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**Impacts of invasive omnivore predators on
plant litter decomposition in streams: the
case of *Procambarus clarkii***

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Impactos de predadores omnívoros invasores na decomposição dos detritos vegetais em rios: o caso do *Procambarus clarkii*

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Impacts of invasive omnivore predators on plant litter decomposition in streams: the case of *Procambarus clarkii*

Abstract

The introduction of non-indigenous invasive species (NIS) has gained momentum in Ecology in recent years mainly because they can cause changes in biodiversity or they may function differently than the native species. In forested streams, the canopy of riparian vegetation decreases the availability of light in freshwater ecosystems and, so, plant litter from riparian vegetation is the main source of energy for microbial communities and macroinvertebrate detritivores. The Louisiana red swamp crayfish, *Procambarus clarkii*, is one of the most problematic NIS in freshwaters and this species is well known by their feeding plasticity that encompass the consumption of plant detritus, submerged vegetation and aquatic invertebrates, being also a possible food resource to higher trophic levels. Given these characteristics, *P. clarkii* is well known by its capacity to change biotic interactions and ecosystem functioning in the invaded areas.

First, we used a laboratory mesocosm approach to assess the effects of *P. clarkii* on the decomposition of alder leaves (*Alnus glutinosa* Gaertn.) in the absence or presence of two abundance levels (6 and 12 individuals) of the invertebrate shredder *Sericostoma* sp. We also assessed, through laboratory mesocosms, the direct and indirect effects of *P. clarkii* on leaf decomposition by providing the chance for invertebrate shredders to avoid predation. Then, we assessed if the effects of the crayfish on leaf decomposition and invertebrate shredders varied with the crayfish sex and size. Finally, we performed a field experiment in a stream of North Portugal manipulating the presence/absence of crayfish, the presence/absence of invertebrate shredders at two different stream sites, in which the presence of *P. clarkii* was already reported (downstream) or not (upstream).

The presence of crayfish affected leaf decomposition and *Sericostoma* sp. abundance as well as the production of fine particulate organic matter (FPOM). The indirect presence of *P. clarkii*, i.e. mesocosms with water in which the crayfish was previously kept, did not affect leaf decomposition by the shredder and FPOM production. However, the direct presence of crayfish increased leaf decomposition and FPOM production. Leaf decomposition and FPOM production were affected by crayfish size but not by the sex. Bigger crayfishes showed higher leaf decomposition and FPOM production than smaller crayfishes. However, the opposite was found when these ecological processes were expressed as g per g of crayfish. In the field experiment, the crayfish affected the structure of the invertebrate community and reduced invertebrate abundance, biomass and diversity. These results were especially significant at the downstream site, where the presence of crayfish was already reported. Leaf mass loss was negatively affected by the presence of crayfish at the downstream site. Upstream, in the presence of *P. clarkii*, the values of leaf mass loss and invertebrate community indicated a naive behavior of the invertebrates.

Our results lend support to the idea that the invader *P. clarkii* is an omnivore predator that may cause changes in aquatic heterotrophic systems. Indeed, the crayfish could predate invertebrate detritivores and, doing so, indirectly affect leaf decomposition. On the other hand, the crayfish was also able to feed on leaf litter accelerating decomposition and reducing the available resources for invertebrate shredders in streams.

Impactos de predadores omnívoros invasores na decomposição dos detritos vegetais em rios: o caso do *Procambarus clarkii*

Resumo

A introdução de espécies invasoras não nativas (NIS) tem ganho importância em ecologia nos últimos anos principalmente pela capacidade destas espécies afectarem a biodiversidade e os processos dos ecossistemas. Em ribeiros florestados, a vegetação ribeirinha diminui a disponibilidade em luz, o que compromete a produção autotrófica, e levando a que os detritos das folhas que caem nos cursos de água sejam a principal fonte de energia para as comunidades microbianas decompositoras e para os macroinvertebrados detritívoros. O lagostim do Louisiana *Procambarus clarkii* é uma das espécies invasoras mais problemáticas nos ecossistemas água doce. Esta espécie é conhecida pela sua plasticidade alimentar que inclui detritos foliares e macroinvertebrados aquáticos constituindo também uma fonte de alimento para os níveis tróficos superiores. Dadas estas características, o *P. clarkii* é conhecido pela sua capacidade de afectar as relações bióticas e o funcionamento dos ecossistemas.

Neste trabalho, em laboratório, usámos uma abordagem em mesocosmos para estudar os efeitos da presença de *P. clarkii* na decomposição da folhada de amieiro (*Alnus glutinosa* Gaertn.) na ausência e na presença de dois níveis de abundância (6 e 12 indivíduos) de invertebrados do género *Sericostoma* sp. Seguidamente, testámos também em laboratório os efeitos directos e indirectos de *P. clarkii* na decomposição de folhas, dando aos invertebrados a possibilidade de evitar a predação. Foi ainda testado se os efeitos do lagostim na decomposição de folhada variavam com o sexo e o tamanho do animal. Por fim, foi realizada uma experiência de campo num ribeiro do Norte de Portugal onde foi manipulada a presença / ausência do lagostim, presença / ausência de invertebrados em dois locais onde foi registada a ocorrência (jusante) ou não (montante) de *P. clarkii*.

A presença do lagostim afectou a decomposição da folhada e a densidade de invertebrados, bem como a produção de matéria orgânica particulada fina (FPOM) nos mesocosmos em laboratório. A presença indirecta do *P. clarkii*, testada por exposição a água onde o lagostim tinha sido mantido, não afectou a decomposição de folhada nem a produção de FPOM pelo *Sericostoma* sp. Pelo contrário, a presença directa do lagostim aumentou a decomposição da folhada e a produção de FPOM. A decomposição da folhada e a produção de FPOM foi afectada pelo tamanho do lagostim, mas não pelo sexo. Os lagostins maiores promoveram maior decomposição da folhada do que os lagostins pequenos. No entanto, o oposto foi observado quando esses valores foram expressos em grama de folha consumida por grama de animal. Na experiência de campo, o lagostim afectou a estrutura da comunidade de macroinvertebrados e reduziu a sua abundância, biomassa e diversidade. Esses resultados foram significativos a jusante onde o lagostim já existe naturalmente. A perda de massa foliar foi afectada negativamente pela presença de lagostim a jusante. A montante, na presença do *P. clarkii* os valores de perda de massa foliar e da comunidade de invertebrados indicaram um comportamento “naive” dos invertebrados.

Os nossos resultados dão suporte à ideia que o lagostim invasor *P. clarkii* é um predador omnívoro que pode causar alterações nos sistemas aquáticos heterotróficos. De facto, o lagostim pode consumir invertebrados detritívoros e afectar, indirectamente, a decomposição da folhada. Por outro lado, o lagostim também é capaz de se alimentar da folhada, acelerando a sua decomposição e reduzindo os recursos alimentares disponíveis para os invertebrados trituradores nos rios.

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1. Introduction

1.1. Biodiversity and ecosystem functioning

Nowadays, a relevant topic in ecology is to understand how biodiversity affect the ecosystem processes and functions. Organisms regulate the flux of energy and carbon uptake, nutrient cycling and oxygen production (Loreau *et al*, 2002; Naeem *et al*, 2009). Ecosystem processes, such as resource consumption, are widely controlled by the density, biomass and metabolic needs of organisms (Reiss *et al*, 2009). Consequently, the number and identity of species within a system are fundamental to maintain those ecosystem processes (Cardinale *et al*, 2002; Reiss *et al*, 2010). Indeed, biodiversity and ecosystem functioning (BEF) relationships have been the focus of great interest and debate among ecologists mainly after the 1990s. BEF is one of the few research topics in ecology that examines how biological variation *per se* acts as an independent variable to regulate communities and key ecosystem-level processes (Naeem, 2002). Understanding the ecological consequences of changes in biodiversity has shown much potential to complement the historical focus on the ecological impacts of highly influential species (Cardinale *et al*, 2009). BEF is one of the few sub-disciplines in ecology that have expanded very quickly over the last two decades and this research has stimulated the emergence of a myriad of empirical and theoretical approaches responsible for advancing our understanding of community and ecosystem ecology (Kinzig *et al*, 2002; Loreau *et al*, 2002; Naeem *et al*, 2009). Only after the 1990's, ecologists recognized that the properties of ecosystems were mediated also by biodiversity itself and not just by abiotic factors (Chapin *et al*, 1992). It is highly recognized that communities with higher number of species perform better than communities with a low number of species. This situation is usually explained by complementarity or facilitative interactions among species (Cardinale *et al*, 2002). Complementarity is generally explained by niche differentiation that leads to a better use of overall resources once species within communities have different resource requirements (Hooper, 1998). On the other hand, facilitation occurs when some species modify the environment and doing so allow others to benefit and increase their biomasses without causing any harm to other species (Vandermeer, 1989; Fridley, 2001).

Nowadays, biodiversity is facing dramatic changes that have resulted in loss of species plus reductions in their distribution and abundance. This has been mainly related to

anthropogenic impacts, such as habitat loss and fragmentation, pollution, climate change and overexploitation of resources (Sala *et al*, 2000). These impacts are responsible for major ecosystem changes also affecting goods and services that are fundamental to humans (Naeem *et al*, 2009). However, some empirical studies have shown that species richness or identity does not always affect ecosystem processes (e.g. Dang *et al*, 2005; Thompson & Starzomski, 2007), suggesting a certain degree of functional redundancy among species within a system. Such redundancy could help to compensate for species loss, if the remaining species respond by increasing their abundance or biomass (Hooper *et al*, 2005). Nevertheless, species contribute to more than a few ecosystem processes at a time and biodiversity becomes more important when more processes are assessed (Gamfeldt *et al*, 2008; Reiss *et al*, 2009; Woodward, 2009). Furthermore, discussions of BEF focus primarily and almost exclusively on what happens to ecosystem processes and ecosystem functioning when we lose species due to extinctions (Hooper *et al*, 2005). There is a clear lack of studies testing what could happen to ecosystem processes and functioning when we add species, i.e., which are the consequence of the introduction of non-indigenous invasive species (NIS). The introduction of NIS has gained momentum on the scientific community in recent years mainly because they can cause a net reduction of species (e.g., introduction of predators, parasites and diseases that are responsible for the extinction of native species) or because they may function differently (e.g., distinct use of nutrients) than the native species (Simberloff *et al*, 2013). In the same vein, species additions can also result in a net increase of species at a local scale, which is an understudied topic in BEF (Sousa *et al*, 2011). According to Sousa *et al* (2011), very few studies analyzed the effect of invasive species on ecosystem functioning and most of these studies were performed in terrestrial ecosystems and in North America. These studies suggested that species additions will affect ecosystem functions like productivity, biogeochemical cycles, fire and hydrologic regimes, decomposition rates and biotic interactions (e.g., predator-prey interactions, introduction of parasites and diseases), with impacts to ecosystem services and human wellbeing (Sousa *et al*, 2011). Once NIS may affect biodiversity and ecosystem functions, it seems important to evaluate those potential direct and indirect effects at local scales in an attempt to predict how these species can affect ecosystems at a regional and global scale.

Currently, 90% of global terrestrial plant production enters the dead organic matter pool (Cebrian, 1999), which means that decomposition and the sequestration of organic carbon in

soils and sediments are key processes in ecosystems. This organic matter consists mainly in leaves that decompose faster than wood detritus and may fall on soil or into streams. Several studies in both terrestrial and aquatic systems have tested whether rates of ecosystem processes decrease when species are lost from decomposer systems (Srivastava *et al*, 2009). Those studies manipulated diversity at one trophic level: resources, i.e. leaf litter diversity (Swan & Palmer, 2004; Madritch & Cardinale, 2007; Lecerf *et al*, 2007; Scherer-Lorenzen *et al*, 2007; Fernandes *et al*, 2013), microbial diversity (Setälä & Mclean, 2004; Dang *et al*, 2005; Duarte *et al*, 2006; Pascoal & Cássio, 2008), or detritivore diversity (Heemsbergen *et al*, 2004; McKie *et al*, 2008) and, in rare cases, biodiversity at several trophic levels was manipulated simultaneously (Hättenschwiler & Gasser, 2005; Bastian *et al*, 2008; Reiss *et al*, 2010).

1.2. Forested streams

In forested streams, the canopy of the riparian vegetation decreases the availability of light which highly decreases the primary production by photosynthetic organisms (Wetzel, 2001; Dodds, 2002). Therefore, these streams depend on allochthonous organic matter from riparian zones which are the major source of energy and carbon to aquatic biota, i.e., these systems are mainly heterotrophic and depend on the organic matter produced elsewhere (Suberkropp, 1998). The decomposition of organic matter in streams is conducted by microbial communities, such as fungi and bacteria, as well as by invertebrate detritivores (Pascoal *et al*, 2005). This process depends on the response of decomposers to environmental conditions (external factors) and the intrinsic quality of the detritus that came from riparian vegetation (internal factors). Both fungi and bacteria convert leaf carbon into microbial biomass, enhancing leaf palatability for invertebrate shredders (Gessner *et al*, 1999). Decomposition of leaf litter includes a range of biotic and abiotic transformations that result in the formation of carbon dioxide and mineral substances, dissolved organic matter (DOM), and fine particulate organic matter (FPOM). The overall process also depends on the biomass produced by microbial decomposers, such as fungi (Gessner *et al*, 1999). The biotic interactions between different groups of decomposers plus interactions between organisms within the same group also have an important role in plant-litter decomposition (Gessner *et al*, 2007). Diversity and quality of leaf detritus are essential to the functioning of freshwater ecosystems and they can change the trophic structure and community

dynamics in a short period of time (Lecerf *et al*, 2005). Thus, these detritus can stabilize energy flow, as well as population dynamics of consumers and change the efficiency of energy and nutrient transfer between trophic levels. In addition, detritus can enhance the persistence of species, but can also be a source of carbon and energy for downstream areas (Vannote *et al*, 1980). At the same time, these detritus can physically alter habitats (Schindler, 1990; Williamson *et al*, 1999) and doing so they can also be responsible for changes at the population or community level (i.e., some species can be favored while others can be in disadvantage) (Harmon *et al*, 1986; Facelli, 1994; Peterson & Pickett, 1995). Plant litter decomposition can be affected by changes in biodiversity (Pascoal & Cássio, 2008; Gessner *et al*, 2010), including changes in species richness and composition of microbial communities (Dang *et al*, 2005; Tiunov & Scheu, 2005; Duarte *et al*, 2006), invertebrate detritivores (Jonsson & Malmqvist, 2000; Ruesink & Srivastava, 2001; Heemsbergen *et al*, 2004; Hatteschwiler & Gasser, 2005; Schadler & Brandl, 2005), predators (Bruno & Cardinale, 2008; Nilson *et al*, 2008) and plant litter itself (Wardle *et al*, 1997; Swan & Palmer, 2004; Kominoski *et al*, 2007; Sherer-Lorenzen *et al*, 2007).

It is crucial to understand the consequences of changes in biodiversity to key ecosystem processes, such as plant litter decomposition, and the mechanisms underlying such *cause-effect* relationships. Furthermore, it is important to understand how species are organized within the trophic chain to better understand possible interactions between species and the overall resulting effects. Food webs based on allocthonous carbon are of major importance in low-order forested streams. Here, subsidies of inland plant detritus and dissolve organic oxygen (DOC) mainly from leaves are the basis of secondary production (Fisher & Likens, 1973; Wallace *et al*, 1997).

1.3. Trophic interactions in aquatic systems

Trophic cascades are defined as reciprocal predator-prey effects that alter the abundance, biomass or productivity of a population or trophic level across more than one level in a food web (Pace *et al*, 1999). Recent meta-analyses showed that aquatic systems, in general, have stronger trophic cascades than terrestrial ecosystems (Shmiltz *et al*, 2000; Shurin *et al*, 2002; Stibor *et al*, 2004). Furthermore, some species (e.g., top predators) may display strong interactions and theoretically may have a disproportional influence over food web dynamics. This idea has drove

much debate over the dominance of omnivory in food webs (Yodzis, 1984; Polis & Strong 1996; Thompson *et al*, 2007) and whether the increased number of feeding links that result from omnivory increases or decreases the stability of energy flow through a food web (MacArthur, 1955; McCann *et al*, 1998). Another current challenge is the identification of species that represent influential nodes in the food web. Usually, these species encompass i) ecosystem engineers, i.e., species that physically modify the environment and provide resources for other species (Jones *et al*, 1994, 1997), ii) keystone species, i.e., species with low abundance that play a key role in the food web, so their presence is important to maintain ecosystem processes (Paine, 1969, Power *et al*, 1996) or iii) foundation species, i.e., species that structure a community by creating locally stable conditions for other species (Ellison *et al*, 2005). Usually, these species might have cascading effects on the abundance, biomass and diversity of other species at different trophic levels (Paine, 1966; Carpenter *et al*, 1987; Elser *et al*, 1988). In theory, it is expected that the increased number of trophic levels (for example, with the introduction of predators) will change food webs from a bottom-up control (based on resources) to a top down control (control by predators and their effects on consumers) (Naeem *et al*, 2009). Studies about trophic cascades have been focused essentially on food chains based on autochthonous resources, in which resources came mainly from primary production *in situ*. However, and since renewal rates of detrital biomass depends on extrinsic factors and are not linked to consumptive effects, the probability of top-down control in plant litter decomposition may be different than in autotrophic systems. Indeed, detrital biomass appears to be more susceptible to top-down control than the biomass of primary producers (Rosemond *et al*, 2001). Thus, predators may affect the dynamic of leaf detritus if they reduce detritus availability or if they influence the abundance and/or the behavior of detritivores (Mancinelli *et al*, 2002). The direct consumptive interaction, i.e., where predator consumes the prey (Taylor, 1984), is recognized as a key biotic interaction in terrestrial, freshwater and marine ecosystems. However, we may have indirect non-consumptive interactions where fierce predators change the behavior of the prey (Lima & Dill, 1990). Preys may consider the risk of being predated as an activity with costs and respond according to that risk. This field of research usually recognized as fear ecology is a recent approach to study the interactions between predators and preys (Brown *et al*, 1999). Over the last years, some studies addressed this subject in terrestrial ecosystems, using wolves and deers (Ripple & Beschta, 2004), birds and bird predators (raccoon, corvid, hawk, owl,

cowbird) (Zanette *et al*, 2011) and lions with the fear of darkness (Packer *et al*, 2011); in marine ecosystems with sharks and dungeons (Wirsing & Ripple, 2010); and in freshwater ecosystems with different fish species (Kuehne & Olden, 2012). An interesting hypothesis that has been raised is that prey behavior and the risk of perception change if the predator is a native or an invasive species. A naivety effect has been found in the responses of preys to non-native predators with great declines in the density and biomass of native preys (mammals: Banks, 1998; Barrio *et al*, 2010; Fey *et al*, 2010; amphibians: Pearl *et al*, 2003; Gomez-Mestre & Diaz-Paniagua, 2011; fishes: McLean *et al*, 2007; Kuehne & Olden 2012; invertebrates: Freeman & Byers, 2006; Edgell & Neufeld, 2008).

1.4. Biological Invasions with emphasis on *Procambarus clarkii*

Ecosystems, at a global scale, show unprecedented rates of species extinctions and invasions. Species introductions outside their native geographical range have been mediated by human activities and this issue emerged recently as one of the five big threats to biodiversity and is a matter of concern in the field of conservation biology (Sala *et al*, 2000). Human activities are clearly related to increasing rates of NIS introductions (Cohen & Carlton, 1998; Vilà *et al*, 2010). Species introductions induced some of the most dramatic changes in biodiversity (Simberloff *et al*, 2013). These invasions conduct to the homogenization of Earth's biota by breakage of dispersal barriers (McKinney & Lockwood, 1999). Freshwater systems may suffer high alterations due to the introduction of NIS, which may interfere with different trophic levels (Sousa *et al*, 2009). NIS modify the structure and functioning of ecosystems because they change the abiotic conditions (light availability, nutrient levels, heat transfer, habitat complexity and physical disturbance) and affect native communities (diversity, spatial distribution, density and biomass of other species) (Grosholz, 2002; Byrnes *et al*, 2007). At the same time, NIS impacts also depend on their position in the trophic chain of the invaded ecosystem. Although ecosystem ecologists do study invasions, the field does not appear to put biological invasions on par with factors such as climate change, nutrient loading, land use change and disturbance when considering what drives the structure and functions of actual ecosystems (e.g. Agren & Anderson, 2012).

In European freshwater ecosystems, the crayfish *Procambarus clarkii* (Girard, 1852) is listed as one of the 100 worst invasive species (DAISIE database), with some authors even

considering this species as one of the ten most problematic invasive species in Europe (Tablado *et al*, 2010). This freshwater crayfish, from the family Cambaridae, is native to the center and south of the United States of America and the northeast of Mexico. This species was introduced in numerous freshwater systems in several parts of the globe, such as in Europe, Africa and Asia. The rapid expansion of *P. clarkii* is related not only to its natural dispersal capabilities but also to human activities. Indeed, many introductions were a result of translocations for commercial or recreational purposes (Geiger *et al*, 2005; Gherardi *et al*, 2006; Sousa *et al*, 2013). This species has been a cause of concern among investigators and managers of natural ecosystems (Gherardi & Holdich, 1999; Rodriguez *et al*, 2005) due to their activities as an ecosystem engineer, and also due to their interference with local biota and ecosystem functioning. In Europe, *P. clarkii* was first imported to Spain in 1972 (Ackefors, 1999) and then introduced in Portugal, Cyprus, England, France, Germany, Netherland (Hobbs *et al*, 1989) and Switzerland (Stucki, 1997). In Portugal, the species is widespread from the north to south and west to east colonizing almost all inland aquatic ecosystems (Sousa *et al*, 2013). *P. clarkii* is omnivorous, highly active, and it is well known for occupying a key position in the food web of native and invaded ecosystems (Holdich, 2002). This NIS has the ability to reproduce more than once per year if conditions are suitable (photoperiod, temperature, hydroperiod and pH) and adapt their size at maturity according to the environmental conditions. This species is recognized as an opportunistic omnivorous with a very high diet plasticity, which can be considered an advantage when invading a new habitat. In the juvenile phase, at least when growth rates are high, crayfishes consume other animals (Hobbs, 1993; Gutiérrez-Yurrita *et al*, 1998) such as arthropods and gastropods (Momot, 1995). On the other hand, adults may consume large quantities of detritus and submerged vegetation (Sousa *et al*, 2013). In the same way, *P. clarkii* may provide an important food source for higher trophic levels such as mammals, birds and fishes (Matthews & Renolds, 1992; Beja, 1996, Correia, 2001; Smart *et al*, 2002; Tablado *et al*, 2010) and they are also a vector of diseases acting as an host for parasites (Kozubiková *et al*, 2009). Reductions in invertebrates due crayfish consumption may have cascade effects on lower trophic levels. Crayfish may also compete with invertebrates for leaf detritus consumption. In laboratory experiments dealing with food preference, *P. clarkii* favored eating animals rather than macrophytes (Ilhéu & Bernardo, 1993), while in the field this species shows preference for vegetation and/or detritus, with no difference in the diet between males and females (Feminella

& Resh, 1986; 1989; Gutiérrez-Yurrita *et al*, 1998). Although *P. clarkii* is widely distributed in Portuguese freshwater ecosystems, there is a lack of studies addressing the possible ecological and economic impacts resulting from this introduction (Sousa *et al*, 2013).

1.5. Objective of the study

In this study, we assessed the impacts of *P. clarkii* on detritus food chains through their influence on the invertebrate detritivores and on the decomposition of leaf detritus in streams through laboratory and field experiments. We wanted to test if this NIS could have a top-down control on a key ecosystem process in forested streams, indirectly by decreasing invertebrate populations and/or directly by consuming leaf litter. The effects were assessed in crayfishes with different traits (sex and size). We also explored the possibility of indirect non-consumptive interactions (fear ecology) by testing the behavior of invertebrate detritivores under the risk of being predated.

2. Materials and Methods

2.1. Effects of *Procambarus clarkii* on invertebrate abundance and leaf decomposition

In a first experiment, we assessed the effects of *P. clarkii* on: i) the abundance of *Sericostoma* sp., an important invertebrate detritivore in streams of North Portugal, and ii) the consumption of leaf litter by *Sericostoma* sp. For this, we manipulated the presence/absence of the crayfish and the abundance of invertebrate shredder *Sericostoma* sp. In total, we had an experiment with six treatments: control with no *Sericostoma* sp. and no crayfish; low abundance of *Sericostoma* sp. (6 individuals) with or without crayfish; high abundance of *Sericostoma* sp. (12 individuals) with or without crayfish; and crayfish with no *Sericostoma* sp. Each treatment was replicated 4 times and the experiment ran for 21 days (N=24).

Mesocosms preparation

Twenty-four aquariums (40 x 23 x 25 cm) were filled with river gravel and pebbles previously washed and autoclaved (120°C, 20 minutes). River gravel and pebbles were sorted previously to include particles with size ranging between 850 µm and 60 mm. Aquariums were filled with 3 L of Fastio water (Table 1) and equipped with an aeration system. Sets of four grams of alder (*Alnus glutinosa* Gaertn.) leaves collected in October 2012 were weighted, placed in separate coarse-mesh bags and submerged in deionized water for 36 hours to promote the leaching of soluble compounds before the beginning of the experiment. After that, leaves were removed from mesh bags and placed in the aquariums. To ensure the presence of natural microbial communities in the aquariums, fine-mesh bags containing 10 discs of alder leaves (12 mm diameter) were previously immersed for one week in a low-order stream to allow microbial colonization. These discs were placed in the aquariums at the beginning of the mesocosm experiment. The experiment was run for 21 days under controlled temperature (15°C) and photoperiod (12 hours in the dark and 12 hours with light).

Table 1- Chemical composition of *Fastio* water used in the mesocosm experiments.

Parameters	
Dry residue (a 180°C)	25.8 ± 4.0 mg L ⁻¹
Silica	9.6 ± 2.0 mg L ⁻¹
pH (at 18°C)	5.8
HCO ₃ ⁻	8.0 ± 0.8 mg L ⁻¹
Cl ⁻	4.2 ± 0.4 mg L ⁻¹
SO ₄ ²⁻	1.0 ± 0.2 mg L ⁻¹
Na ⁺	4.1 ± 0.4 mg L ⁻¹
Ca ²⁺	1.3 ± 0.3 mg L ⁻¹
K ⁺	0.6 ± 0.1 mg L ⁻¹

Animal collection and maintenance

We selected males of *P. clarkii* with approximately 8 cm of total length (from the rostrum tip to the telson rear edge) collected in the Minho River (Portugal) near the village of Vila Nova de Cerveira. Crayfishes were acclimated to the laboratory conditions for a week in aquariums (60 x 30 x 30 cm) with filtrated water under aeration. *Sericostoma* sp. used in the study were collected at the upper reach of the Cávado River, 10 km downstream the town of Montalegre (Portugal). There are no records of *P. clarkii* at the sampling site. Animals were transported to the laboratory in a cool box and acclimated for one week in aquariums. Animals were maintained under controlled temperature (15°C) and allowed to feed ad libitum on alder leaves. Animals were kept under starvation 24 hours before the beginning of the experiment. We measured the total and cephalothorax size of the crayfishes at the beginning and at the end of the experiment to calculate animal growth.

Water chemistry monitoring

On each seven days of the mesocosm experiment, one third of the water was renewed. Water samples were used to determine the concentrations of ammonium (HACH kit, programme 385), nitrate (HACH kit, programme 351), nitrite (HACH kit, programme 371) and phosphate (HACH kit, programme 490) using a HACH DR/2000 photometer (HACH, Loveland, CO).

Leaf mass loss

After 21 days of experiment, leaf mass remaining was carefully washed, dried at 60°C for 48 hours, and weighted to the nearest 0.01 g. Leaf mass loss was quantified by subtracting the final weight to the initial weight of leaves.

FPOM quantification

Water retrieved from each aquarium on every 7 days and at the end of the experiment was filtrated through a 53 µm sieve. FPOM from each replicate was transferred to a 50 mL falcon tube, centrifuged during 10 minutes at 14000 rpm (*Sigma* 4-16 K), the supernatant was removed and the pellet lyophilized for 48 hours. FPOM was weighed to the nearest 0.01 mg.

2.2. Direct and indirect effects of *Procambarus clarkii* on leaf decomposition

In a second experiment, we designed a three-treatment assay (control; 6 *Sericostoma* sp. + crayfish water; 6 *Sericostoma* sp. + 1 crayfish) to assess the *Sericostoma* sp. avoidance behavior in the absence or presence of the predator *P. clarkii*. *Sericostoma* sp. had the possibility to avoid the predator because aquariums were divided transversally by a coarse mesh that separated *Sericostoma* sp. from the crayfish and the leaves. The mesh size allowed the free movement of *Sericostoma* sp. but prevented the passage of the crayfish. Therefore, *Sericostoma* sp. took the risk of being predated when trying to feed on leaves. We also assessed the possible chemical inhibition of *Sericostoma* sp. by *P. clarkii* under the hypothesis that changes in the water chemistry provided by the predator would change the feeding behavior of *Sericostoma* sp. For that, 10 crayfishes were placed in an aquarium with 15 L of Fastio water for five days before the beginning of the experiment. In the mesocosm experiment, invertebrates of the *Sericostoma* sp. were placed in aquariums containing the water that was previously in contact with the crayfish. The experiment ran for 21 days and each treatment had 4 replicates (N=12).

The aquariums were prepared as described for the first mesocosm experiment and contained gravel and pebbles, 3 L of water, 4 g of alder leaves, a fine-mesh bag with 10 discs of alder leaves previously colonized by microbes in a stream, and six invertebrate larvae of

Sericostoma sp. Invertebrates shredders and crayfishes were collected at the sites described above and kept under starvation for 24 hours before the beginning of the experiment.

Water was renewed and used for chemical analysis and quantification of FPOM production as described above. At the end of the experiment, leaf mass loss and the crayfish growth were quantified following the procedure described above.

2.3. Effects of size and sex of *Procambarus clarkii* on leaf decomposition

To test if predation and leaf consumption by *P. clarkii* differed with the crayfish size (small < 7 cm and big > 10 cm) and sex (male/female), mesocosms were prepared with all possible combinations of crayfish size and sex (control – *Sericostoma* sp.; 1 small crayfish male + 6 *Sericostoma* sp.; 1 small crayfish female + 6 *Sericostoma* sp.; 1 big crayfish male + 6 *Sericostoma* sp.; and 1 big crayfish female + 6 *Sericostoma* sp.). The experiment ran for 21 days and each of the five treatments had 4 replicates (N=20).

The aquariums were prepared as described above and contained gravel and pebbles, 4 g of alder leaves, a fine-mesh bag with 10 discs of alder leaves previously colonized by microbes, and six larvae of *Sericostoma* sp. Animals were collected at the same sites described above and kept under starvation for 24 hours before the beginning of the experiment.

Water was renewed every 7 days and used for chemical analysis and quantification of FPOM production as described above. At the end of the experiment, leaf mass loss, FPOM production and crayfish growth were quantified as described in the first mesocosm experiment.

2.4. Field experiment: *in situ* validation

Study site

The field experiment was carried out in the Campos Stream (Vila Nova de Cerveira, North Portugal). This stream is a tributary of the Minho River with a total basin area of nearly 13 km², 7.2 km of main length extension and a maximum altitude of 278.7 m. We selected two sites in a stream stretch with approximately 200 m where a small waterfall divided the upstream from the downstream site. This waterfall acts as a physical barrier to the upstream dispersion of the crayfish *P. clarkii* since there is no records of specimens upstream the waterfall. According to the final report of “Projecto Natura Miño-Minho” (2012), the fish fauna downstream the waterfall includes the non-native Iberian gudgeon (*Gobio lozano*) as well as native species, such as European eel (*Anguilla anguilla*), brown trout (*Salmo trutta*), three-spine stickleback (*Gasterosteus gymnurus*) and Iberian loach (*Cobitis paludica*). In the upper part of the waterfall, there are no records of the presence of non-native species, and fish fauna includes specimens, such as brown trout (*Salmo trutta*), European eel (*Anguilla anguilla*), and ruivaco (*Rutilus macrolepidotus*). In that report, the invertebrate diversity and abundance were higher in the upstream part of the waterfall. The chosen stream stretch is characterized by having typical riparian vegetation of North Portugal dominated by alder and oak (*Quercus robur* L.) trees. Allochthonous plant litter seems to be the principal source of food for stream biota although there are some macrophytes and small segments with no riparian vegetation. In both parts, the stream bottom is mainly constituted by sand, gravel and cobbles.

Experimental design

The field experiment was designed to assess the impacts of the crayfish *P. clarkii* in a real forested stream ecosystem to validate the results obtained in mesocosms with respect to direct predation on invertebrates and effects on leaf decomposition. A four-treatment experiment was designed using rectangular baskets (38 x 29 x 21.5 cm) in which we controlled the presence/absence of the predator and preventing or not the access of invertebrates. For that, half the baskets were covered by a fine mesh (500 µm mesh size) and the other half by coarse mesh (0.5 cm mesh size). The experiment was run in parallel at the upstream and downstream sites. All baskets contained approximately 4 g of alder leaves, 2 pebbles and gravel.

Physical and chemical analysis of the stream water

Temperature, pH, conductivity and dissolved oxygen were measured *in situ* with field probes (Multiline F/set 3 no. 400327, WTW, Weilheim, Germany). Stream water samples were collected with sterile dark glass bottles, transported in a cool box (4°C) to the laboratory to determine the concentrations of inorganic nutrients (phosphate, ammonia and nitrate) as described above.

Sample processing

All samples were transported to the laboratory in separate bags inside cool boxes. The baskets were washed and the invertebrates were separated from the leaves using a battery of sieves from 60 mm to 850 µm. Invertebrates were preserved in falcon tubes containing ethanol (96%, v/v). The leaves were carefully washed and put in separate aluminum boxes for further quantification of leaf mass loss.

Leaf mass loss

After 21 days of experiment, the remaining leaves were carefully washed, dried at 60 °C for 48 hours and weighted to the nearest 0.01 g.

Macroinvertebrate diversity and biomass

The preserved invertebrates were sorted under a dissecting microscope, identified to the lowest possible taxonomic level, according to Tachet *et al.* (2010), and counted. For biomass quantification, macroinvertebrates were oven dried (80°C) for 48 h and weighed to the nearest 0.01 mg.

2.5. Statistical analyses

Two-way analyses of variance (ANOVA) were used (Zar, 2009) to test in mesocosms i) whether the presence of the crayfish and the abundance of invertebrate shredders affected leaf mass loss and FPOM production and ii) whether the crayfish size and sex affected leaf mass loss, FPOM production and crayfish growth. One-way ANOVAs were used (Zar, 2009) to test i) the effect invertebrate abundance on crayfish growth and ii) the effect of the presence (direct or indirect) of the crayfish on leaf mass loss and FPOM production.

In the field experiment, a three-way ANOVA was used (Zar, 2009) to test if the presence of invertebrates, the presence of crayfish and the stream site affected leaf mass loss. Two-way ANOVAs were used to test i) the effect of crayfish presence and stream site on invertebrate abundance, biomass and diversity (Margalef richness, Shannon diversity and Pielou evenness) and ii) the effect of invertebrate presence and stream site on crayfish growth.

All ANOVAs were preceded by Shapiro-Wilk to test if data had a Gaussian distribution and by the Bartlett test to test the homogeneity of variance (Zar, 2009). All the ANOVAs were followed by Tukey post-tests to search for significant differences between treatments (Zar, 2009).

A Multi-Dimensional Scaling (MDS) was performed on invertebrate data. In the MDS ordination, samples are represented as points in a low-dimensional space and the relative distances between points are in the same rank order as the relative dissimilarities of the samples as measured by an appropriate resemblance matrix. Bray-curtis index was used to assess the similarity between invertebrate communities, and crayfish presence at both stream sites. Overlay clusters representing a resemblance level of 50% were superimposed to the MDS diagram. To test if invertebrate assemblages varied with the stream site and the presence of crayfish a PERMANOVA test (Anderson, 2001) was performed with 9999 permutations and Bray-Curtis index was used to quantify the similarity between invertebrate communities and crayfish presence at both stream sites.

Analyses of variance were done with STATISTICA 8 (StatSoft, USA). All graphs were done with GraphPad Prism for Windows (GraphPad software Inc., San Diego). Multivariate analysis (MDS), PERMANOVA and estimation of diversity measures were done with PRIMER 6 (Primer-E, UK) for Windows.

3. Results

3.1. Effects of *Procambarus clarkii* on *Sericostoma* sp. abundance and leaf decomposition

3.1.1. *Sericostoma* sp. consumption

Procambarus clarkii showed a high predation level on larvae of the invertebrate shredder *Sericostoma* sp. Indeed, few days after the beginning of the experiment all invertebrate shredders from high- and low-abundance treatments were eaten by the crayfish.

3.1.2. Leaf mass loss

The effects of *P. clarkii* (presence or absence) and *Sericostoma* sp. abundance (0, 6 or 12 individuals) on leaf mass loss are shown in Fig.1. After 21 days, leaf mass loss varied between 31% in mesocosms without crayfish or *Sericostoma* sp. and 72% in mesocosms containing the crayfish and high *Sericostoma* sp. abundance.

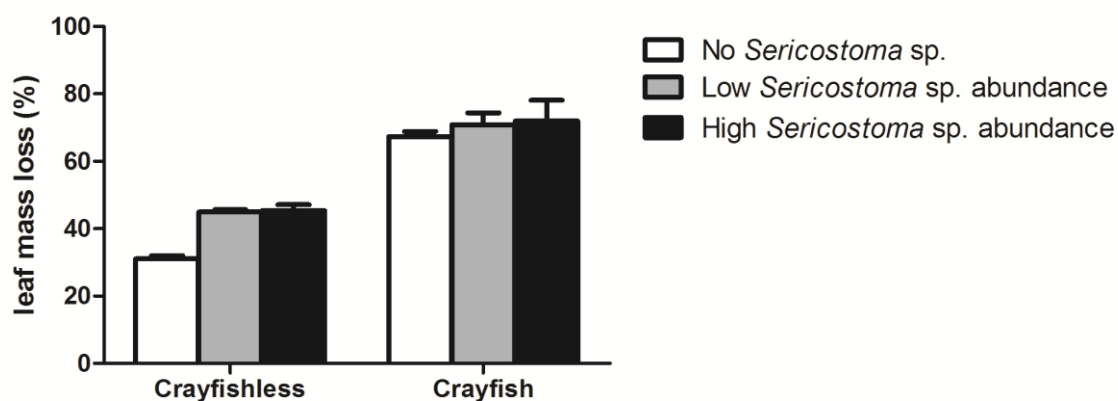


Figure 1- Percentage of leaf mass loss in the presence or absence of *P. clarkii* in mesocosms with different abundance of the invertebrate shredder *Sericostoma* sp. (no *Sericostoma* sp.: 0 individuals; low abundance: 6 individuals; high abundance: 12 individuals). Mean \pm SEM, n=4.

Results showed that the presence of crayfish and the abundance of *Sericostoma* sp. significantly affected leaf mass loss and there was no interaction between those two factors (two-way ANOVA, Table 2). Overall leaf decomposition was higher in the presence of the crayfish and

this factor accounted for 80.45% of the total variance. The invertebrate abundance accounted only for 6.85% of the total variance. In the presence of the crayfish, *Sericostoma* sp. abundance did not significantly affect leaf decomposition (Tukey's tests, $P > 0.05$). In the absence of crayfish, leaf mass loss was higher in the presence than in the absence of *Sericostoma* sp. (Tukey's tests, $P < 0.05$), but no differences in leaf mass loss were found between low and high *Sericostoma* sp. abundances (Tukey's tests, $P > 0.05$).

3.1.3. FPOM production

Results of FPOM produced during leaf decomposition in the presence or absence of the crayfish with different *Sericostoma* sp. abundance are shown in Fig. 2.

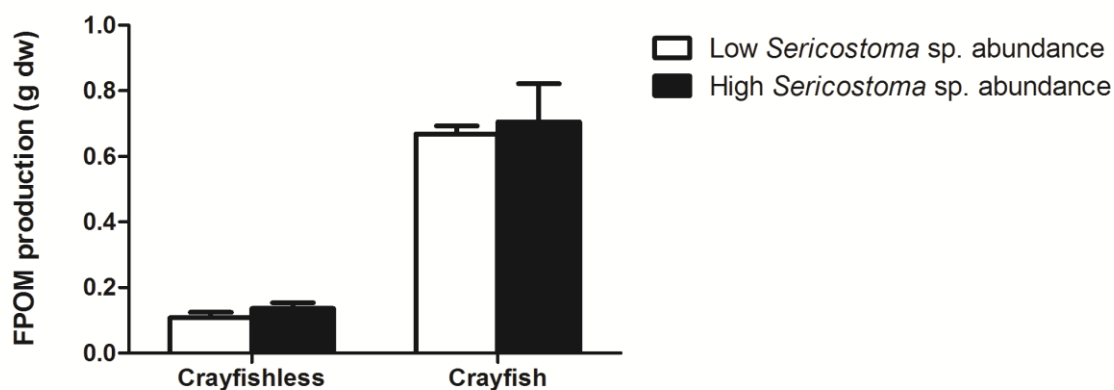


Figure 2- FPOM produced during leaf decomposition in the presence or absence of *P. clarkii* in mesocosms with different abundance of the invertebrate shredder *Sericostoma* sp. (no *Sericostoma* sp.: 0 individuals; low abundance: 6 individuals; high abundance: 12 individuals). Mean \pm SEM, $n=4$.

The presence of crayfish affected significantly the FPOM production (two-way ANOVA, Table 2) and accounted for 87.6% of the variance. FPOM production was significantly higher in the presence than in the absence of the crayfish. The FPOM production varied between 0.1 g in treatments without crayfish and with low invertebrate abundance and 0.7 g in treatments with crayfish and high *Sericostoma* sp. abundance (Fig. 2). The FPOM produced by the crayfish alone (i.e. without *Sericostoma* sp.) corresponded to 0.4 g. The *Sericostoma* sp. abundance did not significantly affect the production of FPOM (two-way ANOVA, Table 2), although values were higher in mesocosms with high than low *Sericostoma* sp. abundances (Fig. 2).

3.1.4. Crayfish growth

During the 21 days of experiment, the crayfish growth varied between 1.6% in mesocosms with high *Sericostoma* sp. abundance and 4.8% in mesocosms with low *Sericostoma* sp. abundance (Fig. 3). There were no significant differences in the growth of the crayfish between treatments with different *Sericostoma* sp. abundance (one-way ANOVA, Table 2).

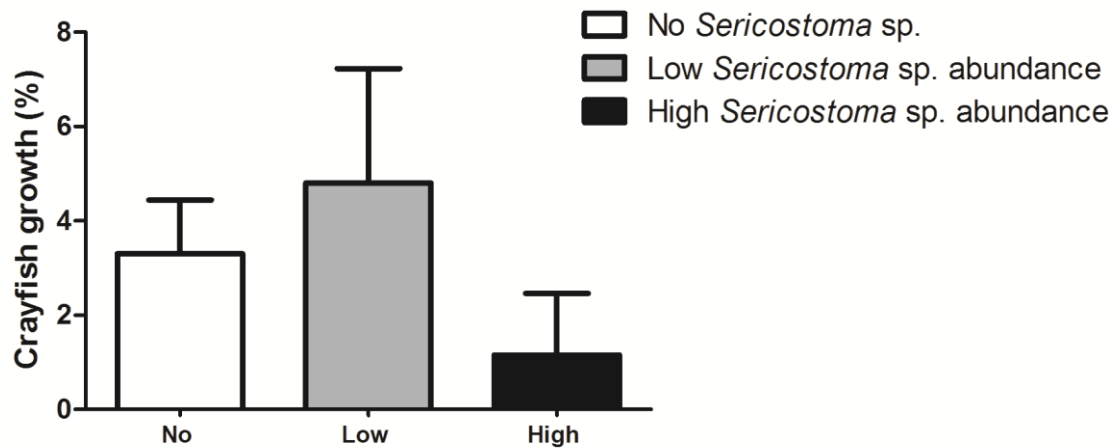


Figure 3- Percentage of *P. clarkii* growth during 21 days in mesocosms with different abundances of the invertebrate shredder *Sericostoma* sp (no *Sericostoma* sp.: 0 individuals; low abundance: 6 individuals; high abundance: 12 individuals). Mean \pm SEM, n=4.

Table 2- Two-way ANOVAs on the effects of *Sericostoma* sp. abundance and crayfish on leaf mass loss and FPOM production; One-way ANOVA on the effect of *Sericostoma* sp. abundance on the crayfish growth.

Parameter	Effect	SS	d.f.	MS	F	P
Leaf mass loss	<i>Sericostoma</i> sp. abundance	447.5	2	223.8	5.80	0.011
	Crayfish	5253.8	1	5253.8	136.26	0.000
	<i>Sericostoma</i> sp. abundance *Crayfish	135.2	2	67.6	1.75	0.201
	Error	694.0	18	38.6		
FPOM production	<i>Sericostoma</i> sp. abundance	0.0	1	0.0	0.29	0.598
	Crayfish	1.3	1	1.3	87.07	0.000
	<i>Sericostoma</i> sp. abundance *Crayfish	0.0	1	0.0	0.00	0.945
	Error	0.2	12	0.0		
Crayfish growth	<i>Sericostoma</i> sp. abundance	26.8	2	13.4	1.13	0.365
	Error	106.8	9	11.9		

3.2. Direct and indirect effects of *Procambarus clarkii* on leaf decomposition

3.2.1. Leaf mass loss

The effects of *P. clarkii* on leaf consumption by the invertebrate shredder *Sericostoma* sp. was tested by direct exposure of shredders to the crayfish or indirectly by exposure of shredders to water in which the crayfish was kept for 5 days (Fig. 4). *Sericostoma* sp. had the possibility to avoid predation but they had to take the risk of predation to feed on leaves. In the presence of *P. clarkii*, all larvae of *Sericostoma* sp. were eaten during the first days of the experiment. After 21 days, leaf mass loss varied between 43.2% in the absence of crayfish and 85.1% in the direct presence of the crayfish (Fig. 4).

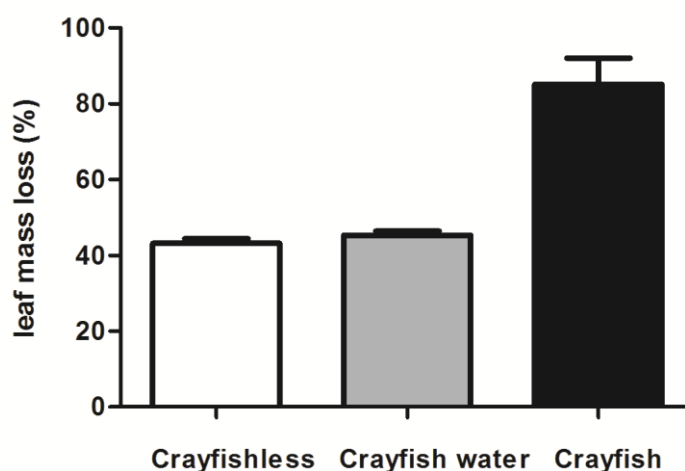


Figure 4- Percentage of leaf mass loss in mesocosms with 6 larvae of the invertebrate shredder *Sericostoma* sp. in the absence of *P. clarkii* (crayfishless) or with indirect (crayfish water) or direct presence (crayfish) of *P. clarkii*. Mean \pm SEM, n=4.

Leaf mass loss was significantly higher in mesocosms with the direct presence of *P. clarkii* than in the other treatments (one-way ANOVA, Tukey's test, $P < 0.0001$; Table 3). No significant differences were found in leaf decomposition by *Sericostoma* sp. in the absence or in the indirect presence of *P. clarkii* (Tukey's test, $P > 0.05$).

3.2.2. FPOM production

Production of FPOM in the absence and presence (direct or indirect) of *P. clarkii* is given in Fig. 5. The FPOM production varied between 0.2 g in the absence of the crayfish and 1.0 g in the direct presence of the crayfish.

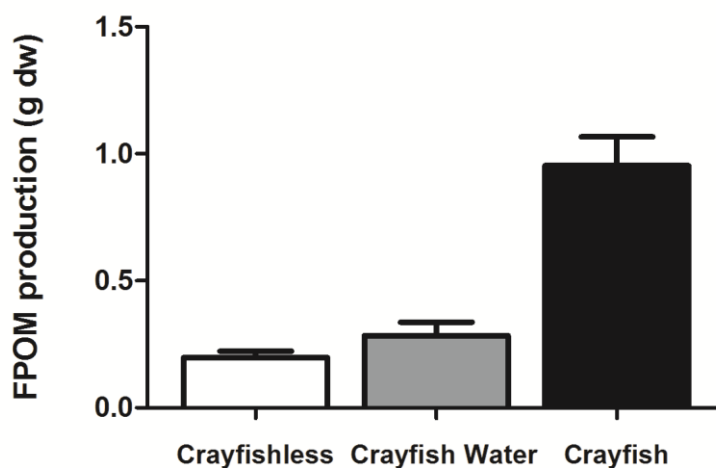


Figure 5- FPOM production in mesocosms with 6 larvae of the invertebrate shredder *Sericostoma* sp. in the absence of *P. clarkii* (crayfishless) or with indirect (crayfish water) or direct (crayfish) presence of *P. clarkii*. Mean \pm SEM, $n=4$.

FPOM production was significantly higher in the direct presence of crayfish than in mesocosms with indirect presence or absence of the crayfish (one-way ANOVA, Tukey's test, $P<0.0001$; Table 3). FPOM production was higher in mesocosms with crayfish water than in those without crayfish, but no significant differences were found (Tukey's test, $P>0.05$).

Table 3- One-way ANOVAs on the effects of crayfish on leaf mass loss and FPOM production. Treatment levels were: absence of crayfish, indirect presence of crayfish, direct presence of crayfish.

Parameter	Effect	SS	d.f.	MS	F	P
Leaf mass loss	Crayfish	4458.1	2	2229.1	33.14	0.0001
	Error	605.4	9	67.3		
FPOM production	Crayfish	1.4	2	0.7	31.40	0.0001
	Error	0.2	9	0.0		

3.3. Effects of size and sex of *Procambarus clarkii* on leaf decomposition

3.3.1. Leaf mass loss

The effects of size and sex of *P. clarkii* on leaf decomposition in the presence of the invertebrate shredder *Sericostoma* sp. are shown in Fig. 6. Leaf mass loss varied between 60.2% in treatments with small male crayfish and 83.5% in treatments containing big male crayfish (Fig. 6A).

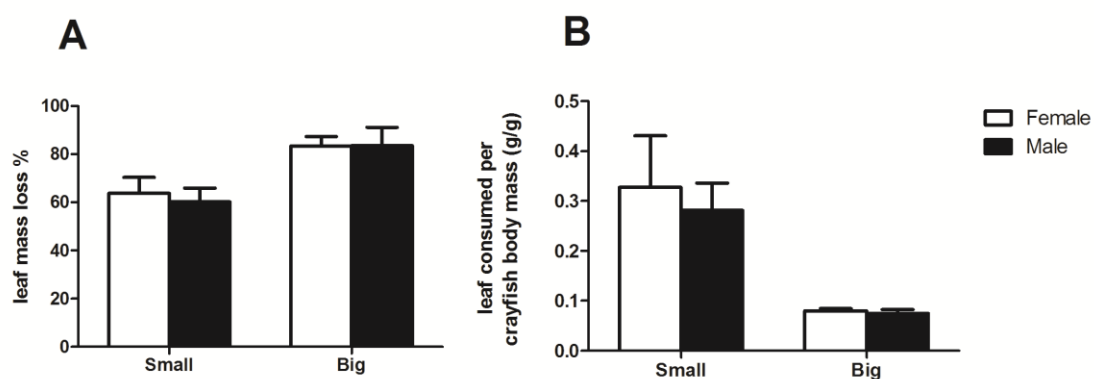


Figure 6- Percentage of leaf mass loss per mesocosm (A) and leaf consumed per crayfish wet biomass (B) in mesocosms with 6 larvae of the invertebrate shredder *Sericostoma* sp. and *P. clarkii* with different sex and size. Mean \pm SEM, $n=4$.

Leaf mass loss was significantly affected by the size of the crayfish but not by the crayfish sex (two-way ANOVA, Table 4). The crayfish size contributed with 50.5% to the total variance in leaf mass loss. No significant differences were found in leaf mass loss between sex within treatments with small or big crayfishes (Table 4).

An opposite trend was found when leaf mass loss was expressed as leaf consumed per crayfish body mass (Fig. 6B). Leaf consumption varied between 0.1 g/g in treatments with big male crayfish and 0.3 g/g in treatments with small male crayfish. Again, the crayfish size had a significant effect on leaf consumption, but crayfish sex had no effect (two-way ANOVA; Table 4).

3.3.2. FPOM production

FPOM production in mesocosms with the invertebrate shredder *Sericostoma* sp. and *P. clarkii* of different sex and size is shown in Fig. 7.

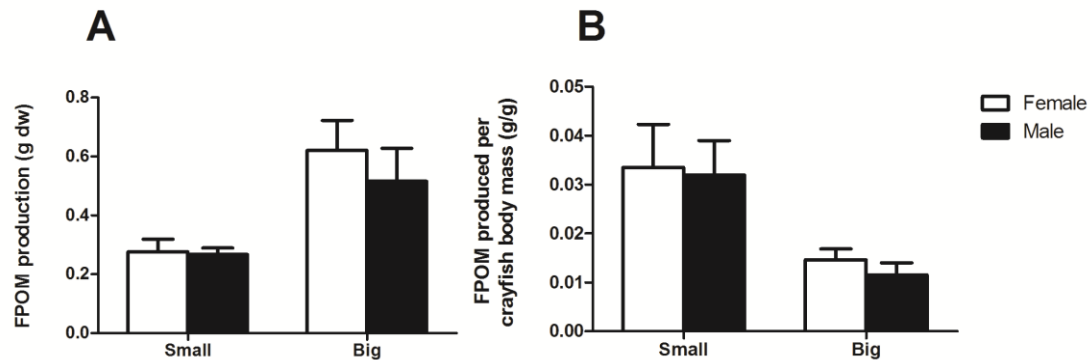


Figure 7- FPOM production per mesocosm (A) and FPOM production per crayfish wet biomass (B) in mesocosms with 6 larvae of the invertebrate shredder *Sericostoma* sp. and *P. clarkii* with different sex and size. Mean \pm SEM, n=4.

FPOM produced per mesocosm varied between 0.3 g in small male crayfish and 0.6 g in big female crayfish (Fig. 7A). FPOM production did not change with crayfish sex, but was affected by the crayfish size (two-way ANOVA, Table 4). The crayfish size accounted for 54.8% of the variance in FPOM production. FPOM production was significantly higher in the presence of big than small crayfish. *Sericostoma* sp. alone produced 0.1 g of FPOM.

A different trend was obtained when FPOM production was expressed in terms of g of FPOM produced per crayfish body mass (Fig. 7B). FPOM production varied between 0.1 g/g in treatments with big male crayfish to 0.3 g/g in treatments with small female crayfish. Again, the crayfish size had a significant effect on FPOM production, but crayfish sex had no effect (two-way ANOVA, Table 4).

3.3.3. Crayfish growth

The crayfish growth varied between 0.7% in big female crayfishes and 7.8% in small male crayfishes after 21 days in the presence of the invertebrate *Sericostoma* sp. (Fig. 8). The crayfish growth varied significantly with body size, but not with the crayfish sex (two-way ANOVA, Table 4).

Indeed, crayfish growth was significantly higher in treatments with small than big crayfishes (Tukey's tests, $p < 0.05$)

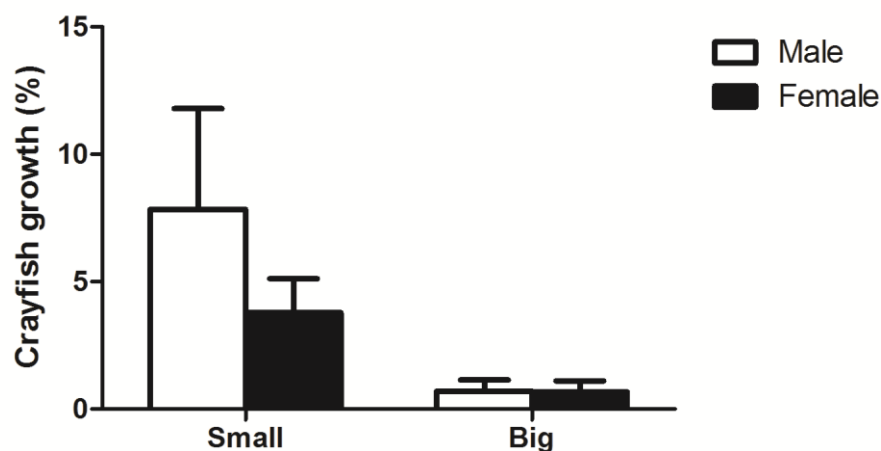


Figure 8- Percentage of *P. clarkii* growth with different size and sex in the presence of 6 larvae of the invertebrate shredder *Sericostoma* sp. Mean \pm SEM, $n=4$.

Table 4- Two-way ANOVAs on the effects of crayfish sex and size on leaf mass loss, leaf mass loss per crayfish biomass, FPOM production and crayfish growth.

Parameter	Effect	SS	d.f.	MS	F	P
Leaf mass loss	Crayfish sex	12.2	1	12.2	0.08	0.7792
	Crayfish size	1848.2	1	1848.2	12.41	0.0042
	Crayfish sex*Crayfish size	14.1	1	14.1	0.09	0.7640
	Error	1787.8	12	149.0		
Leaf mass loss per crayfish biomass	Crayfish sex	0.0	1	0.0	0.19	0.6732
	Crayfish size	0.2	1	0.2	14.92	0.0023
	Crayfish sex*Crayfish size	0.0	1	0.0	0.12	0.7353
	Error	0.2	12	0.0		
FPOM production	Crayfish sex	0.0	1	0.0	0.52	0.4856
	Crayfish size	0.4	1	0.4	14.11	0.0027
	Crayfish sex*Crayfish size	0.0	1	0.0	0.37	0.5563
	Error	0.3	12	0.0		
FPOM production per crayfish biomass	Crayfish sex	0.0	1	0.0	0.16	0.6918
	Crayfish size	0.0	1	0.0	11.25	0.0057
	Crayfish sex*Crayfish size	0.0	1	0.0	0.02	0.8972
	Error	0.0	12	0.0		
Crayfish growth	Crayfish sex	16.7	1	16.7	0.94	0.3519
	Crayfish size	104.9	1	104.9	5.88	0.0320
	Crayfish sex*Crayfish size	16.2	1	16.2	0.91	0.3586
	Error	214.0	12	17.8		

3.4. Field experiment: *in situ* validation

3.4.1. Physical and chemical characterization

The stream water physico-chemical parameters were very similar at upstream and downstream sites. Temperature ranged between 16.3°C at upstream and 16.4°C at downstream site (Table 5). The pH was 6.1 and 6.2 at upstream and downstream site, respectively, while conductivity varied between 76.2 $\mu\text{S}/\text{cm}$ at upstream and 78.5 $\mu\text{S}/\text{cm}$ at downstream site. Dissolved oxygen was 8.6 mg/L at both stream sites. The stream water nutrients showed slightly higher values at the upstream site (phosphate, 0.27 mg/L; ammonia, 0.02 mg/L; nitrate, 0.08 mg/L) than at the downstream site (phosphate, 0.16 mg/L; ammonia, 0.01 mg/L; nitrate, 0.07 mg/L) (Table 5).

Table 5- Stream water parameters at the sampling sites.

Parameter	Upstream	Downstream
Temperature (°C)	16.3	16.4
pH	6.1	6.2
Conductivity ($\mu\text{S}/\text{cm}$)	76.2	78.5
Dissolved oxygen (mg/L)	8.6	8.6
Phosphate P - PO_4^{3-} (mg/L)	0.27	0.16
Ammonia N- NH_4^+ (mg/L)	0.02	0.01
Nitrate N- NO_3^- (mg/L)	0.08	0.07

3.4.2. Leaf mass loss

Differences in leaf decomposition due to the presence/absence of the crayfish, the presence/absence of invertebrates and the stream site (upstream/downstream) are shown in Fig. 9. Results showed that leaf mass loss was significantly affected by the stream site and the crayfish presence, but not by the presence of invertebrates (three-way ANOVA; Table 6).

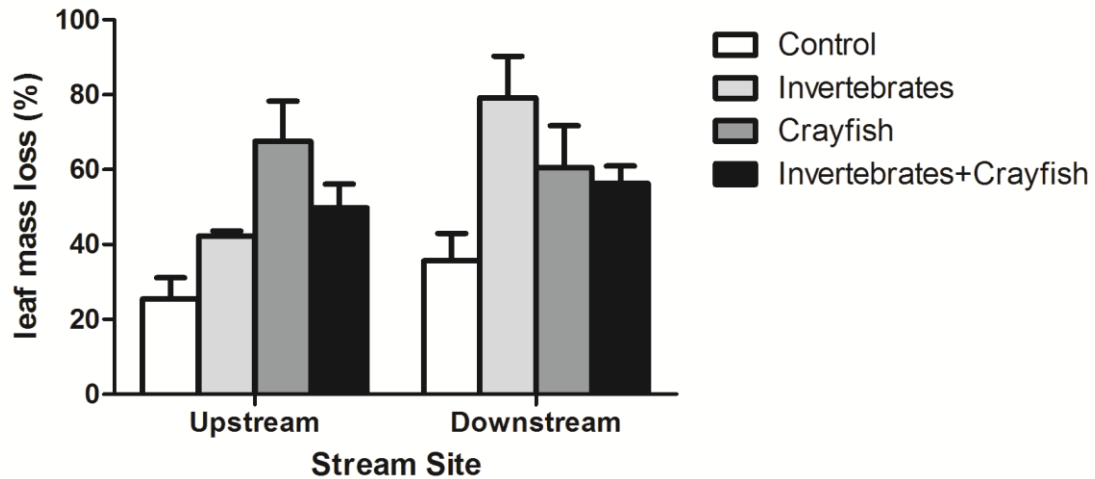


Figure 9- Percentage of leaf mass loss at upstream and downstream sites in the presence or absence of invertebrates and *P. clarkii*. Mean \pm SEM, n=4.

Leaf mass loss varied between 25.5% in the control (without the presence of invertebrates and *P. clarkii*) at the upstream site to 79.2% in treatments with invertebrates at the downstream site. Leaf mass loss differed between control and treatments with crayfish at the upstream site (Tukey's test, $P < 0.05$). At the downstream site, leaf mass loss was significantly different between control and treatments with invertebrates and crayfish (Tukey's test, $P < 0.05$).

Table 6- Three-way ANOVA on the effects of crayfish, invertebrates and stream site on leaf mass loss.

Parameter	Effect	SS	d.f.	MS	F	P
Leaf mass loss	Crayfish	4750.3	1	4750.3	18.61	0.0002
	Invertebrates	732.5	1	732.5	2.87	0.1032
	Stream Site	1087.8	1	1087.8	4.26	0.0500
	Crayfish*Invertebrates	667.2	1	667.2	2.61	0.1191
	Crayfish*Stream site	1.4	1	1.4	0.01	0.9411
	Invertebrates*Stream site	811.0	1	811.0	3.18	0.0874
	Crayfish*Invertebrates*Stream site	533.2	1	533.2	2.09	0.1613
	Error	6127.7	24	255.3		

3.4.3. Characterization of the invertebrate community

The Multi-Dimensional Scaling (MDS) analyses based on the abundance of the leaf-associated invertebrate community showed differences between treatments (presence or absence of crayfish) and stream site (upstream or downstream) (Fig. 10). The analysis allowed the discrimination of 5 groups with overlaid clusters with 50% of similarity in the ordination plot: 1) invertebrate group in the absence of crayfish at the downstream site (DCL2, DCL3, DCL4); 2) invertebrate group in the presence or absence of crayfish at the upstream site (UC1, UC3, UC4, UCL1, UCL3); 3) invertebrate group with two samples from downstream and one from upstream with crayfish (UC2, DC2, DC3); 4) invertebrate group with three samples without crayfish and one sample with crayfish (DC1, DCL1, UCL2, UCL4); and finally 5) invertebrate group with one isolated sample from downstream with crayfish (DC4). Invertebrate community differed between upstream and downstream sites (PERMANOVA, Table 7). The presence of crayfish did not affect the invertebrate community and no interaction between stream site and the crayfish presence was found (PERMANOVA, Table 7).

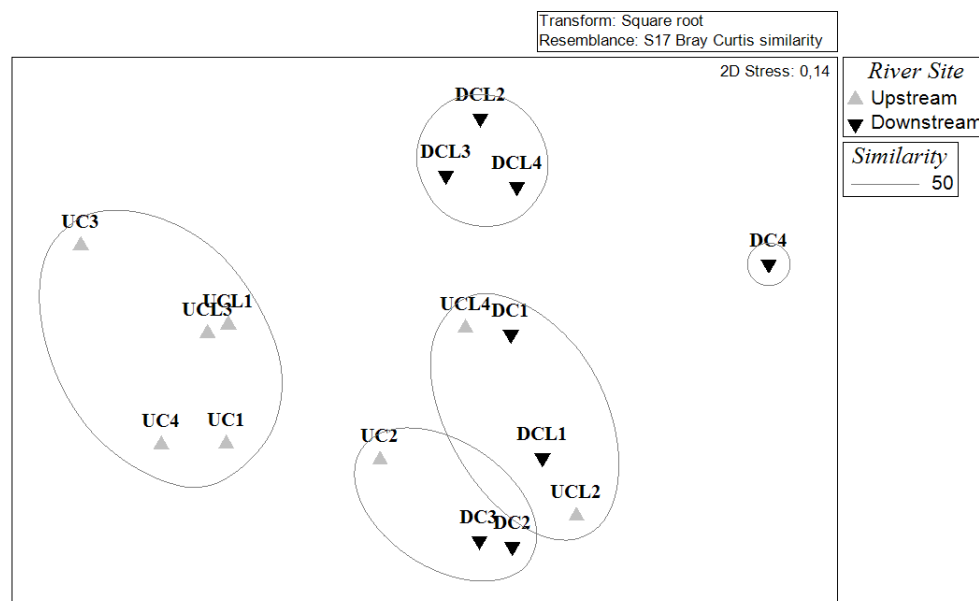
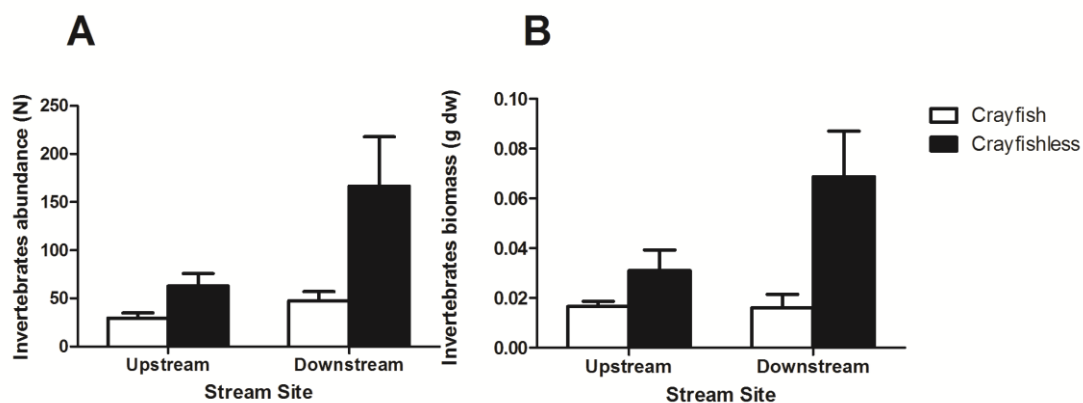


Figure 10- Multi-Dimensional Scaling (MDS) analyzes based on leaf-associated invertebrate community and using the Bray Curtis similarity index (UC: Upstream- Crayfish; UCL: Upstream- Crayfishless; DC: Downstream- Crayfish; DCL: Downstream – Crayfishless).

Table 7- PERMANOVA on the effect of stream sites and crayfish presence on leaf-associated invertebrate community.

Parameter	Effect	SS	df	MS	Pseudo-F	P
	Stream site	71.8	1	71.8	1.74	0.012
Invertebrate community	Crayfish	58.6	1	58.6	1.42	0.065
	Stream site*Crayfish	50.0	1	50.0	1.21	0.224
	Error	494.6	12	41.2		

After 21 days of leaf immersion, the invertebrate abundance varied between 30 individuals at the upstream site in the presence of the crayfish to 167 individuals at the downstream site in the absence of the crayfish (Fig. 11A). Differences in invertebrate abundance were significant between stream sites and also depended on the presence of the crayfish (two-way ANOVA, Table 8).

**Figure 11-** Invertebrates abundance (A) and biomass (B) at upstream and downstream sites in the presence or absence of *P. clarkii*. Mean \pm SEM, n=4.

Invertebrate abundance was higher in the absence of the crayfish and at the downstream site. The presence or absence of *P. clarkii* accounted for 29.0% of the total variance of invertebrate abundance, while the stream site accounted for 18.4%.

The invertebrate biomass after 21 days varied between 0.02 g at downstream site in the presence of crayfish and 0.07 g at downstream site in the absence of crayfish (Fig.11B). The presence of the crayfish affected significantly invertebrate biomass (two-way ANOVA, Table 8) and contributed to 36% of the total variance. Invertebrate biomass also varied with the stream site (two-way ANOVA, Table 8). At the downstream site, the invertebrate biomass was significantly

higher in the absence than in the presence of the crayfish (Tukey's test, $P < 0.05$). At the upstream site, invertebrate biomass was not affected by the presence of *P. clarkii* (Tukey's test, $P > 0.05$).

The presence of crayfish and the stream site affected significantly invertebrate community as indicated by the Margalef richness index, and no interactions between the two factors were found (two-way ANOVA, Table 7). The Margalef index varied between 2.0 at the upstream site in the presence of crayfish and 3.5 at the downstream site without crayfish (Fig. 12A). At the downstream site, the Margalef index was significantly higher in the absence than in the presence of crayfish (Tukey's test, $P < 0.05$). At the upstream site, the presence of the crayfish did not affect the Margalef index (Tukey's test, $P > 0.05$).

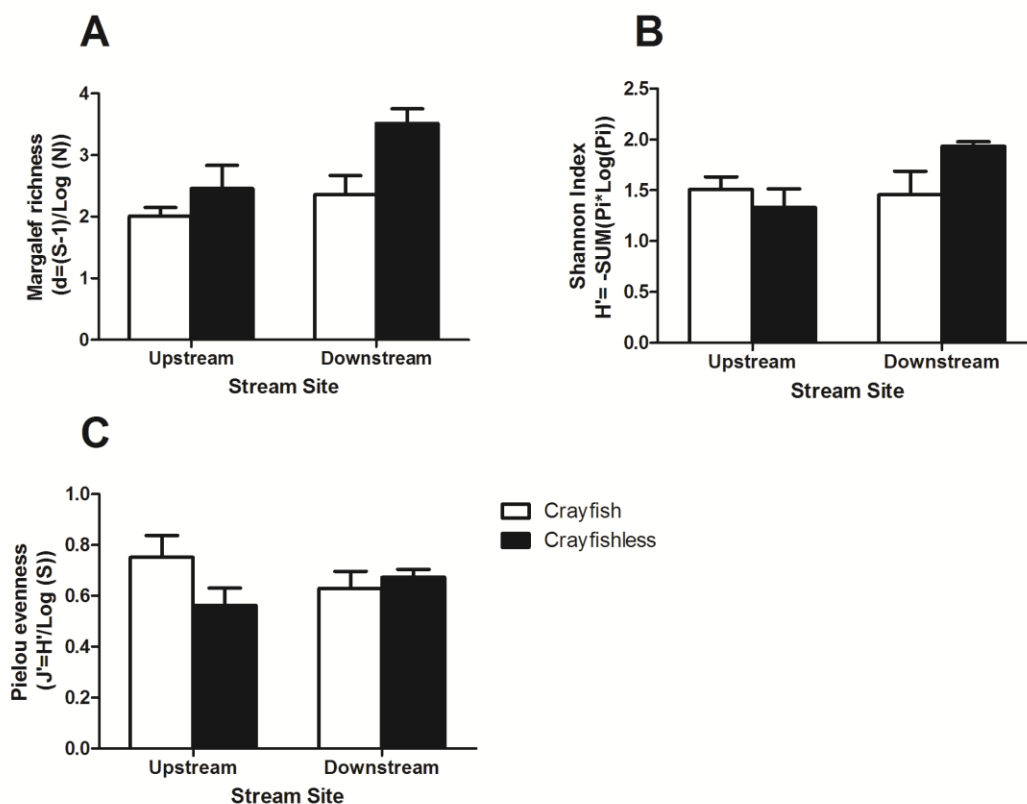


Figure 12- Diversity measures of leaf-associated invertebrate community at upstream and downstream sites in the presence or absence of *P. clarkii*. Margalef richness index (A) Shannon diversity index (B) and Pielou evenness index (C). Mean \pm SEM, $n=4$.

The Shannon diversity index was not significantly affected by the presence of crayfish neither by the stream site (two-way ANOVA, Table 8). The Shannon diversity index varied between 1.3 (upstream – crayfishless) and 1.9 (downstream – crayfishless) (Fig. 12B).

Also, the Pielou evenness was not significantly affected by the presence of crayfish or the stream site. The Pielou evenness varied between 0.6 (upstream – crayfishless) and 0.8 (upstream – Crayfish) (Fig. 12C).

Table 8- Two-way ANOVAs on the effects of stream site and crayfish on invertebrate abundance, invertebrate biomass, Margalef richness index, Shannon diversity index and Pielou evenness index.

Parameter	Effect	SS	d.f.	MS	F	P
Invertebrate abundance	Stream Site	14762.3	1	14762.3	5.09	0.0436
	Crayfish	23256.3	1	23256.3	8.01	0.0152
	Stream site*Crayfish	7396.0	1	7396.0	2.55	0.1364
	Error	34836.5	12	2903.0		
Invertebrate biomass	Stream Site	14786.2	1	14786.2	5.10	0.0434
	Crayfish	23253.4	1	23253.4	8.02	0.0151
	Stream site*Crayfish	7444.0	1	7444.0	2.57	0.1352
	Error	34809.8	12	2900.8		
Margalef Index	Stream Site	2.0	1	2.0	6.38	0.0266
	Crayfish	2.6	1	2.6	8.28	0.0139
	Stream site*Crayfish	0.5	1	0.5	1.61	0.2290
	Error	3.7	12	0.3		
Shannon Index	Stream Site	0.3	1	0.3	2.86	0.1166
	Crayfish	0.1	1	0.1	0.83	0.3802
	Stream site*Crayfish	0.4	1	0.4	4.00	0.0688
	Error	1.3	12	0.1		
Pielou Index	Stream Site	0.0	1	0.0	0.00	0.9455
	Crayfish	0.0	1	0.0	1.22	0.2905
	Stream site*Crayfish	0.1	1	0.1	3.07	0.1052
	Error	0.2	12	0.0		

3.4.4. Crayfish growth

After 21 days of the experiment, the crayfish growth varied between 0.5% at the downstream site in the presence of invertebrates and 1.5% at the upstream in the absence of invertebrates (Fig. 13). The presence of invertebrates and the stream site did not affect significantly the crayfish growth (two-way ANOVA, Table 9).

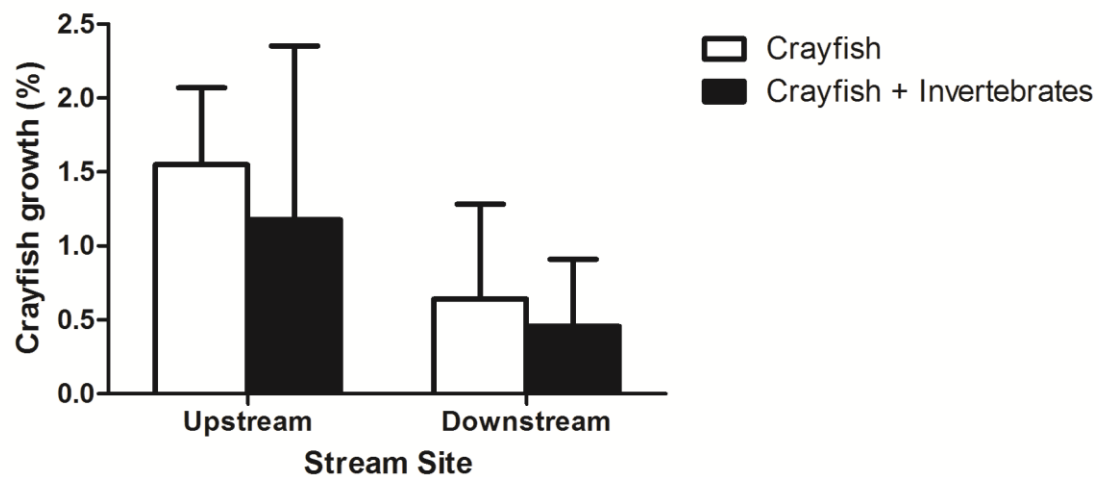


Figure 13- Percentage of *P. clarkii* growth in the presence or absence of invertebrates at upstream and downstream sites. Mean \pm SEM, n=4.

Table 9- Two-way ANOVA on the effects of invertebrate presence and stream site on the crayfish growth.

Parameter	Effect	SS	d.f.	MS	F	P
Crayfish growth	Invertebrates	14.0	1	14.0	0.24	0.6350
	Stream site	31.1	1	31.1	0.53	0.4825
	Invertebrates*Stream site	130.5	1	130.5	2.23	0.1662
	Error	585.3	10	58.5		

4. General discussion

The presence of the omnivore invader *P. clarkii* in freshwater ecosystems may be responsible for changes in detritus food webs. In this study, we showed that this NIS is able to control leaf-litter decomposition directly by consuming the leaves and indirectly by predation on invertebrate shredders that have a key role in the decomposition of leaf litter.

From the first laboratory experiment, we clearly showed that *P. clarkii* can decrease the *Sericostoma* sp. abundance due to predation and affect leaf decomposition process. The crayfish promptly ate all invertebrates in the aquariums in the first days of the experiment. The crayfish diet includes a wide range of animals, plants, detritus and sediments (Gherardi, 2006). *P. clarkii* is well known for its omnivory and shows a potentially high predation rates on aquatic invertebrates, mainly insects, crustaceans and gastropods (Momot, 1995). In addition, *P. clarkii* is known to disturb sediments through bioturbation (Angeler *et al*, 2001) and this behavior may have affected *Sericostoma* sp. once the species belonging to this genus are benthic invertebrates. So, we expected that crayfish engineering activities would dislodge or at least prevent invertebrates from hiding and avoid being predated. The direct effects of crayfish on leaf decomposition were well established in our study: higher leaf decomposition was observed in the presence of crayfish than in the presence of *Sericostoma* sp., regardless the *Sericostoma* sp. abundance (6 or 12 individuals). This can be explained by the body mass of the crayfish that was much higher than *Sericostoma* sp. biomass in the mesocosm experiments, or by higher energy metabolic requirements of *P. clarkii* in relation to *Sericostoma* sp. Although leaf decomposition was higher in the presence of *P. clarkii* comparing to *Sericostoma* sp., leaf decomposition seemed to increase with the increase of *Sericostoma* sp. abundance. This suggests that *Sericostoma* sp. might have affected decomposition of alder leaves before being predated.

The production of FPOM associated with leaf mass loss followed the pattern described above. The crayfish may have a negative effect on other invertebrate species and so contribute to a decrease in FPOM production, but at the same time their direct effect on leaf decomposition led to an increase in the production of FPOM available to other trophic levels. Nevertheless, if the process of leaf decomposition is not compromised but maintained by a restricted number of species, the resilience of the ecosystem may be affected (Peterson *et al*, 1998). Our results seemed to differ from those of Greig & McIntosh (2006) that showed that an invasive fish (*Salmo trutta*) lowered the density of invertebrates and consequently lowered the rates of leaf

decomposition and FPOM production in mesocosms. However, we cannot assume that this is true in our study because all the *Sericostoma* sp. were eaten by the crayfish and so the results of FPOM production are mainly due to crayfish detritivore activities. Furthermore, once that we did not determine the *Sericostoma* sp. biomass we cannot assume that crayfish leads to a higher FPOM production. The growth of the crayfish was not significantly affected by the abundance of *Sericostoma* sp., although growth was lower at higher levels of *Sericostoma* sp. abundance. This may indicate that the prompt predation on *Sericostoma* sp. might have masked the putative effects of invertebrate shredders on leaf decomposition and prevent us to observe competition between *Sericostoma* sp. and crayfish for leaf detritus consumption. Our results supported that crayfish is an important consumer of leaf detritus and may eventually replace large-bodied native detritivores as shown in other studies with other crayfish species such as *Pacifastacus leniusculus* (Dana, 1852) (Moore *et al*, 2012).

To better understand the effects of crayfish on invertebrate shredders, we examined the direct and indirect effects of *P. clarkii* on *Sericostoma* sp. Our results showed that *Sericostoma* sp. did not change its shredding behavior on leaf decomposition in mesocosms filled with water previously exposed to *P. clarkii*. *Sericostoma* sp. had the possibility to avoid predation because they were separated from the predator and alder leaves by a mesh net. However, after a few moments in the mesocosms *Sericostoma* sp. crossed the mesh net to feed on alder leaves. This may happen for two reasons: 1) the shredders were under starvation before starting the experiment and so their need to feed was high and they took the risk of being predated; 2) *Sericostoma* sp. did not recognize *P. clarkii* as a potential threat. The latter hypothesis may happen because the shredders used in the experiment were collected from a stream where *P. clarkii* (or any other crayfish species) was not present and so *Sericostoma* sp. was not able to recognize the crayfish as a predator or as a potential threat. This naivety behavior was already described for other organisms but it is barely known for aquatic macroinvertebrates. Once again, all shredders were eaten under the direct presence of the predator in the first days of the experiment and, so, in the aquariums with the crayfish and *Sericostoma* sp. leaf decomposition was mostly mediated by the crayfish.

In our study, the crayfish produced more FPOM than the invertebrate shredder *Sericostoma* sp. (6 individuals). Although the shredding activities of crayfishes may increase the production of FPOM and facilitate invertebrate collectors to utilize this organic matter (e.g. Huryn

and Wallace, 1987), other studies indicated that crayfishes choose to feed on the most soft and quality leaf parts, reducing the quality of detrital resources to primary consumers (Usio, 2000). Therefore, the FPOM produced by the crayfish may not be of good quality and this may contribute to changes in detritus foodwebs in invaded areas. The indirect presence of crayfish (using water exposed to crayfish) did not decrease the FPOM produced by the *Sericostoma* sp. It is known that populations may take generations to perceive the indirect effects of predators and we may be only quantifying transient dynamics in a population of *Sericostoma* sp. subjected to the presence of a new predator (Yodzis, 1988; Vance-Chalcraft *et al*, 2007). In this regard, and recognizing the widespread distribution of *P. clarkii*, more studies should be done to assess the effects of the direct and indirect presence of the crayfish on other invertebrate shredders from streams where this NIS is already well-established.

Our results did not show any differences in leaf decomposition or shredder predation by crayfishes between males and females under laboratory conditions. Usually, crayfish males are much more aggressive than females (Abrahamsson, 1966; Stein & Magnuson, 1976; Berril & Arsenault, 1984; Usio, 2002) and this activity might contribute to leaf breakdown besides the feeding activity. However, our results agree with other studies showing no differences in diet preferences between males and females of *P. clarkii* (Gutiérrez *et al*, 1998). On the other hand, crayfish size was an important trait for leaf consumption. Although bigger animals consumed higher amounts of leaf litter, smaller animals showed higher levels of leaf consumption per animal biomass. As showed before, smaller animals may have higher metabolic needs and so they can consume higher amounts of leaf litter compared to larger animals. Furthermore, and once the juveniles used in this study were not yet sexually mature, their energetic demands are mainly devoted to rapid growth and so these smaller crayfish may have higher consumption rates than bigger crayfish. Consistently to that found for leaf decomposition, crayfish sex had no effect on FPOM production but bigger animals were able to produce more FPOM. In the same vein, smaller crayfish produced higher FPOM amounts per body mass, and this supports higher metabolic and energetic demands of smaller than bigger animals, as suggested above. As expected, the crayfish growth was affected by the size, and higher growth was observed in smaller animals. Since smaller animals had higher growth this may implicate higher metabolic needs and so explain the higher leaf mass loss and FPOM production by the smaller animals.

Our laboratory mesocosms showed preliminary findings of direct and indirect interactions of *P. clarkii* with leaf decomposition and *Sericostoma* sp. density. However, these findings should be carefully considered because the spatial scale of the experiment is particularly important in freshwater systems since aquatic invertebrates are highly mobile and this mobility may affect our capability to detect how invertebrates respond in nature. Our manipulations in small aquariums are an oversimplification of natural ecosystems that have a much higher complexity and offer, for example, much more opportunities of refuge for highly mobile invertebrates. Indeed, the aquariums used in these mesocosms experiments were small and this could impair the capacity of invertebrate shredders of *Sericostoma* sp. to avoid the predator (Englund & Olsson, 1996; Englund, 1997). On the other hand, mesocosms allowed us to have a highly controlled experiment where we were able to clearly show how the introduction of a predator affect naïve prey which is useful to comprehend mechanisms that control ecological processes in detritus food webs. Given the potential drawbacks of mesocosm experiments it is always important to confirm results in natural ecosystems (Polis, 1994; Polis & Strong, 1996; Persson, 1999).

In this regard, our field experiment was an *in situ* validation of the preliminary results obtained in laboratory mesocosms and with this procedure we were able to show some of the *P. clarkii* effects on stream-dwelling invertebrate communities subjected to natural trophic interactions. Here we had the possibility to understand how different were the effects of the crayfish on leaf decomposition and invertebrate communities at adjacent stream sites where the invasive crayfish was present or absent. The study sites had similar abiotic characteristics what was expected by the proximity of the two stream sites. Stream water chemistry was similar to that found in other oligotrophic streams in north Portugal (Pascoal *et al*, 2003) except for phosphate that showed higher values probably due to a sporadic runoff from upstream areas. In addition, nutrients were quantified only once at the end of the summer when the river flow was low, which may explain the higher levels of phosphate. Temperature in both stream sites was similar to that in natural habitats colonized by *P. clarkii* in the Iberian Peninsula (Gil Sánchez & Alba Torcedor, 2001). On the other hand, the fish species at both sites were very similar which means that potential differences in leaf decomposition between downstream and upstream sites are not related to differences in the fish community (Projecto Natura Miño-Minho, 2012).

Leaf decomposition showed a different pattern at each stream site. At the upstream site (site that never experienced the presence of *P. clarkii*), decomposition of alder leaves was higher

in the presence of the crayfish than in the presence of other invertebrates. This can be explained by the size or the metabolic needs of the crayfish and also because other invertebrates had the possibility to feed inside and outside the baskets, while the crayfish only had the possibility to feed on the leaves inserted in the baskets at the beginning of the experiment. Other studies have shown that leaf consumption by the crayfish is higher in lotic than in lentic systems (Fidalgo *et al*, 2013). This may be explained because water flow washes away leaf material in lotic systems, and the hydrographic conditions in lentic systems are more stable allowing the crayfish to show a coprophagic behavior leading to a reduction of leaf consumption (Fidalgo *et al*, 2013). Leaf decomposition in the presence of crayfish and invertebrates was lower than in treatments with the crayfish alone at the upstream site. This can be partially explained by competition between the crayfish and invertebrates for the available resources (i.e. leaves). On other hand, crayfishes had the possibility to feed directly on leaves or on invertebrates that entered the baskets, reducing in this way leaf decomposition. The effects of the crayfish on leaf mass loss at the upstream site were similar to those found in the laboratory experiment, except when invertebrates and crayfish were together. This can be explained by the higher diversity and biomass of invertebrates in the stream compared to the aquariums where all invertebrate shredders were eaten few days after the beginning of the experiment. These results may indicate that in streams where diversity and biomass of invertebrate shredders are low, *P. clarkii* may play an important role in leaf decomposition as shown for other crayfish species (Usio, 2000).

At the downstream site (site already subjected to the presence of *P. clarkii*), the pattern of leaf mass loss was very different from the upstream site. Leaf mass loss was higher in the presence of invertebrates than in the presence of crayfish alone. In fact, the abundance of invertebrates in the baskets without the crayfish was higher at the downstream site than at upstream site and this may be responsible for higher leaf mass loss at the former site. Although crayfishes are much bigger than the other invertebrates, leaf decomposition mediated by the crayfish was lower than that driven by invertebrates at the downstream site. This situation may corroborate earlier studies that emphasize that richer communities perform better due to complementary or facilitation interactions (Cardinale *et al*, 2002). Despite this, leaf mass loss in the presence of invertebrates and crayfish was lower than that driven by invertebrates alone and was similar to that driven by the crayfish alone. These results may be due to i) competition for resources between invertebrates and crayfish, ii) invertebrates were feeding outside the baskets

because they did not need to enter the basket to feed on leaves, or iii) invertebrates were avoiding the predator. Results of leaf decomposition at the upstream site agree with those obtained in the mesocosm experiment and with those obtained by Usio (2000) with the crayfish *Paranephrops zealandicus*, where the exclusion of the crayfish did not result in cascading effects on basal resources via increased abundance of invertebrates consuming leaves. However, at the downstream site, the exclusion of the crayfish appeared to induce cascading effects and invertebrate abundance and biomass could reach very high levels as found in some tropical streams (Pringle & Hamazaki, 1998; Rosemond *et al*, 1998).

The invertebrate community clearly differed between upstream and downstream sites. The effects of the crayfish on invertebrates were stronger at the downstream site than at the upstream site. At the downstream site, the invertebrate community in the baskets without the crayfish differed from the others as shown by the MDS analysis. Since at the downstream site the crayfish was already well-established, the invertebrate community already had the contact with this species for many years (*P. clarkii* was introduced in the Minho River basin at least at the beginning of the 1990s; Sousa *et al*, 2013). This situation supports the hypothesis that invertebrates may have had enough time to identify the crayfish as a predator, and to adapt their feeding behavior to avoid being predated. At the upstream site, the presence of the crayfish was not reported before, and so invertebrates are expected to have a naïve behavior and ignore that crayfish may predate them. Results of invertebrate abundance and biomass are another indicator of this assumption. Higher abundance and biomass of invertebrates were found downstream. However, the crayfish had a negative effect on invertebrate abundance and biomass, particularly at the downstream site. Here, data from invertebrate abundance and biomass clearly indicated that a large number of invertebrates (that corresponded to a larger biomass of individuals) behaved to avoid the baskets with the crayfish. In temperate streams, other crayfish species (e.g., *Paranephrops zealandicus*) are reported to affect not only leaf decomposition but also the pattern of colonization by invertebrates (Usio, 2000). The Margalef richness index applied to leaf-associated invertebrates followed the same pattern of invertebrate abundance and biomass. Invertebrate richness was higher when the crayfish was absent and the effects of crayfish presence were stronger at the downstream site (i.e., the downstream site had higher invertebrate richness than the upstream site). The Shannon and Pielou indices did not vary with stream site, crayfish presence or invertebrate presence. Even so, the Margalef index, invertebrate abundance

and biomass showed the same pattern at the downstream site, while at the upstream site higher evenness and diversity were found in the presence of the crayfish. Crayfish growth was higher upstream and in the absence of invertebrates at both sites. These results may be explained taking into account our data and previous assumptions that invertebrates may compete with the crayfish for leaf detritus and so crayfish would grow less in the presence of invertebrates.

Overall and following Diehl's (1993) hypothesis, our results suggest that the omnivore invader *P. clarkii* had the potential to affect the aquatic food chain directly through consumption of basal resources (leaf litter) or other invertebrates. This latter interaction may be also responsible for indirect changes in basal resources. Although *P. clarkii* may impact the invertebrate community at the time of its introduction in aquatic ecosystems and may be responsible for reduction in invertebrate abundance, biomass and species richness, it may also happen that after a certain period of time the invertebrate community learns how to adjust their behavior taking in account the presence of *P. clarkii* as a predator. Indeed, our results in the field experiment seemed to corroborate this hypothesis since the invertebrate community at the downstream site showed higher invertebrate diversity, abundance and biomass leading to higher leaf decomposition. Based on results from the downstream site, we hypothesize that crayfish may also have a strong positive indirect effect on leaf litter due to consumption of native invertebrates and those results are coincident with those found for the crayfish *P. leniusculus* in Californian (USA) streams (Moore *et al*, 2012). The invertebrate community seemed to be capable of learning how to recognize the crayfish as a predator after its introduction but more studies are needed to better understand how long this process takes. However, we were not able to find pre-invasion data for a comparative study and to ensure that the patterns shown here are just related to the crayfish presence. Our study and others (e.g. Wyman, 1998; Konishi *et al*, 2001; Mancinelli *et al*, 2002; Greig & McIntosh, 2006) indicate that predators can influence invertebrate densities including detritivores and have also an indirect impact on leaf litter decomposition.

Our data support that the invader *P. clarkii* is an omnivore predator capable of having strong impacts on heterotrophic aquatic systems. This species can affect negatively the detritivore invertebrate community and/or negatively affect leaf decomposition adopting a shredder behavior. At the same time, they may provide an indirect negative effect on the feeding

behavior of invertebrate detritivores and an indirect positive effect on leaf detritus by reducing and balancing leaf mass loss when consuming invertebrate shredders (Fig.14).

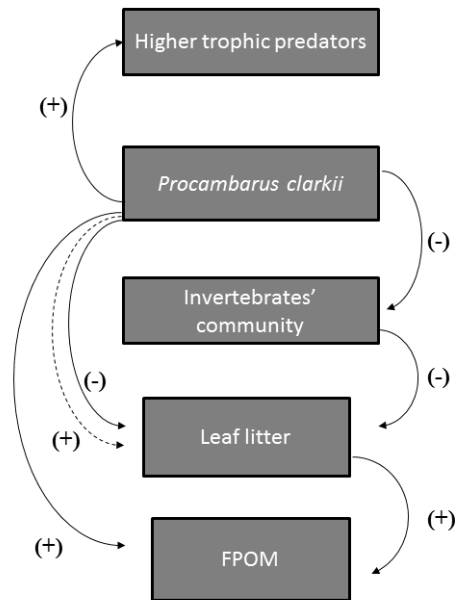


Figure 14 - Scheme of direct and indirect impacts of *Procambarus clarkii* on detritus food webs. Solid arrows show direct impacts and dashed arrows show indirect impacts.

5. Conclusion and future perspectives

The results obtained in this study are an important example of the impacts of an invasive species on detritus food webs in freshwaters using as a model organism the crayfish *P. clarkii* that is nowadays well spread worldwide reaching a high density and biomass in several aquatic ecosystems (Gherardi, 2006). Although our study increased our knowledge about the role of this species in plant litter decomposition, there is a long wide path to explore in the understanding of the mechanisms and dynamics of those impacts. We found that the invertebrate community may react in different ways to the presence of the crayfish and this response may be context dependent, with time after invasion probably playing an important role in the response. In this regard, it will be important to further clarify the naïve behavior of invertebrates that do not recognize *P. clarkii* as a predator using a mix of laboratory and field experiments with naïve and non-naïve populations. Another possible interesting study will be to assess the impacts of crayfishes that loose claws, a regular event especially during reproduction, on litter decomposition and invertebrate communities. Future research based on trophic cascades should include predators that can control the population of *P. clarkii* and reduce their impacts on lower trophic levels. Studies should also include more invertebrate species in laboratory conditions for extended periods of time and other crayfish species to check if they have the same impacts. Once *P. clarkii* (Fidalgo *et al*, 2013) and invertebrates (Kominoski *et al*, 2010) demonstrate preference to feed on certain leaf litter species it is relevant to study if impacts of crayfish on litter decomposition and trophic interactions may change with leaf litter quality and diversity.

Biodiversity and ecosystem functioning is nowadays a very important topic in ecology; however, the major part of studies dealing with BEF emphasizes the possible changes in functions as a result of species loss during the Anthropocene. This could be an oversimplification because nowadays many ecosystems are subjected to species additions due to the introduction of invasive species and this situation may also be responsible for fundamental changes in ecosystem processes and functions. Therefore, it is timely to focus also our attention on the impacts of introducing new species in ecosystems and further understand how the native populations and ecosystem processes will be sustainable through time.

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