ORIGINAL ARTICLE

A curious case: caddisfly cases built from brick and sewage overflow microplastics

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ABSTRACT

Caddisflies larvae have been constructing cases made from vegetation or sediment grains for millions of years, but in the Anthropocene many alternative building materials have become available. We collected caddisfly cases in three distinctive locations in The Netherlands, differing in degree of urbanization and pollution from sewage overflows. This resulted in a total of 1121 caddisfly cases, of which 311 presumably contained artificial material. Our results show that microplastics (MPs) from sewage overflows, along with littered bricks - whether from masonry waste in urban areas or historic dumping for streambank protection- affect how caddisfly larvae construct their cases. In the most polluted stream, more than half of all cases were found to include artificial material. The inclusion of masonry waste and MPs by the larvae as building and ballast material might alter the specific weight of the cases, which may influence the caddisfly larvae's functioning and behaviour. This is the first time a sewage overflow is directly linked to the inclusion of MPs in cases by caddisfly larvae and actions should be taken to minimize pollution from these overflows. Especially the MP load of sewage overflows may impact both the aquatic and the terrestrial ecosystem, as caddisfly larvae have a key role in the food chain and may transport these plastics into a broad and diverse food web. Sewage overflow events are expected to increase due to climate change, and it is expected that there will be three times as much human-made mass than dry biomass on Earth in 2040. This will, besides many other effects, impact caddisfly case construction in an even more dramatic way than is the case today.

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INTRODUCTION

At the beginning of the twentieth century, the combined anthropogenic mass, that is, the mass embedded in solid objects made by humans, was equal to just 3% of the global biomass (Elhacham *et al*., 2020). One hundred and twenty years later, in 2020, anthropogenic mass surpassed the overall biomass in the world (Elhacham *et al*., 2020). This demonstrates the unprecedented impact we have on the world around us, which has led to warnings to humanity (Chapron *et al*., 2018; Ripple *et al*., 2017) and the declaration of the proposed Anthropocene epoch (Crutzen and Stoermer, 2000), characterized as a period defined by humans plundering the earth's resources and dumping excessive amounts of waste products into the environment (Crutzen, 2006; Lewis and Maslin, 2015).

One way in which we pollute our surroundings are sewage overflows (Fig. 1A), which often impact freshwater communities (Balmforth, 1990). Zhou *et al*. (2023) showed that on average nearly 3,500 items of microplastics (MPs) are emitted *per capita* per day in household wastewater. Untreated wastewater from sewage can be released to surface waters when sewage systems are overloaded, for example due to heavy rainfall. In 2023, in the UK waterways, were 14.241 active combined sewer overflows monitored, resulting in 464.056 spills events with 3.6 million h of outflow (Environment Agency, n.d.). Untreated sewage water may include not only human organic debris and toxic materials, but also plastic-containing sanitary waste, such as hygiene products (tampons, sanitary towels; Fig. 1 B,C) or sanitary products like cotton swabs. In addition, rainwater entering the sewage system may also bring artificial material washed from streets (Boon-

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stra and de Winter, 2019). The wastewater being released by a sewage overflow valve is known to be rich in MPs (Chen *et al*., 2020; Di Nunno *et al*., 2021; Dris *et al*., 2015; Treilles *et al*., 2021; Zhou *et al*., 2023). Important sources of MPs in sewage are car tire wear particles, personal care products, laundry fibers, and degraded macroplastics.

Plastic pieces are normally ranked by size: macroplastics (fragments >25 mm in diameter), mesoplastics (5-25 mm), and microplastics (<5 mm). This degradation highlights the distinction between primary plastics, designed to be small (e.g., microbeads), and secondary plastics, which originate from the breakdown of larger items into smaller fragments. Over time, these pieces can become nearly invisible due to their size, yet their collective mass is still twice that of the entire animal kingdom (4 Gt), amounting to 8 Gt (Elhacham *et al*., 2020). Therefore, it may not be surprising that animals in all environments are interacting with plastics and other artificial materials, including freshwater animals (van Emmerik and Schwarz, 2019). While macroplastics in freshwater can cause harm by entangling or entrapping animals (Blettler, 2021; Hiemstra *et al*., 2021), they may also be used as nesting material by birds (Hiemstra *et al*, 2021). Likewise, MPs are known to impact biodiversity (De Sá *et al*., 2018; Quadroni *et al*., 2024), including the use as building material by certain insect larvae (Díaz, 2018; Ehlers *et al*., 2019; Tibbetts *et al*., 2018).

The insect order Trichoptera (Kirby, 1813), or caddisflies, are well-known architects, since many species make intricate protective cases in their aquatic larval stage. Over the past millions of years, their cases were built exclusively from mineral grains, coarse particulate organic matter (detritus, leaf fragments), twigs, mollusc shells and other natural objects, but have recently been found to include a wider selection of materials. Artist Hubert Duprat started in the early 1980s with providing caddisfly larvae with artificial material to make sculptural works, in what Duprat and Besson (1998) refer to as a 'collaboration' between the larvae and the artist. Field experiments on the movement off caddisflies, from the same time period, where self-marked caddisflies with glitter were re-released in a stream so they could be easily followed (Erman, 1986). Currently so much anthropogenic mass is available that these partly-artificial cases are made without the interference of an artist - or scientist. Recently, the first caddisfly cases were reported, found in the wild, that included non-natural material: a river in Spain that suffered from plastic pollution revealed a casing of a *Limnephilus* larva, which included two brightly coloured particles in its case (Díaz, 2018), but also in England, did a study on MPs in the upper River Tame catchment report some artificial looking particles present in caddisfly cases (Tibbetts *et al*., 2018). Ehlers *et al*., (2019) presented the first polymer type analysis of artificial particles in caddisflies, therefore definitively proving that caddisflies incorporate MP.

Caddisfly cases stored in the natural history collection of Naturalis Biodiversity Center already included MPs more than fifty years ago. These MPs were unintentionally collected along with the freshwater specimens and remained undetected for half a century (Hiemstra *et al*., 2025). The earliest example dates back to 1971, the year in which the first MPs in general were discovered, and 47 years before the first field reports of microplastic inclusion by caddisflies (Díaz, 2018; Tibbetts *et al*., 2018).

Besides field studies, multiple laboratorial studies have been conducted to investigate the behaviour of caddisfly larvae in the presence of MP particles (Ehlers *et al*., 2020; Gallitelli *et al*.,


A B C

Fig. 1. A) Sewage overflow valve in Simpelveld, entering the Eyserbeek (B,C) discarded hygiene products littering a nature reserve, indicating overflow activity in the Noor. B) Building brick is visible mixed with the natural streambed substrate. Images credit: Auke-Florian Hiemsta.

2021; Valentine *et al*., 2022). Besides scientific evidence, we also encountered examples of artificial caddisfly cases found in the wild on the social media platform X, formerly known as Twitter (*Appendix 1*). As stated by Holzenthal *et al*. (2015), the 1940s and 1950s were the 'great age of discovery' for caddisfly research, when groundwork was published on these insects and their cases, just before we started to produce synthetic polymers on a bewildering scale. We therefore have some catching up to do, as a changed world requires new groundwork by reporting on how caddisfly larvae now build their cases during the Anthropocene.

Our study is the first to investigate the role of sewage overflow valves, and their load of MP, on cases of caddisflies. Three different study sites were selected in catchments in the Netherlands, with either zero, one, or many overflow valves. Also, the amount of urbanization was considered, as the study sites displayed a range of surrounding urbanisation. The inclusion of other types of anthropogenic materials, like pieces of building brick, were also documented. These may be readily available in the aquatic environment, amongst others, due to historic dumping for streambank protection.

METHODS

Fieldwork was conducted in April and May 2023 at three study sites, each characterized by varying levels of sewage overflow pollution levels and urbanisation. One site was classified as heavily polluted and highly urbanized, the second as slightly polluted with limited urbanization, and the third as neither polluted by overflows nor urbanized. The 'Eyserbeek' stream, which flows through the town of Simpelveld (Limburg, the Netherlands), was the most polluted locality, as it was connected to 21 sewage overflow valves (Fig. 1A). The second study site was the stream 'Noor', located in a natural area next to the village of Noorbeek (Limburg, the Netherlands), which is connected to only one sewage overflow valve (Fig. 1B). We therefore expected the stream to be polluted, but to a lesser extent than the Eyserbeek. The last study site consisted of ditches, which drain fruit growers' orchards in the Betuwe (Gelderland, the Netherlands) (Fig. 1C), without any urbanization or sewage overflow valves. This last locality was used as a baseline for natural cases, yet due to differences in species composition (Tab. 1), a direct comparison on species level cannot be made with the Limburg localities.

Tab. 1. Species identified at the different locations, grouped per family. The number of partly artificial cases is indicated between brackets. Totals represent the total number of species identified, but must be considered as the minimum as a number of cases could not be allocated to a species.

Species identified	Location		
	Betuwe	Noor	Eyserbeek
Family Goeridae			
Goera pilosa	-	-	X
Goeridae	-	X (12 artificial)	X (63 artificial)
Silo nigricornis	-	X	X.
Silo pallipes	-	X	-
Family Leptoceridae			
Athripsodes aterrimus	X (1 artificial)	-	-
Leptocerus tineiformis	X	-	-
Mystacides azurea	-	-	X (24 artificial)
Mystacides longicornis	X	-	-
Triaenodes bicolor	X	-	-
Family Limnephilidae			
Anabolia nervosa	X	-	-
Chaetopteryx villosa	-	X (17 artificial)	X (7 artificial)
Drusus annulatus	-	X (6 artificial)	X (58 artificial)
Glyphotaelius pellucidus	X		-
Limnephilus flavicornis	X	-	-
Limnephilus lunatus	X	-	X (1 artificial)
Potamophylax nigricornis	-	-	X
Potamophylax sp.	-	X	-
One or more additional species of the genus *Limnephilus*	X	-	-
Family Molannidae			
Molana angustata	X	-	-
Family Sericostomatidae			
Sericostoma personatum	-	X (59 artificial)	X (52 artificial)
Unknown			
Unable to identify	X (7 artificial)	X (2 artificial)	X (2 artificial)
Totals			
Species richness	10	7	9
Number of species which included artificial material	1	4	6
Total number of individuals (and the total of cases with artificial particles)	165 (8 with artificial material)	580 (96 with artificial material)	376 (207 with artificial material)

X, presence; -, absence.

Locations description

Betuwe, neither polluted by overflows nor urbanized

Location description: No urbanization and no sewage overflows. Drainage channels were located in orchards where apples and pears are being produced, located in the Betuwe region of the Netherlands. Low gradient, stagnant to slowly flowing system. Some places showed signs of eutrophication, mainly resulting from diffuse nutrient load related to agricultural activities. Coordinates of the exact sample locations can be found in *Appendix 2a*, including a map (*Fig. A1*).

Noor, slightly polluted with limited urbanisation

Location description: Little urbanization and one sewage overflow. This stream, the Noor, has its source at the Brigida Spring in Wesch, north of the village of Noorbeek. Its spring, where the water wells out from the chalk underground, is surrounded by some houses and it crosses a road, after which it flows into a forest. The stream valley is a nature reserve, managed by the Society for Preservation of Nature Monuments in the Netherlands. After fifty meters the spring water is mixed with sewage water from one overflow valve (Fig. 1B). High gradient stream with a high current velocity. Coordinates of the exact sample locations can be found in *Appendix 2b*, including a map (*Fig. A2*).

Eyserbeek, heavily polluted and highly urbanised

Location description: High level of urbanization and many sewage overflows (Fig. 1A). The Eyserbeek is a stream that, after emerging near Bocholtz, flows through the village of Simpelveld. A total of 16 sewage overflows in Simpelveld reaches the stream. Eight of these overflows are classified as 'not ecologically acceptable' by the local water authorities (*pers. com*. waterboard Limburg), as they are utilized more frequently than once every two years - exceeding the recovery period required for macrofauna species (Hofman and Tolkamp, 2008). High gradient stream with a high current velocity. Coordinates of the exact sample locations can be found in *Appendix 2c*, including a map (*Fig. A3*).

Sample collection

At each of the three sites, seven sample locations were visited, and five scoops were collected at each of the seven locations. Sample locations were chosen randomly and on each of these spots, caddisfly larvae were collected with five scoops of 1 m in length with an aquatic hand net (1 mm mesh size; 300 mm wide). Per sample location, the aquatic hand net was moved across the stream or ditch for the different scoops. In the ditches (Betuwe), we collected three scoops from the banks and two from the bottom. In streams (Eyserbeek and Noor) with more diverse habitats, we tried (when present) to sample the dominant substrates in a total of five scoops, for example, sand, pebbles, organic matter or vegetation. Excess mud was removed from the sample by moving the net through the water. Each sample was then divided over six white plastic containers (Kaiser developing trays: 460x350x85mm) filled with a layer of water, for ease of further analysis. Due to the camouflaging abilities of caddisfly larvae, they are almost impossible to spot when they are concealed among plant material, especially those with cases made from plant material. Therefore, the best way to detect them is when they move, as their movement makes them visible and allows for easy collecting. For each scoop, we set a timer for 10 min. If no caddisfly larvae were seen during that time, we moved on to the next scoop. If we spotted at least one caddisfly larva, we extended the search time by another 10 min, spending a total of 20 min on that scoop, picking out all caddisfly larva and storing them on 70% ethanol. As we focused solely on caddisfly larvae, small fishes and other types of macroinvertebrates that also got caught were excluded in the field, as this method allowed us after 10 or 20 min, to immediately release other non-target animal groups that were not required for the study. Cases were studied with non-destructive methods only, for preservation in the scientific collection of Naturalis Biodiversity Center, as new additions to the caddisfly collection from the Anthropocene.

Caddisflies identification and microplastics analysis

Species diversity was determined by morphological identification of larvae, casing, but preferably both, using a microscope (Zeiss, Discovery.V8). When a larva was present in the casing, we separated it gently from its case by feather forceps. When necessary for identification, the head and/or legs were removed from the thorax. Based on Higler (2005) and Waringer and Graf (2011), the larvae were identified to at least genus level.

Each caddisfly case was analysed for the presence of artificial material with a stereo microscope (Zeiss, Discovery.V8). All visible particles on the outer layer of the case were individually inspected, with help of a trained archaeologist, to pass judgement on whether a particle may be artificial or not. Each particle was assessed by multiple criteria: structure (layered, porous, glass-like, entire surface smooth), shape (films, fibres, 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; Moore, 2008). Each presumption of an artificial particle was noted, the casing concerned was stored in an individual tube in 70%-ethanol and marked with a specific ID (1 letter + number).

A subset of four particles that we suspected to be building bricks were examined with X-ray analysis with the EDAX Orbis micro-ED-XRF analyser from EDAX Corporation Mahwah NJ, to determine the composition of the material. The ED-XRF technique was used, with 20 kV, 500 μA and 300 μm spot size for settings. The output was matched with two building brick samples analysed by ED-XRF for reference. While we could not test all particles that we suspected to be microplastics with spectroscopic techniques, a subset of five particles with a range of colours (blue, green and white), patterns (layered and speckled), and structures (both hard and foamy) was analysed. As Raman analysis did not yield usable results, possibly due to degradation, we switched to Energy-Dispersive X-ray Spectroscopy (EDX). We used the integrated Jeol Dry SD100 EDS system connected to the Jeol JSM-IT510 LV Scanning Electron Microscope. With a beam of electrons of 15.0Kv, we scanned the presumed to be plastic particles to see if the elements were indeed of artificial origin, and if these elements matched those as reported for plastic additives (Fries *et al*., 2013; Gniadek and Dąbrowska, 2019).

Statistics

A chi-square test of proportions was used to compare the prevalence of casings with artificial material between locations.

The statistical analyse was performed using R (version 4.3.3), with significance set at $\alpha = 0.05$. Data analysis was performed using Julius (Julius AI, 2024).

RESULTS

Comparison of artificial material presence across the three localities

We collected a total of 1,121 caddisfly cases during our study. A total of 311 caddisfly cases (27.7% of the total) were found for which we presumed incorporation of one or more artificial particles. Of this total, 207 cases were found in Eyserbeek, 96 in Noor, and 8 in Betuwe. On average, 55.1% of the caddisfly cases in the total sample of Eyserbeek were presumed to contain artificial material and for Noor this was 16.6%. We compared the percentages of artificial material use of both Limburg localities, De Noor (96 out of 580, thus 16.6%) and Eyserbeek (207 out of 376, thus 55.1%). A chi-square test shows this difference is highly significant ($p<0.001$). Eyserbeek has a higher proportion of artificial cases, more than three times higher. Even though the Noor has a higher total of caught specimens (580 *vs* 376), Eyserbeek has still more than twice as many artificial cases (207 *vs* 96). For the sampled ditches in Betuwe, only 4.9% of the total number of collected cases were presumed to be containing artificial material. The highest percentage of presumed artificial particle-containing cases was found where the Eyserbeek just left the town of Simpelveld (Location 1, sample 3). Our results show that only caddisfly species which constructed their cases out of mineral grains, or a mix of mineral grains and organic material, are the ones that incorporated artificial materials like brick or plastic in their cases.

The Betuwe, neither polluted by overflows nor urbanised

Seven samples of five scoops resulted in a total of 165 caddisfly cases. At least 10 species could be identified (Tab. 1). Eight caddisfly cases (5%) were found with presumed artificial particles. The species that constructed their cases with artificial material in Betuwe were difficult to identify as, for seven of the eight partly artificial cases, no larvae were present inside. Only a single artificial casing could be attributed to a species with certainty, belonging to *Athripsodes aterrimus* (Stephens, 1836) (Tab. 1).

Noor, slightly polluted with limited urbanisation

Seven samples of five scoops resulted in a total of 580 collected caddisfly cases. Seven species could be identified (Tab. 1). A total of 96 caddisfly cases (16,6%) were found with presumed artificial particles. The species that were constructing cases with artificial material in the Noor can be found in Tab. 1. Cases of the species *Silo nigricornis* (Pictet, 1834), *Silo pallipes* (Fabricius, 1781) and possibly *Goera pilosa* (Fabricius, 1775) were constructed in a very similar way: a mineral grain case with lateral larger stones attached. This made it hard to determine the exact species in the absence of larvae inside the cases. Most of the collected cases with artificial material belonged to *Sericostoma personatum* (Kirby and Spence, 1826; 59 individuals).

Eyserbeek, heavily polluted and highly urbanised

Seven samples of five scoops resulted in 376 collected caddisfly cases. Nine different species could be identified (Tab. 1). The highest species richness was found at the sample location where the stream enters Simpelveld. The lowest species richness was found at the sample location in the outflow of a sewage treatment plant. A total of six empty cases were collected there, which probably all belonged to the same species. Most caddisfly cases were found in location 4 (145 cases), a spot surrounded by houses and backyards. A total of 207 caddisfly cases (55.1%) were found with presumed artificial particles. The species using artificial material in Eyserbeek are listed in Tab. 1. Cases of the species *Silo nigricornis*, *Silo pallipes* and *Goera pilosa* were difficult to distinguish morphologically, as their cases are much alike and often no larvae were present inside. High numbers of Goeridae were collected, as well as *Sericostoma personatum* and *Drusus annulatus* (Stephens, 1837).

Types of artificial materials found in caddisfly cases

Brick

Many particles were encountered with a porous appearance and conspicuously orange, reddish colours (62% of the artificial particles), as seen in Fig. 2. These were presumed to be fragments of building brick. Two reference fragments of building brick, blown from a roof in Leiden, the Netherlands, contained the following eight oxides: Na_2O, Al_2O_3, SiO_2, K_2O, CaO, TiO_2, MnO and FeO. These metal elements all matched with the ED-XRF output of a subsample containing four orange/reddish particles (Fig. 2) detected in three caddisfly cases (*Appendix 3A*). These elements showed a matching compositional similarity with the brick reference samples, visualised in *Appendix 3B*. Additional figures in *Appendix 3C-E* show graphs directly from the ED-XRF output. Out of the 311 collected presumed artificial cases, a total of 193 caddisfly cases were presumed to contain building brick fragments. A total of 136 of these cases were collected in Eyserbeek and 57 cases in the Noor. No cases of brick particles were collected at Betuwe location. Fig. 3 shows a case of *Silo* spp., known for the attachment of larger particles to their casing for additional weight, from which one of these so-called ‘ballast stones’ is a brick particle.

Plastic additives

Some particles showed unnatural colours, had a notably flat shape or had angular edges which caused us to presume that they were MPs (Fig. 4). While Raman analysis did not yield usable spectra, possibly due to degradation, we did manage to melt an indent in some of the particles, disproving them to be ceramics. EDX analysis of a subsample of five of these particles (chosen due to their colour (A,B), patterns (C,D), and foaminess (E) respectively) revealed these as artificial elements. Fig. 5A shows a casing of *Chaetopteryx villosa* (Fabricius, 1798), which was collected in the Noorbeek and included a flat bright-blue coloured particle, that we presumed to be of artificial origin due to its appearance. EDX analysis with a beam of electrons of 15.0Kv revealed a dominant signal for the element titanium (37.37%; Fig. 5C; *Appendix 4*). Another flat particle analysed, green coloured (Fig. 5D), was found to consist of the elements titanium (21.65%) and lead (18.66%), but also chloride (5.61%) and sulphur (4.11%) (Fig. 5 F-H; *Appendix 5*). A white, flat, speckled and layered particle (Fig. 4C) was analysed with EDX spot measuring and was found to consist of titanium (75.01%), calcium (7.31%) and alu-

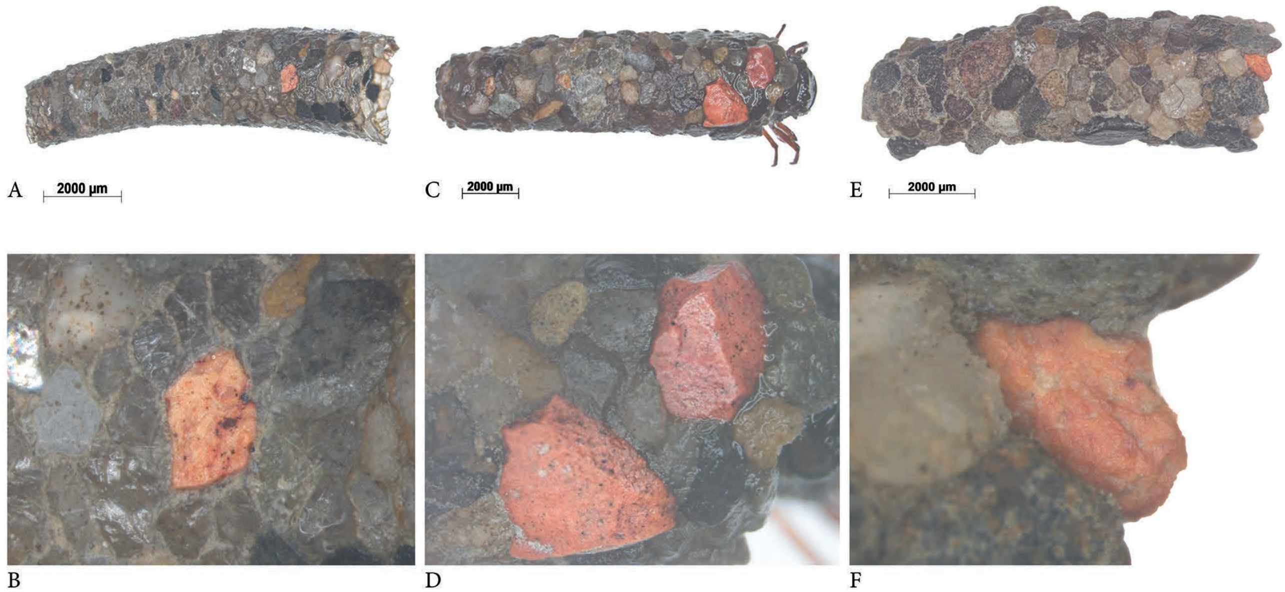

Fig. 2. Examples of brick particles in caddisfly cases, all collected in the Eyserbeek. A) *Sericostoma personatum,* RMNH.INS.1658133 from Simpelveld 4 and detail (B). C) *Drusus annulatus,* RMNH.INS.1658135 from Simpelveld 7, and detail (D). E) *Drusus annulatus,* RMNH.INS.1658134 from Simpelveld 4 and detail (F). All retrievable in jar BE2289653 of the Naturalis Biodiversity Collection. Images credit: Isabel van der Velden and Auke-Florian Hiemstra.

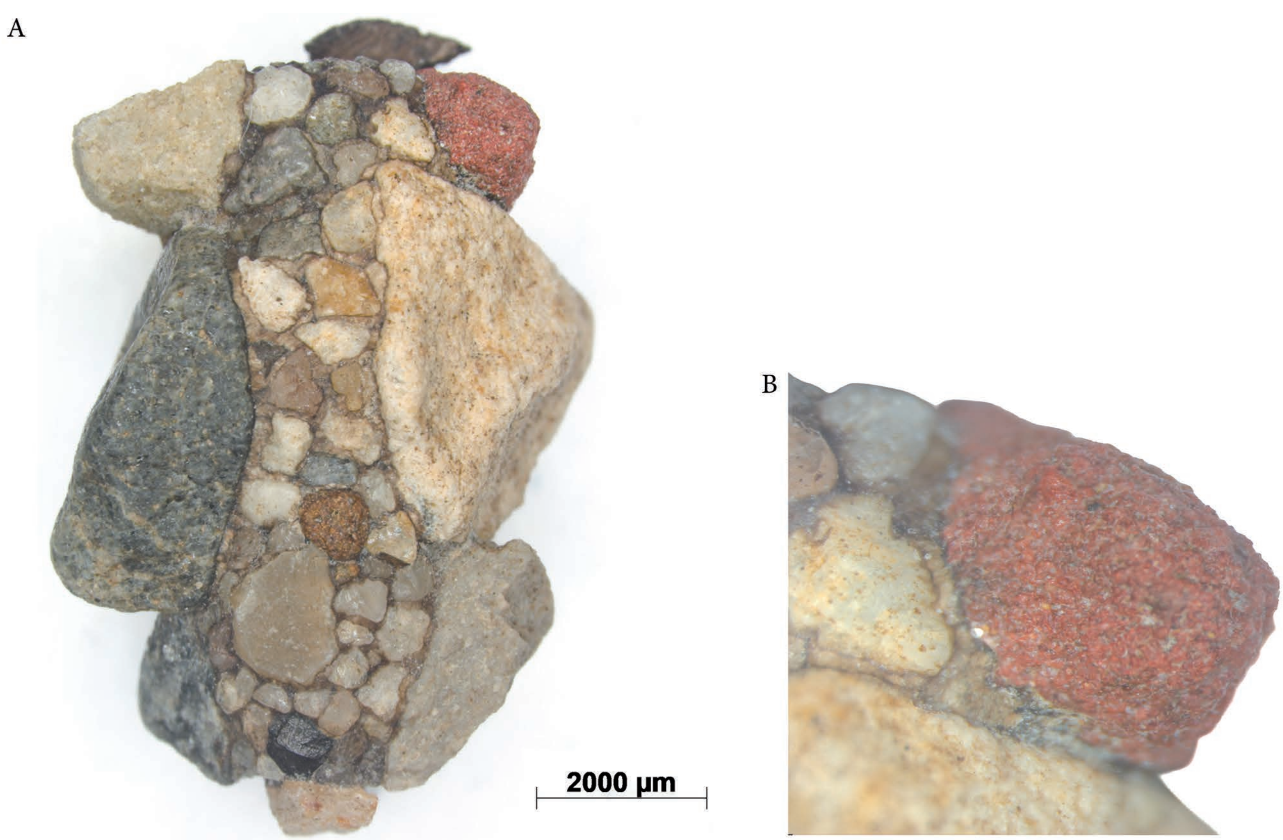

Fig. 3. A) Casing of caddisfly larva from the genus *Silo* with a brick particle as ballast stone, collected in the Noor (RMNH.INS.1658127; Noorbeek 5.1). B) Detail of brick particle as ballast stone. Retrievable in jar BE.2289651 in the collection of Naturalis Biodiversity Center. Images credit: Isabel van der Velden and Auke-Florian Hiemstra.

minium (4.18%), which was further confirmed for the entire particle during a mapping scan, where also zirconium was detected (*Appendix 6*). Another flat and speckled casing, this one more greenish coloured and consisting of a bristle-like structure (Fig. 4D) revealed a strong signal for chloride (46.67%), but also titanium (3.74%) elements (*Appendix 7*). A white and foamy particle (Fig. 4E) showed titanium as the most dominant (66.45%; *Appendix 8*) element. Apart from these suspected plastics, there were also particles detected which were suspected to be pieces of porcelain, ceramic and glass.

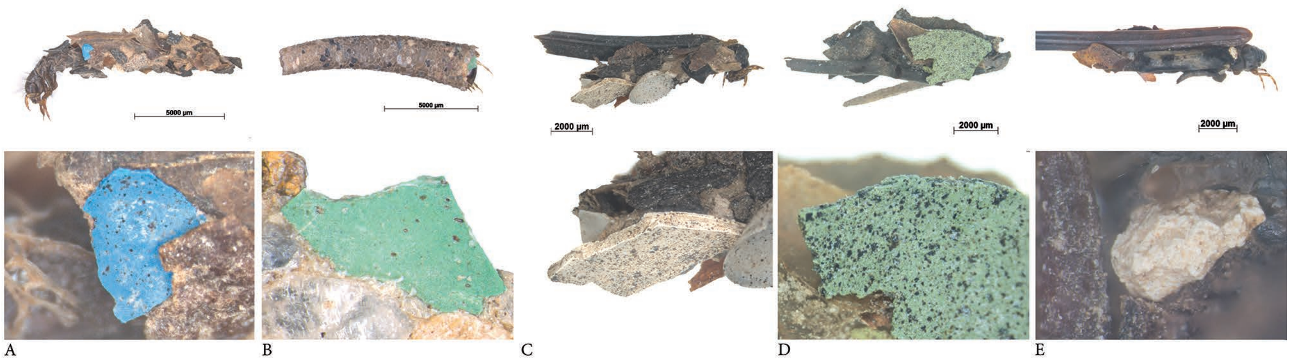

Fig. 4. Artificial particles on caddisfly casings, collected in the Noor (Limburg, The Netherlands). A) *Chaetopteryx villosa* casing with a blue particle incorporated (RMNH.INS1658128, Noorbeek 5.1), and detail. B) *Chaetopteryx villosa* casing with a green and flat particle (RMNH.INS.1658129, Noorbeek 2), and detail. C) *Chaetopteryx villosa* casing with a white, flat, speckled and layered particle, (RMNH.INS.1658130, Noorbeek 5.1), and detail. D) *Chaetopteryx villosa* casing with a flat, speckled and bristle particle (RMNH.INS.1658131, Noorbeek 2) and detail. E) *Chaetopteryx villosa* casing with a piece of white foam (RMNH.INS.1658132, Noorbeek 5.1), and detail. All casings are retrievable in jar BE.2289652 in the collection of Naturalis Biodiversity Center. Images credit: Isabel van der Velden and Auke-Florian Hiemstra.

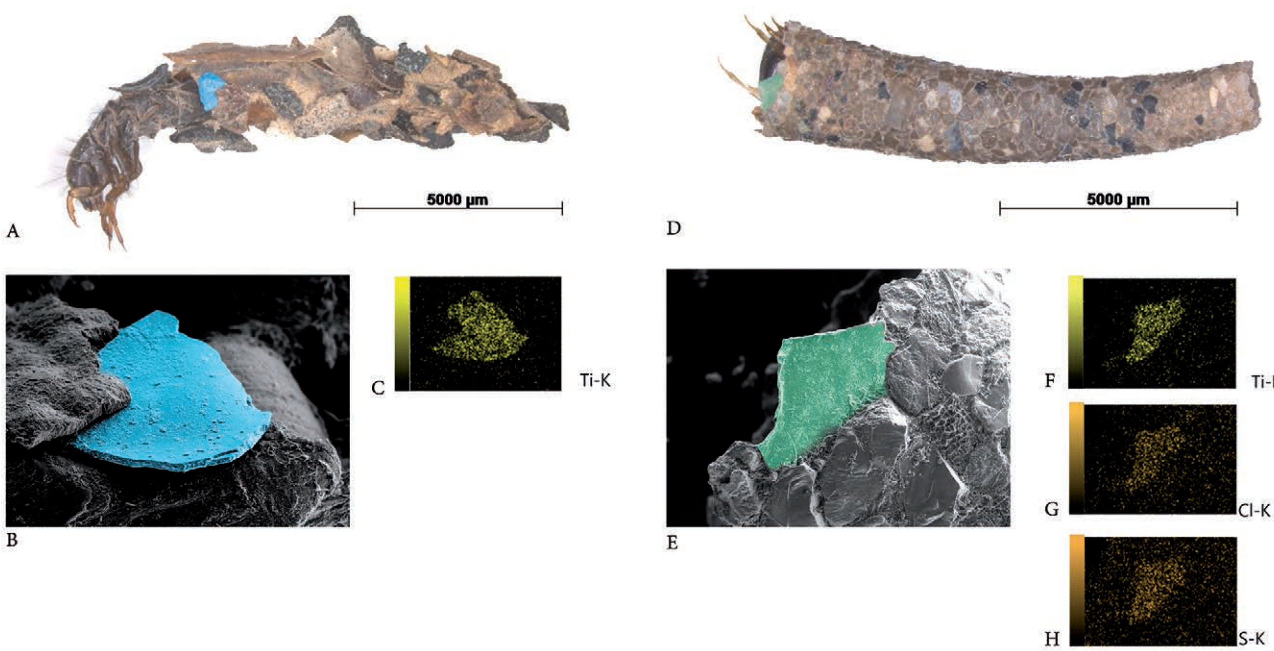

Fig. 5. A) *Chaetopteryx villosa* casing with larva with a blue particle incorporated (RMNH.INS1658128, Noorbeek 5.1). B) Colorized SEM photograph of the artificial particle. C) EDX mapping visualization showing the chemical elements found, in this case titanium. D) *Chaetopteryx villosa* casing with green and flat particle (RMNH.INS.1658129, Noorbeek 2). E) Colorized SEM photograph of the artificial particle (F,G,H) EDX mapping visualization showing the chemical elements found, in this case titanium, chloride and sulphur. Both casings are retrievable in jar BE.2289652 in the collection of Naturalis Biodiversity Center. Images credit: Isabel van der Velden and Auke-Florian Hiemstra.

DISCUSSION

Here we present new findings of caddisfly cases of the Anthropocene. During our study, we collected a total of 1,121 caddisfly cases, of which 311 cases presumably contained artificial materials like building brick fragments and MP particles. To our knowledge, this is the first time that these artificial caddisfly cases are reported from the Netherlands. In stream systems in an urban context with high pollution levels, such as Eyserbeek locality, more than half of all caddisfly cases were presumed to contain artificial material (55.1%). Under these conditions, the inclusion of artificial material by caddisfly larvae appears to be common. Moreover, our numbers must be an underestimate, as there is a high probability that we only detected part of the anthropogenic particles as a consequence of our non-destructive approach. Only the outer layer of particles was visually identified with light microscopy to be natural or artificial, by looking at notable colours, shapes and material types. Additional artificial particles could, however, have been present lacking such notable characteristics. Black, brown, grey or light-yellow coloured particles were often not considered as artificial material. Ehlers *et al* (2019) did find transparent (colourless) plastic particles in *Lepidostoma basale* (Kolenati, 1848) cases, which we might have mistaken for sand particles. The true percentage of casings with artificial material included could thus be considerably higher than the numbers reported here.

Inclusion of building bricks

While humans in Egypt have been building with brick since as early as 14.000 BC (Kadir and Mohajerani, 2011), our study shows that caddisfly larvae have also begun using this material. Of the 311 cases with artificial material, 193 were built with (at least one) building brick fragment. Multiple studies have been focusing on the incorporation of MPs in caddisfly cases (Díaz, 2018; Ehlers *et al*., 2019, 2020; Gallitelli *et al*., 2021; Valentine *et al*., 2022), but to our knowledge no one focused on other anthropogenic materials like brick, pottery and glass yet. The small brick fragments, that we hereby name micro-bricks, in particular stood out in our results as being the dominant artificial material for caddisfly case construction. In total, 136 of the brick-incorporating cases were collected in the stream Eyserbeek, which flows through the town of Simpelveld. Bricks are highly abundant in cities and villages, as they are the primary building material for houses. The study by Elhacham *et al.* (2020) on anthropogenic mass showed that bricks are a big part of our cumulative total, rising from just 11 Gt in 1900, to 16 Gt in 1940, and 28 Gt in 1980. The total mass of bricks on the planet now, around 2020, is estimated at 92 Gt, compared to only 4 Gt for animal biomass. The total amount of brick outweighs the total amount of asphalt (65 Gt) with wood, glass and plastic (23 Gt) combined. Bricks are thus highly abundant, and 1500 billion new bricks are produced each year (Venditti, 2021). When walls are taken down, masonry waste may be crushed and repurposed as recycled aggregate. These fragmented building bricks, often mixed with various other fragmented waste types like plastics, are used in road construction, landscaping or dumped directly in streams as streambank protection. Especially the latter increases the probability for interactions to happen with aquatic organisms, including caddisfly larvae, and construction waste is reported as streambank protection in more Dutch streams (van den Herik and Hoogveld, 1999; Kroodsma and de Vos, 2005; Spikmans, 2013; de Vos, 2012). As a consequence, pieces of brick and other materials mix with natural sediments. If these pieces of brick end up in a stream, they will break down into microparticles through erosion, becoming small enough for a caddisfly larva to work with. Our results show that caddisfly larvae include micro-bricks regularly in their constructions, sometimes even with multiple particles per casing (Fig. 2D). The bigger town of Simpelveld clearly has more caddisflies with micro-brick inclusions (376/136) than the smaller town of Noorbeek (580/57). While Noor may be less impacted by the town of Noorbeek, flowing directly into a forest, the Eyserbeek streams through the middle of the town of Simpelveld. However, there is no data on the historic use of bricks as streambank protection, making further comparisons difficult.

The specific weight of building bricks lies between 1,800 and 2,200 kg/m^3 (Brown, 2023), which is comparatively less than the density of natural stones like the quartz sand which is mainly found in Dutch rivers (De Vries, 1990), weighing 2650 kg/m^3 (Lopes de Leão, 1988). This means that the incorporation of building brick particles in a caddisfly case, made from inorganic material, may potentially lower its weight. During the construction of a caddisfly case, selection of the right building materials is more on size and shape, than on weight or hardness (Dodds and Hisaw, 1925; Hansell, 1968). If pieces are shaped appropriately, this may result in the selection of lighter particles. But weight is also important to prevent a case from being swept away by strong currents, therefore some caddisfly species even incorporate lateral extensions to their casing as ballast stones. One of the *Silo* sp. individuals encountered in the Noor had a lighter brick particle placed on its casing as one of its ballast stones (Fig. 3) and is referred to as a possible ‘evolutionary trap’ (Duncan *et al*., 2021; Robertson and Chalfoun, 2016). In streams with a strong current, having a lighter case may be a disadvantage. Rock fragments make cases stronger and let them sink faster, when they are temporarily disconnected from the bottom (Dodds and Hisaw, 1925). On the other hand, in a stream with a strong current, there may be a pre-selection of found materials, as lighter materials may not be available there. And there may be counteracting natural inclusions as well, like living molluscs or actively moving water mites that occasionally are built into a caddisfly casing, and have a bigger direct impact on the individual, than artificial material.

Inclusion of microplastics

Some caddisfly casings had brightly coloured inclusions, which we presumed to be pieces of MP. All examples featured in Fig. 4 show MP particles found in the Noorbeek, a spring that emerges from limestone and flows through a nature reserve. Just 50 m away from the spring one finds a sewage overflow valve which is connected to the stream. During intense rainfall, when the water volume exceeds the operational load of drainage sewers, the valves are opened, resulting in an overflow and the mixing of untreated wastewater with the natural stream water. During fieldwork in the Noorbeek, traces of these overflow events were visible in the form of discharged women’s hygiene products that littered the creek. The forest through which the creek runs is not accessible for the public, there are no walking paths, and no other litter was observed, except for macro litter in the stream itself, that is clearly linkable to the frequent overflows. Next to these macroplastics, such wastewater contains a high abundance of MP, as previously measured in France (Dris *et al*., 2015; Treilles *et al*., 2021;) Italy (Di Nunno *et al*., 2021), and China (Chen *et al*., 2020; Zhou *et al*., 2023).

A small subset of our presumed MP particles (Fig. 4) could be scanned with EDX microscopy and were confirmed as artificial compounds of titanium, chloride and sulphur. This is an elemental composition that agrees with what has been reported for MPs. Since the particles are not confirmed as MPs by polymer type analysis, they are therefore regarded as suspected MP's. Fries *et al*. (2013) state that titanium and sulphur are common plastic additives. Gniadek and Dąbrowska (2019) report that MPs are rich in titanium, sulphur, and chloride. To our knowledge this is the first time a sewage overflow is directly linked to the inclusion of MPs in cases by caddisfly larvae. The specific conditions of Noor enabled us to establish this connection, further demonstrating how these overflows are impacting freshwater ecosystems.

The density of plastic is significantly lower for the common types of PP and PVC (905 kg/m^3 and 1,384 kg/m^3, respectively) than the density of quartz sand (2,650 kg/m^3), therefore incorporation of these plastics would decrease the weight of caddisfly cases even more than the earlier discussed micro-bricks (Ames Web, https://amesweb.info/Materials/Density-of-Plastics. aspx). Laboratory research by Ehlers *et al.* (2020) showed the effect this may have on case stability of *Lepidostoma basale,* which is reduced by the incorporation of PVC and PET MP particles, but only when this was included in high amounts. Interestingly, examples with 2.90% and 28.67% PVC MP in their case were more stable than 'pure sand' cases, higher percentages of MP did lead to unstable casings (Ehlers *et al*., 2020), but these may not (yet) reflect field conditions.

Ehlers *et al.* (2019) showed that blue-coloured plastic particles (as displayed in Fig. 5A) were most often incorporated in cases of wild *Lepidostoma basale* larva, and brightly coloured plastic particles were more often incorporated in *L. basale* cases in comparison with less notable colours such as black or brown. The inclusion of bright unnatural coloured particles may increase the visibility of caddisfly larvae to visual predators like fish and birds (Otto and Svensson, 1980). However, when also taking their surroundings in consideration, such as the Eyserbeek's riverbed full of 'confetti-sediment' with many artificial elements, including micro-bricks, micro-plastics and other colourful artificial materials, one could argue that a casing including artificial material may stand out less than a fully natural one.

Microplastics flushed into the food chain

To modify artificial materials, caddisflies are known to chew on plastic. As a result, they are considered a driver of MP formation (Valentine *et al*., 2022). However, this behaviour may also lead to the ingestion of plastic particles by the larvae (López-Rojo *et al*., 2020; Windsor *et al*., 2019; Winkler *et al*., 2022). Both the ingestion by the larvae and the inclusion in their casings may be a route for MPs into the food chain. Caddisflies are of ecological importance as they are an important food source and fall prey to a variety of vertebrate and invertebrate predators (Hickin, 1967), such as amphibians, hellgrammites (Corydalidae), fly larvae (Scathophagidae), predatory stoneflies (Systellognatha), dobsonfly larvae (Corydalidae), predaceous diving beetles (Dytiscidae), and dragonfly larvae (Anisoptera) and a few other trichopterans (Holzenthal *et al*., 2015; Rinne and Wiberg-Larsen, 2017; Tempelman *et al*., 2022). They are also preyed upon by bottom-dwelling fish like sculpins (Cottoidea), darters (Etheostomatinae), trout (Salmoninae), and other fishes (Holzenthal *et al*., 2015; Thorp and Rogers, 2011). Some crush the case, eat the larvae and expel the leftovers, others swallow the larva and case altogether (Wiggings, 2004). For birds like dippers (*Cinclus)*, grey wagtail (*Motacilla cinerea;* Tunstall, 1771), waders (Charadriiformes), grebes (Podicipediformes) and ducks (Anatidae), some of which have become specialised predators of aquatic insects, larval caddisflies make up a substantial part of the diet (Wiggings, 2004). While dippers remove the larvae from their cases by repeatedly knocking them against a rock, other birds such as ducks ingest both the larva and case whole (Wiggings, 2004). Once the larvae enter their adult stages, they leave their casing behind but presumably take any ingested MP or transported toxic chemicals with them, into the terrestrial food web, where they are part of the diet of spiders, predaceous beetles, numerous birds and bats, fishes, and other insect species (Rinne and Wiberg-Larsen, 2017; Tempelman *et al*., 2022). Finally, prolonged interaction with plastic once included in a casing, may also result in a higher exposure to the harmful chemicals and toxic metals often associated with plastics. Polycyclic aromatic hydrocarbons, polybrominated diphenyl ethers and heavy metals are known to accumulate in organisms (Rochman, 2015; Wardrop *et al*., 2016). These transported toxic chemicals, and the MP ingested by the caddisfly, and/or the ingesting by a predator of both the caddisfly and its whole casing, including bigger plastic particles, are all routes for MPs to pose a potential risk to animals at high trophic levels, as the ability of MPs to move up in the food chain has been demonstrated (Farrell and Nelson, 2013; Setälä *et al*., 2014; Su *et al*., 2016). Here we warn that the MP load of sewage overflows may impact both the aquatic and the terrestrial ecosystem, as caddisfly larvae act to spread these plastics into a broad and diverse food web.

Limits of the study

From a methodological perspective, the non-destructive approach followed may not be ideal for detailed MP analysis, as a polymer type analysis is lacking. It is advised for future studies to allocate a portion of the sample for destructive MP analyses, while keeping another part for of the sample intact as additions to the timeseries in our natural history collection.

CONCLUSIONS

Dumped masonry waste for streambank protection and MPs from sewage overflow influence how caddisfly larvae are building their casings. For The Netherlands we can conclude that at least seven different Dutch species of caddisflies incorporate artificial particles in their cases. Earlier studies have shown that some caddisfly species are picky about the particles they incorporate in their case construction, while others exhibit more flexibility in their choice of materials (Gaino *et al*., 2002; Hanna, 1961), and so not all species may easily shift to artificial alternatives. But the species that do so, may introduce artificial particles like synthetic polymers into the food web. Action should be taken urgently to minimize the use of these sewage overflows, as they introduce a high MP load into the surroundings. These sewage overflow events are already common and expected to increase due to climate change and the fact that our anthropogenic mass, including our waste, bricks, and plastic, is predicted to exceed 3 Tt by 2040 (Elhacham *et al*., 2020). If realized, there will be three times as much human-made mass than dry biomass on Earth, which will, besides many other effects, impact caddisfly case construction in an even more dramatic way than is the case today.

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Online supplementary material:

Appendices 1-8.