DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

Does stream secondary salinization affect fungal and detritus palatability to shredders?

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 Canhoto (Universidade de Coimbra, Portugal) and Doutora Ana Lúcia Gonçalves (Universidade de Coimbra, Portugal).

Tesfalem Getahun Belihu

2015

ACKNOWLEDGMENTS

It is a pleasure to express my deep sense of gratitude to my advisor Professor Cristina Canhoto for giving me the chance to work and explore the aquatic environment. Your advice and scientific approach have helped me to a very great extent to accomplish this thesis. I would also like to extend my gratitude to Dr. Ana Gonçalves for your valuable comments and keen interest on me in every stage of the research.

I thank Ana Lírio who helped me during laboratory work. Your inspiration with kindness, enthusiasm and dynamics helped me to complete this research.

I thank Leonor Keating for translating the Portuguese version of the abstract and encouragement till the end of this work. I couldn’t have done it without your help; you know my Portuguese.

I want to gratitude my father Getahun Belihu and my mother Zerefe Assefa for teaching me a lot about life. I wish you were alive to see what life brought to me. Your memory will be eternal.

It is my privilege to thank the IMAE family, for sharing a wonderful time with me during my stay in all of the IMAE universities; you were always there for me.

I am also thankful for the European Commission for funding the scholarship and giving me the chance to study this wonderful program. I also want to extend my thankfulness to the coordinators of the IMAE program from each university for their interest on me and giving me an awesome opportunity to study at the best Universities in Europe.

Last but not least, I would like to thank all the people who contributed in this thesis in one way or another.

Above all, I would like to thank my God and saviour Jesus Christ for being with me with all difficulties and sicknesses.

ABSTRACT

Anthropogenic salinization has been considered as one of the main environmental threats leading to water quality degradation and alteration of aquatic biodiversity. However, little is known about its effects on microbial mediated leaf decomposition and leaf palatability to shredders.

In this study we conducted a microcosm experiment to assess the effect of salinity (4g/L NaCL) on individual fungal species' respiration and decomposition activity. We also compared the importance of fungal identity and salinization on leaf quality to the common endemic shredder *Sericostoma vittatum* Rambur (Sericostomatidae) maintained in both contaminated and non-contaminated environments. For this purpose, oak and alder leaves were selected.

We found that salinity at a concentration of 4g/L NaCl did not affect the fungal decomposition activities in oak or alder leaves although a stimulation of alder degradation could be observed in salt-contaminated media. Adverse effects of salinity was observed in microbial respiration on both leaf species. Consumption of alder by *Sericostoma vittatum* larvae was depressed by the presence of salt. On the contrary, a compensatory feeding behaviour of the larvae towards oak leaves conditioned in salt media and when offered in salt media was detected.

The results indicate that the tested concentration (4g/L) does not impair fungal-mediated decomposition. However, the effects of salt contamination on fungal species are species specific and may affect invertebrates feeding behaviour. Dietary and waterborne effects promoted by salt contamination seem able to affect invertebrates' performance particularly if available leaves are more recalcitrant. If generalised the present results indicate that important effects on streams nutrient cycling maybe expected due to salt contamination.

Keywords: *Salinization, aquatic hyphomycetes, shredder, decomposition, feeding behaviour*

RESUMO

A salinização por factores antropogénicos é considerada uma das principais ameaças ambientais que levam à degradação da qualidade da água e à alteração da biodiversidade aquática. No entanto, pouco se sabe acerca dos seus efeitos na decomposição foliar microbiana e na palatabilidade para os trituradores.

Neste estudo, foi usado um microcosmos para avaliar o efeito da salinidade (4 g/L NaCl) na atividade respiratória e de decomposição de espécies de fungos isoladas. Comparámos também a importância do tipo de fungo e da salinização na qualidade foliar para o triturador endémico *Sericostoma vittatum* Rambur (Sericostomatidae), mantido em ambientes diferentes – com e sem contaminação salina. Foram escolhidas folhas de carvalho e de amieiro para a realização do estudo.

A salinidade a uma concentração de 4g/L NaCl não afetou as atividades de decomposição fúngica, nem nas folhas de carvalho nem nas de amieiro, mas foi possível observar uma estimulação da degradação de amieiro no meio com contaminação salina. Foram detetados efeitos adversos da salinidade na respiração microbiana para ambos os tipos de folhas. O consumo de amieiro pelas larvas de *Sericostoma vittatum* foi menor na presença de sal. Pelo contrário, foi detetado um comportamento alimentar compensatório das larvas em relação às folhas de carvalho condicionadas em meio salino quando oferecidas em meio salino.

Os resultados indicam que a concentração testada (4 g/L) não prejudica a decomposição mediada por fungos. No entanto, os efeitos da contaminação salina em fungos são específicos para cada espécie e podem afetar o comportamento alimentar dos invertebrados. As respostas promovidas pela contaminação salina, a nível da água e da dieta, parecem capazes de afetar a performance dos invertebrados, especialmente quando as folhas disponíveis são mais recalcitrantes. Se generalizados, os resultados deste estudo indicam que é possível esperar efeitos importantes nos ciclos de nutrientes de cursos de água devido à contaminação salina.

Palavras-chave: *Salinização, hifomicetes aquáticos, trituradores, decomposição, comportamento alimentar*

ANNEX

CHAPTER 1. INTRODUCTION

Chapter 1

- INTRODUCTION

-

1. General introduction

Freshwater ecosystems are among the most endangered systems in the world (Malmqvist and Rundle 2002; Dudgeon et al., 2006). Anthropogenic influences such as water pollution, overexploitation, habitat degradation, species invasion, flow modification, eutrophication and acidification have been stated as some of the factors for deteriorating the quality of life in aquatic ecosystems and thus, threats the biodiversity in general. Since the knowledge of adverse effects of pollutants in the 1950s (Dodds 2002), freshwater ecologists have made numerous studies on the effect of anthropogenic pressures on the aquatic life and ecosystem processes (Woodward et al., 2009). As we move deeper into the Anthropocene, the scale and magnitude of existing and emerging anthropogenic threats to freshwater ecosystems become even more apparent, yet we are still surprisingly poorly equipped to diagnose causes of adverse change in freshwater ecosystems.

Anthropogenic salinization has been considered as one of the environmental threats leading to the water quality degradation and alteration of the aquatic biodiversity (William 2001, Horrigan et al., 2005). It has been widely acknowledged as one of the most important stressors in stream ecosystem (Millennium Ecosystems Assessment 2005) having a wide range of impacts on organisms from all trophic levels (Piscart et al., 2006; Kefford et al., 2012a).

1.1. Low order streams

Small streams are located at the head of the fluvial continuum and constitute up to 85% of the total length of a lotic system (Allan and Castillo, 2007). In temperate areas, these running waters are heavily shaded by the surrounding riparian vegetation, which limits primary production. The structure and function of these systems are though closely linked with the supply of organic material from the riparian vegetation (Vannote et al., 1980).

The allochthonous organic matter is mainly provided to the stream in autumn and is largely composed of leaf litter supplied directly from the riparian vegetation through vertical or lateral inputs (Petersen and Cummins 1974; Abelho 2001). Besides large amounts of leaves, which accounts more than 60 % of the total litter fall in temperate streams, the inputs of organic matter also include steams, flowers, seeds, and logs (Benfield et al., 1997; Abelho, 2001).

1.2. Leaf litter decomposition

Leaf litter decomposition occurs in three temporarily distinct phases that may overlap in time: leaching, microbial conditioning and fragmentation (Petersen and Cummins 1974; Webster and Benfield, 1988; Gessner et al., 1999; Allan and Castillo 2007). In the early stage of decomposition, the loss of soluble compounds from the leaf occurs by the process called leaching (Abelho 2001; Bärlocher et al., 2005a). After that, its matrix is modified by microbial (aquatic fungi and bacteria) activities. Aquatic hyphomycetes plays a key role in the conversation of leaf litter into more palatable food resource for detritivorous macroinvertebrates (shredders); this process is called conditioning (Boling et al., 1975; Gessner et al., 1999). Lastly, the leaves are fragmented through the consumption and invertebrate manipulation (e.g. to build cases) and by the flow /turbulence (physical fragmentation) (Webster and Benfield 1986; Abelho 2001).

Intrinsic (physical/chemical characteristics of the leaves) such as nutrient concentration (particularly N and P) and lignin (Ostrofsky 1997; Abelho 2001) and external (environmental variables) factors have been considered as determinants for affecting the decomposition process (Webster and Benfield, 1986). Decomposition is slower for leaves with higher lignin, low nitrogen and phosphorous concentrations (e.g. Martinez et al., 2013). External (environmental variables) are related with temperature (e.g. Dang et al. 2009, Gonçalves et al., 2013), pH (e.g. Medeiros et al., 2009) and hydro morphological parameters (e.g. Ferreira et al., 2006).

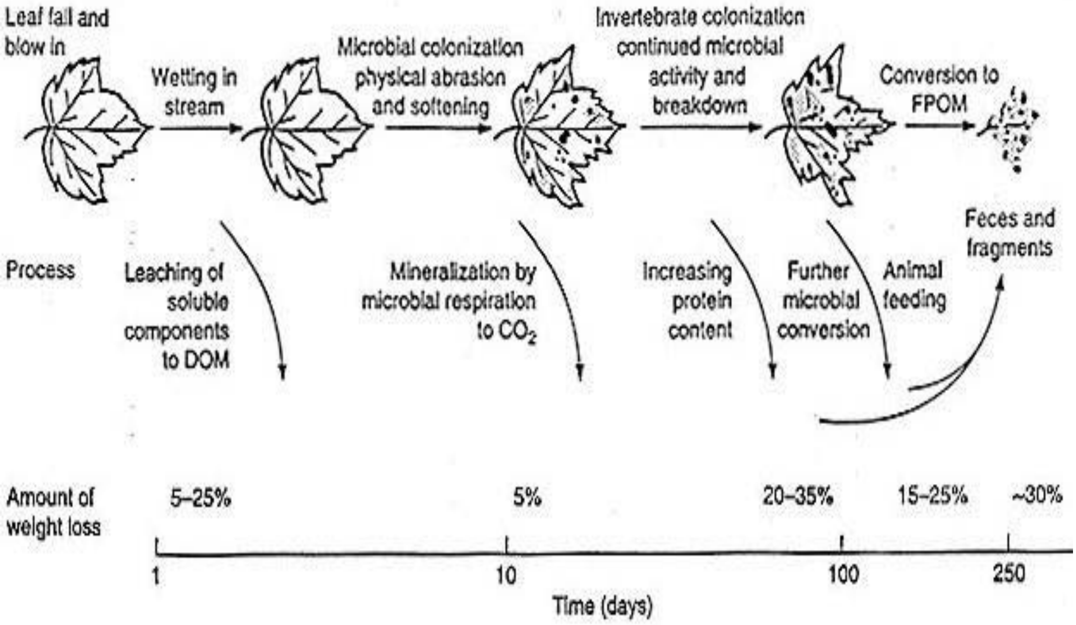


Figure 1: Schematic representation of different phases involved in leaf litter decomposition in stream food web (in Allan and Castillo, 2007)

Leaching: is a process in which the leaves loose a substantial mass immediately after immersion (Gessner 1999). It is a crucial phase for the release of soluble compounds including carbohydrates, amino acids and polyphenols (Graça et al., 2005). During this phase, the leaves may lose up to 42% of their mass (Abelho, 2001). The process usually lasts for 48 h depending on factors such as leaf species and dryness (Maloney and Lamberty, 1995; Taylor and Barlocher, 1996) and environmental factors such as stream water temperature and turbulence (Ferreira et al., 2006a; Ardon and Pringle, 2008). For example, it was found that deciduous leaves leach at higher rates (20-42%) than coniferous leaves (7 %) (Maloney and Lamberty, 1995).

Conditioning: is a phase in leaf break down process in which the leaf material is colonized by microorganisms, namely fungi (aquatic hyphomycetes) and bacteria. During this period, the chemical composition of the leaf tissue is modified and converted to secondary production by microorganisms (Bärlocher and Kendrick, 1975).

Aquatic hyphomycetes are common inhabitants of low order streams and are the main drivers of this phase. This group of fungi is characterized by their ability to sporulate under water. Once the leaf is submerged, large amount of conidia start detaching from the leaves (Krauss et al., 2011). Leaves become softened and its nitrogen and ATP contents rise (Kaushik and Hynes 1968). According to Suberkropp (1991), fresh water hyphomycetes can invest up to 80% of their production into sporulation. They have a capability of producing exo-enzymes that cleave structural leaf polymers such as pectin, xylan, and cellulose (Suberkropp et al., 1983). Due to this, fungi are responsible for the physical and chemical alteration of the leaf resulting the leaf mass loss after all (Gessner, 1999). Different studies suggested that the biomass, productivity and enzymatic substrate degradation of fungi decomposers (aquatic hyphomycetes) is higher than bacteria and play a predominant role in microbial degradation of the leaves (Bärlocher and Kendrick 1974; Gessner et al., 1997; Graça and Canhoto 2006; Krauss et al., 2011).

Based on previous laboratory food preference experiments on shredder invertebrates, leaves conditioned by aquatic hyphomycetes were more palatable than bacterial conditioned leaves (Suberkropp et al., 1983; Graça 2001) and high survival was observed in invertebrates fed fungal over bacterial conditioned leaf material (Kostalos and Seymour, 1976).

For the past years, several studies have been conducted on the effects of environmental factors such as acidity (Bärlocher, 1987), alkalinity (Abel and Bärlocher, 1984), temperature (Chauvet and Suberkropp, 1988), eutrophication (Lecerf and Chauvet, 2008) on survival and growth of aquatic hyphomycetes hence conditioning process (Krauss et al., 2011).

Fragmentation: Leaf biological fragmentation is largely promoted by shredders feeding activities that give origin to fine particulate organic matter (FPOM) (plant fragments and faces) that will be used as a food source for other functional feeding group, the collectors, both *in situ* and further downstream (Cummins 1974, Graça et al., 2001). Discharge, current velocity, turbulence have been reported as some of the environmental factors that may contribute to the leaf physical fragmentation (Abelho, 2001).

1.3. Freshwater salinization

Salinization is defined as an increase in the concentration of dissolved inorganic ions like Na^+, Ca^{2+}, K^+, Cl^-, SO_4, CO_3, HCO_3 in the water (Cañedo-Argüelles et al., 2013). In freshwater ecosystems, an increase in the concentration of chloride anion is often used to measure salinization (Kaushal et al., 2005). According to Venice system 1959, surface waters vary depending on their salt content and the salinity concentration of freshwater was regarded as < 0.5 g/l. Based on the salinity preferences, the biota can be freshwater fauna, brackish water fauna or marine water fauna (Schröder et al., 2015). In fresh water ecosystem, salinization is considered as the main factor for limiting the distribution of biota (Cañedo-Argüelles et al., 2013).

Freshwaters salinization can result from several natural and anthropogenic processes and categorized as primary or secondary salinization (Williams 2001). Primary salinization (natural salinization) is the accumulation of salts originating from natural sources without any human interference. This is due to the geology of the catchment and precipitation; soil rich insoluble salts or shallow saline ground water table leads to the dissolution of salts (Williams 2001). Besides, sea spray and evaporation of seawater are also an important sources of natural salinization (Herczeg et al., 2001).

Salinity from anthropogenic activities are called secondary salinization. Irrigation and rising of ground water are the main causes of secondary salinization in freshwater ecosystems (Williams, 1987). Inflows from irrigation water makes the soil water to be more saline and leaches out through run off and end up in the streams (Lerotholi et al., 2004). In other way, clearing natural and deep-rooted vegetation from the catchment and replacing by shallow rooted plant species is raising ground water tables at rates of 0.05-1.0 m per year (Pinder et al., 2005). This makes the ground water to be saline and leads to flow into the streams (Williams 2001).

Mining activities can salinize freshwater by the release of potash salts from the manufacture of agricultural fertilizers (e.g., Cañedo-Argüelles et al., 2012). In Europe, where potassium and soda industries are developed, salt mining is documented as the main cause of freshwater secondary salinization (Cañedo-Argüelles et al., 2012) especially in the Mediterranean streams, the severe impact of salinity has been reported (Prat and Munné, 2000). Moreover, the use of salts (especially NaCl) as de-icing agent (e.g. Demers and Sage 1989; Williams et al., 2000), discharge from industrial activities (Kefford, 1998), gulp injection (Wood and Dykes, 2002), and sewage treatment plant effluents (Silva et al., 2000; Williams et al., 2003) are also reported as the drivers of fresh water secondary salinization.

1.3.1. Ecological impacts of freshwater salinization

Different studies reported secondary salinization as the main stressors in freshwater ecosystem and yet to increase further due to climatic changes (Millennium Ecosystems Assessment, 2005; Cañedo-Argüelles et al., 2013). In particular, small streams are subject to salinization (William, 2001) leading to substantial changes in flora and fauna (Horrigan et al., 2005).

For many years, extensive studies have been done on the impacts of secondary salinization on different levels of ecosystem organisation. However, most of these studies focused on the community and ecosystem level and little has been said on the role of salinity at organism/individual level (Cañedo-Argüelles et al., 2013).

In ecosystems, increased salinity lead streams to eutrophication (Bernhardt and Palmer, 2001), sedimentation of suspended particles (Cañedo-Argüelles et al, 2013), reduction of dissolved oxygen concentration (James et al., 2003). In the riparian zones, high salinity can reduce the surrounding vegetation, modify the nutrient cycling (Dunlop et al., 2005), and increase the amount of light that enters the stream and may lead to a change in the ecosystem from heterotrophic to autotrophic (Millan et al., 2011). Furthermore, it reduces the carrying capacity of the ecosystem (Schäfer et al., 2012).

High concentration of salinity is associated with a reduction in species richness and diversity (Piscart et al., 2005; Kefford et al., 2011), change in community (Cañedo-Argüelles et al., 2012; Schafer et al., 2012) and modification of a trophic structure of freshwater macroinvertabrates communities (Cañedo-Argüelles et al., 2013). Studies suggested that, freshwater species in general act differently for different salinity levels, where some organisms are regarded as salt- tolerant and other may have sensitive response towards increased salinity (Piscart et al., 2006). For example, Ephemeroptera, Plecoptera and Trichoptera species richness (EPT) have been reported as the most sensitive taxa (Piscart et al., 2006, Kefford et al., 2011) whereas Crustacea, Coleoptera and certain Diptera (e.g. Ceratopogonidae) and Odonata (e.g. Coenagrionidae) are among the most tolerant (Berenzina, 2002; Dunlop et al., 2008). Concerning the effect of salinity on the feeding habits of invertebrates, elevated salinity results in a loss of grazer and shredder species in favour of predators, filter and deposit feeders (Piscart et al., 2006; Kefford et al., 2012b).

1.3.2 The impact of salinization on leaf litter decomposition

Despite salinization has been widely recognized as an environmental threat (Millennium Ecosystems Assessment, 2005), few studies have been conducted on leaf litter decomposition (Schäfer et al., 2012; Cañedo-Argüelles et al., 2014). Studies show that high concentration of salinity leads to a reduction in the breakdown of allochthonous organic matter (e.g., Reice and Herbst 1982, Schäfer et al., 2012). This results in a reduction of the abundance of shredder invertebrate since their growth and survival is dependent on the leaf type (Butler and Suberkropp 1986) and colonisation (Suberkropp 1992). In spite of extensive studies on the effects of salinity on invertebrates, little is known on the physiology and functioning of aquatic hyphomycetes. Studies suggested that freshwater aquatic hyphomycetes have different levels of salinity tolerance (Ristanovic and Miller 1968). Their salinity tolerance is determined by their ability to regulate internal osmotic concentration against external gradient (Hart 1991). Other related inhibition of their reproduction to the increased level of salinity (Byrne and Jones 1975). Moreover, increased salinity was found to reduce leaf associated fungal biomass (Schäfer et al., 2012) and, change the community composition of freshwater aquatic hyphomycetes (Mohamed, 2011). Considering the fact that high preference of shredders for leaf colonized by aquatic hyphomycetes (Graça, 2001), any alteration by increased salinity on aquatic hyphomycetes would affect the feeding preferences of shredders and alter the nutrient cycling and the stream functioning.

Even though numerous studies have focused on the deleterious effects of increased salinity on invertebrates and some ecosystem processes, little information exists on the implication of increased salinity in the colonization of leaf material by aquatic hyphomycetes. Since shredders feed on conditioned leaf and have capabilities to differentiate leaf patches conditioned by different fungi (Arsuffi and Suberkropp 1985; Gonçalves et al., 2014), the effect of increased salinity on their feeding behaviour is clearly unknown. Moreover, less evidence is presented on the effects of increased salinity on microbial-mediated leaf decomposition, invertebrate behaviour and consequent community composition of the ecosystem.

1.4.Main objective

The aim of this study was to assess the effect of increased salinity (NaCl) on individual fungal species respiration and decomposition activity. We also compared the importance of fungal identity and salinization on leaf quality to the common endemic shredder *Sericostoma vittatum* Rambur (Sericostomatidae) maintained in both contaminated and non-contaminated salt rich-environments. We hypothesize that a species specific depressed metabolism will negatively affect decomposition activity of fungi in salt-rich media. The presence of salt in the media will not affect the invertebrates' feeding behaviour. Deleterious consequences will likely be more pronounced in less salt-tolerant fungal species colonizing more recalcitrant leaves, i.e., oak.

Chapter 2

-MATERIALS AND METHODS-

2. Materials and Methods

2.1.Experimental set up

Alder (*Alnus glutinosa*) and oak (*Quercus robur*) leaves were collected immediately after abscission from the forest floor, air-dried and stored, in the dark, at room temperature until needed. The leaves were further cut with a 9mm-diammeter cork borer and oven-dried (105^{o}C for 24 h). For each leaf species, discs were distributed by a total of 12 Erlenmeyer flasks. Each flask contained 36 pairs of leaf discs for consumption tests and 20 discs for the evaluation of microbial respiration and mass loss. In this last case, leaves were previously cut symmetrically in relation to the leaf main vein. Half were oven dried (60^{o}C for 48 h) and used to determine initial dry mass (DM_i). Microcosms were filled with 40 ml of autoclaved (20 min, 121°C) NaCl enriched (4g/L) solution; the other half was filled with the same volume of distilled water.

Three species of aquatic hyphomycetes - *Heliscus lugdunensis* (HELU), *Lemonniera aquatica* (LEAQ) and *Articulospora tetracladia* (ARTE) - were used in this experiments (Fig. 2). Based on previous studies, the palatability of these aquatic hyphomycetes to shredder invertebrates are usually raked as: *Heliscus lugdunensis* (Unpalatable; Butler & Suberkropp, 1986), *Articulospora tetracladia* (Intermediate; Graça et al., 1993), and *Lemonniera aquatica* (Unpalatable; Suberkropp et al., 1983). On the other hand, these species present distinct tolerance towards NaCl: HELU> ARTE> LEAQ (Simões et al, submitted). The species were grown for 3 weeks in Malt Extract Agar (MEA). Discs of each fungal species were used to promote leaf discs colonization in microcosms. The Erlenmeyer flasks were filled with distilled water or distilled water supplemented with the same concentration of NaCl (from now on referred as salt-enriched water). Two microcosms filled with distilled or salt-enriched water, but with no mycelia, were used as controls. The treatments are represented below. Microcosms were incubated on a shaker 120 (rpm) under 12h light: 12 h dark photoperiod at 16°C for 15 days; the media were replaced every 2 days.

Figure 2: Conidia a) *Heliscus lugdunensis* b) *Lemonniera aquatica* and c) *Articulospora tetracladia* (adapted from Gonçalves et al. 2013; 400x amplification)

After two weeks incubation, the leaf discs from each treatment were used for the invertebrates feeding experiment (see below)

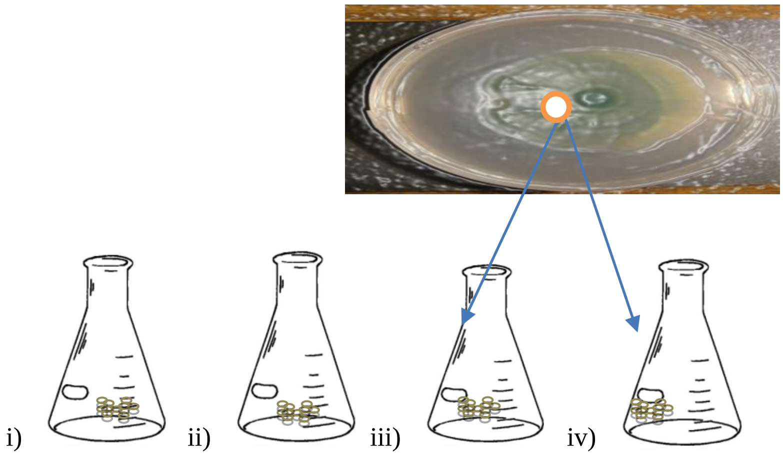


Figure 3: Illustration of the experimental design. Scheme considers one leaf species (alder or oak) and a single fungal species (out of 3): i) Non-conditioned leaves incubated in salt-enriched water, ii) Non-conditioned leaves incubated in distilled water, Species of fungi used for inoculation of microcosm filled with distilled water (iii) and distilled water supplemented with 4g/L NaCl (vi). Additional controls run without inoculation.

2.2.Microbial Respiration

Microbial respiration associated with decomposing the leaf discs were estimated from the measurements of dissolved oxygen before and after discs incubation in oxygen saturated distilled water or salt-enriched water, according to the conditioning treatment (see above). Groups of five alder or oak leaf discs from each treatment were immersed in 50ml falcon tubes filled with the medium of origin (n=4). All the falcon tubes were covered with aluminium foil to promote darkness. Oxygen consumption by the discs' microbial community was allowed for 24 h. The final dissolved oxygen was measured using DO Meter probe (YSI model 55, Yellow Springs, OH, U.S.A). Oxygen consumption of discs were expressed as mg O_2/ g M leaves /h.

2.3.Mass loss

Immediate after the microbial respiration experiment, the leaf discs were oven-dried and reweighted (± 0.1 mg). Loss of dry mass was calculated as the difference between the initial mass of the symmetrical discs (see above) and the final mass of the 20 leaf discs incubated in each flask. The percentage of dry mass (% DM) was calculated as (DM_i - DM_F)/ DM_i · 100%), where DM_i is the initial dry mass of the leaf discs and DM_f, is the final dry mass.

2.4.Invertebrate consumption tests

2.4.1. Test species

In this experiment, we used the caddisfly *Sericostoma vittatum* Rambur (Trichoptera; Sericostomatidae), an endemic species in streams of Iberian Peninsula. According to previous studies, this species plays a key role in the fragmentation of organic matter of streams in central Portugal (Feio and Graca, 2000) and has been previously used as test species (Gonzalez et al., 1992; Graça et al., 2001; Carvalho and Graça 2007; Campos and González 2009).

Larvae were collected in Ribeira de S. João', Lousã (40°05′59″N; 8°14′02″W; drainage basin area – 18 km^2), a 4th order stream in Central Portugal. They were acclimatized to laboratory conditions (12h light:12 dark photoperiod; 16ºC) in a plastic container filled with stream water. The bottom was covered with sediment from the stream of origin (Fig. 4). They were fed *ad libitum* with leaf litter from the stream of origin. The shredders dry mass was calculated according to DM = 0.0136 X CO – 0.0162 (R^2 = 0.83) where DM is dry mass (mg) and CO is the anterior maximum case opening (mm) (Ferreira et al., 2010).

Figure 4: *Sericostoma vittatum* (Trichoptera; Sericostomatidae) (by Ana Gonçalves)

Consumption tests were performed with a total of 9 larvae per treatment (1.37 mg - 14.15mg). Larvae were starved for 24 hours before the experiment. Shredders were individually maintained in cups (70 mm diameter × 85 mm high) filled with 200 ml of stream water or stream water supplemented with salt (4 g/L NaCL) (Fig. 4). A disc from the same treatment was offered to each invertebrate kept in a feeding cup while the pair was placed in a small fine mesh bag (0.55mm mesh) attached to the cup using a plastic clothespin and used as a control. All the cups were covered with a fine layer of ashed (550° C; 6 h) stream sediment.

The cups were continually aerated using aquarium pumps. Experiments run at 16° C with a 12:12 dark: light photoperiod. The experiment was stopped when half of the discs were eaten in 50% of the microcosms, and both leaf discs (Control and Exposed) were collected, oven dried and weighed (± 0.1 mg). Consumption (C) was estimated as the difference between leaf discs dry mass in the control (DM_i) and treatment (DM_f) and expressed per mg dry mass of individual larva (DM_t), per day (d) according to the equation $C = (DM_i – DM_f) / (DM_t \times d)$ (Graça et al., 2005).

The treatments for each leaf species were as follows,

i. Leaf discs conditioned in distilled water by HELU, LEAQ, or ARTE were offered to the invertebrates maintained in microcosms filled with regular stream water or stream water enriched with salt (4g/L NaCL).
ii. Leaf discs conditioned in salt-enriched solution by HELU, LEAQ or ARTE were offered to the invertebrates maintained in microcosms filled with regular stream water or stream water enriched with salt (4g/L NaCL). In all cases parallel experiment for controls were run.

Figure 5. An example of a consumption experiment. Each feeding cup contained one *Sericostoma vittatum* larvae, oak or alder leaf discs conditioned in distilled water or salt enriched water NaCl (4g/L) by distinct species of fungal species. Consumption test was performed by offering the leaf discs to the animals kept in regular stream water or stream water enriched with salt. A) Fine mesh bag containing the control leaf disc B) leaf disc for consumption, the discs for consumption were over the sediment so that the animal could eat them.

2.5.Data analysis

Comparisons of mass loss and microbial respiration associated with each leaf species were done by 2-way ANOVA with fungal species and conditioning media (distilled water or salt-enriched water) as categorical variables.

Leaf consumption of each species was compared among treatments by 3-way ANOVA with fungal species, conditioning media (distilled water or salt-enriched water) and consumption media (regular stream water or stream water enriched with salt) as categorical variables. When significant statistical differences were detected ($P < 0.05$), planned comparisons were used to identify the significant effects of one factor within the other. Tukey's HSD test was performed whenever necessary.

The data was transformed ($\log(x+1)$) when necessary to achieve the assumptions of normality and homoscedasticity of variance. Mean and SE were calculated using non-transformed data. All statistical analysis were performed using STATISTICA 7. The P value of 0.05 was considered as a significance level.

Chapter 3

-RESULTS-

3. Results

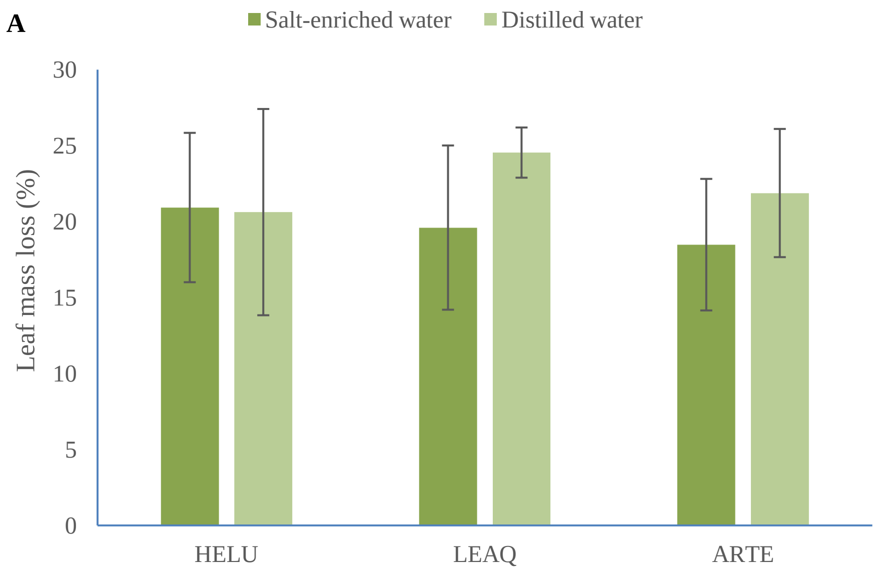
3.1.Leaf mass loss

Oak: The lowest leaf mass loss value (18.47%±4.32) was observed in leaf discs conditioned by *A. tetracladia* in salt-enriched media whereas leaf discs conditioned by *L. aquatica* in distilled water presented the highest mass loss (24.53±1.65).

Leaf mass loss wasn't significantly different among fungi (two-way ANOVA, $F_{0, 01}$ = 1.28, P=0.98; Fig. 6A) or conditioning media (two-way ANOVA, $F_{0, 49}$ =46.27, P=0.48; Fig.6A).

Alder: Mass loss of alder leaf discs wasn't different across fungal species (two-way ANOVA, $F_{2,54}$ = 117.54, P=0.10), but was found to be significantly affected by the conditioning media (two-way ANOVA, $F_{5,17}$ =238.94, P=0.03): higher mass loss was observed in leaf discs conditioned in a salt-enriched water than distilled water. *H. lugdunensis* was the species that induced the highest mass loss (27.93%±3.50) in salt-enriched water, while the opposite occurred with the leaf discs conditioned by *A. tetracladia* in distilled water (13.77%±3.64, Fig. 6B).

In both leaf species, parallel experiments for control were performed at the same time indicated values very close to zero.



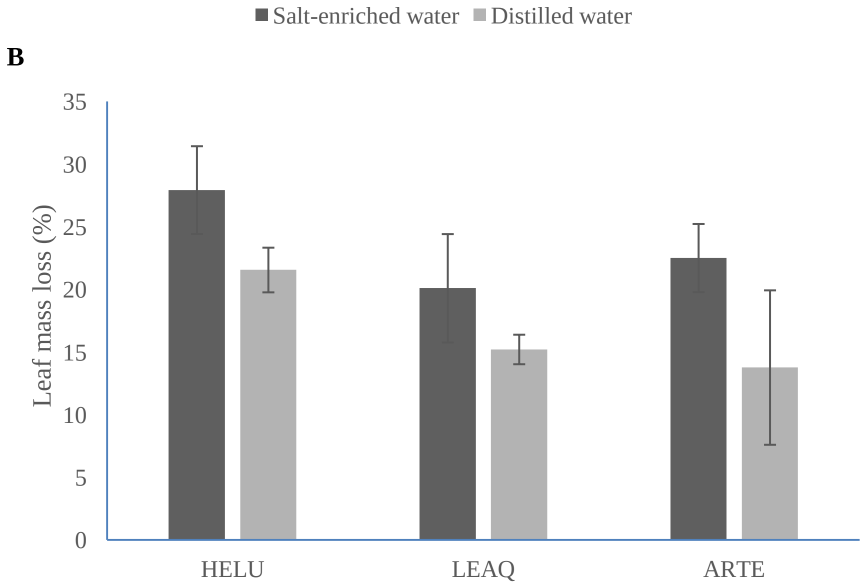


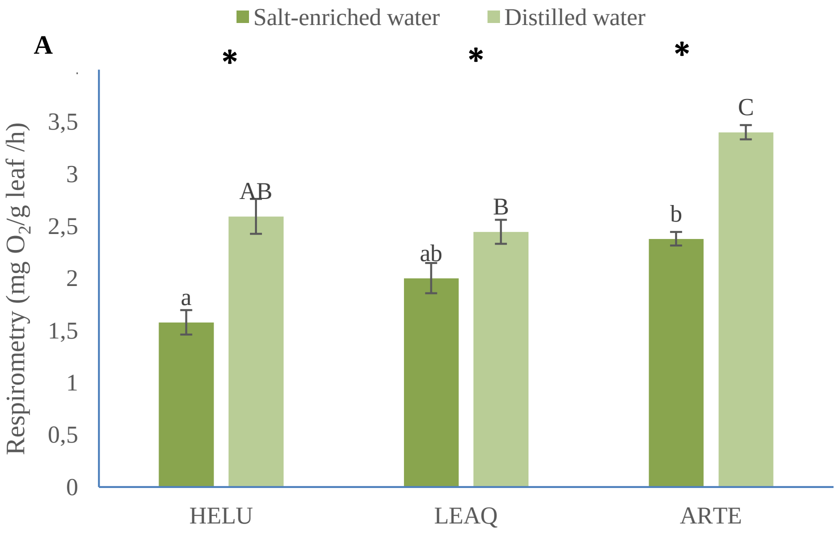
Figure 6: Leaf mass loss (%, ± SE) of oak (A) and alder leaf discs (B) conditioned in distilled and salt-enriched water by three district species of aquatic hyphomycetes *H. lugdunensis* (HELU), *L. aquatica* (LEAQ) and *A. tetracladia* (ARTE). No statistical differences were observed among treatments.

3.2. Microbial respiration

Oak: Microbial respiration associated with oak leaf litter was highly affected by the identity of fungi (two-way ANOVA, $F_{26,13}$=1.47 P<0.00; Fig. 7A) and the conditioning media (two-way ANOVA, $F_{72,47}$ =4.10, P=0.00). Higher values were registered in leaves conditioned in distilled vs. salt-enriched water. The interaction effect between fungal species and the conditioning media was found to be significant (two-way ANOVA $F_{3,88}$=0.22, P=0.03). The highest value (3.39 mg O_2/g leaf /h) was registered in leaf discs conditioned by *A. tetracladia* in distilled water media (Tukey P< 0.00); the lowest value (1.57 mg O_2/g leaf /h) was observed in leaf discs conditioned by *H. lugdunensis* (Tukey P<0.00) in salt-enriched water.

Alder: Respiration associated with alder leaf varied between 2.48 mg O_2/g leaf/h± 0.21 and 1.28 mg O_2/g leaf/h±0.31. Both the identity of fungi (two-way ANOVA, $F_{4,89}$= 1.47, P= 0.02) and the conditioning media (two-way ANOVA, $F_{6,81}$=2.05, P= 0.01, Fig.7B) affected the microbial respiration in alder leaf discs. Microbial respiration was higher in alder leaves conditioned in distilled water than salt-enriched water. However, the interaction effect of fungal species and conditioning media was not significant (Two-way ANOVA, $F_{0,63}$ = 0.19, P > 0.54).

In both leaf species, parallel experiments for control were performed at the same time indicated values very close to zero.



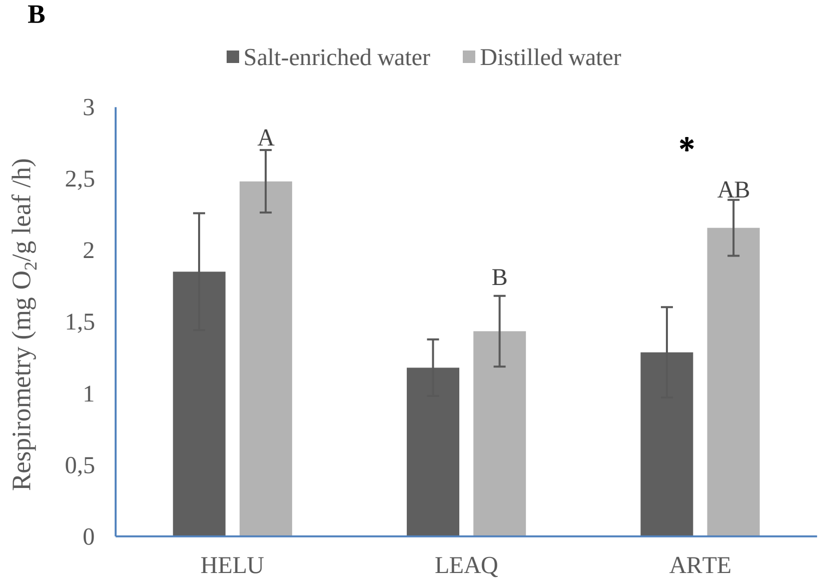


Figure 7: Microbial respiration (Mean ± SE) of oak (A) and alder (B) leaf discs conditioned in distilled and salt-enriched water by three distinct species of aquatic hyphomycetes; *H. lugdunensis* (HELU), *L. aquatica* (LEAQ) and *A. tetracladia* (ARTE). Significant differences within each fungal species between the conditioning media (salt-enriched and distilled water) are represented by (*). Bars with the same letters are not significantly different when tested within each conditioning treatment, salt-enriched water (lowercase letters) and distilled water (capital letters).

3.3 Consumption tests

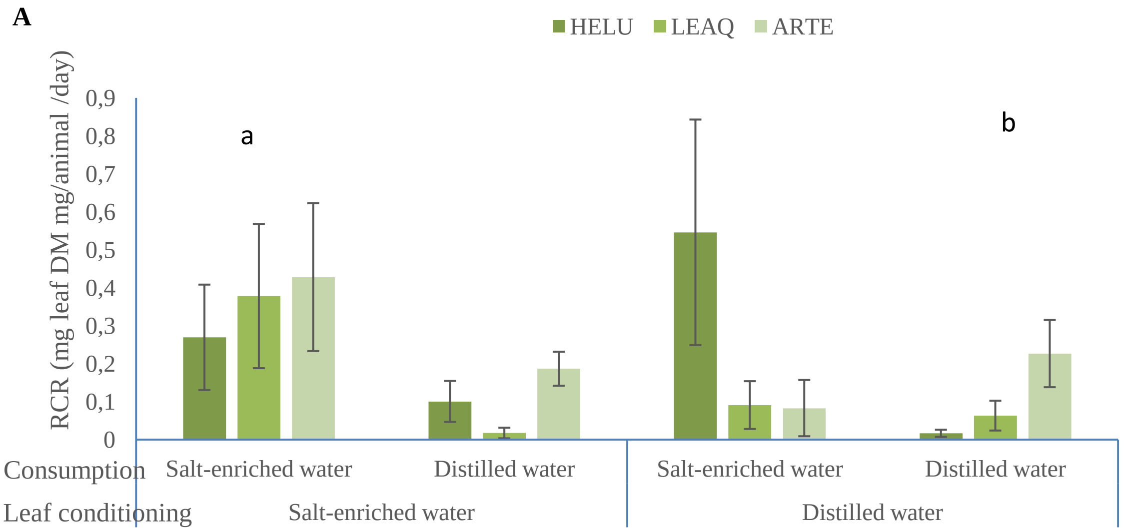
Oak: The lowest consumption value of oak (0.01 mg leaf DM/mg animal) was registered in leaves conditioned in distilled water by *H. lugdunensis* and offered to animals in distilled water whereas the highest (0.54 mg leaf DM/mg animal) was gathered in leaves conditioned by the same species in distilled water and offered to animals in salt enriched water (Planned comparisons, $F_{6,81}$ >0.07, P<0.01, Fig. 8A).

Consumption of leaves was significantly affected by the media where the animals were maintained (salt enriched or regular stream water) (three–way ANOVA, $F_{5,88}$=0.065, P=0.017; Fig. 3). However, the identity of fungi (three–way ANOVA, $F_{0,93}$=0.010, P > 0.39) and the conditioning media of the leaves (salt enriched vs. distilled water) (three–way ANOVA, $F_{1.19}$=0.13, P >0.27) didn't affect the consumption of the invertebrates. No interaction between the three main factors was observed (three–way ANOVA, $F_{2.28}$=0.025, P >0.10). Invertebrates kept in salt-enriched water consumed more leaves conditioned in salt enriched media (0.35 mg leaf DM/mg±0.04) than when they were kept in regular stream water and fed leaves conditioned in distilled water (0.10 mg leaf DM/mg±0.06) (Planned comparisons, $F_{6,19}$ >0.06, P<0.01, Fig. 8A).

Alder: Larvae consumed more alder leaves conditioned in distilled water by *H. lugdunensis* species when maintained in regular stream water (0.49 mg leaf DM/mg animal /day). In opposition, lower consumption (i.e. 0.02 mg leaf DM/mg animal) was observed in leaves conditioned by the same species with salt enriched water and offered to the animals in the same medium (Planned comparisons, F15.99 >0.14 , P<0.00, Fig. 8B)

Consumption rates on alder were significantly affected by the conditioning media (salt enriched vs. distilled water) (three–way, ANOVA, $F_{6,05}$=0.05, P= 0.01), and by the medium were the consumption occurred (salt enriched or regular stream water) (three – way ANOVA, $F_{3,96}$=0.03, P= 0.04). Significant interactions were also detected between fungal species and the consumption media (three – way ANOVA, $F_{3,33}$=0.02, P= 0.03).

Invertebrates kept in regular stream water consumed more leaves conditioned in distilled water than when they were kept in salt-enriched water and offered leaves conditioned in sat-enriched water (Planned comparison , $F_{9,90}$>0.08, P<0.002). No interaction between the three main factors (fungi species, conditioning media and consumption media) was observed (three – way ANOVA, $F_{2.01}$=0.018, P > 0.13).



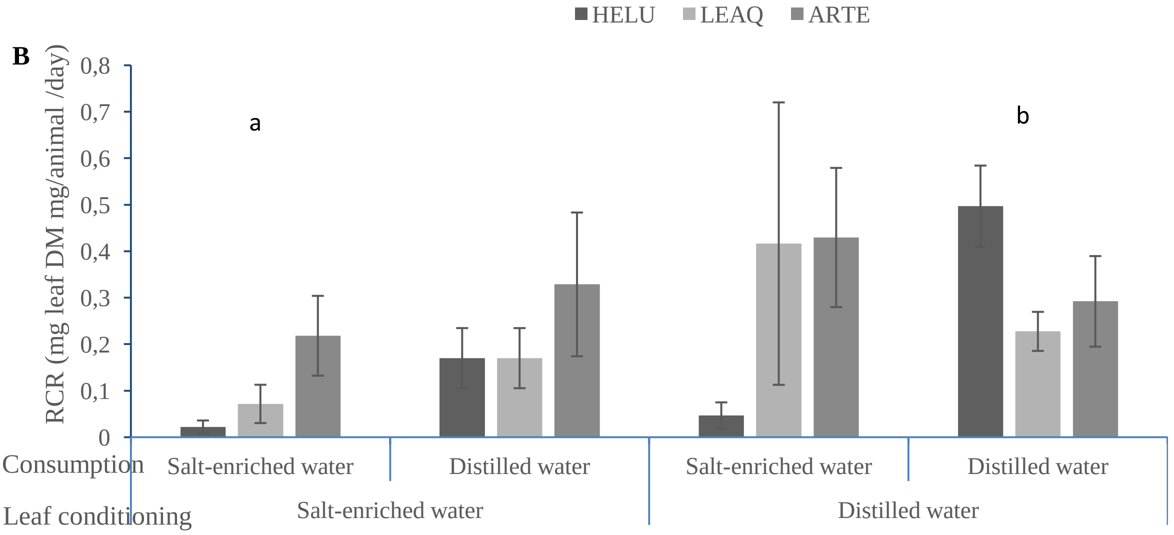


Figure 8: Relative consumption rate (Mean ± SE) of *S. vittatum* fed on oak (A) and alder (B) conditioned in distilled and salt enriched water by three district species of aquatic hyphomycetes; *H. lugdunensis* (HELU), *L. aquatica* (LEAQ) and *A. tetracladia* (ARTE). The consumption took place in cups filled with stream water and salt enriched stream water. Lowercase letters indicate significant differences between treatments (P<0.05).

Chapter 4

-DISCUSSION -

4. Discussion

Little information exists on the relevance of increase salinity on leaf litter decomposition and invertebrates' consumption (Schäfer et al., 2012). Most studies that have been conducted on salinization focused mainly on the structural changes in macroinvertebrates, reduction in diversity and modification in the trophic structure of the stream community (Piscart, et al., 2005; Cañedo-Argüelles et al., 2013). In this study, we examined the effect of increased salinity on individual fungal-mediated decomposition and respiration and, the importance of fungal identity and salinization on conditioned leaf quality to shredder maintained in both contaminated and non-contaminated salt rich environment.

In this study, we expected to observe an effect of salinity on fungal-mediated decomposition; however, the presence of salt (4g/L) during the microbial conditioning did not affect the mass loss of oak leaf. In a previous study (Simões et al., submitted), using fungal assemblages that included HELU and ARTE, a clear depressing effect was observed at this concentration. In alder leaves, even though species capabilities to decompose didn't vary across the treatments, leaves conditioned in salt-enriched media were found to have a consistent higher mass loss than leaves conditioned in distilled water. This suggests that the concentration of 4g/L may stimulate alder leaves decomposition. Whether this is the result of an hormesis effect triggered in the presence of a high quality substratum is not known.

Aquatic hyphomycetes have different responses to salinity; some species may be more salt-tolerant than the others (e.g. Mohamed, 2011). Their tolerance against salinity is determined by their ability to maintain the optimal internal osmotic concentration regardless of external changes (Hart et al., 1991). Simões et al., (submitted), found variability in salinity tolerance of the species based on species-specific growth rate for EC50 (effective concentration that led to a 50% inhibition in growth rates) where, *H. lugdunensis* was the most tolerant species that showed a significant increase in its growth rate at 20 g/L and EC50 value for *A. tetracladia* and *L. Aquatica* was 31.45g/L and 12.57 g/L respectively. In this study, in spite of such distinct tolerances mass loss was not different across species. Although their function seems to remain intact, sporulation is highly affected by the presence of salt which may have serious consequences if salinization is chronic (their reproductive output becomes non-existent and though decomposition).

For the past years, microbial respiration has been considered as a good indicator of microbial utilization of organic matter in streams (Fuss and Smock, 1996; Ramirez et al., 2003; Graça et al., 2005). In this study, microbial respiration was depressed in both alder (even though, the interaction effect of fungal species and conditioning media wasn't significant; $P > 0.54$) and oak leaves where highest microbial respiration was obtained by conditioning leaves in distilled water than salt-enriched water. Our study is in accordance with Simões et al., (submitted) that found significant reduction in respiration rates of oak leaves at lower salt concentrations under laboratory conditions. Connolly et al. (2014) also found a significant decrease in microbial respiration in *Phragmites* sp conditioned in wetlands.

In the case of oak all species are significantly affected by salinity which may be related with more energetic requirements needed to degradate more recalcitrant leaves. In spite of the existence of species-specific deleterious effects on distinct species, the fungal metabolic activity (evaluated through respiration) guaranties in distinct ways and rates) both leaves' degradation. This may indicate investment of reduced energy on growth and enzymatic degradation of leaves in expense of respiration. In this study, however, the investment was clearly seen in oak than alder leaves. This could be due to the difference in the intrinsic characteristics and substratum properties of the two species (Gonçalves et al., 2013).

Most investigation on the importance of salt contamination on stream invertebrates has been centred on water borne exposure rather than dietary exposure. The concentration (4g/L) that is used in this experiment is environmentally realistic and may critically affect the ecosystem functioning; lethal and sub lethal impacts on invertebrates may occur at values as low as 1g/L (Kaushal et al., 2005). Previous studies indicate that the concentration of salt of 1g/L- 3 g/L can change the macroinvertebrates community structure (Piscart et al., 2005).

It is generally accepted that the identity of fungal species, the leaf traits and the conditioning period are main factors for determining palatability of the leaf to the invertebrates (Suberkropp et al, 1983; Gonçalves et al., 2014). In this study, we hypothesized that lower consumption rates will be observed in invertebrates maintained and fed detritus conditioned by fungal species in NaCl rich media. Our finding contradicts the initial hypothesis: oak consumption was promoted by increased salinity; animals fed more leaves conditioned in salt enriched water when maintained in salt enriched stream water. The results from oak leaf consumption suggested that *S. vittatum* may present a compensatory feeding behaviour (e.g., Anderson and Cummins, 1979; Campos and González 2009, Flores et al. 2014) towards oak leaves conditioned in salt media and when offered in salt enriched water. This feeding strategy of the species might determine the energy allocation strategy of the species. (Cornut et al., 2015). Besides, oak was regarded as less palatable than many other leaves in streams' (Butler and Suberkropp 1986; Petersen and Cummins 1974)

In contrast to oak, consumption of alder leaf by *S. vittatum* larvae was reduced by the presence of salt. Animals kept in salt enriched water and fed alder conditioned in salt-enriched water consumed ~31% less than the individuals kept in regular stream water and fed conditioned leaf by distilled water. This maybe related with a compensatory consumption in order to overpass the feeding and waterborne toxicity; such behaviour seems to be triggered only when in the presence of low quality food.

In the case of alder an over conditioning due to a (non-significant) stimulation of decomposition may result in lower consumption in the presence of salt in comparison with the treatment with no salt in any case, even if alder per se is a highly consumed species (e.g. Canhoto and Graça 2006; Foucreau et al., 2013).

The lowest consumption rate (0.1 leaf DM/mg animal /day) of oak was recorded when animals were maintained in stream water and fed leaves conditioned in distilled water by *H. lugdunensis*. The lowest consumption towards leaves conditioned by this species might be due to inherent fungal properties (that may, for instance, be related with their stoichiometric composition (e.g. Gonçalves et al., 2014)) or to the presence of distasteful compounds (Graça 2001). In fact, this behaviour is in accordance with Butler & Suberkropp (1986) that report less palatability of oak leaf conditioned by the same species to *Psychoglypha sp*. (Trichoptera: Limnephilidae) larvae.

In contrast to oak, animals fed higher alder leaves conditioned by *H. lugdunensis* in distilled water and offered in stream water than leaves conditioned in salt enriched water and offered in stream water enriched with salt. Considering that this is a species that can “cope” with salinity, changes in its stoichiometric composition (eventually due to maintenance of osmolarity) may determine changes in preferences.

Globally the present results indicate that responses to contamination by salt, both by fungi and invertebrates, are largely dependent on the leaf quality existent in streams (e.g. Barlocher & Kendrick, 1973; Gonçalves et al., 2014). Consequences may occur in the availability of leaves to invertebrates (faster processing by fungi and/or invertebrates) in salt contaminated streams and cascading effects can be expected through the food chain.

Chapter 5

-FINAL REMARKS-

5. Final remarks

This study concluded that, salinity at a concentration of 4g/L didn’t affect the fungal decomposition activities of oak leaves, yet tend to enhance their activity in alder. The adverse effects of salinity in microbial respiration of both alder and oak leaves were detected. Moreover, the feeding rates of the shredder *Sericostoma vittatum* towards alder leaves was influenced by the presence of salt. On the contrary, likely due to a compensatory feeding behaviour, salinity of 4g/L enhanced their feeding towards oak leaves.

As stated by different studies (e.g. William 2001; Cañedo-Argüelles et al., 20013) salinization can be the result of several ions. Therefore, for a better understanding of the effect of salinization on leaf litter decomposition, nutrient cycling and leaf incorporation into secondary production should consider other ionic compositions, the inclusion of more species along with field approaches.

The present study is ecologically relevant considering the fact that global warming is expected to increase stream salinity (Cañedo-Argüelles et al., 2013). This will result an important effect on streams nutrient cycling and ecosystem functioning.

Chapter 6

-REFERENCES-

6. **References**

Allan, J. D., & Castillo, M. M. (2007). Stream Ecology. 2nd edition. pp. 317–357.Dordrecht: Springer Netherlands

Abel, T.H & Bärlocher, F., (1984). Effects of cadmium on aquatic hyphomycetes. Applied and Environmental Microbiology 48: 245–251.

Abelho, M. (2001). From litter fall to breakdown in streams: a review. The Scientific World Journal, 1, 656–80.

Anderson, N. H. and K. W. Cummins, (1979): Influences of diet on the life histories of aquatic insects. Journal of the Fisheries Board.36: 335–342.

Ardon, M., Pringle, C.M., (2008) Do secondary compounds inhibit microbial- and insect-mediated leaf breakdown in a tropical rainforest stream, Costa Rica? Oecologia, 155:311-323

Arsuffi TL, Suberkropp K (1985).Selective feeding by stream caddisfly (Trichoptera) detritivores on leaves with fungal-colonized patches. Oikos 45:50-58

Barlocher F (1987) Aquatic hyphomycetes spora in 10 streams of New Brunswick and Nova Scotia. Canadian Journal of Botany.65: 76–79.

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

Bärlocher F, Kendrick B, (1973). Fungi and food preferences of Gammarus pseudolimnaeus. Archiv für Hydrobiologie 72: 501-516.

Bärlocher F, Kendrick B, (1974). Dynamics of fungal populations on leaves in a stream. Journal of Ecology. 1974: 62: 761-791.

Bärlocher F, Kendrick B, (1975). Assimilation efficiency of Gammarus pseudolimnaeus (Amphipoda) feeding on fungal mycelium or autumn-shed leaves. Oikos 26:55-59.

Benfield, E.F. (1997) Comparison of litter fall input to streams. Journal of the North American Benthological Society. 16, 104–108.

Berenzina, N.A., (2002). Tolerance of freshwater invertebrates to changes in water salinity. Russian Journal of Ecology 34 (4), 261e266.

Boling, R. H., Goodman, E. D., Van Sickle, J. A., Zimmer, J. O., Cummins, K. W., Petersen, R. C., Reice, S.R. (1975). Toward a model of detritus processing in a woodland stream. Ecology, 56: 141-151.

Butler SK, Suberkropp K (1986) Aquatic hyphomycetes on oak leaves: comparison of growth, degradation and palatability. Mycologia 78:922-928

Byrne P.J, Jones E.B.G (1975) Effect of salinity on the reproduction of terrestrial and marine fungi. Transaction of the British Mycology Society, 65: 185–200.

Campos, J. and González, J. M. (2009), *Sericostoma vittatum* (Trichoptera) Larvae Are Able to Use Pine Litter as an Energy Source. International Review of Hydrobiology, 94: 472–483. doi: 10.1002/iroh.200811155

Cañedo-Argüelles M, Kefford BJ, Piscart C, Prat N, Schäfer RB, Schulz Jr C. (2013). Salinisation of rivers: an urgent ecological issue. Environmental Pollution: 173:157–67.

Cañedo-Argüelles, M., Bundschuh, M., Gutiérrez-Cánovas, C., Kefford, B. J., Prat, N., Trobajo, R., & Schäfer, R. B. (2014). Effects of repeated salt pulses on ecosystem structure and functions in a stream mesocosm. Science of the Total Environment, 476-477(APRIL): 634–642.

Cañedo-Argüelles, M., Grantham, T.E., Perrée, I., Rieradevall, M., Céspedes- Sánchez, R., Prat, N., (2012). Response of stream invertebrates to short-term salinization: a mesocosm approach. Environmental Pollution 166, 144e151.

Canhoto C. & Graça M. A. S. (1999). Leaf barriers to fungal colonization and shredders (Tipula lateralis) consumption of decomposing Eucalyptus globulus. Microbial Ecology, 37: 163–172.

Carvalho, E. M. And M. A. S. Graça, 2007: A laboratory study on feeding plasticity of the shredder Sericostoma vittatum RAMBUR (Sericostomatidae). – Hydrobiologia 575: 353–359.

Chauvet E & Suberkropp K (1998) Temperature and sporulation of aquatic hyphomycetes. Applied Environmental Microbiology. 64: 1522–1525.

Connolly, C. T., Sobczak, W. V., & Findlay, S. E. G. (2014). Salinity effects on phragmites decomposition dynamics among the Hudson River’s freshwater tidal wetlands. Wetlands, 34: 575–582

Cornut, J., Ferreira, V., Gonçalves, A. L., Chauvet, E. and Canhoto, C. (2015). Fungal alteration of the elemental composition of leaf litter affects shredder feeding activity. Freshwater Biology. doi: 10.1111/fwb.12606

Cummins, K. W. (1974). Structure and function of stream ecosystems. BioScience, 24:631-641.

Dang, C.K., Schindler, M., Chauvet, E., Gessner, M.O., (2009). Temperature oscillation coupled with fungal community shifts can modulate warming effects on litter decomposition. Ecology 90, 122e131

Demers, C.L., Sage, R.W., (1990). Effects of road de-icing salt on chloride levels in four adirondack streams. Water, Air, and Soil Pollution 49 (3e4), 369e373.

Dodds W.K (2002). Freshwater Ecology concepts and Environmental Applications. 1st edition. PP269-294. Academic press. An Elsevier science imprint.

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z. I., Knowler, D. J., Lévêque, C., Naiman, R. J., et al. (2006). Freshwater biodiversity: importance, threats, status and conservation challenges. Biological reviews of the Cambridge Philosophical Society, 81, 163–82.

Dunlop, J.E., Horrigan, N., McGregor, G., Kefford, B.J., Choy, S., Prasad, R., (2008). Effect of spatial variation on salinity tolerance of macroinvertebrates in Eastern

Australia and implications for ecosystem protection trigger values. Environmental Pollution 151, 621e630

FEIO, M. J. and M. A. S. GRAÇA, (2000). Food consumption by the larvae of Sericostoma vittatum (Trichoptera), an endemic species from the Iberian Peninsula. – Hydrobiologia 439: 7–11.

Ferreira F., Gulis V. & Graça, M.A.S. (2006b). Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. Oecologia, (in press), 149. doi: 10.1007/s0042-006-0478-0

Ferreira, V., Gonçalves, A. L., Godbold, D. L., & Canhoto, C. (2010). Effect of increased atmospheric CO2 on the performance of an aquatic detritivore through changes in water temperature and litter quality. Global Change Biology, 16, 3284–3296.

Ferreira, V., Graça, M.A.S. de Lima J.L.M.P & Gomes, R. (2006a).Role of physical fragmentation and invertebrate activity in the breakdown rate of leaves. Arch. Hydrobiol. 165:493–513

Ferreira, V., Gulis, V., Grac¸a, M.A.S., (2006). Whole-stream nitrate addition affects litter decomposition and associated fungi but not invertebrates. Oecologia 149, 718e729.

Flores, L., Larrañaga, A., and Elosegi.A. (2014). Compensatory feeding of a stream detritivore alleviates the effects of poor food quality when enough food is supplied. Freshwater Science,33:134-141

Foucreau, N., Puijalon, S., Hervant, F. and Piscart, C. (2013), Effect of leaf litter characteristics on leaf conditioning and on consumption by Gammarus pulex. Freshwater Biology, 58: 1672–1681. doi: 10.1111/fwb.12158

Fuss, C., and Smock, L.A. (1996) Spatial and temporal variation of microbial respiration rates in a blackwater stream. Freshwater Biology 36:339–349

Gessner MO, Suberkropp K & Chauvet E (1997). Decomposition of plant litter by fungi in marine and freshwater ecosystems. The Mycota, Vol. IV (Wicklow DT & S¨oderstr¨om BE, eds), pp. 303–322. Springer, Berlin/Heidelberg, Germany.

Gessner, M.O., Chauvet, E., and Dobson, M. (1999) A perspective on leaf litter breakdown in streams. Oikos 85, 377–384.

Goncalves, A. L., Chauvet, E., Bärlocher, F., Graça, M. A. S., Canhoto, C. (2014) Top-down and bottom-up control of litter decomposers in streams. Freshwater Biology, 59, 10: 2172-2182.

Gonçalves, A. L., Graça, M. a S. & Canhoto, C. (2013). The effect of temperature on leaf decomposition and diversity of associated aquatic hyphomycetes depends on the substrate. Fungal Ecology, 6: 546–553.

González, J. M. and M. A. S. Graça, (2003): Conversion of leaf litter to secondary production by a shredding caddis-fly. Fresh water Biology. 48: 1578–1592.

Graça MAS, Bärlocher F, Gessner MO, (2005). Methods to study litter decomposition. A practical guide. Springer, the Netherlands.

Graça, M. a S. & Canhoto, C. (2006). Leaf litter processing in low order streams. Limnetica, 25: 1–10.

GRAÇA, M. A. S. and C. CANHOTO, (2006): Leaf litter processing in low order streams. – Limnetica 25: 1–10.

Graça, M. A. S., C. Cressa, M. O. Gessner, M. J. Feio, K. A. Callies and C. Barrios, (2001) Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. Fresh water Biology. 46: 947–957.

Graça, M. A. S., L. Maltby and P. Calow, (1993) Importance of fungi in the diet of Gammarus pulex and Asellus aquaticus. II. Effects on growth, reproduction and physiology. – Oecologia 96: 304–309.

Graça, M., Cressa, M., Gessner, M., Feio, M., Callies, K., & Barrios, C. (2001). Food quality, feeding preferences, survival and growth of shredders from temperate and tropical streams. Freshwater Biology, 46, 947–957.

Hart, B. T. et al. (1991). A review of the salt sensitivity of the Australian freshwater biota. Hydrobiologia, 210: 105–144.

Herczeg, A.L., Dogramaci, S.S., Leaney, F.W.J., (2001). Origin of dissolved salts in a large, semi-arid groundwater system: Murray Basin, Australia. Marine and Freshwater Research 52 (1), 41e52

Horrigan, N., Choy, S., Marshall, J., Recknagel, F., (2005). Response of stream macroinvertebrates to changes in salinity and the development of a salinity index. Marine and Freshwater Research 56 (6), 825e833.

James, K. R., Cant, B., & Ryan, T. (2003). Responses of freshwater biota to rising salinity levels and implications for saline water management: a review. Australian Journal of Botany, 51, 703–713

Kaushal, S. S., Groffman, P. M., Likens, G. E., Belt, K. T., Stack, W. P., Kelly, V. R., … Fisher, G. T. (2005). Increased salinization of fresh water in the northeastern United States. Proceedings of the National Academy of Sciences of the United States of America, 102(38), 13517–13520

Kaushik NK & Hynes HBN (1968) Experimental study on the role of autumn-shed leaves in aquatic environments. The Journal of Ecology.56: 229–243.

Kefford, B.J., (1998). Is salinity the only water quality parameter affected when saline water is disposed in rivers? International Journal of Salt Lake Research 7 (4), 285e299.

Kefford, B.J., Hickey, G.L., Gasith, A., Ben-David, E., Dunlop, J.E., Palmer, C.G., Allan, K.,Choy, S.C., Piscart, C., (2012a). Global scale variation in the salinity sensitivity of riverine macroinvertebrates: Eastern Australia, France, Israel and South Africa. PLoS ONE 7 (5), e35224.

Kefford, B.J., Marchant, R., Schäfer, R.B., Metzeling, L., Dunlop, J.E., Choy, S.C., Goonan, P., (2011). The definition of species richness used by species sensitivity distributions approximates observed effects of salinity on stream macroinvertebrates. Environmental Pollution 159 (1), 302e310.

Kefford, B.J., Schäfer, R.B., Metzeling, L., (2012b). Risk assessment of salinity and turbidity in Victoria (Australia) to stream insects' community structure does not always protect functional traits. Science of the Total Environment 415 (0), 61e68.

Kostalos.M, Seymour.R.L. (1976). Role of Microbial Enriched Detritus in the Nutrition of Gammarus Minus (Amphipoda).Oikos 27:512-516

Krauss GJ, Solé M, Krauss G, Schlosser D, Wesenberg D, Bärlocher F, (2011). Fungi in freshwaters: ecology, Physiology and biochemical potential. FEMS Microbiology Reviews 35: 620-651.

Lecerf A & Chauvet E (2008) Diversity and functions of leaf decaying fungi in human-altered streams. Freshwater Biology 53: 1658–1672.

Lerotholi, S., Palmer, C.G., Rowntree, K., (2004). Bio assessment of a River in a Semiarid, Agricultural Catchment, Eastern Cape. In: Proceedings of the 2004 Water Institute of Southern Africa (WISA) Biennial Conference, Cape Town, South Africa, pp. 338e344.

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

Maloney.D.C. & Lamberty. G.A. (1995). Rapid decomposition of summer- input leaves in northern Michigan stream. American midland naturalist, 133, 184-185.

Martínez A., Larrañaga, A., Pérez, J., Basaguren, A., & Pozo J (2013), Leaf-litter quality effects on stream ecosystem functioning: a comparison among five species. Fundamental and Applied Limnology. Vol. 183:239–248.

Medeiros, A.O., Pascal, C. & Graça, M.A.S (2009). Diversity and activity of aquatic fungi under low oxygen conditions. Freshwater Biology, 54:142–149

Millán, A., Velasco, J., Gutiérrez-Cánovas, C., Arribas, P., Picazo, F., Sánchez-Fernández, D., Abellán, P., (2011). Mediterranean saline streams in southeast Spain: what do we know? Journal of Arid Environments 75 (12), 1352e1359.

Millennium Ecosystem Assessment, 2005. Ecosystems and Human Well-being: Synthesis. Island Press, Washington, DC.

Mohamed, D. J. & Martiny, J. B. H. (2011). Patterns of fungal diversity and composition along a salinity gradient. The ISME Journal, 5: 379–388.

Ostrofsky M.L (1997). Relationship between chemical characteristics of autumn-shed leaves and aquatic processing rates. Journal of the North American Benthological Society, 16:4

Petersen, R.C. and Cummins, K.W. (1974) Leaf processing in a woodland stream. Freshwater Biology. 4, 343–368.

Pinder, A.M., Halse, S.A., Mcrae, J.M., Shiel, R.J., (2005). Occurrence of aquatic invertebrates of the wheatbelt region of Western Australia in relation to salinity. Hydrobiologia 543, 1e24

Piscart, C., Moreteau, J.-C., Beisel, J.-N., (2005b). Biodiversity and structure of macroinvertebrate communities along a small permanent salinity gradient (Meurthe river, France). Hydrobiologia 551 (1), 227e236

Piscart, C., Usseglio-Polatera, P., Moreteau, J.-C., Beisel, J.-N., (2006). The role of salinity in the selection of biological traits of freshwater invertebrates. Archiv für hydrobiologie 166, 185e198.

Prat, N., Rieradevall, M., (2006). 25-years of biomonitoring in two Mediterranean streams (Llobregat and Besòs basins, NE Spain). Limnetica 25 (1e2), 541e550.

Ramirez A, Pringle C.M., and Molina L. (2003). Effects of stream phosphorus levels on microbial respiration. Freshwater Biology, 48:88–97

Reice SR, Herbst G. (1982). The role of salinity in decomposition of leaves of Phragmites australis in desert streams. Journal of Arid Environments 5: 361–368.

Ristanovic B. Miller. C.E (1968).Salinity tolerances and ecological aspects of some fungi collected from fresh-water, estuarine and marine habitats. Mycopathologia et mycologia applicata. 37:273-280 (http://link.springer.com/article/10.1007%2FBF02051361)

Schäfer RB, Bundschuh M, Rouch DA, Szöcs E, von der Ohe PC, Pettigrove V, et al (2012). Effects of pesticide toxicity, salinity and other environmental variables on selected ecosystem functions in streams and the relevance for ecosystem services. Science of The Total Environment. 415:69–78.

Schröder, M., Sondermann, M.,Sures, B. & Hering, D., (2015) Effects of salinity gradients on benthic invertebrate and diatom communities in a German lowland river. Ecological Indicators 57: 236–248

Silva, E.I.L., Shimizu, A., Matsunami, H., (2000). Salt pollution in a Japanese stream and its effects on water chemistry and epilithic algal chlorophyll-a. Hydrobiologia437 (1), 139e148.

Simões, S., Gonçalves, A.L., Guilhermino, L., Bärlocher, F. & Canhoto, C. Effects of stream salinization on fungal-mediated leaf decomposition: a microcosm approach (submitted) to be submitted to Fungal Ecology; in preparation)

Suberkropp K (1991) Relationships between growth and sporulation of aquatic hyphomycetes on decomposing leaf litter. Mycological Research95: 843–850.

Suberkropp K, Arsuffi TL, Anderson JP (1983) Comparison of degradative ability, enzymatic activity, and changes in palatability of aquatic hyphomycetes grown on leaf litter. Applied and Environmental Microbiology 46:237-244

Suberkropp K, Arsuffi TL, Anderson JP, (1983). Comparison of degradative ability, enzymatic activity, and palatability of aquatic hyphomycetes grown on leaf litter. Applied and Environmental Microbiology 46: 237-244.

Suberkropp K, Wallace JB, (1992). Aquatic hyphomycetes in insecticide-treated and untreated streams.Journal of the North American Benthologic Society 11: 165-171.

Taylor, B.R. and Bärlocher, F. (1996) Variable effects of air-drying on leaching losses from leaf litter. Hydrobiologia 325, 173–182.

Vannote, R. L., Minshall, G. W., Cummins, K. W., & Cushing, C. E. (1980). The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences, 37, 130-137.

Webster JR, Benfield EF, (1986). Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology, Evolution and Systematics 17: 567-594.

Williams, D.D., Williams, N.E., Cao, Y., (2000). Road salt contamination of groundwater in a major metropolitan area and development of a biological index to monitor its impact. Water Research 34 (1), 127e138.

Williams, M.L., Palmer, C.G., Gordon, A.K., (2003). Riverine macroinvertebrate responses to chlorine and chlorinated sewage effluents e acute chlorine tolerances of Baetis harrisoni (Ephemeroptera) from two rivers in KwaZulu-Natal, South Africa. Water South Africa 29 (4), 483e487.

Williams, W.D., (2001). Anthropogenic salinisation of inland waters. Hydrobiologia 466 (1), 329e337.

Wood, P.J., Dykes, A.P., (2002). The use of salt dilution gauging techniques: ecological considerations and insights. Water Research 36 (12), 3054e3062.

Woodward G, (2009). Biodiversity, ecosystem functioning and food webs in fresh waters: assembling then Jigsaw puzzle. Freshwater Biology 54: 2171-2187