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# Invasive crayfishes as a threat to freshwater bivalves: Interspecific differences and conservation implications

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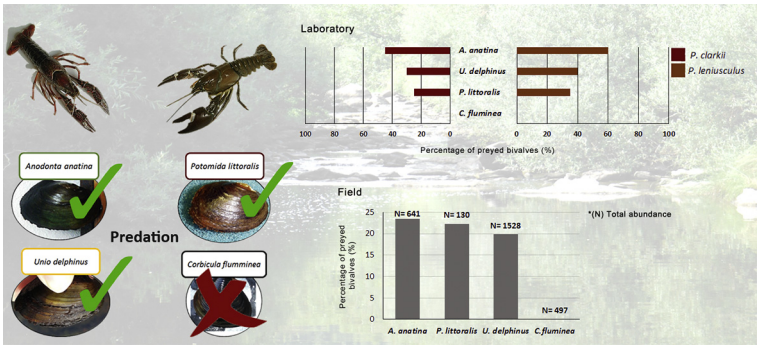
## HIGHLIGHTS

- Invasive crayfishes may threaten native freshwater bivalves.
- Predation of freshwater bivalves by invasive crayfishes was distinct among species.
- *Anodonta anatina* was the most preyed species.
- Future studies should assess predation of invasive crayfishes on freshwater bivalves.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Freshwater bivalves have suffered major global declines, being the introduction of invasive alien species (IAS) an important, but not well studied, mechanism of threat. This study assessed the predator-prey relationship between two non-native crayfish species (*Procambarus clarkii* and *Pacifastacus leniusculus*) and three native (*Anodonta anatina*, *Potomida littoralis* and *Unio delphinus*) and one non-native (*Corbicula fluminea*) freshwater bivalve species through experiments in laboratory and validation under natural conditions (Sabor River basin, Portugal). All native bivalve species were preyed both in laboratory and in the field; however, both crayfish species were unable to prey *C. fluminea*. Predation was dependent on crayfish and bivalve species but was not affected neither by crayfish nor bivalve sizes. In the laboratory, the most preyed species by both crayfishes was *A. anatina*. On average, this species was preyed at least 12% more than other species, when crayfishes had a choice. Similar results were found in the field. We also found signs of competition between both crayfishes, being *P. clarkii* more dominant and aggressive as this species, on average, manipulated the bivalves 63.6% more times and 24:33 min longer than *P. leniusculus*, and initiated 55.8% more agnostic bouts. Our results support the idea that *P. clarkii* and *P. leniusculus* can affect native freshwater bivalves, but clear interspecific differences were detected. Both crayfishes may have direct and indirect impacts on bivalve populations by increasing mortality or by reducing their fitness. In addition, since both crayfishes do not prey *C. fluminea*, they offer this IAS

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another advantage over native bivalves. Given the widespread distribution of both *P. clarkii* and *P. leniusculus* and the threatened status of many freshwater bivalves, the dynamics and impacts of this relationship should be taken in account in the implementation of management measures devoted to the conservation of native freshwater bivalves.



## 1. Introduction

The introduction of invasive alien species (IAS) is among the most important, and sometimes irreversible, human-induced changes on ecosystems, being recognized as one of the biggest threats to biodiversity (Sala et al., 2000; Vitousek et al., 1997). IAS can have numerous impacts in terrestrial and aquatic ecosystems (Olden et al., 2004; Simberloff et al., 2013). For example, 69% of the established aquatic species introduced in six European countries have ecological impacts and these may include changes in biodiversity and ecosystem functioning, trophic interactions and ecosystems' physical properties (García-Berthou et al., 2005; Sousa et al., 2011; Gutiérrez et al., 2014). These changes have possible effects at individual, population, community and ecosystem levels, also leading to high economic losses (Simberloff et al., 2013; Simon and Townsend, 2003; Strayer, 2010; Vilà et al., 2010).

In Europe, numerous crayfish species have been introduced and they can be responsible for several ecological and economic impacts (Gherardi, 2006, Hobbs et al. 1989). Crayfishes can have profound effects in water quality, nutrient dynamics, decomposition and community structure by acting as ecosystem engineers (Carvalho et al., 2016; Creed Jr. and Reed, 2004; Johnson et al., 2010; Matsuzaki et al., 2009; Sousa et al., 2013).

The Signal crayfish *Pacifastacus leniusculus* (Dana, 1852) is a recognized successful invader (Henttonen and Huner, 1999). This species is native to north-western United States of America (USA) and south western Canada (Bondar et al. 2005; Henttonen and Huner, 1999). It was initially introduced in Europe (Sweden in the 1960s) for stocking purposes and as a commercial substitute of the crayfish species *Astacus astacus* (Linnaeus, 1758), which became almost extinct in Europe due to the crayfish plague (Gherardi, 2006). The successful establishment of this species encouraged the later introduction of the Louisiana crayfish, *Procambarus clarkii* (Girard, 1852), in Spain in the 1970s (Gherardi, 2006; Gutiérrez-Yurrita et al., 1999). *Procambarus clarkii* is listed within the top 10 invasive species in Europe with the highest number of impacts on ecosystem services (DAISIE database, 2018; Vilà et al., 2010). This species is native to north-eastern Mexico through south-central USA east to Florida (Gherardi, 2006; Henttonen and Huner, 1999; Hobbs et al., 1989). Both crayfish species spread throughout Europe with considerable success and are now well established. They are also present in Portugal where they occur in sympatry in Sabor River basin (Bernardo et al., 2011; Gutiérrez-Yurrita et al., 1999). *Pacifastacus leniusculus* and *P. clarkii* are known to have a highly plastic diet as they are omnivorous and display generalist and opportunistic feeding habits (Guan and Wiles, 1998; Gutiérrez-Yurrita et al., 1998). *Procambarus clarkii* and *P. leniusculus* may consume large quantities of detritus (e.g. leaf litter) and plants, but they also feed on other animals such as amphibians, fishes, invertebrates, including other crayfishes (Axelsson et al., 1997; Gherardi et al., 2001; Gherardi, 2006; Guan and Wiles, 1998). Their feeding habits are known to have direct and indirect impacts on other species and thus affecting the invaded ecosystems (Axelsson et al., 1997; Gherardi, 2006; Nyström et al., 1996). Even though *P. clarkii* and *P. leniusculus* possible impacts and co-existence in many freshwater ecosystems are recognized, little is known about their role as predators of invertebrate species, such as freshwater bivalves.

Bivalves play a major ecological role in freshwater ecosystems (Howard and Cuffey, 2006; Lopes-Lima et al., 2018; Vaughn and Hakenkamp, 2001; Vaughn et al., 2008); however, in the last decades, freshwater bivalves, especially freshwater mussels from the Unionida order, have suffered a major global decline and are among the most threatened faunal groups in the planet (Lopes-Lima et al., 2014a, 2018; Williams et al., 1993). Since crayfishes can prey on bivalves, the recent introduction of several crayfish species is a possible important threat to the survival of these animals. Nevertheless, very few studies assessed bivalve predation by invasive crayfishes (e.g. Klocker and Strayer, 2004; Machida and Akiyama, 2013) and so the possible consequences of these introductions remain speculative and almost ignored. Given this knowledge gap, the main aim of this study was to describe the potential impacts of *P. clarkii* and *P. leniusculus* on four freshwater bivalve species (the mussels *Anodonta anatina*, *Potomida littoralis* and *Unio delphinus*; and the clam *Corbicula fluminea*) by assessing: i) if predator-prey interactions occur between the invasive crayfishes and bivalves and at what intensity; ii) if some bivalve species are more prone to predation than others; iii) if predation levels depend on predator and prey size; and iv) if *P. clarkii* and *P. leniusculus* compete for the bivalves as a prey. Using laboratory experiments and validations in natural conditions, the null hypothesis of the present study was that the crayfishes have no preference for freshwater bivalve species as prey and both crayfish should have a similar behaviour. However, we predicted that species with thicker shells (*P. littoralis* and *C. fluminea*) will be less prone to predation and *P. clarkii* will present a more aggressive behaviour than *P. leniusculus*.

## 2. Material and methods

### 2.1. Study area and animals' collection and maintenance

Sabor River has its source in Zamora province (Spain) and enters in Portugal by crossing the Montesinho mountain (Bragança). This river is a tributary of Douro River and has a wide range of environmental conditions: elevation range between 100 and 1500 m, total annual precipitation between 443 and 1163 mm, mean annual temperature between 6.9 and 15.6 °C. The flow regime in the Sabor River basin is highly seasonal, having some dried streams or disconnected pools during the summer (Filipe et al., 2017). A large dam was built recently in the lower Sabor River and started to operate in 2016. The basin has overall good ecological quality although some problems related with organic pollution and regulation of river flow have arisen over the last years (Sousa et al., 2012).

Throughout this work, three native (*A. anatina*, *P. littoralis* and *U. delphinus*) and one non-native (*C. fluminea*) bivalve species were studied. The four bivalve species have a sympatric distribution in the Sabor River basin also co-existing with the invasive crayfish species *P. leniusculus* and *P. clarkii* (Filipe et al., 2017; Sousa et al., 2012).

Bivalves were collected in the Sabor River basin by two researchers using snorkelling. Specimens were found visually or by searching through the bottom with hands. Both crayfish species were collected in the Sabor River basin by consistently placing several small baited traps (50 × 30 × 20 cm; 0.5 cm mesh) in the river bottom for 24 h. Only mature males were used in the laboratory experiments in order to minimize possible sex bias in the assessment of the predatory behaviour.

After collection, organisms were immediately transported to the laboratory. Bivalves were maintained in aquariums (60 × 30 × 30 cm) with