



# Caddisfly larvae (Trichoptera: Limnephilidae) facilitate the uptake of microplastics by a freshwater fish (*Ameiurus nebulosus*)<sup>☆</sup>

Hélène Pfister<sup>a,\*</sup> , Geneviève D'Avignon<sup>a,c,\*\*</sup> , Aimy Wang<sup>a</sup> , Miguel Eduardo L. Felismino<sup>a,c</sup> , Anthony Ricciardi<sup>a,b,c</sup> 

<sup>a</sup> Department of Biology, McGill University, Montreal, Quebec, Canada

<sup>b</sup> Bieler School of Environment, McGill University, Montreal, Quebec, Canada

<sup>c</sup> Group for Interuniversity Research in Limnology (GRIL), Canada

## ARTICLE INFO

### Keywords:

Microplastics  
Trichoptera  
Trophic transfer  
Food webs  
Predator-prey interactions  
Freshwater ecosystems

## ABSTRACT

Microplastics are pervasive in inland waters and are entering aquatic food webs through diverse pathways. Caddisflies (Trichoptera) are common prey items for fishes and some aquatic birds; they have been found to incorporate microplastic particles in their larval cases, which are a potential vector of plastic transmission to higher trophic levels. We examined this novel pathway in two sets of experiments. First, we exposed caseless *Limnephilus* larvae to either i) a 50 % mixture of polyethylene terephthalate (PET) microfragments and natural materials, or ii) 100 % natural materials, and compared their subsequent case construction. *Limnephilus* consistently incorporated PET into their cases, even when natural material was available. However, only 5 % of larvae constructed a case completely covering the abdominal region of their body when they used PET fragments. Cases incorporating plastic had a lower mass than those constructed entirely from natural materials, regardless of the mass of the larvae. These findings suggest that the incorporation of microplastics affects case structure and, potentially, functionality. In a subsequent set of experiments, we offered four partially plastic-cased and four natural-cased caddisfly larvae to a benthivorous fish, the brown bullhead (*Ameiurus nebulosus*). Ninety percent (9/10) of the bullheads selected a larva with plastic in its case over a larva with a fully natural case as the first prey they attacked. Overall, consumption of plastic-cased larvae represented 63 % of all prey consumed, suggesting that the incorporation of PET microplastic fragments in case matrices renders larval caddisflies more vulnerable to predation. Case-building caddisflies can thus facilitate the transfer of plastic and associated contaminants into aquatic food webs.

## 1. Introduction

Microplastics (MP) are pervasive novel contaminants in aquatic ecosystems (D'Avignon et al., 2022). MP particles (spheres, fragments, fibers, films) are, by definition, less than 5 mm in size and composed of synthetic polymers. Some are manufactured specifically for commercial or industrial use (e.g., as mild abrasives in cosmetic products), whereas others are derived indirectly from the degradation of larger plastics. Owing to their physical and chemical properties, MP have a propensity to sorb waterborne persistent contaminants, including carcinogenic polycyclic aromatic hydrocarbons, endocrine-disrupting Bisphenol A,

and teratogenic metals such as lead, cadmium, and mercury (Menéndez-Pedriz and Jaumot, 2020). MP particles and their associated contaminants interact with diverse organisms across trophic levels, from plankton to fish, and can threaten the health and function of these organisms (Earn et al., 2021; Bucci and Rochman, 2022; D'Avignon et al., 2022).

Studies of the occurrence and fate of MP in aquatic food webs have identified various pathways of trophic transfer, including direct consumption of environmental-available particles, ingestion of contaminated food items, and uptake via respiration (Wang et al., 2020; Latchere et al., 2021). Often overlooked in these studies are the

<sup>☆</sup> This paper has been recommended for acceptance by Maria Cristina Fossi.

<sup>\*</sup> Corresponding author. Department of Biology, McGill University, 1205 Docteur Penfield, Montreal, Quebec H3A 1B1, Canada.

<sup>\*\*</sup> Corresponding author. FLSH Géomatique appliquée, Université de Sherbrooke, 2500, boul. de l'Université, Sherbrooke (Québec) J1K 2R1, Canada.

E-mail addresses: [helene.pfister@mail.mcgill.ca](mailto:helene.pfister@mail.mcgill.ca) (H. Pfister), [genevieve.davignon@usherbrooke.ca](mailto:genevieve.davignon@usherbrooke.ca) (G. D'Avignon), [aimy.wang@mail.mcgill.ca](mailto:aimy.wang@mail.mcgill.ca) (A. Wang), [miguel.felismino@mail.mcgill.ca](mailto:miguel.felismino@mail.mcgill.ca) (M.E.L. Felismino), [tony.ricciardi@mcgill.ca](mailto:tony.ricciardi@mcgill.ca) (A. Ricciardi).

<https://doi.org/10.1016/j.envpol.2025.127133>

Received 11 April 2025; Received in revised form 29 August 2025; Accepted 16 September 2025

Available online 22 September 2025

0269-7491/© 2025 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC license (<http://creativecommons.org/licenses/by-nc/4.0/>).

non-trophic mechanisms of transfer; for example, MP introduced into food webs by adhering to an organism's body or being caught in their appendages (Cole et al., 2013; Kolandhasamy et al., 2018). Another non-trophic mechanism is derived from the behaviour of caddisfly larvae (Trichoptera) that use environmental materials (e.g., sand grains, macrophyte fragments, molluscan shell debris, mineral grains) bound with secreted silk to construct a portable cylindrical case covering their body, including their entire abdomen, and opened at both ends to facilitate respiration and provide a protective shelter (Cummins and Klug, 1979; Cairns and Pratt, 1993; Morse et al., 2019). Caddisfly larvae can use synthetic material for this purpose (Duprat and Besson, 2004). A recent study examining museum collections confirmed that larval caddisflies have been incorporating plastic into their cases as early as 1971 (Hiemstra et al., 2025a), a time when reports of plastic pollution in aquatic environments were scarce.

Recently, several studies have reported the presence of microplastic in caddisfly cases, suggesting a ubiquitous phenomenon (Hiemstra et al., 2025b). For instance, up to 7 % of larval caddisflies sampled in a British river catchment had MP in their case matrix (Tibbetts et al., 2018), whereas 59 % of larval *Lepidostoma basale* collected from a tributary of the Rhine River had an average load of 0.36 MP particles per milligram of case material (Ehlers et al., 2019). The incorporation of plastics in the larval cases of caddisflies reduces case stability, as found for *L. basale* (Ehlers et al., 2020), suggesting that these added synthetic materials could increase larval vulnerability to predation and other environmental stressors.

Caddisflies comprise a substantive proportion of macroinvertebrate biomass (Wallace and Merritt, 1980) and are a common food source for multiple predators in aquatic habitats including birds (Wiggins, 2005), predaceous insect larvae (e.g., stoneflies, dragonfly, other trichopterans; Holzenthal et al., 2015; Usio et al., 2001), as well as many benthic fishes (Hiemstra et al., 2025b; Holzenthal et al., 2015). Crayfish can crush the case with their chelae to extract and consume the larvae (G.D. & A.R., *personal observation*). Some waterbirds extract and ingest caddisfly larvae from their cases by striking them repeatedly against rocks, while other ducks and trout ingest the larva with its case intact (Wiggins, 2005). Therefore, we suggest that caddisflies that incorporate microplastics to their cases can serve as vectors for the transfer of MPs and associated contaminants to predators. However, evidence has so far not demonstrated this phenomenon.

Here, we test whether the use of polyethylene terephthalate (PET) as case-building material by larval caddisflies affects their risk of predation by brown bullhead (*Ameiurus nebulosus*), a benthivorous fish that is locally abundant in lakes and large rivers throughout eastern North America. Specifically, we tested the following hypotheses: 1) MPs incorporated into larval caddisfly cases compromise case structure and functionality, thereby increasing predation risk; and 2) brown bullheads exhibit a higher propensity to ingest larvae in cases constructed from MPs compared to those built from natural materials.

## 2. Materials and methods

### 2.1. Pilot studies on caddisfly case induction

In 2020, we conducted pilot experiments to test if limnephilid caddisflies with mineral cases could incorporate PET bottle fragments to their case. We sampled a population of limnephilid caddisflies (from the outflow of Lac-des-Deux-Montagnes, Quebec; Fig. S1A–B). Natural cases of these caddisflies were extracted and sieved to identify the size and types of materials they used, namely twigs and mineral grains ~500–5000 µm in size. PET soda bottles (density of 1.38–1.41 g/mL) were cut into pieces of 500–1000 µm maximum dimension to match the size range found in their original case. Results of these pilot experiments showed the larvae integrated PET fragments frequently in their new case even when natural material was available for case-building, and produced a new case within 48 h, a typical timeframe for case construction

(Hinchliffe and Palmer, 2010; Ehlers et al., 2020). Treatments of ~1000 µm PET plastic fragments had the most successful case-building outcome, i.e. highest frequency of completion with microplastic incorporation, so these size fractions were selected for further case-building experiments.

### 2.2. Pilot study caddisfly fish predation

To select fish species for our experiment, we performed a literature search, a stomach content analysis across 52 fish species, and a pilot study (see **Supplementary materials**). Previous studies on the diets of yellow perch, rock bass, and brown bullhead confirmed inclusion of larval caddisflies in their respective diets (Fig. S2). To determine if these fishes are suitable limnephilid caddisfly predators, we captured these fish from local waterbodies in southwestern Quebec: Lac St-Louis, Lac Hertel and Lac des Battures. Our pilot study was designed to validate potential consumption of caddisfly larvae by these three visual benthivorous predators known for selective prey consumption (Michaletz et al., 1987). Only brown bullhead readily hunted limnephilid caddisflies with a mineral grain case whether the larvae were alive and moving, in pupal stage, or frozen and thawed; therefore, this species was selected as a predator for subsequent experiments.

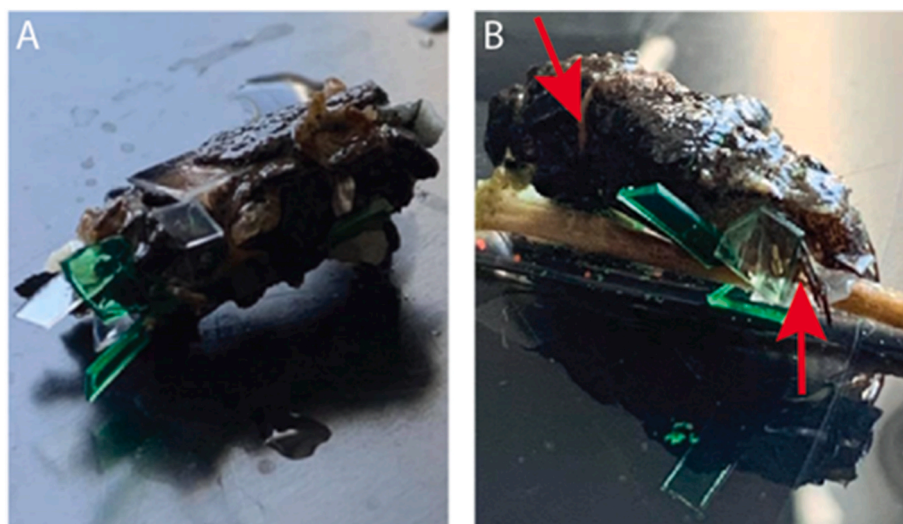
### 2.3. Collection and care of study animals

A total of 236 *Limnephilus* sp. (Family: Limnephilidae) caddisfly larvae (493 mg, range 109.5–920.4 mg) were collected manually from the undersides of rocks along the shores of Lac des Deux-Montagnes, Quebec (45°24'06.2"N, 73°57'33.9"W), from June to August 2020 (Fig. S1A–B) along with water and substrate from their collection sites. All captured specimens were placed into coolers filled with ambient water and transported to our laboratory at McGill University, where they were identified to genus, following Merritt (2008) and Wiggins (1996). They were then placed into groups of five individuals in oxygenated and filtered 8-L aquaria containing a mixture of dechlorinated tap water and water from the collection sites. Unionid mussel shells were added as shelter. The aquaria were held in temperature-controlled chambers set at 18 °C on a 12-h light/12-h dark schedule. Larvae were fed every second day with 70 mg of algae flakes and 1 mL of Kent Marine Phytoplex aqua-cultured phytoplankton solution.

Using seine and fyke nets, we collected 14 brown bullheads (11 from Lac Hertel and three from Lac des Battures, Québec) in July and August 2020. Fish had a mean total length of 18.6 cm (range 14.5 cm to max 23 cm) and a mean mass of 77.5 g (range 30.2 g–156.7 g). They were brought to the outdoor mesocosm array at the Gault Nature Reserve (Mont Saint-Hilaire, Quebec) and acclimated for 14 days. Fish were fed daily with a mixture of fish pellets, bloodworms, dried red shrimp, live and frozen caddisfly larvae. Mesocosms were mounted with a three-layer filtration system and oxygenation. Water temperature was maintained between 18 and 20 °C and water quality (ammonia, nitrate, nitrite, pH) was monitored every day for the first week, then once a week. At the end of the acclimation period, fish were placed individually in oxygenated and filtered 20-L indoor aquaria or coolers held at 18 °C, then bullheads were fasted for four to five days before the onset of the experiment. During fasting, water quality and fish behaviour was monitored to ensure healthy conditions.

### 2.4. Preparation of exposure materials

Natural material used by the larvae to build cases included plant fragments, twigs, sand and mineral grains selected from Lac des Deux-Montagnes collection site (Fig. S3). These natural materials were collected from the field sites, then sieved into standardized size classes of ~500 µm and ~1000 µm (size of material in their original case) to create subsamples of 4000 mg for the natural material treatment. The mixed



**Fig. 1.** Cases constructed by individual larvae. Two separate larvae are shown after a 48 h exposure to 50:50 microplastic to natural building material treatment for case building induction. A) Complete case, B) incomplete case where larvae could not fully retract; red arrows show gaps where larval body is exposed. Integrated plastic can be observed at the posterior end of both cases. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

material treatment consisted of ~2000 mg of natural materials prepared as described above and ~2000 mg of PET fragments cut from plastic soda bottles (density of 1.38–1.41 g/mL) into rough cubes of ~1000  $\mu\text{m}$  in diameter (Fig. S4A). To reduce the buoyancy of our particles, we conditioned them so that a biofilm would form on the surface of the plastic, thus increasing its mass and allowing to sink more readily. The PET fragments produced ranged in mass from 1 to 10 mg per piece, therefore we estimate the total number of pieces of plastics offered per treatment to be between 100 and 1000 fragments per 4000 mg of material. This would yield 25 000 to 250 000 PET fragments per kg of dry mass, representing a concentration range near the extremity end of those observed in the environment, including ~25 000 particles per kg dry mass (Etobicoke Creek, Canada; Ballent et al., 2016; Wen-Rui Tang River, China; Wang et al., 2018) or 72 400 particles per kg dry mass (English rivers; Hurley et al., 2018).

### 2.5. Caddisfly preparation and case building

To prepare caddisflies for experiments, individual larvae were removed from their case manually using thin elongated forceps (Stuart and Currie, 2001). Larvae were then weighed and deposited into 8-L aquaria filled with dechlorinated water and mounted with a filtration unit and an aerator. Caddisflies were initially placed individually ( $n = 54$ ) into each aquarium, then subsequently paired ( $n = 55$ ) because it increased the efficiency of case building (Fig. S5, Table S1). Aquaria were maintained at 18 °C on a 12 h light/12 h dark schedule. Caddisfly larvae (mean 267 mg, range of 32–571 mg) were randomly assigned to either 100 % natural substrate or 50 % synthetic and natural mixture for 48 h. Larvae exposed in pairs were offered 8 g of either mixture to standardize individual exposure (Fig. S4A).

### 2.6. Case structure

At the end of the experiment caddisflies were removed from the aquaria, and we recorded the mass of their new cases (wet mass in mg), their mortality status (alive, dead), and their case construction status. Case construction was considered as successfully induced if the caddisfly began to construct a new case (including both partially completed and completed cases), while a failure was noted where no new case was started. A ‘complete case’ (Fig. 1A) is one in which the larva can fully retract and hide its body, whereas an ‘incomplete case’ (Fig. 1B) is one in

which the case does not fully obscure the abdominal body of the larvae, owing to the case size or to gaps in the matrix. Case building trials were repeated until 40 caddisflies of each treatment had produced a new case—the sample size needed to examine preferential consumption by fish, corresponding to 46 and 66 natural and mixed-cased caddisflies respectively (Fig. S4A).

To examine the effect of MP on case structure and integrity, we measured case mass by subtracting larval wet mass from the total wet mass of the larvae with its case. Differences in case composition were characterized by the proportion of plastic incorporated in constructed cases, as determined by the difference in initial and final mass of natural and synthetic material provided for individual caddisflies ( $n = 8$ , Fig. S4A, Table 1). All caddisflies were euthanized and preserved by placing them in a freezer at –20 °C for 48–72 h and then placed in coolers at 18 °C for 24 h, before being introduced to fish as prey.

### 2.7. Consumption experiment

To compare predation risk per caddisfly case type, four natural encased and four mixed-encased caddisfly larvae resulting from the case induction experiments were randomly assigned to each of 10 bullheads in individual aquaria. To standardize the quality and freshness of the larva after case induction, caddisfly larvae of each case type were kept frozen until needed. The microplastic mass per case accounted for 0.9–72 % of the total mass of the case (Table 1). The larvae were thawed for 24-h, then placed in a 20-L aquarium. Fish were fasted for 96–120 h to standardize hunger and ensure the digestive tract was empty at the start of the experiment (Fig. S4B). Subsequently, a bullhead was added to the aquarium and its behaviour was monitored by direct observation and video recording. Each time trial began when the fish consumed its first caddisfly, and the case type first selected by the fish was noted as its preferred prey. Fish were observed for 10 min, a timeframe allowing the fish to eat a maximum number of larvae without excretion. At the end of the 10-min trial, bullheads were removed from their aquaria and immediately euthanized by immersion in 10 % eugenol solution followed by cervical dislocation (CCAC-AUP: 2017–7913). Their carcasses were individually placed in sealed plastic bags stored at –20 °C. The number and type of caddisflies remaining in each aquarium after exposure to the predator were recorded.

To confirm the ingestion of caddisflies, fish were thawed at room temperature and dissected. The entire digestive tract, from the

**Table 1**

Summary of case reconstruction output in individual caddisfly replicates from the 50:50 MP-to-natural exposure treatment group. All induced cases were considered incomplete as they did not cover the entire body of the larvae. PET represent the plastic material while NAT represent the natural material that was either used by the caddisfly in its case and reported as mass (mg) or proportion of the total mass (%) or the mass (mg) or proportion (%) of the material offered or unused by the caddisfly during case induction.

Larval mass (mg)	Induced case (mg)	PET: case (mg)	PET: case (%)	NAT offered (mg)	PET offered (mg)	Unused NAT (mg)	Unused PET (mg)
206.9	144.2	1.3	0.9	2154.7	2147.5	2011.8	2146.2
210.0	16.5	11.9	72.1	2008.4	2408.2	2003.7	2396.3
215.3	90.7	6.7	7.4	2003.6	2070.3	1919.5	2063.6
251.3	315.8	74.4	23.6	2036.2	1913.3	1794.8	1838.9
292.1	34.3	10.0	29.2	2079.7	1966.1	2055.4	1956.1
421.1	126.7	3.3	2.6	2111.6	1830.7	1988.2	1827.4
438.6	74.6	24.8	33.2	1931.8	2337.0	1881.9	2312.2
531.8	165.7	50.7	30.6	2169.1	2032.8	2054.1	1982.1

esophagus to the anus, was removed and placed in 10 % potassium hydroxide solution in a 40 °C oven for 48 h. Each sample was flushed through a 500 µm stainless-steel sieve and then through a 47 mm A/E PALL glass filter of 1 µm by vacuum filtration. The membrane filters were examined for the presence of larval remains and large PET microplastic fragments under a 20 × stereoscope.

## 2.8. Quality assurance and control

To mitigate microplastic contamination between replicates and across experiments, all tools and equipment were triple rinsed with ultrapure water (0.1 µm) and examined under natural and UV light before dissections and filtration procedures. Researchers wore 100 % cotton lab coats during all experimental procedures, and fish dissections were conducted under a laminar flow hood. Only PET fragments were counted in each animal.

## 3. Statistical analyses

### 3.1. Case building and structure

Using Fisher's Exact Test, we compared the proportion of successful case induction (built case versus no case) and structural completion status (complete versus incomplete) under natural versus 50 % MP-substrate. A one-way analysis of covariance (ANCOVA) was used to assess case structure integrity between microplastic and all-natural treatment groups. The average case mass was compared across treatments with larval mass as a covariate, owing the latter's positive correlation with case mass (Fig. S6). The distribution of residuals met heteroscedasticity and normality assumptions. The emmeans package was used to estimate the mean case mass per case type (natural or MP) using 'R-studio' (version 3.2.3; R Core Team, 2022).

### 3.2. Consumption experiment

Consumption of mixed versus naturally-cased caddisfly was determined by a 2 × 2 contingency analysis. Data of prey proportions consumed by individual fish were pooled and analysed via Fisher's exact test to examine the significance of the association between treatment type (Natural, Mixed) and outcome (consumed, not consumed). All statistical analyses were conducted using 'R-studio' (version 3.2.3; R Core Team, 2022) at a significance level of  $p < 0.05$ .

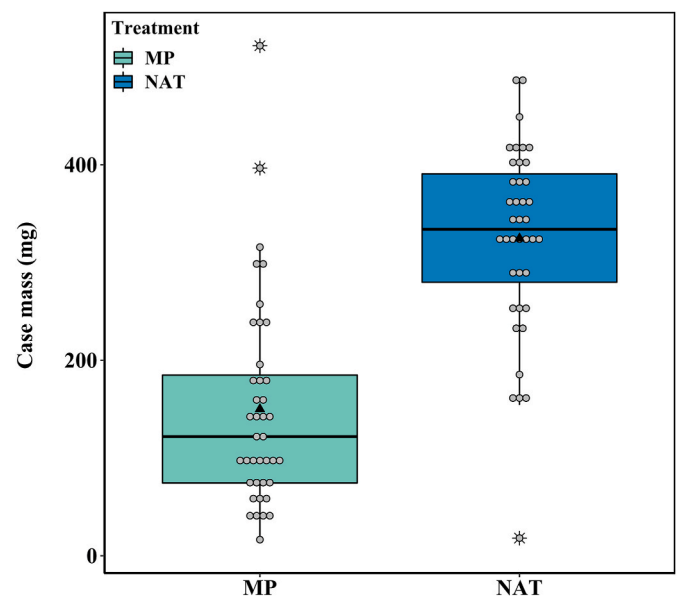
## 4. Results

### 4.1. Case building experiment

Thirteen mortalities occurred among caddisfly larvae during the case-building experiment: two died in the natural treatment group (N = 46), whereas 11 died in the mixed treatment group (n = 66), including eight deaths during case building and three deaths after the larvae built

a case. Across caddisflies that survived long enough for a determination to be made, case-building induction rate of surviving larvae was significantly higher in the natural treatment group (Fisher's exact test,  $p = 0.017$ , Fig. S7) with 93 % of caddisflies inducing a new case (40/44 and 40/54 for natural and mixed treatments, respectively). All caddisflies from the natural treatment group that began building a new case (n = 40) successfully constructed a case covering their entire body (Fig. 1A). Among the caddisflies exposed to MP fragments, 74 % began building a new case during the 48-h period but only 5 % (2/40) produced a complete case (others built emergency cases). Fisher's test revealed that a case completion success rate was significantly lower for larval caddisflies in the mixed treatment than those in the natural treatment ( $p < 0.001$ , Fig. S7).

Only eight caddisfly larvae produced a case when placed alone in their aquarium during case induction, thus allowing us to calculate the mass of MP used per case. Cases built under the 50 % MP treatment varied greatly in mass, with MP comprising 0.9–71.8 % of the total mass (Table 1). The number of PET fragments incorporated by caddisflies in the mixed treatment varied from 1 to 37 per case (estimated from mass and observed -see Fig. S3). However, after the case induction, we recorded 88.1–99.4 % of available natural material and 0.06–2.49 % of



**Fig. 2.** Box-and-whisker plots showing caddisfly case mass discrepancies after 48 h exposure to the 50 % MP treatment (MP; N = 40) in green, or only natural materials (NAT; N = 40) in blue ( $F_{(1, 77)} = 39.19$ ,  $p < 0.001$ ). Case masses in this figure were adjusted for larval size. Individual case masses are shown as grey circles, the mean as a black triangle, and outliers are indicated by asterisks. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

**Table 2**

A chi-square contingency table showing the frequency of observed vs. expected caddisfly prey for each treatment (natural = NAT; mixed = MP) and their consumption status (consumed, unconsumed).

	MP-treated Prey <sup>a</sup>		NAT-treated Prey <sup>a</sup>	
	Obs	Exp	Obs	Exp
Consumed	25	16	7	16
Not Consumed	15	24	33	24
Total	40		40	

<sup>a</sup> To meet minimum expected frequencies for  $X^2$ , data from individual fish were pooled.

PET fragments offered remained unused by caddisflies. Average case mass showed large variation within and between treatments, with MP-incorporated cases generally being lighter than natural ones (Fig. 2). After adjustment for larval mass, there was a significant effect of building material type on the average case mass of *Limnephilus* spp. between treatment groups ( $F_{(1,77)} = 39.19$ ,  $p < 0.001$ ,  $ges = 0.34$ ). Comparing case mass for an average larva of 261 mg, natural cases were significantly heavier ( $316 \text{ mg} \pm 17 \text{ mg}$ ) than cases with MP fragments ( $159 \text{ mg} \pm 17 \text{ mg}$ ).

#### 4.2. Consumption of natural vs MP caddisfly larvae by bullheads

All of Lac Hertel bullheads tested for consumption readily consumed prey in a time frame spanning 1–90 min and showed strong preference for caddisflies from the mixed treatment (Table 2). More caddisflies with mixed cases were readily consumed by fish than expected from a random distribution (Fisher's exact test,  $p < 0.001$ ; Table 2, Fig. S8), with 90 % of replicates involving fish hunting a caddisfly with a mixed case as their first prey (Table 3). One bullhead displaying a distressed behaviour (E12) before the onset of the experimental trial was removed from the trial and euthanized according to CCAC animal use protocol 2017–7913 before the trials. None of Lac des Battures bullheads attempted to consume caddisfly larvae during the trials – a total of four non-exposed fish were therefore used as negative controls for MP contamination of the gastrointestinal tract.

Dissection of fish gastrointestinal tracts confirmed consumption of caddisfly prey; larval remains were found in the stomach of every fish (Fig. 3). One green and two colorless PET fragments were found in fish E7, and two green PET fragments were found in fish replicate E8 (Fig. 3; Table 3). No other artificial or natural case fragments were observed in other bullheads during the dissection. No MP fragments were found in

**Table 3**

Summary of the parameters of the consumption experiment showing the body measurements of *A. nebulosus* ( $N = 14$ ), its choice of prey for its first consumption attempt (Natural vs. Mixed material encased caddisfly), the number of N- and MP-encased caddisfly consumed during the trial (maximum of 4), the time elapsed before the first prey item was selected (foraging time) and the presence of ingested microplastics in the fish digestive tract. The number of PET fragments represents those embedded in the case of the prey.

Fish	Mass (g)	Length (cm)	Prey choice	Total Natural	Consumption PET	Foraging time (min)	Fragments
C1	156.73	23.0	na <sup>a</sup>	na	na	na	0
C2	65.96	18.0	na	na	na	na	0
C3	47.12	16.5	na	na	na	na	0
E4	70.32	18.5	Mixed	0	4	09:26	0
E5	86.18	19.5	Mixed	1	2	9:44	0
E6	102.52	21.0	Mixed	2	2	07:14	0
E7	107.83	20.5	Mixed	2	4	07:49	3
E8	51.32	16.5	Mixed	2	4	16:54	2
E9	117.01	22.5	Mixed	0	2	06:09	0
E10	94.47	20.5	Natural	0	3	03:27	0
E11	30.21	14.5	Mixed	0	1	10:45	0
E12 <sup>a</sup>	38.33	15.0	na	na	na	na	0
E13	35.19	15.0	Mixed	0	2	7:08	0
E14	82.16	20.0	Mixed	0	1	~90:00	0

<sup>a</sup> Non applicable (na) for controls (C1, C2, C3) and fish E12; not exposed to MP-encased preys.

fish that were not exposed to caddisfly prey (see Table S2 for complete data).

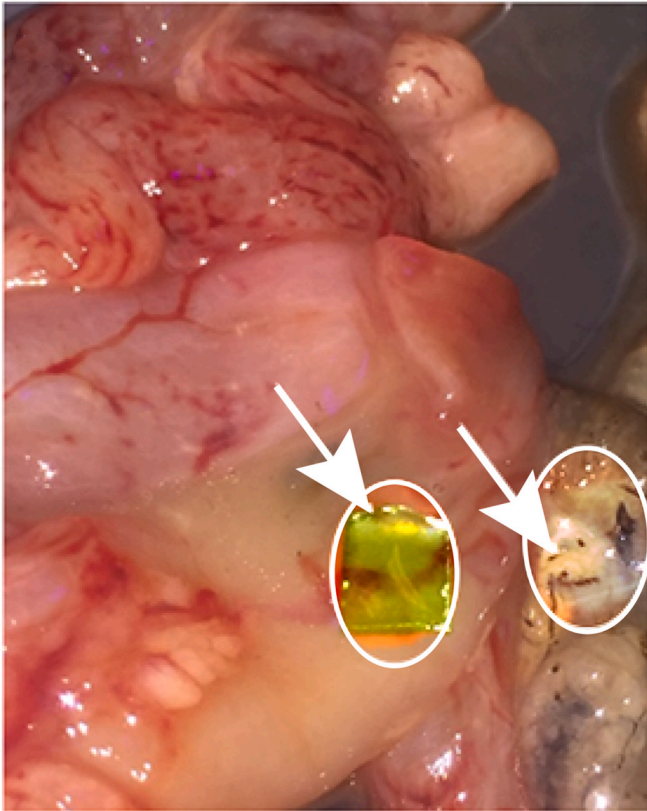
## 5. Discussion

### 5.1. Caddisflies are a vector of microplastic uptake and trophic transfer

Caddisflies are a model organism for the study of aquatic ecosystems because of their abundant representation in lakes and rivers, high species richness (Giller and Malmqvist, 1998) and important role in leaf-litter decomposition and nutrient cycling (Malmqvist and Oberle, 1995). Worldwide, caddisflies are important food sources for waterbirds (Ormerod and Tyler, 1991) and fishes of ecological and recreational importance (Barnes and Hicks, 2003; Hiemstra et al., 2025b; Johansson, 1991), highlighting the need for a better understanding of the role of caddisflies in transferring microplastics through aquatic food webs.

In our study, caddisfly larvae incorporated PET fragments into their case when both artificial and natural materials such as mineral grains and sticks were offered in excess similarly to previous studies (Gallitelli et al., 2021; Valentine et al., 2022; Arias-Paco and Springer, 2025). Larva included a different mass of microplastics, some incorporated a single piece of PET (0.9 % of case mass), while other cases comprised of 72.1 % of the induced case's mass. Similarly, PET fragments of ~1000  $\mu\text{m}$  in size were found in the cases of caddisflies from the Gafos River in Spain (Álvarez-Troncoso et al., 2023) while an average of 20.6 fragments-case<sup>-1</sup> (PVC and PVA) were reported for the limnephilid caddisfly *Hydatophylax nigrovittatus* sampled from Lake Baikal (Karnauchov et al., 2024). Our results align with prior studies showing larval caddisflies adapt to their environment (Williams and Penak, 1980; Eggert and Wallace, 2003; Ajiboye, 2021) by incorporating non-natural materials, including MP, for constructing their cases when suitable natural material is scarce (Fankhauser and Reik, 1935; Tibbetts et al., 2018; Ehlers et al., 2019). Microplastic incorporation to caddisfly cases has been observed for decades, even when environmental microplastics levels were much lower than now (early 1970's; Hiemstra et al., 2025a).

We offered higher concentrations of PET (~25 000–250 000 plastic fragments per kg of dry mass) than the average microplastic concentrations reported along the shoreline and bottom sediments of lakes and rivers (~360–2000 microplastics per kg dry mass; D'Avignon et al., 2022), which may increase the number of particles integrated into the cases of caddisfly in our study. Indeed, in a similar study, Arias-Paco and Springer (2025) observed that the number of particles that were incorporated to caddisfly cases during construction nearly doubled (from 16 to 33 fragments) between their treatment offering 25 % PET: natural



**Fig. 3.** *A. nebulosus* gastrointestinal tracts and their caddisfly content after confirmation of plastic ingestion by dissection. Gastrointestinal tract of fish E7 contained one green and two colorless (one not visible in frame)  $\sim 1000\mu\text{m}$  PET fragments. Green and transparent PET fragments are indicated by arrows. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

materials to that offering 50 % PET: natural materials, but levelled off under higher concentrations (50 %, 75 % and 100 % PET: natural material). Yet, the number of microplastics reported in caddisfly cases sampled from the wild (range 2–54 fibers; Gallitelli et al., 2021; and 10–38 fragments Karnaukhov et al., 2024) is comparable to the number of PET fragments (1–37 per case) observed in this study, suggesting our design supports realistic caddisfly contamination scenarios.

Without global mitigation, plastic production will rise, increasing the concentrations of plastic entering aquatic environments (Borrelle et al., 2020). Microplastics adhered to or incorporated into invertebrate structures—such as moth and caddisfly cases and nets—remain underexplored (Gallitelli et al., 2025; Álvarez-Troncoso et al., 2023; Fritz et al., 2023). Our findings, alongside prior work, highlight the need to better assess plastic presence in invertebrates, especially in polluted areas of rivers and lakes containing caddisflies (e.g., Windsor et al., 2019; Winkler et al., 2022).

### 5.2. Implications of microplastic incorporation in caddisfly cases

The choice of materials by case-building larvae significantly impacts the architecture and integrity of their cases, which regulate oxygen, food uptake and protection from predation (Cummins and Klug, 1979; Cairns and Pratt, 1993; Morse et al., 2019). In our study, we observed that larval cases incorporating PET fragments were more loosely constructed, with more visible gaps than induced cases from natural materials. This observation aligns with Gallitelli et al. (2021), who found that cases built with artificial materials had four times the volume of natural cases. Ehlers et al. (2020) demonstrated that the incorporation of

plastics into larval cases leads to a marked deterioration in case stability, with MP-containing cases requiring significantly less force to dismantle.

Caddisflies from lentic environments can create perforated cases to increase oxygen uptake (Okano and Kikuchi, 2012). Considering that our limnephilid caddisflies were collected from a lotic environment and had previously used mineral grains but were kept under lentic conditions in the lab where the natural materials provided included both twigs and mineral grains, they may have chosen plastic fragments over natural materials to build a looser (perforated) case to increase oxygen uptake in their new environment.

During our mixed treatment experiment, we noted that plastics were integrated primarily at the posterior end of the cases rather than the front (Fig. 1). These traits were also observed in another study where caddisflies were presented with a ratio of 25 % MP to natural materials (Arias-Paco and Springer, 2025). This pattern suggests that larvae initially selected these materials during the early stages of case construction. The initial case matrices may be constructed with readily available materials until the larvae can invest more energy into finding more suitable particles to replace them. This process where material is added to the anterior end and the posterior is shed, has been observed in many case-bearing caddisfly families, including Limnephilidae (Hanna, 1960). We suggest these PET-cases represent incomplete matrices that are pending replacement with materials that can be more easily shaped and embedded into the silk structure of the case (Houghton et al., 2011).

Plastic materials, including PET, differ from natural substrates like leaves and stones in several ways: they are smoother (facilitating silk attachment; Okano & Kikuchi, 2012), sturdier than leaves, and more flexible and buoyant than mineral grains. These traits may influence case-building based on species interactions. Although leaf cases are more susceptible to predation than stone cases (Otto & Svensson, 1980), cases built from any material type (mineral, leaf or stick) increases the survival of caddisfly larvae against predators in comparison to larvae without a case (Ferry et al., 2013). Therefore, when larvae are caseless, they may select the best particles in their vicinity, prioritizing rapid construction of a shelter (e.g., adding more sticks to their case) based on the perceived predation risk (Ajiboye, 2021) or risk of attacks from conspecifics (Wissinger et al., 2004). In some studies, caddisflies strongly selected for colorful plastic particles, such as orange (Arias-Paco & Springer, 2025) or blue (Gallitelli et al., 2021), presumably to increase camouflage with their surroundings. In others, larvae built favoured particles (such as plastic) that appear to be less palatable to other organisms competing for leaf litter in their habitat (Moretti et al., 2009; Rincón & Martínez, 2006).

Observations of different groups of caddisflies incorporating various microplastic polymers (including PET) in diverse laboratory and field studies indicate that the use of plastic by these larval insects is quite common in polluted systems (Ehlers et al., 2018, 2020; Gallitelli et al., 2021; Valentine et al., 2022; Arias-Paco & Springer, 2025; Hiemstra et al., 2025b). Lentic and lotic waters offer different opportunities for caddisfly case-building adaptations, so the addition of plastic can offer different benefits depending on its chemical properties and environmental conditions. In calm lentic waters, swimming freely in a light case is energetically advantageous; whereas, in fast-moving streams, heavier cases made of stones are often more profitable (Peckarsky et al., 1990). Consequently, if the same reduction in mass associated with synthetic case reconstruction (Fig. 2) is observed in wild caddisflies, the replacement of mineral grains (density  $\sim 2\text{--}3.5\text{ g/mL}$ ) with lower density materials such as PET (1.38–1.41 g/mL, this study) can have detrimental implications on their capacity to resist water currents and to withstand buoyancy in fast-flowing lotic environments (König and Waringer, 2008) but could favor caddisflies living in lentic waters. The incorporation of different polymers would influence the rigidity and density of the cases, depending on the abundance of particles and their density in comparison to natural substrates.

The incorporation of synthetic material in the larval case matrix allows for an intimate proximity of MP with the vulnerable larval body

and perhaps increases the risk of leachate absorption by the gills and tissues. Here, we used PET from soda bottles because plastic bottles are common macroplastic debris collected along lake shorelines (Driedger et al., 2015) and therefore readily accessible materials to caddisflies. PET is also amongst the most reported compounds in MP studies (Rochman, 2015). The fragments from polyethylene bottles used in our experiment are typically enhanced with toxic phthalates and UV stabilizers (Rani et al., 2015). It is conceivable that the presence of phthalates, stabilizers or their leachates from PET is responsible for the higher mortality rate (16 %) of caddisfly larvae during the case-building phase of our study compared with the natural materials treatment (6.5 %). Chemical additives have been shown to drive MP toxicity and can sometimes have a larger impact than the MP particles themselves (Zimmermann et al., 2020). Although our study targeted a single polymer and size fraction of MP, caddisflies in natural environments are found to incorporate a variety of polymers to their cases (Ehlers et al., 2018; Hiemstra et al., 2025b). Each MP type can have a unique mixture of toxic substances (polymer composition, additives, sorbed contaminants) whose toxicity is mediated by MP size and shape (Rochman et al., 2019; Bucci and Rochman, 2022), which can further influence case-building behaviour and the toxic load transferred in the food web.

### 5.3. Microplastic transfer from caddisflies to brown bullheads

We have shown that caddisflies with MP fragments in their cases are consumed by fish in laboratory settings. PET fragments from case matrices were found in 20 % (2/10) of brown bullheads digestive tract, three PET fragments in E7 and two fragments in E8 (Table 3). We observed fish picking up and breaking larval cases to access the larvae (see Video S1). In the process of handling the cases, the fish would occasionally ingest case fragments along with the larvae. Even if the predator does not intentionally ingest the case, the PET fragments are more buoyant than the natural materials in the caddisfly cases, so instead of sinking they were observed floating near the oral cavity of the fish while it was feeding (Video S1). This property can explain why plastic particles were found in the digestive system of brown bullheads that attempted feeding on caddisflies, even if they were observed to selectively consume the larvae while expelling case particles.

The presence of PET in caddisfly cases reduced survivorship of caddisfly larvae in our consumption experiment (Table 3), owing to direct predation by fish. We speculate that this enhanced predation was the result of i) cases built with MP being more easily broken, or ii) increased attraction by the fish to MP because of chemosensory or visual cues. It is plausible that fish learn to associate plastic cases with more accessible larvae especially if PET fragments have a distinct chemical cue or bioelectric field from natural materials (Valenticic et al., 1996; Eeuwes et al., 2008). Although all induced cases had more perforation (spaces) than their original mineral grain cases, caddisfly larva from the mixed treatment, reconstructed cases were more loosely structured, which could have facilitated larval extraction; therefore, fish may have consumed more larvae from the MP treatment because it required a reduced prey handling time to extract the larva from its case. MP particles can be conspicuously colorful and thus increase the larva's visibility to predators when incorporated in the case matrix (Otto and Svensson, 1980). The synergistic combination of reduced physical protection and loss of camouflage could put caddisflies at greater predatory risk from fish relying on visual cues when hunting (Lynch et al., 2002; Mogren et al., 2013) like brown bullheads (Michaletz et al., 1987). Indeed, cases with PET fragments increased predation risk in our study for the encased larvae as bullheads consumed more caddisflies in mixed cases than natural cases, and 90 % of the fish targeted caddisfly with PET fragments in their case first (Table 3).

In nature, context-dependent factors may affect the frequency of trophic transfer via this mechanism such as predator gape size, dietary preferences, the availability of alternate sources of prey and whether they consume caddisflies with their case or shred them open and only

consume the larvae. In our study, when offered cased *Limnephilus* larvae, brown bullheads from one lake (Lac Hertel) hunted and ingested them, whereas brown bullheads of overlapping size from another lake (Lac des Battures) did not do so. Lac Hertel fish likely had prior exposure to limnephilid caddisflies that are commonly found in nearshore areas of the lake and connecting streams (H.P. personal observations), likely facilitating their recognition as prey items.

While specimens from our single test population of brown bullheads readily consumed caddisfly larvae during our trials, we expect these results to be relevant to other fish species and populations. For example, rock bass (*Ambloplites rupestris*), like brown bullheads, are opportunistic feeders in that they will likely pursue any suitable prey (Cressman & Garay, 2011). Both fish species likely select caddisfly larvae according to measured opportunity of feeding (e.g., proximity) rather than matrix type.

For fish that hunt visually, the movement of prey increases predation risk. In our pilot experiments, we observed rock bass hunting only moving caddisfly larvae. In our consumption experiment, all caddisflies were offered frozen and thawed so the preference observed by the bullheads is not influenced by differences in the condition, health or the movement potential of the larvae. Under field conditions, the number and type of plastic particles incorporated in the case of wild caddisflies could potentially alter their mobility and thus influence the response of caddisfly predators.

Our study shows evidence that microplastic incorporated in caddisfly cases can be ingested (whether intentionally or accidentally) by a predatory fish, even if it does not consume the entire case in its attack. As mechanisms of predation vary across species, so do the risks of trophic transfer. Certain fishes (e.g. brown bullheads; this study), birds (e.g., dippers, wagtails, waders, and grebes; Wiggins, 2005), and other invertebrates crush the case to extract the larvae and expel the case fragments, thus could be subjected to this transfer accidentally, whereas other predators (such as trout and ducks) that consume the case and larvae in their entirety (Wiggins, 2005) could ingest microplastics more readily and therefore be more vulnerable to their ecotoxicological effects.

We suggest that transfers of MP to higher trophic level are common and diverse. The digestive tracts of 20–96 % of species of freshwater fishes collected worldwide have been found to contain MP (Jabeen et al., 2017; Munno et al., 2022). Up to  $10^3$  MP particles per fish have been recorded in various water bodies such as the Rhine River, the Pearl River, and the Laurentian Great Lakes (D'Avignon et al., 2022). Since the brown bullhead population from Lac Hertel occurs at sites where MPs were found in sediments (Brown et al., 2020), we can presume that they too are exposed to prey (including caddisflies) that have either ingested or incorporated MPs.

### 5.4. Implications for the fate of microplastics in food webs

The non-trophic uptake of MP particles by a common invertebrate prey item and subsequent MP transfer to a predator are novel findings for freshwater benthic communities. The selection by case-building caddisfly larvae of microplastic particles from common anthropogenic waste (plastic soda bottles) resulted in cases of poorer quality and increased risk of predation by brown bullheads, which consumed both case material and caddisfly tissue. Thus, increasing availability of microplastics in the environment generates greater opportunities for primary and secondary uptake that exposed predators to MP and associated contaminants.

Considering that caddisfly larvae are consumed by a broad diversity of fishes, waterbirds, amphibians, crayfish and predaceous insects (Wiggins, 2005; Ajiboye, 2021; Hiemstra et al., 2025b), trophic transfer of microplastics and their associated contaminants from caddisfly cases into the food chain has widespread and important implications in aquatic and terrestrial food webs. Therefore, models of the cycling and fate of plastic in ecosystems should consider both trophic and

non-trophic organismal behavior that can affect MP transfer through aquatic and terrestrial ecological communities and may increase the risk of MP contamination and the accumulation of associated contaminants in these food webs.

#### CRediT authorship contribution statement

**Hélène Pfister:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Geneviève D'Avignon:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Aimy Wang:** Writing – original draft, Visualization, Investigation, Conceptualization. **Miguel Eduardo L. Felismino:** Writing – review & editing. **Anthony Ricciardi:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization.

#### Ethical statement

All animal care and use were performed in compliance with the Animal Use Protocol 2017–7913 revised by McGill University Animal Care Committee (ACC) following the guidelines on the care and use of fish in research, teaching, and testing from the Canadian Council on Animal Care (CCAC). All researchers and animal care givers received appropriate training on ethical care practices based on aforementioned guidelines. Ministerial guidelines for the collection and transport of live animals were applied as described by the SEG permit 2020-07-13-2862-05-06-16-SP granted by the Ministère de l'Environnement, de la Lutte contre les changements climatiques, de la Faune et des Parcs de Québec.

#### Declaration of generative AI in scientific writing

The authors did not use any AI and AI-assisted technologies in the writing process of this manuscript.

#### Funding

GD received financial support from the Interuniversity Research Group in Limnology, the Natural Sciences and Engineering Research Council of Canada (NSERC) CREATE Ecolac Program, the McGill Arthur Willey Memorial Fellowship, and the McGill University Trotter Graduate Fellowship. AR acknowledges support from the McGill Trotter Institute for Science and Public Policy, and the Natural Sciences and Engineering Research Council of Canada (2016–03918).

#### 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.

#### Acknowledgements

McGill University in Montréal (Canada) is located on land which has long served as a site of meeting and exchange amongst Indigenous peoples, including the Haudenosaunee and Anishinabeg nations. We also acknowledge that the Gault Nature Reserve (Mont-St-Hilaire, Canada) is situated on the ancestral and unceded territory of the Abenaki Nation, specifically the Odanak and Wôlinak communities, who are part of the Waban-Aki people. We would honour, recognize, and respect these nations as the traditional stewards of the lands and waters where our work has been conducted. We also thank Jessamine Trueman and Eveline Bérubé for their assistance, the Gault Nature Reserve for hosting our project, and Duncan Wang for advice and support. We would like to thank the reviewers and editors for their suggestions and advice upon revision of this manuscript.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.envpol.2025.127133>.

#### Data availability

I have shared my data link via my GitHub account

#### References

- Ajiboye, M.O., 2021. The Role of Conspecific Damage-Released Alarm Cue and Plastic Pollution on Caddisfly Larvae Case Construction Behaviour. Master Thesis. University of Saskatchewan. <https://hdl.handle.net/10388/13422>.
- Álvarez-Troncoso, R., Gutiérrez, D., Villar, I., Ehlers, S.M., Soto, B., Mato, S., Garrido, J., 2023. Microplastics in water, sediments and macroinvertebrates in a small river of NW Spain. *Limnética* 43, 1. <https://doi.org/10.23818/limn.43.13>.
- Arias-Paco, A., Springer, M., 2025. Patterns of microplastic incorporation in larval and pupal cases of *Limnephilus hamifer* (Trichoptera: Limnephilidae). *Rev. Biol. Trop.* 73, e63696. <https://doi.org/10.15517/rev.biol.trop.v73iS1.63696>.
- Ballent, A., et al., 2016. Sources and sinks of microplastics in Canadian Lake Ontario nearshore, tributary and beach sediments. *Mar. Pollut. Bull.* 110 (1), 383–395. <https://doi.org/10.1016/j.marpolbul.2016.06.037>.
- Barnes, G.E., Hicks, B.J., 2003. Brown bullhead catfish (*Ameiurus nebulosus*) in Lake Taupo. Department of Conservation, Conference held at Hamilton 27–35. <https://hdl.handle.net/10289/1747>.
- Borrelle, S.B., Ringma, J., Law, K.L., Monahan, C.C., Lebreton, L., McGivern, A., Murphy, E., Jambek, J., Leonard, G.H., Hilleary, M.A., Eriksen, M., Possingham, H. P., De Frond, H., Gerber, L.R., Polidoro, B., Tahir, A., Bernard, M., Mallos, N., Barnes, M., Rochman, C.M., 2020. Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. *Science* 369, 1515–1518. <https://doi.org/10.1126/science.aba3656>.
- Brown, E., Mackey, L., Rothberg, L., Burnett, M., Bergeron, N., Lewis, Y., 2020. An evaluation of microplastics in Lac Hertel sediment over time. *McGill Science Undergraduate Research Journal* 15 (1), 38–44. <https://doi.org/10.26443/msurj.v15i1.8>.
- Bucci, K., Rochman, C.M., 2022. Microplastics: a multidimensional contaminant requires a multidimensional framework for assessing risk. *Microplastics and Nanoplastics* 2 (1), 7. <https://doi.org/10.1186/s43591-022-00028-0>.
- Cairns, J.J., Pratt, J.R., 1993. A history of biological monitoring using benthic macroinvertebrates. In: Rosenberg, D.M., Resh, V.H. (Eds.), *Freshwater Biomonitoring and Benthic Macroinvertebrates*. Chapman/Hall, New York, pp. 10–27.
- Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T. S., 2013. Microplastic ingestion by zooplankton. *Environ. Sci. Technol.* 47 (12), 6646–6655. <https://doi.org/10.1021/es400663f>.
- Cressman, R., Garay, J., 2011. The effects of opportunistic and intentional predators on the herding behavior of prey. *Ecology* 92 (2), 432–440. <https://doi.org/10.1890/10-0199.1>.
- Cummins, K.W., Klug, M.J., 1979. Feeding ecology of stream invertebrates. *Annu. Rev. Ecol. Systemat.* 10, 147–172. <https://www.jstor.org/stable/2096788>.
- D'Avignon, G., Gregory-Eaves, I., Ricciardi, A., 2022. Microplastics in lakes and rivers: an issue of emerging significance to limnology. *Environ. Rev.* 30 (2), 228–244. <https://doi.org/10.1139/er-2021-0048>.
- Driedger, A.G.J., Dürr, H.H., Mitchell, K., Van Cappellen, P., 2015. Plastic debris in the Laurentian Great lakes: a review. *J. Great Lake. Res.* 41, 9–19. <https://doi.org/10.1016/j.jglr.2014.12.020>.
- Duprat, H., Besson, C., 2004. The Wonderful caddis Worm: Sculptural work in Collaboration with Trichoptera. Available from: <https://leonardo.info/isast/articles/duprat/duprat.html>, 30-05-2022 2022.
- Earn, A., Bucci, K., Rochman, C.M., 2021. A systematic review of the literature on plastic pollution in the Laurentian Great Lakes and its effects on freshwater biota. *J. Great Lake. Res.* 47 (1), 120–133. <https://doi.org/10.1016/j.jglr.2020.11.001>.
- Eggert, S.L., Wallace, J.B., 2003. Reduced detrital resources limit *Pycnopsyche gentilis* (Trichoptera:Limnephilidae) production and growth. *J. North Am. Benthol. Soc.* 22 (3), 388–400. <https://doi.org/10.2307/1468269>.
- Ehlers, S.M., Manz, W., Koop, J.H.E., 2019. Microplastics of different characteristics are incorporated into the larval cases of the freshwater caddisfly *Lepidostoma basale*. *Aquat. Biol.* 28, 67–77. <https://doi.org/10.3354/ab00711>.
- Ehlers, S.M., Al Najjar, T., Taupp, T., Koop, J.H.E., 2020. PVC and PET microplastics in caddisfly (*Lepidostoma basale*) cases reduce case stability. *Environ. Sci. Pollut. Control Ser.* 27 (18), 22380–22389. <https://doi.org/10.1007/s11356-020-08790-5>.
- Euweus, L., Peters, R., Bretschneider, F., 2008. Behavioural relevance of AC and DC in prey detection by the brown bullhead, *Ameiurus nebulosus*. *Anim. Biol.* 58, 321–336. <https://doi.org/10.1163/157075608X344640>.
- Fankhauser, G., Reik, L.E., 1935. Experiments on the case-building of the caddisfly larva, *Neuronia postica* Walker. *Physiol. Zool.* 8 (3), 337–359. <https://doi.org/10.1086/physzool.8.3.30151253>.
- Ferry, E.E., Hopkins, G.R., Stokes, A.N., Mohammadi, S., Brodie Jr., E.D., Gall, B.G., 2013. Do all portable cases constructed by caddisfly larvae function in defense? *J. Insect Sci.* 13, 5. <https://doi.org/10.1673/031.013.0501>.

- Fritz, S.F., Albertson, L.K., Hobgood, J.L., Mohr, E.J., Oakland, H.C., Poole, G.C., 2023. Macroinvertebrate ecosystem engineering affects streambed retention of microplastics. *Freshw. Sci.* 42, 133–145. <https://doi.org/10.1086/724584>.
- Gallitelli, L., Cera, A., Cesarini, G., Pietrelli, L., Scalici, M., 2021. Preliminary indoor evidences of microplastic effects on freshwater benthic macroinvertebrates. *Sci. Rep.* 11 (1), 720. <https://doi.org/10.1038/s41598-020-80606-5>.
- Gallitelli, L., Ceschin, S., Mariani, F., Pietrelli, L., Scalici, M., 2025. Preliminary observations on the use of microplastics by aquatic larvae of the moth *Cataclysta lemnata* (Linnaeus, 1758). *Environments* 12, 80. <https://doi.org/10.3390/environments12030080>.
- Giller, P.S., Malmqvist, B., 1998. *The Biology of Streams and Rivers*. Oxford University Press, Oxford, p. 480. *Biology of habitats*.
- Hanna, H.M., 1960. Methods of case-building and repair by larvae of caddisflies. In: *Proceedings of the Royal Entomological Society of London. Series A, General Entomology*, pp. 97–106. <https://doi.org/10.1111/j.1365-3032.1960.tb00676.x>, 35 (7–9).
- Hiemstra, et al., 2025a. Half a Century of caddisfly Casings (Trichoptera) with microplastic from natural history collections. *Sci. Total Environ.* 974, 178947. <https://doi.org/10.1016/j.scitotenv.2025.178947>.
- Hiemstra, A.F., Van der Velden, I., Koese, B., Verdonschot, R., Gravendeel, B., Schilthuis, M., 2025b. A curious case: caddisfly cases built from brick and sewage overflow microplastics. *J. Limnol.* 84, 2212. <https://doi.org/10.4081/jlimnol.2025.2212>.
- Hinchliffe, R., Palmer, A.R., 2010. Curious chiral cases of caddisfly larvae: Handed behavior, asymmetric forms, evolutionary history. *Integr. Comp. Biol.* 50 (4), 606–618. Available from: <http://www.jstor.org/proxy3.library.mcgill.ca/stable/40863408>, 2022/05/30/.
- Houghton, D.C., Rogers, S.E., Hocquard, K., Wolfe, C.I., 2011. Case-building behavior, persistence, and emergence success of *Pycnopsyche guttifer* (Walker) (Trichoptera: Limnephilidae) in laboratory and in situ environments: potential trade-offs of maternal preference. *Gt. Lakes Entomol.* 44 (2), 103–116. <https://scholar.valpo.edu/tgle/vol44/iss2/1>.
- Holzenthal, R.W., Thomson, R.E., Ríos-Touma, B., 2015. Order trichoptera. In: *Thorpe and Covich's Freshwater Invertebrates*. Academic Press, pp. 965–1002.
- Hurley, R., Woodward, J., Rothwell, J.J., 2018. Microplastic contamination of riverbeds significantly reduced by catchment-wide flooding. *Nat. Geosci.* 11, 251–257. <https://doi.org/10.1038/s41561-018-0080-1>.
- Jabeen, K., Su, L., Li, J.N., Yang, D.Q., Tong, C.F., Mu, J.L., Shi, H.H., 2017. Microplastics and mesoplastics in fish from coastal and fresh waters of China. *Environ. Pollut.* 221, 141–149. <https://doi.org/10.1016/j.envpol.2016.11.055>.
- Johansson, A., 1991. Caddis larvae cases (Trichoptera, Limnephilidae) as anti-predatory devices against brown trout and sculpin. *Hydrobiologia* 211, 185–194. <https://doi.org/10.1007/BF00008534>.
- Karnaukhov, D.Yu., Lavnikova, A.V., Nepokrytykh, A.V., Zhdanov, I.A., Salovarov, K.V., Gulighev, A.T., Osadch, B.V., Biritskaya, S.A., Ermolaeva, Y.K., Maslennikova, M. A., Bukhaeva, L.B., Golubets, D.I., Okholina, A.I., Kulbachnaya, N.A., Milovidova, I. V., Silow, E.A., 2024. Baikal endemic and Palearctic species of caddisflies (Trichoptera) build cases from microplastics. *Acta Biologica Sibirica* 10, 649–659. <https://doi.org/10.5281/zenodo.12670062>.
- Kolandhasamy, P., Su, L., Li, J.N., Qu, X.Y., Jabeen, K., Shi, H.H., 2018. Adherence of microplastics to soft tissue of mussels: a novel way to uptake microplastics beyond ingestion. *Sci. Total Environ.* 610, 635–640. <https://doi.org/10.1016/j.scitotenv.2017.08.053>.
- König, E., Waringer, J., 2008. The ballast stones in *Silo nigricornis* cases (Insecta: Trichoptera): drift resistance and ecological benefits, investigated by acoustic Doppler velocimetry. *Large Rivers* 18, 311–328. <https://doi.org/10.1127/lr/18/2008/311>.
- Latchere, O., Audroin, T., Hétier, J., Métails, I., Châtel, A., 2021. The need to investigate continuums of plastic particle diversity, brackish environments and trophic transfer to assess the risk of micro and nanoplastics on aquatic organisms. *Environ. Pollut.* 273, 116449. <https://doi.org/10.1016/j.envpol.2021.116449>.
- Lynch, R.J., Bunn, S.E., Catterall, C.P., 2002. Adult aquatic insects: potential contributors to riparian food webs in Australia's wet-dry tropics. *Austral Ecol.* 27 (5), 515–526. <https://doi.org/10.1046/j.1442-9993.2002.01208.x>.
- Malmqvist, B., Oberle, D., 1995. Macroinvertebrate effects on leaf pack decomposition in a lake outlet stream in northern Sweden. *Nord. J. Freshw. Res.* 70, 12–20.
- Menéndez-Pedriz, A., Jaumot, J., 2020. Interaction of environmental pollutants with microplastics: a critical review of sorption factors, bioaccumulation and ecotoxicological effects. *Toxics* 8 (2), 40. <https://doi.org/10.3390/toxics8020040>.
- Merritt, R.W.C.K.W., 2008. *An Introduction to the Aquatic Insects of North America*. Kendall/Hunt Pub. Co, Dubuque, Iowa, p. 1498.
- Michaelitz, P.H., Unkenholz, D.G., Stone, C.C., 1987. Prey size selectivity and food partitioning among zooplanktivorous age-0 fishes in Lake Francis Case, South Dakota. *Am. Midl. Nat.* 117 (1), 138–146. <https://doi.org/10.2307/2425714>.
- Mogren, C.L., Walton, W.E., Parker, D.R., Trumble, J.T., 2013. Trophic transfer of arsenic from an aquatic insect to terrestrial insect predators. *PLoS One* 8 (6), e67817. <https://doi.org/10.1371/journal.pone.0067817>.
- Moretti, M.S., Loyola, R.D., Becker, B., Callisto, M., 2009. Leaf abundance and phenolic concentrations codetermine the selection of case-building materials by *Phylloicus* sp. (Trichoptera, Calamoceratidae). *Hydrobiologia* 630 (1), 199–206. <https://doi.org/10.1007/s10750-009-9792-y>.
- Morse, J.C., Frandsen, P.B., Graf, W., Thomas, J.A., 2019. Diversity and ecosystem services of Trichoptera. *Insects* 10 (5), 125. <https://doi.org/10.3390/insects10050125>.
- Munno, K., Helm, P.A., Rochman, C., George, T., Jackson, D.A., 2022. Microplastic contamination in Great lakes fish. *Conserv. Biol.* 36, e13794. <https://doi.org/10.1111/cobi.13794>.
- Okano, J., Kikuchi, E., 2012. Effect of current velocity and case adaptations on the distribution of caddisfly larvae (Glossosoma, Trichoptera). *Limnology* 13, 37–43. <https://doi.org/10.1007/s10201-011-0353-5>.
- Ormerod, S.J., Tyler, S.J., 1991. Exploitation of prey by a river bird, the dipper *Cinclus cinclus* (L.), along acidic and circumneutral streams in upland Wales. *Freshw. Biol.* 25, 105–116. <https://doi.org/10.1111/j.1365-2427.1991.tb00477.x>.
- Otto, C., Svensson, B.S., 1980. The significance of case material selection for the survival of caddis larvae. *J. Anim. Ecol.* 49, 855–865. <https://doi.org/10.2307/4231>.
- Peckarsky, B.L., Fraissinet, P.R., Penton, M., Conklin, D.J., 1990. *Freshwater Macroinvertebrates of Northeastern North America*. Cornell University Press, p. 442.
- Rani, M., Shim, W.J., Han, G.M., Jang, M., Al-Odaini, N.A., Song, Y.K., Hong, S.H., 2015. Qualitative analysis of additives in plastic marine debris and its new products. *Arch. Environ. Contam. Toxicol.* 69 (3), 352–366. <https://doi.org/10.1007/s00244-015-0224-x>.
- Rincón, J., Martínez, I., 2006. Food quality and feeding preferences of *Phylloicus* sp. (Trichoptera: Calamoceratidae). *J. North Am. Benthol. Soc.* 25 (1), 209–215. [https://doi.org/10.1899/0887-3593\(2006\)25\[209:FQAFPO\]2.0.CO;2](https://doi.org/10.1899/0887-3593(2006)25[209:FQAFPO]2.0.CO;2).
- Rochman, C.M., 2015. The complex mixture, fate and toxicity of chemicals associated with plastic debris in the marine environment. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), *Marine Anthropogenic Litter*. Springer, Cham. [https://doi.org/10.1007/978-3-319-16510-3\\_5](https://doi.org/10.1007/978-3-319-16510-3_5).
- Rochman, C.M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Buccì, K., Athey, S., Huntington, A., McIlwraith, H., Munno, K., De Frond, H., Kolomijec, A., Erdle, L., Grbic, J., Bayoumi, M., Borrelle, S.B., Wu, T., Santoro, S., Werbowsk, L.M., Zhu, X., Giles, R.K., Hamilton, B.M., Thaysen, C., Kaura, A., Klasios, N., Ead, L., Kim, J., Sherlock, C., Ho, A., Hung, C., 2019. Rethinking microplastics as a diverse contaminant suite. *Environ. Toxicol. Chem.* 38, 703–711. <https://doi.org/10.1002/etc.4371>.
- Stuart, Currie, 2001. Using caddisfly (Trichoptera) case-building behaviour in higher level phylogeny reconstruction. *Can. J. Zool.* 79 (10), 1842–1854. <https://doi.org/10.1139/z01-14>.
- Tibbetts, J., Krause, S., Lynch, I., Sambrook Smith, G., 2018. Abundance, distribution, and drivers of microplastic contamination in urban river environments. *Water* 10, 1597. <https://doi.org/10.3390/w10111597>.
- Usio, N., Townsend, C.R., Death, R.G., 2001. Resource use by crayfish in a stream food web: influence of omnivory and cannibalism. *J. North Am. Benthol. Soc.* 20 (4), 506–520. <https://doi.org/10.2307/1468086>.
- Valenticic, T., Kralj, J., Stenovac, M., Koce, A., 1996. Learned olfactory discrimination of amino acids and their binary mixtures in bullhead catfish (*Ameiurus nebulosus*). *Physiol. Arch. Eur. J. Physiol.* 431 (6), R313–R314. <https://doi.org/10.1007/BF02346394>.
- Valentine, K., Cross, R., Cox, R., Woodmancy, G., Boxall, A.B.A., 2022. Caddisfly larvae are a driver of plastic litter Breakdown and microplastic formation in freshwater environments. *Environ. Toxicol. Chem.* 41, 3058–3069. <https://doi.org/10.1002/etc.5496>.
- Wallace, J.B., Merritt, R.W., 1980. Filter-feeding ecology of aquatici. *Annu. Rev. Entomol.* 25 (1), 103–132. <https://doi.org/10.1146/annurev.en.25.010180.000535>.
- Wang, Z., Su, B., Xu, X., Di, D., Huang, H., Mei, K., Dahlgren, R.A., Zhang, M., Shang, X., 2018. Preferential accumulation of small (<300 μm) microplastics in the sediments of a coastal plain river network in eastern China. *Water Res.* 144, 393–401. <https://doi.org/10.1016/j.watres.2018.07.050>.
- Wang, W.F., Ge, J., Yu, X.Y., 2020. Bioavailability and toxicity of microplastics to fish species: a review. *Ecotoxicol. Environ. Saf.* 189, 109913. <https://doi.org/10.1016/j.ecoenv.2019.109913>.
- Wiggins, G.B., 1996. *Larvae of the North American Caddisfly Genera (Trichoptera)*. University of Toronto Press, Toronto, Ontario, Canada, p. 400. <https://doi.org/10.3138/9781442623606>.
- Wiggins, G.B., 2005. *Caddisflies: the Underwater Architects*. University of Toronto Press, p. 292.
- Williams, D.D., Penak, B.L., 1980. Some aspects of case building in *Phryganea cinerea* Walker (Trichoptera: Phryganeidae). *Anim. Behav.* 28 (1), 103–110. [https://doi.org/10.1016/S0003-3472\(80\)80013-3](https://doi.org/10.1016/S0003-3472(80)80013-3).
- Windsor, F.M., Tilley, R.M., Tyler, C.R., Ormerod, S.J., 2019. Microplastic ingestion by riverine macroinvertebrates. *Sci. Total Environ.* 646, 68–74. <https://doi.org/10.1016/j.scitotenv.2018.07.271>.
- Winkler, A., Antonelli, D., Masseroni, A., Chiarcos, R., Laus, M., Tremolada, P., 2022. Following the fate of microplastic in four abiotic and biotic matrices along the Ticino River (North Italy). *Sci. Total Environ.* 823, 153638. <https://doi.org/10.1016/j.scitotenv.2022.153638>.
- Wissinger, S.A., Eldermire, C., Whissel, J.C., 2004. The role of larval cases in reducing aggression and cannibalism among caddisflies in temporary wetlands. *Wetlands* 24, 777–783. [https://doi.org/10.1672/0277-5212\(2004\)024\[0777:TROLCl\]2.0.CO;2](https://doi.org/10.1672/0277-5212(2004)024[0777:TROLCl]2.0.CO;2).
- Zimmermann, L., Göttlich, S., Oehlmann, J., Wagner, M., Völker, C., 2020. What are the drivers of microplastic toxicity? Comparing the toxicity of plastic chemicals and particles to *Daphnia magna*. *Environ. Pollut.* 267, 115392. <https://doi.org/10.1016/j.envpol.2020.115392>.