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Effects of light intensity on leaf litter quality for the shredder *Sericostoma vittatum*

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Resumo

Os ribeiros florestados são altamente heterotróficos dependendo da matéria orgânica de origem terrestre para suportar as cadeias alimentares aquáticas. A cobertura ripícola, além de fornecer grande parte dessa matéria orgânica, determina o grau de insolação dos cursos de água reduzindo a quantidade de radiação solar que chega à superfície da água e criando uma distribuição desigual de zonas de luz (L) e de sombra (S). Ao longo da decomposição foliar, desenvolve-se um biofilme (camada composta maioritariamente por fungos, bactérias e algas embebidas numa matriz mucilaginosa) na superfície das folhas que vai enriquecer a matéria orgânica para os invertebrados. A luz pode afectar o desenvolvimento e a proporção dos componentes autotróficos presentes nesse biofilme (perifíton) condicionando a qualidade do alimento para os trituradores.

O principal objectivo deste estudo foi avaliar *in situ* os efeitos da intensidade da luz (L 35.653 µmol m^{-2} s^{-1} vs. S 0.281 µmol m^{-2} s^{-1}) na alimentação e *performance* do triturador endémico *Sericostoma vittatum*, através de alterações na qualidade da folhada. Adicionalmente, foi realizada uma experiência em microcosmos para testar a discriminação de folhas condicionadas à L ou S pelos invertebrados (mantidos num fotoperíodo de 12h luz : 12 escuro ou continuamente em condições de sombra). A folhada de carvalho (*Quercus robur*), condicionada durante três semanas nas condições de L e S, foi avaliada em termos de perda de massa e qualidade foliar: C, N, P, lenhina, dureza, biomassa fúngica, concentração de clorofila-*a* no biofilme assim como a sua biomassa. Estas folhas foram fornecidas semanalmente a oito grupos de 10 larvas cada, mantidos em jaulas de malha de 0.5 mm, nas zonas correspondentes de luz ou sombra do curso de água. O consumo e massa dos invertebrados foram avaliados a cada semana, ao longo de um mês.

A biomassa do biofilme e a qualidade (conteúdo em algas) assim como as características físico-químicas da folhada (N, P e dureza) foram significativamente

diferentes entre as folhas condicionadas no ambiente de L ou S; no entanto, a decomposição foliar não foi afectada pela intensidade da luz. As folhas expostas ao sol indicaram maior qualidade, foram preferidas pelos trituradores e permitiram uma sobrevivência mais alta. O consumo de folhas de S foi maior indicando um mecanismo de alimentação compensatório, dado que não houve diferenças nas taxas de crescimento relativo das larvas. De acordo com os resultados, os padrões de luz num curso de água podem afectar o comportamento de forrageamento e a aptidão dos trituradores através de alterações na qualidade do biofilme da folhada.

Cursos de água com coberturas vegetais distintas (em qualidade e quantidade) ou zonas diferencialmente ensolaradas poderão apresentar taxas de reciclagem de nutrientes diferentes; a quantidade de luz parece afectar a qualidade foliar (através da qualidade do seu biofilme) e o seu processamento pelos trituradores.

Os resultados demonstraram a importância e alguns dos efeitos da luz nos ecossistemas ribeirinhos. A gestão das áreas ripícolas deve considerar a disponibilidade de luz para o curso de água, visto que, alterações na cobertura vegetal poderão ter consequências ténues, mas significativas na dinâmica dos detritos em ribeiros.

Abstract

Forested streams are highly heterotrophic relying on organic matter from terrestrial origin to support the aquatic food webs. Riparian canopy, besides providing most of this organic matter, determine the insolation degree of the stream channel, reducing the amount of solar radiation that reaches the water surface and creating a patchy distribution of unshaded (U) and shaded (S) areas. In the course of leaves decomposition, a biofilm (a layer mainly composed of fungi, bacteria and algae embedded in a mucilaginous matrix) develops in leaf's surface enriching the organic matter to invertebrates. Sunlight may affect the development and autotroph proportion present in that biofilm determining food quality for shredders.

The main objective of this study was to evaluate *in situ* the effects of light intensity (U 35.653 µmol m^{-2} s^{-1} vs. S 0.281 µmol m^{-2} s^{-1}) on the endemic shredder *Sericostoma vittatum* feeding and performance through changes in leaf litter quality. Additionally, a microcosm experiment was conducted to test the selectivity of these invertebrates (kept under a 12h light : 12h dark photoperiod or continuous dark conditions) for unshaded or shaded conditioned leaves. Oak (*Quercus robur*) leaf litter conditioned for three weeks under U and S conditions were assessed in terms of mass loss and litter quality: C, N, P, lignin, toughness, fungal biomass, chlorophyll-*a* concentration in the biofilm and its biomass. These leaves were weekly provided to eight groups of 10 larvae, kept in 0.5 mm mesh cages, in correspondent unshaded or shaded areas of the watercourse. Consumption and invertebrates mass were evaluated each week for one month.

Biofilm biomass and quality (algae content) and leaf litter physic-chemical characteristics (N, P and toughness) significantly differed between leaves conditioned in U and S environment, although leaves decomposition was not affected by light intensity. Leaves conditioned in U area indicated higher quality food, were further preferred by shredders and allowed greater survival. Consumption was higher in S

leaves indicating a compensatory feeding as no differences were found in relative growth rate of the larvae. According to the results, light patterns in the stream channel may affect shredder foraging behaviour and fitness through changes in the quality of the leaf litter biofilm. Streams with distinct canopy cover (in quality and quantity) or areas differentially illuminated may have different nutrient recycling rates; the amount of sunlight seems to affect leaf litter quality (through quality of its biofilm) and processing by shredders.

Results showed evidence of the importance and some effects of sunlight in stream ecosystem. Riparian areas management should take in to account the light availability to the streambed since changes in canopy cover may have subtle but, significant consequences on detritus dynamics in streams.

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Chapter 1

Introduction

1. Introduction

1.1. General introduction

In our planet, most of the water is concentrated in oceans: only about 2.5% is freshwater. From this reduced fraction, only a small percentage (0.3%) is easily available as large part of it is frozen or trapped in glaciers (Shiklamanov 1993; Oki & Kanae 2006). Only 0.00014% of Earth's freshwater occurs in river channels (Shiklamanov 1993; Oki & Kanae 2006) main suppliers of water to men.

Freshwater ecosystems, namely lotic systems, represent a vital resource for the increasing human population providing goods and services as cleaning water, waste assimilation, recreation and economic productivity (e.g. fisheries). Nonetheless, water quality is threatened all over the world due to anthropogenic activities such as catchment disturbance, intensive agriculture, pollution, water resource management (dams and river fragmentation), over-exploitation, introduction of non-native species and destruction of habitats (Vörösmarty et al. 2010; Dudgeon et al. 2006). The impact of these stressors contributes to low biodiversity and undermines water security to humankind, which is increasing substantially (Malmqvist & Rundle 2002; Vörösmarty et al. 2010). Nevertheless, the human pressure can be mitigated if conservation and management strategies are applied so that threats to running waters are minimized (Malmqvist & Rundle 2002).

1.2. Low order streams

Low order streams are located at the head of the drainage network and constitute about 85% of the total length of a lotic system (Anderson & Sedell 1979). Their importance largely derives from their extension and upstream position in the fluvial *continuum*.

In temperate areas a large number of streams are lined by deciduous trees. The riparian cover constitutes the main supply of organic matter to the stream, particularly

in autumn. This allochthonous material constitutes an important source of energy (up to 99%) transformed by aquatic organisms and it is mainly composed of leaves but also flowers, fruits and branches (Fisher & Likens 1972). Riparian canopies limit light supply to the stream channel; shading results on low primary production although an heterogeneous pattern of light incidence may allow the development of algae frequently present in biofilms (Franken et al. 2005).

Once in the stream, leaves suffer a series of biochemical and physical transformations before being converted into living biomass by stream communities.

1.3. Leaf decomposition

Leaf litter breakdown is a key ecosystem level process in lotic system (Gessner et al. 1999). The breakdown of leaves occur in three sequential phases that can overlap in time: leaching, conditioning and fragmentation. The duration and extension of each phase depends on intrinsic properties of leaves (e.g. chemical characteristics and toughness) and environmental factors (e.g. temperature, pH, current, water chemistry). Among these, light is frequently neglected, but an important direct and indirect abiotic factor in leaf litter decomposition (Franken et al. 2005; Albariño et al. 2008; Fanta et al. 2010).

Leaching. This phase corresponds to a release of soluble inorganic and organic compounds such as phosphorus, potassium, carbohydrates, aminoacids, polyphenols (Suberkropp et al. 1976). This process usually lasts for 48h after leaves immersion, although it can occur throughout all litter decomposition, and cause a rapid decrease in leaf mass up to 42% (reviewed by Abelho 2001). Leaching quantity and quality is species specific, depends on leaf condition (e.g. leaf dryness, integrity) (Gessner 1991), and is largely influenced by stream characteristics as flow, temperature or nutrients in the water.

Conditioning. In this phase leaves are colonized by aquatic fungi (Aquatic Hyphomycetes) and bacteria that enhance leaf palatability to detritivores. The early stages of leaf degradation are dominated by fungi while bacteria involvement tends to increase over time (Bärlocher 2005; Hieber & Gessner 2002). Algae can also be present in this aggregate (Golladay & Sinsabaugh 1991). After a few weeks, depending on leaf species, most leaves reach the peak of fungal biomass being fully conditioned (Boling et al. 1975).

Leaf colonization determines an increase in fungal biomass and enzymes that allow degradation of recalcitrant material, induce changes in leaf texture (softening) and increase their content in nitrogen and phosphorous (Suberkropp & Klug 1980; Bärlocher & Brendelberger 2004). Invertebrates profit from the fungal biomass itself, from the increase of the leaf nutritional value and frequently from the fungal enzymes further used in their digestive processes (Canhoto & Graça 2008).

Fragmentation. Leaves are fragmented into smaller pieces by physical fragmentation/abrasion or biological activity. Physical fragmentation/abrasion may result from turbulence, discharge and floods; thus, leaf resistance can influence the susceptibility to degradation. Biological fragmentation is largely promoted by invertebrates, mainly shredders (i.e. functional feeding group that consume coarse particulate organic matter (CPOM; > 1 mm) (Cummins 1974)). This group convert CPOM into fine particulate organic matter (FPOM; 0.45 µm - 1 mm) and dissolved organic matter (DOM; < 0.45 µm) that will be available to other invertebrates (i.e. collectors) in the aquatic system. This feeding functional group may represent 20% of the total biomass present in stream (Cummins et al. 1989).

Shredders prefer and consume more conditioned leaf litter material being able to discriminate between conditioned and unconditioned leaves (e.g. Graça et al. 2001). When these invertebrates are allowed to choose between different leaf kinds they prefer and consume the item with higher nutritional value, i.e., softer leaves, richer in N and poor in secondary compounds such as phenolics. Subsequently, food preference

and consumption influence growth rate, survival, larval development and survivorship of shredders (Canhoto & Graça 2008).

1.4. Biofilms

Biofilms can be an import food source for stream invertebrates, as grazers (Allan 2007). They are organic layers that develop on submerged surfaces present in water (cobbles, stems or leaves) and it is largely composed by algae, bacteria, fungi and organic matter embedded in a mucilaginous matrix called glycocalyx (Golladay & Sinsabaugh 1991). Each group can respond differentially to variations in abiotic and biotic conditions (Fuller et al. 2007) such as discharge, temperature, nutrients and light. Nevertheless, the organic matter on which biofilm evolves can determine the extent of its development (Ardón & Pringle 2007).

The autotrophs (algae) and heterotrophic microbial communities (fungi and bacteria) present in biofilm can interact. In the course of algal photosynthesis, labile organic compounds (such as lipids, proteins and carbohydrates) are released and used by bacteria; heterotrophs activities provide to algae N and P (during mineralization) and CO_2 (during respiration) and, in turn, receive O_2 produced by algal photosynthesis (Cole 1982; Wetzel 1993). Algal exudates may promote the growth of fungi and bacteria (Franken et al. 2005).

Bacteria, fungi and algae produce extracellular enzymes that facilitate the uptake of dissolved organic matter and mineralization of organic N and P (Rier et al. 2007). Therefore communities that constitute the biofilm are also decomposers that play an essential role in the flow of nutrients to higher trophic levels and serve as important obligate (grazers) and/or additional (shredders) food source to macroinvertebrates in streams. Biofilms can also retain pollutants such as heavy metals and herbicides improving water quality (Sabater et al. 2002).

1.5. Light heterogeneity – Consequences in leaf biofilm development and subsequent food quality for shredders

Light is one of the most important environment factors regulating stream ecosystem structure and function (Kiffney et al. 2004). In order to understand this subject several studies have compared closed-canopy and open-canopy streams or investigated each individually by changing light availability (Kiffney et al. 2004; Li & Dudgeon 2008; Lagrue et al. 2011; Sturt et al. 2011).

In forested streams light is heterogeneous due to the canopy; it also varies daily and seasonally (annual cycle of leaf growth and abscission) limiting the amount of light reaching the water and thus restricts primary production. Some streams (e.g. some tropical areas) are so heavily shaded by riparian canopy that light penetrance almost does not occur (e.g. Larned & Santos, 2000).

In the course of leaves conditioning by fungi and bacteria, if the conditions are favourable, algae will develop and enrich epiphyton composition, a food source accessible to shredders which in turn can stimulate litter breakdown (Franken et al. 2005; Dangles 2002). Sunlight can affect autotroph/heterotroph proportions. Stream patches where light is incident will tend to develop biofilms with a high component of autotrophs; in contrast, in shaded areas, biofilm composition tend to exhibit a higher proportion of heterotrophic components.

Light has been referred as a factor able to accelerate litter breakdown in streams by photodegradation (Denward & Tranvik 1998) releasing organic matter that will stimulate decomposer activity. Rier et al. (2007) investigated the effects of light on decomposition rates of organic detritus. They concluded that in high-light conditions decomposition of leaf substrata was faster due to algal stimulation of extracellular enzymes, mainly phenol oxidase (POA) responsible for degradation of lignin and tannins. Consequently, decomposition rates may increase nutrient availability (N and P) enhancing heterotrophic pathways (Rier et al. 2007).

Light can also act as a stimulus to sporulation of aquatic hyphomycetes, although mycelial growth is higher in continuous darkness than in light (Rajashekhar & Kaveriappa 2000). Fungal growth can be diminished by sunlight possibly due to negative effects of UV radiation and probably competition between algae and fungi (Franken et al. 2005). But the former outcome in aquatic hyphomycetes is not consensual. Díaz Villanueva et al. (2010) found that neither UV radiation, PAR light (photosynthetically active radiation) nor shade effects affect growth or change litter decomposition rates, although sporulation rate increased in presence of light. These results may be attributable to the presence of photoprotective molecules (Díaz Villanueva et al. 2010).

Shredder feeding activities are also influenced by light/shadow patterns. Some shredders consume more in dark conditions (Feio & Graça 2000) and leaf litter biofilm composition seem to determine discriminative feeding behaviour, consumption (and though decomposition) and to affect invertebrates performance. Lagrue et al. (2011) found out that small changes in light supply to the stream have profound impacts in litter breakdown rates by means of lower contribution of shredders to leaf degradation due to altered quality food.

Shredders are capable to feed on algae (Leberfinger & Bohman 2010) and exploit this resource especially in open sites. This indicates that at least some species are able to choose and include it in their diet depending on the availability of food types (Leberfinger et al. 2011) and, eventually, stage of the life cycle. However, not all authors agree that shredders benefit from algae rich biofilms. For instance, Franken et al. (2005) found that increases in light intensity had positive effects on growth of shredders directly related with contribution of biofilm rich in algae. In contrast, Albariño et al. (2008) indicated that the shredder *Klapopteryx kuscheli*, although revealing an absence of preference for leaves incubated under shade or light conditions, presented

a higher fitness when fed leaves incubated under shade; this was probably due to the higher dependence of this species on fungal items as food source.

In forested streams allochthonous material is the main source of energy. Nevertheless, in moderately open canopies, shredders may benefit from autochthonous energy especially during spring and summer after the peak availability of well-conditioned leaf detritus (Cummins et al. 1989). The types and quantity of available energy may regulate secondary production, food webs and ultimately the dynamics of ecosystem (Hall et al. 2001).

Studies that address light issues have been increasing as it become a factor which proved to be of relevance in community dynamics and ecosystem functioning. Interest on the effects of light also emerged from the recognition that forestations, afforestation and replacement of riparian cover, with consequent changes in light availability to the stream channel, might affect stream diversity (impacts on community structure) and processes (e.g. metabolism and litter breakdown rates) (e.g. Abelho & Graça 1996; Riipinen et al. 2010).

1.6. Objectives

The main objective of this study was to evaluate *in situ* the effects of light intensity (i.e. unshaded vs. shaded environment) on the shredders *Sericostoma vittatum* Rambur (Tricoptera; Sericostomidae) feeding behaviour and performance through changes in leaf litter quality. This caddisfly is common and abundant in low order forested streams of the Iberian Peninsula and is an endemic species.

Additionally, we conducted a microcosm experiment to assess the effect of invertebrates habitat characteristics (i.e. light intensity) on their feeding behaviour; we tested whether invertebrates maintained in unshaded or shaded environments presented distinct preferences for leaves conditioned in either condition.

We hypothesized that (a) leaf litter conditioned in unshaded conditions will produce better quality food (richer in algae) compared to leaves conditioned in shaded conditions, (b) leaves conditioned in unshaded conditions will enhance shredders performance and that (c) *S. vittatum* larvae will be able to detect and discriminate items of higher food quality (leaves conditioned in unshaded conditions), in spite of their original habitat.

Chapter 2

Methods

2. Methods

2.1. Study area and water parameters

The study was carried out between April and June 2010, in a second order stream, Ribeira do Candal, Serra da Lousã (40º04'48.10''N, 8º12'11.16''W; 634 m a.s.l). The stream is lined by a mixed native riparian forest mainly composed of oak (*Quercus* sp.) and chestnut (*Castanea sativa* Mill.) trees. Water pH (Wissenschaftlich Technische Werkstätten 537), conductivity and temperature (WTW LF 92), dissolved oxygen (Oxi 3210 SET 1, WTW, Weilheim, Germany) and current velocity (Novalynx, 280-FP111 Water Flow Probe) were recorded, *in situ*, weekly (Table 1). Water samples were also taken on a week base, filtered through fiber glass filters (Millipore APFF04700, MA, USA, 47 mm Ø), and frozen at -20 ºC for later determination of cations and anions by ion chromatography (Dionex DX–120, Sunnyvale, Calif., USA). Soluble reactive phosphorus (SRP) was determined by the ascorbic acid method (APHA, 1995) and alkalinity by titration (APHA, 1995).

The study reach was about 21 m long and comprised an unshaded (U) and shaded (S) area. The S area were established artificially using dark PVC shade cloths (1.5 x 2 m) hanged about 40 cm above the stream line with the help of ropes and tied to the riparian trees and rocks. Light intensity in U and S near the water surface was measured with a luminometer (Delta OHM HD 9221). Light intensity of S area (0.281 ± 0.144 µmol m^{-2} s^{-1}) was about 99% lower than U (35.653 ± 2.814 µmol m^{-2} s^{-1}) (average ± SE).

2.2. Experimental set up

A total of 32 bags (fine mesh; 10 x 15 cm, 0.5 mm mesh) were filled weekly with approximately 1g of air dried senescent oak leaves (*Quercus robur* L.). Leaves were carefully disposed inside the bags minimizing overlap. The bags were divided in sets of 4 and immersed each week in the stream; two sets were fixed in the stream bed under

unshaded conditions and the other two under shaded conditions. Conditioning was allowed for 3 weeks. After this period, bags conditioned in U or S areas were used for the experiments with the invertebrates. Four additional sets of 4 bags were weekly placed in each stream U and S areas (8 + 8 bags for each) as controls for mass loss, chlorophyll evaluation, biofilm biomass and to assess leaf litter quality after the conditioning period (section 3).

2.3. Leaf litter

Physic-chemical parameters. Bags conditioned for 3 weeks in U (n=8) and S (n=8) areas of the stream were used to determine mass loss and leaf litter quality after each conditioning period. Bags from each treatment were retrieved from the stream, placed in individual zip lock bags, and transported to the laboratory in a cooler for immediate processing. Each sample was gently rinsed with distilled water. Leaf material contained in randomly chosen 4 bags per treatment was processed by punching out 3 leaf discs with a cork borer (12 mm Ø) which were frozen at -20ºC and further used for microbial determinations. The remaining material was oven-dried at 105ºC, for 24h, and reweighted to calculate remaining dry mass (DMr) after corrected for the disks that were retrieved for ergosterol. The material was then used for determination of carbon, nitrogen (IRMS Thermo Delta V advantage with a Flash EA 1112 series), lignin (Goering & Van Soest 1970) and phosphorus (Graça et al. 2005) (Table 2). Before drying the leaves leaf toughness was also determined by measuring the weight required to push a 1.55 mm diameter metal shaft through the wet leaves (3 per bag) while avoiding the veins, and mass units transformed into penetration pressure (kPa) = water mass (g) x gravity (m s^{-2}) / area of the rod penetrating the leaf (mm^2) (Graça et al. 2005).

The leaf material contained in the remaining set of bags, 4 from U and 4 from S conditions, was used for biofilm biomass and chlorophyll-*a* evaluation (see below).

Biofilm biomass and Chlorophyll-*a*. Biofilm of the leaves conditioned in U or S areas were gently scraped with a toothbrush into a tray. The suspended material was filtrated through ignited, pre-weighed fiberglass filters (Millipore APFF04700, Millipore, MA, USA). Half of the filters obtained were placed, individually, in Petri dishes, frozen at -18 ºC, then lyophilized and weighed to assess biofilm biomass per leaf surface area (mg cm^{-2}). The other half was used to determine chlorophyll-*a* concentration spectrophotometrically after extraction in 90% acetone (Jeffrey & Humphrey 1975; Gómes et al. 2009). The values of chorophyll-*a* were expressed in concentration of chlorophyll-*a* per leaf surface area (µg Chl-*a* cm^{-2}).

Ergosterol. The 3 leaf discs obtained from each replicate were used to determine ergosterol concentration as a measure of fungal biomass. The discs were lyophilized, weighed and ergosterol extracted according to Gonçalves et al. (2011). Ergosterol concentration was quantified by high performance liquid chromatography (HPLC) using a Merck LiChroCART 250-4 (LiChrospher 100) RP-18 column by measuring absorbance at 282 nm (Young 1995; Gessner & Schmitt 1996). Ergosterol was converted to fungal biomass using a conversion factor of 5.5 µg ergosterol mg^{-1} fungal dry mass (Gessner & Chauvet 1993). Results were expressed as mg fungal biomass per g DM (mg g^{-1} DM).

2.4. Invertebrate experiments

General. *Sericostoma vittatum* is a common endemic shredder species in low order streams of Central Portugal. In this study we collected a total of 150 individuals from Ribeira de S. João, Lousã (40°5′57.74” N, 8°14′02.55”W) for consumption and growth tests performed in the field and feeding preference trials conducted in the laboratory. All larvae were previously measured; individual dry mass (DM) was estimated by the expression DM = 0.0136 * CO – 0.0162 (R^2 = 0.83; $p < 0.001$; n = 35)

where DM is expressed in mg and case opening (CO) expressed in mm (Gonçalves et al. 2011).

Consumption and growth. In order to assess the effects of light intensity (U vs. S) on invertebrates consumption and growth we used a total of 80 individuals (11.443 ± 0.227 mg; average ± SE) distributed in U and S areas of Candal stream and feed them with 3 weeks leaves conditioned in the same conditions.

Invertebrates of similar mass were randomly distributed by 8 marked cages. The cage had a tetrahedral form (10 x 15 cm) and was made of 0.5 mm mesh allowing water flow inside but excluding other invertebrates. Each cage (i.e. replicate) contained a similar amount of local ashed stream sand (~ 55 g), 10 invertebrates of known mass and a known mass of leaf litter material previously conditioned in U or S conditions (see above). Cages were assembled in 2 groups of 4 and fixed to the stream in the correspondent U and S areas. In parallel, 4 cages per treatment, without animals, were also provided with U and S oak leaves and ashed stream sand. These control cages were distributed as above in order to assess mass loss not promoted by invertebrates feeding activities.

Animals were allowed to feed for a maximum period of one month. Each week the remains of the offered leaves from each cage were brought to the lab for mass loss evaluation and cages refilled with the correspondent U or S 3 weeks freshly conditioned oak leaves (see above). The groups of 10 invertebrates from each cage were also measured in the field, with the help of a stereo microscope, and immediately returned to the original cage.

Consumption was assessed as the difference between leaves initial mass and final mass after seven days and expressed as mg leaf consumed per mg of animal per day (mg mg^{-1} d^{-1}). The mean ratio of initial to final mass in bags of the same treatment (i.e. U and or S) without animals were used to correct changes in mass due to factors other than consumption.

Relative growth rate (RGR) was calculated as the mean larvae DM increment in each cage, after the total growth period (i.e. 2 weeks, following an acclimation period of 2 weeks), by mean initial DM and expressed per day (mg mg^{-1} d^{-1}) (Albariño et al. 2008). Animal survival was assessed each week.

Feeding preference. In order to evaluate the capacity of the invertebrates to choose between leaves conditioned in U and S conditions, a total of 55 larvae of *S. vittatum* (8.857 ± 0.192 mg; average ± SE), were acclimatized to laboratorial conditions (15 ºC; 12h light : 12h dark or 24h dark) and fed *ad libitum* with leaves randomly collected in the stream of origin. After this period, specimens were individually allocated in plastic cups (70 mm diameter x 85 mm high) filled with aerated stream water. The bottom of the containers was covered with a fine layer of ignited stream sand (550 ºC; 6h). Leaves, conditioned as above (i.e. U and S conditions), were used to cut pairs of discs symmetrically in relation to the main vein. Two leaf discs, one of each type (U or S) were marked with coloured pins and offered to each larva. The correspondent pair was attached to the edge of cup inside a 0.5 mm mesh bag as controls. Experiments run under a 12h light : 12h dark (n=32) or 24h dark (n=23) photoperiod, until at least one of the disc was half eaten in half the cups of one treatment, i.e., 2 days.

Discs were then retrieved, dried (105 ºC, 24h), and weighed (0.1 mg). Individual consumption was calculated as the difference between DM of each control (U or S) and DM of the correspondent disc (U or S) exposed to shredder. Results were expressed as mg leaf consumed per mg of animal per day (mg mg^{-1} d^{-1}) (Graça et al. 2005).

2.5. Statistical analysis

Leaf mass loss, leaf litter physic-chemical parameters (nitrogen, phosphorus, carbon and toughness), fungal and biofilm biomass and Chl-*a* concentration were analysed by two-way ANOVA with light intensity and time as categorical factors,

followed by the Tukey HSD test when necessary (Zar 1999). Lignin was compared between treatments by a t-test (in this case, was used a mixed sample of each treatment so time was not taken into account).

Invertebrate's consumption and relative growth rate (RGR) were compared between treatments with ANOVA repeated measures and t-test, respectively (Zar 1999). Animal survival was compared between treatments by two-way ANOVA with light intensity (U or S) and time as categorical variables. Consumption rates of U or S leaves in feeding preference trial was evaluated by a paired t-test. Data were transformed when necessary to achieve the assumptions, normality and equality of variances. All the statistical test were performed to a significance level of $p = 0.05$.

Chapter 3

Results

3. Results

3.1. Field conditions

During the study period, the stream reach had a mean discharge of 0.108 ± 0.023 $m^3 s^{-1}$ (average ± SE). Temperatures did not differ between U and S areas through time (11.5 ± 0.27 ºC). During the experimental time the water was well oxygenated, circum-neutral and oligotrophic (Table 1).

Table 1 Physic-chemical characteristics of the stream water during the experiment (average ± SE).

Parameter	
Temperature (ºC)	11.5 ± 0.27
Conductivity (µS/cm)	27.82 ± 0.63
O_2 (mg/L)	9.61 ± 0.19
NO_3 (mg/L)	0.09 ± 0.01
SRP (µg/L)	29.47 ± 8.76
TDS (mg/L)	30.20 ± 0.37
pH	6.99 ± 0.04
Alkalinity (mg $CaCO_3$/L)	4.73 ± 0.07

3.2. Leaf litter

After 3 weeks conditioning, leaves content in lignin (t-test; p = 0.459) and carbon (two-way ANOVA; $F_{1,24}$ = 4.080; p = 0.055) did not show differences between treatments; on the contrary nitrogen (two-way ANOVA; $F_{1,23}$ = 9.138; p = 0.006) and phosphorus (two-way ANOVA; $F_{1,24}$ = 10.822; p = 0.003) were significantly higher in U leaves (Table 2). No significant differences were found through time (p > 0.05).

Table 2 Lignin, phosphorus, nitrogen and carbon contents in leaves conditioned for 3 weeks in unshaded and shaded conditions (average ± SE). Two-way ANOVA; different letters indicate significant differences between treatments (p < 0.05).

	Lignin (% leaf DM)	P (% leaf DM)	N (% leaf DM)	C (% leaf DM)
Unshaded	37.314 ± 3.022 [a]	0.047 ± 0.004 [a]	1.257 ± 0.024 [a]	47.380 ± 0.193 [a]
Shaded	39.619 ± 0.418 [a]	0.034 ± 0.002 [b]	1.158 ± 0.022 [b]	46.861 ± 0.169 [a]

Biofilm biomass was higher in U (63.308 ± 1.842 mg cm^{-2}) than in S leaves (56.525 ± 2.359 mg cm^{-2}) (two-way ANOVA; $F_{1,15}$ = 11.134; p = 0.005) (Fig. 1). Light intensity had a significant positive effect in chlorophyll-*a* concentrations in the biofilm (two-way ANOVA; $F_{1,17}$ = 36.149; p<0.001). In U leaves chlorophyll-*a* was 2.4 times higher (0.294 ± 0.030 µg Chl-*a* cm^{-2}) than in S leaves (0.122 ± 0.011 µg Chl-*a* cm^{-2}) (Fig. 2).

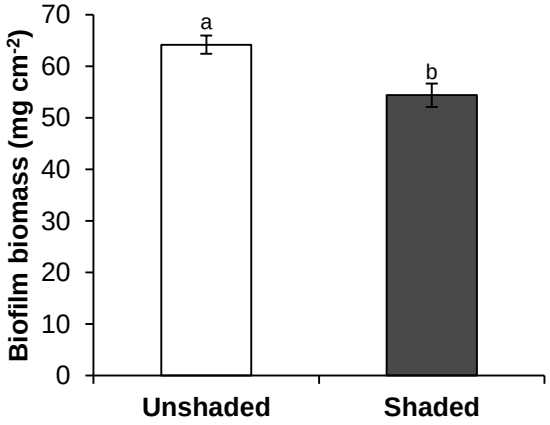

Fig. 1 Biomass of the biofilm (mg cm^{-2}) developed in leaves conditioned, for 3 weeks, in unshaded and shaded areas (average ± SE). Two-way ANOVA; different letters indicate significant differences between treatments (p < 0.05).

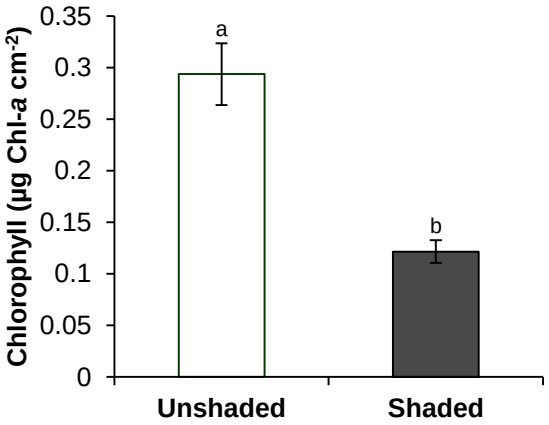

Fig. 2 Chlorophyll-*a* concentration (µg Chl-*a* cm^{-2}) in the biofilm of leaves conditioned, for 3 weeks, in unshaded and shaded areas (average ± SE). Two-way ANOVA; different letters indicate significant differences between treatments (p < 0.05).

Mass loss in the two conditioning areas were not different (two-way ANOVA; $F_{1,23}$ = 0.343; p = 0.564) (Fig. 3a) and varied between 26.35% and 27.50%. Similarly, leaves fungal biomass did not differ between treatments (two-way ANOVA; $F_{1,23}$ = 3.437; p = 0.077) (Fig. 3b). However, leaf toughness was higher in S leaves (two-way ANOVA; $F_{1,22}$ = 16.215; p < 0.001) (Fig. 3c). Time effect was not significant in any of these parameters (p > 0.05).

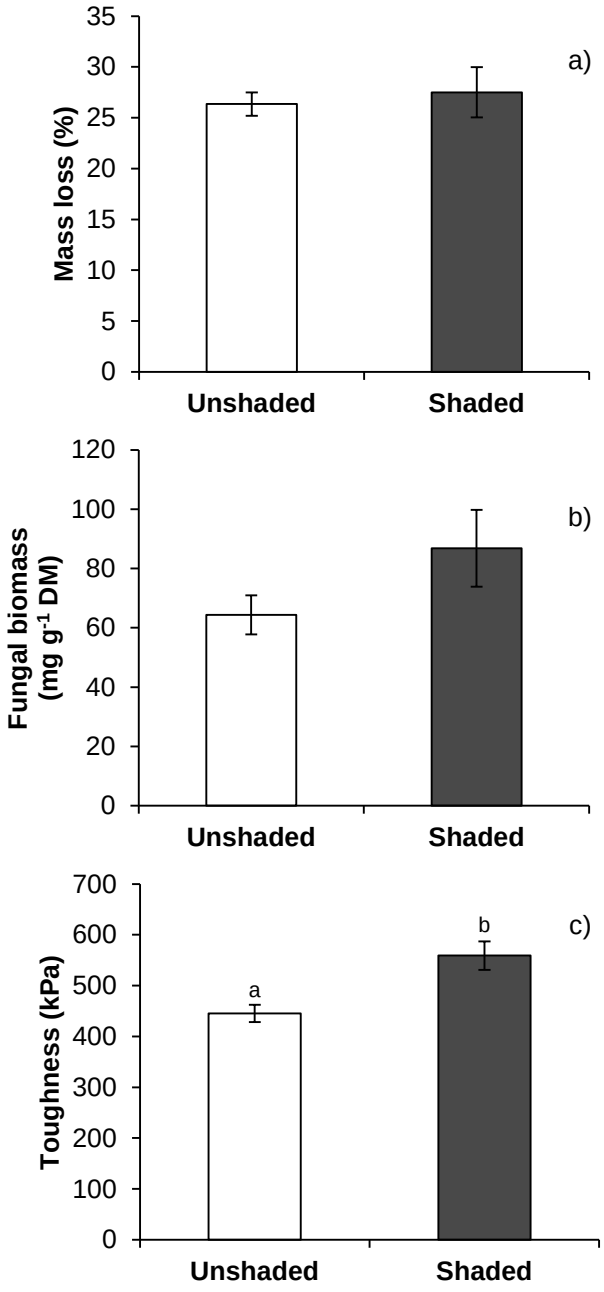

Fig. 3 a) Mass loss (%), b) fungal biomass (mg g^{-1} DM) and c) toughness (kPa) of oak leaves conditioned in unshaded and shaded conditions for 3 weeks (average ± SE). Two-way ANOVA; different letters indicate significant differences between treatments (p < 0.05).

3.3. Invertebrates

Invertebrates consumption was significantly different between treatments (ANOVA Repeated measures; $F_{4,3} = 16.067$; $p = 0.023$) (Fig. 4). Growth started after 2 weeks of acclimatization in which the invertebrates suffered a decrease in weight. RGR (Fig. 5), subsequent to T2, were not significantly different between treatments and varied between 11.594 mg (± 0,334 SE) (U) and 11.122 mg (± 0,350 SE) (S) (t-test; $p = 0.791$). Survival was significantly lower in shredders fed and maintained in the stream shaded area (two-way ANOVA; $F_{1,4} = 17.286$; $p < 0.001$) (Fig. 6).

In feeding preference tests, all invertebrates consistently chose and consumed more leaves conditioned at the U area (Fig. 7) independently of the photoperiod maintained during the experiment 12h light : 12 dark vs. 24h dark (paired t-test; $p = 0.014$ and $p = 0.027$, respectively).

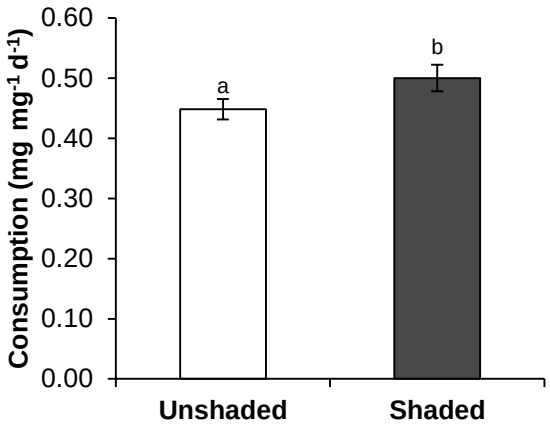

Fig. 4 Consumption rates by *S. vittatum* larvae kept in unshaded and shaded areas of the stream and fed leaves conditioned in the same conditions for 3 weeks (average ± SE). ANOVA Repeated measures; different letters indicate significant differences between treatments ($p < 0.05$).

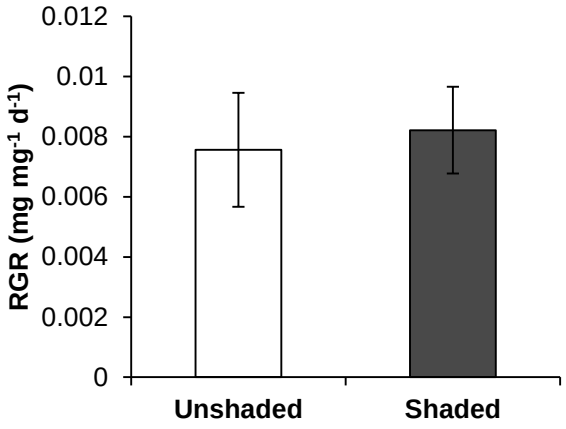

Fig. 5 Relative growth rates (RGR) of *S. vittatum* larvae kept in unshaded and shaded areas and fed leaves conditioned in correspondent areas, after 2 weeks of acclimatization (average ± SE). T-test; ($p < 0.05$).

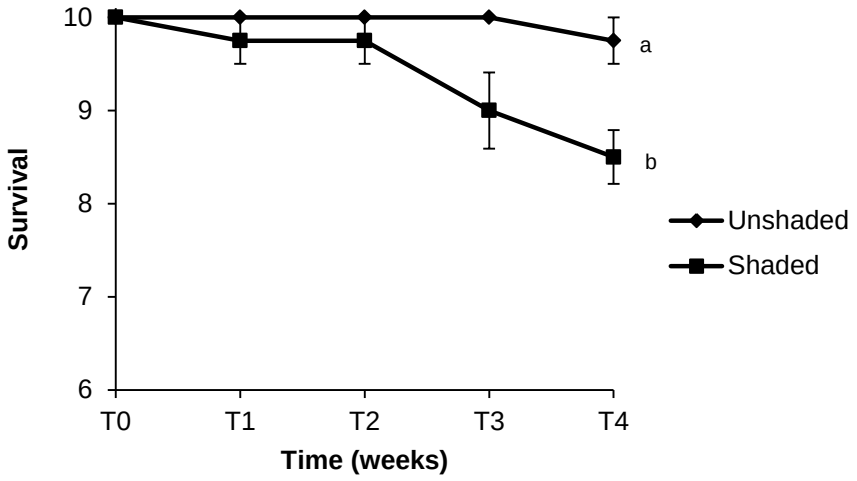

Fig. 6 Survival of *S. vittatum* larvae kept in cages (10 larvae/cage) in unshaded and shaded areas and provided leaves conditioned in similar areas, for a maximum period of 4 weeks (average ± SE). Two-way ANOVA; different letters indicate significant differences between treatments ($p < 0.05$).

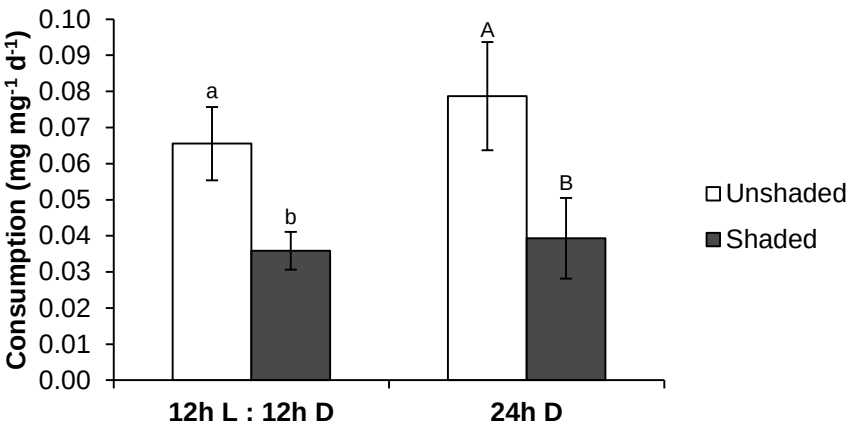

Fig. 7 Feeding preference of *S. vittatum* fed with oak leaf discs conditioned in unshaded or shaded stream areas for 3 weeks. Consumption was allowed in laboratorial conditions in 12 light : 12 dark (12h L: 12h D) or 24h dark (24h D) conditions, for 2 days (average ± SE). Paired t-test; different letters indicate significant differences between treatments ($p < 0.05$).

Chapter 4

Discussion and Conclusions

4. Discussion and Conclusions

4.1. Discussion

In this study, we explored two distinct light conditions that can occur in forested streams depending on the type (i.e. tree species) and density of the riparian canopy. A pattern of light/shadow is also characteristic of woodland streams where canopy cover is heterogeneous allowing some areas to receive sunlight while others may be continuously (or alternately) shaded. This is, as far as we know, the first study that assessed the effects of light intensity on invertebrate's performance *in situ.*

Leaves decomposition was not affected by light intensity although it seems that the quantity (biomass) and quality (algae content) of the biofilm, as well as the detritus physic-chemical characteristics (namely N, P and toughness), differed among leaves conditioned in unshaded and shaded areas. Such differences, although not translated in differences in RGR of *S. vittatum* larvae, could be detected by these shredders in feeding preference trials and affected consumption and survival of the invertebrates in the field.

Previous studies indicated that light intensity may affect leaf litter quality through effects on leaf's biofilm development (Hill et al. 2011), autotrophs proportion (Golladay & Sinsabaugh 1991; Franken et al. 2005; Albariño et al. 2008; Gjerløv & Richardson 2010) and also through the relationships established between biofilm components as fungi, bacteria and algae (Romaní et al. 2004; Rusanov et al. 2009). Our study corroborate these findings: biofilm biomass was higher in unshaded areas and leaves conditioned in these environment showed 2.4 times more chlorophyll-*a* content (and tough algae) than those conditioned in the shadow. It is generally accepted that chlorophyll-*a* determination is a reliable proxy of the algal content of the biofilm (Schiller et al. 2007). The light intensity values in our field experiment were not high by the fact that it was a forested stream, however the chlorophyll-*a* concentration in unshaded

areas where within the range of values previously found in similar studies in forested streams (Stephens et al. 2012)

Regardless of the difference among unshaded and shaded leaves and epiphyton quality, leaves mass loss and fungal biomass were not different between treatments which is in agreement with Díaz Villanueva et al. (2010) that found no differences in litter mass loss or fungal biomass, using different levels of solar radiation (direct exposition to sunlight radiation, protection from UV radiation and shade). It seems that the observed higher algal biomass (or algae activity (Romaní et al. 2004)) was not enough to increase the biofilm C availability to fungi stimulating mycelium growth (Rier et al. 2007; Hill et al. 2011). Moreover, in our study, a (non-significant) tendency for higher fungal biomass was even observed in leaves conditioned in shade conditions. This may be related with the fact that a higher mycelia growth usually occurs in dark conditions (Rajashekhar & Kaveriappa 2000). It seems possible that the stream oligotrophic environment and the recalcitrance of the oak leaves might have limited the mycelial growth in these conditions. The paramount importance of water nutrients and substratum in the quality of the periphyton has been largely stated (Sinsabaugh & Foreman 2001; Ardón & Pringle 2007). Discrepancies also exist in previous studies considering fungal biomass: some authors found a higher fungal biomass in leaves conditioned in shadow (Rajashekhar & Kaveriappa 2000; Albariño et al. 2008) while others had the opposite results, higher fungal biomass in non-shaded reaches (Lagrue et al. 2011).

In spite of the likely no effect of increased biofilm and algae biomass on leaves mass loss, it seems that the greater quality of the biofilm might have been responsible for the non-shaded leaves higher concentrations of N and P and softness. It is reasonable to assume that a higher uptake of nutrients, mainly from the oak leaves themselves, promoted by a more developed biofilm and a higher algae biomass, might also have stimulated fungal, and eventually bacterial (Rusanov et al. 2009), activities. Some studies indicate that light-exposed periphyton preferentially use substrata rather

than water as nutrient source (Schiller et al. 2007) and that biofilm heterotrophs may benefit from algal exudates (Burrell & Ledger 2003).

The biofilm is considered a valuable food source for stream invertebrates, namely grazers, as it is easily assimilated promoting a faster growth and maturation of some invertebrates (Lieske & Zwick 2007). In poor-quality food resource, as wood, biofilm is even the major source of energy to detritivores, especially after leaf detritus turn out to be scarce (Eggert & Wallace 2007). Algae in particular, important component in well-lit biofilms, is considered to have higher nutritional value to invertebrates (Leberfinger et al. 2011). These autotrophs comprise essential nutrients in high concentrations, reduced structural compounds (that are difficult to digest) and low concentration of phenols (which may restrain consumption and limit digestion). It is also the main provider of essential fatty acids to invertebrates frequently needed for the completion of their life cycles (Hill et al. 2011). In view of that, biofilms has been shown to constitute an alternative and/or additional food source to shredders (Dangles 2002; Franken et al. 2005).

In this study, shaded leaves were more consumed than unshaded leaves. It seems possible that this may be the result of a compensation mechanism for their lower quality: larvae need to consume more leaves conditioned in the shadow to offset the same energy and nutrients supplied by the more nutritious unshaded detritus. This compensation mechanism seems to have allowed the larvae to grow at similar rates in both treatments. Such feeding behaviour has been reported by several authors (Albariño & Balseiro 2001; Eggert & Wallace 2007; Campos & González 2009) including by Friberg & Jacobsen (1999) in a study with *S. personatum*, a species that occupies the same ecological niche as *S. vittatum*. They also concluded that larval consumption was more correlated with the nutrient value of food items than growth, due to the use of the same compensatory feeding strategy. However, Franken et al. (2005), also in a laboratory study, showed that changes in light availability had a

positive effect in growth of shredders *Asellus aquaticus* and *Gammarus pulex* through increases of algal content in leaf's biofilm. Such discrepancies may be justified by different species strategies or by several unknown factors as, in our case, the invertebrates were kept in variable field (vs. laboratory) conditions and under constant obligated unshaded or shaded regimes.

Larval survival was higher in shredders fed and maintained in unshaded conditions. It was interesting to notice that the differences occurred after the 2 weeks of acclimatization, when invertebrates started to grow and was accentuated along the experimental period, indicating that an extended single diet of leaves conditioned in the shadow (e.g. overshadowed streams) may affect the performance of these invertebrates. In forested streams with alternating light/shadow patches, *S. vitattum* may be capable of move between areas of different food quality including in their diet a variety of items which would allow compensating for lower nutritional food in shaded patches. Our short-term (4 weeks) study added knowledge about the effects of light intensity on leaf-biofilm quality and shredders (*S.vitattum*) feeding behaviour, but a prolonged field experiment would had given a wider understanding of the impacts in the entire life cycle of the species.

Invertebrates revealed a marked preference for unshaded higher quality leaves independently of the photoperiod to which they were subjected in the laboratory. Higher total leaf consumption (in particularly unshaded leaves, which were 50.1% more consumed than shaded leaves) in the 24h D trial most likely resulted from the fact that these larvae largely forage during the night (Feio & Graça 2000). The present results are consistent with other studies that showed that invertebrates usually select resources of higher quality (e.g. Friberg & Jacobsen 1994; Lieske & Zwick 2007). This may indicate that, even in heavily shaded streams, invertebrates recognise higher

quality leaves that may be produced in stream bed patches of light or be transported from other unshaded areas compensating the lower poor quality food produced locally.

Sericostoma vittatum feeding plasticity or generalist diet, also demonstrated by Carvalho & Graça (2007), may favour the presence of this species in streams with distinct riparian covers. On the other hand, a synchronization of the life cycle with the seasonal light patterns may support an increase of leaf quality through time, avoiding the observed mortality.

4.2. Conclusions

In conclusion, our results show that the light/shadow patterns in the stream channel can affect shredders fitness and foraging behaviour through changes in the quality of the leaf litter. Leaves conditioned in well-lit areas seem to produce a better food quality, as algae enriched the biofilm promoting changes in the physic-chemical characteristics of leaves. This biofilm response to light is likely more important in nutrient poor streams lined by recalcitrant leaves, as oak. In this case, the quality and quantity of biofilm may constitute an additional important food source for detritivores, increasing their performance.

Human activities such as clear cutting, logging and changes in canopy cover alter sunlight availability reaching the stream bed, changing primary production and consequently the stream invertebrate community (Mckie & Malmqvist 2009; Gjerløv & Richardson 2010). Streams with dense canopy cover or with heterogeneous distribution of light may have distinct and/or patchy nutrient recycling rates as organic matter will support biofilms with distinct qualities determining distinct behavioural responses by invertebrates. If the observed pattern is generalized, leaf litter processing and energy flow will be faster in the shadowed streams/areas. Whether this is quantitatively relevant or not, is still unknown. In any case, light limited streams by

dense canopies will potentially have higher organic matter inputs that may compensate higher feeding rates.

The success of stream management practices should consider the quality of the organic matter inputs, insolation possibilities of the riparian areas; effects may be subtle, but determinant of streams biodiversity and detritus dynamics.

Chapter 5

References

5. References

Abelho, M., 2001. From litterfall to breakdown in streams: a review. *TheScientificWorld*, 1, pp.656-680.

Abelho, M. & Graça, M., 1996. Effects of eucalyptus afforestation on leaf litter dynamics and macroinvertebrate community structure of streams in Central Portugal. *Hydrobiologia*, 324, pp.195-204.

Albariño, R. & Balseiro, E., 2001. Food quality, larval consumption, and growth of *Klapopteryx kuscheli* (Plecoptera: Austroperlidae) from a South Andes stream. *Journal of Freshwater Ecology*, 16(4), pp.517-526.

Albariño, R., Villanueva, V.D. & Canhoto, C., 2008. The effect of sunlight on leaf litter quality reduces growth of the shredder *Klapopteryx kuscheli*. *Freshwater Biology*, 53(9), pp.1881-1889.

Allan, J., Castillo, M., 2007. *Stream Ecology: Structure and Function of Running Waters.* 2nd edition. Springer, The Netherlands.

Anderson, N.H. & Sedell, J.R., 1979. Detritus processing by macroinvertebrates in stream ecosystems. *Annual Review of Entomology*, 24, pp.351-377.

APHA, 1995. Standard methods for the examination of water and wastewater. 19th edition. American Public Health Association. Washington, DC.

Ardón, M. & Pringle, C.M., 2007. The quality of organic matter mediates the response of heterotrophic biofilms to phosphorus enrichment of the water column and substratum. *Freshwater Biology*, 52, pp.1762-1772.

Bärlocher, F., 2005. Freshwater fungal communities. In Deighton, J., White, Jr. J. F. & Oudemans, P. eds. *The fungal community: its organization and role in ecosystem.* Taylor & Francis, CRC Press, pp. 39-60.

Bärlocher, F. & Brendelberger, H., 2004. Clearance of aquatic hyphomycete spores by a benthic suspension feeder. *Limnology and Oceanography*, 49(6), pp.2292-2296.

Boling, R.H., Googman, E. D., Van Sickle, J. A., Zimmer, J. O., Cummins, K. W., Peterson, R. C., Reice, S. R., 1975. Toward a model of detritus processing in a woodland stream. *Ecology*, 56, pp.141-151.

Burrell, G.P. & Ledger, M.E., 2003. Growth of a stream-dwelling caddisfly (*Olinga feredayi*: Conoesucidae) on surface and hyporheic food resources. *Journal of the North American Benthological Society*, 22(1), pp.92-104.

Campos, J. & González, J.M., 2009. *Sericostoma vittatum* (Trichoptera) larvae are able to use pine litter as an energy source. *International Review of Hydrobiology*, 94(4), pp.472-483.

Canhoto, C. & Graça, M., 2008. Interactions between fungi and stream invertebrates: back to the future. In K. R. Sridhar, F. Bärlocher, & K. D. Hyde, eds. *Novel Techniques and Ideas in Mycology, Fungal Diversity Research Series.* pp. 305-325.

Carvalho, E. & Graça, M., 2007. A laboratory study on feeding plasticity of the shredder *Sericostoma vittatum* Rambur (Sericostomatidae). *Hydrobiologia*, 575, pp.353-359.

Cole, J.J., 1982. Interactions between bacteria and algae in aquatic ecosystems. *Annual Review of Ecology and Systematics*, 13, pp.291-314.

Cummins, K.W.,Wilzbach, M. A., Gates, D. M., Perry, J. B., Taliaferro, W. B., 1989. Riparian and shredders vegetation - leaf litter that falls into streams influences communities of stream invertebrates. *BioScience*, 39(1), pp.24-30.

Cummins, K.W., 1974. Structure and Function of Stream Ecosystems. *BioScienc*e, 24(11), pp.63-640.

Dangles, O., 2002. Functional plasticity of benthic macroinvertebrates : implications for trophic dynamics in acid streams. *Canadian Journal of Fisheries and Aquatic Sciences*, 59, pp.1563-1573.

Denward, C.M.T. & Tranvik, L.J., 1998. Effects of solar radiation on aquatic macrophyte decomposition. *Oikos*, 82, pp.51-58.

Dudgeon, D. Arthington, A. H., Gessner, M. O., Kawabata, Z-I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A-H., Soto, D., Stiassny, M. L. J., Sullivan, C. A. 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological reviews of the Cambridge Philosophical Society*, 81(2), pp.163-82.

Díaz Villanueva, V., Albariño, R. & Graça, M., 2010. Natural UVR does not affect decomposition by aquatic hyphomycetes. *International Review of Hydrobiology*, 95(1), pp.1-11.

Eggert, S.L. & Wallace, J.B., 2007. Wood biofilm as a food resource for stream detritivores. *Limnology and Oceanography*, 52(3), pp.1239-1245.

Fanta, S.E. Hill, W. R., Smith, T. B., Roberts, B. J., 2010. Applying the light : nutrient hypothesis to stream periphyton. *Freshwater Biology*, 55(5), pp.931-940.

Feio, M.J. & Graça, M., 2000. Food consumption by the larvae of *Sericostoma vittatum* (Trichoptera), an endemic species from the Iberian Peninsula. *Hydrobiologia*, 439, pp.7-11.

Fisher, S.G. & Likens, G. E., 1972. Stream ecosystems: organic energy budget. *BioScience*, 22, pp.33-35.

Franken, R.J.M. Waluto, B., Peeters, E. T. H. M., Gardeniers, J. J. P., Beijer, J. A. J., Scheffer, M., 2005. Growth of shredders on leaf litter biofilms: the effect of light intensity. *Freshwater Biology*, 50(3), pp.459-466.

Friberg, N. & Jacobsen, D., 1994. Feeding plasticity of two detritivore-shredders. *Freshwater Biology*, 32, pp.133-142.

Friberg, N. & Jacobsen, D., 1999. Variation in growth of the detritivore-shredder *Sericostoma personatum* (Trichoptera). *Freshwater Biology*, 42, pp.625-636.

Fuller, R.L. LaFave, C., Anastasi, M., Molina, J., Salcedo, H., Ward, S., 2007. The role of canopy cover on the recovery of periphyton and macroinvertebrate communities after a month-long flood. *Hydrobiologia*, 598(1), pp.47-57.

Gessner, M. O. & Chauvet, E., 1993. Ergosterol to biomass conversion factors for aquatic hyphomycetes. *Applied and Environmental Microbiology*, 59, pp.502-507.

Gessner, M. O. & Schmitt, A.L., 1996. Use of solid-phase extraction to determine ergosterol concentrations in plant tissue colonized by fungi. *Applied and Environmental Microbiology*, 62, pp.415-419.

Gessner, M. O., 1991. Differences in processing dynamics of fresh and dried leaf litter in a stream ecosystem. *Freshwater Biology*, 26, pp.387-398.

Gessner, M. O., Chauvet, E. & Dobson, M., 1999. A perspective on leaf litter breakdown in streams. *Oikos*, 85(2), pp.377-384.

Gjerløv, C. & Richardson, J.S., 2010. Experimental increases and reductions of light to streams: effects on periphyton and macroinvertebrate assemblages in a coniferous forest landscape. *Hydrobiologia*, 652, pp.195-206.

Goering, H.K. & Van Soest, P.J., 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). *In Agricultural Handbook 379*. Washington DC: US Department of Agriculture, pp. 1-20.

Golladay, S.W. & Sinsabaugh, R.L., 1991. Biofilm development on leaf and wood surfaces in a boreal river. *Freshwater Biology*, 25, pp.437-450.

Gómes, N., Donato, J. C., Giorgi, A., Guasch, H., Mateo, P., Sabater, S., 2009. La biota de los ríos: los microorganismos autótrofos. In A. Elosegi & S. Sabater, eds. *Conceptos y técnicas en ecología fluvial.* España: Fundacion BBVA, pp. 234-236.

Gonçalves, A.L., Lírio, A. V., Pratas, J., Canhoto, C., 2011. Uranium contaminated water does not affect microbial activity but decreases feeding by the shredder *Sericostoma vittatum*. *Fundamental and Applied Limnology / Archiv für Hydrobiologie*, 179(1), pp.17-25.

Graça, M. Cressa, C., Gessner, M. O., Feio, M. J., Callies, K. A., Barrios, C., 2001. Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. *Freshwater Biology*, 46, pp.947-957.

Graça, M., Barlocher, F. & Gessner, M. O., 2005. *Methods to study litter decomposition - A pratical guide.* Springer The Netherlands.

Hall, R.O., Likens, Gene E. & Malcom, H.M., 2001. Trophic basis of invertebrate production in 2 streams at the Hubbard Brook Experimental Forest. *Journal of the North American Benthological Society*, 20(3), pp.432-447.

Hieber, M. & Gessner, Mark O., 2002. Contribution of stream detrivores, fungi, and bacteria to leaf breakdown based on biomass estimates. *Ecology*, 83(4), pp.1026-1038.

Hill, W.R., Rinchard, J. & Czesny, S., 2011. Light, nutrients and the fatty acid composition of stream periphyton. *Freshwater Biology*, 56, pp.1825-1836.

Jeffrey, S.W. & Humphrey, G.F., 1975. New spectophotometric equations for determining chlorophylls a, b and c in higher plants, algae and natural phytoplankton. *Biochemic und Physiologie der Pflazen*, 167, pp.191-194.

Kiffney, P.M., Richardson, J.S. & Bull, J.P., 2004. Establishing light as a causal mechanism structuring stream communities in response to experimental manipulation of riparian buffer width. *Journal of the North American Benthological* Society, 23(3), pp.542-555.

Lagrue, C. Kominoski, J. S., Danger, M., Baudoin, J-M., Lamothe, S., Lambrigot, D., Lecerf, A., 2011. Experimental shading alters leaf litter breakdown in streams of contrasting riparian canopy cover. *Freshwater Biology*, 56, pp.2059-2069.

Larned, S.T. & Santos, S.R., 2000. Light and nutrient-limited periphyton in low order streams of Oahu, Hawaii. *Hydrobiologia*, 432, pp.101-111.

Leberfinger, K. & Bohman, I., 2010. Grass, mosses, algae, or leaves? Food preference among shredders from open-canopy streams. *Aquatic Ecology*, 44(1), pp.195-203.

Leberfinger, K., Bohman, I. & Herrmann, J., 2011. The importance of terrestrial resource subsidies for shredders in open-canopy streams revealed by stable isotope analysis. *Freshwater Biology*, 56, pp.470-480.

Li, A.O.Y. & Dudgeon, D., 2008. Food resources of shredders and other benthic macroinvertebrates in relation to shading conditions in tropical Hong Kong streams. *Freshwater Biology*, 53, pp.2011-2025.

Lieske, R. & Zwick, P., 2007. Food preference, growth and maturation of *Nemurella pictetii* (Plecoptera: Nemouridae). *Freshwater Biology*, 52, pp.1187-1197.

Malmqvist, B. & Rundle, S., 2002. Threats to the running water ecosystems of the world. *Environmental Conservation*, 29(2), pp.134-153.

Mckie, B.G. & Malmqvist, B., 2009. Assessing ecosystem functioning in streams affected by forest management: increased leaf decomposition occurs without changes to the composition of benthic assemblages. *Freshwater Biology*, 54, pp.2086-2100.

Oki, T. & Kanae, S., 2006. Global hydrological cycles and world water resources. *Science*, 313, pp.1068-1072.

Rajashekhar, M. & Kaveriappa, K.M., 2000. Effects of temperature and light on growth and sporulation of aquatic hyphomycetes. *Hydrobiologia*, 441, pp.149-153.

Rier, S.T., Kuehn, K. A. & Francoeur, S.N., 2007. Algal regulation of extracellular enzyme activity in stream microbial communities associated with inert substrata and detritus. *Journal of the North American Benthological Society*, 26(3), pp.439-449.

Riipinen, M.P. Fleituch, T., Hladyz, S., Woodward, G., Giller, P., Dobson, M., 2010. Invertebrate community structure and ecosystem functioning in European conifer plantation streams. *Freshwater Biology*, 55, pp.346-359.

Romaní, M. uasch, H., Muñoz, I., Ruana, J., Vilalta, E., Schwartz, T., Emtiazi, F., 2004. Biofilm structure and function and possible implications for riverine DOC dynamics. *Microbial Ecology*, 47(4), pp.316-328.

Rusanov, A.G., Grossart, H-P. & Pusch, M.T., 2009. Periphytic bacterial and algal response to a hydraulic gradient under different light levels: Test of algal-bacterial coupling in the laboratory. *Fundamental and Applied Limnology / Archiv für Hydrobiologie*, 175(4), pp.339-353.

Sabater, S. Guasch, H., Roman, A., Muñoz., 2002. The effect of biological factors on the efficiency of river biofilms in improving water quality. *Hydrobiologia*, 469, pp.149-156.

Schiller, D.V. Martí, E., Riera, J. L., Sabater, F., 2007. Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. *Freshwater Biology*, 52, pp.891-906.

Shiklamanov, I.A., 1993. *Water in Crisis: A guide to the world's fresh water resources.* Oxford University Press, New York, pp.13-24.

Sinsabaugh, R.L. & Foreman, C.M., 2001. Activity profiles of bacterioplankton in a eutrophic river. *Freshwater Biology*, 46, pp.1239-1249.

Stephens, S.H., Brasher, A.M.D. & Smith, C.M., 2012. Response of an algal assemblage to nutrient enrichment and shading in a Hawaiian stream. *Hydrobiologia*, 683, pp.135-150.

Sturt, M.M., Jansen, M. a. K. & Harrison, S.S.C., 2011. Invertebrate grazing and riparian shade as controllers of nuisance algae in a eutrophic river. *Freshwater Biology*, 56, pp.2580-2593.

Suberkropp, K. & Klug, M.J., 1980. The maceration of deciduous leaf litter by aquatic hyphomycetes. *Canadian Journal of Botany*, 58, pp.1025-1031.

Suberkropp, K., Godshalk, G.L. & Klug, M.J., 1976. Change in the chemical composition of leaves during processing in a woodland stream. *Ecology*, 57, pp.720-727.

Vörösmarty, C.J. McIntyre, P. B., Gessner, M. O., Dudgeon, D., Prusevich, A., Green, P., Glidden, S., Bunn, S. E., Sullivan, C. A., Liermann, C. R., Davies, P. M., 2010. Global threats to human water security and river biodiversity. *Nature*, 467, pp.555-561.

Wetzel, R.G., 1993. Microcommunitites and Micrograd ientes: Linking nutrient regeneration, microbial mutualism, and high sustained aquatic primary production. *Netherlands Journal of Aquatic Ecology*, 27(1), pp.3-9.

Young, J.C., 1995. Microwave assisted extraction of the fungal metabolite ergosterol and total fatty acids. *Journal of Agriculture and Food Chemistry*, 43, pp.2904-2910.

Zar, J.H., 1999. *Biostatistical Analysis*, 4th edition., Prentice-Hall, Englewood Cliffs, N J.