



Universidade de Aveiro Departamento de Biologia

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**DIANA MARINA
GOMES DE CAMPOS**

**EFEITOS DO CÁDMIO EM CADEIAS TRÓFICAS DE
PROCESSAMENTO DE DETRITOS**

**EFFECTS OF CADMIUM ON A MODEL STREAM
DETRITUS PROCESSING CHAIN**



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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Aplicada, ramo de Microbiologia Clínica e Ambiental realizada sob a orientação científica do Doutor João Luís Teixeira Pestana, Investigador do Departamento de Biologia e CESAM - Centro de Estudos do Ambiente e do Mar, Universidade de Aveiro e co-orientação do Professor Doutor Marco Filipe Loureiro Lemos, Professor Adjunto da Escola Superior de Turismo e Tecnologias do Mar de Peniche - Instituto Politécnico de Leiria) e do Doutor Artur Jorge da Costa Peixoto Alves, Investigador Auxiliar do CESAM - Centro de Estudos do Ambiente e do Mar - Universidade de Aveiro.

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“Acredita que o tempo em que estamos com aqueles que nos querem bem é sempre um tempo ganho, como quem acumula pontos de felicidade para o futuro.”

Margarida Rebelo Pinto

Aos meus pais que todos os dias lutam para que o meu futuro seja sempre risonho. Ao Tiago e à minha irmã que me enchem o coração de amor.

o júri

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palavras-chave

cádmio, detritos, efeito indiretos, Espécies exóticas, interações entre fragmentadores – colectores, DGGE, cascatas tróficas

resumo

A integridade ecológica dos ecossistemas é na maior parte das vezes avaliada em termos de ocorrência de espécies e composição das comunidades e não em termos de relações tróficas entre espécies ou parâmetros funcionais. O processamento de detritos é um processo ecológico vital para os ecossistemas lóticos, mediado por microrganismos e invertebrados aquáticos. Nestas cadeias tróficas, os invertebrados fragmentadores contribuem para a ciclo de nutrientes através da transformação de matéria orgânica particulada grosseira em matéria orgânica particulada fina que serve de alimento para os invertebrados coletores. O nosso objetivo passou por avaliar os efeitos combinados do cádmio e da alteração na qualidade dos recursos (espécie de folha) na cadeia trófica de detritos e na interação entre fragmentadores – coletores.

Em laboratório folhas de amieiro (espécie nativa) e de eucalipto (espécie exótica) foram condicionadas em diferentes concentrações de cádmio (0, 50 e 200 µg/L). A comunidade microbiana presente nas folhas provenientes de diferentes tratamentos foi analisada através da técnica molecular DGGE e a taxa de respiração microbiana foi medida. *Sericostoma vittatum* (tricóptero fragmentador) e *Chironomus riparius* (um díptero coletor) foram expostos às mesmas concentrações de cádmio e alimentados com discos de folhas correspondentes. Foram avaliados, em microcosmos, o crescimento de *C. riparius* e a perda de peso das folhas.

O cádmio afetou o condicionamento das folhas e provocou alterações na diversidade de fungos em ambas as espécies de folhas. A comunidade microbiana presente no amieiro e no eucalipto mostrou reagir de maneira diferente ao cádmio. Este apenas afetou a decomposição das folhas de amieiro através da redução da alimentação dos invertebrados e também afetou o crescimento larvar de *C. riparius*. Esta espécie mostrou não ser exclusivamente coletora sendo capaz de se alimentar de folhas de amieiro na ausência de invertebrados fragmentadores. Contudo os fragmentadores mostraram promover o crescimento dos coletores no tratamento com eucalipto como fonte de alimentação. Estes resultados levam-nos a crer que a hipótese de facilitação entre fragmentadores - coletores pode ser tida como verdadeira mas que depende da qualidade dos recursos bem como das espécies de detritívoros presentes. Os resultados sugerem ainda que as cadeias de processamento de detritos podem ser ferramentas valiosas em ecotoxicologia de comunidades.

Mais estudos com a utilização de diferentes espécies de detritívoros e de outros stressores (naturais e antropogénicos) deverão continuar para melhor avaliar possíveis efeitos indiretos de contaminantes ao longo destas cadeias de processamento de detritos sob diferentes cenários ecológicos.

keywords

cadmium, indirect effects, exotic species, shredder-collector interactions, DGGE, trophic cascades

abstract

The ecological integrity of ecosystems is often evaluated in terms of species occurrence and composition of communities and not in terms of trophic relationships between species or functional parameters. The processing of detritus is a vital ecological process for freshwater ecosystems, mediated by microorganisms and aquatic invertebrates. Here, shredders contribute to the nutrient cycle, transforming coarse into fine particulate organic matter which serve as food for collectors. The aim of this work was to evaluate the combined effects of cadmium exposure and changes in resource quality (leaf species) on detritus trophic chain and on shredder–collector interactions.

In the laboratory, alder leaves (native species) and eucalyptus (exotic species) were conditioned at different cadmium concentrations (0, 50 and 200 µg/L). The microbial community present in leaves from the different treatments was analyzed by molecular DGGE technique and microbial respiration rates were measured. *Sericostoma vittatum* (a caddisfly shredder) and *Chironomus riparius* (a midge collector) were exposed to the same concentrations of cadmium and fed the corresponding leaf discs. *C. riparius* growth and leaf weight loss were evaluated in these multispecies microcosms.

Cadmium exposure affected the leaf conditioning and the reduction in fungal diversity in both leaf species was clear. Cadmium affected the decomposition of alder leaves through reductions in invertebrate feeding and also impaired *C. riparius*' growth.

C. riparius showed to be a non-exclusive collector and capable of feeding on alder leaf discs in the absence of shredders. However shredders appear to promote collectors growth in treatments with eucalyptus as food source.. The observed pattern suggest that shredder-collector facilitation hypothesis can hold true and also that it might be dependent on resource quality and detritivore species present.

The results suggest that detritus processing chains can be valuable tools to community ecotoxicology. We advocate that these investigations should continue with the use of different detritivore species and stressors (natural and anthropogenic) to improve our current understanding of indirect effects of contaminants in detritus based food webs under different ecological scenarios.

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Chapter I: General Introduction

General Introduction

1. Trophic cascades and indirect effects of contaminants

The ultimate goal for ecotoxicologists is to understand the effects of anthropogenic toxicants on the ecosystem structure and function. However, most ecotoxicological studies are focused on the direct effects of contaminants on single species in order to estimate permissible levels of contamination (Long et al. 1995; Rohr et al. 2006). These maximum accepted levels of contamination are usually derived from laboratory toxicity tests using a limited number of model species in single toxicant exposures (Fleeger et al. 2003). The direct effects of toxicants are typically assessed in terms of changes in mortality or sub-lethal endpoints (e.g. physiology, fecundity, behavior) and there is a wide range of tolerances to a particular toxicant (Fleeger et al. 2003). Nevertheless, contaminants may exert effects on tolerant species through their interactions with other species that are directly affected by toxicity (Fleeger et al. 2003; Rohr et al. 2006). Such effects are called indirect effects of contaminants and may be difficult to predict (Pace et al. 1999; Rohr and Crumrine 2005; Rohr et al. 2006). For instance, toxicant-induced suppression of competition or predation may have a beneficial effect for a particular species (Pace et al. 1999), while on the other hand, a reduction in prey density can cause a decline in predator populations despite of their tolerance to the toxicant (Hanazato and Yasuno 1987).

By definition, single species toxicity tests are unable to detect and evaluate indirect effects of contaminants since these result from complex ecological mechanisms such as interspecific interactions like predation or competition (Fleeger et al. 2003).

Indirect effects of contaminants are now the focus of many ecotoxicology and ecological risk assessment studies and modern experimental ecotoxicology should be focused on characterizing common indirect effects resulting from ecosystem disturbances and quantifying the relative magnitude of direct and indirect effects of contaminants (Fleeger et al. 2003). Progress in this research field is urgent since investigations using a combination of organismal, population and community responses will enable scientists to evaluate how community context alters toxicity and a contribute to reliable predictions of

anthropogenic impacts on natural ecosystems (Fleeger et al. 2003; Clements and Rohr 2009).

Trophic cascades are examples of indirect effects mediated through consumer-resource interactions and are often considered in terms of "top-down" (influence of predators on lower trophic levels) and "bottom-up" (nutrient / food / prey influence on higher trophic levels) effects (Pace et al. 1999; Schmitz et al. 2004). Thus, integrating food-web ecology into the design and implementation of ecotoxicological research is essential for the understanding and prediction of indirect effects of contaminant within natural ecosystems (Relyea and Hoverman 2006; Rohr et al. 2006).

Studies of trophic cascades have focused mainly on autochthonous resource-based food webs, in which basal resources are derived from *in situ* primary production (Huryn 1998; Narwani and Mazumder 2012). However, many aquatic food webs receive energy from allochthonous matter which is vital for ecosystem functioning since terrestrial detritus derived mainly from leaves from riparian vegetation form the basis of secondary production in headwater streams (Vannote et al. 1980; Abelho 2001).

Moreover, the decomposition of organic matter and detritivorous macroinvertebrates have been widely used to monitor the ecological status of freshwaters (Naddafi et al. 2007). As such, biotic interactions between detritivore species can mediate a large number of indirect effects of contaminants in freshwater ecosystems and are ideal for community ecotoxicological manipulative experiments.

2. Detritus processing chains

Autochthonous matter from primary production of the stream and allochthonous matter from the surrounding riparian vegetation are the two main sources of organic matter in freshwater ecosystems (Abelho 2001). When headwater streams are flanked by dense riparian vegetation, light penetration becomes which, together with low water temperatures, limit the primary production (Friberg et al. 1997) making allochthonous matter extremely important for this type of ecosystems.

The allochthonous organic matter supplied to headwater streams is variable and may include leaves and leaf fragments, floral parts, bark, wood (branches and twigs), cones and nuts, fruits, and other plant parts. Most of the litterfall that reaches streams is however composed of leaves (Abelho 2001).

These leaves after entering the streams suffer decomposition by different physical, chemical and biological processes such as leaching, physical abrasion, microbial colonization (conditioning), and fragmentation by invertebrate shredding (fig.1) (Feio and Graça 2000; Abelho 2001; Allan and Castillo 2007).

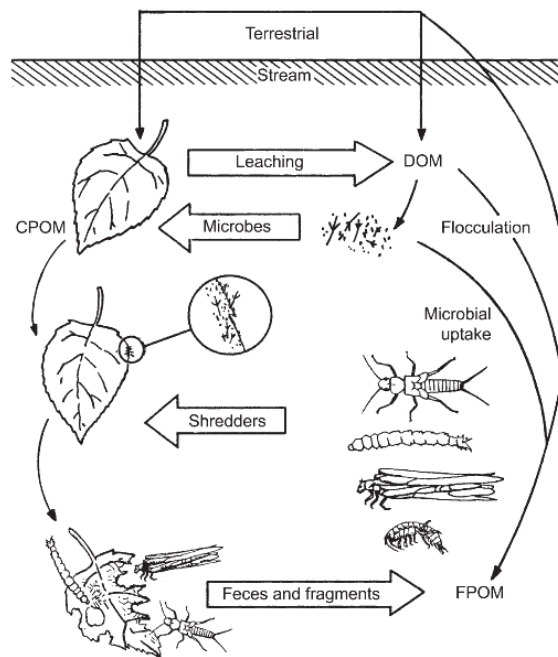


Figure 1 – Representation of different process involved in decomposition of leaf litter (adapted from Allan and Castillo 2007).

Leaching can be relatively fast (from 2 to 7 days) and can cause a substantial decrease in initial weight of the leaves. However, some soluble compounds are released over longer periods of time (Abelho 2001). The leaf litter is then colonized by microorganisms (conditioning). The conditioning is often considered one of the most important processes that determine organic matter degradation rates (Rader et al. 1994; Abelho 2001). Lastly the leaves suffer fragmentation. This process can occur by physical abrasion, by action of fungi, and by feeding activities of invertebrates, consisting on the transformation of the leaf litter into smaller particles (Gessner et al. 1999; Abelho 2001). Although shredders activity is important mechanism for the litter breakdown in low-order

streams (Graça et al. 2001), their importance change according to the latitude and altitude of ecosystems in question (Irons III et al. 1994).

All these processes are affected by several factors such as the structure and chemical composition of the leaf and the temperature and chemical composition of the water (i.e., alkalinity, pH and nutrient concentration) (Abelho 2001; Allan and Castillo 2007).

The processes involved are thus sequential being described as a processing chain (Heard 1994). During conditioning, the microbiota is an important mediator of detritus processing. The leaves that fall in the stream are quickly colonized by bacteria and fungi, especially aquatic hyphomycetes (Bärlocher and Kendrick 1975). These aquatic microorganisms are crucial in many biochemical processes, where they play an important role in the recycling of organic matter and also contribute to food web energy transfer (Duarte et al. 2012). During the conditioning stage, microorganisms have the ability to increase the palatability of the leaves since they excrete enzymes capable of degrading molecules such as cellulose and lignin into simpler compounds (Sinsabaugh and Linkins 1990). Thus microorganisms make the leaf litter more appropriate and nutritious for detritivores invertebrates (Abelho 2001; Krauss et al. 2011). Within microorganisms, fungi play the most important role in the degradation of leaf litter ((Kaushik and Hynes 1968). Detritivore invertebrates have a clear preference for leaves incubated under conditions which allow fungal colonization since fungi conditioned leaves are more palatable than the sterile leaves or leaves conditioned only with bacteria (Mackay and Kalff 1973). Moreover fungi increase the protein content of leaf litter. In fact, several studies have shown that the growth of fungi on recently fallen leaves generally improves the growth of aquatic invertebrates (Krauss et al. 2011). The mycelium itself is usually more efficiently assimilated than the digested leaf material (Krauss et al. 2011).

The fungal community present in leaf detritus may be evaluated by molecular techniques, such as Denaturing Gradient Gel Electrophoresis (DGGE) since traditional methods can leave gaps in our knowledge. The community of fungi present on the leaves has been traditionally evaluated by identification of their characteristic conidia, which were then stained and identified under a light microscope (Gessner et al. 2003; Bärlocher 2005). Moreover the community of bacteria has been evaluated using methods of cultivation, or by staining bacterial cells with fluorescent dyes (Suberkropp and Klug 1976; Hieber and Gessner 2002). Thus a large part of the microbial biodiversity assessment

could be underestimated since non-sporulating fungi as well as the non-cultivable bacteria were not addressed, thus showing that the conventional microscopy and the traditional culture-dependent techniques are not sufficient for the correct assessment of microbial communities (Duarte et al. 2012). Molecular approaches come fill such limitations since they are independent of the cultivation and do not require the presence of reproductive stages to evaluate microbial communities (Bärlocher 2007). For the evaluation of environmental samples various techniques based on 16S rRNA gene of bacteria and the 18S rRNA gene and ITS region (Internal Transcribed Spacer) of fungi have been used. The DGGE technique has been applied to microbial ecology for almost 20 years. However only more recently this technique was applied to evaluate the diversity of microorganisms in decomposition of leaf-litter in freshwaters (Duarte et al. 2012). Used for this purpose, the DGGE has been shown to be a sensitive tool to discriminate microbial communities from reference and impacted sites (e. g. (Duarte et al. 2009; Sridhar et al. 2009)). Moreover, it has proven useful for detecting changes in the structure of aquatic microbial communities exposed to anthropogenic stressors in microcosms (Duarte et al. 2012).

The technique consists in the separation of fragments that have the same size but have different nucleotide sequences. This separation of DNA fragments occurs in a denaturing gradient polyacrylamide gel and is based on their profile of denaturation (Fischer and Lerman 1983; Muyzer et al. 1993). During the process the DNA fragments will be denatured, i.e. the double chain will be separated until a determinate specific concentration of denaturant and stops its migration in the gel. The double chain do not denature completely, since in the 5' end it is connected to a GC clamp (DNA sequence of 40 to 60 nucleotides) that has very high melting domain. At the end the fragments with different melting points will migrate in different ways and occupy different positions in the gel (Muyzer et al. 1993). Each gel band corresponds to a phylotype or operational taxonomic unit (OTU) or a unique "sequence type" and is treated as a group of fungal cells that belong to the same type (Duarte et al. 2012). This technique has the advantage of "taking a picture" of the diversity and structure of microbial communities in various environmental samples in only a few hours. Furthermore, the DGGE allows simultaneously to process and compare many samples facilitating the evaluation of locations with different environmental conditions. Moreover, the DGGE allows us to obtain taxonomic information, because bands can be excised, re-amplified, sequenced and specific bands can hybridize with specific oligonucleotides probes (Heuer et al. 1999; Riemann and Winding 2001). As other techniques based on DNA, this technique reveals

some limitations such as the separation of fragments that have only up to 500bp; different sequences can take identical positions in the gel due to the similarity in GC content and thereby a band may not represent a single species.

The chemical composition and structure of the leaf itself cause variations in its breakdown rates (Allan and Castillo 2007). Therefore, in streams, the leaf processing is dependent of the leaf species. According to leaf species, the nutrient content, toughness and secondary compounds are different. These three factors are very important for the colonization of leaves by microorganisms and by invertebrates (Graça 2001). For example, the toughness of leaves can be a physical barrier for invertebrates, because tougher leaves are more difficult to break than softer leaves and consequently invertebrates have greater difficulty to feed on them. The nutrient content of leaves can also affect the leaf processing in streams. In general detritivores show preference for leaves with higher nutrient content (Irons et al. 1988). In laboratory conditions authors such as Canhoto and Graça (1995), Schulze and Walker (1997), and Graça and Cressa (2010) gave shredders a choice between different leaves types and observed that they preferred to feed on certain leaf types rejecting others. These preferences are usually related to nutrient content and with leaf colonization by microorganisms (Graça 2001). Feeding preference can also be dependent on the fungal species present (Arsuffi and Suberkropp 1989). On the other hand, secondary compounds present in leaves (polyphenolics, flavonoid pigments, saponins, others) can be toxic and interfere with organisms digestion (Graça 2001). For example, eucalyptus has been shown to be responsible for affecting macroinvertebrate communities as well as microbial communities, due to polyphenols, essential oils and surface waxes in its constitution (Graça et al. 2002). The polyphenols are considered important defense in the leaves against herbivores, since they bind to proteins, including enzymes, causing digestion blocking (Graça et al. 2002).

Detritivore species richness and density also affect leaf processing in streams. Dangles and Malmqvist (2004) observed that “diversity– function relationship” is influenced by the composition of the shredders community and that decomposition rates are dependent on the identity of the dominant species (Dangles and Malmqvist 2004).

Due to the factors described above different types of leaves have different rates of degradation in streams (Canhoto and Graça 1996) and can affect detritivores growth (Abelho 2008; Graça and Cressa 2010).

3. Shredders collector interactions and the facilitation hypothesis

The forest streams tend to be dominated by detritivorous organisms that feed on leaf litter derived organic matter. Within these organisms two large functional groups can be distinguished: shredders and collectors (Cummins 1974). Shredders consume coarse particulate organic matter (CPOM), turn it into fine particulate organic matter (FPOM) which serves as food for the collectors (Cummins 1974; Vannote et al. 1980). Shredders thus contribute to the processing of CPOM and by producing non-ingested particles and by faeces, promote the collectors' performance. This shredder-collector facilitation hypothesis (Heard and Richardson 1995) states that in the presence of shredders, growth or survival of collectors is favored either on site or downstream. This facilitation hypothesis can be formulated at the population level involving particular species of shredders and collectors, at the ecosystem level involving shredder and collector functional groups in the aggregate. It should be noted that the assumption of this connection as direct and positive although generally and theoretically accepted is not well founded, existing few studies focused on testing this hypothesis (Heard and Richardson 1995).

4. Thesis objectives and Model trophic chain used

The aim of this thesis was thus to evaluate the combined effects of cadmium and changes in resource quality (leaf species), on freshwater detritus based food-webs, and assess how these factors may compromise the leaf litter decomposition. Laboratory assays were done with a model detritus food chain where leaf species are manipulated to assess effects of cadmium on shredder-collector interactions under different ecological scenarios.

Cadmium behavior and toxicity to aquatic life is well documented, so it was chosen as a model toxicant in this study (Planelló et al. 2010). Sources of cadmium are varied, for

example: mining, wastewaters discharges and agriculture runoffs (Eaton et al. 2001). Cadmium accumulates in sediment and may therefore affect the benthic biota with potential consequences to higher levels of the food chain (Oskarsson et al. 2004). Exposure to cadmium has been reported to cause adverse effects on detritivore invertebrates (Vogt et al. 2010) and impair the processing of detritus in freshwaters (Giesy Jr 1978; Schaller et al. 2011).

The proposed simplified detritivore food-web is composed of leaf detritus (*Alnus glutinosa*), one shredder species (*Sericostoma vittatum*) and a collector species (*Chironomus riparius*). Alder was chosen because is a native species common to many portuguese headwater streams (Canhoto and Graça 1996). Both organisms are detritivore macroinvertebrates common in portuguese streams and have been used to monitor the accumulation of metals (Vogt et al. 2010) and insecticide in freshwaters (Rasmussen et al. 2012). Due to their behavior and characteristics, these organisms have an important influence on nutrient cycles, translocation of materials, decomposition and primary productivity (Wallace and Webster 1996). The caddisfly *Sericostoma vittatum* Rambur (Trichoptera: Sericostomatidae) is an endemic species of the Iberian Peninsula that occurs throughout the year, feeding on conditioned leaves and is among the most abundant consumers in many streams in the region (Feio and Graça 1997). These organisms have relatively long life-cycles with several aquatic stages (eggs, larvae and pupae) and an aerial phase (adult). These aquatic larvae may play an important role in the fragmentation of allochthonous matter of streams (González and Graça 2003). Caddisflies are important preys for many species of fish and were previously used in toxicological studies (Schulz and Liess 1999, 2000; Berra et al. 2006; Pestana et al. 2009). The midge *Chironomus riparius* Meigen (Diptera: Chironomidae) has a short life-cycle, contrary to the Sericostomatidae, and includes aquatic stages (eggs, four larval stages and pupa) and an aerial phase (adult), being the larval phase the most enduring. The Chironomidae are extremely important organisms in freshwater ecosystems, and they dominate the benthic communities of lotic and lentic environments both in number and in biomass (Merritt and Cummins 1996). Chironomids can be found in different aquatic environments due to their capacity to adapt to environments with extreme conditions of pH, temperature, depth and salinity (Armitage et al. 1995). Chironomids are easy to grow and maintain in the laboratory, and because of their direct contact with the sediment *C. riparius* larvae have been used as a model organism in many sediment toxicity studies both in the laboratory as *in situ* where endpoints such as larval behavior, growth, survival and emergency are

evaluated (Chappie and Burton Jr 1997; OECD 2001; Soares et al. 2005; Faria et al. 2006; Domingues et al. 2007; Pestana et al. 2009).

This simplified detritus based food web was also used to test the effects of changing resource quality. The introduction and spread of exotic species can cause impacts on native ecosystems, and this impact ranks second as a threat to biodiversity, being surpassed only by habitat loss (Sala et al. 2000). The chosen species was *Eucalyptus globulus* Labill. This exotic species from Australia was introduced in Portugal in 1829. Today vast areas of the Iberian Peninsula are covered with eucalyptus monocultures and this species is already part of the riparian vegetation. This species is known to adversely affect aquatic ecosystems causing problems to organic matter dynamics when replacing the native vegetation (Graça et al. 2002).

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**Chapter II: Effects of Cadmium and changes in resource quality
(leaf species) on freshwater detritus based food webs**

Effects of Cadmium and changes in resource quality (leaf species) on freshwater detritus based food webs

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Abstract

Detritus processing is a vital ecological process for freshwater ecosystems being dependent on the leaf litter from riparian vegetation and mediated by microorganisms and aquatic invertebrates. Detritivorous shredders contribute to the nutrient cycle, transforming coarse organic matter into fine particulated organic matter which serves as food for collectors. Direct and indirect effects of contaminants and natural stressors can impair the processing of detritus and affect the functioning of these ecosystems. The aim of this work was to evaluate the combined effects of cadmium exposure and changes in resource quality (leaf species) in detritus trophic chain and on shredder – collector interactions. In the laboratory, alder leaves (native species) and eucalyptus (exotic species) were conditioned at different cadmium concentrations (0, 50 and 200 µg/L). The microbial community present in leaves from the different treatments was analyzed by DGGE and microbial respiration rates were measured. *Sericostoma vittatum* (a caddisfly shredder) and *Chironomus riparius* (a midge collector) were exposed to the same concentrations of cadmium and fed the corresponding leaf discs. *C. riparius* growth and leaf weight loss were evaluated in these multispecies microcosms. Cadmium exposure affected the leaf conditioning and the reduction in fungal diversity in both leaf species was clear. Cadmium exposure affected the decomposition of alder leaves through reductions in shredder feeding and also impaired *C. riparius* growth. *Chironomus riparius* showed to be a non-exclusive collector and capable of feeding on alder leaf discs in the absence of shredders. However, shredders appear to promote collectors growth in treatments with eucalyptus as food source. The observed results support the shredder-collector facilitation hypothesis but also that it is dependent on resource quality and detritivore species present.

Key words: leaf decomposition, trophic cascades, indirect effects, exotic species, shredder-collector interactions

Introduction

Trophic cascades resulting from predator-prey interactions result in inverse patterns of abundance, biomass or productivity of a population community or trophic level across trophic links in food webs, can have strong impacts on ecosystems (Pace et al. 1999). Trophic cascades arise due to strong species interactions within food webs and are generally considered in terms of top-down (predator influence on lower trophic levels) and bottom-up effects (nutrient /food / prey influence on higher trophic levels). The majority of studies on trophic cascades have been based on autochthonous resource-based food webs with primary producers at the base of the food chain (Dieterich et al. 1997). However, one of the major energy sources in low order streams are detritus from the surrounding riparian vegetation (Wallace et al. 1997). Detritus, mainly leaf litter are of extreme importance since dense riparian corridors cause low water temperature (Friberg et al. 1997) and insufficient light penetration which compromises the primary production (Abelho 2001). Leaf litter constitute the primary source of food for many organisms in many stream and rivers (Graça 2001). These include microorganisms colonizing detritus and benthic detritivore macroinvertebrate species (Wallace et al. 1997; Graça 2001).

The decomposition of this organic matter can be considered a processing chain since the resource (leaf litter) passes through a temporal sequence of condition or quality changes, that is used by different consumers (bacteria, fungi and invertebrate detritivores) (Heard 1994). Moreover, within the detritivore species two large functional groups can be distinguished in these chains: shredders and collectors (Heard and Richardson 1995). Shredders feed on conditioned leaf litter and convert coarse particulate organic matter (CPOM) into fine particulate organic matter (FPOM) that can be used by collector species which are particle limited (Cummins 1974; Vannote et al. 1980). Shredders can thus promote growth or survival of collector organisms within these detritus processing chains. This shredder-collector facilitation hypothesis may be placed at the population level or community levels and can be important on local and larger spatial scales in terms of stream food webs (Heard and Richardson 1995).

Few studies have addressed trophic cascades on detritus based food webs and are focused on the effects of variation on the quality of resources and on performance of single detritivore species (Canhoto and Graça 1999; Campos and González 2009), or effects of predators on detritus processing (Ruetz et al. 2002). A small number of studies have considered possible indirect effects of contaminants or changes of detritus quality

along these processing chains with possible consequences for secondary production and ecosystem functioning (Narwani and Mazumder, 2012).

Different leaf species have different decomposition rates and nutritional value for invertebrate detritivores (Graça 2001; Graça and Cressa 2010). For instance *Eucalyptus globulus*, an Australian species now occupies vast areas of the Iberian Peninsula and it is becoming a common riparian tree species (Graça et al. 2002; Larrañaga et al. 2009). The impact of these plantations on stream invertebrates has been the focus of ecological studies in recent years (see review (Graça et al. 2002)). However, to our knowledge there is no existing research concerning trophic cascades caused by this alteration of resource quality. The same can be said for effects of anthropogenic chemical stressors and their possible indirect effects on collectors along detritus processing chains.

Based on these research gaps, with this work, the study of the possible effects of eucalyptus leaves and metal exposure along detritus processing chains is addressed. A simplified model detritus processing chain composed of alder leaves, a caddisfly shredder and a dipteran collector species was used. *Sericostoma vittatum* Rambur (Trichoptera: Sericostomatidae) was chosen as a model shredder detritivore since it is an endemic species of the Iberian Peninsula, and are among the most abundant detritivore consumers in many streams, playing an important role in the fragmentation of allochthonous organic matter (Feio and Graça 2000). *Chironomus riparius* Meigen (Diptera: Chironomidae) was chosen as the model collector species due to its high ecological relevance in terms of biomass within benthic communities in all freshwater systems (Stief and de Beer 2002). Moreover both species are already used in aquatic toxicology studies (OECD 2001; Soares et al. 2005; Faria et al. 2006). The main objective of this work was to assess the effects of metal exposure on detritus processing and shredder-collector interactions and evaluate how the change in leaf species can mediate these effects. Microcosms tests were performed where the effects of cadmium and leaf species were evaluated in terms of *C. riparius* growth and leaf processing with or without the presence of the caddisfly shredder.

Methods

2.1 – Test Organisms

Sericostoma vittatum Rambur (Trichoptera: Sericostomatidae) were collected at the S. João stream, Lousã, Portugal (40°06'N, 8°14'W). The organisms were acclimated in the laboratory for at least one week, in ASTM at 20°C with light-dark regime/cycle of 16:8h and fed *ad libitum* with alder leaves (*Alnus* sp.).

Egg masses of *Chironomus riparius* Meigen (Diptera: Chironomidae) used for the experiment were obtained from a laboratory culture at the University of Aveiro, Portugal, and established for more than 10 years. They were maintained under standard hard water ASTM (ASTM 2000) conditions, at a temperature of 20°C and with light-dark regime of 16:8h and fed with a suspension of ground commercial fish food (TetraMin®, Melle, Germany). Two-day-old larvae (1st stage) were used throughout the experiments.

2.2 - Leaf Conditioning

Eucalyptus and alder leaves were collected in autumn after abscission in reference locations, near Aveiro. Leaves were air dried and stored in the dark. Leaves were soaked in distilled water and leaf discs (Ø 10mm) were produced with a cork borer.

For the conditioning we used an inoculum composed of 400mL of distilled water with 50 gr of grinded eucalyptus alder leaves collected in a reference stream. Conditioning was performed by adding 25 ml of inoculum to 1L of enriched water (Fuller 1978) with 200 leaf discs in separated batches during 15 days at 20°C with strong aeration. Cadmium chloride (technical grade, CASNo. 10108-64-2, Sigma–Aldrich, USA) was used to obtain three concentrations (0, 50 and 200 µg/ L).

2.3 - Analysis of leaf microbial community

2.3.1 – Microbial respiration (measure of oxygen consumption)

From each conditioning treatment, four sets of five leaf discs were placed into gastight syringes (Hamilton Co., Reno, Nevada, USA) with 50 ml of previously oxygenated

ASTM at 20 ° C. Four sets of syringes without leaf discs were used as a control for each treatment to correct the oxygen depletions due to factors other than microbial conditioning of leaves. After one hour, the initial concentration of oxygen was measured with an oxygen meter (Model 782, Strathkelvin Instruments, Glasgow). The final concentration of oxygen was measured 6 hours after the beginning of the test following same procedure. The respiration rate is expressed in $\mu\text{g O}_2/\text{mg of leaf / hour/ ml}$ using the results obtained in controls (no leaf discs) as correction factors in each treatment (leaf species and cadmium concentration).

2.3.2 – DNA extraction, amplification and DGGE

For DNA extraction, after conditioning, five leaf disks were frozen (-80°C), and DNA was isolated with the UltraClean® Soil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's instructions.

DNA isolated from each sample was amplified by PCR with primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2 (5'-GCTGCGTTCTTCATCGATGC-3') directed to the ribosomal ITS region. A GC clamp (5'-CGCCCGCCGCGCGCGGGCGGGGCGGGGGCACGGG-3') was attached to the 5' end of the forward primer. PCR reactions were carried out with reagents supplied by Promega (Madison, WI USA). The PCR reaction mixture contained 1X GoTaq buffer, 3 mM MgCl₂, 0.2 mM of dNTPs, 5% DMSO (dimethylsulfoxide), 10 pmol of each primer, 1U of *GoTaq* polymerase and 1 μL of template DNA in a final volume of 25 μL . The PCR amplification was performed on a Mycycler (Bio-Rad, Laboratories, Hercules, CA, USA) under the following conditions: 95 ° C for 3 min, followed by 35 cycles of 94°C for 30 sec (denaturation), 50°C for 30 sec (annealing), 72°C for 30 sec (extension) and a final extension at 72°C for 30 min. In order to accomplish a suitable amount of amplicon it was necessary to perform a second round of PCR amplification using as template 1 μL of the first PCR amplification.

DGGE was performed on a DCode™ Universal Mutation Detection System (Bio-Rad). The PCR amplicons were loaded on 8% polyacrylamide gel in 1 X Tris-acetate-EDTA (TAE) with a denaturing gradient of 20-50% (100% denaturant corresponds to 40% formamide and 7 M urea). The electrophoresis was performed in 1X TAE at 20V for 15 min and after at 75 V for 16h. The gel was stained 5 min with 1 $\mu\text{g/mL}$ EtBr (ethidium

bromide) solution, then rinsed 20 min in distilled water and visualized under UV light using a Gel Doc imaging system (Bio- Rad Laboratories, Hercules, CA). DGGE banding profiles were analyzed with GelComparII software (Applied Maths). Gels were normalized using the same standard loaded at both sides. The “rolling disk” background subtraction option was applied. DNA bands detected by the software were carefully verified by visual examination to correct unsatisfactory detection. Variations in band intensity were not considered to be differences. Levels of similarity between the profiles were calculated with the band matching Dice coefficient. Cluster analysis of similarity matrices was performed by the unweighted pair group method using arithmetic averages (UPGMA).

2.4 – Detritivore trials

For the leaf processing experiments, testing for leaf species, cadmium exposure and the presence/ absence of shredders, seven replicates with one caddisfly and 10 *C. riparius* larvae (1st stage larvae) and seven replicates with only 10 *C. riparius* larvae were used. Animals were allocated to plastic vessels, containing a 1 cm layer of inorganic fine sediment (<1 mm), 150 mL of experimental medium (same Cd concentrations as in the conditioning) and food in the form of 10 alder or eucalyptus leaf discs (Ø 10mm). Leaf discs from the respective conditioning treatments in each replicate were dried at 50° C for 2 days and weighed. They were soaked in the respective cadmium solutions for 96 h prior to use. The *S. vittatum* was placed one day before the *C. riparius*. After 10 days animals and the respective remaining food (leaf discs) were removed, dried at 50° C for 4 days and reweighed. Caddisflies were removed and *C. riparius* larvae were counted and kept in ethanol 70% for measurement. *C. riparius* growth was estimated by measuring the total length and head capsule width of each larva at day 10 with a stereo dissecting microscope fitted with a calibrated eye-piece micrometer.

Leaf weight loss was calculated as the difference between the initial and final leaf disc dry mass (mg). Three control cages per treatment with leaf discs and no animals (shredders or collectors) were used to correct for weight change due to factors other than feeding. All tests were conducted at 20 ± 1° C with a photoperiod of 16 h light: 8 h dark.

2.5 – Statistical analysis

Two-way analysis of variance (ANOVA) with Holm-Sidak multiple comparison of group means was employed to determine significant differences relatively to control

treatment. Where applicable, results are presented as mean±SE. For all statistical tests the significance level was set at $P \leq 0.05$. All calculations were performed with SigmaStat (Systat Software Inc. 2006).

Results

3.1 Effects of cadmium on microbial conditioning.

Microbial respiration in alder leaf discs was significantly different compared to eucalyptus leaf discs across all cadmium treatments (fig 1, table1) revealing higher microbial biomass conditioning alder leaf discs. However for each leaf species a clear trend in terms of effects of cadmium exposure on oxygen consumption rates was not observed. For alder, the lowest concentration of cadmium caused a significant increase in the rate of microbial respiration and for eucalyptus increasing cadmium concentrations reduced, although not significantly, microbial respiration of leaf discs.

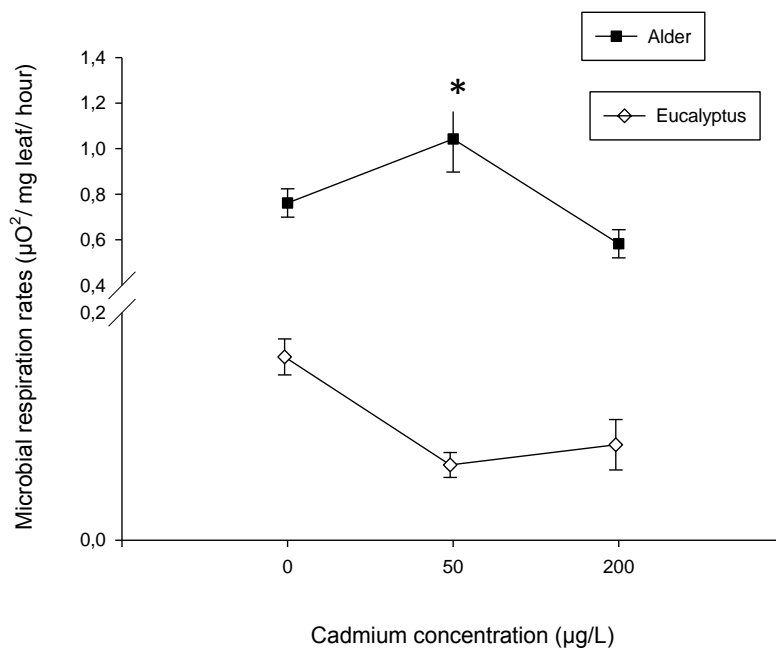


Figure 1- Microbial respiration rate (mean +/- SE) in two different species of leaves (alder: black square and eucalyptus: white diamond) according with no (control), low (50 $\mu\text{g/L}$) or high (200 $\mu\text{g/L}$) cadmium concentration.

Asterisks denote statistically significant differences ($P < 0.05$) compared to the control treatment (no cadmium).

Table 1 - Two-Way ANOVA results testing for effects of leaf species, cadmium concentrations and their interaction on microbial respiration rates.

Factor	Df	F	P-value
Species of leaf	1	144,672	<0,001
[Cd]	2	5,002	0,019
Species of leaf × [Cd]	2	6,424	0,008

DGGE profiles obtained from our conditioned leaf discs are represented in figure 2. Eucalyptus and alder showed different fungal communities, which were altered in the presence of cadmium (Fig 2). By analysis of the dendrogram it can be observe that fungal communities of conditioned alder leaf discs under cadmium exposure are considerably different from fungal communities of control leaf discs (no cadmium exposure). In alder leaf discs, a clear reduction of the number of bands, from 39 in the control treatment (AC0) to 25 in the conditioning treatment with high cadmium concentrations is observed. In eucalyptus leaf discs a higher concentration of cadmium is necessary to significantly affect its fungal conditioning (figure 2).

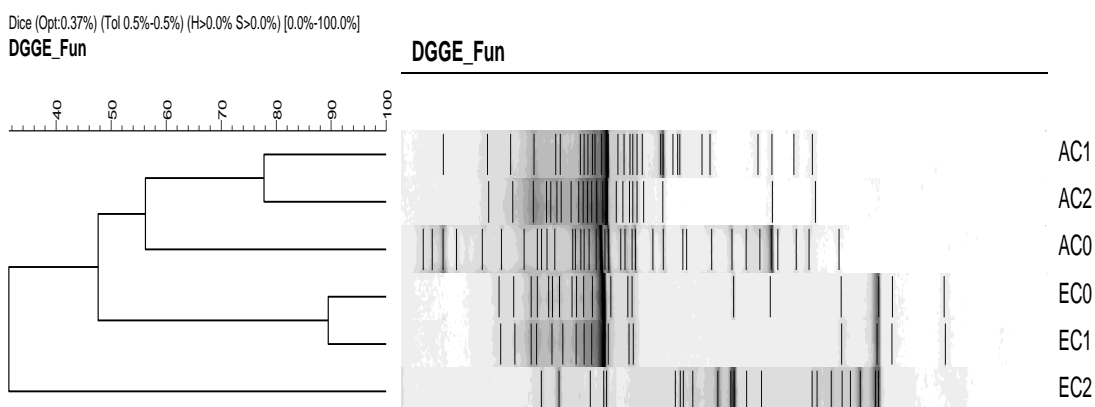


Figure 2- Fungal ITS DGGE band profiles and cluster analysis of DGGE patterns of leaves (A- alder, E- eucalyptus) samples from different treatments (C0- control, C1- [50 µg/L] C2- [200 µg/L] cadmium concentration). Similarities were calculated using Dice coefficient.

3.2 Effects of cadmium on leaf discs weight loss (leaf decomposition)

A significant higher leaf decomposition rate was observed in the treatments with shredders both with alder and eucalyptus as food (figure 3 and 4, table 2 and 3) revealing the expected differences between shredders and collectors in term of feeding rates. Exposure to cadmium caused a significant reduction of leaf decomposition when detritivore organisms were fed alder leaves. This pattern is observed both in the presence and absence of shredders (figure 3, table 2). However, when eucalyptus leaf discs were offered as food, decomposition rates were not significantly affected by cadmium exposure (figure 4, table 3). As expected the decomposition rate of alder leaf discs was much higher compared to eucalyptus leaf discs revealing that alder leaves are preferred by detritivores.

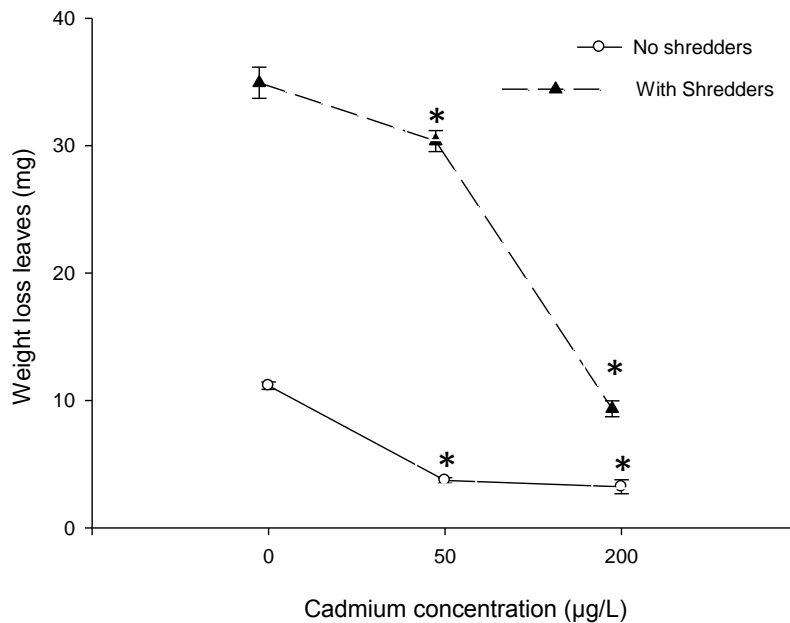


Figure 3 - Alder weight loss of the leaves (mean +/- SE) when the organisms are exposed to a concentration gradient of cadmium according to presence (black triangle) or absence (white circle) of shredders. Asterisks denote statistically significant differences ($P < 0.05$) compared to the control treatment (no cadmium).

Table 2- Two-Way ANOVA results testing for effects of presence/absence (P/A) of shredder, cadmium concentrations and their interaction for weight loss of the Alder leaves.

Factor	df	F	P-value	LOEC for Cadmium
P/A Shredder	1	1036,074	<0,001	50 µg/L
[Cd]	2	287,932	<0,001	
P/A Shredder ×[Cd]	2	123,354	<0,001	

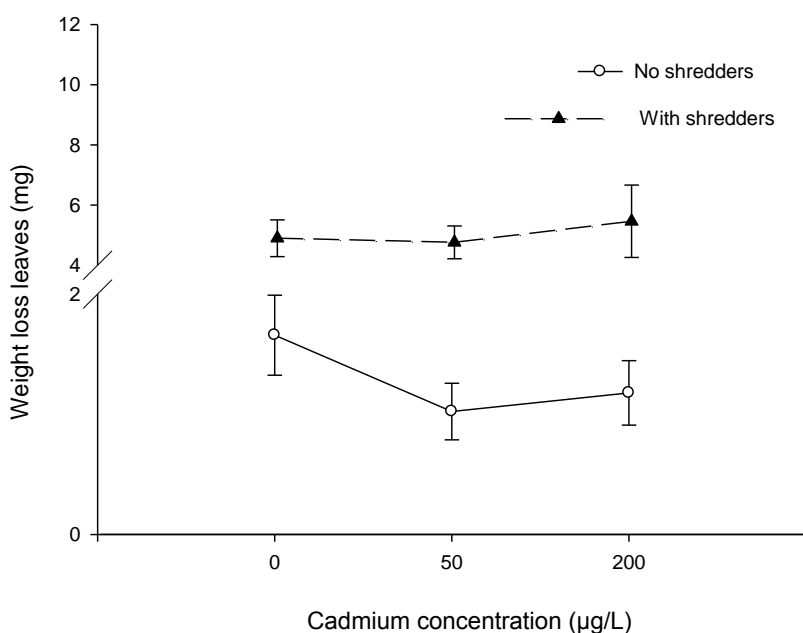


Figure 4 – Eucalyptus weight loss of the leaves (mean +/- SE) when the organisms are exposed to a concentration gradient of cadmium according to presence (black triangle) or absence (white circle) of shredders.

Table 3- Two-Way ANOVA results testing for effects of presence/absence (P/A) of shredder, cadmium concentrations and their interaction for weight loss of the Eucalyptus leaves.

Factor	df	F	P-value	LOEC for Cadmium
P/A Shredder	1	53,732	<0,001	
[Cd]	2	0,282	0,756	
P/A Shredder ×[Cd]	2	0,347	0,709	

3.3 *Chironomus riparius* growth

Cadmium exposure significantly reduced *C. riparius*' growth both in the presence of alder and eucalyptus leaf discs (fig 5 and 6, table 4 and 5). The presence of shredders affected *C. riparius* only when they were fed eucalyptus (fig 6, table 5). However, this effect was only observed in control treatments where *C. riparius* growth was significantly higher in the presence of shredders and thus a significant interaction between both factors was observed. Moreover there was a significant effect of leaf species and eucalyptus fed *C. riparius* grew almost half of those fed with alder leaves (fig 5 and 6, table 4 and 5).

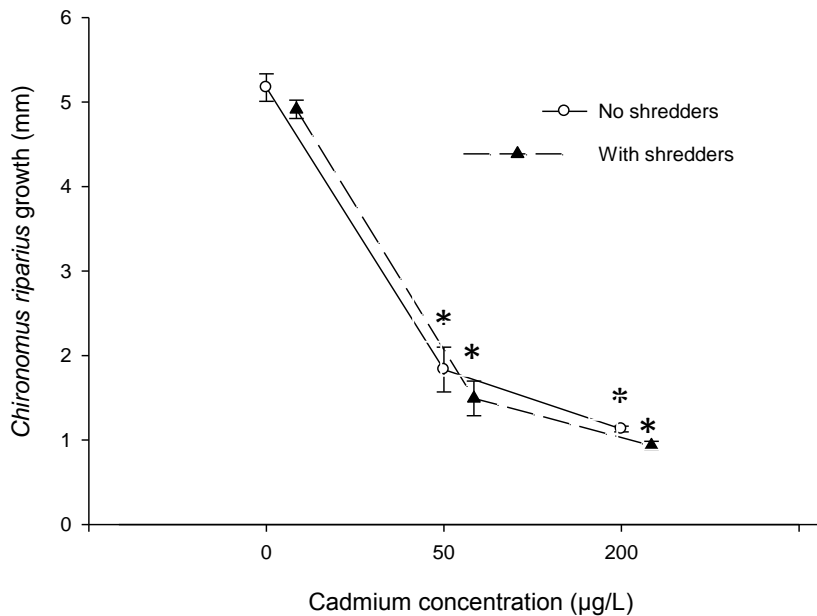


Figure 5 - Growth measurements (mean +/- SE) of *C. riparius* under exposure to a gradient of cadmium concentrations and feeding with alder leaves according to presence (black triangle) or absence (white circle) of shredders. Asterisks denote statistically significant differences ($P < 0.05$) compared to the control treatment (no cadmium).

Table 4 - Two-Way ANOVA results testing for effects of presence/absence (P/A) of shredder, cadmium concentrations and their interaction for *C. riparius* growth when feeding on alder leaves.

Factor	Df	F	P-value	LOEC for Cadmium
P/A Shredder	1	4,098	0,050	
[Cd]	2	362,786	<0,001	50 µg/L
P/A Shredder x [Cd]	2	0,101	0,904	

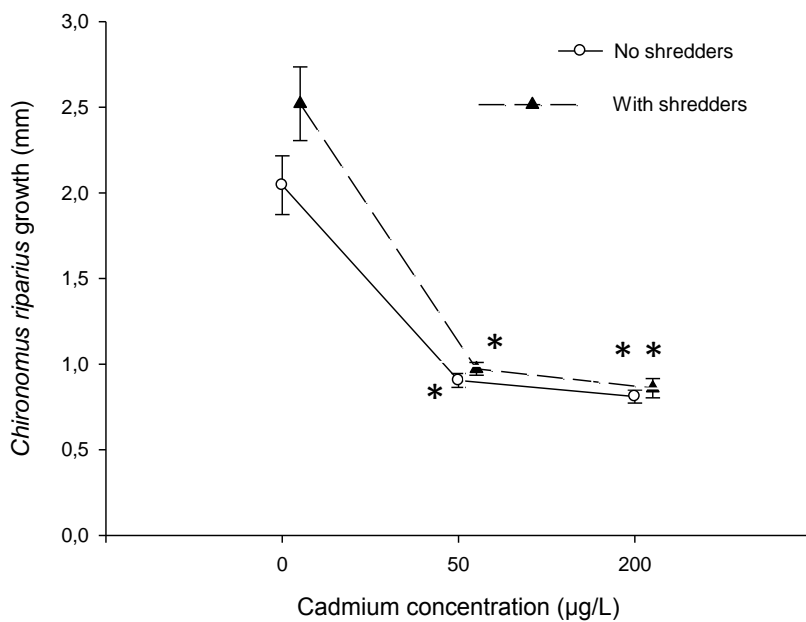


Figure 6 - Growth measurements, (mean +/- SE) of *C. riparius* exposed to a gradient of cadmium concentrations and fed with eucalyptus leaves according to presence (black triangle) or absence (white circle) of shredders. Asterisks denote statistically significant differences ($P < 0.05$) compared to the control treatment (no cadmium).

Table 5 - Two-Way ANOVA results testing for effects of presence/absence (P/A) of shredder, cadmium concentrations and their interaction for *C. riparius* growth when feeding with eucalyptus leaves.

Factor	Df	F	P-value	LOEC for Cadmium
P/A Shredder	1	22,235	<0,001	
[Cd]	2	89,891	<0,001	50 µg/L
P/A Shredder x[Cd]	2	11,072	<0,001	

Discussion

Detritus processing is vital for river ecosystems with shredders contributing for the decomposition of conditioned coarse particulate organic matter into fine particulate matter. Moreover it is also widely believed that shredders facilitate collectors as a result of this particle production (Heard and Richardson 1995). This shredder collector facilitation hypothesis can be posited at the population level, involving particular species of shredders and collectors, or at a systems level involving the shredder and collector functional groups in the community (Heard and Richardson 1995; Dieterich et al. 1997). This way, detritus processing chains and its intervenients (microbes, shredders and collectors) offer great potential to be used in the field of community ecotoxicology aiming to study how contaminants affect ecological integrity of river ecosystems. Here a simplified tri trophic detritivore food web was used, composed of leaf detritus, a caddisfly shredder and a dipteran collector species, all chosen based on their co-occurrence in Iberian streams, to study the effects of cadmium on these shredder collector interactions. By manipulating the presence and absence of shredders the effects of cadmium exposure on collectors growth was evaluated. Moreover, by using eucalyptus or alder leaves, it was assessed how the effects of metal exposure and strength of this interspecific interaction are mediated by environmental context in terms of changes of resource quality.

Initially, the effects of cadmium exposure on fungal community structure conditioning was assessed for both types of leaves and also the respiration rates of these microbial communities. Results from DGGE showed that cadmium can, in fact, alter the fungal communities present in leaves and a decrease in the diversity of fungal species when leaves are conditioned under cadmium exposure was observed. Results are also in

accordance with previous studies showing different fungal communities in different leaf species (Canhoto and Graça 1996). In fact, alder leaves, a fast decomposition rate leaf species, showed much higher microbial respiration than the conditioned eucalyptus leaf discs. Eucalyptus leaves physical and chemical properties are known to limit fungal colonization and decomposition (Suberkropp 1992; Graça et al. 2002) Phenolic compounds and lipids (essential oils and waxes) as well as physical barriers, such as the cuticle, inhibit the enzymatic activity of fungi and retard their colonization in eucalyptus leaf litter (Canhoto and Graça 1996).

In contrast, results from microbial respiration assays, used as a proxy for microbial biomass present, revealed no significant effects for cadmium exposure. This apparent contradiction can be explained by the different methods used. For the molecular analysis (DGGE) it was decided to target communities only since fungi and Hyphomycetes in particular, are considered the main leaf litter microbial decomposers in streams in terms of biomass and enzymatic potential (Abelho 2001). The oxygen consumption results show the respiration rates of microbial communities (fungi and bacteria) as a whole, and evidently bacterial communities could have had different responses to cadmium exposure. However, and due to the minimal contribution of bacteria on leaf decomposition, these differences are probably due to effects of some differential sensitivity to cadmium and functional redundancy between fungal species colonizing leaves in the different cadmium treatments.

Concerning the leaf discs weight loss, results showed that cadmium exposure reduces alder leaf decomposition through toxic effects on shredder and collector larvae. In fact many investigations have already shown feeding inhibition of invertebrate detritivores under cadmium exposure (Pestana et al. 2007; Coulaud et al. 2011). Surprisingly cadmium exposure had no effects on eucalyptus leaf discs weight loss. Although detritivores generally show feeding preferences for different leaf species and even for different conditioning conditions (Arsuffi and Suberkropp 1989; Irons et al. 1988; Graça 2001; Abelho 2008; Graça and Cressa 2010) probably the low feeding rates observed for eucalyptus leaves conditions even in the control (i.e. no cadmium) treatment, may have prevented the observation of any differences in leaf discs weight loss. It is also clear in alder and eucalyptus treatments, that the higher decomposition rates of leaf discs occurs when in the presence of *S. vittatum* known to be a very efficient shredder (Feio and Graça 2000).

Cadmium exposure also reduced significantly the growth of *C. riparius larvae* fed on alder or eucalyptus. Since no significant reductions of feeding (leaf weight loss) were observed for eucalyptus treatments these differences are also probably due to effects of cadmium on altered assimilation efficiencies or on energetic expenditure. Metals are known to increase metabolic expenditures on invertebrates and reductions of energetic reserves, which will ultimately affect growth (De Coen and Janssen 2003; Vandenbrouck et al. 2009). However, the possibility of differences of nutritional values of the different batches of leaves conditioned under different cadmium treatments cannot be excluded. This different conditioning could be a source of possible indirect effects of contaminants along detritus chains, but to test for that a refined analysis would be necessary with cross treatments where detritivores under different exposure conditions are offered leaves from different conditioning conditions. As expected *C. riparius* feeding on eucalyptus leaf discs grew much less than larvae fed on alder leaf discs. This was expected based on the lower nutritional value of eucalyptus leaves compared to alder (Pozo 1993; Graça et al. 2002) even if microbial conditioned leaves are used as in the present study.

The growth of *C. riparius* was also used to evaluate shredder-collector facilitation hypothesis and assess possible indirect effects of cadmium along this simplified detritus processing chain. Results showed beneficial effects for the presence of shredders on collectors' performance (*C. riparius* growth) but only in control treatment in the presence of eucalyptus leaves. This means that when fed with alder leaves *S. vittatum* presence does not promote *C. riparius* performance. It should be noted that *C. riparius* larvae feeding in the absence of the caddisfly shredder caused measurable effects on leaf discs weight revealing that they are not exclusive collectors and that even first stage midge larvae used in the present tests can feed and grow on coarse organic matter - even if they feed preferentially on the fungal communities colonizing the leaf. This is in accordance with previous studies on feeding plasticity of chironomidae (Callisto et al. 2007) and field collections of chironomids in streams where they are usually found on leaf litter depositional areas (Callisto et al. 2007). In fact, when alder is used as food, the effects of the presence of shredders were marginally statistically significant but contrary to the facilitation hypothesis *C. riparius* grew less with the presence of *S. vittatum* possibly an indication of competition for food. In the presence of eucalyptus, a much tougher leaf species (which has also less microbial biomass - see respiration results), the presence and feeding activity of *S. vittatum* significantly promoted *C. riparius* growth through FPOM

production. Moreover this positive effect was lost under cadmium exposure probably due to effects of cadmium on both species and meaning that indirect effects of contaminants can arise along detritus processing chains depending on the environmental context.

The results presented indicate that it is advisable to consider the possibility of indirect effects of contaminants along these detritus processing chains through effects on shredder collector interactions. This will surely increase our knowledge on ecological effects of contaminants on secondary production and ecosystem functioning. In detritus processing chains, these effects are dependent on resource quality of detritus and sensitivity and feeding plasticity of detritivores involved. These characteristics will determine the strength of the interaction and thus the magnitude of trophic cascades arising from exposure to contaminants. Because these shredders collector interactions can be conjectured at the community level, an effort to study possible indirect effects of detritus processing should be placed on by using more complex detritus based food webs.

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Chapter III: General Discussion

General Discussion

Indirect effects of contaminants may be a significant factor influencing the manner in which the ecosystem structure and function respond to anthropogenic stressors. Subsequently, failure to incorporate indirect effects into risk assessment may be a significant source of uncertainty in risk estimates. Single species, laboratory-based toxicity tests cannot detect indirect contaminant effects; studies at the population, community or ecosystem level are thus required.

Trophic cascades are a well-studied type of indirect effect and are generally considered in terms of 'top-down' (predator influence on lower trophic levels) and 'bottom-up' (nutrient/ food/ prey influence on higher trophic levels) effects (Schmitz et al. 2004). However, despite many aquatic food webs receiving considerable energy from allochthonous resources, investigations of trophic cascades have focused largely on autochthonous resource-based food webs (Schmitz et al. 2004; Naddafi et al. 2007). This is surprising since detritivore macroinvertebrates and organic matter decomposition have now been extensively used to monitor the ecological status of freshwaters (Pestana et al. 2009). These heterotrophic food chains are vital for ecosystem functioning since allochthonous terrestrial detritus derived mainly from leaves from riparian vegetation form the basis of much secondary production in headwater streams. Moreover, shredder-collector interactions can mediate a large number of indirect effects of contaminants in these ecosystems.

Studying these interactions is of utmost importance, since it allows us to study the direct and indirect effects of chemical stressors on secondary production and ecosystem functioning. Largely overlooked, however, is the potential for alien species to play an important role in altering the community context and thus become themselves an important mediator of contaminant effects on natural communities (Mack et al. 2000).

The work developed in this thesis focused on detritivore processing chains and studied the effects of cadmium and of changes of resource quality (leaf species) along these trophic chains, considering microbial conditioning and the action of detritivore invertebrates. Specifically this work intended to study how cadmium exposure mediated shredder-collector interactions and if this effect was in turn altered by resource quality - testing the presence of eucalyptus as leaf litter. For that it was decided to use a simplified food web composed of detritus (leaf discs), *S. vittatum*, an efficient caddisfly shredder,

and *C. riparius* larvae, a midge collector. The effects of cadmium were assessed in terms of microbial leaf conditioning, leaf weight loss and midge growth used as a measure of secondary production. To assess effects on leaf conditioning we chose the DGGE technique, widely used for similar purposes (Duarte et al. 2008; Batista et al. 2012), and respiration rates of conditioned leaf discs was used as a measure of microbial biomass (Bergfur and Friberg 2012).

Cadmium exposure affected microbial conditioning altering the structure of fungal communities in leaves, reducing the decomposition of alder leaves, and also impairing *C. riparius* growth. No significant effects of cadmium exposure were however observed on microbial respiration and weight loss in eucalyptus leaves. As expected, the effects of changing leaf species affected invertebrates detritivores in accordance with studies stating that the presence eucalyptus in riparian corridors may impair secondary production and stream ecosystem functioning. It was also demonstrated here that *C. riparius* larvae can feed and grow on alder leaves easily, in the absence of shredders producing fine particulate matter. *C. riparius* is thus not an exclusive collector species, not even in the first larval stages, and so we can conclude that there is not a strong interaction between both species used in our feeding trials. Still, we managed to prove the existence of this interaction when detritivores were offered eucalyptus as leaf litter showing that in this case shredder activity promoted collector's growth. Moreover we demonstrated that this interaction can be compromised with cadmium exposure.

We provided evidence that sub-lethal concentrations of cadmium can have significant detrimental consequences not only because of their direct toxic effects but also through effects on shredder-collector interactions and that this effects is dependent on resource quality in detritus processing chains.

To conclude, while we continue to gain a basic understanding of the indirect effects of contaminants on species interactions, future studies should incorporate field-relevant scenarios such as different shredders and collector species, relevant contaminant concentrations tested in complex environments and varying in the level of resource quality and preferably accounting for long-term effects. This would be extremely valuable since the strength of shredder collector interaction is mediated itself by the different species and their sensitivity towards specific contaminants.

The methodologies presented can be used to incorporate ecological complexity into experiments using detritus processing chains and address the indirect effects of

contaminants on relevant endpoints such as secondary production and leaf litter decomposition, and thus to better predict the potential ecological impacts that these compounds might be having in freshwater ecosystems.

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