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Original Article

Feeding preference of the shredder *Phylloicus* sp. for plant leaves of *Chrysophyllum oliviforme* or *Miconia chartacea* after conditioning in streams from different biomes

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Abstract

Macroinvertebrate shredders consume preferably leaves conditioned by fungi and bacteria which offer greater palatability to them. Plant species in Cerrado present high concentration of chemical elements such as lignin and cellulose, phenols and tanins thus making them less attractive for shredders consumption and limiting the palatability. This study aimed to evaluate the feeding preference of a macroinvertebrate shredder of the genus *Phylloicus* for plant material from two different biomes (Cerrado and Mata Atlântica), after conditioning in a stream of Mata Atlântica and observing their physical and chemical characteristics. Senescent leaves were collected, monthly from the litterfall of riparian vegetation in a 500 m stretch of a stream in each biome from August 2014 to January 201. The most abundant species in each stream was selected for the experiment. The experimental design consisted in with two treatments. The first (T1) comprised leaf discs from *Chrysophyllum oliviforme* (Cerrado species) together with leaf discs of *Miconia chartacea* (Atlantic Forest species) which were conditioned in the Atlantic Forest stream. The second treatment (T2) involved leaf discs of *Miconia chartacea* conditioned in Mata Altlântica and Cerrado streams. Both tests had showed significant differences between the two treatments (T1 and T2). For T1, there was consumption of *M. chartacea* leaf discs by *Phylloicus* sp., but there was no consumption of *C. oliviforme* discs. For T2, there was preference for *M. chartaceae* leaves conditioned in a stream of Mata Atlântica than in Cerrado stream. The results showed that *Phylloicus* sp., had presented preference for food detritus of the Mata Antlântica biome and rejection to the one from Cerrado biome.

Keywords: aquatic macroinvertebrates, plant detritus, Mata Atlantica, Cerrado.

Preferência alimentar do fragmentador *Phylloicus* sp. por folhas de *Chrysophyllum oliviforme* e *Miconia chartacea* após condicionadas em riachos de dois biomas

Resumo

Macroinvertebrados fragmentadores consomem folhas preferencialmente condicionadas por fungos e bactérias que lhes oferece uma maior palatabilidade. Nas espécies do cerrado esse condicionamento está também associado às altas concentrações de elementos químicos limitantes à palatabilidade como alto teor de lignina e celulose, que tornam as folhas menos atrativas para os fragmentadores. O trabalho teve como objetivo avaliar a preferência alimentar de macroinvertebrados fragmentador (*Phylloicus sp.*) por material vegetal de dois diferentes biomas (Cerrado e Mata Atlântica), após condicionamento em riacho de Mata Atlântica, observando suas características físicas e químicas. Foram coletadas folhas senescentes do aporte vegetal (AV) de espécies nativas da vegetação ripária nesses dois biomas, com periodicidade mensal em um trecho de 500 m de um córrego em cada bioma. O experimento foi delineado com dois tratamentos. O primeiro (T1) compreendeu discos de folhas do Cerrado (*Chrysophyllum oliviforme)* mais discos de folhas da Mata Atlântica (*Miconia chartacea*) que foram condicionadas em córrego de Mata Atlântica. O segundo tratamento (T2) envolveu discos de folhas da Mata Atlântica condicionadas em córrego da Mata Atlântica mais discos de folhas da Mata Atlântica condicionadas em córrego do Cerrado. Os dois testes apontaram diferenças significativas entre os dois tratamentos (T1 e T2). Para T1 houve consumo de discos de folha de *M. chartacea* por *Phylloicus sp*, mas não houve consumo dos discos de *C. oliviforme*, de Cerrado. Para T2, houve o consumo, porém a preferência pelas folhas de *M. chartacea*e condicionadas no córrego da Mata Atlantica foi consideravelmete maior. Os resultados apontam que *Phylloicus* sp, apresentou preferência alimentar pelo detrito de bioma de Mata Atlântica e rejeição pelo detrito do bioma Cerrado.

Palavras-chave: macroinvertebrados aquáticos, detritos vegetais, Mata Atlântica, Cerrado.

1. Introduction

The decomposition process is one of the most important processes in nutrient cycling and energy transformation in aquatic ecosystems (Gimenes et al., 2010), and it begins by the action of physical forces of leaching and the macroinvertebrate shredders that transform detritus into particulate organic matter (Ward and Stanford, 1995; Callisto and Esteves, 1998; Carvalho and Uieda, 2009). The detritus from riparian forest, especially in shaded parts of rivers of small orders is transformed from coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM), by macroinvertebrate shredders allowing a great source of food for others organisms (Graça, 2001; Benfield, 2007).

The denomination of the term "shredder" is attributed to the aquatic macroinvertebrates that chew leaves, sticks and other organic detritus that enter in the water systems (Merritt and Cummins, 1996). Such organisms have mouthparts morphological adaptations to macerate and shred large particles of organic matter (Cushing and Allan, 2001).

The genus *Phylloicus* (Calamoceratidae: Trichoptera) has been considered the most common shredder among those that are part of this trophic guild (Moretti and Callisto, 2005), because they are exclusively shredders of leaf detritus and use these resources both for food and for the construction of shelters contributing to the leaf decomposition process in streams (Wantzen and Wagner, 2006; Moretti, 2009). As reported by Jinggut and Yule (2015), these shredders are less abundant in tropical streams because the detritus in temperate streams are of better quality, despite their preference for milder temperatures. However, Tonello et al. (2014) have shown that typical shredders are important as detritus consumers in subtropical streams even when they represent less than 10% of the total invertebrate community. According to Tonello et al. (2016), the increase of the relative abundance of *Phylloicus* at certain times of the year boost the fragmentation of the detritus about 4x. Also, Ferreira et al. (2006) had showed an increase in the loss of leaf biomass in the presence of shredders. Gonçalves Junior et al. (2007) observed that a high density of shredders was responsible for high loss of leaf weight of *Protium brasiliense* in a tropical stream. Thus, the shredding activity is important because it promotes the fragmentation of leaf detritus and contribute to facilitate microbial colonization (Graça, 2001).

The food preference of shredders is closely associated with the quality of the leaves as measured by the physical (e.g. hardness) and chemical (e.g. nutritional content and soluble and insoluble organic compounds) characteristics of the detritus (Gessner and Chauvet, 1994; Hladyz et al., 2009). The leaf palatability is enhanced by fungal and bacterial activity on this material, which is considered the conditioning of the detritus by microbiota (Graça and Cressa, 2010).

According to Wantzen and Junk (2000) the quality of the plant material from the riparian area in acid soils of Cerrado makes the establishment and colonization by fungi and bacteria difficult, which consequently reduces the attraction of shredders due to lack of palatability, causing the decomposition of riparian vegetation to become slower. This study aimed to evaluate the feeding preference of a shredder macroinvertebrate (*Phylloicus* sp.) for plant material from two different biomes (Cerrado and Mata Atlantica), when conditioning in streams of these two biomes.

2. Methodology

2.1. Obtention and conditioning of plant material

The plant material was obtained in headwater streams of first order. The Buritizal stream is located in an Environmental Protection Area of Cerrado (10° 15' 35"S, 48° 07' 54") and the Banana stream is inserted in a secondary forest fragment in Mata Atlantica biome (20° 02' 21"S, 40° 31' 54") (Figure 1). Both streams presented shadowed riparian vegetation.

Senescent leaves from the litterfall of riparian vegetation were collected in the two biomes from August 2014 to January 2015. Five interception nets (1m × 1m) were fixed and distributed equidistantly to the stream margin along a 500 m stretch. Each month, the plant material that was retained in the nets was collected, placed in individual plastic bags and taken to the laboratory. The most abundant species was selected for the experiment: *Chrysophyllum oliviforme* from Cerrado and *Miconia chartacea* from Mata Atlantica.

For the experiment of food preference, leaves of *Miconia chartacea* from Mata Atlantica were conditioned in the Cerrado stream and also in Mata Atlantica stream, and leaves of *Chrysophyllum oliviforme* from Cerrado were conditioned in the Mata Atlantica stream. The litterbags containing leaves arranged in area of moderate stream flow. After 10 days of conditioning in litterbargs of 30 × 30cm, with mesh of 10 µm, the leaves were removed and sent to the laboratory for use in the food preference experiment.

Figure 1. Location of the studied areas.

2.2. Invertebrate collection

For the experiment, there were captured 40 larvae of the shredder *Phylloicus* sp. in the Banana stream and then taken to the laboratory. The larvae were placed in plastics bottles and substrates were added (gravel, leaves and water) from the collection site to simulate the natural environment and reduce the stress of the collection in the organisms.

2.3. Experimental design

The study was conducted in the laboratorial complex BIOPRATICAS of the Universidade Vila Velha (UVV-ES). The experiment was design with two treatments. The first treatment (T1) was composed of leaf disks of *Chrysophyllum oliviforme* from Cerrado and *Miconia chartacea* from Mata Atlantica that were conditioned in the Mata

Atlantica stream. The second treatment (T2) was composed of leaf disks of *Miconia chartacea* from Mata Atlantica conditioned in Mata Atlantica and Cerrado streams.

Larvae were selected by size and placed separated during a 24 hour period to start the procedure. Later, they were placed individually in aquariums containing 400 mL of filtered water from the Mata Atlantica stream in 20 replicates for each treatment. The water of the aquariums was oxigenated during the experiment in order to maintain an oxygenation similar to the stream. At the bottom of the aquariums, two pins were placed and each pin with a leaf disk for each treatment. These disks were offered to shredders. For each replicate, a pair of small litterbags containing control disks was placed submerged in the aquarium wall (Graça et al., 2005).

After the period of consumption of two thirds of the area of each disk, the larvae of the shredder and leaf disks were removed, the discs dried in an oven at 60 °C for a period of 48 hours and consumption estimate was calculated by the difference between the weight of control disks and the disks consumed by the larvae (Graça et al., 2005).

2.4. Determination of leaf hardness

To obtain the leaf hardness of the leaves, a device described by Graça (2001) was used to verify the resistance of the leaf disks by disruption force. A total of nine leaf disks were used for each plant species, previously moisturized with distilled water. Hardness was estimated as the force per gram of sand needed to break the leaf disc and expressed in kilo Newtons (kN) of weight resistance.

2.5. Determination of tannins

In order to determine the content of tannins, 250 mg of leaves (*Chrysophyllum oliviforme* and *Miconia chartacea*) were dried at 60 °C during 48 hours in an oven, crushed and then 3 mL of extraction solution of acetone 70% were added. The sample was homogenized using a vortex for thirty seconds. After a one hour rest, the sample was placed in a centrifuge at 5000 RPM for twenty minutes. In a petri dish with agarose gel and acetic acid, circles were made with a cork cutter (cork borer) of 4mm diameter. Using an automatic pipette, 30 µL of the supernatant were pipetted and carefully put in the circles, then the plate was put in the fridge for 48 hours. This procedure was done in triplicate. After 48 hours, precipitation rings were compared to standard rings of known tannin concentration by using digital images read by the software *ImageJ* (Rasband, 2008). The calibration curve was made with solutions with different concentrations of tannic acid (1, 2, 3, 5, 10, 15, 20, 25 mg/µL).

2.6. Determination of lignin and cellulose

An amount of 250 mg of leaves of each species was crushed and the structural compounds were removed after washing in a solution of acid detergent and sulfuric acid 72%. The first compounds removed were the fibers, then cellulose and at last lignin. For the last two compounds the results were obtained through the weight difference after burning the leaves in a muffle at 550 °C for 5 hours (Graça et al., 2005).

3. Results

3.1. Feeding preference

The larvae of *Phylloicus* sp. showed feeding preference for *Miconia chartacea* disks conditioned in stream of Mata Atlantica biome. In the first treatment (T1), leaf disks from *Miconia chartacea* were consumed but not disks from *Chrysophyllum oliviforme* (Figure 2A). In treatment 2 (T2), leaves from *Miconia chartacea* conditioned in both biomes were consumed, however there was a significant preference for the leaves that were conditioned in a stream of Mata Atlantica (Figure 2B).

3.2. Physical and chemical characterization of plant material

The tannin concentration was higher in leaves of *Chrysophyllum oliviforme* (0.121 mg/g), compared to leaves of *Miconia chartacea* (0.071 mg/g) (Figure 3). After the conditioning period in the stream of Mata Atlantica, the tannin concentrations were higher in *C. oliviforme* leaves

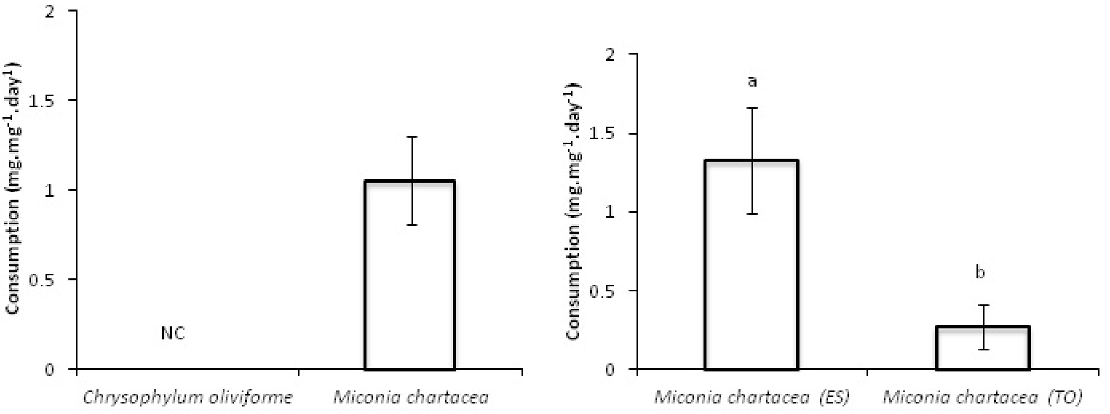

Figure 2. Feeding preference of *Phylloicus* sp. for leaves of *Miconia chartacea* and *Chrysophylum* oliviforme when conditioned in stream waters of Cerrado and Mata Atlantica biomes. (a) represents T1 and (b) represents T2.

(0.044 mg/g) than in M. *chartacea* (0.033 mg/g) (Figure 3). For *M. chartacea*, there was no significant difference in the concentration of tannins after incubation in the two different biomes (Figure 3).

The hardness was higher in *C. oliviforme* leaves than in *M. chartacea* (Table 1) before and after the conditioning in the streams. Comparing the hardness values in *M. chartacea* leaves after the incubation in the two different biomes, it is possible to notice that the hardness was lower after the conditioning in the Cerrado stream (0.113 g against 0.146 g in Mata Atlantica).

The percentage content of cellulose and lignin is greater in *C. oliviforme* leaves (Table 1), both before and after conditioning. It is interesting to note that lignin content is higher in *C. oliviforme* leaves after conditioning (65% after and 60% prior conditioning), although the cellulose content decreased from 26 to 23% of the total weight of the leaf. For the leaves of *M. chartacea*, there were differences in the results of conditioning in different biomes. The cellulose content increased its percentage after conditioning in a stream of Mata Atlantica, while it decreased after conditioning in a stream of Cerrado. Also, the lignin content increased after conditioning in Cerrado, similar to *C. oliviforme*.

4. Discussion

The shredder *Phylloicus* sp. of Mata Atlantica streams had showed feeding preference for leaves of *M. chartacea* rather than the exogenous plant species *C. oliviforme* which was collected in Cerrado. Invertebrates clearly prefer certain plants species (e.g., ash leaves are preferred than the ones of *Acer* plants and those are preferred than oak leaves (Bärlocher and Kendrick, 1973); alder leaves are preferred over any other species from temperate biomes, according to Anderson and Sedell (1979) and to Bärlocher and Sridhar (2014). In tropical ecosystems, the introduction of exotic leaves and the changes in nutrient availability affect shredder activity in streams, and therefore the processing of organic matter and ecosystem functioning (Casotti et al., 2015).

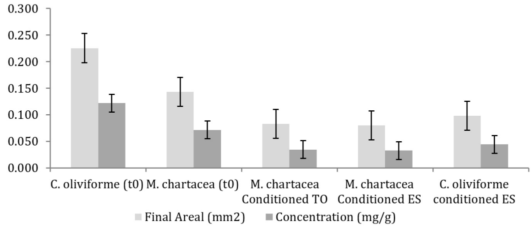

Figure 3. Tannin concentration (mg/g) per area (mm^2).

Table 1. Comparison of the characteristics of hardness (g) cellulose content (%) and lignin (%) of the species before and after conditioning at different biomes.

Plant specie	Hardness (g)	Cellulose content (%)	Lignin content (%)
Chrysophyllum oliviforme	0.431	26	60
Miconia chartacea	0.159	14	51
*Chrysophyllum oliviforme**	0.373	23	65
*Miconia chartacea**	0.146	16	50
*Miconia chartacea***	0.113	12	54

*After ten days of incubation in a stream of Mata Atlantica – ES; **After ten days of incubation in a stream of Cerrado – TO.

The characteristics of the leaves had influenced the palatability. The higher hardness and tannin concentrations of *C. oliviforme* leaves were probably inhibitory and influenced the non-feeding behavior by the shredder when offered as the only source of food. Also the higher lignin content may have influenced the unpalatability of *C. oliviforme* leaves, as the larvae of *Phyloicus sp*. escaped from the aquariums that contained only leaves of *C. oliviforme* (personal observations). These structural elements are component of the leaves and are considered important features that determine the feeding preference of the invertebrates (Gessner and Chauvet, 1994; Hoorens et al., 2003; Balseiro and Albariño, 2006). Navarro (2014) showed that the fragmentation behavior of *Phylloicus* sp. is mainly dependent on the concentration of lignin and cellulose of plant species used as food resource.

Secondary compounds, such as tannins from plant species confer the structural hardness, and may act as repellents for herbivores, rendering them unpalatable to invertebrates (Gonçalves Junior et al., 2007). The tannin content of the leaves of *C. oliviforme* was higher than the leaves of *M. chartacea,* which can also indicate that the unpalatability of the Cerrado leaves may be related to the high concentration of tannin, which increases the hardness of the leaves. Such characteristics of the Cerrado plant species made it less attractive for macroinvetebrates and may deter or slow down the decomposition process of detritus. These results are similar to those found by Konig et al. (2014), which had showed that detritivore groups prefer leaves of better quality, and this can be influenced by nutritional content and low content of compounds which complicate the fragmentation such as tannin, lignin and cellulose. Our study corroborates to the findings of Wantzen et al. (2002), of Yule and Gomez (2009) and Gonçalves Junior et al. (2012), which indicate that, in tropical streams of Cerrado, the low palatability of the leaves occurs due to the high amount of secondary and structural compounds that are used by plants as a defense against herbivores and/ or due to water stress, which remains after the abscission of the leaf.

The conditioning in streams of different biomes resulted in preference for leaves of the same plant species *M. chartacea* conditioned in Mata Atlantica than in Cerrado. According to Barlochër and Sridhar (2014), the hypothesis that the conditioning by fungi increases the acceptability of leaves for invertebrate is very robust, while some leaves are well accepted without conditioning. Invertebrates also differ between combinations of foliar species and fungal species, and the choice of a fungal species masks and overcomes the choice for a plant species. For example, oak leaves conditioned by *Anguillospora longissima* are preferred than the ash leaves colonized by *Tetracladium marchalianum* (Bärlocher and Kendrick, 1973). Arsuffi and Suberkropp (1989) showed that the composition of fungal community is as important as the degree of conditioning in determining the selection of food items by shredders. Thus, the fungal community of Mata Atlantica streams probably colonized the substrate more quickly and efficiently, which consequently produced the enzymatic attack necessary for palatability. In contrast, in the Cerrado stream, the fungal community produced no major change in the substrate within 10 days. Marques et al. (2015) show that fungal colonization in streams of that Cerrado region, including Buritizal stream occurs more effectively in 30 days of conditioning, when the fungal counts reach maximum values and then decays.

During conditioning, the leaves lose 10-30% of its original mass, becoming softer as the polymers are attacked by fungal enzymes (Suberkropp and Klug, 1980; Chamier, 1985). The conditioning in Cerrado led to an increase in lignin content, although the hardness was lower and the tannin content was similar. Thus, the lignin content seems to have greater influence in determining the hardness of the preference of *Phylloicus* sp. for leaves of *M. chartacea*.

5. Conclusion

The shredder *Phylloicus* sp. is selective in its choice of substrate, preferring leaves of a native species of Mata Atlantica rather than an exogenous species of Cerrado, which has less palatable leaves and lignin content that increased after the conditioning time. Feeding preference for detritus of *Miconia chartacea*, which was conditioned in the stream in Mata Atlantica biome, indicates that the characteristics of the aquatic ecosystem affect the conditioning and this is probably because the fungal communities differ between the two ecosystems of these two tropical biomes.

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