Limnologica 94 (2022) 125969

Contents lists available at ScienceDirect

Limnologica

journal homepage: www.elsevier.com/locate/limno

# Influence of leaf detritus quality and risk of predation on the behavioral patterns of *Phylloicus* sp. (Trichoptera: Calamoceratidae)

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ARTICLE INFO

*Keywords:*
Shredders
Chemical composition
Stream
Natural selection
Top-down effects
Bottom-up control

ABSTRACT

Predation can generate a cascading effect (top-down control) within the aquatic food web. On the other hand, the bottom-up control can also significantly influence the behavior patterns of shredders, especially on their leaf-shredding activity. We aimed to assess the individual and interactive effects of the predation risk by fishes from the genus *Astyanax* and the quality of leaf discs in the behavioral patterns of shredders from the genus *Phylloicus* (Trichoptera). The experimental design consisted of two levels of predation risk (absence and presence) and two levels of quality of leaf discs (high and low) in three experimental blocks. All experiments were run for nine days. Our results showed that the quality of the leaf discs affected the percentage of the general body movements of *Phylloicus* sp, with these caddisflies foraging for more extended periods in the presence of high-quality leaf discs. Moreover, the presence of invertebrates in cases can be influenced by the detritus quality and the interaction between predation risk and detritus quality. Therefore, the predation associated with low-quality nutrition might affect the behavioral patterns of *Phylloicus* sp., reflecting a shorter time spent in the construction of its larval case, which might lead to higher predation. This might explain this functional feeding group's low abundance and density in tropical streams. On the other hand, their capacity to consume their larval case could be an adaptative advantage over other shredders. Still, more field and microcosm experiments are necessary to arrive reality of tropical streams. We showed a way to explain the natural selection of this species and how interactions are running now.

## 1. Introduction

Predation and primary production exert top-down and bottom-up controls, respectively, with their interactions resulting in a cascade effect in the aquatic ecosystem food web (Carpenter et al., 2001; Schmitz, 2006, 2008; Stief and Holker, 2006; Frank, 2008; Schmitz et al., 2010). The trophic cascade includes two effects from predation: (i) lethal predation effects, which is characterized by consumptive interactions in which predators kill and consume their prey, thus altering their abundance; and (ii) non-lethal predation effects, in which non-consumptive interactions can induce defensive phenotypic responses upon exposure to signs of predation risk (Tollrian and Harvell, 1999; Werner and Peacor, 2003; Preisser et al., 2005; Abrams, 2007), such as changes in behavior, morphology, and life history (Fraser et al., 2004; Dalton and Flecker, 2014). Changes in the behavioral and morphological characteristics of the caddisflies can occur within or across generations, directly affecting their susceptibility to predation (Van Buskirk and McCollum, 2000). Among these effects, the risk of predation might result in physiological stress in the prey (Dalton et al., 2018); for example, it could lead to increased synthesis and release of the glucocorticoid hormone cortisol (Martínez-Porchas et al., 2009). This hormone activates the processes of glycogenolysis (the breakdown of glycogen) and gluconeogenesis (the production of sugars from non-sugar and non-carbohydrate compounds), resulting in higher blood

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https://doi.org/10.1016/j.limno.2022.125969
Received 2 November 2021; Received in revised form 14 February 2022; Accepted 27 February 2022
Available online 14 April 2022

glucose levels to meet the increased energetic demands due to the accelerated metabolism and increased respiratory rates. (Martínez-Porchas et al., 2009; Reid et al., 1998).

There is little knowledge about the top-down effects on detrital food chains, although detritus is essential in the energy flow of aquatic ecosystems (Polis and Strong, 1996). Detrital food webs depend on the allochthonous matter input, with consumers regulating the amount of energy available in the ecosystem through their assimilation rate (Polis and Strong, 1996). The studies of detrital food webs in soil (Santos et al., 1981; Wyman, 1998) and streams (Oberndorfer et al., 1984; Malmqvist, 1993; Konishi et al., 2001) have shown that predators indirectly can control rates of release of the nutrients in ecosystems (Dalton et al., 2018). In turn, bottom-up control affects primary consumers, particularly invertebrate shredders. The quality of leaf litter for shredders depends on its chemical composition, physical structure, microbial colonization, and the level of litter degradation, which can vary according to leaf species and the period of water exposure (Ligeiro et al., 2010). These characteristics can influence these invertebrate shredders' feeding preferences and case-building behavior (Graça, 2001; Moretti et al., 2009).

In general, invertebrate shredders play a key role in the process of leaf decomposition in temperate streams (being responsible for approximately 25% of the loss of leaf mass), converting coarse particulate organic matter into fine particulate organic matter, which other organisms can then use (Petersen and Cummins, 1974; Wallace et al., 1982; Cuffney et al., 1990; Newman, 1990; Allan and Castilho, 2007; Graça et al., 2015). In contrast, other studies have shown few or no effects of shredders in tropical streams (Boyero et al., 2012; Tonin et al., 2021). However, the main issue in the tropics might be using parameters like abundance or density instead of shredder biomass to study their influence on leaf breakdown, with large shredders presenting a low density and having a more substantial effect on fragmentation (Tonin et al., 2017). Therefore, in the last ten years, some studies with shredders have been conducted to improve the knowledge about their relationship with detritus quality (Rezende et al., 2018), competition and predation (Rezende et al., 2015), and their behavior under climate change (Martins et al., 2017a, 2017b, 2016; Navarro et al., 2013). However, there are still many issues left to be studied or clarified through experimental and field studies, like the behavior of shredders (Moretti et al., 2009; Navarro et al., 2013).

The most common genus of shredders in the Brazilian Savanna is *Phylloicus* sp. (Mueller, 1880). Given the lack of studies in the tropical regions on the effects of top-down and bottom-up controls on the behavioral patterns of *Phylloicus* sp., this study had aimed to assess the impact of predation and leaf quality on their behavior. Our hypotheses: (1) *Phylloicus* sp. in the treatment with high-quality leaf-disks will show more behavioral plasticity and activity due to higher consumption and use as case-building material; (2) The risk of predation (non-lethal) will induce behavior responses (indicating stress) in *Phylloicus* sp. larvae, leading to increase body movements, mainly the undulating ones; (3) The combination of low-quality resources with the presence of (non-lethal) predation will lead to a negative synergistic effect, resulting in an overall reduction of movement patterns.

## 2. Material and methods

### 2.1. Selection and characterization of plant species

The plant species selected were *Maprounea guianensis* and *Inga laurina*, both collected by nets within an area of 1 $m^2$, 1 m above the soil, at the Capetinga stream. Each leaf species was pre-colonized by microorganisms (conditioning) for 11 days at the Capetinga stream, and we incubated 15 g of each leaf species in litterbags (0.5 mm). Then, we chose good quality leaves and cut the disks using a cork borer with 1.8 cm diameter, avoiding damage to the primary and secondary veins. Both selected species are dominant in the phytophysiognomy of the riparian zone of the Cerrado biome. Physicochemical characteristics were used to classify the quality of the leaf-discs, with *I. laurina* being considered low-quality (lignin = 44% $g^{-1}$, hardness = 364 g, C:N 32, and C:P 986) and *M. guianensis* high-quality leaf sources (lignin = 22% $g^{-1}$, hardness = 146 g, C: N 70, C:P 1956, more details in Navarro, 2014).

### 2.2. Experimental design

Invertebrate shredders of the genus *Phylloicus* (Trichoptera) were exposed to the risk of non-lethal predation by exposing them to the chemical signals of *Astyanax* sp. (Characiformes, Characidae). This fish species is considered a natural predator of these shredders in the Cerrado rivers (Leite, 2012). The larvae of *Phylloicus* sp. were collected between August and October 2021 by "active search" with the aid of a sieve and later placed in a glass containing stream water. The Capetinga is a headwater stream with 18 ± 1°C water temperature, pH 7 ± 1, 7.5 ± 2 mg $L^{-1}$ of dissolved oxygen, and electric conductivity of 7 ± 4 μS.$cm^{-1}$ (Bambi et al., 2017). The *Astyanax* sp. specimens were collected at the same site between August and October 2012 using a dragnet and transferred to plastic bags with the stream water. The aquatic organisms were transported to the Laboratory of Limnology of the Department of Ecology. They were kept in aquaria for acclimatization (room temperature 20° C, photoperiod 12/12 h; in mineral water; Table 1) period of 24 h.

In the laboratory, experiments were set up in 60 L aquaria (50 cm × 35 cm x 34 cm) filled with standard mineral water to exclude the presence of chemical signals from other aquatic organisms that could affect the behavioral pattern of *Phylloicus* sp. specimens. We have done the same procedure with fish. Water quality parameters (dissolved oxygen, pH, temperature, and conductivity) were measured daily. The dissolved oxygen content and temperature were measured using a digital oxygen meter (Jenway 970), the pH was measured with a digital pH meter (PHTEK®), and the conductivity was read with a conductivity meter (Quimis®) (Table 1). Water flow was simulated using submerged pumps to mimic the lotic environment's natural flow. The oxygen levels were kept using aerators, and the photoperiod was set at 12 h of light and 12 h of darkness. The chemistry and physical characteristics of the mineral water used in the aquariums of the three blocks have low standard-deviation indicating similarity among blocks (Table 1, more details in Navarro, 2014).

The experimental design was composed of 1 block with four treatments with two replicates. This design was repeated three times (between August and October) to make independent samples totaling six replicates for each treatment (Fig. 1). The experimental time was set at nine days, enough to keep the microcosm in good conditions. Each block contained the following treatments: 80 low-quality leaf litter discs + 8 invertebrate shredders with their larval cases; 80 low-quality leaf litter discs + 8 invertebrate shredders with their larval cases + 3 plastic cages with one fish in each one; 80 high-quality leaf litter discs + 8 invertebrate shredders with their larval cases; 80 high-quality leaf litter discs + 8 invertebrate shredders with their larval cases + 3 plastic cages with one fish in each one (Fig. 1).

The cages were made of transparent polyethylene terephthalate (PET) bottles (≈ 10 cm in diameter and 15 cm in height). The end parts were sealed using 1-mm mesh screens, and the cages were kept at the aquaria's surface through polystyrene foam buoys.

**Table 1**
Mean and standard deviation values of the physical-chemical parameters of the water of the experimental containers.

| Parameters | (Mean ± SD) |
|---|---|
| pH | 7.92 ± 0.07 |
| Dissolved Oxygen (mg/L) | 11.89 ± 0.99 |
| Electrical Condutivity (μS/cm) | 11.57 ± 3.41 |
| Temperature | 20.39 ± 0.27 |


**Fig. 1.** 2 × 2 factorial design consisting of two levels of non-lethal risk of predation (absence and presence) and two levels of leaf detritus quality (high and low quality), replicated in the time, making up three experimental blocks regardless.

We feed fishes daily with commercially available feed flakes containing 28% crude protein. The fishes were removed from the cages and transferred to a 60-L reserve aquarium for feeding purposes. They were fed during 12–24 h (totaling eight changes for each aquarium for nine days), and after that, they were transferred back to the cages. This procedure aimed to prevent the nutrients in the feed and feces from affecting the water quality and the variables analyzed in this study. The group of fishes that took part in this transfer varied over time since we used more fishes than the number required for the experiment in order to reduce the effect of stress on them.

### 2.3. Invertebrate shredder behavior

An ethogram was elaborated based on previous observations of *Phylloicus* sp. that was carried out for nine days (see details in Tables 2 and 3). The undulatory movement was featured only when the body moved without larval dislocation in the aquarium. Moreover, we have proposed some categories described in Table 2. The displacement was associated with a change of place in the aquarium, which was revealed in detail by the ethogram (Table 2). However, to facilitate the comparison between treatments, the subcategories described for undulatory movement and displacement were not considered in this study, which assessed only the total percentage of each category. The behavior of three randomly selected *Phylloicus* sp. specimens was quantified at each replicate of the different treatments. These measurements were taken once each day during the nine day-period for each experimental block (pseudo-replication) on an alternating schedule (9:00 am or 4:00 pm). We used the focal-animal sampling method with an instantaneous recording (*scan sampling*) and 1-minute intervals for 20 min (Altmann, 1974). Qualitative analyses were also performed through visual observations during the assessment periods to complement the behavioral patterns of the *Phylloicus* sp. specimens.

**Table 2**
Behavioral repertoire of Caddisfly larvae (*Phylloicus* sp.) in the experimental containers.

| Category | Subcategories (Siglas) | Description |
|---|---|---|
| Body movements without displacement | MH | Undulatory movement horizontal body |
| | ML | Undulatory movement lateral body |
| | SBO | Body stretching |
| | RBO | Body retraction |
| | ULBO | Uplift lateral body |
| | LTA | Lower tail |
| | UMP | Uplift of the previous members |
| Body position | VPB | Vertical body position |
| | HPBBa | Horizontal body position with back up |
| | HPBBe | Horizontal body position with belly up |
| Displacement | FD | Forward displacement |
| | RD | Rearward displacement |
| | UD | Upward displacement |
| Rest | RS | Invertebrate stopped without perceptible body movements |
| Feeding | F | Invertebrate consuming leaf resource to meet their energy demands |
| Building cocoon | BC | Invertebrate using leaf resource to build its cocoon |
| Behaviour no perceptible | BNP | Invertebrate outside the focus of observation of the researcher which prevents the verification its behavior |

**Table 3**
Position of *Phylloicus sp* larvae into experimental containers during the study.

| Category (Sigla) | Description |
|---|---|
| ONFD | Caddisfly without larval case on the leaf disc |
| UNFD | Caddisfly without larval case under the leaf disc |
| BFD | Caddisfly between leaf disc, ie inside the larval case |
| ONGR | Caddisfly without larval case on the gravel |
| FDGR | Caddisfly inside the larval case with previous members on the gravel |
| GL | Caddisfly without larval case in contact with the aquarium glass |
| GRGL | Caddisfly without larval case in contact with gravel and aquarium glass, simultaneously |
| FDGL | Caddisfly inside the larval case and in contact with the aquarium glass |
| FDGRGL | Caddisfly inside the larval case and in contact with gravel and glass, through previous members |
| DWC | Displacement in the water column |

### 2.4. Statistical analyses

The behavioral parameters (response variables) were tested considering the leaf litter quality, the risk of predation, and the interaction between these factors (higher level explanatory variables) using a SPLIT-PLOT Analysis of Variance (SPANOVA) at a significance level of $p < 0.05$ (Crawler, 2007). We performed a random effect SPANOVA considering the same organism measured for nine days in each treatment. We have accounted for the specific features of organism behavior in each aquarium. The experimental block was included as a lower-level explanatory variable. The normality of the data was assessed using the Kolmogorov-Smirnov test, with the data being log-transformed whenever necessary (Massey Junior, 1951). All analyzes were performed using the R software (R Core Team, 2020).

## 3. Results

The behavioral repertoire found for *Phylloicus* sp. were similar under the different treatments. The leaf litter quality significantly affected the leaf litter with high quality has had a significantly lower affected percentage of body movements (SPANOVA, $F_{1,18} = 5.832$, $p = 0.027$). However, it had no influence on the percentage of displacement (SPANOVA, $F_{1,18} = 0.566$, $p = 0.462$), rest (SPANOVA, $F_{1,18} = 0.222$, $p = 0.643$), feeding (SPANOVA, $F_{1,18} = 2.295$, $p = 0.147$), case-building (SPANOVA, $F_{1,18} = 1.469$, $p = 0.241$), or imperceptible behavior (SPANOVA, $F_{1,18} = 1.666$, $p = 0.2132$) of the *Phylloicus* sp. specimens under the different treatments (Table 4). Predation risk (SPANOVA, $F_{1,18}$ = 0001, p = 0.971) and the interaction between predation risk and detritus quality (SPANOVA, $F_{1,18}$ = 2424, p = 0.139) did not significantly influence behavioral patterns.

The leaf litter quality has significantly affected the vertical body position of the larvae (SPANOVA, $F_{1,18}$ = 9.670, p < 0.01), with the *Phylloicus* sp. specimens under the treatments with *M. guianensis* (high-quality litter) remaining in this position for a longer time compared with those under the treatments with *I. laurina* (low-quality litter) Further, the *Phylloicus* sp. specimens exposed to *I. laurina* spent more time with the ventral side facing up (SPANOVA, $F_{1,18}$ = 19.427, p < 0.001) compared to those exposed to *M. guianensis.* In contrast, the horizontal position, with the dorsal side facing up, was not significantly affected by the leaf litter quality (SPANOVA, $F_{1,18}$ = 0.152, p = 0.702; Table 5).

Mostly, the location of the *Phylloicus* sp. specimens in the different microcosms was not affected by the kind of plant species offered, risk of predation, or their interactions. We have not found caddisflies without larval cases in contact with the aquarium glass during the experimental period. Besides, these specimens have rarely been observed in touch with the gravel substrate and the glass wall using their front legs and in the water column, indicating they little-explored these positions in microcosms (Table 6).

We have found that leaf litter quality significantly affects the duration of the permanence of the caddisfly larvae between the leaf discs (SPANOVA, F1,18 = 6.615, p = 0.019). The shredders exposed to *M. guianensis* spend more extended periods inside the leaf larval case than under those treatments with *I. laurina*. Further, an interaction between the nutritional quality and predation risk was confirmed regarding the residence time inside the leaf larval case (SPANOVA, F1,18 = 17.865, p < 0.001). The *Phylloicus* sp. specimens exposed to (non-lethal) predation and low-quality litter spent less time inside their larval cases (Table 6).

We must register that we have observed the consumption of caddisfly larval cases (formed by detritus from the natural environment) by different individuals of *Phylloicus* sp. in the experiments. This ingestion of larval cases led to a considerable reduction in their size. However, the larval cases were more consumed in low leaf-quality treatments (*I. laurina*) than treatments containing high-quality leaf (*M. guianensis*).

## 4. Discussion

Our results indicated the caddisfly might adapt their behavior under the influence of leaf quality and during grazing, keeping it safe from predators. However, our study was not enough to test all effects, indicating the necessity of future studies. Studies like this, carried out in microcosms, allow us to expand our knowledge about the ecological niche of shredders invertebrates in tropical streams and its relationship with the riparian zone (high plant diversity). Moreover, it is essential to broaden the understanding of the functionality of these ecosystems, viewing their preservation, restoration, and management.

The low-quality litter of I. *laurina* led *Phylloicus* sp. to increase the percentage of body movements, mainly the undulating ones (rejecting our first hypothesis). This increase in movements probably occurred due to the respiratory rate caused by the nutritional deficiency that resulted from the difficulty in shredding this food resource. The insect larvae breathe voluntarily through these undulating bodily movements that create water flow over the abdominal gills (Milne, 1938; Wiggins, 2004). Lukas and Wacker (2014) have shown that the metabolism o *Daphnia magna* can be adjusted with increased efficiency assimilation of cholesterol to control its respiration rate. However, when under low food quality cannot make this adjustment, they observed an increase in respiration rate. Moreover, other studies with *Daphnia magna* also showed an increase in the respiration rate when fed on diets lacking certain minerals (Darchambeau et al., 2003; Beckerman et al., 2007). It is still unclear if this metabolic adjustment is a transitory change or not and how it might affect organisms in the long term since an increase in the respiratory rate resulting from a nutritional deficiency can lead to carbon loss and a reduction in the allocation of food to growth and reproduction (Darchambeau et al., 2003). This could be why shredders have lower abundance and limited distribution in tropical streams, characterized by a high number of riparian plants with high levels of refractory compounds (Ribeiro and Walter, 2001; Gonçalves et al., 2006, 2007). These results have brought evidence about the necessity of comprehensive studies considering shredders' metabolism to understand their relationship with food quality.

A reduction in the rate of displacement and food intake might be a behavioral strategy of preys exposed to the risk of predation (Relyea, 2001; Fraser et al., 2004). Moreover, aquatic insects (e.g., *Chironomus riparius*) can sense fish predators (*Rutilus rutilus*) through chemical signals (kairomones) and spend less time foraging on the sediment surface (Stief and Holker, 2006). However, in our study using *Phylloicus* sp., the specimens that received high-quality resources (*M. guianensis*) exhibited increased shredding behavior regardless of the presence or absence of predation. Besides, the shredding (consumption) of the *I. laurina* leaf discs (low quality) was restricted to their edges, which are the parts more likely to be ingested. This limitation to the edge of the discs probably resulted in the similar times spent on feeding under both

**Table 5**
Mean and standard deviation values of body position *Phylloicus* sp. larvae (Vertical body position (VBP), horizontal body position with back up (HBPBa), horizontal body position with belly up (HBPBe) of different treatments and statistical results SPANOVA.

| Leaf species | Presence/Absence of predation | VPB | HPBBa | HPBBe |
|---|---|---|---|---|
| *M.guianensis* | Absence | 36.67 ± 9.76 | 48.41 ± 6.74 | 11.92 ± 8.56 |
| | Presence | 36.43 ± 5.22 | 55.02 ± 5.94 | 8.52 ± 3.83 |
| *I.laurina* | Absence | 23.53 ± 11.02 | 57.17 ± 8.08 | 19.30 ± 7.97 |
| | Presence | 28.49 ± 16.31 | 49.60 ± 17.32 | 21.91 ± 2.76 |
| Statistics | | | | |
| $P_{leaf\ spec.}$ | | **< 0.010** | 0.702 | **< 0.001** |
| $P_{absence/presence}$ | 0.826 | 0.914 | 0.867 | |
| $P_{leaf\ specie\ X\ absence/presence}$ | 0.303 | 0.111 | 0.219 | |

**Table 4**
Mean and standard deviation values of the behavioral repertoire (%) of different treatments and statistical results SPANOVA.

| Leaf species | Presence/Absence of predation | Movements Body | Displacement | Rest | Feeding | Building Larval Case | Behaviour no perceptible |
|---|---|---|---|---|---|---|---|
| *M.guianensis* | Absence | 7.25 ± 4.01 | 5.27 ± 6.63 | 48.89 ± 12.28 | 28.85 ± 9.65 | 6.39 ± 10.16 | 3.35 ± 5.00 |
| | Presence | 5.48 ± 12.17 | 3.82 ± 2.33 | 44.86 ± 17.23 | 35.31 ± 17.85 | 2.81 ± 3.17 | 7.70 ± 3.29 |
| *I.laurina* | Absence | 8.24 ± 3.03 | 7.89 ± 3.43 | 44.90 ± 8.17 | 26.42 ± 7.54 | 2.58 ± 4.45 | 9.96 ± 6.81 |
| | Presence | 10.09 ± 0.87 | 4.27 ± 5.63 | 53.95 ± 14.15 | 24.19 ± 8.48 | 0.88 ± 2.15 | 6.60 ± 4.74 |
| Statistics | | | | | | | |
| $P_{leaf\ spec.}$ | | **0.027** | 0.462 | 0.643 | 0.147 | 0.241 | 0.213 |
| $P_{absence/presence}$ | | 0.971 | 0.231 | 0.648 | 0.642 | 0.281 | 0.819 |
| $P_{leaf\ specie\ X\ absence/presence}$ | | 0.139 | 0.603 | 0.242 | 0.344 | 0.697 | 0.081 |

**Table 6**
Mean and standard deviation values of the position of *Phylloicus* sp. larvae of different treatments and statistical results SPANOVA.

| Leaf species | Presence/Absence of predation | ONFD | UNFD | BFD | ONGR | FDGR | GRGL | GL | FDGL | FDGRGL | DWC |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *M.guianensis* | Absence | 0.00 ± 0.00 | 0.00 ± 0.00 | 67.12 ± 6,98 | 3.68 ± 12.59 | 6.39 ± 10.16 | 0.00 ± 0.00 | 0.00 ± 0.00 | 24.29 ± 15.71 | -0.25 ± 0.62 | 0.40 ± 1.59 |
| | Presence | 0.00 ± 0.00 | 0.00 ± 0.00 | 86.23 ± 9.57 | 0.81 ± 1.97 | 2.81 ± 3.17 | 0.00 ± 0.00 | 0.00 ± 0.00 | 9.00 ± 14.04 | 0.00 ± 0.00 | 0.16 ± 0.79 |
| *I.laurina* | Absence | 0.00 ± 0.00 | 4.43 ± 10.8 | 74.80 ± 8.70 | 4.21 ± 10.30 | 2.58 ± 4.45 | 0.00 ± 0.00 | 0.00 ± 0.00 | 9.23 ± 16.21 | 0.00 ± 0.00 | 0.00 ± 0.00 |
| | Presence | 1.16 ± 3.67 | 4.98 ± 8.01 | 54.69 ± 18.10 | 12.29 ± 9.95 | 0.88 ± 2.15 | -0.82 ± 2.00 | 0.00 ± 0.00 | 20.97 ± 19.62 | 1.27 ± 3.18 | 0.00 ± 0.00 |
| Statistics | | | | | | | | | | | |
| $P_{leaf\ spec.}$ | | 0.444 | 0.104 | **0.019** | 0.153 | 0.352 | 0.331 | – | 0.813 | 0.251 | 0.139 |
| $P_{absence/presence}$ | | 0.444 | 0.922 | 0.915 | 0.525 | 0.647 | 0.331 | – | 0.786 | 0.251 | 0.887 |
| $P_{leaf\ specie\ X\ absence/presence}$ | | 0.444 | 0.922 | **< 0.001** | 0.190 | 0.876 | 0.331 | – | 0.050 | 0.436 | 0.846 |

Acronymous see Table (3).

treatments but different forms of shredding considering each leaf resource. Besides, our study has the leaf quality could be stronger effects than predation for the behavior of caddisfly.

We did not find direct effects of the predation in the behavior of *Phyloicus* sp. (the second hypothesis was rejected). Although, studies with Trichoptera larvae had also observed the consumption of larval cases belonging to other individuals when they presented diatoms on their surface (Williams and Penak, 1980). We also observed the consumption of caddisfly larval cases from the natural environment by different *Phylloicus* sp. mainly in the treatments with low leaf quality (probably with microbial colonization increasing detritus quality; Rezende et al., 2018). The larval case has a primary protected function (as observed in Rezende et al., 2021) but could also be a reserve of food resources. This secondary function could explain their survivor success (natural selection) in the Cerrado streams.

The possibility of rebuilding the larval case appears to be related to the quality of the leaf resource (Sena et al., 2020). Our results revealed an increase in the use of low-quality leaf discs in the presence of predation risk, which could protect the larvae from predators. Case-building materials selected by caddisfly could be related to chemical leaf contents, such as higher concentrations of phenolic and lignin compounds, as observed in other studies with Phylloicus larvae (Moretti et al., 2009; Rincón and Martínez, 2006).

The caddisfly larvae without a larval case and under predation risk in the treatments with *I. laurina* used strategies such as hiding under the leaf discs or even trying to burrow inside the larval cases containing other larvae to ensure their survival. The last strategy was not previously reported in the literature. This temporary shelter may be a strategy to ensure survival and to reduce the energy costs associated with rebuilding a new larval case. Moreover, we observed that most of the invertebrates exhibited a horizontal position, with higher exposure of the ventral side, in the treatments with *I. laurina* and fewer individuals between the leaf discs. The cost of building a larval case includes the energy spent collecting materials and producing silk by the labial glands of the larvae (Okano and Kikuchi, 2009). The building of temporary larval cases is expected since the larvae need to quickly shelter themselves to ensure survival (Lepneva, 1964). Moreover, studies show that the prey subjected to predation pressure increases their energy demands and induces phenotypic defense responses, such as habitat changes (Holomuzki and Short, 1988; Fraser et al., 2004; Hawlena and Schmitz, 2010; Hawlena et al., 2012).

Most of the larvae in the different treatments were found in the horizontal body position with the dorsal side up. This positioning reflects their preference for remaining inside the larval case rather than on the gravel substrate while at rest or during foraging activities. This trait may ease their movements, crawling, and the adherence of their front legs to the substrate (Gall et al., 2011). In addition, the presence of the caddisflies inside their larval cases adhered to the dorsal side of another shredder, feeding or not on this resource, also contributed to the increased periods spent by the *Phylloicus* sp. specimens in this horizontal position.

The nutritional quality did not change the overall behavioral patterns of the *Phylloicus* sp. specimens and, in addition, revealed some peculiarities. The low-quality leaf discs were also responsible for increasing the percentage of the undulating body movements, in opposition to the third hypothesis, which expected that this behavior would increase with the risk of non-lethal predation. Moreover, a higher concentration of polyphenols in *M. guianensis* might provide an antimicrobial effect associated with lower hardness and a lower percentage of refractory compounds, leading *Phylloicus* sp. to use these leaves to rebuild their larval cases (Sena et al., 2020; Navarro and Gonçalves, 2020). Behavioral ethograms can be used as a quantitative methodological tool and increase the knowledge about the biology of shredder caddisfly larvae. However, it is necessary to combine the results from ethograms with film or visual observations, which would allow a more descriptive qualitative analysis of certain behavioral details (e.g., intraspecific interactions, feeding types dependent on the leaf resource type, anti-predation mechanisms) and thus be able to provide evidence that could help explain why there is a low abundance and density of shredders in tropical streams.

## CRediT authorship contribution statement

**Fernanda Keley Silva Pereira Navarro:** Conceptualization, Data curation, Methodology, Formal analysis, Resources, Investigation, Writing – original draft, Writing – review & editing, Visualization. **Luciana Silva Carneiro:** Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Mariana Caldeira:** Methodology, Resources, Data curation. **José Francisco Gonçalves Júnior:** Conceptualization, Data curation, Methodology, Formal analysis, Resources, Investigation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration.

## Author statement

This statement came to certify that all authors have seen and approved the final version of the manuscript being submitted. They warrant that the article is the authors' original work, has not received prior publication, and isn't under consideration for publication elsewhere.

## Declaration of Competing Interest

We have no conflict of interest, and we followed all predicted ethical concepts.

## Acknowledgments

We are grateful to PROCAD-NF/CAPES (No. 173/2010), to PELD/CNPq (No. 558233/2009-0), to FAPEMIG (No. APQ-00274-12), to the Graduate Program in Biological Sciences/Unimontes for the grant provided (PROIC/2012 – Recém-Doutor /DPP), and to the University of Brasília (DPP; No. 121366/2011) for the financial support. In addition, to the REUNI Program for granting a Ph.D. Scholarship to Fernanda Navarro (No. 07/2011). Prof. Renan S. Rezende for the statistical analysis consultancy. Finally, we would like to express our special gratitude Aquariparia group for their massive support throughout this study.

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