplastic beads were less inhibitory. The potential of microplastic to act as vector for hydrophobic pollutants was examined using [^{14}C]phenanthrene as tracer. Polyethylene microplastic sorbed less [^{14}C]phenanthrene compared to natural plankton organisms (bacteria, algae, yeast). As microplastics are much less abundant in most aquatic ecosystems compared to plankton organisms this suggests a limited role as vector for hydrophobic pollutants under current environmental conditions.

Keywords *Daphnia magna* · Polyethylene microplastics · Phenanthrene · Sorption · Acute toxicity

Pollution of aquatic ecosystems with plastic debris is of increasing global concern. An important category of plastic debris is microplastics (< 5 mm) which has shown an increase in occurrence and abundance in most surface waters (Browne et al. 2007; Eerkes-Medrano et al. 2015; Lassen et al. 2015; Galloway and Lewis 2016; Duis and Coors 2016). Microplastic pollution include (i) primary microplastic originating from pellets and resins used in the plastic industry, and plastic exfoliators in face and body scrubs, and (ii) secondary microplastic generated during fragmentation and weathering of larger plastic pieces (Napper et al. 2015; Eerkes-Medrano et al. 2015; Ziccardi et al. 2016; Duis and Coors 2016). Lightweight microplastic particles tend to initially accumulate in the surface microlayer of aquatic ecosystems due to the surface tension of the water and the low density of some microplastics (Song et al. 2014). Microplastics can interact with hydrophobic organic contaminants (HOCs) in the surface microlayer or in the water column, due to the hydrophobicity of both microplastic and HOCs (Teuten et al. 2007; Cunliffe et al. 2013; Koelmans et al. 2016; Ziccardi et al. 2016). Microplastics have a large surface area and can potentially accumulate high concentrations of HOCs. If microplastics are ingested by aquatic organisms they may subsequently act as a source of toxic HOCs (vector) and contribute to HOCs' bioaccumulation (Teuten et al. 2007; Engler 2012; Bakir et al. 2016; Koelmans et al. 2016; Ziccardi et al. 2016).

Ingestion of microplastics has been observed in a range of marine and freshwater zooplankton species thereby introducing microplastic into the aquatic food web (e.g., Cole et al. 2013; Setälä et al. 2014). Ingestion of microplastics, however, depends on several factors including plastic type, weight, concentration, and frequency at which microplastic particles are within the range of ingestible particles naturally consumed by the organism (Wright et al. 2013; Galloway and Lewis 2016). A key zooplankton species that may ingest microplastics in aquatic ecosystems is the Cladoceran *Daphnia magna*, which is a filter feeder that

✉ Peter Roslev
pr@bio.aau.dk

[1] Section of Biology and Environmental Science, Department of Chemistry and Bioscience, Aalborg University, Frederik Bajers Vej 7H, DK 9220 Aalborg, Denmark

forages nonselectively on particles with sizes from < 1 µm to approximately 70 µm (Ebert 2005; Rosenkranz et al. 2009; Rehse et al. 2016; Nørgaard and Roslev 2016). However, little is known about the uptake and adverse effects to daphnids of different microplastic morphologies and whether microplastics are important vectors for hydrophobic pollutants.

The purpose of this study was to investigate: (i) the amount of regular and irregular shaped microplastic particles ingested and egested by *D. magna* during short term exposure and gut clearance; (ii) the adverse effect of microplastic with and without sorbed phenanthrene; (iii) the significance of phenanthrene sorption by microplastic particles compared to sorption by naturally occurring plankton (i.e., bacteria, algae and yeast). We hypothezise that (i) plastic morphology will affect egestion and adverse effects of ingested microplastic particles in *D. magna*, and (ii) polyethylene microplastic will sorb phenanthrene at levels comparable to plankton organisms.

Materials and Methods

Two types of polyethylene microplastic particles were included in this study: regular shaped round microplastic consisting of pristine white polyethylene beads with sizes of 10–106 µm (Cospheric, USA), and irregular shaped recycled microplastic particles consisting of black polyethylene fragments with sizes between 10 and 75 µm obtained from a plastic recycling company (Aage Vestergaard Larsen A/S, Denmark).

Unlabelled phenanthrene (CAS 85-01-8) and ^{14}C-labelled phenanthrene ([$^{14}C_9$]phenanthrene; 57 mCi/mmol) was obtained from Sigma-Aldrich (Denmark) and American Radiolabeled Chemicals (USA), respectively. Phenanthrene was dissolved in DMSO before addition to aqueous samples with *D. magna*. [^{14}C]phenanthrene was used for sorption experiments with microplastic and plankton, and was dissolved in hexane to facilitate evaporation of the solvent. [^{14}C]phenanthrene stocks had a specific radioactivity of 0.35 µCi/mL corresponding to 1.09 µg/mL.

The model organism *D. magna* originated from a laboratory clone cultivated from pure-culture ephippia (MicroBioTests Inc., Belgium). Female *D. magna* were grown and maintained at 20 ± 1 °C in aquaria with light:dark cycles of 16:8 h. Aquaria water consisted of high-quality groundwater (16 °dH; pH 7.8; O_2 > 8 mg/L). *D. magna* were fed a 1:1 mixture of dried organically grown *Chlorella pyrenoidosa* (Naturland, Denmark) and dry yeast (Malteserkors, Denmark) at levels corresponding to 0.1–0.2 mg C day^{-1} daphnid^{-1}.

Daphnia magna was exposed to either regular shaped microplastic beads or irregular shaped microplastic fragments in parallel experiments. The same concentrations of the two microplastic types were used and all incubation was carried out in sterile 20 mL glass vials containing autoclaved filter sterilized (0.22 µm) artificial freshwater medium (ISO 6341 2012). Each exposure concentration consisted of 5 vials with 4 *D. magna* in each (20 animals in total per concentration). Vials with *D. magna* and microplastic beads or microplastic fragments were incubated in the dark at 20 ± 1 °C on a plankton wheel at 5 rpm. No food was added in experiments with microplastics.

Ingestion and egestion of microplastics was investigated at six different initial concentrations between 0.0001 and 10 g/L. Tween 20 (CAS: 9005-64-5, Merck KGaA, Germany) equal to 0.001 vol% was added to facilitate mixing of microplastic and water. Ingestion of microplastics by *D. magna* was determined for each animal after 24 h of incubation. The animals were then transferred to fresh medium without microplastics and the concentration of microplastic particles in the organisms was determined again after 24 h of gut clearance (egestion). Microplastic in animal biomass was visualized by microscopy at ×10 magnification using a Carl Zeiss Axioskop 2 plus (Germany).

Toxicity of microplastics and phenanthrene to *D. magna* was determined using immobility as response variable (ISO 6341 2012). Tests were performed with the following combinations: microplastic (0.01–5 g/L), phenanthrene (0.008–5 mg/L), and microplastic-phenanthrene mixtures with variable phenanthrene concentrations (0.008–5 mg/L) combined with a fixed microplastic concentration of 0.05 g/L. In experiments with microplastic-phenanthrene mixtures, vials were pre-incubated for 24 h without *D. magna* to allow chemical sorption of phenanthrene before the animals were added. This procedure resulted in co-exposure to phenanthrene from microplastic and the aqueous phase. Mobility-immobility of *D. magna* was determined after 48 h of incubation (ISO 6341 2012).

Sorption of phenanthrene by microplastics and different plankton organisms was compared for irregular microplastic [10–75 µm], natural plankton [0.2–80 µm] obtained from an estuary (Limfjorden, Denmark), the bacterium *Vibrio fischeri* [≈ 1.2 µm] (DSMZ, Germany), and the yeast *Saccharomyces cerevisiae* [≈ 3.6 µm] (Malteserkors, Denmark). The concentrations, diameters and volumes of microplastic particles and different cell types were determined with a Multisizer™ 4e Coulter Counter® equipped with 20 and 100 µm apertures (Beckman-Coulter, USA). Particle concentrations (numbers/mL) were used to calculate approximate particle volumes and weight. Spherical particle geometry and uniform density (1 g/cm^3) was assumed to simplify the calculations. Irregular microplastic at a concentration of 0.05 g/L was used for the sorption experiments, and the amount of natural plankton, *V. fischeri* and *S. cerevisiae* used in the experiment was scaled according to the surface area of the microplastics. All particle types were incubated in

triplicates in 20 mL medium on a plankton wheel for 24 h at 20 ± 1°C (5 rpm). The initial [^{14}C]phenanthrene concentration corresponded to 12 µg/L. After 24 h, particles were filtered onto nitrate cellulose membrane filters, and washed with distilled water. Sorption of [^{14}C]phenanthrene was determined by dissolving the filter in Filter-Count scintillation cocktail (Perkin-Elmer, USA) followed by counting in a Tri-Carb 1600 TR Liquid Scintillation Analyzer (Packard, USA). The LOD and LOQ of the liquid scintillation analysis corresponded to 0.003 and 0.06 ng of [^{14}C]phenanthrene, respectively. Sorption of phenanthrene by microplastic and plankton organisms was estimated relative to particle mass (wet weight) to reflect that phenanthrene will likely be distributed on both the surface and inside particles due to intraparticle diffusion (Ahn et al. 2005; Fries and Zarfl 2012). An apparent distribution coefficient (K_P) was estimated as C_s/C_w where C_s and C_w is the concentration of [^{14}C]phenanthrene in the sorbent and water, respectively (Fries and Zarfl 2012).

The Mann Whitney *U* test (Wilcoxon rank sum test) with a significance level of $p < 0.05$ was used for evaluating differences in ingestion and egestion of different microplastic types and different exposure concentrations. The statistical analyses were carried out using Microsoft Excel 2007 (USA). EC50 values for concentration–response curves were calculated using KaleidaGraph 4.5.1 and a log-logistic equation as described previously (Ørsted and Roslev 2015). The 95% confidence interval (CI) was calculated as described in ISO 6341 (2012).

Results and Discussion

Microplastic particles in nature display a range of different morphologies including regular shaped spheres and irregular shaped fragments (Helm 2017). Microscopic images of the regular and irregular shaped microplastics used in this study are shown in Fig. 1a, b. Some of the irregular shaped microplastic fragments contained noticeable appendages (Fig. 1b). Microplastic particles collected in an estuary (Limfjorden, Denmark) are shown for comparison (Fig. 1c). The two morphological types of microplastics used in the present study (Fig. 1a, b) show resemblance to environmental microplastic types described as primary and secondary microplastics (Eerkes-Medrano et al. 2015; Duis and Coors 2016; Helm 2017).

The model organism *D. magna* rapidly ingested both regular and irregular shaped microplastic, but the concentration in the animals after 24 h was strongly dependent on the initial microplastic concentration in the water (Fig. 2a, b). At low initial microplastic concentrations (≤ 0.001 g/L), the ingestion was somewhat comparable for regular and irregular microplastic and corresponded to an average of 0.7–1.4 plastic particles/animal. Maximum biomass accumulation occurred at high microplastic concentrations > 0.01 g/L, and resulted in 33–50 plastic particles/animal. These results are in accordance with previous studies suggesting ingestion of microplastic by zooplankton organisms likely driven by the fact that the size range of microplastics overlaps with that of natural food items (Cole et al. 2013; Setälä et al. 2014; Galloway and Lewis 2016; Rehse et al. 2016). A significantly lower ingestion of irregular microplastic fragments was observed at 10 g/L compared to 1.0 and 0.1 g/L ($p < 0.05$; Mann Whitney *U* test). This difference may in part be due to considerable agglomeration of this microplastic type at high concentrations (i.e., occurrence of large microplastic aggregates).

Significant differences ($p \leq 0.05$; Mann Whitney *U* test) were observed in the ability of *D. magna* to egest regular and irregular shaped microplastic (Fig. 2). After 24 h of microplastic ingestion, each animal was followed for an additional 24 h to study egestion in microplastic free medium. Forty-nine percent of test organisms fed with regular microplastic completely emptied their gut within 24 h whereas only < 1% of organisms fed with irregular microplastic were able to clear their gut completely. In addition, the average post depuration concentration of microplastic in biomass

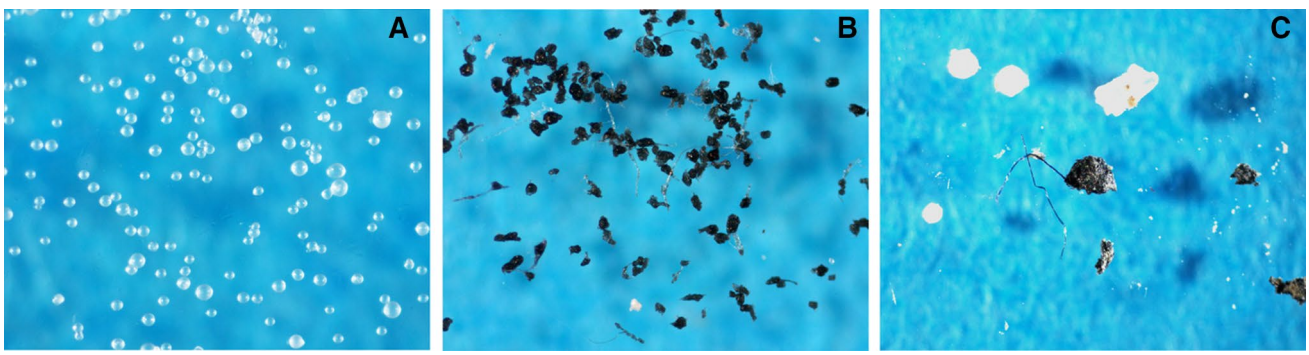

Fig. 1 **a** regular shaped polyethylene microplatic (10–106 µm); **b** irregular shaped polyethylene microplastic (10–75 µm); **c** mixed microplatic collected in Limforden, Denmark. The microplastic shown in **a** and **b** were used in experiments in the present study

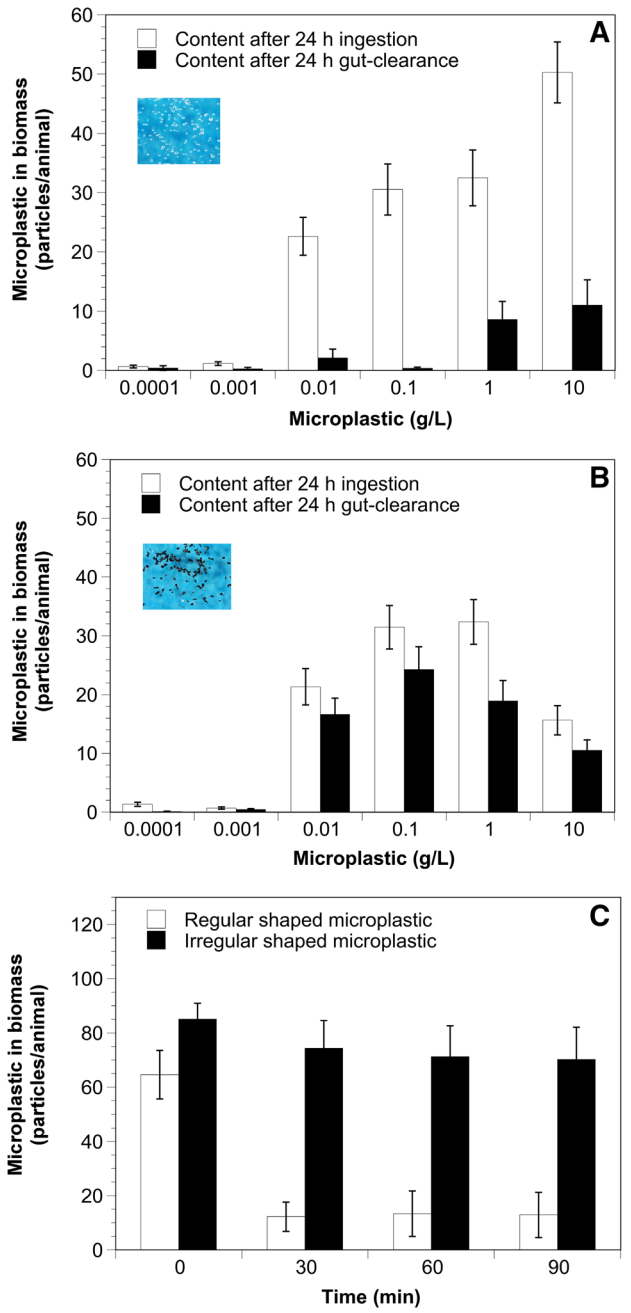

Fig. 2 Ingestion and egestion of regular (**a**) and irregular (**b**) shaped polyethylene microplastic by *D. magna* during 24 h uptake at different initial microplastic concentrations followed by 24 h egestion in microplastic free medium (depuration). An example of egestion of regular and irregular shaped polyethylene microplastic during the initial 90 min of depuration is shown in **c**. Data represent means for 20 animals ± standard error

was much higher for animals exposed to irregular shaped microplastic. The effect of microplastic morphology was also evident during the initial 90 min of depuration where biomass concentrations remained 5–6 times higher in animals previously exposed to 0.05 g/L irregular compared to 0.05 g/L regular shaped microplastic (Fig. 2c). Eighty-three percent of *D. magna* fed with regular microplastic emptied the gut during the initial depuration whereas none of the organisms fed irregular microplastic where able to clear their gut within 90 min. The rapid depuration of regular shaped plastic particles is in accordance with other studies suggesting limited bioaccumulation in animal GI tracts of microbeads (Rosenkranz et al. 2009; Grigorakis et al. 2017). In the preset study, all animals were alive and mobile during the depuration experiment, and the significant difference ($p = 0.001$) in egestion of regular shaped and irregular microplastic was likely due to the smoother surface of the regular shaped spherical beads compared to the somewhat spiky structure of the irregular microplastic particles (Fig. 1). Irregular microplastics are believed to be the most abundant microplastic particles in aquatic ecosystems (Duis and Coors 2016; Helm 2017). As irregular microplastics are also for a longer period of time retained within the guts of organisms, gut blockages with implications for the nutrition of organisms is a potential scenario (Wright et al. 2013). Hence, microplastic morphology is a factor that should be considered when conducting experiments with microplastics and filter feeding zooplankton.

Elevated microplastic concentrations affected the mobility of *D. magna* (Fig. 2). However, immobilization was different for regular and irregular microplastic. The immobility caused by regular shaped microplastic was very low, and less than 50% inhibition was observed after 48 h of exposure to concentrations as high as 5 g/L. In contrast, a clear immobilization was observed in response to irregular microplastic, and the 48 h EC50 was estimated to 0.065 g/L (0.021–0.192 g/L CI). This EC50 value for irregular polyethylene microplastic is comparable to an EC50 value of 0.057 g/L reported for *D. magna* after exposure to small polyethylene particles for 96 h (Rehse et al. 2016). Interestingly, these authors found noticeable size dependent differences in inhibitory effects of polyethylene with no observable effect of 100 µm particles compared to 1-µm particles (Rehse et al. 2016). Related results have been observed for polystyrene plastic where small particles (50 nm) were more toxic to *D. magna* than larger particles (Ma et al. 2016). It has been suggested that small microplastic particles may decrease fitness of zooplankton such as *D. magna* by adhering to inner and outer surfaces, impairing filtering activity, compromising the gut integrity, and entering tissue and cells (Cole et al. 2013; Eerkes-Medrano et al. 2015; Rehse et al. 2016; Ma et al. 2016). However, it should be noted that acute microplastic EC50 concentrations in the mg/L range are clearly above typical concentrations (ng/L to µg/L) most often encountered in aquatic ecosystems (e.g., Lassen et al. 2015; Duis and Coors 2016; Fischer et al. 2016). Hence, acute lethal effects of current microplastic concentrations on zooplankton such as *Daphnia* are likely rare whereas

the potential for sub-lethal effects after long-term exposure needs further investigations.

It has been suggested that microplastics in aquatic ecosystems may increase exposure of zooplankton to harmful chemicals by serving as a vector for HOCs (Teuten et al. 2007; Engler 2012; Eerkes-Medrano et al. 2015; Koelmans et al. 2016; Ziccardi et al. 2016). When zooplankton ingests microplastics with sorbed HOCs, these chemicals may desorb in the gut and increase the risk of bioaccumulation and toxic effects. In our study, irregular microplastic preincubated with phenanthrene for 24 h to facilitate HOC sorption was more toxic to *D. magna* than microplastic alone. However, the inhibitory effect of microplastic with phenanthrene was not unambiguously more toxic than the same phenanthrene concentration without microplastic. Exposure in the absence of microplastic resulted in an EC50 of 0.47 mg/L for phenanthrene (0.23–0.91 mg/L CI). In comparison, incubation of *D. magna* with phenanthrene in the presence of irregular microplastic (0.05 g/L) resulted in an EC50 of 0.14 mg/L for phenanthrene (0.01–0.34 mg/L CI).

The role of microplastics as a potential vector for HOCs compared to the role of natural plankton organisms has been the source of some debate (Teuten et al. 2007; Bakir et al. 2016; Koelmans et al. 2016; Ziccardi et al. 2016). In our study, simple sorption experiments with [^{14}C]phenanthrene showed that irregular polyethylene microplastic (10–75 µm) was a good sorbent for phenanthrene (Fig. 4). The phenanthrene sorption estimated for polyethylene microplastic is in line with results from related studies (Karapanagioti and Klontza 2008; Fries and Zarfl 2012; Napper et al. 2015). Sorption by our irregular polyethylene fragments corresponded to an apparent distribution coefficient (K_P) of 6696 ± 946 L/kg (24 h). This is in the same range as K_P values estimated in more comprehensive studies by Karapanagioti and Klontza (2008) and Fries and Zarfl (2012).

[^{14}C]phenanthrene sorption by irregular polyethylene microplastic estimated in the current study was 8–35 times lower per mass (weight) compared to sorption by different plankton organisms (Fig. 3). Mixed plankton (0.2–80 µm) from an estuary showed the greatest sorption of [^{14}C]phenanthrene (Fig. 3). These results confirmed that microplastics are potential environmental sorbents of phenanthrene but the sorption is not greater than that for different plankton organisms when based on mass. Furthermore, the abundance of microplastics compared to plankton organisms should also be considered when evaluating their potential role as HOC vectors (Koelmans et al. 2016; Ziccardi et al. 2016). Examples of the abundance of microplastics and different plankton organisms in aquatic ecosystems are shown in Table 1. Calculation of simple ratios between microplastics, phytoplankton and bacterioplankton abundances suggest that current concentrations of microplastics in both freshwater and marine ecosystems are much lower than that of natural

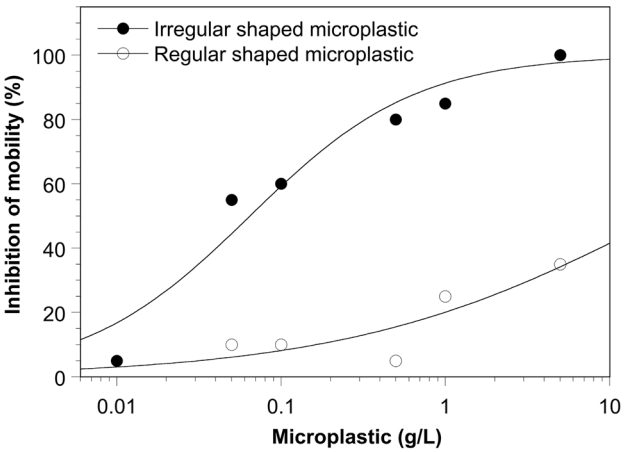

Fig. 3 Inhibitory effect of regular and irregular shaped polyethylene microplastic on the mobility of *D. magna* after 48 h exposure. Data represent means for 20 animals

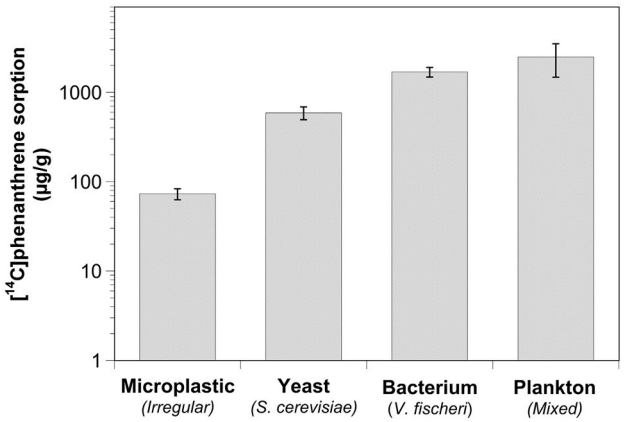

Fig. 4 Sorption of [^{14}C]phenanthrene (24 h) by irregular shaped microplastic particles, yeast (*S. cerevisiae)*, a bacterium (*V. fischeri*), and mixed plankton. [^{14}C]phenanthrene sorption is expressed relative to the weight of the different particles ± standard error

plankton organisms (by a factor of at least 10^3). Furthermore, significant amounts of HOC may also be associated with dead organic mater including dissolved and particulate organic carbon. This suggests a more limited role of microplastics as HOC vector compared to live and dead plankton organisms given the current microplastic concentrations in most aquatic ecosystems. This conclusion support previous studies suggesting that microplastic ingestion is not likely to significantly increase HOC exposure and risk compared to other exposure pathways (Bakir et al. 2016; Koelmans et al. 2016; Ziccardi et al. 2016). To examine these aspects further, future studies may include sorption and desorption of HOCs from microplastics and different plankton fractions by de facto measuring HOC bioavailability and bioaccumulation.

Table 1 Examples of abundances of microplastics, phytoplankton and bacterioplankton in aquatic ecosystems

Aquatic environment	Abundance in water column (numbers m^{-3})			Ratio
	Microplastics (M)	Phytoplankton (P)	Bacterioplankton (B)	M:P:B
Rivers	0.9–17.9	0.8×10^{8}–8×10^{10}	4×10^{11}–1.2×10^{14}	$1:10^{9}:10^{12}$
Lakes	0.82–65	6.4×10^{5}–5.9×10^{8}	9.5×10^{10}–2.2×10^{11}	$1:10^{6}:10^{9}$
Marine (coastal)	4.0×10^{3}–2.6×10^{5}	1×10^{6}–1×10^{8}	5×10^{11}–2.5×10^{12}	$1:10^{3}:10^{7}$
Marine (open waters)	0.004–4	6×10^{4}–8×10^{8}	4.9×10^{11}–2.5×10^{12}	$1:10^{8}:10^{11}$

The ratios between microplastics, phytoplankton and bacterioplankton were calculated from the average abundance of plastic particles and plankton organisms in the water column. Data are based on Heinanen (1992), de Ruyter van Steveninck et al. (1992), Bratbak et al. (2011), Lassen et al. (2015), Davidson et al. (2013), Duis and Coors (2016), and Fischer et al. 2016

In summary, this study showed that *D. magna* rapidly ingested regular and irregular shaped polyethylene microplastic particles but egestion of regular microplastic was faster than egestion of irregular microplastic. Gut clearance and apparent gut residence time was longer for irregular shaped microplastic, and acute inhibitory effects were more pronounced for irregular microplastic compared to regular shaped microplastic. Microplastic morphology is therefore a factor that should be considered in experiments with microplastics and filter feeders because most microplastics in the environment may be irregular in shape. The long-term effects of different irregular microplastic morphologies warrant further studies. Irregular polyethylene microplastic sorbed less phenanthrene compared to different plankton organisms, and the abundance of phytoplankton and bacterioplankton is often much greater than the present number of microplastic particles in aquatic ecosystems. Hence, live and dead plankton organisms are likely more critical carriers of HOCs than microplastics under current environmental conditions.

Acknowledgements We thank Helle Blendstrup and Jens Eiby Hansen for laboratory assistance. This work was supported in part by an equipment grant from the Danish trust fond COWIfonden.

References

Ahn S, Werner D, Karapanagioti HK, McGlothlin DR, Zare RN, Luthy RG (2005) Phenanthrene and pyrene sorption and intraparticle diffusion in polyoxymethylene, coke, and activated carbon. Environ Sci Technol 39:6516–6526. doi:10.1021/es050113o

Bakir A, O'Connor IA, Rowland SJ, Hendriks AJ, Thompson RC (2016) Relative importance of microplastics as a parthway for the transfer of hydrophobic organic chemicals to marine life. Environ Pollut 219:56–65. doi:10.1016/j.envpol.2016.09.046

Bratbak G, Jacquet S, Larsen A, Pettersson LH, Sazhin AF, Thyrhaug R (2011) The plankton community in Norwegian coastal waters-abundance, composition, spatial distribution and diel variation. Continent Shelf Res 31:1500–1514. doi:10.1016/j.csr.2011.06.014

Browne MA, Galloway T, Thompson R (2007) Microplastic—an emerging contaminant of potential concern? Integr Environ Assess Manag 3:559–561. doi:10.1002/ieam.5630030412

Cole M, Lindeque P, Fileman E, Halsband C, Goodhead R, Moger J, Galloway T (2013) Microplastic ingestion by zooplankton. Environ Sci Technol 47:6646–6655. doi:10.1021/es400663f

Cunliffe M, Engel A, Frka S, Gašparović B, Guitart C, Murrell JC et al (2013) Sea surface microlayers: a unified physicochemical and biological perspective of the air–ocean interface. Prog Oceanogr 109:104–116. doi:10.1016/j.pocean.2012.08.004

Davidson K, Gilpin LC, Pete R, Brennan D, McNeill S, Moschonas G, Sharples J (2013) Phytoplankton and bacterial distribution and productivity on and around Jones Bank in the Celtic Sea. Prog Oceanogr 117:48–63. doi:10.1016/j.pocean.2013.04.001

de Ruyter van Steveninck ED, Admiraal W, Breebaart L, Tubbing GMJ, van Zanten B (1992) Plankton in the River Rhine: structural and functional changes observed during downstream transport. J Plankton Res 14:1351–1368. doi:10.1093/plankt/14.10.1351

Duis K, Coors A (2016) Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ Sci Europe 28:2. doi:10.1186/s12302-015-0069-y

Ebert D (2005) Ecology, epidemiology, and evolution of parasitism in *Daphnia*. National Library of Medicine (US) and National Center for Biotechnology Information (US), Bethesda. ISBN-10: 1-932811-06-0

Eerkes-Medrano D, Thompson RT, Aldridge DC (2015) Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. Water Res 75:63–82. doi:10.1016/j.watres.2015.02.012

Engler RE (2012) The complex interaction between marine debris and toxic chemicals in the ocean. Environ Sci Technol 46:12302–12315. doi:10.1021/es3027105

Fischer EH, Paglialonga L, Czech E, Taminga M (2016) Microplastic pollution in lakes and lake shoreline sediments—a case study on Lake Bolsena and Lake Chiusi (central Italy). Environ Pollut 213:648–657. doi:10.1016/j.envpol.2016.03.012

Fries E, Zarfl C (2012) Sorption of polycyclic aromatic hydrocarbons (PAHs) to low and high density polyethylene (PE). Environ Sci Pollut Res 19:1296–1304. doi:10.1007/s11356-011-0655-5

Galloway TS, Lewis CN (2016) Marine microplastics spell big problems for future generations. PNAS 113:2331–2333. doi:10.1073/pnas.1600715113

Grigorakis S, Mason SA, Drouillard KG (2017) Determination of the gut retention of plastic microbeads andmicrofibers in goldfish (*Carassius auratus)*. Chemosphere 169:233–238. doi:10.1016/j.chemosphere.2016.11.055s

Heinanen A (1992) Bacterioplankton in a sub-arctic estuary—the Gulf of Bothnia (Baltic Sea). Mar Ecol Prog Ser 86:123–131. doi:10.3354/meps086123

Helm PA (2017) Improving microplatic sources apportionment: a role for microplatic morphology and taxonomy? Anal Methods 9:1328–1331. doi:10.1039/C7AY90016C

ISO 6341 (2012) Water quality—determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Custacea)—acute toxicity test. International Organization for Standardization, Geneva

Karapanagioti HK, Klontza I (2008) Testing phenanthrene distribution properties of virgin plastic pellets and plastic eroded pellets found on Lesvos island beaches (Greece). Mar Environ Res 65:283–290. doi:10.1016/j.marenvres.2007.11.005

Koelmans AA, Bakir A, Burton GA, Janssen CR (2016) Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. Environ Sci Technol 50:3315–3326. doi:10.1021/acs.est.5b06069

Lassen C, Hansen SF, Magnusson K, Hartmann NB, Rehne Jensen P, Nielsen TG, Brinch A (2015) Microplastics—occurrence, effects and sources of releases to the environment in Denmark. The Danish Environmental Protection Agency, Environmental project No. 1793

Ma Y, Huang A, Cao S, Sun F, Wang L, Guo H, Ji R (2016) Effects of nanoplastics and microplastics on toxicity, bioaccumulation, and environmental fate of phenanthrene in fresh water. Environ Pollut 219:166–173. doi:10.1016/j.envpol.2016.10.061

Napper IE, Bakir A, Rowland SJ, Thompson RC (2015) Characterisation, quantity and sorptive properties of microplastics extracted from cosmetics. Mar Pollut Bull 99:178–185. doi:10.1016/j.marpolbul.2015.07.029

Nørgaard LS, Roslev P (2016) Effects of ammonia and density on filtering of commensal and pathogenic *Escherichia coli* by the cladoceran *Daphnia magna*. Bull Environ Contam Toxicol 6:848–854. doi:10.1007/s00128-016-1963-8

Ørsted M, Roslev P (2015) A fluorescence based hydrolytic enzyme activity assay for quantifying toxic effects of Roundup® to *D. magna*. Environ Toxicol Chem 34:1841–1850. doi:10.1002/etc.2997

Rehse S, Kloas W, Zarfl C (2016) Short-term exposure with high concentrations of pristine microplastic particles leads to immobilization of *Daphnia magna*. Chemosphere 153:91–99. doi:10.1016/j.chemosphere.2016.02.133

Rosenkranz P, Qasim C, Stone V, Fernandes TF (*2009*). A comparison of nanoparticle and fine particle uptake by *Daphnia magna*.Environ Toxicol Chem 28:2142–2149. doi:10.1897/08-559.1

Setälä O, Fleming-Lehtinen V, Lehtiniemi M (2014) Ingestion and transfer of microplastics in the planktonic food web. Environ Pollut 185:77–83. doi:10.1016/j.envpol.2013.10.013

Song YK, Hong SH, Jang M, Kang JH, Kwon OY, Han GM, Shim WJ (2014) Large accumulation of micro-sized synthetic polymer particles in the sea surface microlayer. Environ Sci Technol 48:9014–9021. doi:10.1021/es501757s

Teuten EL, Rowland SJ, Galloway TS, Thompson RC (2007) Potential for plastics to transport hydrophobic contaminants. Environ Sci Technol 41:7759–7764. doi:10.1021/es071737s

Wright SL, Thompson RC, Galloway TS (2013) The physical impacts of microplastics on marine organisms: a review. Environ Pollut 178:483–492. doi:10.1016/j.envpol.2013.02.031

Ziccardi LM, Edgington A, Hentz K, Kulacki K, Driscoll SK (2016) Microplastics as vectors for bioaccumulation of hydrophobic organic chemicals in the marine environment: a state-of-the-science review. Environ Toxicol Chem 35:1667–1676. doi:10.1002/etc.3461