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Microplastics of different characteristics are incorporated into the larval cases of the freshwater caddisfly *Lepidostoma basale*

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ABSTRACT: Plastic pollution is present in aquatic systems worldwide. While numerous studies have investigated microplastic interactions with marine organisms, microplastic effects on freshwater organisms, especially insects, have been rarely studied. Previous studies have mainly focused on dietary uptake of microplastics, but the presence of microplastics in animal constructions is largely unknown. To date, microplastics have only been observed in the tubes of a marine polychaete species. In freshwater systems, common caddisfly (Trichoptera) larvae build cases by using larval silk and mineral grains from benthic sediments, which are known microplastic sinks. Therefore, we examined caddisfly cases for microplastic presence. We collected caddisfly *Lepidostoma basale* cases in the field, disintegrated them using hydrogen peroxide, and determined microplastic polymer type through micro-Fourier-transform infrared spectroscopy. We found primary and secondary microplastics of different shapes, colors, sizes and chemical compositions (e.g. polypropylene, polyethylene, polyvinyl chloride). Thus, this is the first study to show that microplastics are present in the biological construction of a freshwater organism. Larval stages are usually more vulnerable than adult individuals, and microplastics can transport persistent organic pollutants and emit toxic leachates. In the caddisfly larval case, those substances are in close proximity to the sensitive larval body, which may be harmful for the larva and may eventually impede its development. We discuss the potential of caddisfly larval cases to act as microplastic bioindicators in freshwater habitats.

KEY WORDS: Synthetic polymers · Freshwater insects · Trichoptera · Case construction · Stream

1. INTRODUCTION

Freshwater ecosystems worldwide, including streams and rivers, are polluted with microplastic debris (Eerkes-Medrano et al. 2015). Such microplastics (plastic particles <5 mm, Moore 2008) vary in shape (fragment, film, pellet [spherical], foam and fiber; Free et al. 2014), color and chemical composition, and are classified as primary and secondary microplastics (Cole et al. 2011). Primary microplastics such as spherical microbeads are manufactured to be of a very small size. These spheres have multiple applications, e.g. as exfoliating agents in cosmetics, and may reach the environment because waste water treatment plants are often inefficient in removing them (Rochman et al. 2015). In contrast, secondary microplastics, such as fragments, films and fibers, are created when larger plastic debris becomes (e.g. mechanically or photolytically) degraded into smaller plastic pieces (Cole et al. 2011). Plastic items from land-based sources can be transported to streams and rivers by wind, rain drainage, waste water and improper disposal of plastic waste (Duis & Coors 2016). The industrial mass production of plastics

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started in the 1940s (Cole et al. 2011), and microplastics were reported in the oceans as early as the 1970s (Carpenter et al. 1972, Carpenter & Smith 1972). Since then, numerous studies have examined the impact of microplastics on marine organisms (Erren et al. 2013, Wright et al. 2013, Egbeocha et al. 2018), including vertebrates (Germanov et al. 2018) and invertebrates (Cole et al. 2013, Kaposi et al. 2014). Rivers are important sources of marine microplastics, as they transport plastic debris from inland waters to the ocean (Lebreton et al. 2017). Despite this important pathway, freshwater microplastics have been considerably less studied than microplastics in the marine environment (Eerkes-Medrano et al. 2015). Regarding the interactions of microplastics with freshwater organisms, recent studies have mainly focused on microplastic ingestion by vertebrates, e.g. fish (Horton et al. 2018, McNeish et al. 2018), and invertebrates, such as mollusks (Su et al. 2018) and crustaceans (Weber et al. 2018), and on the resulting physiological effects (Ding et al. 2018, Redondo-Hasselerharm et al. 2018). Despite the great abundance and ecological importance of aquatic insects (Suter & Cormier 2015), microplastic studies on those organisms are just emerging and largely focus on microplastic dietary uptake (Kim et al. 2018, Nel et al. 2018, Windsor et al. 2019).

Besides microplastic ingestion, microplastics may be incorporated into the structures built by animals. For instance, the marine tube-building polychaete *Gunnarea gaimardi* (Quatrefages, 1848) incorporates microplastics into its housing (Nel & Froneman 2018), and thereby fixes microplastic particles in a biological construction. Similar biological structures in freshwater habitats are larval cases that several epibenthic caddisfly (Trichoptera) species build. It has recently been suggested that microplastics may be fixed in caddisfly larval cases, but no analysis such as micro-Fourier-transform infrared (μFTIR) spectroscopy has been performed to verify that the particles which were observed in caddisfly larval cases were microplastics and that they were not, for example, parts of ceramics or cardboard (Tibbetts et al. 2018). Hence, to investigate if freshwater microplastics may be incorporated into caddisfly larval cases, we analyzed whether microplastics are present in those cases and which characteristics (shape, polymer type, color, size) such microplastics possess.

After hatching from the eggs, the larvae of many caddisfly species start building cases to protect themselves from predators (Boyero et al. 2006) and from environmental influences (Zamora Muñoz & Svensson 1996). As the larvae grow, new material is added to the anterior end of the case and secured with silk filaments produced by the larvae (Stewart & Wang 2010). For case building, caddisfly larvae actively collect different biotic (e.g. leaves; Sheath et al. 1995, Moretti et al. 2009) and abiotic (e.g. sediment grains; Gaino et al. 2002, Okano et al. 2012) materials, and some species use both material types (Hansell 1972). Different caddisfly species differ in their preferences for certain case-building materials (Hanna 1961).

Recently, a few potential sentinel species for microplastic pollution in freshwater ecosystems have been suggested, including tadpoles (Hu et al. 2018) and Asian clams (Su et al. 2018), but none has been established so far. Therefore, we discuss the potential role of caddisfly larval cases as bioindicators for freshwater microplastics which might facilitate microplastic assessment in streams and rivers.

Given that a marine polychaete species incorporates microplastics into its tube (Nel & Froneman 2018), and freshwater sediments are a sink for microplastics (Castañeda et al. 2014), we hypothesized that microplastics would be fixed in the larval cases of the caddisfly *Lepidostoma basale*, which uses sediment grains for case building (Skuja 2010). We tested our hypothesis through an observational field study.

2. MATERIALS AND METHODS

2.1. Study site

We conducted our study in the Saynbach stream (50.438399° N, 7.5732430° E), located in the Schlosspark Sayn, a public park in the town of Bendorf (Rhineland-Palatinate, Germany). This stream originates in Himburg (Rhineland-Palatinate) and flows directly into the Rhine River. The stream has a length of 43.7 km, a width of 3 to 4 m, a depth of 6 to 40 cm and a catchment area of 219 km^2 (Beckmann et al. 2005). Downstream of our study site in the Schlosspark Sayn, the Saynbach passes the Bendorf wastewater treatment plant before leading into the Rhine.

The abiotic conditions of the Saynbach are regularly measured by the Rhineland-Palatinate State Office for the Environment (Landesamt für Umwelt) at a nearby location (50.429° N, 7.565° E). On 10 April 2018 at 15:40 h Central European Summer Time (1 d before we collected caddisfly larvae; see Section 2.2), water temperature was 13.6°C, turbidity was 8 formazin nephelometric units, pH was 8.4, conductivity (at 20°C) was 264 μS cm^{-1}, and oxygen concentration was 10.9 mg l^{-1}. The Saynbach sediment at the study site consisted of sand and fine gravel.

2.2. Caddisfly larvae collection and identification

On 11 April 2018, we manually collected case-bearing caddisfly larvae in individual glass vials (Wheaton, DWK Life Sciences) that contained 70% ethanol. All larvae were collected at random and had intact cases. To avoid airborne microplastic contamination of the larvae, we wore cotton clothes. Furthermore, the vials used for sampling were previously rinsed with ultrapure water.

At the lab, we identified 29 of the 30 caddisfly larvae as *Lepidostoma basale* (Kolenati, 1848) under a binocular microscope according to morphological species keys (Wallace et al. 1990, Waringer & Graf 2011). This caddisfly (former synonym: *Lasiocephala basalis*) occurs in streams and rivers across Europe (Moretti et al. 1981, Beisel et al. 1998, Hoffmann 2000, Chadd & Extence 2004, Bonada et al. 2008, Skuja 2010, Verdonschot et al. 2010). It lives on woody debris, shows facultative xylophagy (Hoffmann & Hering 2000) and feeds on biofilms (Schulte et al. 2003). *L. basale* larvae can feed on invertebrates (Schulte et al. 2003) and leaf litter (Hoffmann 2000). Annual *L. basale* development proceeds through 5 larval stages and 1 pupal stage (Verdonschot et al. 2010). In all larval stages (i.e. instars), *L. basale* constructs mineral cases (Skuja 2010); in the fifth-instar larva, the case has a maximum length of ca. 1.5 cm (Hoffmann 2000). In *L. basale*, individuals of different larval stages (and thereby of different sizes) can be found simultaneously at the same location (Verdonschot et al. 2010).

We identified the remaining caddisfly larva as either *Sericostoma personatum* (Spence in Kirby & Spence, 1826) or *Sericostoma flavicorne* Schneider, 1845, whose taxonomic differentiation is not fully understood (Waringer 1987, Malicky 2005), but has recently been clarified to some extent by genetical analyses (Weigand et al. 2017). *S. personatum* larvae are detritivore-shredders that feed on leaves (Friberg & Jacobsen 1999). As we only found 1 individual of this species, we present information on microplastics in its larval case as an additional observation but focus on the larval cases of *L. basale*.

2.3. Preparation of caddisfly larval cases

We measured caddisfly larval case length along the convex case side using a digital microscope (VHX-2000, Keyence). We then removed the larvae from their cases using metal forceps. To remove any particles that adhered to the case surface but were not fixed in the case matrix, we carefully rinsed all cases in ultrapure water and put them into individual glass Petri dishes. Next, we dried the cases at 40°C for 4 h in a drying cabinet and immediately measured their dry weight using an analytical balance (XS205 Dual-Range Analytical Balance, Mettler Toledo).

To prevent microplastic contamination, we thoroughly cleaned all lab surfaces and glassware using 70% ethanol and ultrapure water before starting lab-work. Furthermore, to prevent microplastic cross-contamination between the caddisfly larval cases, we carefully rinsed our forceps between samples. Finally, to prevent airborne microplastic contamination, we immediately covered all Petri dishes containing caddisfly larval cases with aluminum foil.

2.4. Caddisfly larval case oxidation and density separation

For case disintegration, we transferred all rinsed caddisfly larval cases into individual glass beakers in which we submerged each case in 20 ml of a hydrogen peroxide solution (34.5–36.5% H_2O_2) to disintegrate them and remove any organic substances present. All beakers were then covered with parafilm and left on a shaking table at 150 rpm for 7 d until the cases were completely disintegrated. Nuelle et al. (2014) previously showed that such H_2O_2 solutions can successfully reduce organics in sediment sample matrices similar to caddisfly case matrices. Occasionally, stirring the samples with a glass rod can help in case disintegration. We ran blanks parallel to the case disintegration and scanned them for microplastics, but did not detect any parafilm or microplastic particles in the blanks. However, we found natural fibers in the blanks which we identified using µFTIR.

After case disintegration, we separated the resulting minerals from the microplastics through density separation. To do so, we transferred each sample to a glass separation funnel to which we added potassium formate (99%) until the solution was saturated at about 1.6 g ml^{-1}, a similar approach to that used to separate microplastics from sediment grains in sediment samples (Zhang et al. 2016). Again, we ran blanks with the density separation to exclude microplastic contamination from, for example, the lid of the container in which the potassium formate was stored. After approximately 3 h, the mineral grains had settled to the bottom of the funnel. We then drained the grains and filtered the supernatant onto aluminum oxide filters (Anodisc filter; pore size 0.2 µm; diameter 47 mm, Whatman) using a pressure filtration unit (model 16249, Sartorius). We chose these filters to en-

able µFTIR measurements in transmission mode during further microplastic analyses (Löder et al. 2015).

We placed the filters in small aluminum bowls, covered them with aluminum foil and placed them in a drying cabinet (50°C) for 2 d. We then visually inspected each filter for microplastics using a digital microscope (VHX-2000, Keyence) and identified all microplastics by their unnatural color and shape (Hidalgo-Ruz et al. 2012). The shapes for microplastic particle classification were sphere, film (thin and small layer), fragment (part of a larger plastic item) and fiber (Su et al. 2016, 2018). We recorded the occurrence of microplastics and measured their dimensions (maximum length of fragments, films and fibers, and the diameter of the spheres).

2.5. µFTIR analyses of microplastics found in caddisfly larval cases

We manually analyzed all microplastics found in the *L. basale* cases using a Hyperion 2000 FTIR microscope equipped with a mercury-cadmium telluride detector (Bruker) in a wavenumber range of 4000–600 cm^{-1} with 32 co-added scans and a spectral resolution of 4 cm^{-1}. The software used was OPUS 7.5, and we compared the obtained spectra with the Bruker database. As in previous studies, only particles with a hit quality of over 700 were considered as microplastics (Bergmann et al. 2017). We analyzed the microplastics using attenuated total reflectance (µATR) with a germanium crystal and an ATR 20× objective. For some particles that were difficult to analyze, we used µATR and transmission mode (with a 15× infrared objective). For measurements in transmission mode, the blank aluminum oxide filter was used for background measurements. Measuring the blank filter material as a background in transmission measurements is a common procedure for µFTIR measurements in microplastics research (Löder et al. 2015).

2.6. Sediment and water samples

We manually collected 5 sediment samples within meters of the caddisfly collection site using yellow containers. As in previous studies, sediment was sampled to a depth of ca. 10 cm and as little surface water as possible was collected along with the sediment (Tibbetts et al. 2018). At the laboratory, we freeze-dried the wet sediment samples, a common procedure in microplastic research (Matsuguma et al. 2017), and took subsamples with a dry weight of 15.64 ± 0.17 g (mean ± SE, n = 5 sediment subsamples, range: 15.16–16.15 g). We then transferred each subsample to an individual glass beaker which we covered with aluminum foil to avoid airborne microplastic contamination of our samples. To facilitate the sediment analysis, we digested organic matter using 20 ml 10 M KOH and 20 ml H_2O_2 (34.5–36.5%) on a shaking table. The beakers were covered with parafilm, and controls were run in parallel to the digestion. KOH was then neutralized with formic acid, as aluminum oxide membrane filters are sensitive to alkaline conditions. Afterwards, sediment grains were separated from microplastics using density separation with potassium formate. Again, blanks were run during digestion and density separation. Moreover, we took 4 water samples (again using yellow containers) with a volume of 467.5 ± 11.09 ml (mean ± SE, n = 4 water samples, range: 450–500 ml) each close to the caddisfly collection site. In the laboratory, the water samples were transferred to glass beakers and freeze-dried. Afterwards, we digested organic materials inside the suspended sediment fraction using 10 ml 10 M KOH and 20 ml H_2O_2 (34.5–36.5%), neutralized the KOH with formic acid and performed density separation as well as filtration onto aluminum oxide membrane filters. Again, no microplastic particles were found in the blanks. After transferring the samples onto aluminum oxide membrane filters, sediment and water microplastic loads were analyzed under the digital microscope as well as under the µFTIR. Again, µFTIR measurements were conducted using µATR, and only particles with a hit quality greater than 700 were considered as microplastics. Furthermore, special care was taken to avoid contact between the germanium crystal and sand grains to prevent any damage to the crystal. No yellow particles that might have come from the containers for sediment and water sampling were found inside the samples.

3. RESULTS

3.1. Microplastics in caddisfly cases

In *Lepidostoma basale*, the average larval case length was 0.85 ± 0.04 cm (mean ± SE, n = 29 cases, range: 0.65–1.40 cm) and average larval case dry weight was 6.40 ± 0.63 mg (mean ± SE). The *Sericostoma personatum/flavicorne* larval case had a length of 1.26 cm and a dry weight of 34.07 mg.

Out of the 29 *L. basale* larvae, 17 individuals (59%) had microplastics of different shapes fixed into their cases (Fig. 1). The average microplastic load per indi-

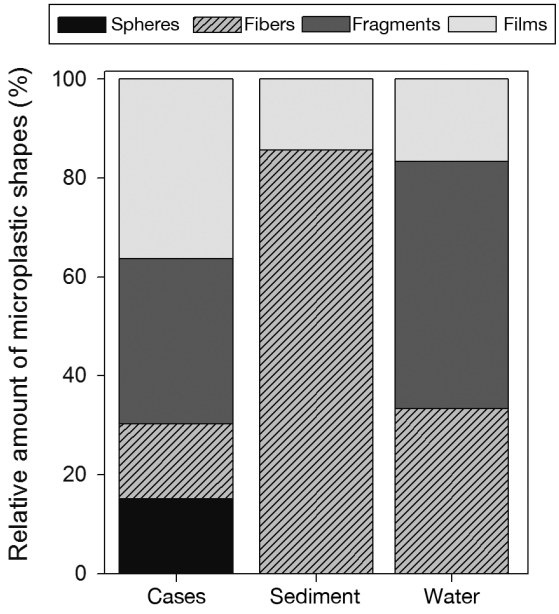

Fig. 1. Relative amount (in %) of different microplastic shapes (sphere, fiber, fragment, film) in *Lepidostoma basale* cases (n = 17), sediment (n = 5 samples) and water (n = 4 samples)

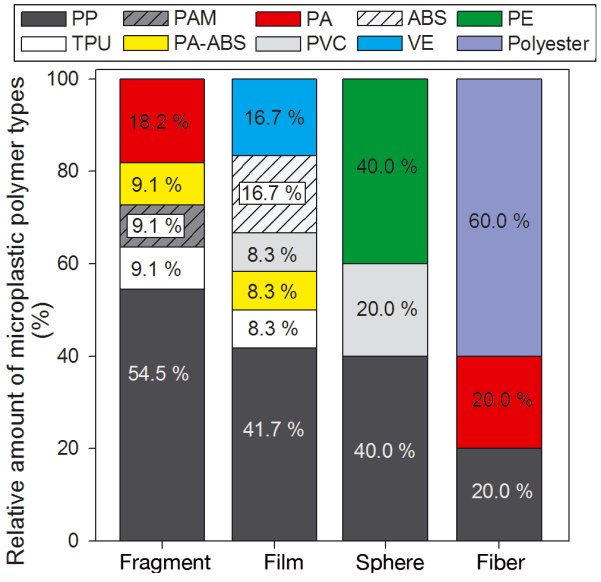

Fig. 2. Relative amount (%) of microplastic polymer types (PP: polypropylene; TPU: thermoplastic polyurethane; PAM: polyacrylamide; PA: polyamide; ABS: acrylonitrile-butadiene-styrene; PVC: polyvinyl chloride; VE: vinyl ester resin; PE: polyethylene) of microplastic fragments, films, spheres and fibers found in *Lepidostoma basale* caddisfly cases

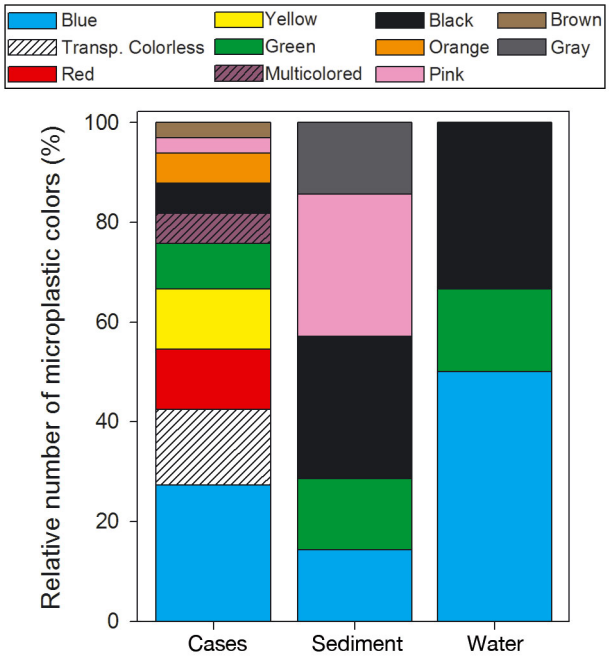

Fig. 3. Relative number (in %) of differently colored microplastics found in *Lepidostoma basale* cases (n = 17), sediment (n = 5 samples) and water (n = 4 samples). Transp.: transparent

vidual was 1.14 ± 0.28 microplastics per caddisfly larval case (mean ± SE, n = 29 caddisfly larval cases, range: 0–6 microplastics per individual case). The average microplastic load was 0.36 ± 0.09 microplastics mg^{-1} case dry weight (mean ± SE, n = 17 caddisfly larval cases, range: 0.07 – 1.44 microplastics mg^{-1} case dry weight). In the *S. personatum/flavicorne* case we detected 2 polyamide (PA) microplastics.

µFTIR spectroscopy revealed that microplastics in the *L. basale* cases consisted of the polymers polypropylene (PP), PA, acrylonitrile-butadiene-styrene (ABS), a blend of PA and ABS, polyacrylamide (PAM), thermoplastic polyurethane (TPU), vinyl ester resin (VE), polyvinyl chloride (PVC), polyethylene (PE) and polyester (Fig. 2). The microplastic particles incorporated into the caddisfly cases also showed a wide spectrum of colors (Fig. 3).

Furthermore, we found rayon, a cellulose-based, semi-synthetic material, in the cases. It is debated as to whether rayon should be counted as microplastic, considering its highly processed structure and potential environmental effects (Song et al. 2015). In our study, rayon was rare, so we did not consider it as a microplastic.

The microplastic fragments had an average length of 236.09 ± 33.44 µm (mean ± SE, n = 11 microplastic fragments, range: 40–375 µm). The films had an average length of 98.92 ± 18.57 µm (n = 12 films, range: 27–191 µm). The plastic spheres had an average diameter of 91.20 ± 25.88 µm (n = 5 spheres, range: 29–163 µm) and the microplastic fibers had an average length of 1872.40 ± 1434.03 µm (n = 5 fibers, range: 295–7601 µm).

3.2. Analyses of sediment and water samples

The sediment subsamples had a microplastic load of 0.09 ± 0.05 microplastics g^{-1} (mean ± SE, n = 5 sediment subsamples, range: 0–0.255 microplastics g^{-1}). Most of the sediment microplastics were fibers (Fig. 1). According to µFTIR measurements, sediment microplastics consisted of polyethylene, acryl and polyester. The 4 water samples contained a microplastic load of 0.003 ± 0.001 microplastics ml^{-1} (n = 4 water samples, range: 0.002–0.007 microplastics ml^{-1}). Among these microplastics, we found urea formaldehyde resin and silicone rubber.

4. DISCUSSION

In our study, we found primary (spheres) as well as secondary (fibers, films, fragments) microplastics incorporated into the larval cases of *Lepidostoma basale*. The spheres accounted for 15.2% of the microplastics found and resemble those that are commonly used in cosmetics (Tanaka & Takada 2016). In contrast, secondary microplastics, such as the fragments, films and fibers in the caddisfly cases, are created when larger plastic debris becomes degraded into smaller plastic pieces (Cole et al. 2011). The *L. basale* individuals that we analyzed had primary and secondary microplastics of different shapes, polymer types, sizes and colors fixed in their cases (see Figs. A1 & A2 in the Appendix). Those results were verified by µFTIR analysis, a crucial step in microplastic analysis, as up to 70% of particles found in the environment can visually be misidentified as microplastics (Hidalgo-Ruz et al. 2012). Hence, we showed that freshwater microplastics of different characteristics are incorporated into *L. basale* larval cases.

As caddisfly larval cases contain material available in the water body the larva inhabits, the microplastics found inside the *L. basale* case matrix in this study must be a subset of the microplastics suspended in the Saynbach. Thus, caddisfly case analysis may provide information on the diversity of microplastics present in a caddisfly's habitat. Inside the caddisfly larval case matrix, the microplastic particles are fixed with silk, and mineral cases show a particularly high stability (Otto & Svensson 1980). As *L. basale* mineral cases contain a low fraction of organic material and lower amounts of sand grains than sediment samples, a filter resulting from the caddisfly case processing is easier to visually analyze than a filter with a sediment microplastic fraction. The latter is often characterized by not fully digested organic residues and small sand grains which could not fully be removed by density separation (Mathalon & Hill 2014). For instance, we found a small yellow PP sphere with a diameter of 43 µm and a small orange PP film with a length of 73 µm in the caddisfly cases (see Fig. A1F) which might have been easily overlooked in visual sediment analysis due to their similarity to sediment sand grains.

The disintegration of the cases enabled us to analyze all microplastics present in the caddisfly cases. Some microplastics which might have been part of the inner case wall or were previously covered by sediment grains inside the case matrix (see Fig. A3 in the Appendix) would have been overlooked without dissolving the cases. Also, the usage of the digital microscope allowed us to record microplastic particles which are normally not visible to the naked eye. Nuelle et al. (2014) showed that a 7 d long treatment with 35% H_2O_2 successfully digests organic materials but might lead to color and size loss in some polymer types. The strong colors of the microplastics that we found in the caddisfly cases (see Fig. A1) suggest that there was no bleaching of microplastics due to the usage of H_2O_2, perhaps aided by microplastic protection inside the caddisfly case matrix during digestion.

The cases exhibited microplastics with a range of chemical compositions (PE, PP, TPU, PA, PAM, VE, PVC, ABS, polyester). Low-density microplastics such as ABS (density 1.02–1.07 g cm^{-3}) can float in the water column (McCormick et al. 2016), whereas high-density microplastics such as PVC (density ca. 1.20–1.45 g cm^{-3}, Avio et al. 2017) are denser than freshwater and accumulate in sediments (Wright et al. 2013). In the caddisfly cases, we found not only plastics with high densities such as PVC, but also microplastics with low density such as ABS, PE (density 0.93–0.98 g cm^{-3}, Avio et al. 2017) and PP (density 0.89–0.91 g cm^{-3}, Avio et al. 2017). Hence, it is likely that factors such as turbulence (McCormick et al. 2016) or an increase in microplastic density caused by a biofilm cover (Lagarde et al. 2016, Rummel et al. 2017) led to low-density microplastic sedimentation and made it available for caddisfly larvae. Similarly, we found PE in the sediment samples. The fact that the sediment samples showed higher microplastic loads than the water samples corroborates that the microplastics in the sampled caddisfly cases stem from the stream sediments. However, as water flows through the caddisfly case (Williams et al. 1987), it cannot be ruled out that some microplastics inside the caddisfly case may come from the water column. The fact that the microplastic load in the caddisfly cases was higher than in the sediment and water samples

indicates that caddisfly cases may act as microplastic sinks in freshwater habitats.

The µFTIR analysis revealed that a high proportion of the microplastics were made of PP and that many spheres consisted of PE, which are the most commonly used plastics (Zhang et al. 2016). They serve as material for different single-use items such as plastic bags, bottle caps and drinking straws which are used in high quantities and regularly accumulate in the environment after disposal (Andrady 2011).

Our results suggest that the plastic types which are most frequently used, and are therefore probably the most prevalent in the environment, are incorporated into caddisfly cases. This finding implies that caddisfly cases may potentially be used as quantitative bioindicators for microplastics. However, future studies should test the potential of caddisfly larval cases as quantitative freshwater microplastic bioindicators by comparing microplastic loads in caddisfly cases from streams differing in plastic pollution. Furthermore, future studies could examine if microplastic loads in caddisfly larval cases may change according to seasonal flow rates. For instance, amounts of microplastics ingested by chironomid larvae show the same seasonal fluctuations as sediment microplastic loads (Nel et al. 2018).

In our study, the only polymers which we found in the caddisfly cases as well as in the sediment samples were PE and polyester, suggesting that *L. basale* cases may have the potential to act as qualitative microplastic bioindicators. The reason why we did not find more similarities between microplastics in caddisfly cases and in the sediment and water samples may be the limited number of specimens from just 1 species which was analyzed in our study. Different caddisfly species use different case building materials and may therefore more likely represent the variety of microplastics present in their habitats when analyzed together.

In our study, all sediment and water microplastic shapes were reflected in the *L. basale* cases. However, microplastic spheres were only found in the caddisfly cases and not in the sediment and water samples. An explanation for this may be that spheres were present in the sediment and water samples, but could not be detected as they were covered by residues which are characteristic for filters from sediment and water samples. In the caddisfly cases, the spheres were as small as 29 µm and were yellow or transparent and colorless, which may further restrict the chances of finding them on filters covered by residues. Therefore, analyzing caddisfly cases might help identify microplastics which are present in an aquatic habitat but cannot easily be detected in sediment and water samples.

The disintegration of caddisfly cases used in our study does not require extensive amounts of chemicals and sieving and is therefore less costly and less time consuming than sediment analyses (Klein et al. 2015). Hence, screening of caddisfly cases might be a cost- and time-effective first step in assessing if microplastics of different characteristics are present in a freshwater habitat.

As the case-building larvae of some caddisfly species occur in brackish and marine environments (Haage 1968, Mouro et al. 2016), future studies should investigate if their cases contain microplastics and may thereby provide information on microplastic characteristics in marine habitats. The fact that a marine polychaete species (Nel & Froneman 2018) and freshwater caddisfly larvae incorporate microplastics inside their tube-like structures may indicate a general microplastic fixation process present across different ecosystems. Other freshwater organisms such as dipterans also construct tube-like structures (Brennan & McLachlan 1979), and in terrestrial systems, termites use soil particles for building mounds which have sizes similar to the microplastics found in our study (Jouquet et al. 2002). Furthermore, bagworm moth larvae construct self-enclosing bags using organic and inorganic materials (Rhainds et al. 2009). These biological constructions could be analyzed for microplastics in future studies and potentially provide information on ecosystem-wide processes of microplastic fixation.

Future research should also examine the effects of microplastics in insect constructions as indirect stressors. As plastics often contain harmful additives (Hermabessiere et al. 2017), might increase the risk of cancer (Erren et al. 2013) and may transport pathogenic bacteria (McCormick et al. 2014), microplastics in caddisfly cases may affect caddisfly larvae. For instance, plastic leachates harm brown mussel larvae (Gandara e Silva et al. 2016), and PVC (which was observed in the caddisfly cases) leads to increased mortality in barnacle larvae (Li et al. 2016). We conclude that caddisfly larval cases may have the potential to be used as freshwater microplastic bioindicators. However, future studies should analyze microplastic contents of cases from different caddisfly species and from habitats differing in plastic pollution levels.

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Appendix. Examples of microplastics, µFTIR spectra, and a picture of the analyzed caddisfly species.

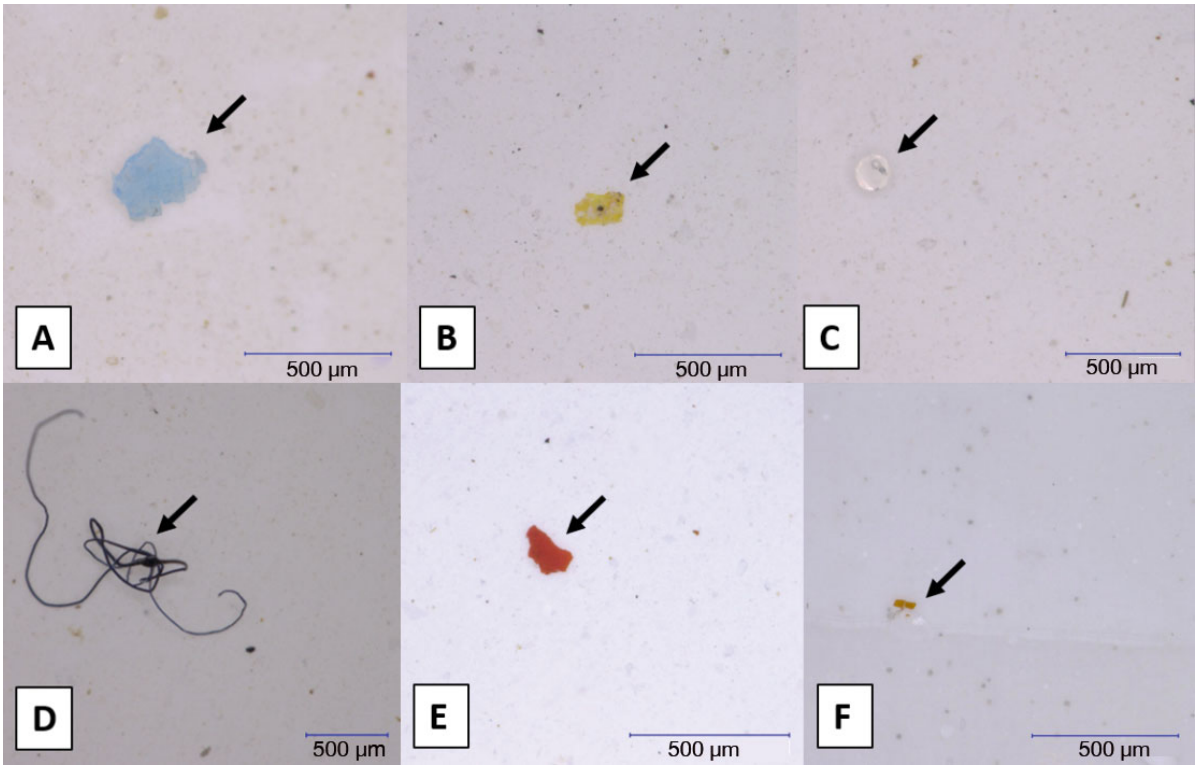

Fig. A1. Examples of microplastics found in *Lepidostoma basale* cases. (A) Blue polypropylene (PP) fragment, (B) yellow acrylonitrile-butadiene-styrene (ABS) film, (C) transparent polyethylene (PE) sphere, (D) black PP fiber, (E) red thermoplastic polyurethane (TPU) film, (F) orange PP film

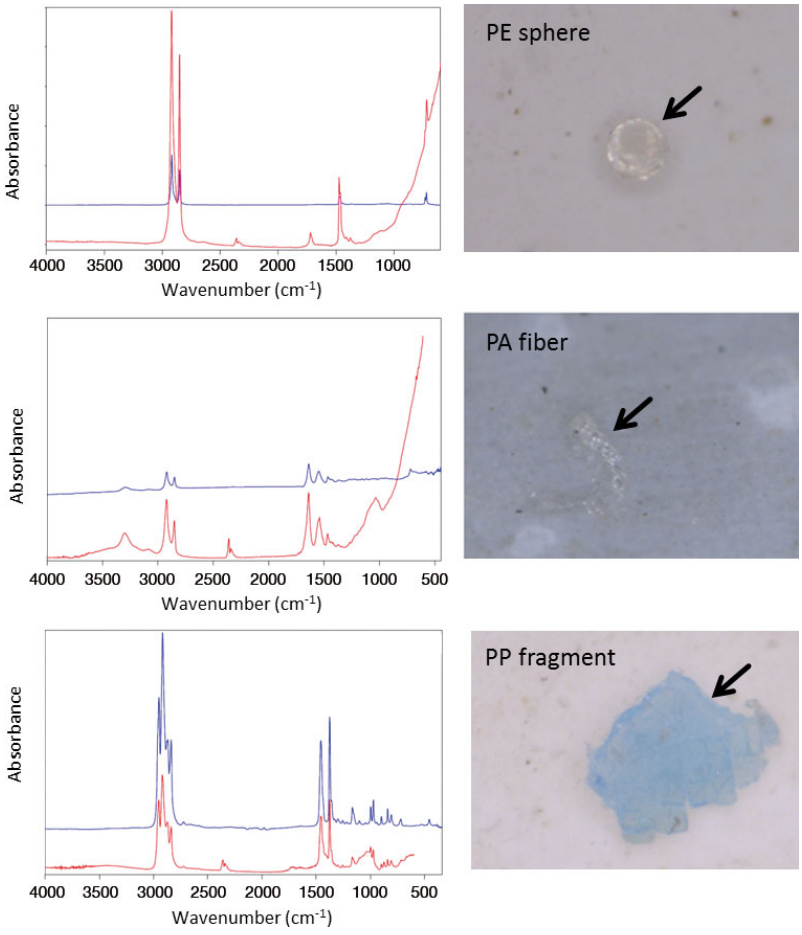

Fig. A2. Spectra of microplastics (red spectra) measured with micro-Fourier-transform infrared spectroscopy (μFTIR) in attenuated total reflectance (μATR) mode. The blue spectra are reference spectra from the Bruker spectra database. PE: polyethylene; PA: polyamide; PP: polypropylene

Fig. A3. Case-bearing *Lepidostoma basale* larva that had microplastics fixed in its case which are not visible to the naked eye

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