

Baikal endemic and Palearctic species of caddisflies (Trichoptera) build cases from microplastics

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Pollution of freshwater ecosystems by microplastics is a serious problem. Many studies are related to the pelagic zone and the organisms living in it. However, benthic organisms are most susceptible to this problem in aquatic ecosystems. Benthic organisms can both consume microplastics and incorporate them into their cases. The last statement concerns caddisflies. In this study, cases and larvae of two caddisfly species were analyzed. The first species is endemic to Lake Baikal (*Baicalina thamastoides*), the second species is a Palearctic species (*Hydatophylax nigrovittatus*). Microplastic fragments were found in both species. However, we are the first to show that microplastic fragments can be embedded in cases in large numbers (from 10 to 38, with an average value of 20.56 ± 1.91 specimens/case in *H. nigrovittatus*). For this species, there is a negative significant ($p < 0.05$) correlation between the length of the larvae and the number of microplastic particles (fragments). In addition,

mesoplastics with a length of more than 5 mm were found among the embedded particles.

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Keywords

Baicalina thamastoides, *Hydatophylax nigrovittatus*, Lake Baikal, microplastic, mesoplastic, plastic pollution

Introduction

Microplastic pollution of the natural environment is currently a widespread problem. The term microplastics refers to particles of artificial polymers with an upper limit of 5 mm (Free et al. 2014; Gomiero et al. 2019; Thomas et al. 2024). Microplastic particles are found in a variety of places (Enyoh et al. 2019; Corradini et al. 2019; Napper et al. 2020; Zhang et al. 2020). Thus, there is a large amount of data on microplastic pollution of marine ecosystems (Issac and Kandasubramanian 2021; Sanchez-Vidal et al. 2021; Zavala-Alarcón et al. 2023). At the same time, pollution of the surface layer of water has been studied to the greatest extent (Free et al. 2014). However, it is now clear that freshwater bodies are susceptible to microplastic pollution to an even greater extent, because have much greater contact with urbanized areas as opposed to marine ecosystems (Windsor et al. 2019; Frank et al. 2022). In addition, it became obvious that microplastics settle over time and its concentration must be assessed not only at the surface of the water, but also at different horizons, as well as at the bottom. It is worth considering that the final place for the accumulation of microplastic particles is the bottom of the reservoir (Claessens et al. 2013; Pinheiro et al. 2020; Sanchez-Vidal et al. 2021; Frank et al. 2022; Pastorino et al. 2023).

Microplastics have now been found in the natural environment in many organisms from zooplankton to aquatic mammals (Winkler et al. 2022; Pastorino et al. 2023). Basically, again, the emphasis was on marine organisms (Gutow et al. 2016; Zavala-Alarcón et al. 2023). Microplastic particles are transmitted through the food chain (Windsor et al. 2019; Pinheiro et al. 2020; Winkler et al. 2022; Pastorino et al. 2023), where higher numbers of particles are found in organisms at higher levels. Moreover, in the same ecosystems, fish, for example, those associated with the bottom, have a larger number of particles than pelagic fish (Goss et al. 2018). This confirms the fact that plastic accumulates at the bottom, where it enters the appropriate trophic chain. Accordingly, organisms from these chains may be the main ones for assessing microplastics in aquatic communities (Ehlers et al. 2019).

The problem of microplastics in the unique ancient Lake Baikal is still poorly understood. At the moment there are only a few studies that relate to the lake (Karnaukhov et al. 2020; Il'ina et al. 2021; Karnaukhov et al. 2022; Moore et al. 2022; Meyer et al. 2022a; Meyer et al. 2022b). Some

studies refer to the Lake Baikal basin (Free et al. 2014; Battulga et al. 2019; Karnaukhov et al. 2020). It should be borne in mind that these studies mainly refer to the upper layer of water. Currently, studies of microplastics on the bottom and in the organisms of Lake Baikal are not carried out. Taking this into account, we decided to look at microplastic particles in caddisfly cases. The aim of this study was to analyze the incorporation of microplastic particles by different caddisfly species from Lake Baikal.

Materials and methods

In this study, we analyzed the available collections of two species of representatives of Trichoptera. The first species was the Baikal endemic *Baicalina thamastoides* Martynov, 1914 (227 individuals), the second species was the larger and more widespread *Hydatophylax nigrovittatus* (McLachlan, 1872) (18 individuals), which is an eastern Palearctic species (Lepneva 1966). The analysis consisted of counting microplastic particles embedded in the cases, measuring them, determining the type of polymer, and the color of the particles. Individuals of both species were collected near settlements near piers (*B. thamastoides* – Bolshiye Koty village (51°54'11.5"N, 105°04'09.4"E), *H. nigrovittatus*– Listvyanka village (51°50'53.2"N, 104°52'16.7"E)). Moreover, the anthropogenic impact in the second site is noticeably higher than in the first (Timoshkin et al. 2016).

Identification of microplastic particles was carried out visually (color, shape) using the “hot needle” method (Masura et al. 2015). Particle counting and measurement of the length of cases and larvae were carried out using a stereomicroscope (UNITRON Z850). The area was measured from the photographs taken using ImageJ software. Weighing of cases and larvae was carried out on an OHAUS AX224 analytical balance. The polymer type was determined using a Perkin Elmer FT-IR ATR Spectrum Two spectrometer.

Results and discussion

The analysis showed the following results (Tab. 1). In caddisflies of the species *B. thamastoides* (Fig. 1), the number of microplastic particles per case was 0.18 ± 0.02 . This number is close to, but significantly less than that of caddisflies of the species *Lepidostoma basale* (Kolenati, 1848) in the Saynbach stream (Ehlers et al. 2019). However, in the caddisfly species *H. nigrovittatus* (Fig. 2), the number of particles per case at the sampling site is 20.56 ± 1.91 . This number exceeds the available data on the inclusion of microplastic fragments in cases of caddisflies in the natural environment (Tibbetts et al. 2018; Ehlers et al. 2019; Gallitelli et al. 2020; Gallitelli et al. 2021; Alvarez Troncoso et al. 2022). In addition, inclusions of mesoplastic particles were observed in individuals of this species ($n=3$, length – $8,27 \pm 0,62$ mm, area – $32,61 \pm 3,35$ mm²). It is worth noting that some studies have observed a similar or greater number of particles per case (Gallitelli et al. 2020; Gallitelli et al. 2021), but these particles were fibers, and in our study we see large fragments (Fig. 2).

In addition to the available data, we conducted Spearman correlation analyzes (R_s , this correlation test was used due to the fact that the data turned out to be nonparametric). Relationships were considered statistically significant at $p < 0.05$. The strength of the connection was assessed using the Chaddock scale. Thus, no relationships were found between the number of particles and the length of the case, the number of particles and the length of the larva, the number of particles and the mass of the case, and the number of particles and the mass of the larva in the species *B. thamastoides*. In turn, for the species *H. nigrovittatus* there is no relationships between the number of particles and the mass of the case. Between the number of particles and the length of the case, R_s is equal to -0.41 , and between the number of particles and the mass of the larva, $R_s = -0.31$. At the same time, relationships in comparisons are statistically unreliable. However, there is a statistically significant average negative relationship between the number of particles and the length of the larva in *H. nigrovittatus* ($R_s = -0.62$; $p = 0.03$).

Species name	Parameters	Length case, mm	Length larva, mm	Weight case, g	Weight larva, g	M/p, unit	Length m/p, mm	M/p area, mm ²
<i>Baicalina thamastoides</i>	Mean	8.76	7.07	0.0268	0.0032	0.18	0.86	0.86
	SE	0.07	0.09	0.0008	0.0001	0.02	0.07	0.04
	Min	6	5	0.0119	0.0018	0	0.42	0.09
	Max	12	10	0.0442	0.0052	2	1.78	1.009
<i>Hydatophyla x nigrovittatus</i>	Mean	22.67	16	0.0748	0.0050	20.56	1.66	1.65
	SE	0.73	0.85	0.0113	0.0007	1.91	0.16	0.50
	Min	19	10	0.0154	0.0027	10	0.55	0.21
	Max	30	20	0.1644	0.0076	38	3.49	13.33

Table 1. Results of measurements and calculations of available samples of caddisflies

From the data obtained it is clear that in the endemic species *B. thamastoides*, microplastic particles are found almost sporadically in cases. This can justify the absence of any correlations. At the same time, the widespread *H. nigrovittatus* exhibits a large number of particles embedded in the case. It is possible that microplastic particles being in close proximity to the larval covers have a negative effect on its growth, or caddisflies spend more energy maintaining themselves at the bottom (due to the greater buoyancy of microplastic particles compared to sand). The fact that this can happen has been discussed many times before, including for caddisflies (Ehlers et al. 2019; Ehlers et al. 2020).

Another important parameter that we took into account was the color of the particles built into the case. For both species, the largest percentage was white particles (Fig. 3; Tab. 2). The most common particles are red, gray, azure, blue and orange. It is likely that in the areas where these species are found, microplastic particles of these colors predominate at the bottom. The greatest variety of colors of microplastic particles was recorded for *H. nigrovittatus*. We believe that this is due both to the size of these individuals and their cases, and to the greater anthropogenic load at the place of their collection (compared to *B. thamastoides*) (Timoshkin et al. 2016), because caddisflies tend to mimic the surrounding biotope. It is worth noting that particles of similar colors have been recorded in other studies (Ehlers et al. 2019; Gallitelli et al. 2020).

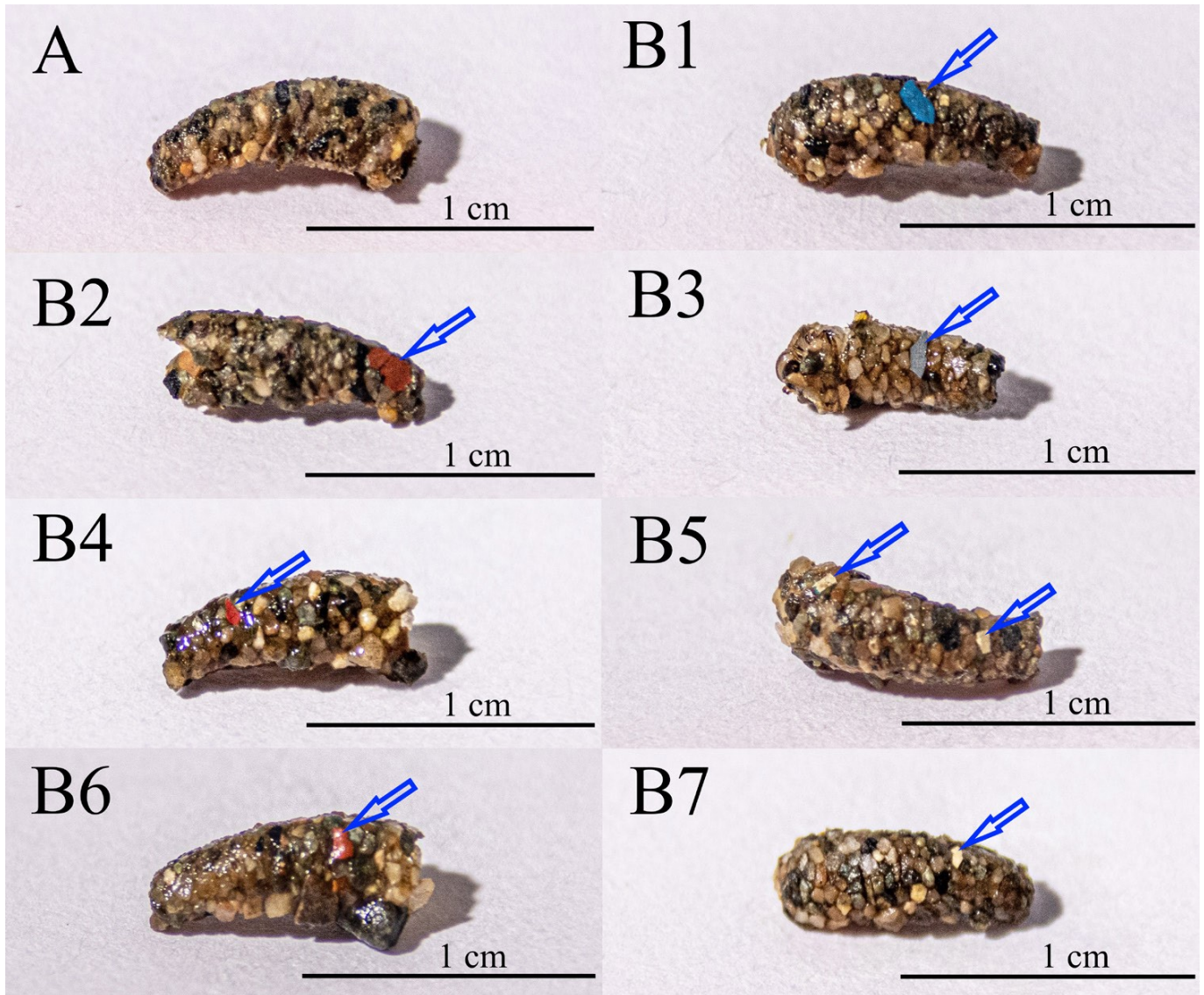


Figure 1. Examples of inclusion of microplastics in cases of caddisflies of the species *B. thamastoides*: A - case without microplastic, B1-7 - cases with microplastic (microplastic particles are marked with arrows).

Identification of particles using spectroscopy showed that the detected fragments belong to such types of polymers as alkyd resin, PVA (polyvinyl alcohol), PVC (polyvinyl chloride). This composition of polymers is consistent with the habitat of these caddisfly individuals (the area of the pier and the mooring of ships for decades). It is likely that caddisflies in other areas of Lake Baikal will use microplastic particles to a lesser extent (due to the lower availability of particles in other areas). It is worth noting that these artificial polymers are found in large quantities in other ecosystems (Gomiero et al. 2019; Alvarez Troncoso et al. 2022; Thomas et al. 2024).



Figure 2. Examples of the inclusion of microplastics in cases of caddisflies of the species *H. nigrovittatus*: A – case without microplastic (from another sampling point in Lake Baikal), B1-7 – cases with microplastic and mesoplastic (mesoplastic particles are marked with arrows).

No	Colors	Species	
		<i>H. nigrovittatus</i>	<i>B. thamastoides</i>

1	White	166	19
2	Red	46	13
3	Orange	3	5
4	Grey	63	3
5	Blue	13	1
6	Azure	54	0
7	Green	16	0
8	Wheat	3	0
9	Aquamarine	3	0
10	Black	2	0
11	Yellow	1	0

Table 2. Total number of microplastic particles and their color

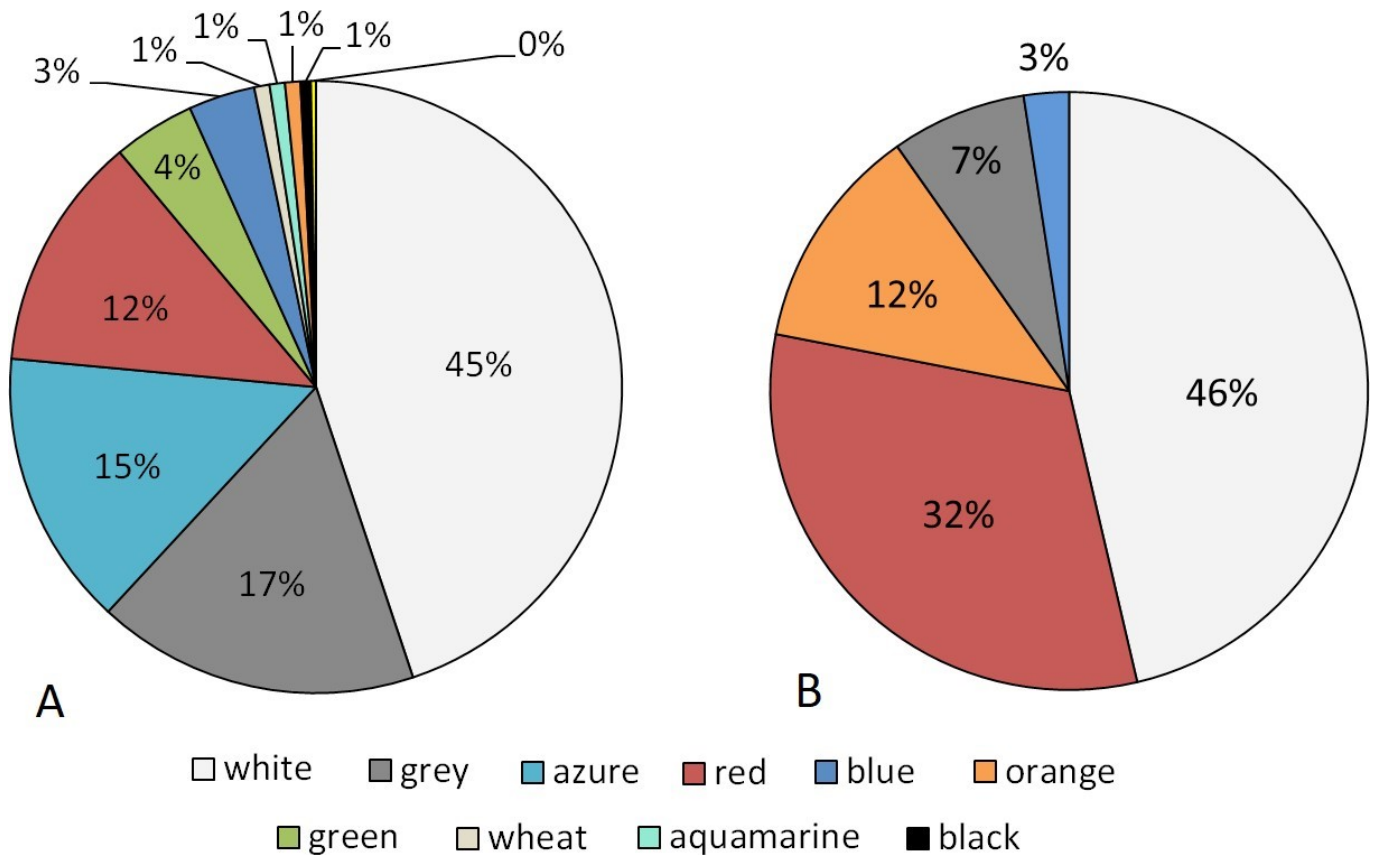


Figure 3. Percentage of microplastic particles of different colors: A - *H. nigrovittatus*, B - *B. thamastoides*.

Conclusion

Based on the presented work, it is clear that the issue of including microplastic particles in caddisfly cases has been little studied. Apparently, the incorporation of particles will depend, on the one hand, on the species of caddisfly and its size, and on the other hand, on the level of pollution of the territory with microplastics. However, this requires additional research. Special attention should be paid to the influence of embedded particles on the growth of caddisfly larvae and the further formation of adult individuals from them.

Author contributions

Conceptualization and validation, D.Y.K. and A.V.L.; investigation and resources, D.Y.K.; writing–original draft preparation, D.Y.K. and A.V.L.; writing–review and editing, E.A.S.; visualization, A.V.L. and B.V.O.; supervision and project administration, D.Y.K. and E.A.S.; collected samples in the field, A.T.G., K.V.S., A.V.N. and D.I.G.; processed laboratory samples, I.A.Z., Y.K.E., S.A.B., A.I.O., I.V.M., N.A.K., L.B.B. and M.A.M.; funding acquisition, D.Y.K. All authors have read and agreed to the published version of the manuscript.

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