Science of the Total Environment 757 (2021) 143735

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Transfer and effects of PET microfibers in *Chironomus riparius*

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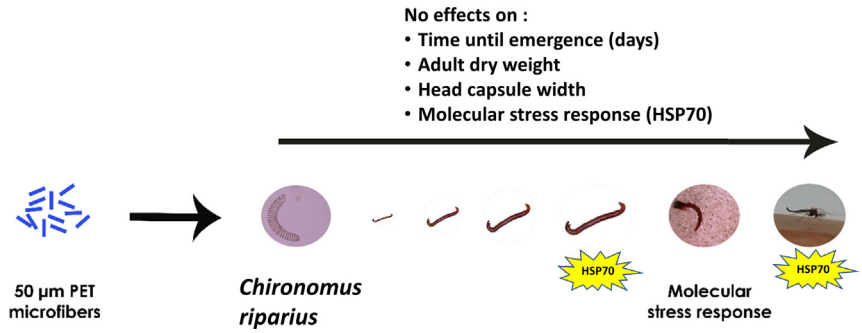
HIGHLIGHTS

- *C. riparius* were exposed to artificial sediment spiked with PET microfibers.
- PET microfibers were ingested by the chironomids and transferred to the next life stages.
- PET microfibers did not adversely affect the growth and development of chironomids.
- Stress response in same life stages were not affected by PET microfibers.
- Transgenerational effects of PET microfibers should be further studied.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 14 July 2020
Received in revised form 8 October 2020
Accepted 9 November 2020
Available online 25 November 2020

Editor: Kevin V. Thomas

Keywords:
Transfer of microplastic
Chironomus
Sediment
Toxicity
Polyethylene terephthalate
Microfibers

ABSTRACT

Multiple studies in freshwater environments have verified that microplastic particles are present in water columns, sediment, and aquatic organisms. These studies indicated that certain freshwater ecosystems may act as temporary sinks of microplastic particles, leading to accumulation in the sediment and the ingestion by benthic organisms. Polyethylene terephthalate (PET) is one of the non-buoyant polymers that has been frequently found in aquatic sediments. This study aims to investigate a possible transfer of PET microfibers from aquatic to the terrestrial habitats and addressed selected effects (i.e. survival, general stress response, and growth) of PET microfibers using *Chironomus riparius*, a frequently applied model organism in ecotoxicological research. To assess the growth and development of *C. riparius*, a modified 28-day sediment chronic toxicity test was conducted, in which the main endpoint is time until emergence of the larvae. In this assay, *C. riparius* were exposed to artificial sediments spiked with PET microfibers. In addition, weight and head capsule lengths of the larvae were also measured. As a general stress response marker on the molecular level, Heat Shock Protein 70 (HSP70) levels were measured in two involved life stages, i.e. larvae and adults. Using staining method, ingestion of PET microfibers was verified in the adult sample. Our results clearly demonstrated that ingested microfibers by *C. riparius* larvae can be carried through subsequent life stages and end up in the adults. Accordingly, this is the first proof of aquatic-terrestrial transfer of PET microfibers for *C. riparius*. However, toxicity test results showed that there was no significant effect on the time until emergence, weight or head capsule lengths in the organisms exposed to PET microfibers compared to control organisms. HSP70 measurements showed no significant effects between control and exposure groups in the same life stage. The result suggests that PET microfibers in the applied concentration do not exert adverse effects both on organism and subcellular level in one generation.

1. Introduction

More than 4 million tons of plastic debris have been estimated to annually enter and pollute the oceans, mainly via rivers (Lebreton et al.,

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https://doi.org/10.1016/j.scitotenv.2020.143735
0048-9697/

2017; Schmidt et al., 2017). However, recent studies have detected fluctuations of the number of plastic particles in the water column along rivers, such as the river Rhine (Heß et al., 2018; Mani et al., 2015), suggesting that rivers may act not only as carriers but also as sinks for microplastic particles (Nel et al., 2018). This has been verified by frequent findings of microplastic particles in freshwater sediments, representing potential risks to benthic invertebrates (Horton et al., 2017; Vermaire et al., 2017; Mani et al., 2019). Certain plastic polymers such as polyethylene terephthalate (PET) have a higher density than water and are likely to deposit if flow velocities or discharge are not too high (Claessens et al., 2013; Hübner et al., 2020). Even though some polymers have a lower density than water, biofouling and aggregation with organic materials or decreased flow velocities due to impoundments enhance the possibility of microplastic particles sedimentation.

The number of studies on the biological effects of microplastic particles to freshwater and sediment-dwelling organisms is growing, but the results are ambiguous and offer no clear trends on the toxicity of these pollutants, as reviewed in Haegerbaeumer et al. (2019). Microplastics particles that are ingested by organisms may adhere to intestinal tissues causing inflammation (Lei et al., 2018) or accumulate inside the gut (Jemec et al., 2016). Further risks are posed by additives and adsorbents on the particles such as organic chemicals, toxic metals, or biofilm harboring pathogenic or invasive microorganisms (Ivleva et al., 2017) which suggests that potential adverse effects might be due to both physical and chemical characteristics of microplastic particles.

Microfibers are among the most abundant plastic forms found in water and sediments (Gago et al., 2018; Haave et al., 2019; Hurley et al., 2017). These fibers mostly derive from synthetic garments manufactured from polymer fibers that are shed during the washing process and enter the environment via wastewater (Carney Almroth et al., 2018; Hartline et al., 2016). Microfibers have been found to exert negative effects on organisms, e.g. deformities in *Ceriodaphnia dubia* carapaces (Ziajahromi et al., 2017) and higher mortality in *Daphnia magna* (Jemec et al., 2016). However, these studies were conducted on pelagic filter-feeding organisms, leaving a lack of data on organisms from other feeding groups and habitats. We therefore tested the effects of PET microfibers on a sediment-dwelling organism, which is classified as a collector-gatherer.

Chironomus riparius (Meigen 1804) is an invertebrate species belonging to the Nematoceran Diptera, whose larvae are frequently used to determine ecological quality and have been extensively studied in ecotoxicological research. They have been proven to be suitable for acute and chronic toxicity testing due to their sensitivity to chemical substances, their wide geographic distribution, and their short life span (Watts and Pascoe, 1996; Simpson and Kumar, 2016). Previous studies showed that exposure of *Chironomus tepperi* larvae to polyethylene (PE) microspheres negatively affected their time of emergence and body weights (Ziajahromi et al., 2018). Moreover, recent results using another mosquito species, *Culex pipiens*, revealed that polystyrene (PS) particles were transferred from the aquatic larvae to the adult stage, thereby presenting a potential pathway for contamination of terrestrial environments and food webs by aquatic sources (Al-jaibachi et al., 2018).

Thus, we hypothesize that microfibers ingested by *C. riparius* larvae can also be carried through subsequent life stages up to the adults and therefore represent a transfer of microplastic particles from aquatic to terrestrial ecosystems. Furthermore, we hypothesize that the uptake of the microfibers will exert negative effects on the physiology and the life cycle of the test organisms, using time until emergence as the main endpoint. In addition to the main endpoint, dry weight, head capsule width, and heat shock protein 70 (HSP70) concentrations were measured to provide complementary data on the sublethal effects that might affect the development process. Since the toxicity of microplastic particles depends on several variables such as polymer type, sizes, and shapes (Lehtiniemi et al., 2018), HSP70 levels were measured to indicate a general stress response that can be expressed after long-term exposure to physical and chemical stressors (Frank et al., 2013; Imhof et al., 2017; Rhee et al., 2009). A previous study on *Lumbricus terrestris* showed altered stress response shown by significant difference of HSP70 levels after exposure to microfibers (Prendergast-Miller et al., 2019). We used fibers of polyethylene terephthalate (PET), a type of non-buoyant polymer frequently found in freshwater sediments (Horton et al., 2017; Li et al., 2019), which has not yet been very often used as test substance compared to other polymers such as polyethylene (PE) and polystyrene (Triebskorn et al., 2018).

2. Material and methods

2.1. Test organisms

Chironomus riparius (Meigen 1804) egg ropes were obtained from an in-house culture from the Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam. The larvae were hatched by soaking eggs in reconstituted water (Marinkovic et al., 2011) and transferred to an 15 × 25 × 50 cm glass aquarium filled with rinsed sand (WECO GmbH, Germany; grain size <200 μm) and reconstituted water. Constant aeration was provided using an air pump and food of 600 mg ground fish flakes (TetraMin, Germany) was administered every second day. The culture was kept in a temperature-controlled room at 20 °C with a light cycle of 16 h:0.5 h:7.5 h (light:dim:dark). Emerged individuals were transferred to another glass box covered with insect nets and equipped with a glass beaker filled with reconstituted water for the individuals to lay eggs so that <24 h old larvae could be reared. According to the OECD guideline 218 (OECD, 2004), the larvae that are used in the test should hatch in 2–3 days.

2.2. Experiment set-up

Freshly produced PET was obtained as a spool of fibers with a diameter of 14 μm (Goodfellows Cambridge Limited, England; ES305710/1; density 1.38 g/cm^3). The fibers were processed to microfibers at the Helmholtz Centre for Environmental Research (UFZ), Magdeburg, according to a novel protocol (Cole, 2016). Briefly, the fibers were wound tightly around a spool, and subsequently frozen using freezing agent to form blocks of fibers. The blocks were subsequently mounted on the cryotome and cut to 50 μm microfibers, then diluted with hot water to make a stock solution. The concentration of the solution was measured in triplicate using a counting chamber (Marienfeld Neubauer, Carl Roth GmbH, Germany). The size of 50 μm was chosen based on the ingested microplastic particle size preference of *C. riparius* (Silva et al., 2019). The concentrations of microfibers in the treatments are 500 particles/kg dry weight sediment, 5000 particles/kg dry weight sediment, and 50,000 particles/kg dry weight sediment. The concentrations were chosen in accordance with a recommendation for microplastic particles concentrations in sediment to be applied when testing effects on organisms (Triebskorn et al., 2018). Moreover, these concentrations are in accordance with in situ levels, 500 particles/kg dry weight sediment in Lake Ontario, Canada (Ballent et al., 2016) and 4900 particles/kg dry weight sediment in Rhine river (Leslie et al., 2017). The lowest concentration was already applied in a previous study exposing *Chironomus tepperi* to PE microspheres (Ziajahromi et al., 2018). The higher concentrations were prepared to resemble more extreme pollution events, adding up to a total of three exposure concentrations and a control, each consisting of five replicates.

2.3. Sediment preparation and toxicity test procedure

The chronic sediment toxicity test was conducted in the Institute of Landscape Ecology, University of Münster. Formulated sediment was prepared according to OECD guideline 218 (OECD, 2004). A microfiber stock solution was prepared to further be spiked into

100 g of sand per treatment according to the concentration series (see Supplementary Table 1). The mixing of the formulated sediment was done in the following order: dry materials, i.e. sand, spiked sand, and kaolinite clay (Acros Organics, Belgium, CAS 1332-58-7) were prepared first in a ceramic mixing bowl. 35 g of peat were gently added to 350 mL deionized water and mixed thoroughly. The pH in each beaker was adjusted to 5.5 ± 0.5 using a suspension of $CaCO_3$ powder in deionized water. The peat mixture was then covered with parafilm to avoid evaporation and homogenized using a horizontal shaker with the speed of 120 rpm (SM–30, Edmund Bühler GmbH, Germany). The appropriate final pH of the mixture was 6.0 ± 0.5, which was achieved after homogenization for 7 days. To compensate for the different volumes of $CaCO_3$ solution added depending on the pH of each peat mixture, deionized water was adjusted to all beakers so that each mixture has the same water content. The peat mixtures were subsequently added to the mixing bowl and mixed using a stainless steel spoon. Finished formulated sediment mixture was divided evenly to each 600-mL beaker with 9.5 cm in diameter (Fisherbrand, Germany), the base of which was regularly tapped to remove air bubbles. Then, 300 mL reconstituted water was added to each beaker, fulfilling the water-sediment height ratio of 1:4 according to the OECD guideline 218 (OECD, 2004). An aeration system using silicone pipes was inserted to each beaker and this system was left for three days to stabilize.

Prior to the start of the test, five *C. riparius* egg ropes, each consisting of roughly 200–300 eggs, were isolated in beakers filled with reconstituted water to hatch in order to obtain larval organisms less than 3 days old. Before insertion of the larval organisms, the aeration system was turned off and the dissolved oxygen, temperature, and pH were measured from random beakers from each concentration. Into each beaker, 35 individuals were gently inserted using a Pasteur pipette. The aeration system was turned on 4 h after insertion. Larvae were fed 0.6 mg Tetramin/larvae/day, as recommended in the OECD guideline 218 (OECD, 2004), which were added to each beaker in the form of suspension of the ground flakes and deionized water. Each beaker was covered with perforated cling film to minimize evaporation and contamination. The water level was refilled every three days with deionized water to compensate for evaporated water.

The test system was inspected every day for 28 days. As soon as the chironomid emerged, the day of emergence was recorded, the sex was visually identified based on morphological parameters, i.e. males have a slender body and plumose antennae (Armitage et al., 1995). The organisms were stored in ethanol as a preservation measure prior to staining and weight measurement. For weight analysis, preserved individuals were dried at 70 °C for 24 h and weighted separately based on sex. Head capsule widths of exuviae from emerged individuals were measured using a digital microscope (Keyence VHX 900 F, Japan) and ImageJ (National Institute of Health, USA).

2.4. Verification of microplastic particles ingestion

In order to verify the ingestion of microfibers by *C. riparius*, a staining method was performed as described by Erdbeer and Kiel (2019). A subsample of 12 individuals (2 larvae and 10 imagines) per concentration was used. The larval subsample was kept small, to not interfere with the other analyses. The individuals were cleared using KOH 13% (Merck, Germany, CAS 1310 – 58 – 3) for 24 h. Then, the samples were washed to remove microfibers that might have attached to the outer part of the individuals using 2 different concentration series of ethanol, 70% and 96%, each for 24 h. After the clearing, the body and head capsule remain intact and become translucent. For staining, 0.001 g of the dye powder and 0.75 μL of dye intensifier (iDye Poly, Jacquard Products, USA) were added to 150 mL deionized water and heated to 80 °C in a water bath. The samples were then inserted to this solution and heated for 4 h. The solution was stirred regularly using a glass rod to avoid the samples from sticking to the wall of the beakers. Afterwards, the stained specimens were briefly and carefully washed in deionized water and inspected under the microscope. PET microfibers appear as thick blue spots (see Fig. 1) and could be distinguished from the natural particles in the sediment because the dye specifically attached to the PET. Using this method, it is possible to detect both the presence and the position of microfibers inside the gut without destroying the specimens.

2.5. HSP70 measurement using western blot

HSP70 measurements and analyses were conducted in the Department of Aquatic Ecology, University of Duisburg-Essen. At day 14 of the toxicity test (Marinković et al., 2012), four larval individuals from each test vessel were euthanized using liquid nitrogen and stored at −80 °C. All larvae were already in the fourth instar stage. To compare HSP70 levels in larval and adult individuals, additionally 2–3 adults from each test vessel were also euthanized at the day of emergence using the same procedure. Frozen samples were thawed and mechanically homogenized using a stainless steel micropestle in 1.5 mL tubes and 100 μL extraction buffer (25 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 5% glycerin, pH 7.4). The samples were centrifuged for 15 min in 4 °C and 14,000 RCF. Supernatant from each sample was subsequently extracted. 20 μL supernatant and 1.8 μL protease inhibitor (P8340, Sigma-Aldrich, USA) were added to 180 μL extraction buffer for further total protein content measurement using Pierce™ BCA Protein Assay Kit (Thermo Fischer Scientific, USA) and albumin standard (Thermo Scientific, USA) in accordance with Chen et al. (2015). Subsequently, constant protein weights were separated by discontinuous SDS- polyacrylamide gel electrophoresis (see also Frank et al., 2013). In detail, 50 μL supernatant were added to 50 μL Laemmli – buffer (20% glycerol, 3% sodium dodecyl sulfate (SDS), 0.3% mercaptoethanol, 10 mM Tris, 0.005% bromphenole blue) and boiled at 95 °C for 5 min in a thermal block (Ditabis, Germany). 4 μL protein ladder (PageRuler™ Plus, 10 to 250 kDa, Thermo Fischer Scientific, USA) and 20 μg total protein from each sample were subjected to discontinuous SDS-PAGE (6% and 12% acrylamide -biacrylamide gel) for 50 min at 180 Volt. The electrophoresized protein was transferred onto nitrocellulose membrane covered with Whatman paper using a semidry blotting chamber (Bio-Rad Laboratory Inc., USA) for 60 min at 14 V, corresponding to 0.4 A. Afterwards, protein was blocked in 50% horse serum (Cedarlane Labs, Canada) and 50% tris buffer saline (TBS) for 1 h. Subsequently, protein was incubated in 9 mL TBS, 1 mL horse serum and 5 μL monoclonal anti HSP70 (H1547, Sigma-Aldrich, USA) for 2 h. After washing the membrane in TBS for 5 min, the membrane was incubated in secondary antibody solution (Anti-Mouse IgG, Sigma A4416, Sigma-Aldrich, USA) for 1 h. Following incubation, the membrane was washed for 5 min in TBS. HSP70 was further visualized using 2 mL chloronaphthol (3 mg/mL in ethanol, Sigma – Aldrich, USA, CAS 604-44-4) and 40 μL H_2O_2 (30% wt with inhibitor, Sigma-Aldrich, USA, CAS 7722-84-1) for 3 min. Staining reaction was stopped using bidest water. After drying between Whatman papers, each membrane was scanned using conventional image scanner (CanonScan LiDE700F, Japan). HSP70 appears as black bands on the nitrocellulose membrane. To determine HSP70 levels, scanned images were processed using the function “Gels” in the software ImageJ, which quantifies the size and density of the gray bands in the form of peaks. The integrated area of each peak represents the amount of proteins.

2.6. Data processing

For the chronic sediment toxicity test, the number and sex of emerged individuals from each test beaker were recorded every day. Prior to statistical analysis, each dataset was tested for normal

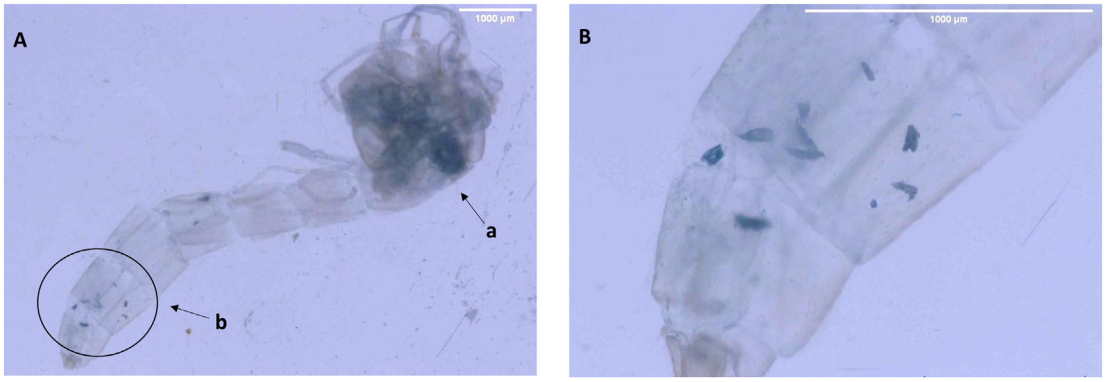

Fig. 1. Aggregated PET microfibers in adult *C. riparius* in the subsample from the concentration of 5000 particles/kg dry weight sediment (A) 50 times magnification, with head (a) and gut (b) intact, black circle points to PET microfibers (B) 200 times magnification.

distribution in every individual endpoint. For sample sizes smaller than 50, a Shapiro-Wilk test was performed, while the Kolmogorov-Smirnov test was performed for sample size larger than 50 but less than 100 (Ghasemi and Zahediasl, 2012). If the data were normally distributed, significant differences among the concentration groups were tested by Student's *t*-test, while on data without normal distribution, Mann-Whitney *U* test was performed between control and each treatment group. Statistical analysis was performed using the software R Studio version 1.2.1335.

3. Results

3.1. Microplastic ingestion and emergence of C. riparius

PET microfibers could be verified in two out of 10 adult individuals from the concentration of 5000 particles/kg dry weight. Some of the microfibers formed aggregates and were clearly visible within the digestive tract (see Fig. 1). However, ingestion of the microfibers was not associated with obvious adverse effects according to the tested parameters. No mortality was recorded in either treatments or control groups. Since male individuals emerged earlier (see Supplementary Fig. 1), emergence data are presented separately for male and female individuals (Fig. 2). Neither for males nor for females, significant differences for the average day of emergence among the different treatments were detected ($p > 0.05$, Mann-Whitney *U* test for male individuals, *t*-test for female individuals). However, individuals of the highest plastic concentration (50,000 particles/kg sediment) tended to emerge 1 to 3 days earlier than individuals from the other treatments in comparison to the control (Fig. 2).

3.2. Dry weight and head capsule width

No significant effects of PET microfibers concentration on the dry weight of male individuals were found (Fig. 3, $p > 0.05$, Mann-Whitney *U* test). However, female individuals of the lowest concentration group (500 particles/kg) were significantly heavier than control females ($p > 0.05$, Mann-Whitney *U* test), although no significant differences occurred for the higher concentration groups. Head capsule width was also not affected by exposure to PET microfibers, at any concentration (Fig. 4).

3.3. Heat shock protein 70 (HSP70)

No significant effects of PET microfibers exposure could be detected on HSP70 level, neither in larval nor adult samples (Fig. 5, $p > 0.05$, Mann-Whitney *U* test for larval samples, Student's *t*-test for adult samples). In adult *C. riparius*, HSP70 levels appear to be lower in the 500 and 5000 particles/kg sediment treatments than in the control.

4. Discussion

In the present study, aggregates of PET microfibers were clearly detected in the digestive tract of *C. riparius* after their emergence. This confirmed previous studies on the ingestion of microplastics by *C. riparius* larvae (Scherer et al., 2017). This observation is also in accordance with a study in which *Culex pipiens* were exposed to polystyrene (PS) particles within a size range of 2–15 µm (Al-jaibachi et al., 2018). The authors showed that the particles were transferred from the feeding stage (larvae) to the pupa and subsequently to the adult stage after

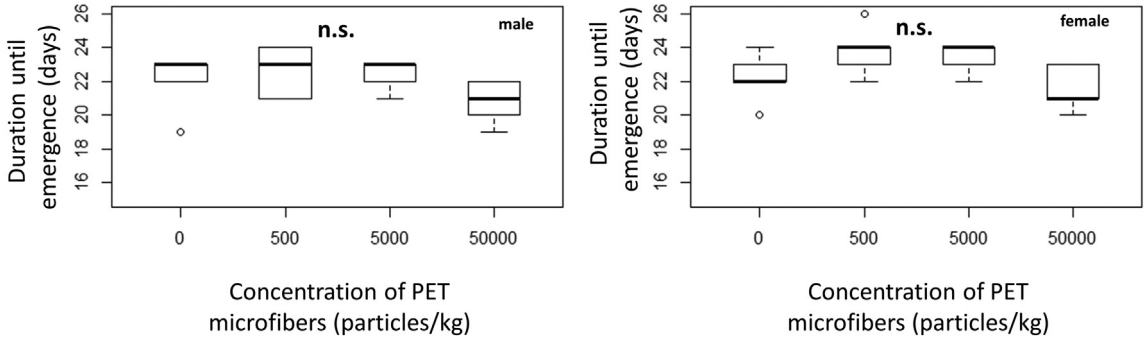

Fig. 2. Average day of emergence of male and female *C. riparius* individuals. No significant differences were detected ($p > 0.05$; Mann-Whitney *U* test for male individuals, *t*-test for female individuals; $n = 31$ for each replicate).

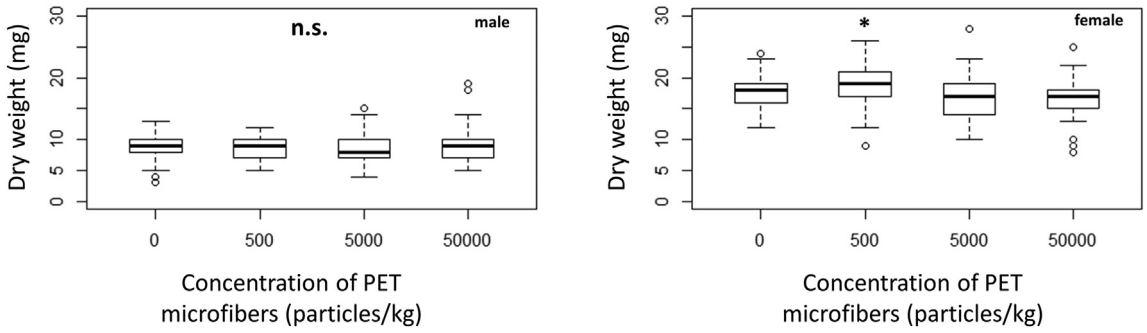

Fig. 3. Dry weights of male and female *C. riparius*. Significant differences between control and each of the exposure group were indicated by asterisks ($p > 0.05$; Mann-Whitney *U* test; in each concentration, $n = 69$ for male individuals, $n = 58$ for female individuals).

metamorphosis. In the present study, the transferability of microfibers from larval aquatic stages to terrestrial adult stages for a different species, different polymer type, and shape was shown for the first time. The emerged imagines may serve as a food resource for terrestrial food webs, and therefore allow for a transfer of aquatic pollutants to terrestrial ecosystems. However, ingestion of microfibers was only found in 4% of the subsample, all of which were exposed to the high concentration. This might indicate either a fast egestion or a low availability of the microfibers applied in environmentally relevant concentrations.

Results of the present study showed that 50 μm PET fibers did not significantly affect time until emergence, growth (body mass or head capsule width), nor the sublethal stress markers (HSP70) in larval *C. riparius*. Hence, this set up of parameters (plastic size, shape, concentration, polymer type and investigated species) most likely does not exert any evident adverse effects on this species. The results are in line with findings of Khosrovyan and Kahru (2020) whose results showed also no significant effects on emergence, time to emergence, sex ratio of imagines nor the number of egg clutches per female when exposing larvae of *C. riparius* to irregularly-shaped co-polyamide (PA, 10–180 μm) particles. Furthermore, another study revealed that *C. riparius* is insensitive to PVC (polyvinyl chloride) particles as only very high (and therefore environmentally irrelevant) concentrations induced adverse effects of reduced emergence and reduced mass on this species (Scherer et al., 2019). Reductions of larval growth and delayed emergence of *C. riparius* was also detected at a concentration range much higher than this present study for PE-particles (Silva et al., 2019). Another study found longer development time in *C. riparius* after exposure to a mixture of PET, PS, PVC, PA through three different exposure routes: sediment, water column, and water surface (Stankovic et al., 2020). Experiments with a related species (*C. tepperi*) indicated that PE-particles negatively affected the survival, growth (i.e. body length and head capsule) and emergence of *C. tepperi* (Ziajahromi et al., 2018).

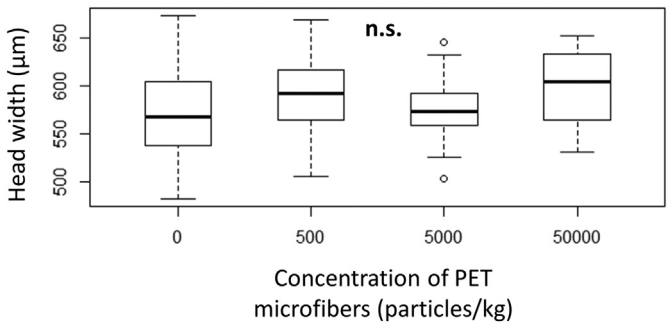

Fig. 4. *C. riparius* head capsule widths showing no significant differences ($p > 0.05$; *t*-test; $n = 20$ for each concentration).

The fibers used in our study were made of PET, which was shown to have ambiguous effects on the aquatic fauna based on the following studies. PET fibers fed to daphnids lead to increased mortality after 48 h when daphnids were not pre-fed with algae prior to the experiment whereas no effect was reported when daphnids were fed before the experiments (Jemec et al., 2016). PET particles also increased mortality of daphnids in comparison with naturally-occurring particles (Gerdes et al., 2019). Marine copepods (*Parvocalanus crassirotris*) were shown to have reduced population size and Histone3 gene expression (Heindler et al., 2017) and *Calanus helgolandicus* expressed reduced clearance rate and increased fecal pellets sinking rate (Coppock et al., 2019). Exposure of *Ruditapes philippinarum* to irregular-shaped PET showed altered oxidative status on the organisms' gills (Parolini et al., 2020a). There are implications that the effects of PET might be specific to certain feeding groups and exposure scenarios. In contrast to filter feeders, benthic feeding groups are so far not known to be adversely affected. Neither the amphipod *Gammarus pulex* (mostly shredder; Weber et al., 2018) nor the snail *Potamopyrgus antipodarum* (grazer; Imhof and Laforsch, 2016) were adversely affected in their development until maturity. However, effects on the oxidative status of the sea urchin *Paracentrotus lividus* was found after exposure to irregular-shaped PET (Parolini et al., 2020b). In terrestrial animals, exposure to PET microfibers reduced food intake and induced oxidative stress in the terrestrial snail *Achatina fulica* (Song et al., 2019). Hence, the benthic collector-gatherer *C. riparius* can be added to those feeding groups in which no taxon was affected. Collector-gatherers feed on detritus, which contains all non-living particulate organic matter and non-photosynthetic microorganism and has been found to be the most frequent food type in the gut beside sediment (Ristola et al., 1999) and silt particles (Osborne et al., 2000). PET microfibers would fit in with the gut content in terms of material hardness and the mechanism of ingestion would not be different to that of detritus and woody debris because of the nonselective feeding mechanism in the older instar stages (Armitage et al., 1995; Scherer et al., 2017). Furthermore, benthic invertebrates have peritrophic membranes that cover the inner lining of the gut and protect the gut from abrasive damage to the tissue through different types of food including woody debris and materials with hard surfaces (Jarial and Engstrom, 1997; Weber et al., 2018). One further explanation of these results could also be the chemical characteristics of PET, which is highly inert and has been originally engineered for food purpose even though nowadays its usage also covered non-food purpose, therefore the amount of colorants, antioxidants, plasticizers, and printed ink are minimal in the events of migration and leaching (Capolupo et al., 2020; Welle, 2011; Zimmermann et al., 2019).

Although there is only limited knowledge of the plastic shape, plastic fibers presumably induce more adverse effects on aquatic fauna than other shapes of plastic particles. Polypropylene fibers reside longer in

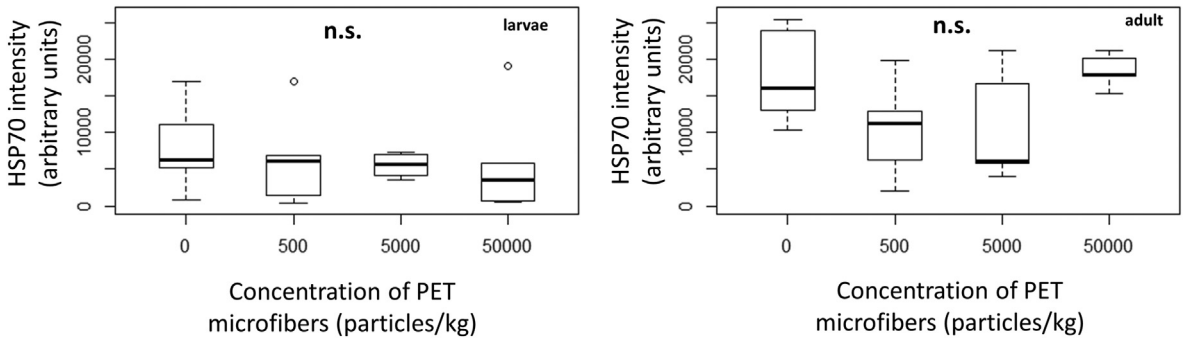

Fig. 5. HSP70 expression in larvae and adult individuals of *C. riparius* ($p > 0.05$; Mann-Whitney *U* test for larvae sample, *t*-test for adult samples; $n = 5$ for each concentration). Units of HSP70 are arbitrarily assigned based on area width and density from western blot measurements.

the gut of the amphipod *Hyallela azteca* than PE fragments leading to decreased growth, reproduction, and higher mortality (Au et al., 2015). Furthermore, polyester fibers induce carapace and antenna deformities in *Ceriodaphnia dubia* while no deformities were observed after exposure to PE beads. In the same study, a significant reduction in neonate numbers and adult body size was observed after exposure to polyester fibers, while higher exposure to PE microbeads was needed to produce a similar effect for neonate numbers and adult body size, respectively (Ziajahromi et al., 2017). Hence, PET particles might also have little effects on *C. riparius*. However, future studies should focus on the potential of different effects of particles and fibers of the same plastic type to evaluate the impacts.

The present study showed that HSP70 levels did not differ significantly after exposure to 500–50,000 particles/kg dry weight sediment both in larval and adult chironomids. Different levels of HSP70 in adult and larval samples were most likely attributed to the level of heat shock proteins that are produced through different life stages (Morales et al., 2011; Moreira-de-Sousa et al., 2018). Previous studies showed alteration of HSP70 levels after exposure to different types of microplastic particles in *M. galloprovincialis* (Détrée and Gallardo-Escárate, 2017) and *D. magna* (Imhof et al., 2017). However, studies measuring HSP70 after exposure to microplastic particles are relatively rare in comparison to other biomarkers. Therefore, further studies should corporate a battery of biomarkers to see the sublethal effects of microplastic particles on the oxidative stress, neurotoxicity, and energy metabolism to shed light on the mechanisms of toxicity (Prokić et al., 2019).

In this present study, only one generation of chironomids was investigated. However, there are still very few studies on the transgenerational effects of microplastic particles on invertebrates. Currently available studies suggested that exposure of the filter-feeder *D. magna* to microplastic particles exerted transgenerational effects, such as reduced growth, reproduction, population growth rate, and recovery (Martins and Guilhermino, 2018). Another study showed significant survival reduction of *D. magna* within four generations after being exposed to very high concentration of irregular polystyrene in comparison to exposure to natural particles (Schür et al., 2019). Transgenerational effects of microplastic particles on benthic collector-gatherers are similarly possible and therefore need to be further studied.

5. Conclusions

This study shows that exposure of *Chironomus riparius* to 50 μm PET microfibers does not cause evident adverse effects on selected organism and molecular markers, shown by insignificant differences in all endpoints. Biomarker measurements of HSP70 showed that PET microfibers do not exert clear stress responses in the organisms, but further studies using more biomarkers indicating different toxic mechanisms could provide different results and should not be neglected. However, we could confirm that microfibers can be retained through different life stages, suggesting effects on terrestrial food webs and calling for further studies on multigenerational toxicity. Future research should also focus on the effects of other types of non-buoyant polymers to the growth of *C. riparius* to mimic real-life scenarios in freshwater sediments. *Chironomus* sp. could be incorporated more frequently in studies on microplastic particles toxicity since it is a highly abundant benthic invertebrate genus and an essential part in the assessment of the ecological status of water bodies (European Union Water Framework Directive). Based on current knowledge, it is suggested that toxicity of microplastic particles cannot be generalized, because the umbrella term "microplastics" encompasses numerous polymers with different characteristics just as the term "chemicals" contains a very wide variation of different substances with different characteristics (Rochman et al., 2019). Future studies will have to continue adding information on the impacts of the vide variety of plastic types and shapes on organisms, food webs and ecosystems. This includes results with no significant effects within the tested parameters, as it still contributes important knowledge on this highly relevant environmental problem.

CRediT authorship contribution statement

Lydia Setyorini: Conceptualization, Methodology, Investigation, Writing – original draft. **Diana Michler-Kozma:** Conceptualization, Methodology, Validation, Writing – review & editing. **Bernd Sures:** Supervision, Writing – review & editing. **Friederike Gabel:** Supervision, Writing – review & editing.

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.

Acknowledgments

We are highly grateful to Helmholtz Centre for Environmental Research (UFZ) Magdeburg for the assistance with the preparation of microfibers. We want to particularly thank Alessandro Manfrin for the support in establishing *C. riparius* culture, Marion Woermann and Julios Kontchou for the guidance in the sediment toxicity test, Daniel Grabner for the invaluable support in HSP70 measurements. We also thank Titus Rombouts and Taymaz Azarno from Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam for their support in *C. riparius* culture maintenance. We want also to thank Rense Jongsma from Institute of Molecular Microbiology and Biotechnology, University of Münster for the support in sample storage and handling.

Funding

This study was supported by the German Federal Ministry of Education and Research (BMBF) as part of the MikroPlaTaS (Microplastics in Dams and Reservoirs: Sedimentation, Spread, Effects) project (BMBF grant no. 02WPL1448B).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.143735.

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