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Assessing the lack of intolerant macroinvertebrate species in a low order peri-urban stream

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica da Professora Doutora Cristina Maria M. Monteiro Leal Canhoto (Universidade de Coimbra) e da Professora Doutora Manuela Abelho (Instituto Politécnico de Coimbra)

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Abstract

More than half of the world's population lives in urban zones (54%) and the value is expected to increase in a near future. The increase in urbanization led to increasing pressures on streams resulting in physicochemical and ecological degradation, a phenomenon known as the 'Urban Stream Syndrome'. Aquatic macroinvertebrates, especially the more pollution intolerant orders Ephemeroptera, Plecoptera and Trichoptera, are often used as bioindicators. In this study I assessed, via 7 days laboratory experiments, the quality of water and food of a low order urban stream using the macroinvertebrate shredders Leuctridae, Sericostomatidae and Tipulidae. There was no effect of water or food quality in survival. Except for the more tolerant Tipulidae, consumption rates of urban food were higher than consumption rates of control food, especially for macroinvertebrates kept in urban water. The obtained results suggest that poor food quality, probably due to lower microbial colonization, and water quality degradation, probably due to non-point pollution sources, prevent the establishment of intolerant shredders in the stream.

Key words: Ribeira dos Covões; urban streams; macroinvertebrates; Leuctridae, Sericostomatidae, Tipulidae; shredders; survival; consumption; environmental quality.

Resumo

Mais de metade da população mundial vive em zonas urbanas (54%) e é previsto um aumento neste valor num futuro próximo. O aumento da urbanização leva a uma maior pressão exercida sobre os cursos de água, resultando numa degradação fisico-quimica e ecológica, um fenómeno conhecido como “Urban Stream Syndrome”. Os macroinvertebrados aquáticos, especialmente os pertencentes a ordens mais intolerantes à poluição como os Ephemeroptera, Plecoptera e Trichoptera são muitas vezes usados como indicadores biológicos. Neste estudo avaliei durante 7 dias em experiências laboratoriais, a qualidade da água e alimento de um curso de água de pequena ordem usando os macroinvertebrados trituradores Leuctridae, Sericostomatidae e Tipulidae. Não houve efeito da água e alimento na sobrevivência dos macroinvertebrados. Excepto para os mais tolerantes (Tipulidae), as taxas de consumo do alimento proveniente do curso de água urbano foram maiores que as taxas de consumo do alimento proveniente do curso de água controlo, especialmente para os macroinvertebrados mantidos em água do curso de água urbano. Os resultados obtidos sugerem que a fraca qualidade do alimento, provavelmente devido a uma baixa colonização microbial e a degradação da qualidade da água, provavelmente devido a fontes de poluição não pontuais, impedem o estabelecimento destes trituradores intolerantes à poluição no curso de água em estudo.

Palavras chave: Ribeira dos Covões; cursos de água urbanos; macroinvertebrados; Leuctridae, Sericostomatidae, Tipulidae;detritívoros fragmentadores; sobrevivência; consumo; qualidade ambiental.

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1 INTRODUCTION

1.1 URBANIZATION, AN INCREASING PRESSURE ON CITY STREAMS

Urbanization occupies a prominent area of land surface. According to the United Nations Population Division (UNPD 2014), more than half of the world's population (54%) already lives in urban zones and it is expected that these numbers will increase by 66% in 2050. For example, both in Europe and in North America the percentage of the population that live in urban areas reached 74% in 2014.

The increasingly urbanization occurs mainly due to the migration from the rural zones and to the transformation of rural into cities (UNPD 2014). Although urban areas represent only 2% of the total Earth's land surface occupied by humans (Paul & Meyer 2001), the need for productive ecosystems outside city borders to produce food, water, and renewable resources for city consumption leads to an ecological footprint much higher, with ecosystem supporting areas of 500 to 1000 times their size in Baltic cities (Folke et al. 1997).

The continuous growth of urban zones and the extensive use of adjacent lands turn the riparian ecosystems increasingly vulnerable. In fact, only agriculture, due to its higher covered area, overpasses the global deleterious effects of urbanization on stream ecosystems (Paul & Meyer 2001). Worldwide, urban expansion is one of the main drivers of habitat loss and species extinction (Seto et al. 2011). The transition of forest and unpopulated lands to urban lands influences the climate and air quality, and changes the energy and nutrient flows leading to a decrease of native biodiversity (Dudgeon et al. 2006, Bernhardt et al. 2007). By providing habitats for biota, carrying water and processing materials, streams play an important role in the dynamics of urban ecosystems and are important socially and culturally for the human inhabitants of their catchments (Walsh et al. 2005). However, streams in urban areas are also particularly vulnerable to impacts associated with land cover change.

1.2 THE URBAN STREAM SYNDROME – CAUSES AND EFFECTS

Due to the intensification of urbanization, streams are increasingly flowing through urbanized catchments leading to an increase of literature concerning urban streams. A new term, the "urban stream syndrome", first used by Meyer et al. (2005) describes the consistent patterns of physicochemical and biological degradation of stream conditions associated with urban land use (Feminella & Walsh 2005). There are three main causes leading to the degradation of urban streams: the increase of impervious surface cover, the absence or alteration of riparian vegetation and direct modification of streams (Wenger et al. 2009). The subsequent effects are diverse and can be grouped in three interdependent categories: physical, chemical and ecological.

1.2.1 PHYSICAL EFFECTS

Physical effects can be hydrological, geomorphologic and climatic. The changes concerning hydrology are mostly due to the impervious surfaces cover characteristic of urban zones, due to the construction of highways, rooftops and parking lots. These structures cause a decrease of water infiltration on soils and an increase in surface runoffs directly into streams (Walsh et al. 2000; Paul & Meyer 2001; Bernhardt et al. 2007), decreasing groundwater recharge, and potentially reducing surface water withdrawals, especially on summer months (Konrad & Booth 2005), thus changing the hydrology of urban streams and increasing the frequency and magnitude of high-flow events (Wenger et al. 2009). As a result of hydrological alterations, banks and beds of urban streams normally exhibit an erosion increment leading to the increase in the width and depth compared with non-urban streams (Paul and Meyer 2001; Bernhardt et al. 2007). However, in the early stages of development, initial construction in a watershed provides the stream with larger quantities of substrate than forested catchments, but this tends to be a short-term effect being surpassed by the long-term decrease of sediment supply due to the increase in impervious surfaces in the watershed (Doyle et al. 2000; Paul and Meyer 2001). .

The geomorphology of streams may be also affected by direct modification of the stream beds (Paul & Meyer 2001, Walsh 2005), when they are made into concrete, non-erosive beds to stabilize the stream channel (Walsh et al. 2005). Concrete walls simplify habitats in an extreme way resulting in the disruption of the riffle/pool sequences, important for the diversity of habitats (Nolan & Guthrie 1998). It will also amplify hydrologic and geomorphic impacts downstream. Furthermore, the floodplain and the hyporheic zone will be separated from the stream causing a loss of important microbial processing locations (Wenger et al. 2009).

Urban streams present increased temperature in summer and decrease in the winter months in comparison to their forested counterparts. This may be related with the release of stored water from shallow detention ponds, point discharges from wastewater treatment plants, decreased groundwater recharge and increased insolation from removal of riparian vegetation (Paul & Meyer 2001; Pusey & Arthington 2003; Wenger et al. 2009). Differences of temperature between urban zones and surrounding rural and forested zones are usually referred as the “urban heat island” (Kleerekoper et al. 2012). Temperature changes can affect respiration due to the lower concentration of dissolved oxygen in higher temperatures, causing metabolic changes and the composition of native fish and macroinvertebrate species less tolerant to higher temperatures (Paul & Meyer 2001; Wenger et al. 2009).

1.2.2 CHEMICAL EFFECTS

The presence of contaminants is very common in urban streams, and most of these toxic components reach the streams by wastewater systems (Kolpin et al. 2002). However, the amount of contaminants reaching the streams through storm water runoff increases with the amount of impervious surface (Paul & Meyer 2001; Hatt et al. 2004). The lack of riparian vegetation also contributes to the presence of contaminants in urban streams since these areas act as agents for trapping and processing nutrients and contaminants (Pusey & Arthington 2003).

Inorganic nutrients exhibit higher concentrations in urban catchments than in forest streams, and sometimes have the same level of concentration as the agricultural streams (Wenger et al. 2009). The level of concentration of these compounds is dependent of wastewater treatment technology, the degree of illegal discharges and leakage of sewer pipes as well as the use of lawn and garden fertilizers by homeowners (Hatt et al. 2004; Bernhardt et al. 2008; Wenger et al. 2009). Other ions such as calcium, sodium, potassium, and magnesium are also found in high amounts due to land use intensity and waste water flow (Zampella 1994) and may lead, for example, to an increase of water conductivity (Paul & Meyer 2001).

The metals found in higher concentrations in urban streams are lead, zinc, cadmium, copper, chromium, manganese and nickel. Mercury is also found in high concentrations, especially after storm events (Paul & Meyer 2001; Wenger et al. 2009). Apart from the usual industrial discharges and roadways that are the principal cause of pollution by toxic metals, there are also other significant sources that contribute to the increase of these metals in urban streams, such as metallic roofs, parking lots, gutters and downspouts, metallic corrugated pipes, old lead pipes, storage areas, scrap yards and landfill sites (Beasley & Kneale 2002).

Common xenobiotics in water include drugs, carcinogens and industrial chemicals and reach urban streams through industrial discharges (Kolpin et al. 2002). . Household chemicals and pharmaceuticals are released to streams after passing through wastewater treatments processes that generally are inefficient in the removal of these components from the effluent (Kolpin et al. 2002). . Due to their polarity, some pharmaceutical contaminants are poorly retained in sludge and sediments and could contaminate groundwater besides common endocrine active contaminants are lipophilic and can accumulate in biofilms and organic sediments (Reinstorf et al. 2007). Xenobiotics characteristic of human activity like pharmaceuticals such as lipid regulators, analgesics, β-blockers and antiepileptics and fragrances are very common in surface waters and increasingly abundant in urban streams (Reinstorf et al. 2007). Since some of these contaminants were already found in food, fish tissue, human milk and blood plasma, the concern regarding their presence in water is increasing (Kolpin et al. 2004).

Pesticides including insecticides, herbicides and fungicides commonly used in domestic homes as well as commercial and industrial zones are also a concern in urban streams since their concentrations in certain areas are already higher than those observed in agricultural zones (Paul & Meyer 2001).

1.2.3 ECOLOGICAL EFFECTS

The effects of urbanization on algal diversity and biomass are very complex and can vary with the characteristics of the stream. Nutrients like nitrogen (N) and phosphorus (P) as well as sunlight limit the growth of stream algae (Busse et al. 2006); an increase in urbanization may lead to an increase of algal biomass due to high levels of nutrients (Hatt et al. 2004) and light inputs (Walsh et al. 2005; Busse et al. 2006). On the other hand some studies report that the constant change of stream bed sediments and the high turbidity of water courses, also characteristic of urban streams, can limit the abundance of algae (Paul & Meyer 2001, Konrad & Booth 2005) even with high N and P in stream water (Walsh et al. 2005). Direct anthropologic intervention can also limit and reduce the algal biomass despite high concentrations of nitrogen and phosphorus. According to Grimm et al. (2005) the direct use of algaecides and herbicides may prevent algae and plant growth. Many species of algae are also sensitive to heavy metals and their accumulation in stream sediments can contribute to the reduction of algal biomass (Olguin et al. 2000).

Catchment imperviousness and storm water runoff quantity and quality have important effects on macroinvertebrate communities in urban streams. An increase of impervious surfaces on the catchment area is directly related with a decrease on taxon richness, especially Plecoptera, Trichoptera and Ephemeroptera (EPT) (Walsh et al. 2001). In fact, macroinvertebrate species more sensitive to pollution are almost always less abundant or absent in urban streams (Couceiro et al. 2006; Miserendino et al. 2008) due to the transport of pollutants from the catchment area and to storm water runoff that can also move the bed sediment affecting the benthic macroinvertebrates. According to Pesacreta (1997), smothering by sand and silt could be a reason to a high mortality of the stonefly *Pteronarcys dorsata* in two urban streams in North Carolina. Couceiro et al. (2006) indicated that the lack of substrates can affect the movement of macroinvertebrates, facilitating drift due to absence of interstitial substrates for shelter. On the other hand, species of oligochaetes and chironomids, more tolerant to environment changes have increased abundances and usually show increased abundances in urban streams (Couceiro et al. 2006; Wenn 2008; Kripa et al. 2013). Riparian zones can act as a buffer between urban areas and streams helping the retention of contaminants that otherwise would go directly to water (Paul & Meyer 2001) and stabilizing the soils avoiding sediments to be transported to watercourses from stream banks (Couceiro et al. 2006). Riparian deforestation linked to urban zones leads to a reduction of allocthonous material (Uieda & Kikuchi 1995) decreasing the food sources for most macroinvertebrates (Cummins & Klug 1979; Wallace & Webster 1996) and has an important role on the decrease of macroinvertebrate richness (Couceiro et al. 2006). Higher temperatures related to riparian deforested streams can accelerate macroinvertebrates life cycle (Couceiro et al. 2006) and decrease dissolved oxygen in water (Connolly et al. 2004), increasing macroinvertebrates effort to obtain oxygen and thereby leading to an increased absorption of contaminants affecting the production of biotic communities (Couceiro et al. 2006).

Fish species more sensitive to pollution are usually replaced by more tolerant species as the level of urbanization increases. Typically both diversity and abundance of fish decrease although in some cases high abundances of tolerant populations of fishes can be supported in degraded streams (Wolter et al. 2000). Invasive species typically are more able to populate these areas and increase their abundance (Paul & Meyer 2001; Walsh et al. 2005) due to their eurytropic character (Wolter 2008).

1.3 AQUATIC MACROINVERTEBRATES

Stream macroinvertebrates play an important role in the ecosystem, mainly in low order streams, and can influence the nutrient cycles, primary production, decomposition and translocation of materials (Wallace and Webster 1996). According to Metcalf (1989) a preference for using benthic macroinvertebrates as biological indicators over periphyton, plankton and fish is due to the following reasons: (i) they are ubiquitous, abundant and easy to collect; (ii) they are representative of local conditions due to their relatively sedentary lifestyle; (iii) they exhibit differential sensitivities do pollutants and react relatively fast to pollution; (iv) their life span is long enough to provide a record of environmental quality, and (v) macroinvertebrate communities are very heterogeneous increasing the probability that some of these organisms will react to some environmental conditions.

1.3.1 FUNCTIONAL FEEDING GROUPS

The functional feeding groups classify macroinvertebrates by the type of food ingested and the way they acquire food (Cummins & Klug 1979). This type of classification was implemented because a large portion of stream macroinvertebrates feed not only vegetal matter but also on fungi, bacteria and even animals attached to the vegetal material (Cummins & Klug 1979; Wallace & Webster 1996). Stream macroinvertebrates are classified in five functional feeding groups: scrappers, collector-filterers, collector-gatherers, predators and shredders (Cummins & Klug 1979).

Scrapers feed by scraping periphyton / epilithic algae attached to stones and submerged objects but when scraping the surfaces they can also ingest organic matter and animals (Cummins & Klug 1979; Wallace & Webster 1996). Scrapers exhibit several morphologic characteristics that allow them to obtain food like scoop-shaped mandibles with a cutting edge, the inner grinding surfaces of many mayfly mandibles and the rasping radular structure of gastropods (Cummins & Klug 1979). Usually the abundance of scrapers is associated with increased light levels and algal biomass. Collectors feed on fine particulate organic matter (<1mm; FPOM), and can harvest FPOM in suspension (collector-filterers) or feed on FPOM deposited on surfaces (collector-gatherers) (Cummins & Klug 1979). Shredders feed mainly on coarse particulate organic matter (>1 mm diameter; CPOM) preferring CPOM well-conditioned by microorganisms (Cummins & Klug 1979; Wallace & Webster 1996). Normally, CPOM-shredding insects have low assimilation efficiencies and thus are able to transform large amounts of CPOM into FPOM, facilitating downstream transport and providing food for FPOM-feeding organisms (Wallace & Webster 1996). Predators are all the macroinvertebrates that are adapted to feed on living prey and have an important role on regulating the abundance of their prey, composed mainly by other groups of macroinvertebrates (Cummins & Klug 1979).

1.3.2 EPT AS BIOINDICATORS

The orders Trichoptera, Plecoptera and Ephemeroptera are the groups of aquatic insect most used in programs of aquatic environment assessment, mainly due to their high richness, ecological diversity and abundance in several types of aquatic habitats (Pereira et al. 2012). Most of the EPT families are intolerant to pollution and have the maximum rank on the BMWP (Biological Monitoring Working Party) index (Jáimez-Cuéllar et al. 2002). The number of species of Ephemeropthera, Plecoptera and Trichoptera found in a sample (the EPT index) is considered one of the best indicators of water quality degradation and human intervention on streams (Somers et al. 1994; Krno 2007). A study by Baker & Sharp (1998) showed that upstream from the urban zone the benthic macroinvertebrate community included large numbers of EPT larvae and was relatively not impacted from urban pollution. However, at the urban zone, the stream was virtually striped of these taxa leading to the conclusion that nonpoint source pollution could be the cause for the EPT reduction (Baker & Sharp 1998).

Almost 80% of Trichoptera *taxa* exhibit intolerance to organic pollution; the *taxa* more tolerant to pollution are normally collector-filterers caddisflies. A loss of caddisflies species richness can indicate habitat disturbance due to agriculture or urbanization that increase habitat organic pollution. The increase of organic material promotes an increase of abundance on some of collector-filterers and a decrease on overall species richness (Houghtona 2004). Plecoptera is the most sensitive order of aquatic macroinvertebrates, tending to decline with the increasing levels of human disturbance (Gage et al. 2004). Plecoptera in general are sensitive to organic pollution and low oxygen concentration (Törnblom et al. 2011) providing a high bioindication potential especially for the assessment of small streams (Krno 2007). The Ephemeroptera are sensitive to acidification, oxygen depletion and presence of various contaminants such metals and ammonia (Savić et al. 2011), being highly successful to predict water quality in degraded streams (Edsall et al. 2004).

1.4 OBJECTIVES

Biomonitoring of urban streams reveals relatively poor macroinvertebrate communities and lack of the most sensitive families (Isidoro 2014). However, from the study of local communities it is impossible to assess which of the multiple stressors associated with urbanization are responsible for the disappearance of intolerant *taxa*. The goal of this work was to assess the factors (water quality, food quality or habitat quality) responsible for the absence of macroivertebrate *taxa* intolerant to disturbance in a low order peri-urban stream near Coimbra, Central Portugal. The factors water quality (control vs. urban) and food quality (control vs. urban) were tested in full-factorial laboratory experiments with the following rationale: (i) if animals in urban stream water perform badly irrespective of food origin, water quality is the factor preventing the establishment of intolerant macroinvertebrates; (ii) if animals fed with urban food perform badly irrespective of water origin, food quality is the factor preventing the establishment of intolerant macroinvertebrates; (iii) if all animals perform equally well irrespective of water or food origin, habitat alteration is the factor preventing the establishment of intolerant macroinvertebrates.

2 MATERIALS AND METHODS

2.1 STUDY STREAM

The study stream was Ribeira dos Covões (40º21’26”N, -08º44’92”W, elevation 25 m) a fourth order peri-urban stream with a draining area of 7 km^2 with the source located near Hospital dos Covões and the mouth at the Mondego River, near the urban center of Coimbra, Portugal (Pato et al. 2011). The area is characterized by a humid Mediterranean climate, with an average temperature of +15 ºC and total precipitation of 980 mm in an average year, with strong seasonal and inter-annual variation. The basin is predominantly covered with forest (55.5%) and farmland (13%) with an estimated population of 7000 inhabitants by 2001 and a significant presence of artificial structures (31.5%) (Ferreira et al. 2011). From mouth to source, the stream crosses both urban and agricultural areas as well as a highway which cause several characteristic effects of urban zones.

The control stream was Ribeira do Botão (40º18’23”N, 08º23’55”W, elevation 30 m), a third order stream located near Coimbra, central Portugal. The site is characterized for having a streambed mainly composed of cobble and gravel. The riparian corridor exhibits no major alterations being composed essentially of mixed deciduous trees where the most dominant trees are alder, willow and poplar. The basin area is mostly used for eucalypt forestry, nonetheless small agricultural areas and the town of Botão are presented downstream of macroinvertebrate collection point. This site has high macroinvertebrate richness including a high number of sensitive families and very good water quality according to the IBMWP biotic index (Castela et al. 2007).

2.2 MACROINVERTEBRATE *TAXA*

The macroinvertebrates used in the experiments were the families Leuctridae (order Plecoptera), Sericostomatidae (order Trichoptera) and Tipulidae (order Diptera), and were chosen based on three criteria: (1) availability; (2) to be leaf-shredding macroinvertebrates; (3) to belong to medium - high pollution intolerant groups.

The Plecoptera Leuctridae were collected on two different occasions, the first in December and the second in January. Plecoptera nymphs (stoneflies) are found in running waters, especially in small cold streams, associated with coarse substrates such as cobble, leaf packs and large woody debris. The stoneflies Leuctridae are shredders and highly intolerant to disturbance (IBMWP rank: 10/10; Jáimez-Cuéllar et al. 2002). The Trichoptera Sericostomatidae were collected in January. Some Trichoptera *taxa* have the ability to spin silk, this silk is used to build retreats, nets for collecting food, for construction of cases, for anchoring to the substrate, and to spin a cocoon for the pupa (Bouchard 2004). The caddisflies Sericostomatidae use silk to make a protective case of sand or of silk alone (Holzenthal et al. 2007), are shredders and highly intolerant to disturbance (IBMWP rank: 10/10; Jáimez-Cuéllar et al. 2002). The Diptera Tipulidae were collected in March. Larval Tipulidae (crane flies) are shredders (Cummins 1973), feeding and growing during autumn, winter and spring (Martin 1980). These organisms have a foregut and midgut strongly alkaline where pH can reach up to 11.5 exhibiting very high proteolytic activity (Martin et al. 1980; Bärlocher & Porter 1986); the high alkalinity present in the midgut would be expected to dissociate protein-tannin complexes or preventing the formation of these complexes between polyphenols and protein liberated during maceration of ingested food or between polyphenols and digestive enzymes present in the tipulid’s gut making this a highly efficient protein-digesting system (Martin 1980). Crane flies are moderately intolerant to disturbance with an IBMWP rank of 5/10 (Jáimez-Cuéllar et al. 2002).

The macroinvertebrates were collected from Ribeira do Botão with a surber net at random locations. Each sample was placed in trays and the macroinvertebrates needed for the experiment were selected. Collection of macroinvertebrates ended when enough animals were selected. Macroinvertebrates were transported in containers filled with stream water to the laboratory where they were kept in aerated conditions at room temperature with alder leaves collected in the same stream as food source for at least 2 days before being used in the experiments.

2.3 FOOD PREPARATION AND CONDITIONING

Oak leaves (*Quercus robur*) were used as a food source for the macroinvertebrates. Senescent leaves were collected in the previous autumn and air dried at room temperature until needed. Five pairs of leaf discs with 8 mm of diameter were cut symmetrically on each side of the leaves to ensure a uniform mass in the two leaf discs. One of the two groups of five leaf discs was used to feed the macroinvertebrates and the other was used to determine initial dry mass. Each group was needled together; the two groups were connected with a nylon wire and sewn to the inner face of fine-mesh (2 mm) pyramidal-shaped bags (***Figure 1***).

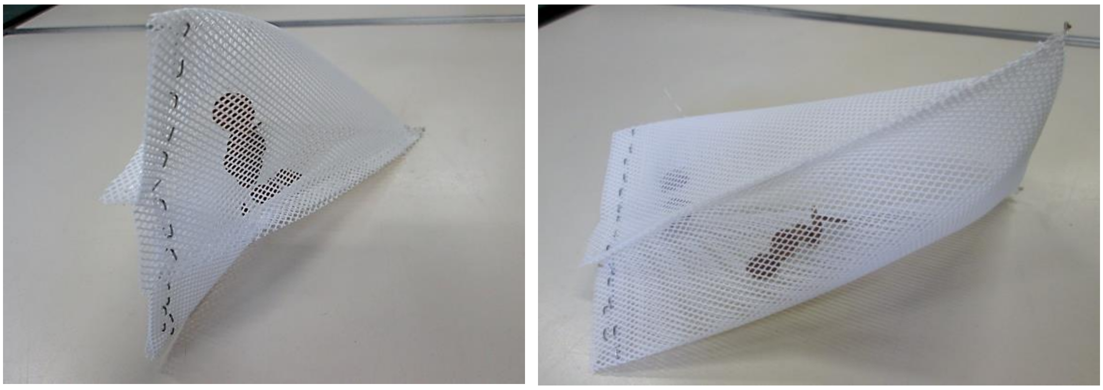

Figure 1 Fine mesh (2 mm) pyramidal-shaped plastic net bags with two pairs of five oak leaf discs.

Half of the bags with the two duplicate sets of leaf discs were placed in a container with 30 L of water from the urban stream (Ribeira dos Covões) and the other half was placed in a container with 30 L of control water from Ribeira do Botão (***Figure 2***). The conditioning of the leaf discs was carried out at room temperature (circa 20ºC) for a period of 21 days. The water was aerated continuously with air pumps. After 21 days, the bags with the conditioned leaf discs were frozen at -4ºC until needed.

Figure 2 Conditioning of oak leaf discs with water from the urban stream (Ribeira dos Covões; left) and from the control stream (Ribeira do Botão; right) during a period of 21 days at room temperature.

2.4 SURVIVAL AND CONSUMPTION RATES

Survival and consumption rates of the macroinvertebrates as well as the mass loss of the leaf discs were assessed after seven days in four treatments: (1) control (water and food from the control stream), (2) poor-water quality (water from the urban stream; food from the control stream), (3) poor-food quality (water from the control stream; food from the urban stream), and (4) poor-water and food quality (water and food from the urban stream). Each of the treatments had five replicates in a total of 20 chambers. The chambers were kept at room temperature (circa 20ºC) and aerated continuously throughout the experiments (**Figure 3**). The two groups of leaf discs in each bag were separated, one was oven-dried at 60ºC during 3 days to determine initial dry mass and the other was used to feed the macroinvertebrates.

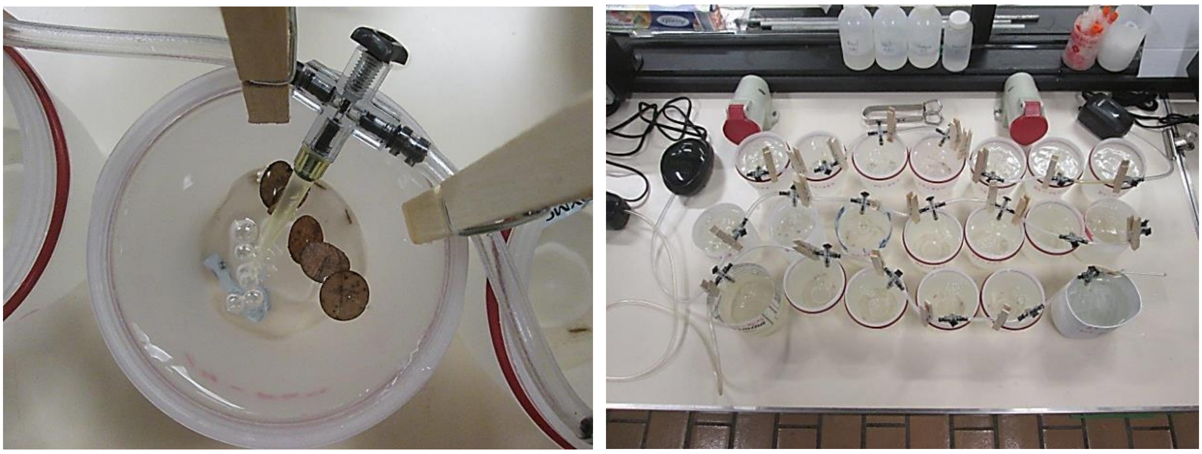

Figure 3 Oxygenated container with the conditioned oak leaf discs and the stoneflies Leuctridae (left) and overall view of the oxygenated containers (right).

Due to their small size and mass, the experiments with the stoneflies Leuctridae were made using five individuals per replicate. The stoneflies were randomly allocated to groups of five individuals, each of the individuals was placed for three seconds on absorbent paper to remove the excess of water, and the group of the five individuals was weighted (wet weight) on an analytical balance (4 decimal places). Each group of five individuals was allocated to a container with approximately 350 mL of the urban or the control stream water. The caddisflies (Sericostomatidae) and the dipterans (Tipulidae) had enough mass to be weighed individually; a single individual was allocated in each container. Fresh mass of the Tipulidae was determined as above and fresh mass of the caddisflies Sericostomatidae was estimated from measurements of the width of the case opening (Ferreira et al. 2010):

$$\text{Dry mass} = 0.0136 \times \text{case opening width (mm)} - 0.0162$$

Mean initial mass of the five Leuctridae per replicate was 0.0118 g (range 0.0083 g – 0.0158 g) for the first experiment and 0.0131 g (range 0.0098 g – 0.0170 g) for the second experiment, mean individual initial mass of the Sericostomatidae was 0.0217 g (range 0.0164 g – 0.0273 g) and mean individual initial mass of the Tipulidae was 0.6793 g (range 0.3680 g – 1.0750 g). At the end of the experiment, the macroinvertebrates still alive were weighted as above. The leaf discs were removed, oven-dried (60ºC, 3 days) and weighed to determine dry mass remaining.

2.5 CALCULATIONS AND DATA ANALYSIS

Survival in each replicate was expressed as the percentage of invertebrates alive at the end of each experiment. Leaf mass loss was calculated as the difference between initial and final leaf disc mass and expressed as a percentage of the initial mass. Consumption rate was calculated as the leaf mass consumed per unit body mass per day, where average body mass is the average of initial and final fresh mass:

$$\text{Consumption rate (mg g}^{-1}\text{ d}^{-1}\text{)} = \left(\frac{\text{mg consumed}}{\text{average body mass} \times \text{time}}\right)$$

Data was tested for homogeneity of variances and ln-transformed whenever required. Two-way factorial ANOVA was used to test for the effect of the origin of food and the origin of water on survival and consumption rate of the macroinvertebrates and on mass loss of the leaf discs. All statistical tests were performed with the software STATISTICA 7.0 with the level of significance set at $\alpha=0.05$.

3 RESULTS

3.1 SURVIVAL

Survival of the stoneflies Leuctridae in the first experiment ranged from 68% to 88% being highest with urban stream water and control food and lowest in the urban treatment (***Figure 4 A***). In the second experiment, survival ranged from 56% to 84% being highest with control water and urban food and lowest in the control treatment (***Figure 4 B***).

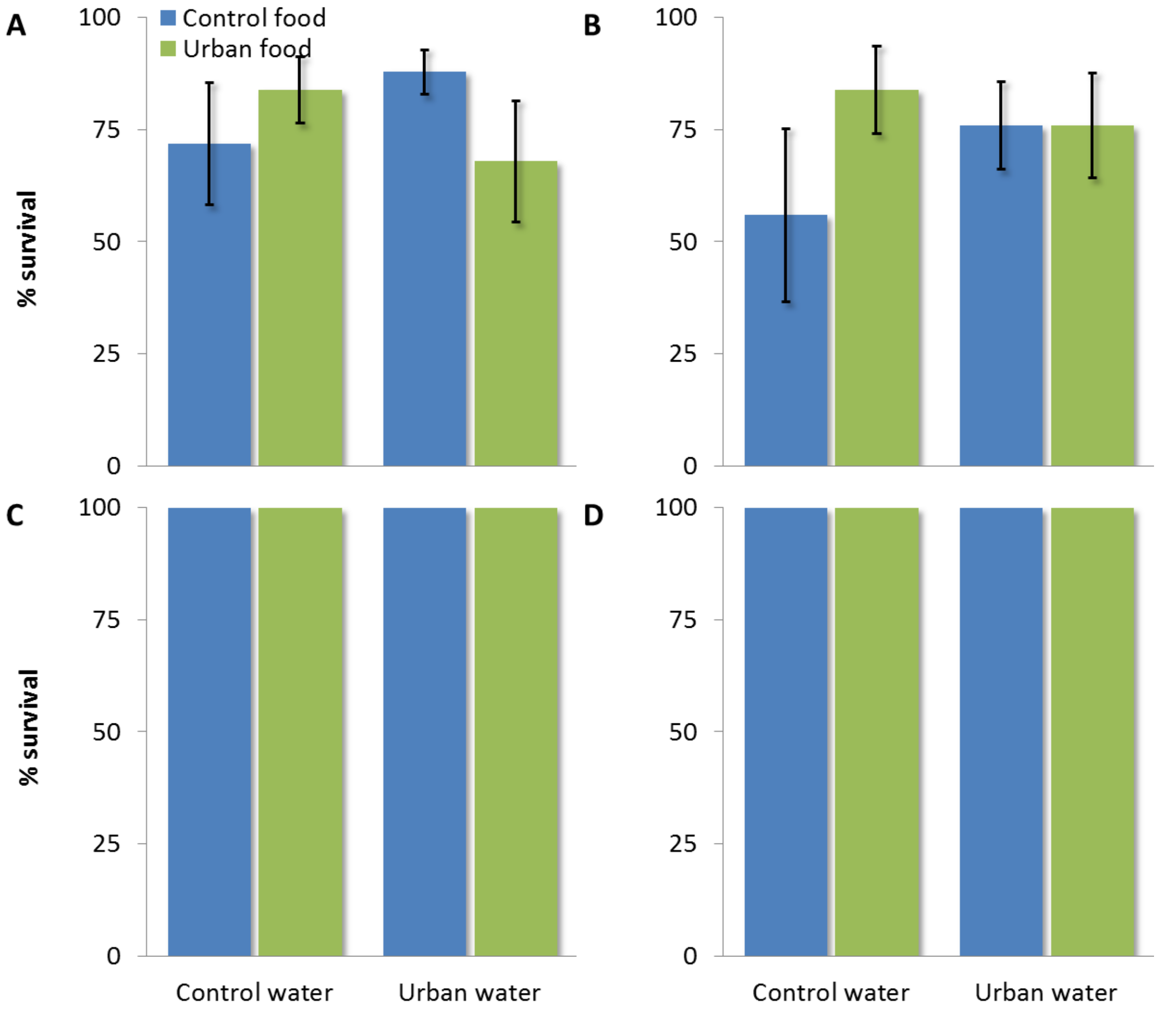

Figure 4 Percentage survival of the Plecoptera Leuctridae (A, B), the Trichoptera Sericostomatidae (C) and the Diptera Tipulidae (D) during seven days in the four treatments (mean ± SE; n=5).

There was a high within-treatment variation and no significant effect of water or food origin on survival and no significant interaction between the factors on experiment 1 (2-way ANOVA: respectively $F_{0.05,1,16}$=0.14, P=0.7; $F_{0.05,1,16}$=0.0, P=1.0; $F_{0.05,1,16}$=2.29, P=0.15) or experiment 2 (2-way ANOVA: respectively $F_{0.05,1,16}$=2.75, P=0.12; $F_{0.05,1,16}$=3.60, P=0.08;

$F_{0.05,1,16}$=0.14, P=0.7). All the caddisfly Sericostomatidade and the crane fly Tipulidae survived the seven days of the experiment (***Figure 4 C, D***).

3.2 CONSUMPTION RATES

Consumption rates of the stoneflies Leuctridae ranged from 0.038 mg g^{-1} d^{-1} to 0.085 mg g^{-1} d^{-1} in the first experiment being highest in the urban and lowest in the control treatment (***Figure 5 A***). Consumption rates in the second experiment ranged from 0.047 mg g^{-1} d^{-1} to 0.084 mg g^{-1} d^{-1} being highest with urban water and control food and lowest with control water and urban food (***Figure 5 B***). There was no significant effect of water and no interaction between the factors (2-way ANOVA: respectively $F_{0.05,1,16}$=4.40, P=0.05; $F_{0.05,1,16}$=1.98, P=0.18) but there was a significant effect of food origin $F_{0.05,1,16}$=5.41, P=0.03 with highest consumption of food incubated in the urban stream. In the second experiment consumption rates were highest in urban water with control food and lowest in control water with urban food. There were no significant effects of water or food origin and no interaction between the factors on consumption rates in experiment 2 (2-way ANOVA: respectively $F_{0.05,1,16}$=2.63, P=0.12; $F_{0.05,1,16}$=2.53, P=0.13; $F_{0.05,1,16}$=0.01, P=0.94).

Consumption rates of the caddisflies Sericostomatidade ranged from 0.060 mg g^{-1} d^{-1} to 0.128 mg g^{-1} d^{-1}, being highest in the urban and lowest in the control treatment (***Figure 5 C***). There was a significant effect of water (2-way ANOVA: $F_{0.05,1,16}$=13.85, P=0.002) with consumption rates highest in urban water from. There was no significant effect of food and no interaction between the factors on consumption rates of the caddisflies (2-way ANOVA: $F_{0.05,1,16}$=1.56, P=0.23; $F_{0.05,1,16}$=0.00, P=0.97, respectively).

Consumption rates of the crane flies Tipulidae ranged from 0.004 mg g^{-1} d^{-1} to 0.007 mg g^{-1} d^{-1}, being highest with control water and urban food and lowest in the control (***Figure 5 D***). There were no significant effects of water or food origin and no interaction between the factors on consumption rates (2-way ANOVA: respectively $F_{0.05,1,16}$=0.00, P=0.95; $F_{0.05,1,16}$=0.17, P=0.69; $F_{0.05,1,16}$=0.74, P=0.40).

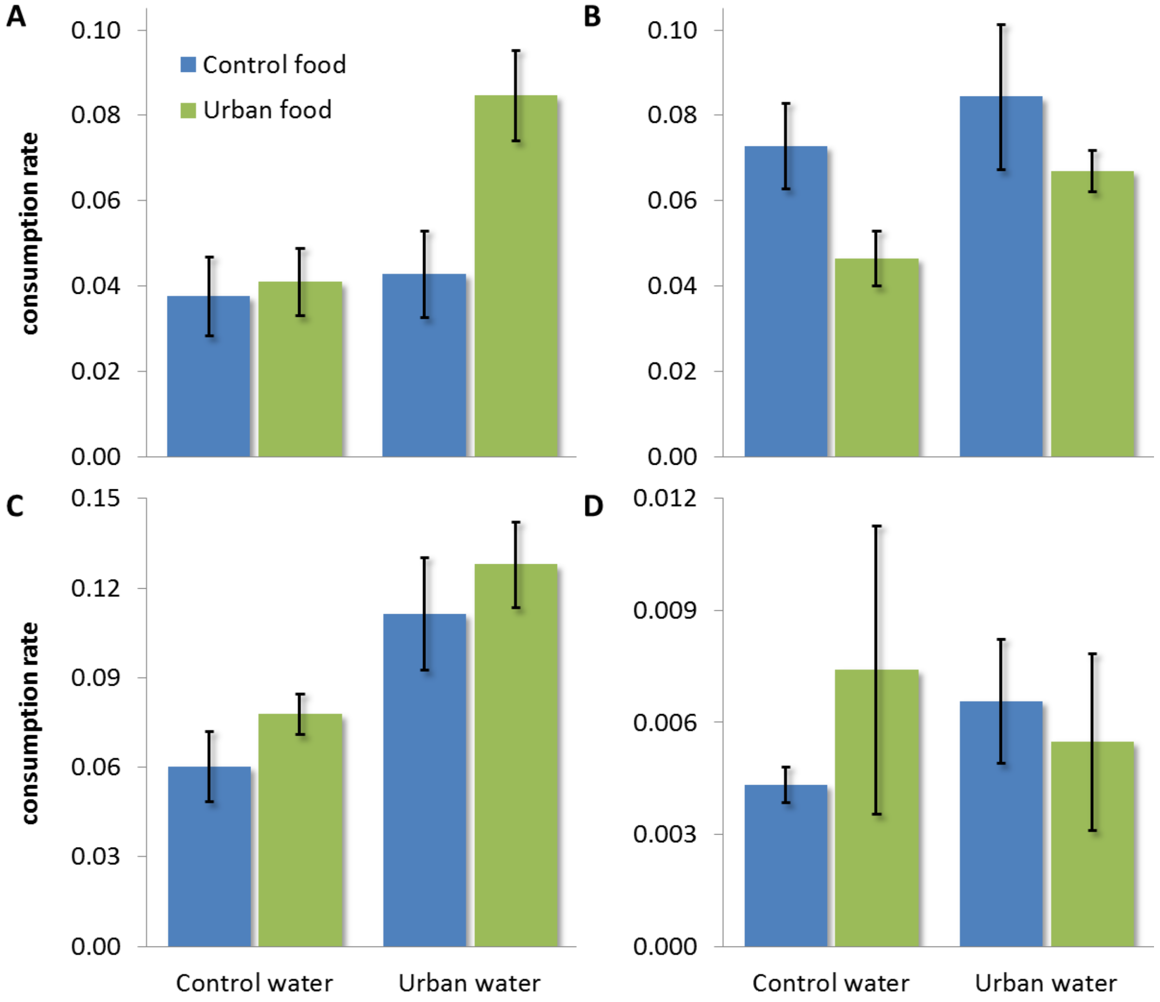

Figure 5 Consumption rates of the Plecoptera Leuctridae (A, B), the Trichoptera Sericostomatidae (C) and the Diptera Tipulidae (D) during seven days in the four treatments (mean ± SE; n=5).

3.3 LEAF MASS LOSS

Leaf mass loss by the Plecoptera Leuctridae in the first experiment ranged from 7% with highest values in the urban and lowest values in the control treatment (***Figure 6 A***). Leaf mass loss by the Plecoptera Leuctridae in the second experiment ranged from 13% to 16% with highest values in the treatment with urban water and control food and lowest values in the treatment with control water and urban food (***Figure 6 B***). There was no significant effect of water or food origin on percentage on leaf mass loss and no interaction between the factors on experiment 1 (2-way ANOVA: $F_{0.05,1,16}$=2.75, P=0.12; $F_{0.05,1,16}$=3.60, P=0.08; $F_{0.05,1,16}$=0.14, P=0.72, respectively) or experiment 2 (2-way ANOVA: $F_{0.05,1,16}$=1.00, P=0.33; $F_{0.05,1,16}$=1.47, P=0.24; $F_{0.05,1,16}$=1.21, P=0.29, respectively).

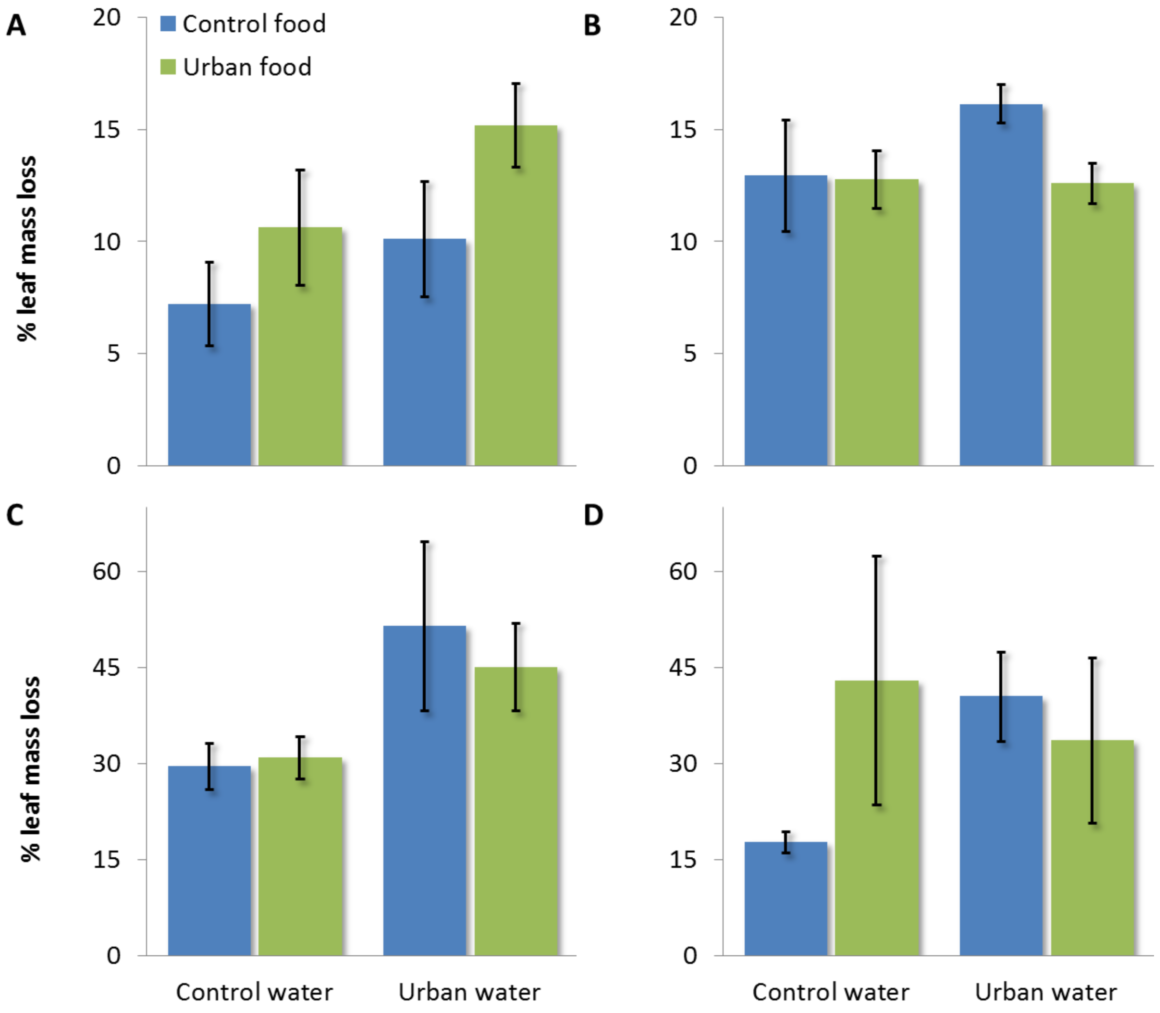

Figure 6 Percentage leaf mass loss promoted by the Plecoptera Leuctridae (A, B), the Trichoptera Sericostomatidae (C) and the Diptera Tipulidae (D) during seven days in the four treatments (mean ± SE; n=5).

Leaf mass loss by the Trichoptera Sericostomatidade ranged from 30% to 52%, with highest values in urban water with control food and lowest values in the control (***Figure 6 C***). There was a significant effect of water with highest leaf mass loss in urban water (2-way ANOVA: $F_{0.05,1,16}$=5.27, P=0,04). There was no significant effect of food and no interaction between the factors on leaf mass loss by the Sericostomatidae (2-way ANOVA: respectively $F_{0.05,1,16}$=0.00, P=0.97; $F_{0.05,1,16}$=0.08, P=0.78).

Leaf mass loss by the Diptera Tipulide ranged from 18% to 43% with highest values in control water with urban food and lowest values in the control (***Figure 6 D***). There were no significant effects of water or food origin and there was no significant interaction between the factors leaf mass loss by the Tipulidae (2-way ANOVA: respectively $F_{0.05,1,16}$=0.25, P=0.62; $F_{0.05,1,16}$=0.10, P=0.76; $F_{0.05,1,16}$=1.49, P=0.24).

4 DISCUSSION

Leaf mass loss may occur by macroinvertebrate ingestion but also by microbial and abiotic factors (Graça 2001). A comparison between leaf mass loss and consumption rate is advised to check if the leaf mass loss was all ingested by macroinvertebrates and not just lost by microbial and abiotic decomposition. There was a general agreement between consumption rates and mass loss; however, in the case of the Leuctridae in control water fed with urban food and the Sericostomatidae in urban water fed with control food, mass loss was higher than expected on basis of consumption rates. This may have been due to fragmentation into fine particles not weighed at the end of the experiment or to mass loss due to microbial breakdown.

Globally all species of studied macroinvertebrates, with the exception of the second experiment with the stoneflies, had a higher consumption rate of leaves incubated in the urban stream and/or kept in urban water, suggesting a possible compensatory feeding behavior to overcome poor-food quality. Compensatory feeding has been reported for several macroinvertebrates (Friberg & Jacobsen 1999; Graça et al. 2001; Albariño & Balseiro 2011). The caddisfly *Sericostoma vittatum* has been shown to increase consumption rate with lower quality food (Flores et al. 2014) in a pattern similar to the one observed in the present study. Although food quality was not actually assessed, the Diptera Tipulidae lost weight in all treatments except the control, where the body mass increased. Since the only differences between foods were related to the incubation water, probably microbial colonization (Suberkropp & Chauvet 1995) was lower in the urban stream thus constituting a lower quality food source for the macroinvertebrates.

The performance of macroinvertebrates showing a compensatory feeding behavior may depend on their relative mobility, with more sedentary species being able to circumvent the effects of low nutritional quality, while the more mobile species are not (Cruz-Rivera & Hay 2000). In the present study, all Trichoptera and all Tipulidae survived the experiments.

Although, of the tested *taxa* Tipulidae is the only present in the urban stream (Isidoro 2014) and the animals performed worst in treatments with urban water and/or with urban food. However, in the stream the animals have access to more diverse food sources while in the experiment only oak leaves were available. It is impossible to evaluate the same effect on the Trichoptera because body mass was assessed from the case opening width, but Flores et al. (2014) showed that the compensatory mechanism exhibited by *Sericostoma vittatum* was not enough to compensate growth in the case of lowest-quality food, which could explain the absence of this animals in the urban stream. On the other hand, Friberg and Jacobsen (1994) showed that the shredder *Sericostoma personatum* had almost the same consumption rate for two contrasting food types, suggesting feeding plasticity.

Regarding the Plecoptera, the two experiments showed different results. In the first experiment, consumption was highest for the urban stream food suggesting the existence of a compensatory feeding behavior. However, both the individuals who received water and food from the control stream and the ones who received water and food from the urban stream (despite the highest consumption) showed the lowest survival. Lieske & Zwick (2007) showed that the larvae of the stonefly *Nemurella pictetii* developed significantly faster when grown on their preferred (more suitable) food. In the present study the experimental containers did not have a net cover to prevent eventual emerged adults to fly away and several plecopterans were seen flying in the laboratory. If the results obtained by Lieske & Zwick (2007) that larval growth and maturation are related to available food qualities apply to the Leutridae, the animals fed the control food emerged as adults during the experiment while animals fed the urban stream food were not able to survive despite the compensatory feeding observed.

4.1.1 CONCLUSION

In conclusion, the tested macroinvertebrates seems to be affected by both water and food quality in Ribeira dos Covões. Being a peri-urban stream also affected by agriculture, nonpoint source pollution may be affecting water quality and thus the survival of the studied macroinvertebrates (Carpenter et al. 1998). The quality of food conditioned in the stream seems to be low probably due to low microbial colonization.

The bad performance exhibited by the macroinvertebrates in urban stream water as well as the compensatory feeding behavior exhibited by all studied taxa in urban stream water treatments confirms the first hypothesis that water quality could be the factor preventing the establishment of intolerant macroinvertebrates in Ribeira dos Covões.. The results obtained with the macroinvertebrates fed with urban stream food also confirms the second hypothesis that food quality prevents the establishment of intolerant shredders in the urban stream; this poor food quality seems to be related to stream water quality at the time of microbial colonization. . Since macroinvertebrates did not perform equally well at all treatments, habitat quality seems not to be the main factor preventing establishment in Ribeira dos Covões.

4.1.2 FURTHER WORK

Although the results of this study suggest that both water and food quality are responsible for the lack of intolerant macroinvertebrate *taxa* in Ribeira do Covões, in order to identify the specific causes involved, further investigation should be carried out in order to assess: (i) microbial colonization of leaves conditioned in both types of water and of leaves naturally occurring in the urban stream; (ii) chemistry and nutritional value of leaves incubated in both conditions; and (iii) to repeat the same experimental design in the field.

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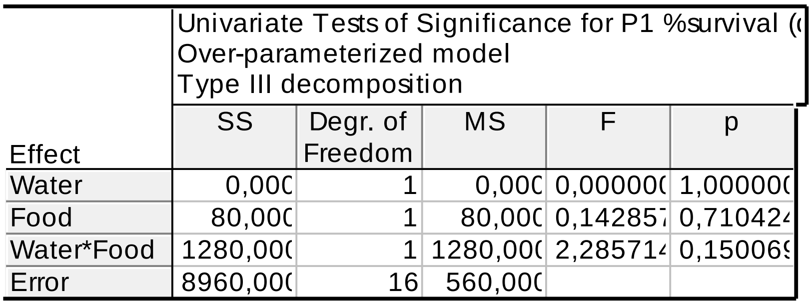
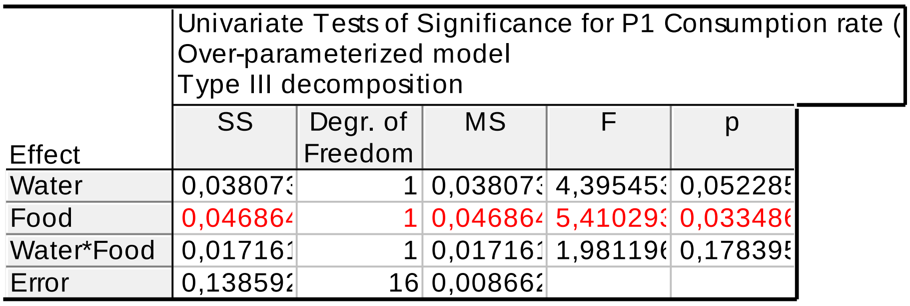
6 APPENDIX

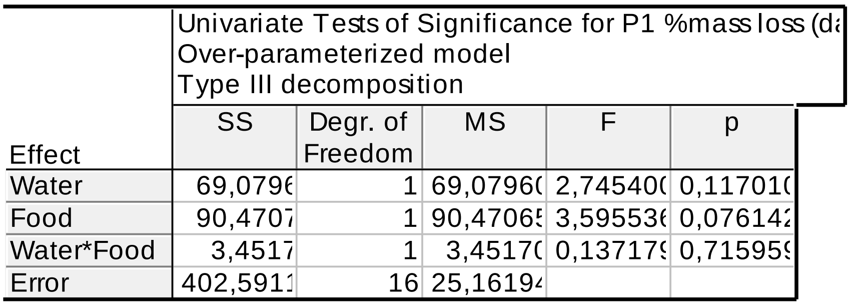
Appendix Table 1 Results of two-way ANOVA for survival, consumption rate, and leaf mass loss, respectively, in the first experiment with the Plecoptera *Leuctridae.*



	Univariate Tests of Significance for P1 %survival (Over-parameterized model Type III decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Water	0,000	1	0,000	0,000000	1,000000
Food	80,000	1	80,000	0,142857	0,710424
Water*Food	1280,000	1	1280,000	2,285714	0,150069
Error	8960,000	16	560,000		

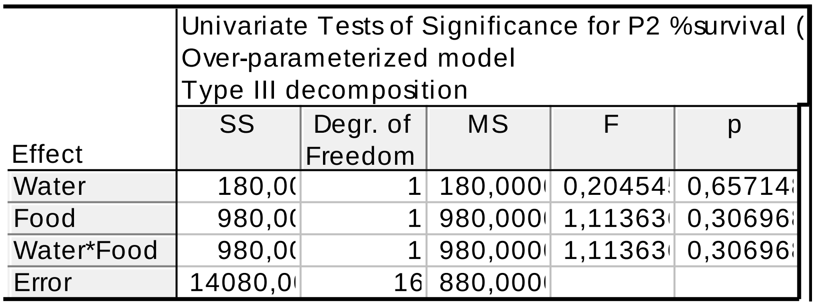


	Univariate Tests of Significance for P1 Consumption rate (Over-parameterized model Type III decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Water	0,038073	1	0,038073	4,395453	0,052285
Food	0,046864	1	0,046864	5,410293	0,033486
Water*Food	0,017161	1	0,017161	1,981196	0,178395
Error	0,138592	16	0,008662		

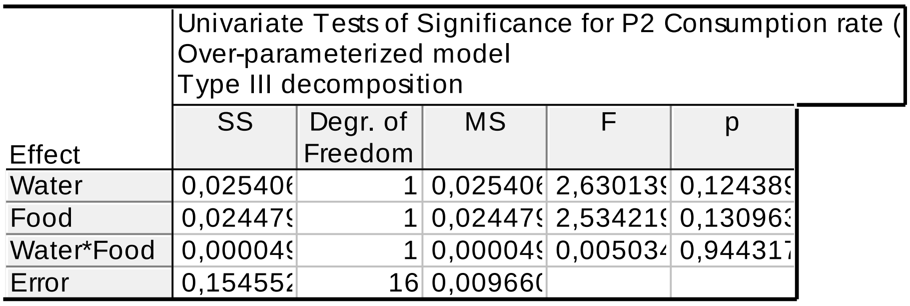


	Univariate Tests of Significance for P1 %mass loss (da Over-parameterized model Type III decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Water	69,0796	1	69,07960	2,745400	0,117010
Food	90,4707	1	90,47065	3,595536	0,076142
Water*Food	3,4517	1	3,45170	0,137179	0,715959
Error	402,5911	16	25,16194		

Appendix Table 2 Results of two-way ANOVA for survival, consumption rate, and leaf mass loss, respectively, in the second experiment with the Plecoptera *Leuctridae.*



	Univariate Tests of Significance for P2 %survival (Over-parameterized model Type III decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Water	180,000	1	180,000	0,204545	0,657143
Food	980,000	1	980,000	1,11363	0,306968
Water*Food	980,000	1	980,000	1,11363	0,306968
Error	14080,00	16	880,000		



	Univariate Tests of Significance for P2 Consumption rate (Over-parameterized model Type III decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Water	0,025406	1	0,025406	2,630139	0,124389
Food	0,024479	1	0,024479	2,534219	0,130963
Water*Food	0,000049	1	0,000049	0,005034	0,944317
Error	0,154552	16	0,009660		

Univariate Tests of Significance for P2 %mass loss (da
Over-parameterized model
Type III decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Water	11,6544	1	11,65443	0,995397	0,333277
Food	17,2364	1	17,23637	1,472147	0,242616
Water*Food	14,1191	1	14,11914	1,205907	0,288396
Error	187,3332	16	11,70832		

Appendix Table 3 Results of two-way ANOVA for consumption rate and leaf mass loss, respectively, in the experiment with the Trichoptera Sericostomatidae.

Univariate Tests of Significance for T Consumption rate (
Over-parameterized model
Type III decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Water	0,012855	1	0,012855	13,85266	0,001855
Food	0,001449	1	0,001449	1,56098	0,229490
Water*Food	0,000001	1	0,000001	0,00145	0,970047
Error	0,014848	16	0,000928		

Univariate Tests of Significance for ln(%ML) (data
Over-parameterized model
Type III decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Water	0,817132	1	0,817132	5,268174	0,035576
Food	0,000172	1	0,000172	0,001112	0,973813
Water*Food	0,012769	1	0,012769	0,082321	0,777858
Error	2,481716	16	0,155107		

Appendix Table 4 Results of two-way ANOVA for consumption rate and leaf mass loss, respectively, in the experiment with the Diptera Tipulidae.

Univariate Tests of Significance for Tip Consumption rate
Over-parameterized model
Type III decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Water	0,000000	1	0,000000	0,004263	0,948753
Food	0,000005	1	0,000005	0,168359	0,687016
Water*Food	0,000022	1	0,000022	0,739233	0,402606
Error	0,000469	16	0,000029		

Univariate Tests of Significance for ln(%ML) (data
Over-parameterized model
Type III decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Water	0,24720	1	0,247196	0,248606	0,624843
Food	0,09480	1	0,094803	0,095344	0,761475
Water*Food	1,48051	1	1,480514	1,488954	0,240059
Error	15,90930	16	0,994331		