PET microplastics do not negatively affect the survival, development, metabolism and feeding activity of the freshwater invertebrate *Gammarus pulex*.

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**Abstract**

Over the past decade, microscopic plastic debris, known as microplastics, emerged as a contaminant of concern in marine and freshwater ecosystems. Although regularly detected in aquatic environments, the toxicity of those synthetic particles is not well understood. To address this, we investigated whether the exposure to microplastics adversely affects the amphipod *Gammarus pulex*, a key freshwater invertebrate. Juvenile (6–9 mm) and adult (12–17 mm) individuals were exposed to irregular, fluorescent polyethylene terephthalate fragments (PET, 10–150 μm; 0.8–4,000 particles mL⁻¹) for 24 h. Results show that body burden after 24 h depends on the dose and age of *G. pulex* with juveniles ingesting more microplastics than adults. After chronic exposure over 48 d, microplastics did not significantly affect survival, development (molting), metabolism (glycogen, lipid storage) and feeding activity of *G. pulex*. This demonstrates that even high concentrations of PET particles did not negatively interfere with the analyzed endpoints. These results contradict previous research on marine crustaceans. Differences may result from variations in the exposure regimes (e.g., duration, particle concentrations), plastic characteristics (e.g., type, size, shape, additives) as well as the species-specific morphological, physiological and behavioral traits. As a detritivorous shredder *G. pulex* is adapted to feed on non-digestible materials and might, therefore, be less sensitive towards exposure to synthetic particles. Accordingly, we argue that the autecology needs to be taken into account and that research should focus on identifying traits that render species susceptible to microplastic exposure.

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1. **Introduction**

Plastic debris, especially its microscopic form termed microplastics (MP, commonly defined as < 5 mm in diameter), accumulates in aquatic ecosystems and is of concern due to their persistence, mobility and increasing abundance in marine and freshwater waterbodies (Auta et al., 2017; Eerkes-Medrano et al., 2015; Thompson et al., 2004). Due to their capacity to interact with or to be ingested by aquatic organisms, MP might be able to negatively affect ecosystem (reviewed by Cole et al., 2011; Eerkes-Medrano et al., 2015). So far, laboratory and field studies reported that over 160 different marine species ingest MP, including invertebrates, reptiles, fish, birds and mammals (reviewed by Lusher, 2015). In contrast, MP ingestion was confirmed in 39 freshwater species only, including crustaceans, annelids, insects, mollusks and fish (Scherer et al., 2017).

Ingested MP can trigger molecular, cellular or physiological effects (Browne et al., 2015). First investigations in freshwater amphipods and fish point towards particle-induced alterations of behavior, physiology and reproduction (Au et al., 2015; Carlos de Sa et al., 2015; Mattsson et al., 2015). Still, knowledge on the biological impact of MP is limited and sometimes conflicting, preventing a science-based risk assessment of synthetic polymers in freshwater systems (Wagner et al., 2014). To address this gap of knowledge, we investigated the uptake (i.e., body burden) as well as the effects of MP in the freshwater amphipod *Gammarus pulex*. The species is
vastly distributed throughout European rivers and lakes (Engelhardt et al., 2015; Hynes, 1955) and widely used in ecotoxicological studies (De Lange et al., 2006; McCaon and Pascoe, 1988).

Laboratory studies have mainly analyzed the uptake and toxicity of uniform, spherical MP made of polyethylene (PE) or polystyrene (PS, Lusher, 2015). While PE and PS are among the most abundant of uniform, spherical MP made of polyethylene (PE) or polystyrene (PET MP have been detected in several lake systems worldwide (Corcoran et al., 2015; Imhof et al., 2016; Zbyszewski and Corcoran, 2014). Due to its density (Corcoran et al., 2015; Imhof et al., 2016; Zbyszewski and Corcoran, 2014) and widely used in ecotoxicology details see 2.3.1 in SD). The lysates were directly frozen at -80 °C. We determined the abundance of MP in the digestive tract of G. pulex qualitatively by direct examination under the fluorescent microscope (Olympus, BX50, Hamburg, Germany, Narrow Band (NB) filter, 100× magnification) as well as quantitatively by lysing the individuals enzymatically (for methodological details see 2.3.1 in SD). The larvae were filtered on black PES membrane filters (see 2.2), fixed on microscope slides and analyzed under a fluorescent microscope with the image analyzer software ImageJ (National Institute of Health, version: 1.46r, Rockville Pike, Maryland, USA). We examined both the total particle number on the filter surface as well as the size of the particles. Due to high concentrations of heterogeneously distributed particles overlapping each other on the filter surface, we could not accurately determine particle abundance in six individuals with the analyzer software. These replicates were consequently excluded from analysis (one adult in the 0.4 p mL⁻¹ treatment, three juveniles in the 440 p mL⁻¹ treatment, two adults in the 4,000 p mL⁻¹ treatment, compare Table S1).

In the same way, we lysed four G. pulex individuals which were not previously exposed to PET particles to determine background contamination caused by lysis and analysis. In average, four green fluorescent particles per individual were observed in unexposed animals. Subsequently, all particle abundance results in the study were corrected by this blank value. In addition, the size distribution of particles in the 40,000 p mL⁻¹ stock suspension was examined using the same analytical method as for lysates (see 2.3.1 in SD).

2.4. Effects of chronic microplastic exposure

In the effect study we tested the same variables as in the uptake study (particle concentration, age), but we used five MP concentrations (0.4, 4, 40, 400 and 4,000 p mL⁻¹) as well as a negative and
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