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Plastic bag and facial cleanser derived microplastic do not affect feeding behaviour and energy reserves of terrestrial isopods

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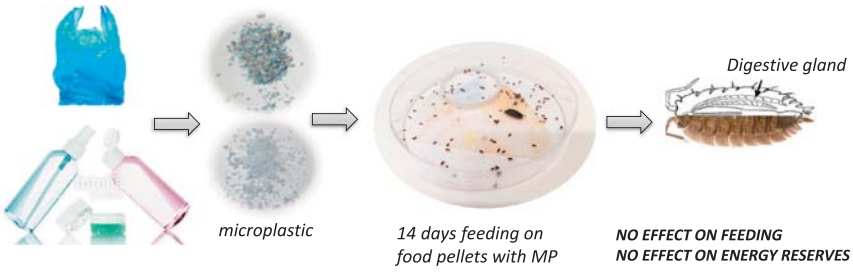
HIGHLIGHTS

- Plastic bag and facial cleanser microplastic had no affect on the feeding rate of isopods.
- No alterations in isopod body mass were found.
- Microplastic had no effect on energy reserves in digestive glands of isopods.
- 14 days exposure to tested microplastic is not severely hazardous to isopods.

GRAPHICAL ABSTRACT



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ABSTRACT

Current data regarding the effects of microplastic (MP) on terrestrial organisms are very scarce. Isopods play an important role in plant litter decomposition processes and are commonly used test species in terrestrial ecotoxicity studies. Their altered feeding behaviour and energy reserves are established biomarkers of adverse effects upon stressor exposure. For this study we assessed the effects of MP derived from plastic bag film (mean size 183 ± 93 μm) and particles from a facial cleanser (mean size 137 ± 51 μm) on the terrestrial isopod, *Porcellio scaber*. Isopods were exposed to MP via feeding on food pellets (4 mg g^{-1} dry weight; 0.4% w w^{-1}) for 14 days under laboratory conditions. A control group was exposed to food pellets with no MP added. In line with previously suggested modes of MP action on animal ingestion, we assessed the food ingestion rate, defecation rate, food assimilation rate and efficiency, body mass change, mortality and energy reserves (proteins, carbohydrates, and triglycerides) in the digestive glands (hepatopancreas) of individual isopods. Contrary to our expectations, no effects on either end-point were observed under the given exposure conditions. Further work should be carried out to investigate the potential longer-term effects of such exposure. We conclude that 14 days exposure to plastic bag and facial cleanser MP is not severely hazardous to isopods.

1. Introduction

Over the past years, the presence of small plastic particles (<5 mm) – microplastics (MPs) in the environment have been attracting increasing attention and MPs now represent an active emerging area of research (da Costa et al., 2016; GESAMP, 2015). A recent review showed that the extent of microplastic pollution in the terrestrial environment is largely still unknown and remains a fundamental gap in our knowledge (Horton et al., 2017). Microplastics can potentially enter the terrestrial environment via sewage sludge deposition on agricultural land, since plastic particles present in sewage are mostly retained in the sludge (Horton et al., 2017). The concentrations of MP extracted from seven sewage sludge samples in Ireland were in the range of 4196–15,385

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particles kg^{-1} dry mass (250 μm sieve) (Mahon et al., 2016). Similarly, in agricultural sites and landfills in Europe, a concentration in the range of 4000 microplastic particles kg^{-1} dry mass has been reported (Zubris and Richards, 2005). The majority of MPs identified in different wastewater effluents were similar to polyethylene particles found in cosmetic products (Carr et al., 2016). Plastic films used as soil cover in gardens and mulch film on agricultural land fragment to yield so called secondary MPs (Huerta Lwanga et al., 2016; Steinmetz et al., 2016). These particles may enter the soil and can be further transported along the soil column by bioturbation (Huerta Lwanga et al., 2016). In another study, analysis of industrial compost samples showed that they contained up to 0.2% w w^{-1} of plastic particles. Of these ~20% were smaller than 5 mm giving an estimated MP concentration of 0.04% w w^{-1} or 1800 particles kg^{-1} compost (Gajšt, 2016). Additional proof that the terrestrial environment is polluted by plastic particles was reported by Zhao et al. (2016) who showed that microscopic anthropogenic litter is abundant in the gastrointestinal tracts of 12 species of terrestrial birds in China (Zhao et al., 2016).

Despite the presence of MP in terrestrial environments, data regarding the effects of MP on terrestrial organisms are very scarce. To our knowledge, only four studies are available, three used the earthworm *Lumbriculus terrestris* (Huerta Lwanga et al., 2016, 2017; Hodson et al., 2017) and one used the earthworm *Eisenia andrei* (Rodriguez-Seijo et al., 2017). Rodriguez-Seijo et al. (2017) demonstrated that polyethylene particles (250–1000 μm; 1000 mg kg^{-1} soil or 3960 ± 520 particles kg^{-1} soil) had no effect on *E. andrei* survival, reproduction and weight gain after 28 days of exposure, but histopathological alterations of the gut epithelium and immune response in coelomocytes were noted. Huerta Lwanga et al. (2016, 2017) highlighted that polyethylene particles (200–300 μm) caused increase in mortality of *L. terrestris* after 60 days exposure to 60% of MPs in the topsoil layer. Using the same exposure procedure but a lower concentration of MPs (28%) in the topsoil layer resulted in an increased ingestion rate and a decreased body weight of *L. terrestris*, but no effect on the reproduction was seen (Huerta Lwanga et al., 2016).

In the present study, we investigated the effects of MPs on the terrestrial isopod *Porcellio scaber*. These organisms are abundantly found under stones and dead wood, particularly in compost heaps, gardens, and marginal grassland. They play an important role in plant litter decomposition by breaking down organic materials such as fallen leaves into smaller fragments, physically transporting litter materials in the soil column, and via the alteration of microbial activity (David, 2014). Terrestrial isopods are commonly used as test organisms in ecotoxicological studies and altered feeding behaviour and energy reserves have been recognised as relevant biomarkers of the adverse effects of pollutants (Ferreira et al., 2015; Loureiro et al., 2005). Measurements of energy reserves in isopods are done by either using the whole animal (Ferreira et al., 2015) or just the midgut digestive gland (hepatopancreas), which is the main metabolic organ (Schill and Köhler, 2004).

In line with previously suggested modes of MP action on animal feeding (Huerta Lwanga et al., 2016), the focus of this work was to investigate whether MP affects food ingestion rate, defecation rate, assimilation rate and efficiency, body mass, mortality and energy reserves (proteins, carbohydrates, and triglycerides) in the digestive glands of *P. scaber*. We tested two types of MP - one derived from plastic bag film and the other from a facial cleanser. The two types of MP were selected due to their reported occurrence in terrestrial environments (Carr et al., 2016; Huerta Lwanga et al., 2016).

2. Materials and methods

2.1. Test organisms

The isopods *Porcellio scaber* were collected from the laboratory culture originally derived from individuals collected from a compost heap in a non-polluted garden in Ljubljana, Slovenia in the spring of 2016. The terrarium was kept in a controlled chamber under constant temperature (20 ± 2 °C) and illumination (16:8 h light: dark photoperiod) regimes (LI-1000 Data Logger, LI – COR, Nebraska, USA). Isopods were kept in glass containers with moist loamy sand and peat at the bottom, and fed on dry fallen leaves from common hazel (*Corylus avellana*), common alder (*Alnus glutinosa*) and carrots.

2.2. Extraction and characterisation of MP from facial cleanser and from plastic bag

Microplastic particles from a commercial facial cleanser were extracted by gradient filtration: three filters were used in a cascade starting with a 300 μm mesh size, followed by 120 and 20 μm. A small amount of facial cleanser was poured on the filter with the largest mesh size and the soluble ingredients were washed away using warm (40 °C) deionized water. The washing was repeated until no foam formation was observed in the effluent water. After filtering and rinsing, the particles were left to dry in a Petri dish at room temperature and stored in a sealed container.

Microplastic particles from a plastic shopping bag were prepared by grinding the already fragmented plastic shopping bag using an agate mortar and sodium chloride (NaCl) to enhance the effect of grinding. The sample was then poured on a 120 μm mesh filter and was washed with deionized water to remove NaCl. After washing the particles were left to dry in a Petri dish at room temperature and stored in a sealed container.

The polymer composition of particles was characterized using a FTIR spectrometer (Spectrum One, Perkin Elmer), and the size/shape of particles by a field emission scanning electron microscope (FE-SEM, Zeiss ULTRA plus, Carl Zeiss, Germany). The number and volume particle size distributions of prepared MP samples were measured using a Microtrac S3500 Bluewave laser diffraction particle size analyser. Particle size distributions were obtained in a dry sample disperser coupled with a three-laser system with a detection range between 0.25 μm and 2000 μm. Each sample was recorded in three dry runs, where the refractive index was set as 1.51 (Fluid reference table, Microtrac document SI-RT-01, Revision A, Microtrac Inc.). The presented particle size distributions were calculated as an average value of all three measurements.

2.3. Food pellet preparation

Food pellets were prepared as in previous studies involving isopods (Žižek et al., 2011). They were made of ground maple leaves (42 wt%), ground commercial rabbit food (25 wt%) and potato powder (33 wt%), with or without (control group) added MP. All ingredients were mixed with demineralised water and heated for 10 min at 60–65 °C. The hot mixture was poured into plastic blisters and left to solidify and dry for 24 h at room temperature. The dry food pellets were approximately 0.5 cm in diameter. They were weighed and stored in a desiccator until use. Typically, the pellets were up to 3 days old before use in the experiment. The nominal mass concentration of MP in the food pellet was 4 mg g^{-1} dry weight (0.4% w w^{-1}) which is in the range of realistic mass concentrations of total plastic particles in industrial compost (0.2%), but approximately 10 times higher than the concentration of particles below 5 mm (0.04% w w^{-1}) (Gajšt, 2016). The average number concentration of particles was 496 of particles g^{-1} food in the case of facial cleanser particles and 1084 particles g^{-1} food in the case of plastic bag particles.

2.4. Experiments with isopods

2.4.1. Experimental design

The feeding experiment with isopods was performed according to a previously applied protocol where animals were exposed individually in a Petri dish (Golobič et al., 2012; Jemec et al., 2016a, 2016b)