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Half a century of caddisfly casings (Trichoptera) with microplastic from natural history collections

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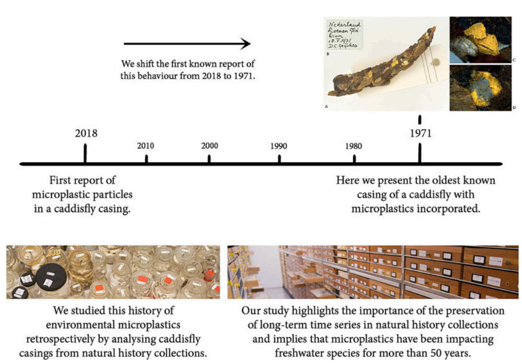
HIGHLIGHTS

- We studied environmental microplastics retrospectively by analysing caddisfly casings from natural history collections.
- Here we present the oldest known casing of a caddisfly with microplastics incorporated.
- We shift the first known occurrence of this behaviour from 2018 to 1971, 47 years earlier than the previous first reported finding.
- Our study highlights the importance of the preservation of long-term time series in natural history collections.
- Microplastics have been impacting freshwater species for more than 50 years.

GRAPHICAL ABSTRACT



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ABSTRACT

Since the start of the production of plastic, synthetic polymers have littered our world. Early observations of this pollution, however, are scarce, and little is known of the history of environmental microplastics, especially in freshwater ecosystems. We studied this history retrospectively by analysing material from natural history collections. Since the 1950s, pieces of microplastic may have unintentionally been collected along with freshwater specimens. In the collection of Naturalis Biodiversity Center, Leiden, The Netherlands, we found early cases of animals that incorporated bits of plastic in their structures. Caddisflies are known to construct larval casings, normally made from fragments of vegetation or grains of sediment, but now that we live in the Anthropocene, caddisflies also have microplastics at their disposal as building materials.

Here we present the oldest known casing of a caddisfly with microplastics incorporated, shifting the first known occurrence of this behaviour from 2018 to 1971, 47 years before the previous first reported finding. Tellingly, 1971 was also the year in which the first microplastics were discovered. The caddisfly casings examined here were not found in heavily urbanised places or polluted rivers, but in the springs of small natural streams with pristine groundwater, indicating that creeks were already polluted with microplastic right at the source. The particles suspected to be artificial were analysed with Energy Dispersive X-rays (EDX); this revealed

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titanium, barium, sulphur, zinc, chlorine, and lead – an elemental composition of common plastic additives in agreement with what has been previously reported for microplastics. Our study highlights the importance of the preservation of long-term time series in natural history collections and implies that microplastics have been impacting freshwater species for more than 50 years, and continue to do so on an increasing scale, as the amount of produced plastic keeps rising.

1. Introduction

Plastic production reached almost 500 million metric tons (Mt) in 2022 (Williams and Rangel-Buitrago, 2022). As a result, extrapolated projections show continued exponential growth of the amount of plastic waste generated, quadrupling to more than 25,000 Mt. by 2050 (Geyer et al., 2017). Macro-sized littered items may fragment over time due to processes such as photo-degradation, thereby degrading into in small, ubiquitous plastic particles. Such synthetic polymers, less than 5 mm in size, are widely defined as microplastics (Moore, 2008; Thompson et al., 2009).

Plastic particles are nowadays found throughout the abiotic and biotic components of the environment (Besseling et al., 2015; Van Cauwenberghe et al., 2013; Napper et al., 2020; Thrift et al., 2022). For example, the 5.25 trillion or more plastic particles that are floating at sea, 92.4 % consists of microplastic (Eriksen et al., 2014). Only recently have researchers recognized the comparative lack of studies on microplastics in freshwater environments (Horton et al., 2017). Less than 4 % of microplastics-related studies are reportedly associated with freshwaters (Lambert and Wagner, 2018), even though rivers are one of the largest contributors of microplastics to marine systems (Lebreton et al., 2017). While recently more studies have focused on freshwater, early observations are scarce and little is known of the history of freshwater microplastic pollution, as this is difficult to investigate retrospectively. Fortunately, museum collections can provide insight into freshwater pollution levels of the past (Ilechukwu et al., 2023; Hou et al., 2021; Toner and Midway, 2021), and all reported microplastic particles from the past help piece together the history of this pollutant.

Since the 1950's, pieces of microplastic have unintentionally been collected along with natural history specimens for research. Here we looked at collections in search of early cases of animals that gathered bits of plastic.

The insect order Trichoptera, or caddisflies, are known for the casings they make in their aquatic larval stage. Many caddisfly larvae build these portable casings of hard particles like sand grains and small rocks, as well as organic components such as leaves, bark or other plant material (Holzenthal et al., 2015). Only recently, the first caddisfly casings were found in the wild that included non-natural materials (Díaz, 2018; Tibbetts et al., 2018; Ehlers et al., 2019). A *Limnephilus* larva, collected in a polluted river section in Spain, included two brightly coloured particles in its casing, which are referred to as 'plastic fragments', as reported in the thesis of Díaz (2018). During the same period, microplastics were also reported in caddisfly cases in England, specifically found in the upper River Tame catchment, as first published in a peer-reviewed journal by Tibbetts et al. (2018). Ehlers et al. (2019) presented the first polymer type analysis of artificial particles in caddisflies, therefore definitively proving that caddisfly larvae incorporate microplastic in their structures. Caddisfly casings with presumed plastic particles are also being shared on the social media platform Twitter by citizens and scientists (Kennedy, 2018; South East Rivers Trust, 2018; Perks, 2019; Álvarez Troncoso, 2020). Besides field studies, multiple laboratory studies have been conducted to investigate the behaviour of caddisfly larvae when presented with microplastic particles (Ehlers et al., 2020; Gallitelli et al., 2021; Valentine et al., 2022).

The artist Hubert Duprat has been experimenting with "the insect's aesthetic behaviour" since the 1980s, showing that caddisfly larvae will make casings from, e.g., pearls, diamonds, and tiny rods of gold (Duprat et al., 1998). During the same time as these indoor experiments took place, field studies by Erman (1986) and Jackson et al. (1999) on the movement of caddisfly larvae showed also evidence for the incorporation of plastic particles and glitter in caddisfly casings. In the field experiments by Erman (1986) were in a creek in California, plastic particles of different colours placed, each colour indicating a different part of the watercourse. When a caddisfly larva crossed a station, it could incorporate the plastic particle in its casing. On the basis of the incorporation of the different coloured plastic particles, the movement of the caddisfly larvae could be monitored. Also in California, Jackson et al. (1999) used caddisfly larvae with self-marked glitter casings to be able to easily track their movement. These studies provided early evidence for the incorporation of artificial particles in caddisfly casings. However, in these studies, plastic was either actively added to the stream or to the streamside where they were reared, the question remains when wild caddisfly larvae started integrating microplastics unintentionally introduced into their habitat by human activities.

This study aims to analyse caddisfly casings preserved in a natural history museum to identify early instances of wild caddisfly larvae incorporating microplastics into their structures and to explore the potential of Trichoptera collections for retrospective studies on environmental microplastic pollution.

2. Methods

Naturalis Biodiversity Center in Leiden, the Netherlands, owns a large historical caddisfly collection of primarily species collected in the Netherlands, encompassing specimens either stored dry or preserved in ethanol. We manually screened caddisfly casings for the presence of artificial material by passing specimens under a stereomicroscope (Zeiss, Discovery.V8). All particles on the outer layer of a casing were visually inspected. Each particle was assessed for potential artificiality by applying multiple criteria: particle structure (layered, porous, glass-like, entire surface smooth), size (large or small), shape (films, fibers, fragments, foam, pellets, spheres), and colours uncommon in nature (e.g. light blue, bright red, bright orange, bright yellow, turquoise, clear white) (Anderson et al., 2017; Ehlers et al., 2019). Casings for which we suspected the incorporation of microplastic particles were separated.

We visually checked each of the 359 caddisfly drawers of the dry collection to assess if they contained juveniles. When suspected artificial particles were noted, a focus-stacked photograph of the notable particle was produced. As Raman analysis did not yield usable results to determine the polymer type, possibly due to degradation, we switched our analysis to Energy-Dispersive X-ray Spectroscopy (EDX). Individual casings were mounted on an aluminium SEM stub coated with conductive adhesive tape to perform chemical characterisation using an energy-dispersive X-ray microanalyzer (EDX) attached to a JEOL-IT510 LV SEM. We scanned the candidate particles to see if the detected elements matched known artificial materials. We used an accelerating voltage of 15 kV, a probe current of 65.0 nA, a work distance of 10 mm and a pressure of 50 Pa. As a detector we used the Low Vacuum Secondary Electron Detector: LSED-E with a point measurement duration of 30 s; the samples were not sputter coated.

Although the caddisfly collection of 1615 jars of caddisflies and larvae stored on ethanol were not screened systematically, here, too, we found a sample with multiple casings containing artificial material, for which we followed the same steps as described above.

3. Results

The dry collection of Naturalis Biodiversity Center contains 549 caddisfly casings, all of which were visually screened for potential microplastics. A distribution graph of caddisflies grouped per decade is given in Appendix 1. The casings from the dry collection were partly without a collection date ($n = 198$, 36,1 %) and mostly collected from before the mass production of plastics began, in the 1950s ($n = 261$, 47,5 %). A total of 90 casings (16,4 %) were collected between 1951 and 2000.

Fig. 1. a) Casing of *Ironoquia dubia* (RMNH.INS.1544419) collected on May 18th 1971 in Loenen, The Netherlands. b) The label of the specimen. c) Detail of the artificial items. d) Detail of the artificial items. Photographs: (A) Auke-Florian Hiemstra, (B—D) Pasquale Ciliberti.

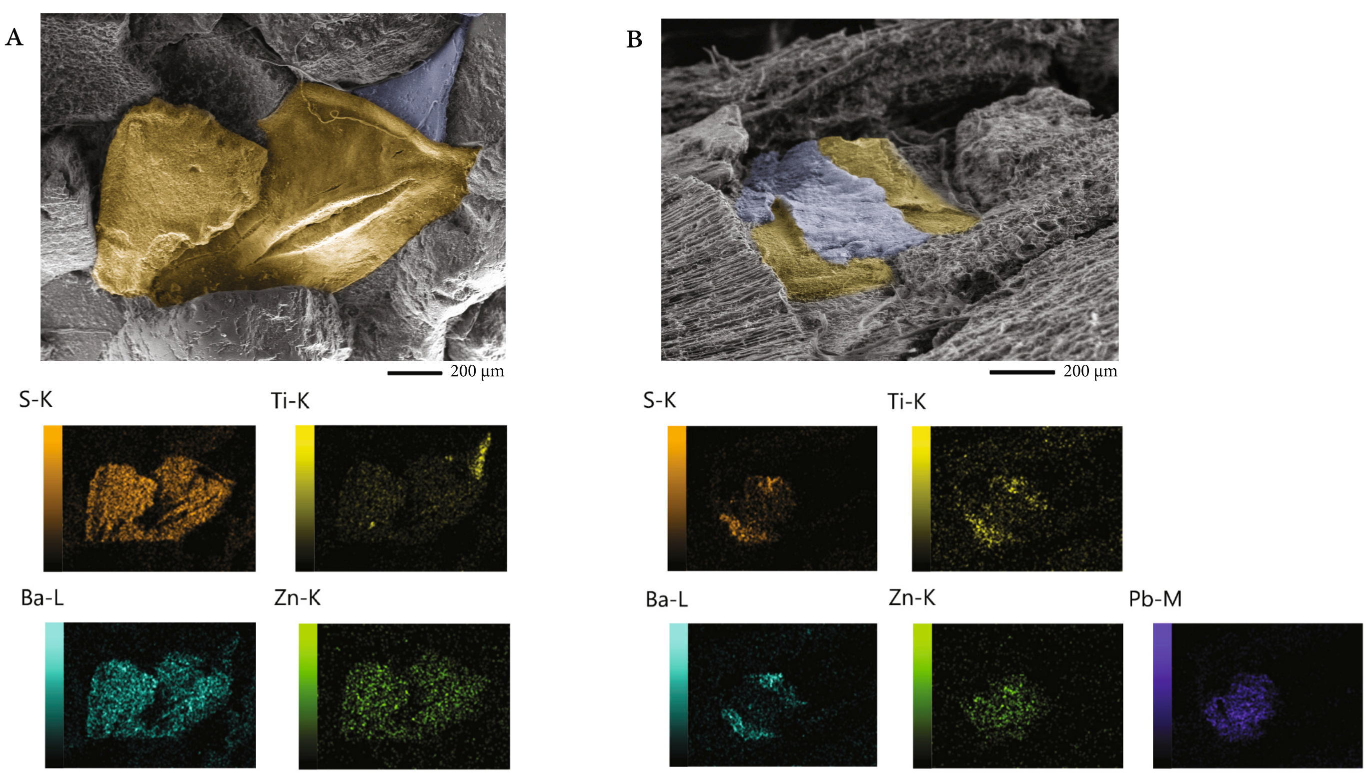

Fig. 2. Colorized SEM photographs of the artificial particles from the casing of *Ironoquia dubia* (RMNH.INS.1544419) collected in Loenen, The Netherlands, followed by EDX mapping visualization of the chemical elements found. The notation '-K' refers to the closest shell to the nucleus, '-L' and '-M' refer to the second and third shells, respectively. A) Yellow and grey elements, one with a striking fissure, as seen in Fig. 1c. B) The yellow/Gy layered elements, as seen in Fig. 1d. Photographs: Bertie Joan van Heuven & Auke-Florian Hiemstra.

Just one casing, in this last category, of *Ironoquia dubia* (Stephens, 1837), registered with collection number RMNH.INS.1544419 stood out as anomaly due to striking yellow particles. It was collected by D.C. Geijkes in Loenen, in the Loenense Beek (Stouten, 1982: 88), Gelderland, the Netherlands, (see Appendix 2 for a detailed location description). The casing was collected more than 50 years ago, on May 18th, 1971.

A total of three notable yellow particles were found to be incorporated in this casing (Fig. 1a). The two particles in Fig. 1c show a striking shape, a large fissure is present and the edges are straight at one side and frayed on the other sides. Behind the particle with the fissure (Fig. 1c), a flat, smooth grey particle is visible. The bright colours, straight edges and smooth material provide a first indication that those particles could be plastic. EDX revealed the particles to be rich in sulphur, titanium, zinc, and barium. A 75× close-up of both the grey and yellow particles, together with the EDX mapping for all these elements can be seen in Fig. 2a.

Fig. 1d shows a yellow foamy particle, with a layer of grey material on top. The particle is irregularly spherical and tiny holes are present in the material, resembling a type of foam. The appearance of the particle suggested it to be artificial due to the colouration, the layered build-up,

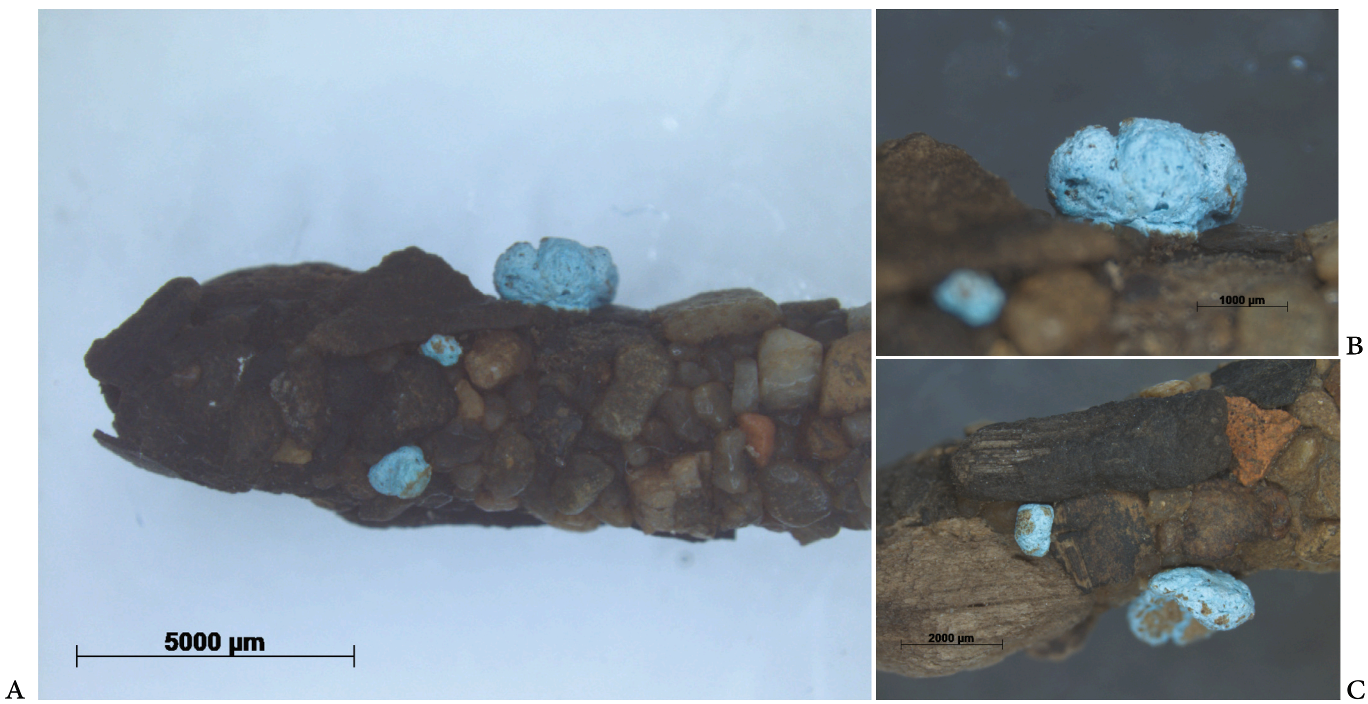

Fig. 3. a) Close-up of casing of *Chaetopteryx villosa* (RMNH.INS.1635762) collected on May 8th 1986 in Oosterbeek, The Netherlands. b) Detail of the artificial items. c) Detail of the artificial items. Photographs: Pasquale Ciliberti & Isabel van der Velden.

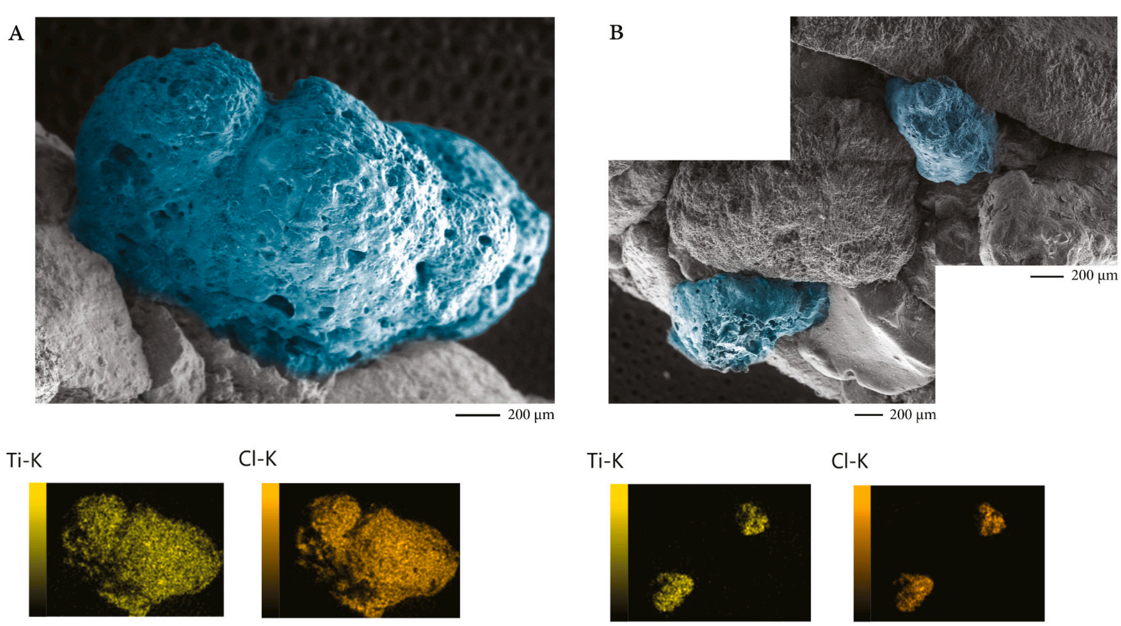

Fig. 4. Colorized SEM photographs of an artificial particle from the casing of *Chaetopteryx villosa* (RMNH.INS.1635762) collected in Oosterbeek, The Netherlands, followed by EDX mapping visualization of the chemical elements found. The notation '-K' refers to the closest shell to the nucleus. A) Blue elements as seen in Fig. 3b. B) The blue elements, as seen in Fig. 3c. Photographs: Bertie Joan van Heuven & Auke-Florian Hiemstra.

and its structure. EDX analysis revealed it to contain sulphur, titanium, zinc, barium, and lead. A 90× close-up of the particle, together with the EDX mapping for all these elements can be seen in Fig. 2b.

Inspection of the wet collection of Naturalis Biodiversity Center revealed, serendipitously, a peculiar jar (BE.2512473) with *Chaetopteryx villosa* (Fabricius, 1798) casings, one of which is depicted in Fig. 3. This anomaly casing, registered with collection number RMNH. INS.1635762, was collected in the spring of the Oosterbeek, Gelderland, the Netherlands, on May 8th 1986, by G.L.N. van Alewijk and R. de Vos (see Appendix 2 for a detailed location description). Here, Alewijk & de Vos collected 15 caddisfly casings, stored in a phenoxyethanol jar registered with collection number BE.100976. In eight of these casings, one or more blue particles are visible. These other casings are retrievable with collection number RMNH.INS.1635763, in the collection of Naturalis Biodiversity Center, also in jar BE.2512473. Based on the coloration, shape, and foamy structure (Fig. 3), these incorporated particles were suspected to be pieces of plastic, possibly originating from packing or insulation material (Turner, 2020). One of the casings has a large particle like this attached laterally (Fig. 3b) and two smaller particles of the same material are incorporated on the ventral side (Fig. 3c). EDX analysis revealed the particles to contain chlorine and titanium. A 35× close-up of the particle, together with the EDX mapping for these elements can be seen in Fig. 4. It should be noted that while Raman analysis did not yield usable spectra, possibly due to degradation, we did manage to melt indents in multiple artificial particles, disproving them to be ceramics, see Appendix 3.

4. Discussion

Here we present, as far as we know, the oldest casing of a caddisfly with microplastics incorporated, shifting the first known occurrence of this behaviour from 2018 back to 1971, 47 years before the previous first reported findings (Díaz, 2018; Ehlers et al., 2019; Tibbetts et al., 2018). Tellingly, 1971 is the first year in which microplastics were found (Buchanan, 1971), namely, in water samples from the North Sea containing “embarrassing proportions” of colourful synthetic fibers. Two subsequent publications in *Science*, one on the discovery of plastic pallets in the Sargasso Sea, and a second on high densities of these pellets in coastal waters of New England, fuelled scientific attention to this new phenomenon (Carpenter and Smith Jr, 1972; Carpenter et al., 1972) and speculation that such particles could become a significant problem if plastic production continued to increase. Plastic production increased; in fact, during that decade, plastics would become the most used material worldwide (Millet et al., 2018). The term ‘microplastic’ that is now used to describe such particles was only coined a few decades later, in 2004 (Thompson et al., 2004). This historical timeline is important to show that during the time of collection of the 1971 caddisfly, microplastic had not yet been reported for freshwater, and the material had thus unintentionally found its way into the natural history collection.

The particles here described in caddisfly casings show striking bright colours that EDX microscopy confirmed as inorganic components. We found titanium, barium, sulphur, zinc, chlorine, and lead in the blue and yellow particles, an elemental composition that is in agreement with what has been reported for microplastics. Fries et al. (2013) describe titanium, barium, sulphur, and zinc as common plastic additives. Gniadek and Dąbrowska (2019) report that microplastics are rich in titanium, sulphur, and chloride.

We are the first to discover historic microplastic in freshwater invertebrates from natural history collections. To the best of our knowledge, only two previous papers addressed collection-based research on freshwater microplastic (Ilechukwu et al., 2023), but both focused on American riverine fishes (Hou et al., 2021; Toner and Midway, 2021). The first study showed two fish species that have been collected with microfibers and microplastic particles since the 1950s (Hou et al., 2021). A second study resulted in only three pieces of microplastic, encountered in fishes from Louisiana, collected in 1982, 1996, and 2006 (Toner and Midway, 2021); however, the plastic found in our study precedes all three pieces mentioned. Our current knowledge on environmental microplastic pollution in freshwater systems is thus very limited, and more studies like ours on caddisfly larvae may help to further enhance our perception of this retrospectively.

What makes this freshwater study unique, is that it reflects a first look at the environmental microplastic pollution of two non-urban sites. Hou et al. (2021) studied water bodies situated in Chicago's urban and suburban areas, and the rivers examined by Toner and Midway (2021) ran through various urban and developed areas, which increased the likelihood of finding microplastics. In our study, we present freshwater microplastics in small natural streams and springs (‘sprengen’), that have provided pristine groundwater to surrounding small towns and watermills since the 14th century (IJzerman, 1979; IJzerman, 1982). The label of the casings of caddisfly larvae collected in the vicinity of Loenen mentions the word ‘bron’ (Fig. 1B), meaning ‘spring’, so it was found at one of the origins (‘sprengkoppen’) of the Loenense beek (Stouten, 1982:88). Also the caddisflies collected near Oosterbeek (Fig. 3) are from Hoog Oorsprong, the spring of the Oorsprongbeek, indicating that both creeks were already polluted with microplastic at the source.

Based on just one or a few casings, it is difficult to assess the degree of microplastic contamination, as each caddisfly species may react differently to artificial material. Some species prefer natural material, while other caddisflies preferentially selected plastic material, even in presence of sufficient natural material (Gallitelli et al., 2021; Valentine et al., 2022). As for both species presented in the present paper, it is the first record of building casings with artificial material, it is currently unclear which category they will ultimately belong to. However, some traits may hint in a certain direction. The larvae of *Chaetopteryx villosa* live in springs, streams, and small rivers, both on stony substrates as well as between water plants, rough detritus or sand (Wallace et al., 2003; Schmedtje and Colling, 1996). They are mainly cutters, partly scrapers, and may occasionally prey on mosquito larvae (Otto, 1993; Speth et al., 2006). When their preferred leaves are not available, they can also eat other riparian plants (Wagner, 1991). Interestingly enough, they are described to be opportunistic, not only in food sources, but also in construction materials, as their casings are built from rough detritus and each is formed very differently and irregularly (Higler, 2005; Tempelman et al., 2022). This opportunistic behaviour may increase the chances of a larva incorporating artificial material in its casings. This is not clearly stated about the larvae of *Ironoquia dubia*, which live in small streams that may run dry in summer (Sommerhäuser, 1995; Hiley, 1976) and are considered rheophiles (Schmedtje and Colling, 1996). They are found between water plants, feed by chopping up plant material, and normally build their cylindrical, strongly curved casings from leaves (Graf et al., 2008; Speth et al., 2006; Wallace, 2011; Higler, 2005; Tempelman et al., 2022), yet appear now to also include artificial material.

The attachment of artificial material may affect the caddisfly larvae in different ways. The inclusion of brightly coloured particles (like the yellow and blue particles reported here) may increase their visibility to visual predators like fish (Otto and Svensson, 1980). Also, the structural strength of a mineral grain casing is reduced when partly made with microplastics (Ehlers et al., 2020). On the other hand, casings made from leaf litter (like *Ironoquia dubia* does) may be more robust when constructed with polymer particles, providing increased protection (Valentine et al., 2022). As plastic is a less dense material than mineral grains, an artificial casing may become more buoyant. This may reduce their stability in streams with fast-moving water (König and Waringer, 2008) where *Ironoquia dubia* is naturally found and may make larvae more prone to predation (Ehlers et al., 2020). A more buoyant casing may, however, offer advantages while capturing prey (Otto, 1987). Modifying the artificial material, caddisflies are known to chew on plastic and are even referred to as a driver of microplastic formation (Valentine et al., 2022), which may result in the ingestion of plastic particles (López-Rojo et al., 2020; Windsor et al., 2019; Winkler et al., 2022). Finally, the prolonged interaction with the plastic once included in its casing may also result in exposure risk of harmful chemicals and toxic metals often associated with plastics (Rochman, 2015). How all these factors balance out for the here reported species is still unknown, and would require additional research and experiments.

Studying these casings non-destructively, and focussing on bright colours may imply that we easily could have missed artificial particles that were natural-coloured, and artificial transparent (colourless) particles as reported upon in caddisfly cases by Ehlers (et al., 2019) may have been mistaken here for sand. As we also took the shape and structure of the particle in account, we hope to have declined this margin of error, but a visual inspection will likely result in an underestimation. As environmental scientists have begun only recently to study freshwater pollution on the basis of collection material (Hou et al., 2021; Toner and Midway, 2021), all observations are valuable. We urge that caddisfly casings from more natural history collections may be studied, as this may uncover further instances of cases that include artificial particles. Together, these findings may provide a valuable repository of historical microplastic data. And following Hou et al. (2021), incorporation of plastic in caddisfly casings might be traced all the way back to the 1950s. Our study highlights the importance of the preservation of long-term time series in natural history collections, and implies that microplastics have been impacting freshwater species for more than 50 years, and continue to do so on an increasing scale, as the amount of produced plastics keeps rising.

CRediT authorship contribution statement

Auke-Florian Hiemstra: Writing – original draft, Visualization, Investigation, Conceptualization. **Isabel van der Velden:** Writing – original draft, Visualization, Investigation. **Pasquale Ciliberti:** Visualization, Resources. **Liliana D'Alba:** Methodology. **Barbara Gravendeel:** Writing – review & editing, Supervision. **Menno Schilthuizen:** Writing – review & editing, Supervision.

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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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.178947.

Data availability

The caddisfly specimen referred to in this study are retrievable in the scientific collection of Naturalis Biodiversity Center, Leiden, The Netherlands.

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