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Consumption, growth and survival of the endemic stream shredder *Limnephilus atlanticus* (Trichoptera, Limnephilidae) fed with distinct leaf species

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ABSTRACT

Oceanic freshwater communities tend to be species poor but rich in endemism due to their physical isolation. The ecology of endemic freshwater species is, however, poorly known. This study assessed allometric relationships, feeding preferences, growth and survival of larvae of the endemic stream insect *Limnephilus atlanticus* (Trichoptera, Limnephilidae) exposed to four leaf species differing in their physical and chemical characteristics (*Ilex perado*, *Morella faya*, *Alnus glutinosa* and *Clethra arborea*), in laboratory trials. All regression models used to estimate *L. atlanticus* dry mass from body and case dimensions and wet mass were significant, but wet mass and body length were the best predictors. *Limnephilus atlanticus* consumed all the four leaf species offered, but when given a choice, shredders significantly preferred *A. glutinosa* over the other three leaf species. Relative larval growth rate was significantly higher when *L. atlanticus* fed on *A. glutinosa* and *I. perado* leaves in comparison with the other leaf species. Survival of 95% was found when individuals fed on *A. glutinosa* leaves while it decreased to 75% when they fed on the other leaf species. Our results suggest that *L. atlanticus* can be an active shredder and that it exhibits the same basic patterns of food exploitation as its continental counterparts. The lack of an effect of shredders on litter decomposition in Azorean streams revealed by previous studies may thus be due to low densities or to a preference for food resources other than the low quality native litter species.

1. Introduction

Organic matter derived from the riparian vegetation is a key source of energy for forest streams (Cummins et al., 1989; Wallace et al., 1997; Webster and Benfield, 1986). Once in water, this organic matter, generally in the form of leaf litter (Abelho, 2001), is decomposed by microbial decomposers and macroinvertebrate detritivores (Graça, 2001; Graça and Canhoto, 2006; Webster and Benfield, 1986). Microbes colonize leaf litter soon after leaf immersion and as microbial biomass accumulates the nutrient concentration in the litter increases (Gulis et al., 2006; Gulis and Suberkropp, 2003), which improves its nutritional value to invertebrate consumers (Graça, 2001; Graça and Cressa, 2010). Also, the activities of fungal exoenzymes promote leaf softening making feeding activities by invertebrates easier (Arsuffi and Suberkropp, 1989). In continental temperate streams, macroinvertebrate detritivores generally play an important role in leaf decomposition (Cornut et al., 2010; Gulis et al., 2006; Hieber and Gessner, 2002), while in islands leaf decomposition seems to be essentially driven by microbes (Benstead et al., 2009; Ferreira et al., 2016; Raposeiro et al., 2014). The low contribution of the macroinvertebrate community to leaf decomposition in island streams may be related with the overall depauperate and disharmonic nature of the insular assemblages (Raposeiro et al., 2012; Smith et al., 2003).

The caddisfly *Limnephilus atlanticus* Nybom (1948) (Trichoptera, Limnephilidae) was described during the Finnish expedition to the Azores islands in 1938 (Nybom, 1948) and was found to be endemic of this archipelago. This taxon is known to be well distributed in the Azores archipelago (Borges, 2010; Raposeiro et al., 2012), especially in streams at high elevations flowing through native vegetation (Raposeiro et al., 2013). Because it belongs to the Limnephilidae family and has typical detritivore mouthparts, it is expected that this taxon be a shredder. Although shredders are less abundant and diverse in oceanic islands (Raposeiro et al., 2012) than in mainland streams, there is no a priori reason why insular shredders should have a feeding strategy much different from their continental counterparts. However, stream macroinvertebrate have little influence on the decomposition of

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leaf litter in insular freshwater ecosystems (Ferreira et al., 2016; Raposeiro et al., 2014). One possible explanation could be that most native plant species (e.g. Lauraceae, Aquifoliaceae) synthesize a large number of secondary substances that act as natural insecticide and likely diminish the palatability of the leaves to consumers (Rosa et al., 2010). Also, native species, especially the endemic ones, have a waxy cuticle and a thick palisade cell layer that protect the leaf mesophyll from consumers (Raposeiro et al., 2014).

Due to the difficulty of finding good quality leaves (i.e. soft with high nutrient concentration) in island streams, would *L. atlanticus* have adapted to poor quality food resources or is it using alternative food resources? The aim of this study was to determine whether *L. atlanticus* individuals use coarse organic matter as a food resource by assessing food preferences, consumption and growth rates and survival of *L. atlanticus* individuals fed with distinct leaf species.

2. Materials and methods

2.1. Collection of *Limnephilus atlanticus* larvae

Experiments were performed with early-stage larvae of *L. atlanticus*, a stream caddisfly endemic to the Azores archipelago and common in the upstream sections of some Azorean streams (Raposeiro et al., 2012, 2013). Individuals were collected in a single day from depositional areas in a first order reach of Ribeira do Folhado, São Miguel Island (37°48′48″N, 25°14′47″W; 729 m above sea level), in spring 2015, and transported to the laboratory in a cooler. Ribeira do Folhado is a narrow (1 m wide) and short (~7 km long) stream that drains an area < 10 km^2. Predominant substrates comprise mixed gravel/cobbles with occasional large, submerged boulders. The stream water is circumneutral, has low conductivity and low nutrient concentration (Table 1). The riparian forest is dominated by *Cryptomeria japonica* (L.f) D. Don and *Clethra arborea* Aiton trees.

2.2. Estimation of *Limnephilus atlanticus* dry mass

Individuals were maintained in plastic containers with aerated stream water and sediment, at 12 °C, and measured within 48 h after collection. Five case and body dimensions were taken with a stereomicroscope and used as predictors of biomass: case length (CL), case opening width (CW), body length (BL), head length (HL) and interocular distance (ID). Additionally, wet mass (body + case; WM) was determined after drying the individuals with paper (0.1 mg precision). Then, individuals were removed from their cases, placed individually in pre-weighed aluminum cups, dried at 60 °C for 24 h and weighed (0.1 mg precision) to determine the dry mass (DM). CW, BL and HL measurements were obtained for 74 larvae; CL, ID, WM and DM were obtained from 120 larvae.

Three regression models were used to determine the relationships between case and body measurements and DM. The data were fitted to the following models in order to determine which best described the relationship: linear ($y = a + b \times x$), power ($y = a \times x^b$; in linear form: $\ln(y) = \ln(a) + b \times \ln(x)$) and exponential ($y = a \times e^{xb}$; in linear form: $\ln(y) = \ln(a) + b \times x$), where **y** is DM (mg), **x** is case or body measurement (mm; mg for WM) and **a** and **b** are regression constants.

Table 1
Physical and chemical characteristics of Ribeira do Folhado between 2014 and 2015 (n = 5).

Water variables	Mean ± SE
Temperature (°C)	9.3 ± 0.3
pH	6.7 ± 0.4
Conductivity (μS/cm)	53.7 ± 3.1
Nitrate (μg NO_3^-/L)	119.1 ± 21.6
Nitrite (μg NO_2^-/L)	0.4 ± 0.4
Total nitrogen (μg N/L)	190.4 ± 36.3
Soluble reactive phosphorus (μg PO_4^{2-}/L)	37.3 ± 11.5
Total phosphorus (μg P/L)	14.6 ± 44.9

2.3. Leaves

Leaves from four tree species were used: *Ilex perado* Aiton and *Morella faya* Aiton are two native broadleaf perennial tree species commonly present in the riparian area of streams flowing through native vegetation, *Clethra arborea* Aiton is an invasive broadleaf perennial tree species also common in the riparian vegetation of Azorean streams, and *Alnus glutinosa* (L) Gaertn. is an exotic broadleaf deciduous tree species. *Alnus glutinosa* trees are rare in the Azores archipelago but previous studies have shown this to be a highly palatable leaf species to shredders (Friberg and Jacobsen, 1994; Graça and Cressa, 2010) and thus it was used here for comparative purposes. Leaves from the perennial species were collected directly from the trees since these shed leaves at a low rate, incompatible with the experimental needs for leaf litter. Alder leaves were collected after natural senescence in autumn. All leaves were transported to the laboratory where they were air dried at ambient conditions and stored in the dark until used.

Leaf species were characterized regarding toughness and chemical composition. Leaf toughness was determined using a penetrometer after leaves had been soaked in distilled water for 1 h and results were expressed as the force (g/mm^2) needed to perforate the leaves with an iron rod (Graça et al., 2005). Subsamples of dry leaves were ground to fine powder (< 1 mm) and used for the determination of nitrogen (IRMS Thermo Delta V advantage with a Flash EA − 1112 series), phosphorus (Apha, 1995), lignin (Goering and Van Soest, 1970) and polyphenol concentration (Graça et al., 2005). Results were expressed as % DM. Comparison of physical and chemical characteristics among leaf species were done by one-way ANOVA, followed by Tukey's test.

Leaves were offered to individuals as 12 mm diameter leaf discs, extracted with a cork borer avoiding the main vein. Leaf discs were enclosed in 0.5 mm mesh bags (3.5 × 4.0 cm) and incubated for seven days in 25-L laboratory containers filled with water from Ribeira do Folhado that was continuously aerated. The water was renewed each 3 days to ensure the leaching of leaf soluble compounds and the colonization of leaf discs by microbial decomposers that increase litter palatability to shredders (Graça and Cressa, 2010; Graça et al., 2001). Discs were oven dried (60 °C for 48 h) and weighed (0.1 mg precision) to determine initial mass before being used in the experiments.

2.4. Experimental chambers

Experimental chambers consisted of 8.5 × 8.0 × 6.5 cm containers with 250 mL of filtered water (0.45 μm-pore membrane filter; GF/C, Whatman) and 10 g of ignited (8 h at 500 °C) sand (200 μm) from Ribeira do Folhado. Chambers were kept inside a Sanyo versatile Environmental Test Chamber, MLR-351-H (Japan), maintained at 12.0 ± 0.5 °C with a 10:14 h light:dark photoperiod, and aerated for the duration of the experiments. A single individual was weighed (WM; 0.1 mg precision) and added to each chamber. Larvae with similar size (40.0–50.0 mg) were selected to be used.

2.5. Feeding preferences

Limnephilus atlanticus were presented with: (i) a choice among the four leaf species, to provide a direct comparison of species preferences, and (ii) a no-choice situation with each leaf species being given individually. Twenty chambers were set up for the choice (total n = 20) and 40 chambers for the no-choice experiment (10 chambers × 4 species). In the choice experiment, each chamber received the four leaf species with the four leaf discs (one per leaf species) being