

Universidade do Minho
Escola de Ciências

Alexandra Gonçalves Meira

**Predation of freshwater bivalves by
invasive crayfishes**

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invasive crayfishes**

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Predation of freshwater bivalves by invasive crayfishes

Abstract

The introduction of non-native species is one of the main threats to biodiversity and freshwater ecosystems are extremely vulnerable to biological invasions. *Procambarus clarkii* and *Pacifastacus leniusculus* are problematic aquatic invasive species and are well known for their feeding plasticity. These species are capable of preying bivalves. In the last decades, freshwater bivalves have suffered major global declines, being the introduction of invasive species a possible important, but not well studied, threat. Given this perspective, the aim of this study was to assess the potential impacts of *P. clarkii* and *P. leniusculus* on freshwater bivalve populations.

For this it was verified whether there is a predator-prey relationship between these crayfish species and native (*Anodonta anatina*, *Potomida littoralis* e *Unio delphinus*) and invasive (*Corbicula fluminea*) bivalve species through experiments in laboratory and sampling two rivers (Sabor and Maçãs) in the north of Portugal where all species live in sympatry. In the laboratory, crayfish feeding preferences in terms of bivalve species and competition between the two crayfish species were also assessed. In the field, abundances and length of the specimens of each bivalve species were recorded, crayfish predation marks on bivalves were quantified and crayfish distribution, abundance and sex were determined.

All native bivalve species were preyed both in laboratory and in the field; however, *P. clarkii* and *P. leniusculus* were unable to prey the non-native *C. fluminea*. Bivalve predation was not affected neither by the lengths or sex of the crayfish. The most preyed native species by both crayfishes was *A. anatina*, while *P. littoralis* was the least preyed species. We also found competition for bivalves between the crayfishes, being *P. clarkii* the dominant species and displaying a more aggressive behaviour than *P. leniusculus*. In addition to direct mortality on native bivalves (which in the mesocosm experiments was extremely low) indirect effects can also be important (although not studied) because it is expected that animals with predation marks may have their fitness reduced causing impacts on bivalve populations and consequent effects on ecosystem functions and services (e.g. filtration, nutrient cycling, bioturbation).

The results of this study support the idea that *P. clarkii* and *P. leniusculus* can affect native bivalves. These predators may have direct and indirect impacts on bivalve populations by reducing the number of effectives by increasing mortality and by reducing their fitness, which may have indirect impacts on freshwater ecosystems. On the other hand, the fact that these crayfishes do not prey *C. fluminea* offers this invasive bivalve another advantage over native species. Therefore, future management actions devoted to the conservation of freshwater bivalves should have in account the possible effects of invasive crayfishes on these organisms.

Predação de bivalves de água doce por lagostins invasores

Resumo

A introdução de espécies não nativas é uma das principais ameaças à biodiversidade e os ecossistemas de água doce são extremamente vulneráveis a invasões biológicas. *Procambarus clarkii* e *Pacifastacus leniusculus* são espécies invasoras problemáticas e conhecidas pela sua grande plasticidade alimentar. Estas espécies são capazes de predação de bivalves. Nas últimas décadas os bivalves de água doce sofreram grandes declínios a nível global, sendo a introdução de espécies invasoras uma possível ameaça importante, mas não muito bem estudada. Dada esta perspectiva, o objetivo deste estudo foi avaliar os potenciais impactos de *P. clarkii* e *P. leniusculus* nas populações de bivalves de água doce.

Para tal, verificou-se se existe uma relação predador-presa entre estas espécies de lagostim e espécies de bivalve nativas (*Anodonta anatina*, *Potomida littoralis* e *Unio delphinus*) e invasoras (*Corbicula fluminea*) através de experiências de predação em laboratório e amostragens de campo em dois rios (Sabor e Maçãs) do Norte de Portugal onde todas as espécies vivem em simpatria. No laboratório, as preferências alimentares dos lagostins em relação às espécies de bivalve e a competição entre as duas espécies de lagostim foram avaliadas. No campo, as abundâncias e comprimentos dos espécimes de cada uma das espécies de bivalves foram registadas, as marcas de predação dos lagostins nos bivalves quantificadas e a distribuição, abundância e sexo dos lagostins determinados.

Todas as espécies de bivalves nativas foram predadas tanto em laboratório como no campo; no entanto, *P. clarkii* e *P. leniusculus* foram incapazes de predação da espécie não-nativa *C. fluminea*. A predação dos bivalves não foi afetada nem pelo comprimento nem pelo sexo dos lagostins. A espécie nativa mais predada e preferida por ambos lagostins foi *A. anatina*, enquanto que *P. littoralis* foi a espécie menos predada. Foi também verificada competição pelos bivalves entre os lagostins, sendo *P. clarkii* a espécie dominante e apresentando um comportamento mais agressivo que *P. leniusculus*. Para além da mortalidade direta nos bivalves nativos (que foi extremamente baixa nas experiências em mesocosmo), efeitos indiretos podem ser também importantes (contudo não estudados), uma vez que, é esperado que animais com marcas de predação possam ter o seu *fitness* reduzido causando impactos nas populações e consequente efeito nas funções e serviços dos ecossistemas (e.g. filtração, ciclo de nutrientes, bio-turbação).

Os resultados deste estudo suportam a ideia que *P. clarkii* e *P. leniusculus* podem afetar bivalves nativos. Estes predadores podem ter impactos diretos e indiretos nas populações de bivalves pela redução do número de efetivos devido ao aumento da mortalidade e pela redução do seu *fitness*, que podem ter impactos indiretos nos ecossistemas de água doce. Por outro lado, o facto destes lagostins não predarem *C. fluminea* oferece a este bivalve invasor outra vantagem em relação às espécies nativas. Assim sendo, futuras ações de gestão que visem a conservação de bivalves de água doce nativos devem ter em conta os possíveis efeitos dos lagostins invasores nestes organismos.

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1. Introduction

1.1. Trophic interactions and biological invasions

Ecosystems are subjected to natural changes over time; however, these changes are currently intensified by anthropogenic activities (Hobbs et al. 2009). Biodiversity in terrestrial and aquatic ecosystems are responsible for several functions and provide numerous services such as, provisioning services (e.g. water for consumption), regulatory services (e.g. maintenance of water quality), cultural services (e.g. aesthetics) and supporting services (e.g. primary production) (Constanza et al. 1997, Aylward et al. 2005). Therefore, all these functions and services can be altered or even lost due to increasing human disturbance.

Biological invasions are one of the major threats to biodiversity in addition to habitat loss and fragmentation, overexploitation of resources, climate change and pollution (Dudgeon et al. 2006, Vörösmarty et al. 2010, Simberloff et al. 2013). Nowadays, freshwater ecosystems are specially threatened, suffering higher biodiversity losses than terrestrial ecosystems (Sala et al. 2000), turning conservation of freshwater ecosystems a management priority (Dudgeon et al. 2006). The introduction of invasive species can be responsible for numerous impacts in freshwater ecosystems (e.g. changes in biodiversity, changes in trophic interactions, modifications of physical properties, and introduction of pathogens, parasites and diseases) with possible effects at individual, population, community and ecosystem levels, also leading to substantial economic losses (Simon & Townsend 2003, Strayer 2010, Vilà et al. 2010, Simberloff et al. 2013). These impacts are usually higher in modified and/or disturbed ecosystems in which invasive species are more successful (Bunn & Arthington 2002, Havel et al. 2005).

Biological interactions have a major role in ecosystems as they influence population structure, community properties and may also have evolutionary effects (Molles 2008). Predation is an important biological interaction that can have significant cascading effects on different trophic levels by regulating populations through direct preying (Estes et al. 2011, Ripple et al. 2014). Predation may also have indirect effects due to, for example, non-consumptive interactions (usually known as fear ecology) that change prey behaviour (e.g. feeding behaviour and habitat use) (Paine 1980, Brönmark et al. 1992, Lima 1998). Ecological effects mediated by predators will depend on their trophic level (apex or mesopredators), level of specialization, diet (e.g. omnivorous, carnivorous, scavengers), prey behaviour (e.g. alert and activity level), intensity of predation and community complexity (Virnstein 1977, Holt & Lawton 1994, Lima 1998). For example, as demonstrated by

Paine (1966), predation may increase species diversity by decreasing the density of a dominant species allowing less competitive species to increase their density.

Due to the important role of predation on ecosystems the introduction of invasive predators is expected to have important ecological impacts (Crooks 2002). For example, in Florida, recent declines in mammals are associated with the establishment and expansion of the Burmese python (*Python bivittatus* Kuhl, 1820) within the Everglades National Park (ENP) (Snow et al. 2007, Dorcas et al. 2012). The Burmese python is known for its ability to consume large preys and a great variety of species and it has been shown to prey preferentially on mammals (rabbits and other rodents, bobcats, racoons and large American opossums) in the ENP. In addition, it also feeds on various terrestrial and aquatic bird species, suggesting that many native species on ENP are highly vulnerable to predation by this invasive species (Snow et al. 2007). On the other hand, and by severely reducing the abundance of their prey, *P. bivittatus* may induce a cascading effect on other species. For instance, by preying on racoons, the Burmese python may increase the nesting success and recruitment of oviparous amniotes (e.g. birds, turtles and crocodiles) as their eggs are frequently preyed by racoons (Dorcas et al. 2012, Willson 2017). Salmonids are another group of invasive predators known to have cascading effects on the invaded ecosystems. Experiments with the invasive brown trout (*Salmo trutta* Linnaeus, 1758) in New Zealand and rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)) in Sweden and Japan, have shown that these salmonids can indirectly increase algal biomass by preying on invertebrate grazers. The reduction of grazers biomass and activity releases the algae from grazers top-down regulation, ultimately increasing algal biomass (Flecker & Townsend 1994, Nyström et al. 2001, Baxter et al. 2004). Baxter et al. (2004), also found that the rainbow trout can indirectly affect adjacent terrestrial ecosystems. By feeding on terrestrial prey that fell into the stream, the rainbow trout forced the native Dolly Varden char (*Salvelinus malma* (Walbaum, 1792)) to start foraging insects that graze algae. This situation contributes to an increase in algae biomass and a decrease in the biomass of adult insects that emerge from the stream to the forest, also reducing the density of specialist spiders in the surrounding riparian forest. The introduction of muskrats (*Ondatra zibethicus* (Linnaeus, 1766)) in Europe is another clear example of the extensive damage an invasive predator can cause. This species has a diverse diet, feeding on riparian vegetation, crayfishes and bivalves resulting in the decrease of their density. It may also cause indirect impacts because fish species that need to deposit their eggs in bivalves (i.e. bitterlings) will face more difficulties to find their hosts (Vaughn & Hakenkamp 2001, Skyrienė & Paulauskas 2013). In addition to the above mentioned examples, many more exist in the literature, being the impacts of invasive predators on islands and freshwater

ecosystems well known (e.g. the introduction of *Boiga irregularis* (Bechstein, 1802) in Guam, the introduction of *Euglandina rosea* (Férussac, 1818) in Hawaii and other Polynesian islands, the introduction of *Lates niloticus* (Linnaeus, 1758) in Lake Victoria), where the majority of the prey behave naively to the presence of introduced predators and are subjected to great declines and even extinction (Davis 2009, Lockwood et al. 2013). All these studies demonstrated the strong direct and indirect impacts that invasive predators may have on ecosystems showing the importance of studying and preventing biological invasions.

1.2. Invasive crayfishes: *Procambarus clarkii* and *Pacifastacus leniusculus*

Human activities accelerate the rates of species extinctions and invasions at a global scale. The introduction of species outside their native range is one of the most important and irreversible human-induced changes on ecosystems, being recognized as one of the biggest threats to biodiversity (Vitousek et al. 1997, Sala et al. 2000, Simberloff et al. 2013). As referred in the previous section, the introduction of invasive species can have numerous impacts in terrestrial and aquatic ecosystems. In Europe, for example, 69% of the established aquatic species introduced in six European countries are known to have ecological impacts (García-Berthou et al. 2005). This large quantity of introduced species in freshwater systems include plants, fishes, mammals and invertebrates such as crayfishes.

In Europe, numerous crayfish species have been introduced throughout the years. For instance, in the Iberian Peninsula, introductions of six crayfish species have been reported (Gutiérrez-Yurrita et al. 1999): *Astacus astacus* (Linnaeus, 1758), introduced in 1963-1964; *Procambarus clarkii* (Girard, 1852), introduced in 1973; *Procambarus zonangulus* Hobbs & Hobbs, 1990, introduced in 1974; *Pacifastacus leniusculus* (Dana, 1852), also introduced in 1974; *Astacus leptodactylus* (Eschscholtz, 1823), introduced in 1975; and lastly *Cherax destructor* Clark, 1936 introduced in 1983. Out of these six species, only *P. clarkii*, *P. leniusculus* and *C. destructor* were successfully introduced and are now established in Spain but only the first two spread with considerable success and is now present in Portugal (Gutiérrez-Yurrita et al. 1999, Oscoz et al. 2010, Bernardo et al. 2011).

P. clarkii, the Louisiana crayfish, is listed in the top 10 invasive species in Europe with the highest number of different type of impacts on ecosystem services (Vilà et al 2010, DAISIE database 2017). This species is native to north-eastern Mexico and south-central United States of America (USA) and was introduced in several states of USA outside its native range and central and south

America, eastern Asia, Caribbean and Pacific islands, eastern and southern Africa, and central and western Europe (Hobbs et al 1989, Henttonen & Huner 1999, Gherardi 2006). *P. clarkii* was initially introduced in Europe, more specifically in Spain for stocking purposes and as a commercial substitute of the crayfish species *Austropotamobius pallipes* (Lereboullet, 1858), which has become almost extinct in Europe due to the crayfish plague, a disease caused by the fungus *Aphanomyces astaci* Schikora, 1906 (Gutiérrez-Yurrita et al. 1999, Henttonen & Huner 1999, Gherardi 2006). After the initial introduction, *P. clarkii* spread quickly throughout western Europe either by its natural dispersal abilities or by human intervention due to economic and recreational reasons (Henttonen & Huner 1999, Gherardi 2006). The occurrence of this species in Portugal was first recorded in 1979 at the Caia River (Ramos & Pereira 1981). Since then, the Portuguese populations have increased rapidly, and its presence has been recorded in the entire country (Anastácio & Marques 1995, Fidalgo et al. 2001). This rapid spread is mainly explained by its high adaptive capacity, high reproductive success associated with the low density of predators and the non-exploitation of freshwater crayfish for human consumption (Anastácio & Marques 1995, Gutiérrez-Yurrita et al. 1999, Fidalgo et al. 2001).

P. leniusculus, also known as the signal crayfish, is native to north-western USA and south-western Canada (Henttonen & Huner 1999, Bondar et al. 2005). In Europe, this species was first introduced in Sweden in 1960, for stocking purposes and replacement of the native species *A. astacus*, which, like *A. pallipes*, suffered great declines due to the crayfish plague (Gherardi 2006). In Spain, the signal crayfish was introduced mainly to replace *A. pallipes* and the successful establishment encouraged the later introduction of *P. clarkii* (Gutiérrez-Yurrita et al. 1999, Gherardi 2006). Nowadays, *P. leniusculus* is well established throughout central and western Europe and Japan (Henttonen & Huner 1999, Nakata et al. 2010). In Portugal, this species was first detected in 1997 in Maçãs River. The arrival of the signal crayfish in Portugal may be explained by its natural dispersal after being introduced in 1994 in two Spanish rivers, San Mamed and Arriba Rivers, which are tributaries of Arbedal River that flows into Maçãs River (Bernardo et al. 2011).

P. leniusculus is the most widespread non-native crayfish in Europe, especially in the north, while *P. clarkii* is more common in southern Europe (Holdich et al. 2009). Even though *P. clarkii* and *P. leniusculus* have slightly different ecological niches, both species present high reproductive success, fast growing populations, wide environmental preferences and high dispersal abilities, making them successful invaders (Bondar et al. 2005, Gherardi 2006, Loureiro et al. 2015). The signal crayfish has a wide temperature tolerance, being able to establish in areas where the water temperature may vary between 5 °C during a few months and 25 °C (Westman 1999, Bernardo et

al. 2011). Although this species generally prefers temperatures lower than 25 °C it has been shown that it can survive in temperatures up to 32 °C (Becker et al. 1975, Nakata et al. 2002). The Louisiana crayfish has been shown to have preference for temperatures around 23 °C but can withstand temperatures as low as 2.5 °C and as high as 38 °C (Espina & Herrera 1993, Ramírez et al. 1994, Gutiérrez-Yurrita et al. 1999, Veselý et al. 2015). As the temperature range of both species overlap, they may be able to share the same habitat which happens in some countries, including Portugal (Nakata et al. 2005, Bernardo et al. 2011, Hanshew & Garcia 2012). Temperature is also a key abiotic factor in the reproduction of these crayfish species and sometimes, mainly in laboratory conditions, is the only stimulus required to initiate mating (Westin & Gydemo 1986). Several studies reported that the temperature needed for reproduction in *P. leniusculus* range between 13-15 °C (Reynolds et al. 1992, Guan & Wiles 1999, Nakata et al. 2004). *P. clarkii* generally reproduce from May to late October (at temperatures between 14 °C and 24 °C) when water temperature is higher (Dörr et al. 2006, Mueller 2007, Chucholl 2011). Both species can generate high numbers of offspring. Signal crayfish females can produce more than 450 eggs and it has been reported that they can produce up to 90% more eggs than *A. astacus* females (Abrahamsson 1971, Guan & Wiles 1999, Nakata et al. 2004). As for the Louisiana crayfish, females can produce more than 600 eggs (Reynolds et al. 1992, Chucholl 2011). Both species present parental care in the early stages of development of the offspring and it has been observed behavioural differences between *P. clarkii* females tending their offspring and other adult conspecifics, being more aggressive and more likely to win combats against non-reproductive individuals (Reynolds et al. 1992, Figler et al. 1997, Thiel 2000). The great number of eggs and the parental care indicate a potential high reproductive success for both species, which may play an important role in their invasive success.

Besides these ecological features, both species are also known to have a high diet plasticity, which may be an advantage when invading new habitats. Both crayfish species are omnivorous and display generalist and opportunistic feeding habits enabling dietary shifts (Guan & Wiles 1998, Gutiérrez-Yurrita et al. 1998). *P. 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 tadpoles, fishes, benthic invertebrates and other crayfishes (Axelsson et al. 1997, Guan & Wiles 1998, Gherardi et al. 2001, Gherardi 2006). On the other hand, *P. clarkii* and *P. leniusculus* may be a food source for higher trophic levels such as birds (e.g. white stork), fishes (e.g. largemouth bass, eels) and mammals (e.g. otter and American mink) (Godinho & Ferreira 1994, Guan & Wiles 1998, Correia 2001, Tablado et al. 2010, Mortimer et al. 2012).

Due to the above-mentioned characteristics and the widespread distribution and high abundances both species are responsible for several ecological and economic impacts in many invaded ecosystems. Crayfishes can act as ecosystem engineers changing physically the habitats they invade and the resources available for other species, which can lead to profound impacts in water quality and transparency, nutrient dynamics and community structure (Creed Jr & Reed 2004, Shin-ichiro et al. 2009, Johnson et al. 2010). *P. clarkii* and *P. leniusculus* are also known to be somewhat resistant vectors of the crayfish plague, which is lethal to native crayfish species (Diéguez-Uribeondo & Söderhäll 1993, Kozubíková et al. 2009). In addition, *P. clarkii* and *P. leniusculus* affect native species like fishes and other crayfishes by competing for resources and shelter (Guan & Wiles 1997, Gherardi 2006). Besides the known direct impacts, their feeding habits can have indirect impacts on other species. For example, by feeding on macrophytes they can affect the reproduction of amphibians that depend on macrophytes as a substrate for the deposition of their eggs or affect the invertebrates that use these plants as shelter (Nyström et al. 1996, Axelsson et al. 1997, Gherardi 2006). At last, and as referred before, *P. clarkii* and *P. leniusculus* occupy a central position in food webs in many ecosystems acting simultaneously as predators and preys, affecting different trophic levels and being able to have bottom-up and top-down cascading effects (Geiger et al. 2005). In short, the introduction of these species can reduce biodiversity and increase biotic homogenisation, thereby leading to possible problems in the invaded ecosystems, including economic impacts due to, for example, increased erosion and consumption of rice plants (Anastácio et al. 2005a, Anastácio et al. 2005b). Even though *P. clarkii* and *P. leniusculus* possible impacts and co-existence in Portuguese freshwater ecosystems is acknowledged, little is known about their role as predators of invertebrate species, such as freshwater bivalves.

1.3. Freshwater Bivalves

Bivalves play a major role in freshwater ecosystems providing several important functions and services (e.g. water purification, nutrient cycling, bioturbation) (Vaughn & Hakenkamp 2001, Howard & Cuffey 2006, Vaughn et al. 2008). They constitute the majority of zoobenthic biomass in many freshwater ecosystems and an important part of their role on ecosystems processes result directly from their feeding behaviour (Strayer et al. 1994, Vaughn et al. 2008). Freshwater bivalves are filter feeders, feeding on phytoplankton, bacteria and particulate organic matter from the water column, and/or deposit feeders, feeding on organic matter from the sediment (Hakenkamp & Palmer 1999, Vaughn & Hakenkamp 2001). By filter feeding, bivalves can control phytoplankton production and consequently influence populations of consumers that depend on phytoplankton (Strayer et al.

1999). Water filtration reduce eutrophication and turbidity, increasing water clarity and quality and thus affecting the biological communities (Soto & Mena 1999, Nakamura & Kerciku 2000). Deposit feeding decrease organic matter and bioturbate the sediments (Vaughn & Hakenkamp 2001). Bivalves can also bioturbate the sediments by moving and burrowing themselves, which has been shown to release nutrients from the sediment to the water column and to increase sediment water content and the depth of oxygen penetration (McCall et al. 1979, Vaughn & Hakenkamp 2001). In freshwater ecosystems, nutrient dynamics can be also affected by bivalves through the excretion of nutrients and biodeposition of faeces and pseudofaeces, which may also alter the composition of benthic communities (Sephton et al. 1980, Spooner & Vaughn 2006). At last, bivalves can act as ecosystem engineers and introduce complexity and heterogeneity to ecosystems and provide shelter to other species through their shells (Gutiérrez et al. 2003).

In the last decades, freshwater bivalves, especially freshwater mussels from the Unionoida order (unionoids), have suffered a major global decline and are one of the most threatened faunal groups in the planet (IUCN 2017, Williams et al. 1993; Lopes-Lima et al. 2014a). The main threats to freshwater bivalves are pollution, habitat loss and fragmentation, loss of host fishes, climate change and the introduction of non-native species (Bogan 1993, Strayer et al. 2004, Lopes-Lima et al. 2017).

Nowadays, there are six species of native unionoids known to occur in Portugal, *Anodonta anatina* (Linnaeus, 1758), *Anodonta cygnea* (Linnaeus, 1758), *Margaritifera margaritifera* (Linnaeus, 1758), *Potomida littoralis* (Cuvier, 1798), *Unio delphinus* Spengler, 1793 and *Unio tumidiformis* Castro, 1885 (Lopes-Lima et al. 2017). *M. margaritifera* and *P. littoralis* are assessed as Endangered species by the IUCN Red List. Only the classification of the first species is due to their localised and global declines, while *P. littoralis* was recently classified as endangered based on a suspected decline of more than 30% in the past generations and suspected future declines (Lopes-Lima 2014b, Moorkens et al. 2017). In addition, *A. anatina* and *A. cygnea* are assessed as Least Concern, *U. delphinus* is assessed as Near Threatened and *U. tumidiformis* as Vulnerable. An invasive freshwater bivalve species also occurs in Portugal, the Asian clam *Corbicula fluminea* (Müller, 1774), which is known for its high population growth rates and impacts on freshwater ecosystems (McMahon 2002, Sousa et al. 2008). As other invasive species, *C. fluminea* may have important ecological and economic impacts by acting as an ecosystem engineer, competing for resources and space with native species, growing and establishing dense populations on underwater structures and equipment (biofouling), altering water quality and affecting the survival of other species through massive die-offs (Sousa et al. 2008, Rosa et al. 2011, Ilarri & Sousa 2012).

1.4. Statement of the problem and objective of the study

As stated before freshwater bivalves are extremely threatened and have suffered major declines throughout the world and the introduction and expansion of invasive species are one of the causes of these declines. Although invasive species may have major impacts on bivalve populations, indirectly by affecting bivalves' host fish species and directly through introduction of new parasites and diseases, competition and predation (Bogan 1993, Sousa et al. 2008, Skyrienė & Paulauskas 2013), many of these impacts (and responsible mechanisms) are highly speculative with an almost completely lack of quantitative data concerning this topic. Since crayfishes can prey on bivalves, a possible important threat to the conservation of freshwater bivalves is the recent introduction of several crayfish in aquatic ecosystems. However, very few studies assessed bivalve predation by invasive crayfishes (e.g. Klocker & Strayer 2004, Machida & Akiyama 2013) and so the possible consequences of these introductions on the conservation of freshwater bivalves remain speculative and almost ignored. Given these gaps, the central aim of this study is to assess the potential impacts of *P. clarkii* and *P. leniusculus* on bivalve populations. For this, we used a set of laboratory experiments and assessments in natural ecosystems to test if predator-prey interactions occur between the invasive crayfishes and bivalves, and evaluate if some bivalve species are more prone to predation than others. In addition, this study also intends to determine if these invasive crayfishes can act as control agents of the invasive *C. fluminea*, to investigate the competition and behaviour differences between *P. clarkii* and *P. leniusculus* and evaluate if they have prey and prey-size preferences. Finally, the results obtained in the laboratory experiments were validated *in situ* using the Sabor River basin as a case study.

2. Material and Methods

2.1. Animal collection and maintenance

Throughout this work three native (*A. anatina*, *P. littoralis* and *U. delphinus*) and one non-native (*C. fluminea*) bivalve species were studied. The four species have a sympatric distribution in the Sabor River (Portugal) also co-existing with the crayfish *P. clarkii* and *P. leniusculus*. Bivalves were collected in the Sabor River and maintained in aquariums (60 x 30 x 30 cm), filled with 20 L of water under aeration and fed on microalgae. Both crayfish species were also collected in the Sabor River. For this, several small baited traps were consistently put in the river bottom for 24 h of fishing. Only mature males were collected and used in the experiments in order to minimize possible bias in the assessment of the predatory behaviour. The specimens of each crayfish species were maintained in separate aquariums (60 x 30 x 30 cm) in individual small cages with filtrated water under aeration and were fed daily with freshwater fish food sticks. Animals (both bivalves and crayfish) were always maintained under controlled temperature (15 °C).

2.2. Predation of bivalves by *Procambarus clarkii* and *Pacifastacus leniusculus*

In a first experiment it was determined if: i) *P. clarkii* and *P. leniusculus* recognized the bivalve species as prey; ii) predation success depends on bivalve species and/or the crayfish species; and iii) predation was size dependent. In this experiment various sizes of each bivalve and crayfish species were used (Table 1).

Table 1: Size range used for each bivalve and crayfish species.

Bivalve species	Size range (mm)
<i>Unio delphinus</i>	36 – 97
<i>Potomida littoralis</i>	44 – 78
<i>Anodonta anatina</i>	35 – 126
<i>Corbicula fluminea</i>	15 – 37
Crayfish species	Size range (mm)
<i>Procambarus clarkii</i>	71 – 120
<i>Pacifastacus leniusculus</i>	74 – 110

A single crayfish was placed in a mesocosm, with 2 cm of fine sand, previously washed, and filled with 10 L of water, under controlled temperature (15 °C), in combination with a single specimen of one of the bivalve species (Figure 1). Bivalves were given to the crayfishes randomly to reduce possible bias and each species combination was replicated 20 times (N=160) and ran for 72 h.

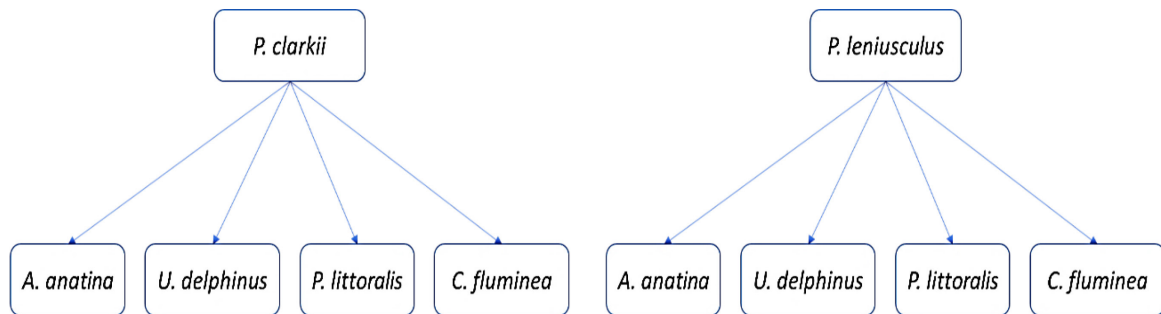


Figure 1: Experimental design of the predation experiments. Each bivalve species was tested as prey for each crayfish species. Only two specimens were placed in each mesocosm (one bivalve and one crayfish).

Before the experiments, crayfishes were starved for 3 days to encourage predation on bivalves. The length of all animals was measured (Figure 2) and bivalves were also weighed before and after each experiment. Predation was assessed by checking for the presence of predation marks on bivalve shells and the percentage of predation was visually estimated (Figure 3).

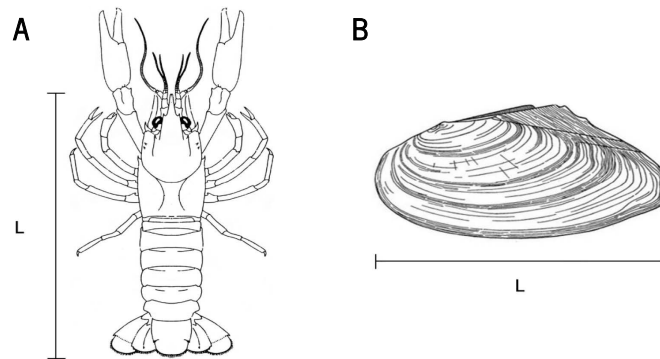


Figure 2: Schematic representation of length (L) measurements of crayfishes (A) and bivalves (B).



Figure 3: Dead specimen of *Unio delphinus* with crayfish predation marks.

2.3. Prey preferences of *Procambarus clarkii* and *Pacifastacus leniusculus*

In a second experiment, it was determined if *P. clarkii* and *P. leniusculus* prefer to prey on certain bivalve species when they have a choice. For this, one crayfish of each species was placed on a mesocosm with one specimen of each of the four bivalve species.

As in the previous experiment, the mesocosms had 2 cm of fine sand and were filled with 10 L of water, under controlled temperature (15 °C). Crayfishes were starved for 3 days before the beginning of the experiment and length of all animals was measured and bivalves were also weighed before and after the experiment. Each treatment was replicated 10 times (N=20 for both crayfish species) and ran for 72 h. Predation marks were visually inspected, and their percentage was assessed.

2.4. Competition between *Procambarus clarkii* and *Pacifastacus leniusculus*

To test if there is competition between *P. clarkii* and *P. leniusculus* one specimen of each crayfish species was placed in a mesocosm with one specimen of *A. anatina*. The choice of *A. anatina* was based in earlier experiments since this species was the most preyed bivalve by both crayfish species. The experiment had the same conditions as described above, the length of all animals was measured, and bivalves were also weighed before and after the experiment. The experiment was replicated 10 times (N=10) and ran for 72 h, marks were visually inspected and their percentage was assessed. All the experiments were recorded using a webcam (HP® WebCam HD 2300) to observe crayfish behaviour. The time of beginning and ending of all interactions was registered to determine bivalve handling time, the duration of aggressive interactions, and to assess the number of times each of these interactions occurred; subsequently assessing which crayfish species was dominant.

2.5. Predation of bivalves by *Procambarus clarkii* and *Pacifastacus leniusculus*: *in situ* validation

Study area

The field survey was carried out in Sabor River (North-eastern Portugal). This river has its source in Zamora province (Spain) and enters in Portugal by crossing Montesinho mountain (Bragança). Sabor River is a tributary of Douro River and has a wide range of environmental conditions: elevation range between 100 and 1500 m above sea level, the total annual precipitation varies between 443-1163 mm, the mean annual temperature ranges between 6.9 to 15.6 °C and the flow regime is highly seasonal, having some dried streams or disconnected pools during the summer (Filipe et al. 2017). A dam was built in this river even though the surrounding region is part of Natura 2000 network. Sabor River still has overall good ecological quality although some problems related with organic pollution and regulation of river flow have arisen in the last years. As referred before, the crayfishes *P. clarkii* and *P. leniusculus* and the bivalves *A. anatina*, *P. littoralis*, *U. delphinus* and *C. fluminea* co-exist in sympatry in this river basin.

Sampling and *in situ* validation

To assess if predation occurs in the natural environment, a total of 51 sites were sampled during July 2017, 45 in Sabor River and 6 in Maçãs River (Figure 4). For each site, and for freshwater bivalve ecological characterization, a river stretch with a minimum of 100 m was surveyed covering the maximum of different habitats as possible (i.e. riffles and pools, banks and center of the channel). These surveys were always performed by a minimum of two divers and freshwater bivalves were found visually or by hand-searching through the bottom since visibility at many sites was low. A total of four replicates lasting 15 minutes were performed totalizing 60 minutes of surveyed time per site. In each site, bivalves were collected, including empty shells, and identified to the species level, their length was measured and the percentage of predation by crayfish was estimated using the same methodology as described above (i.e. percentage of predation in the shells).

Crayfishes were also collected in 15 sites of the 51 initial sites in an attempt to cover all the river section surveyed for bivalves. Crayfishes were captured by placing funnel traps, 5 rectangular (50 x 30 x 20 cm; 0.5 cm mesh) and 5 cylindrical (43 cm diameter; 22 cm height; 1.5 cm mesh) per site for 24 h. Sites were chosen based on the predation estimates and percentage of preyed bivalves to evaluate if there is a relationship between crayfish population abundance and bivalve

distribution, abundance and levels of predation. The crayfishes collected were identified to the species level, their sex was determined and their length was measured.

Sample sites:

- Only bivalve sampling
- Crayfish and bivalve sampling

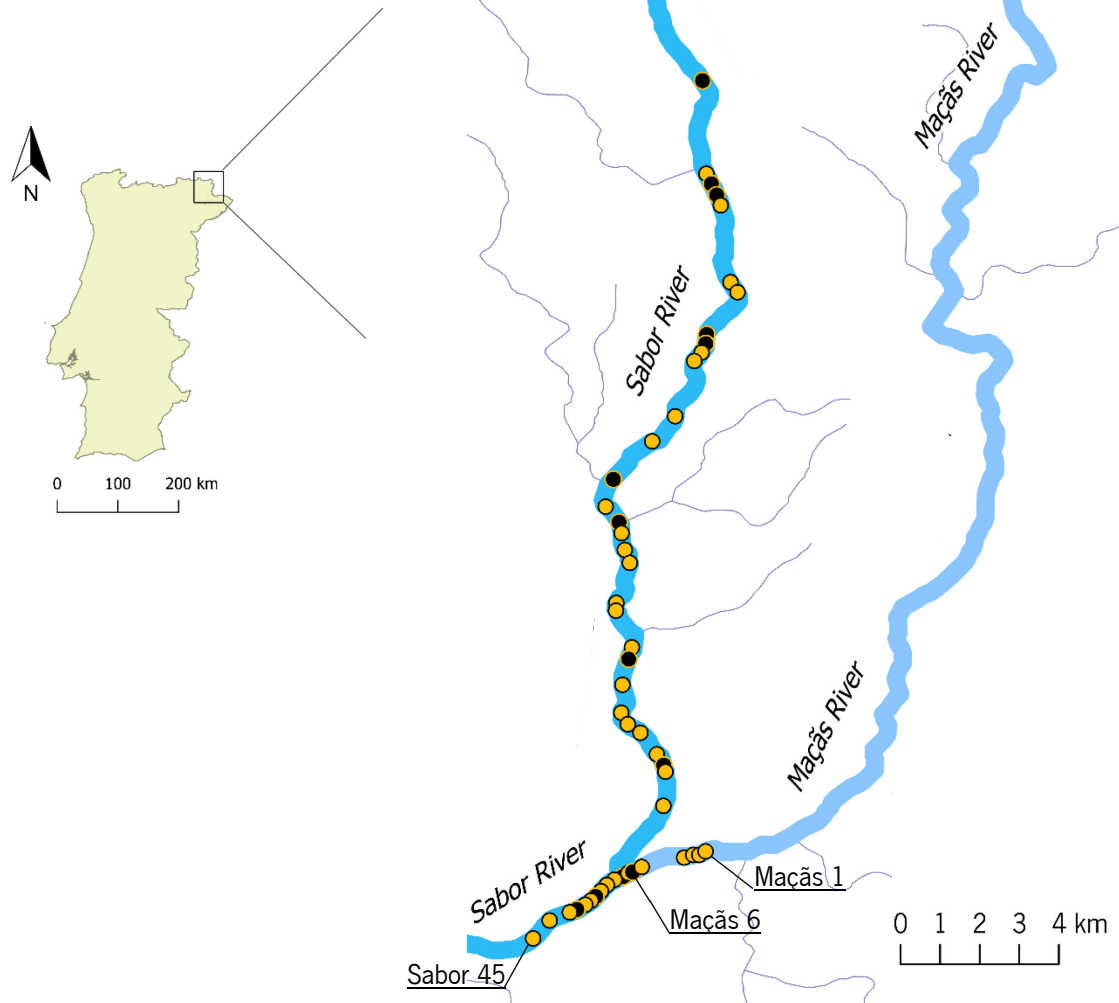


Figure 4: Map of the sampling sites.

2.6. Data analysis

All statistical analyses performed were preceded by Shapiro-Wilk to test if data had a Gaussian distribution and by the Bartlett test to test the homogeneity of variance.

In the first experiment a 2-way permutational multivariate analysis of variance (PERMANOVA) (type-III), with crayfish (2 levels: *P. clarkii* and *P. leniusculus*) and bivalve species (4 levels: *A. anatina*, *U. delphinus*, *P. littoralis* and *C. fluminea*) as fixed factors was used to assess the effect of predator and/or prey species on bivalve predation, after which, pairwise comparisons were used to analyse how predation varied between bivalve species. Pearson's chi-squared and Fisher's exact test of independence tests were used to test if the percentage of preyed bivalves was significantly different between *P. clarkii* and *P. leniusculus* and between bivalve species. In addition, a Mann-Whitney U and Kruskal-Wallis tests were performed to, respectively, assess if the crayfishes consumed different percentage of preyed bivalves shell and if that percentage was different between bivalve species. Welch's t-test was used to evaluate if predation success depends on bivalve and crayfish lengths, while Spearman's correlations were used to assess the degree of association between predation rate and these lengths.

In the second experiment, a Pearson's chi-squared test was performed between the expected and observed percentage of preyed bivalves when crayfishes had prey choice. Additionally, the percentage of preyed bivalves when crayfishes had prey choice was also compared to the percentage of preyed bivalves of the first experiment.

In the third laboratorial experiment, the differences between *P. clarkii* and *P. leniusculus* on bivalve handling time, percentage of times the bivalve was handled, and percentage of initiated agonistics bouts were examined using, respectively, a PERMANOVA (type-III), with crayfish species (2 levels: *P. clarkii* and *P. leniusculus*) as fixed factors, a Wilcoxon matched-pairs signed ranks test and a paired t-test to assess if there is interspecific competition between the crayfishes and what species is dominant.

The data collected in the field was analysed using PERMANOVAs (type-III), with sampling site (24 levels: Sabor 2, Sabor 5, Sabor 7, Sabor 10, Sabor 14 – Sabor 21, Sabor 24 – 34, Sabor 41) and bivalve species (2 levels: *A. anatina* and *U. delphinus*) as fixed factors, to examine the effect of river site and bivalve species on bivalve predation. Additional PERMANOVA pairwise comparisons were used to assess which bivalve species was the most preyed within each site and which sites had higher predation of each bivalve species. Spearman's correlations were used to test the relationship between bivalve predation rate and bivalve length and the relationship between the percentage of preyed bivalves and crayfish abundance. Finally, a Wilcoxon matched-pairs signed

ranks test was performed to assess differences in abundance between crayfish species, paired t-tests compared differences in the abundance of female and male crayfishes and a Spearman's correlation was used to test the association of sex ratio and the percentage of preyed bivalves. As *P. littoralis* overall abundance was low and its distribution very restricted, no statistical analyses were done with this species to assess predation differences between sites. Additionally, to assess predation differences between sites, only sites where at least 4 specimens of each species were found were considered.

All PERMANOVAs were performed with 9999 permutations on the basis of Euclidean distances (Anderson 2001). PERMANOVAs were done with PRIMER 6 (Primer-E, UK) for Windows. All other analyses were done with R software 3.2.2 for Windows (R Development Core Team, 2015).

3. Results

3.1. Predation of bivalves by *Procambarus clarkii* and *Pacifastacus leniusculus*

3.1.1. Species effect on bivalve predation

P. clarkii and *P. leniusculus* preyed on all bivalve species except *C. fluminea*. Results showed that bivalve predation was dependent on crayfish and bivalve species and there was no interaction between these two factors (PERMANOVA, Table 2). Despite this, pairwise comparisons showed significant differences only between the native bivalves and *C. fluminea*. On average, *P. leniusculus* consumed a higher percentage of bivalve shells than *P. clarkii* and one signal crayfish was able to eat 44.4% of a *U. delphinus* specimen. Besides this 44.4% preyed specimen the maximum shell percentage consumed by *P. leniusculus* was 5% for *A. anatina*, 7% for *U. delphinus* and 6.5% for *P. littoralis*. The maximum shell percentage consumed by *P. clarkii* was 10% for *A. anatina*, 5% for *U. delphinus* and 2% for *P. littoralis*.

Table 2: Summary of the PERMANOVA results on the effect of crayfish and bivalve species on bivalve shells predation.

Parameter	Effect	df	SS	MS	Pseudo F	P
Shell consumption	Crayfish species	1	44.3	44.3	3.164	0.020
	Bivalve species	3	79.76	26.6	1.898	0.049
	Crayfish species x Bivalve species	3	48.6	16.2	1.157	0.339
	Error	152	2128.9	14.0		

After 72 hours in the presence of bivalves, and when only considering preyed bivalves, average shell predation rate was 2.7% in treatments with *P. leniusculus* and 2.6% with *P. clarkii* for *A. anatina*; 9% and 1.5% for *U. delphinus*; and 3% and 1% for *P. littoralis*, respectively (Figure 5). On average, *P. leniusculus* consumed a higher percentage of shell than *P. clarkii*, but significant differences were only found regarding *U. delphinus* (Mann-Whitney U test, U=8, p=0.036). The percentage of shell consumption of preyed bivalves was not significantly different between bivalve species regardless the crayfish species (Kruskal-Wallis test, p>0.05).

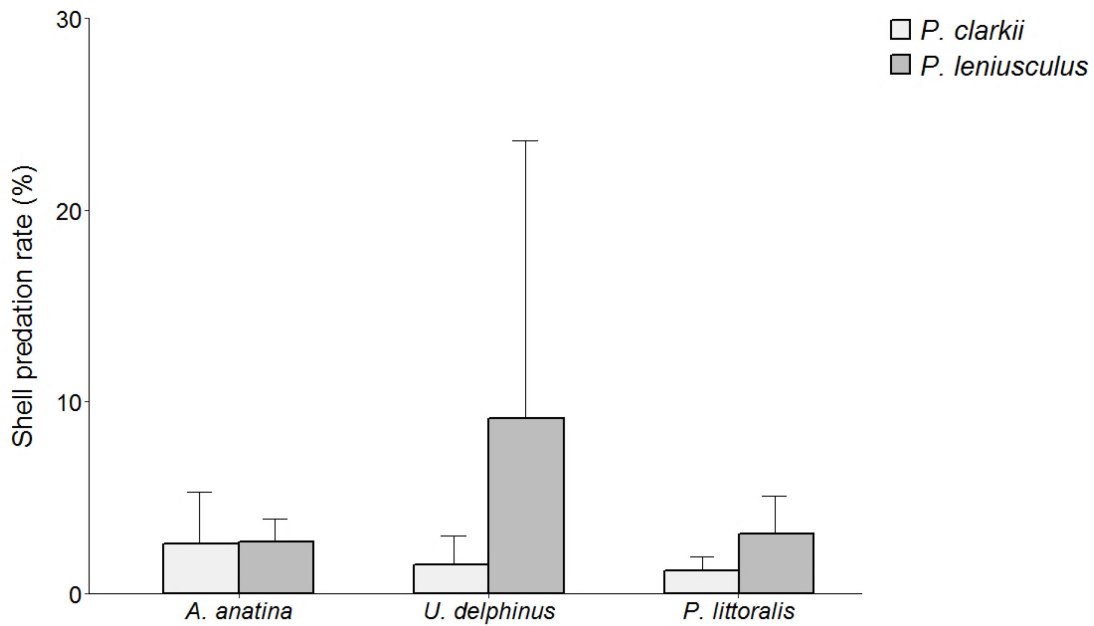


Figure 5: Average (\pm SD) shell predation rate of *Anodonta anatina*, *Unio delphinus* and *Potomida littoralis* by *Procambarus clarkii* and *Pacifastacus leniusculus* (only considering preyed bivalves).

Regarding the percentage of preyed individuals by both crayfish species *A. anatina* was the bivalve species with higher values, followed by *U. delphinus*, *P. littoralis* and *C. fluminea* (Table 3). Although the results suggested that *P. leniusculus* is capable of preying more bivalves than *P. clarkii*, differences between both crayfishes were not significant (Pearson's chi-squared test, $p > 0.05$). In addition, the percentage of preyed individuals was not significantly different between native bivalve species (Pearson's chi-squared test, $p > 0.05$) but was significantly different between these and *C. fluminea* (Fisher's exact test of independence, $p < 0.05$).

Table 3: Percentage (%) of preyed bivalves (*Anodonta anatina*, *Unio delphinus*, *Potomida littoralis* and *Corbicula fluminea*) by *Pacifastacus leniusculus* and *Procambarus clarkii*.

	Percentage of individuals preyed (%)			
	<i>A. anatina</i>	<i>U. delphinus</i>	<i>P. littoralis</i>	<i>C. fluminea</i>
<i>Pacifastacus leniusculus</i>	60	40	35	0
<i>Procambarus clarkii</i>	45	30	25	0

3.1.2. Size effect on bivalve predation

No significant correlation was found between crayfish and bivalve lengths (Spearman's correlation, $p > 0.05$). Regarding the predation status (i.e. preyed or not preyed), no significant differences were found between the lengths of the animals when bivalves were preyed or not. Therefore, the success of predation did not depend on crayfish length nor bivalve length (Welch t-test, $p > 0.05$) (Figure 6).

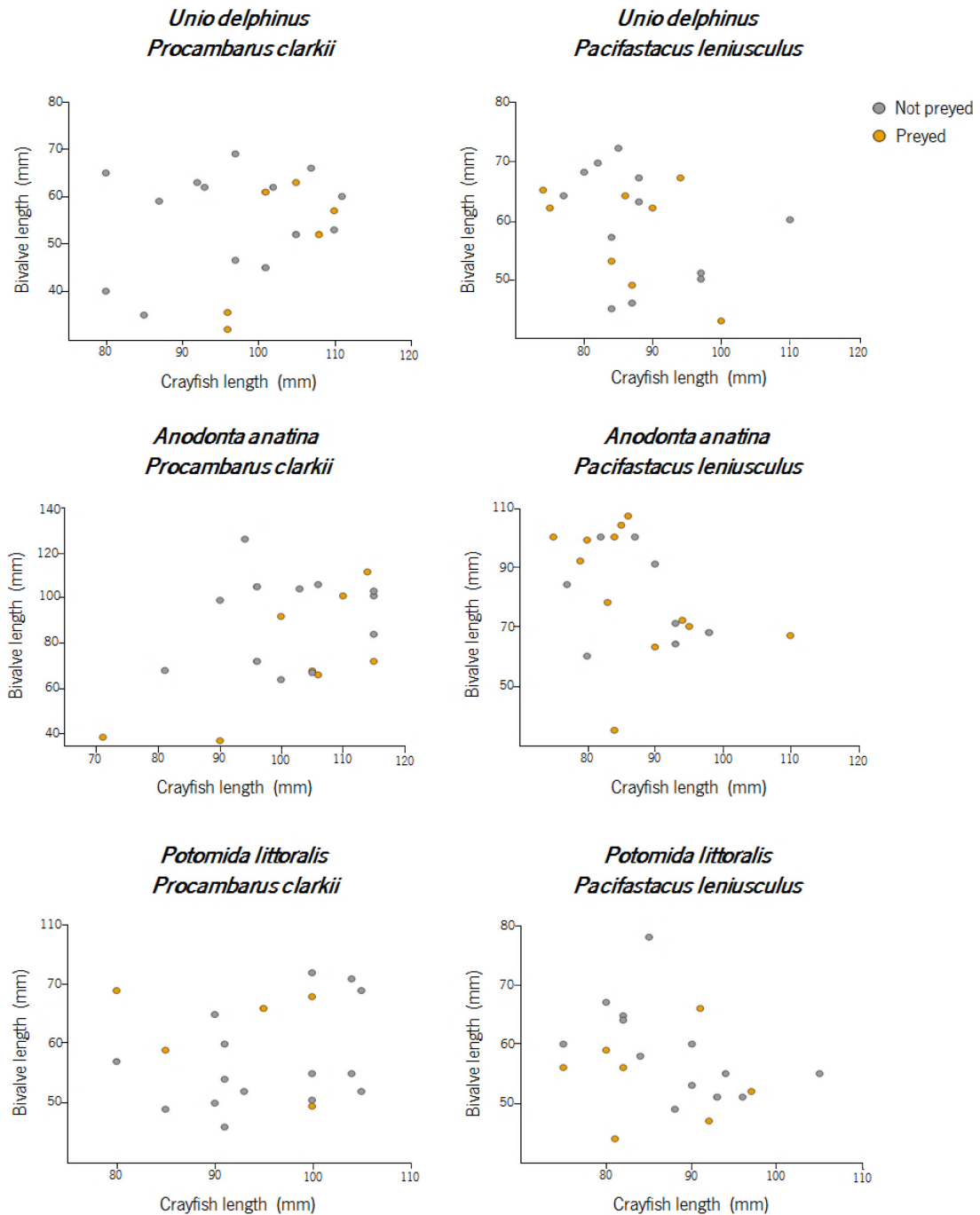


Figure 6: Relationship between bivalve predation status (preyed or not preyed) and crayfish and bivalve length. Bivalve and crayfish species represented are indicated on the top of each plot. None of the relationships were significant ($p > 0.05$).

In addition, bivalve predation did not depend on crayfish length (Figure 7) nor bivalve length (Figure 8), regardless of crayfish and bivalve species (Spearman's correlation, $p > 0.05$). When only considering bivalves that were preyed, results showed that neither crayfish nor bivalve lengths affected bivalve shell predation rate (Spearman's correlation, $p > 0.05$).

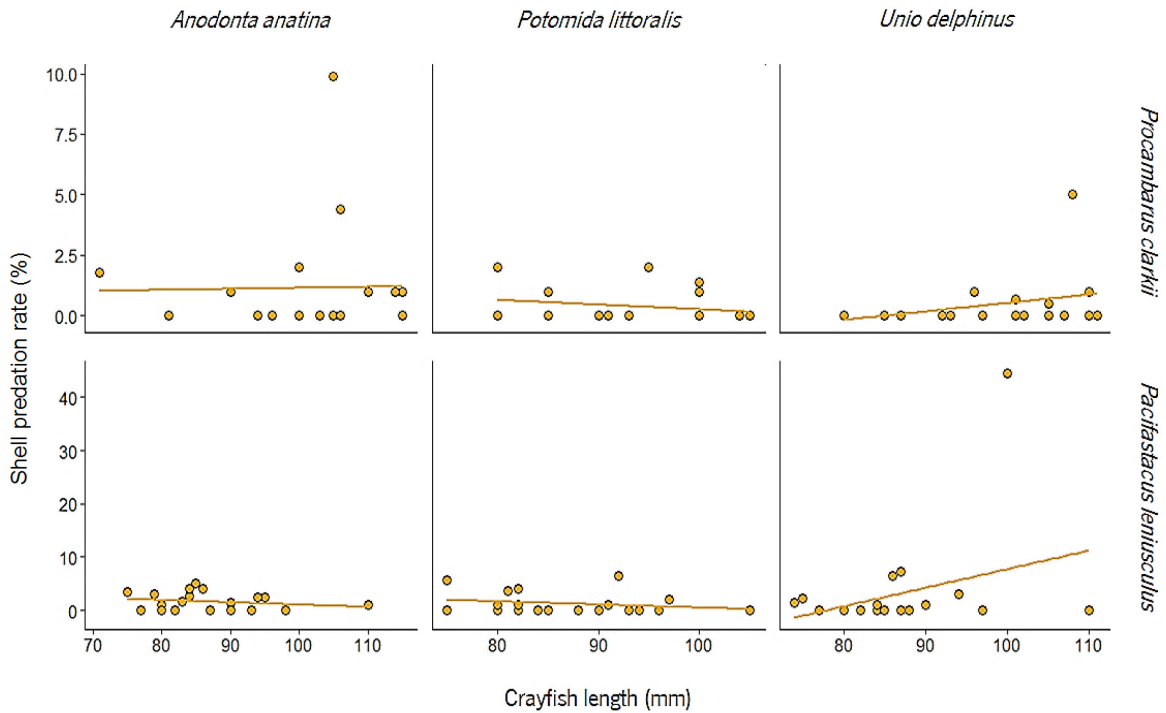


Figure 7: Relationship between shell predation rate and crayfish length. Relationships of bivalve and crayfish species represented are indicated on the top and right side of the plots. None of the relationships were significant ($p > 0.05$).

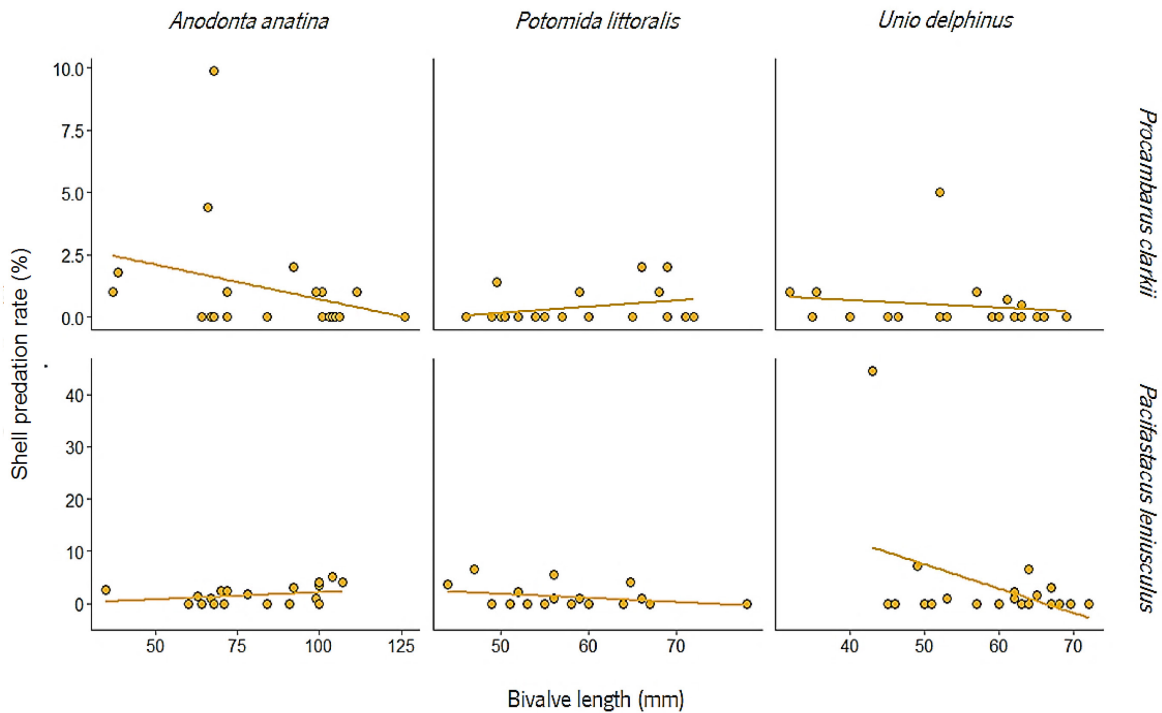


Figure 8: Relationship between shell predation rate and bivalve length. Relationships of bivalve and crayfish species represented are indicated on the top and right side of the plots. None of the relationships were significant ($p > 0.05$).

3.2. Prey preferences of *Procambarus clarkii* and *Pacifastacus leniusculus*

When given a choice, *P. clarkii* and *P. leniusculus* preyed on the same bivalve species as in the first experiment. The percentages of preyed bivalves (Table 4) was different than the expected if crayfishes had no prey preferences (Pearson's chi-squared test *P. clarkii*: $\chi^2(2) = 22.248$, $p < 0.001$; *P. leniusculus*: $\chi^2(2) = 14.465$, $p = 0.001$). Additionally, the percentages of preyed bivalves when crayfishes had prey choice were different from the ones of preyed bivalves when crayfishes had no choice (Pearson's chi-squared test *P. clarkii*: $\chi^2(2) = 12.203$, $p = 0.002$; *P. leniusculus*: $\chi^2(2) = 6.140$, $p = 0.046$). These results showed that both crayfish species have prey preferences in the presence of all four bivalve species, being *A. anatina* the most preyed bivalve species by both crayfish species, with 90% of individuals having marks of predation in both cases. *P. leniusculus* preyed on 50% of *P. littoralis* and 30% of *U. delphinus* individuals. In the case of *P. clarkii*, 80% of *U. delphinus* individuals had marks of predation while only 20% of *P. littoralis* individuals suffered predation. None of the crayfish species preyed *C. fluminea* individuals.

Table 4: Average percentage (%) of preyed bivalves (*Anodonta anatina*, *Unio delphinus*, *Potomida littoralis* and *Corbicula fluminea*) by *Pacifastacus leniusculus* and *Procambarus clarkii* when having prey choice.

	Average percentage of individuals preyed (%)			
	<i>A. anatina</i>	<i>U. delphinus</i>	<i>P. littoralis</i>	<i>C. fluminea</i>
<i>Pacifastacus leniusculus</i>	53.33	13.33	23.33	0
<i>Procambarus clarkii</i>	51.67	39.58	6.67	0

3.3. Competition between *Procambarus clarkii* and *Pacifastacus leniusculus*

When sharing the same space and prey, no significant differences were found between bivalve handling time between *P. clarkii* and *P. leniusculus* (PERMANOVA, $p > 0.05$), although *P. clarkii* present higher values (Figure 9). On average, and in 72 hours, *P. clarkii* handled bivalves for 27:26 minutes while *P. leniusculus* did it only for 3:06 minutes. During the experiments, two Louisiana crayfishes surpassed two hours of handling whereas their opponents handled those bivalves for, approximately, 14 and 6 minutes. In one replicate none of the crayfishes handled the bivalve and the signal crayfish also did not handle the bivalve in other three replicates.

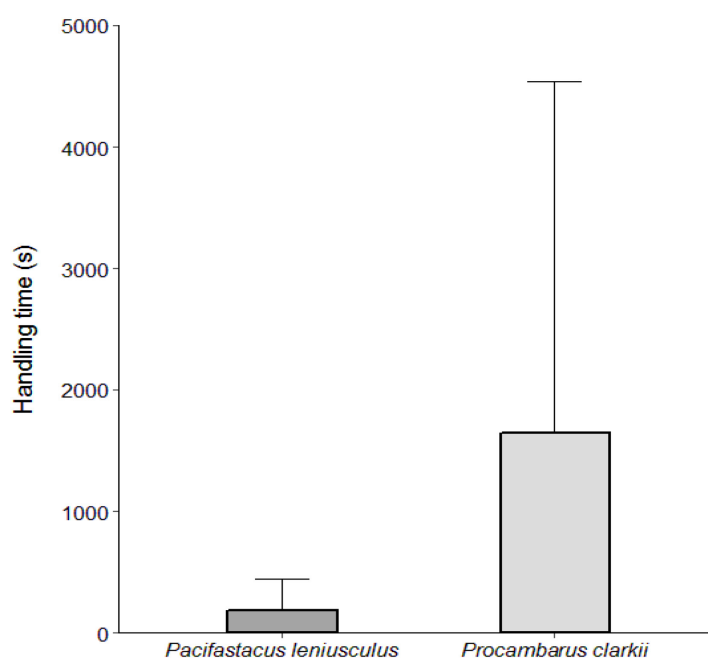


Figure 9: Average (+SD) of bivalve handling time by *Pacifastacus leniusculus* and *Procambarus clarkii*.

Although there were no differences on handling time, *P. clarkii* was found to manipulate bivalves a number of times significantly higher (63.6% on average) than *P. leniusculus* (Wilcoxon matched-pairs signed ranks test, $W=50$, $p=0.008$, $N=10$) (Figure 10A). When sharing the same space and prey, *P. clarkii* was also the most aggressive crayfish species, initiating more agonistic bouts than *P. leniusculus* (Paired t-test, $t(9) = -6.334$, $p < 0.001$) (Figure 10B). On average, the Louisiana crayfish initiated 77.9% of the fights, which translates to 55.8% more initiated agonistic bouts than the signal crayfish. There was one case where *P. leniusculus* initiated 50.0% of the agonistic bouts but on average this species initiated only 22.1% of the aggressive interactions.

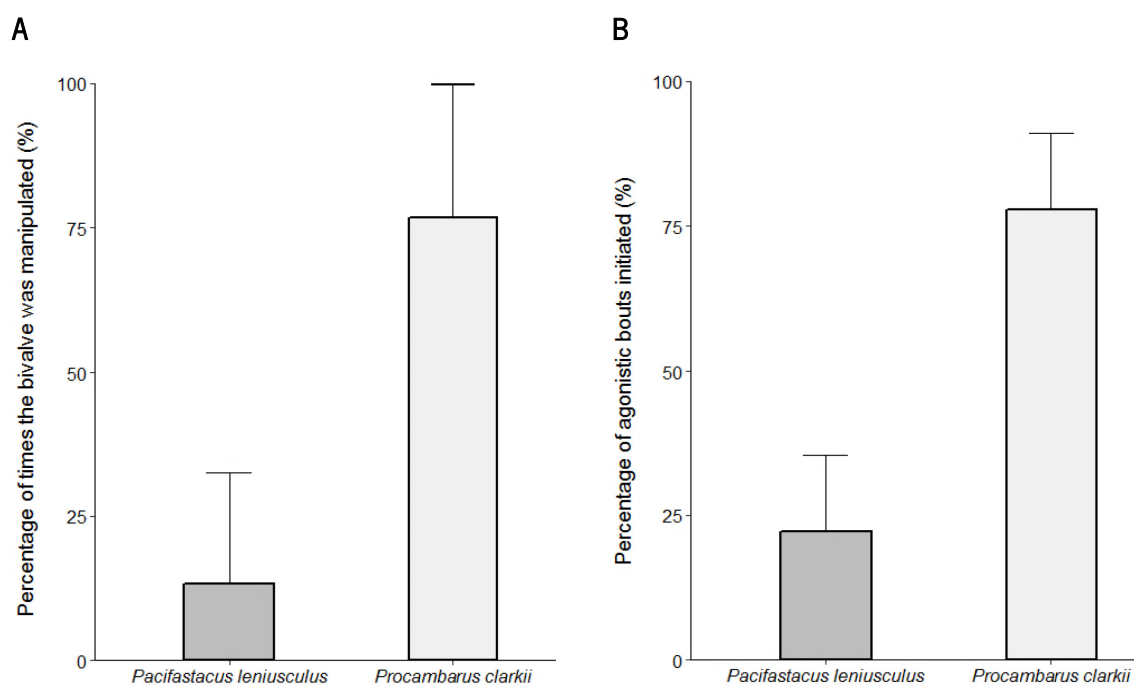


Figure 10: Average (+SD) of (A) the percentage of times the bivalve was manipulated and of (B) the percentage of times agonistic bouts were initiated by *Pacifastacus leniusculus* and *Procambarus clarkii* when sharing the same space and prey.

3.4. Predation of bivalves by *Procambarus clarkii* and *Pacifastacus leniusculus*. *in situ* validation

3.4.1. Predation of bivalves

Throughout the 51 sampling sites in Sabor and Maçãs Rivers, 2800 bivalves were found wherein 1531 were identified as *U. delphinus*, 642 as *A. anatina*, 130 as *P. littoralis* and 497 as *C. fluminea* (Figure 11). The invasive *C. fluminea* was only found in 14 sampling sites, which include all sites in Maçãs River and sites 26, 35, 36, 37, 38, 39, 40 and 42 in Sabor River (Appendix 1). Native bivalves were found in all sampling sites except sites 12, 13 and 44 in Sabor River and sites 1, 2 and 3 in Maçãs River (Appendix 1). Besides being the most abundant species, *U. delphinus* was also the most widespread, being present in 45 of the 51 sampling sites. *A. anatina* was found in 36 of the 51 sampling sites and *P. littoralis* was present in only five of the sampling sites (Sabor River sites 34, 36 and 39 and Maçãs River sites 4 and 6).

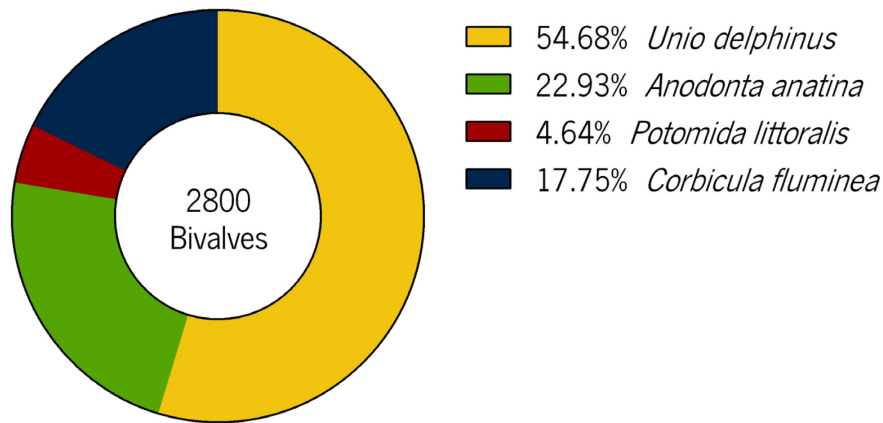


Figure 11: Relative abundance of *Unio delphinus*, *Anodonta anatina*, *Potomida littoralis* and *Corbicula fluminea* throughout all sampling sites.

No predation marks were found in any of *C. fluminea* specimens collected. On the other hand, all native species were found to be preyed by crayfishes, which corroborates the results of the laboratorial experiments. Only 156 of the 2800 bivalves collected were found dead and presented predation marks. *P. littoralis* only showed signs of predation in Maçãs River site 6, which is the site with higher abundance of this species. In this site, the percentage of *P. littoralis* preyed bivalves was 22.7% and the average percentage of shell predation was 3.8%. *A. anatina* was most abundant in Sabor River site 28 where 153 individuals were collected and 36.6% of these individuals had crayfish predation marks. As for *U. delphinus*, it was most abundant in Sabor River site 33 with 198 specimens collected and 29.7% of these individuals had marks of predation. All the collected bivalves with more than 15% of their shell preyed were found dead except one *U. delphinus* specimen that was found alive with 25% of its shell preyed.

When comparing sites where both *U. delphinus* and *A. anatina* were present, results showed that bivalve predation was dependent on the river site and bivalve species, having a significant interaction between both factors (PERMANOVA, Table 5). PERMANOVA pair-wise comparisons showed differences on bivalve predation between *A. Anatina* and *U. delphinus* in five of the analysed sites (Appendix 2). On average, *A. anatina* had significantly higher percentage of shell predation in Sabor River sites 16 and 17, while *U. delphinus* had significantly higher percentage of preyed shell in Sabor River sites 19, 20 and 28.

Table 5: Summary of PERMANOVA results on the effect of river site and bivalve species on bivalve's shell predation.

Parameter	Effect	df	SS	MS	Pseudo F	P
Shell consumption	River site	23	2483.2	107.97	11.555	0.001
	Bivalve species	1	43.86	43.86	4.694	0.034
	River site x Bivalve species	23	1413.3	61.45	6.576	0.001
	Error	1796	16781	9.34		

There were significant differences on the percentage of bivalve shell predation between sampling sites for both *A. anatina* (Figure 12, Appendix 3) and *U. delphinus* (Figure 13, Appendix 4). On average, the percentage of preyed bivalves was 23.36% for *A. anatina* and 22.85% for *U. delphinus*. When analysing sites where both species were present no significant differences were found between the percentage of preyed bivalves (Wilcoxon matched-pairs signed ranks test, $p > 0.05$).

There was a weak negative correlation between shell predation rate and bivalve length for *U. delphinus* (Spearman's correlation, $r_s = -0.25$, $p < 0.001$), but only when all preyed bivalves were considered. When only considering alive preyed bivalves, no significant correlation was found between the shell predation rate and length of this species (Spearman's correlation, $p > 0.05$). No significant correlations were found between shell predation rate and bivalve length for *A. anatina* regardless of whether all or only alive preyed bivalves were considered.

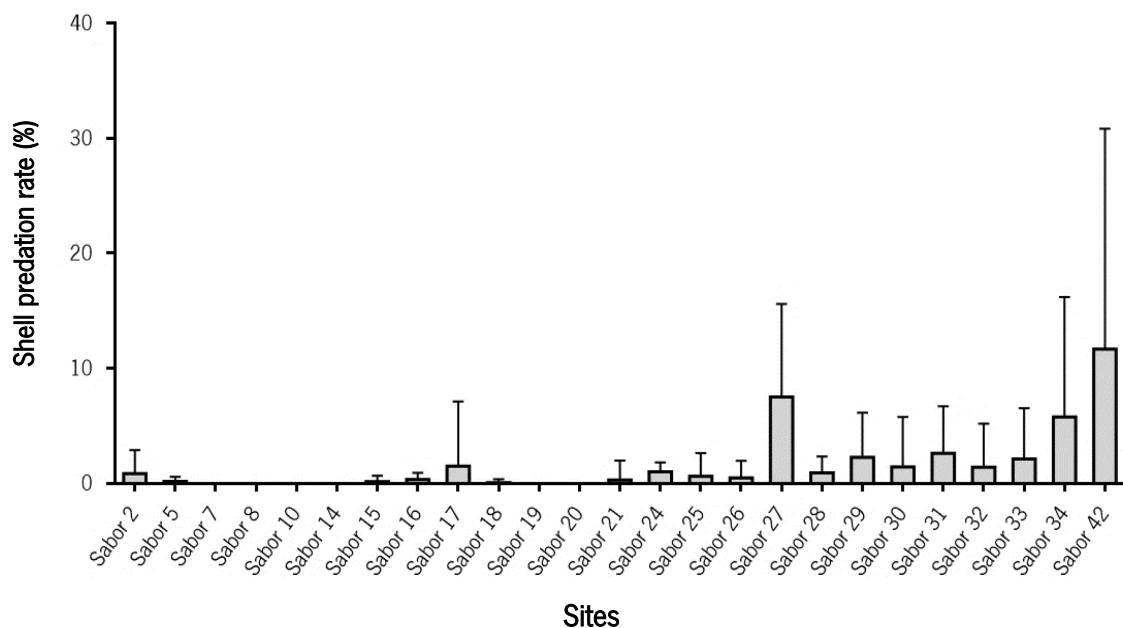


Figure 12: Average (+SD) of shell predation of *Anodonta anatina* by sampling site.

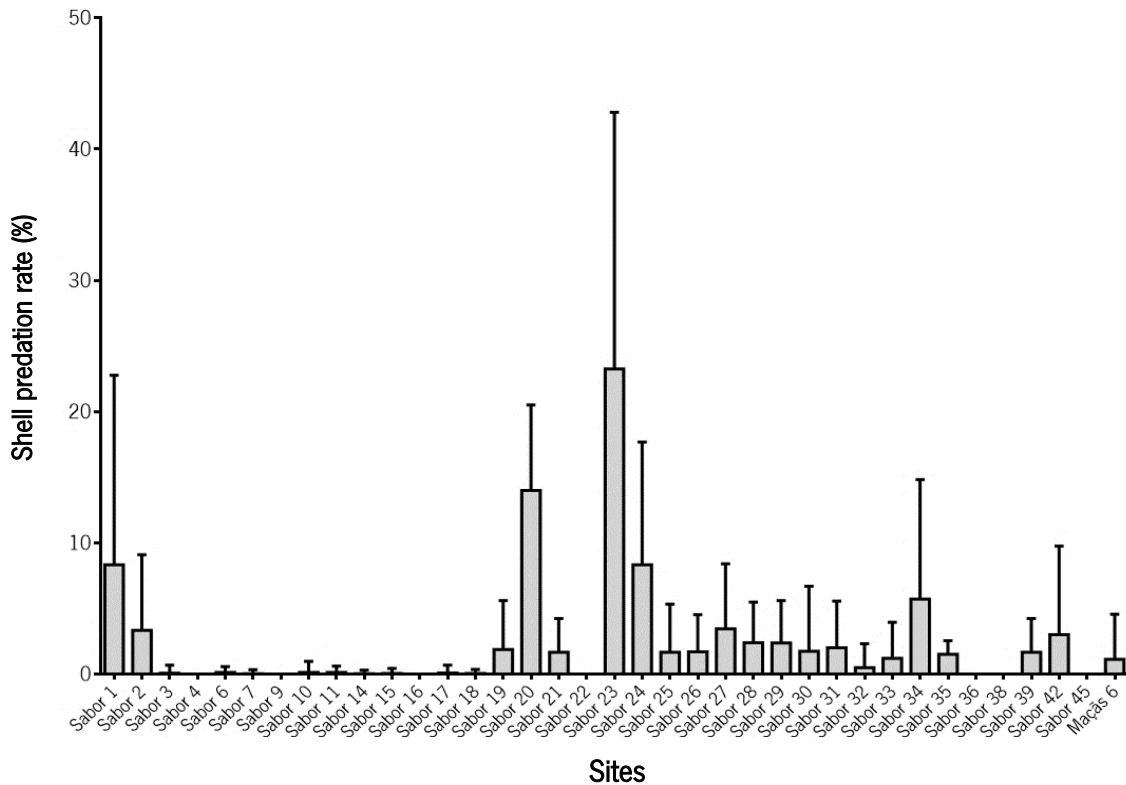


Figure 13: Average (+SD) of shell predation of *Unio delphinus* by sampling site.

3.4.2. Crayfish sex and abundance effect on bivalve predation

In total, 1320 crayfishes were collected and 92.1% of them were identified as *P. leniusculus* and 7.9% as *P. clarkii*, being *P. leniusculus* significantly more abundant than *P. clarkii* (Wilcoxon matched-pairs signed ranks test, $W=75$, $p=0.016$, $N=15$). Despite this, the signal crayfish was not found in four of the 15 surveyed crayfish sampling sites, while *P. clarkii* was found in all sampling sites except one (Figure 14).

Overall, there were no significant differences between the number of female and male crayfishes collected (Paired t-test, $p>0.05$). However, when analysing each crayfish species individually differences were found between female and male abundance. Average female signal crayfish abundance was 5.8% higher than its male counterpart (Paired t-test, $t(14) = 2.367$, $p=0.033$), while in the case of the Louisiana crayfish, male crayfishes were 1.78% more abundant than female crayfishes (Wilcoxon matched-pairs signed ranks test, $W=61$, $p=0.0493$, $N=15$).

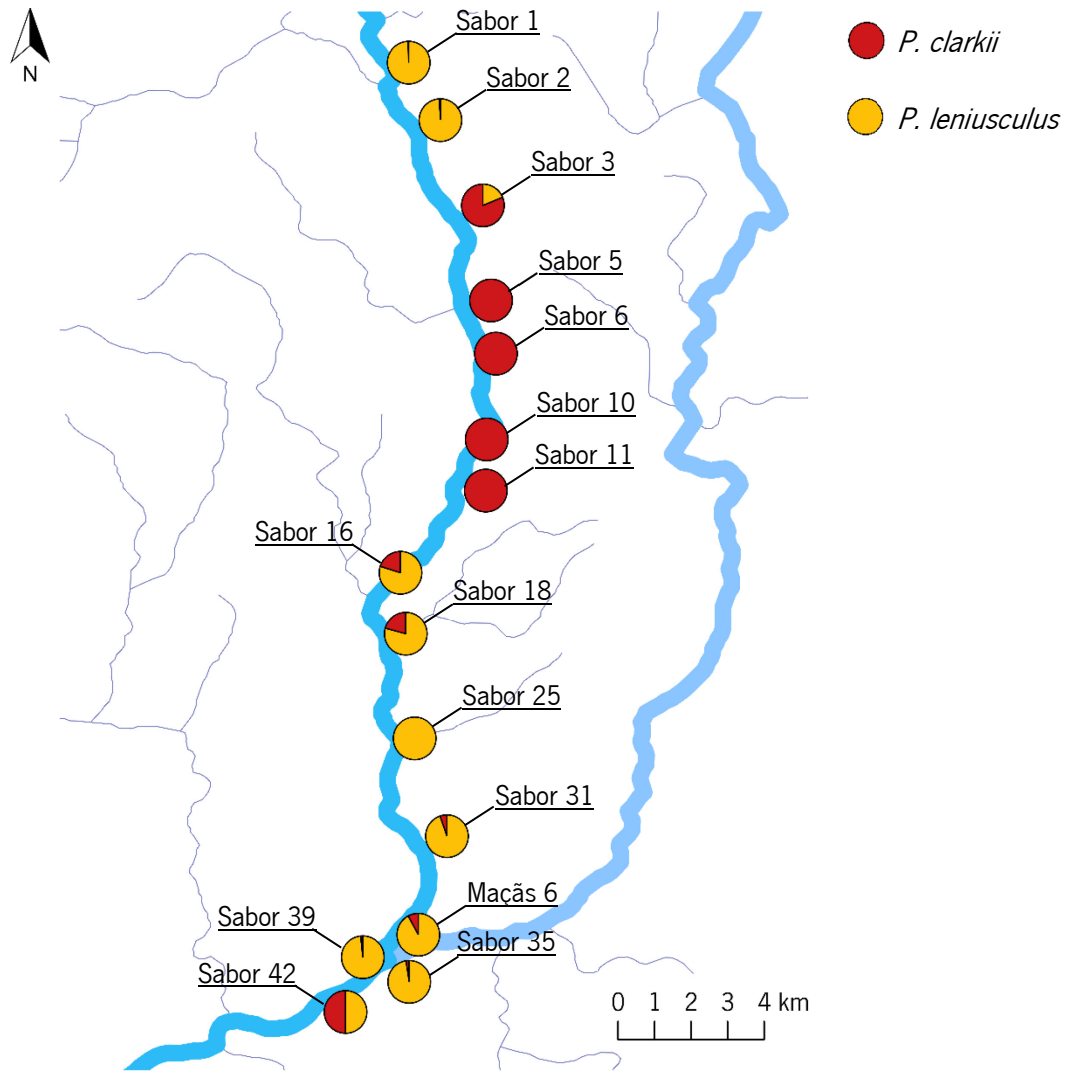


Figure 14: Distribution and relative abundance of *Procambarus clarkii* and *Pacifastacus leniusculus* across sampling sites.

The percentage of preyed bivalves was not related to crayfish sex ratio neither for *A. anatina* nor *U. delphinus* (Spearman's correlation, $p > 0.05$). A moderate correlation was found between the average abundance of crayfish and the percentage of preyed bivalves (Spearman's correlation, $r_s = 0.57$, $p = 0.034$). Despite this, when specifying crayfish and bivalve species, significant correlations were only found between crayfish abundance and the percentage of preyed *U. delphinus* (Figure 15A). No significant correlation was found between crayfish abundance and the percentage of preyed *A. anatina* (Figure 15B) (Table 6).

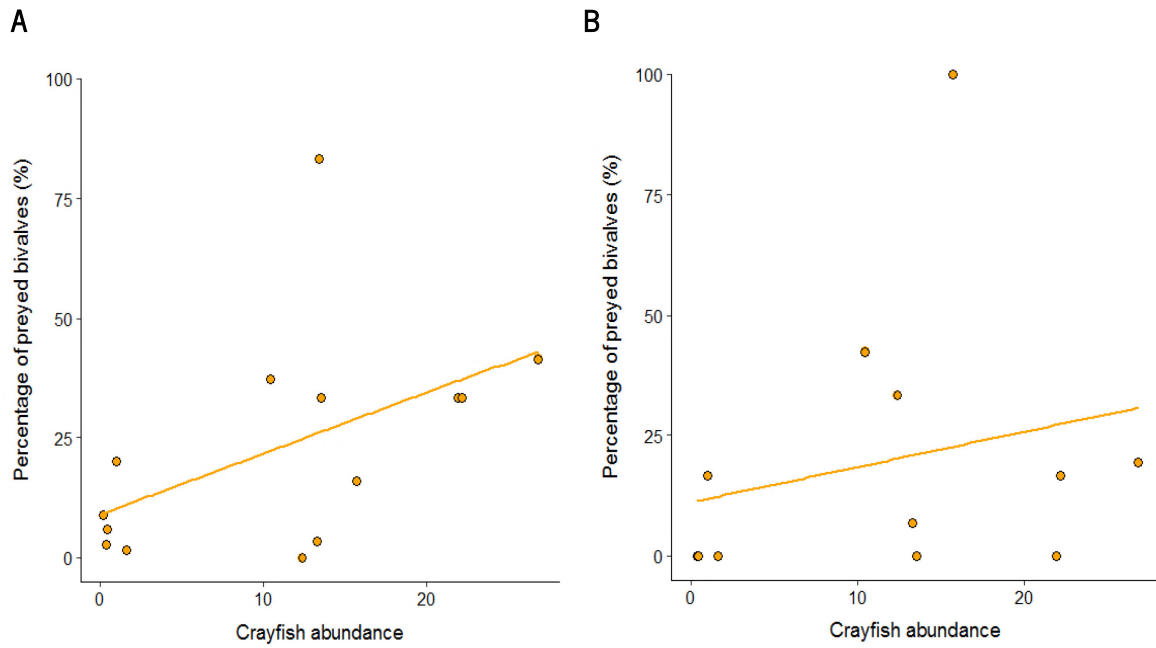


Figure 15: Relationship between crayfish abundance and the percentage of preyed (A) *Unio delphinus* and (B) *Anodonta anatina* bivalves. Only the relationship between percentage of *U. delphinus* preyed bivalves and and crayfish abundance was significant ($p < 0.05$).

Table 6: Spearman's rank correlation (r_s) coefficients between crayfish abundance and the percentage of preyed bivalves.

	<i>Unio delphinus</i>		<i>Anodonta anatina</i>	
	r_s	p	r_s	p
<i>Procambarus clarkii</i>	-0.64	0.013	0.24	0.46
<i>Pacifastacus leniusculus</i>	0.55	0.04	0.30	0.337

4. Discussion

In this study we showed that the invasive crayfishes *P. clarkii* and *P. leniusculus* are able to prey and kill native freshwater bivalves. This comes as no surprise since other crayfish species are known to prey freshwater bivalves (e.g. Perry et al. 1997, Klocker & Strayer 2004) and Machida & Akiyama (2013) showed that *P. leniusculus* was capable of preying two endangered *Margaritifera* species.

All native bivalve species used in this study were preyed and results showed that *P. leniusculus* is a more capable predator than *P. clarkii*. On average, *P. leniusculus* consumed a higher percentage of shell of *U. delphinus* than *P. clarkii* but this result was highly influenced by one specimen that was killed, which resulted in 44.4% of its shell eaten. If we exclude this replicate from the analyses, the average percentage of shell consumption of *U. delphinus* by the signal crayfish would have been 3% (and not 9%), which was not significantly higher than the average percentage consumed by *P. clarkii* (1.5%). Therefore, these possible differences between crayfish species should be interpreted with some caution. Anyway, several morphological and taxonomical studies showed clear differences in the morphology of the mandibles between crayfish species (e.g. Capelli & Capelli 1980, Kwai 2012). For example, Harlioğlu (1996) studied *P. leniusculus* and *Astacus leptodactylus* Eschscholtz, 1823) and found differences in their morphology and biology. This author suggested that by having more teeth on its mandibles and crista dentata, long and abundant setae on the second maxilliped, and differences in the form of the chelipeds' dactylus and propodus, *P. leniusculus* had a feeding advantage over *A. leptodactylus*. Therefore, the possible differences in bivalve predation between *P. leniusculus* and *P. clarkii* might be explained by differences in morphology; however, future studies must be conducted to address this hypothesis.

When given a choice, both crayfish species preferred *A. anatina* as a prey. It should be easier, and thus require less energy, for crayfishes to handle and break *A. anatina* shells, since this bivalve species has the most fragile and thin shell (Ilarri et al. 2015). It should be energetically more profitable for crayfishes to prey *A. anatina* instead of the other bivalve species. *P. littoralis* was the less preyed native species in both choice and non-choice experiments and this may be also related with the shell thickness since this species presented the most robust and thick shell (Ilarri et al. 2015). Therefore, *P. littoralis* should be more resistant to crayfish predation and less profitable, in terms of energy yield. In addition, and for all native bivalve species, the percentage of bivalves preyed by *P. leniusculus* was lower than in the non-choice experiments. This suggests that bivalve species richness may reduce the impact of this crayfish, especially in the case of

U. delphinus, which had, in the choice experiments, 26.7% less preyed bivalves than in the non-choice experiments. Both crayfish species preyed less on *P. littoralis* individuals when they could choose their prey, which indicate that this bivalve species may be less affected by both *P. clarkii* and *P. leniusculus* when other bivalve species are present. Again, these results suggest that shell thickness may be key a factor explaining overall results in the different native bivalve species.

The results of the laboratorial experiments matched those found in the field, as only native bivalve species were preyed. In the field, predation was dependent on bivalve species and there was an interaction between this factor and the river sites, which means that the most preyed bivalve species differ from site to site. However, in the laboratorial experiments predation was not significantly different between native bivalve species. These contradictory results should be interpreted with some caution since in the field the differences on bivalve predation were only significant in 5 out of the 24 sites analysed and the most preyed species was not the same throughout those five sites. Overall bivalve predation in the field was not related to the bivalve species but mostly to distribution and abundance in addition to other possible not studied factors that may also change along river sites (for example the availability of other food sources for crayfish). Therefore, field results somehow match the results gathered in the laboratorial experiments.

Potomida littoralis is the most endangered native bivalve species since its populations in Portugal are very fragmented and have suffered major declines (Lopes-Lima 2014b) and this reality was seen in the field as this species was only found in five of the 51 sites and its abundance was always low. Despite this, *P. littoralis* was the least preyed bivalve species in the laboratorial experiments. Additionally, only two specimens collected in the field were found dead and the cause of death did not appear to be directly related to crayfish predation due to the absence of the characteristic marks in the shells. This situation seems to indicate that crayfish predation pressure may not play a major role on the decline of *P. littoralis* due to the strong shells of this bivalve species (as referred above), which probably turn the predation by both invasive crayfish more challenging.

Even though the results suggest that *P. clarkii* and *P. leniusculus* predation behaviour did not directly cause a great reduction in bivalve abundance on the native populations in Sabor and Maçãs Rivers, given the low number of found empty shells with predation marks, these results should be interpreted with some caution because many empty shells may have disappeared (due to shell decay or washed to downstream areas). Anyway, and given the high number of alive bivalves that presented predation marks it is possible that these bivalves can still suffer non-lethal effects from these invasive crayfishes. The damage caused to shells should have a physiological cost for bivalves as they spend

energy repairing those damages instead of spending it on growth and/or reproduction. In addition, every prey has to deal with the risk of being predated when feeding, thus having to balance the trade-off between predation risk and energy intake (Lima 1998). In extreme situations, where animals are hungry and face a risk of starvation, this trade-off may force preys to be more vulnerable to predators as they will be less alert (Godin & Smith 1988, Pettersson & Brönmark 1993). Anti-predator behaviour may decrease prey energy intake and thus reduce its fitness and alter resource allocation, which can impact growth, reproduction and/or long-term survival (Lima 1998, Johnson & Smee 2012). Naddafi et al. (2007) showed that predator presence had a negative effect on the filtration rate of the invasive zebra mussel *Dreissena polymorpha* (Pallas, 1771). Additionally, Maire et al. (2010) showed that in the presence of a predatory shrimp (*Crangon crangon* (Linnaeus, 1758)), *Limecola balthica* (Linnaeus, 1758), a burrowing marine clam, retracted its siphon and never expelled faeces and pseudofaeces. In addition, and when *C. crangon* was not actively foraging and was well distanced from it, *L. balthica* was observed to deposit feed but less actively than in the absence of *C. crangon* (Maire et al. 2010). Therefore, it may be reasonable to consider that freshwater bivalves used in the present study may also be affected in their filter feeding activity, due to the presence of *P. clarkii* and *P. leniusculus*, thus having lower fitness and consequently lower growth and reproduction rates. Anyway, no signs of anti-predator behaviour were observed in *A. anatina* during the competition experiment, as the specimens used seemed to move in the sediment regardless of the presence and distance of predators, sometimes even moving towards the crayfishes. This could be explained by prey naivety that is associated with the lack of previous experience with non-native predators as it has been shown that prey behaviour and perception of risk may change depending on whether the prey is dealing with a native or a non-native predator (Cox & Lima 2006, Sih et al. 2010). However, *A. anatina* specimens were collected from a river where both crayfish species are present from more than a decade and prey on this species at least in the last five years (Ronaldo Sousa, personal communication); therefore, this naïve behaviour is probably not a good explanation for these results and other hypotheses have to be tested in the future.

Most predators prefer small-sized prey when offered a range of different sizes (Juanes 1992). However, in the present study, *P. clarkii* and *P. leniusculus* did not show any preferences regarding the prey size. Machida & Akiyama (2013) observed that crayfishes particularly injured *Margaritifera* spp. of medium size and suggested that this size class was the most suitable for crayfish manipulation. This may indicate that these invasive crayfishes will not necessarily affect bivalve recruitment by preying on sexually immature individuals. Nonetheless, based on

Machida & Akiyama (2013) results, these crayfishes may kill small immature bivalves. Besides, crayfish size did not affect bivalve predation, which shows that smaller crayfishes are as capable of preying bivalves of various sizes as their larger counterparts. In addition, in the field study no correlation was found between crayfish sex ratio and bivalve predation suggesting that female and male crayfishes have the same level of impact on native freshwater bivalves.

P. clarkii and *P. leniusculus* predatory behaviour is known to have many indirect impacts on invaded ecosystems (Nyström et al. 1996, Geiger et al. 2005, Carvalho et al. 2016). Therefore, by preying on bivalves, these species should also indirectly impact ecosystems as their non-lethal effects could affect the functional role of bivalves. As referred before, bivalves filter phytoplankton, bacteria and particulate organic matter, remove organic matter from the sediment and transfer high-quality pelagic resources to the benthos through the biodeposition of faeces and pseudofaeces (Vaughn et al. 2008). These processes affect nutrient dynamics and water quality in freshwater ecosystems, thus indirectly affecting numerous species (Vaughn & Hakenkamp 2001, Vaughn et al. 2008). Since bivalve predation can cause bivalve mortality or feeding inhibition, *P. clarkii* and *P. leniusculus* may indirectly increase the abundance of phytoplankton, increasing eutrophication, and they may reduce the abundance of chironomids and other detritivores that comes with the accumulation of organic matter between shells and the biodeposition of faeces and pseudofaeces by bivalves (Vaughn & Hakenkamp 2001). However, these and other indirect effects mediated by crayfish predation on freshwater bivalves remain speculative and future manipulative studies should assess these changes on ecosystem functioning.

Zu Ermgassen & Aldridge (2011) described the signal crayfish feeding behaviour on bivalves stating that crayfishes search the benthos randomly with their second and third pair of pereopods until a prey is found, picking the prey up and bringing it to their mouth where it is held by the maxillipeds and chipped with their mandibles. This feeding behaviour was observed during the laboratorial experiments by both crayfish species and suggests that even if bivalves burrow into substrate they are still vulnerable to predation by crayfishes. Regardless of this, on the laboratorial experiments only *C. fluminea* specimens were able to fully burrow into substrate, disappearing from substrate surface, and were not preyed, which may indicate that the burrowing behaviour can be an effective protection against predation. Machida and Akiyama (2013) showed that small mussel individuals disappeared from the surface of the pebbles used as substrate in their experiments, and that the damage rate on these individuals was lower than on larger individuals that were unable to fully burrow. Despite this, small mussels were still injured by crayfishes, hinting that only some of these small mussels could not be found by crayfishes. This situation may be also valid for the

burrowed *C. fluminea* individuals, which strengthens the idea that there is another explanation to why *C. fluminea* was not preyed in laboratory or field.

No studies were found about the ability of *P. leniusculus* to prey *C. fluminea*, but a few demonstrated that *P. clarkii* was able to consume this invasive bivalve (e.g. Covich et al. 1981, Pereira et al. 2016). These earlier results on *P. clarkii* differs from the ones reported here that suggested that neither crayfish species was able to prey *C. fluminea*. The lack of information about predator-prey interactions between *P. leniusculus* and *C. fluminea* impair us of further comparisons and the incoherence between the results of this study and those of others means that the absence of predation of the Asian clam is not necessarily due to the lack of ability of these crayfish species to prey it. Indeed, shell morphology and size may also affect the predation on *C. fluminea*. For example, Pereira et al. (2016) have shown that *P. clarkii* was only able to eat Asian clams with less than 10 mm, which was also demonstrated by Covich et al. (1981). In our study, the smallest *C. fluminea* individual used measured 15 mm, which may explain why no crayfish was able to prey this species. Besides, *C. fluminea* shell is thicker (Ilarri et al. 2015) and has a globelike shape, probably making it harder for crayfishes to manipulate and open it, unlike the other studied bivalves that have elongated and thin shells, especially *A. anatina* and *U. delphinus*. Based on the available information (Bernardo et al. 2011, Crespo et al. 2015), the co-existence of these crayfish species and *C. fluminea* in Sabor River is relatively recent and thus, the absence of predation of *C. fluminea* may be explained by the fact that both crayfish populations have not recognized this species as prey yet. However, both *P. leniusculus* and *P. clarkii* have been able to identify and use new introduced species as preys, like the invasive zebra mussel (Zu Ermgassen & Aldridge 2011, Gonçalves et al. 2016) and the Chinese mystery snail, *Bellamya chinensis* (Gray 1834) (Olden et al. 2009) turning the hypothesis of no recognition of *C. fluminea* as a possible prey very unlikely.

Summing up, and although very unlikely, crayfishes in the Sabor River may still not recognize *C. fluminea* as prey but if they do they should only be able to prey on smaller specimens (<10 mm). This means that *P. clarkii* and *P. leniusculus* may be able to affect *C. fluminea* recruitment as this species only becomes sexually mature when shell length reaches 6 to 10 mm (Sousa et al. 2008). However, our results clearly showed that both crayfish species have prey preferences when it comes to bivalve species, and as generalist omnivores the probability of them preying *C. fluminea* in natural environments should be very low, as they have various and much easier alternative feeding sources, including other bivalves. Thus, *P. clarkii* and *P. leniusculus* would probably not significantly reduce *C. fluminea* populations and should not be expected to act as control agents of this species in invaded ecosystems.

The aggressive behaviour of *P. leniusculus* and *P. clarkii* associated with interspecific and intraspecific competition has been widely studied (e.g. Söderbäck 1991, Blank & Figler 1996, Vorburger & Ribi 1999). In the present study, none of the crayfish species was dominant in terms of handling time; however, during the 72 hours of the experiment, *P. clarkii* manipulated bivalves 63.6% more times and initiated 55.8% more agonistic bouts than *P. leniusculus*. Additionally, video records showed that in most of the cases the Louisiana crayfish had an extremely aggressive behaviour towards the signal crayfish as it tried to initiate fights even when they were in opposite sides of the mesocosms, while the signal crayfish avoided confrontation and always tried to avoid *P. clarkii* except when the agonistic bouts were associated to the use of the bivalve as a feeding source. Some of the confrontations were started as a feeding territory defence as, when distanced from the bivalve and with no signs of interest in it, *P. clarkii* attacked *P. leniusculus* when it was approaching the bivalve. These results show that *P. clarkii* is the dominant species when both crayfish species have similar sizes. This is consistent with the results of Pearl et al. (2013), which showed that *P. clarkii* has the potential to locally displace *P. leniusculus* and that *P. leniusculus* avoided areas where *P. clarkii* was present. However, in the field, *P. leniusculus* was more abundant than *P. clarkii*, which suggests that, besides aggressiveness, other factors might be important (for example, *P. clarkii* may be more prone to predation than *P. leniusculus* or the environmental conditions are more favourable to *P. leniusculus*, among other hypotheses). Although competition between *P. clarkii* and *P. leniusculus* was confirmed in the laboratorial experiments, and their niches probably overlap since both species are omnivorous generalists, these crayfishes have numerous feeding sources (Axelsson et al. 1997, Guan & Wiles 1998, Gherardi et al. 2001, Gherardi 2006), which means that competition may be minimized in the field. As a result, and as suggested by Jackson et al. (2014), due to their niche overlap *P. clarkii* and *P. leniusculus* may have either an additive effect of their independent impacts or an amplified effect on the native bivalve populations. Although competition for food may be low in the Sabor River, these crayfish species may still compete for shelter in order to hide from predators. In fact, remains of both crayfish species were found regularly in otter (*Lutra lutra* (Linnaeus, 1758)) spraints and American mink (*Neovison vison* (Schreber, 1777)) scats, which means that otters and minks may act as a control agent of both crayfish populations. In addition, and as stated above, these predators may have a different pressure in both species being *P. clarkii* more prone to predation; however, this situation remains speculative and should be further investigated in the future.

This study supports the idea that *P. clarkii* and *P. leniusculus* have mostly non-lethal effects on native bivalve species and those effects on overall bivalve populations can indirectly impact

ecosystem functions and properties such as nutrient dynamics, water quality and transparency, species abundances, among others. Additionally, these crayfishes prefer preying on *A. anatina*, which is the least threatened native bivalve species of this study (IUCN 2017), and cannot act as *C. fluminea* control agent. Therefore, and in addition to other possible advantages of *C. fluminea* over the native species (e.g. rapid growth, early sexual maturity, more plastic feeding; Sousa et al. 2008), this invasive clam may also benefit of being much less predated. This advantage may increase the impacts of *C. fluminea* on native bivalve populations and invaded ecosystems. Our results also suggest that *P. clarkii* causes less damage to native bivalves; and thus, native bivalves present in ecosystems where only *P. clarkii* exists will suffer less impacts than those where *P. leniusculus* occurs. However, this situation may be highly context and density dependent and deserves further investigation.

5. Conclusion and future perspectives

The results of this study are an example of the possible direct impacts that invasive predators can have on freshwater bivalves. In addition, and although not studied, indirect effects in freshwater ecosystems resulting from these predator-prey interactions may be also possible. *P. clarkii* and *P. leniusculus* are nowadays well spread worldwide (Holdich et al. 2009) and can affect numerous bivalve species, which is alarming as these animals are one of the most threatened faunal groups on the planet and have an important role in freshwater ecosystems (Vaughn & Hakenkamp 2001, IUCN 2017).

Even though the present study may increase the knowledge about the behaviour of these invasive crayfishes on freshwater bivalves, there is still a lot to explore in order to better understand the dynamics of these relationships and their impacts. Bivalves may react in different ways to the presence of *P. clarkii* and *P. leniusculus* and be more or less vulnerable to crayfish predation depending on how long they have been co-existing. Regarding this, it is important to further study the naïve behaviour of some bivalves and anti-predator mechanisms that bivalves might develop to protect themselves from these crayfishes. This line of thought could be very interesting to pursue as some studies have demonstrated that the presence of predators, and even waterborne cues from them, may induce shell thickening and avoidance behaviour of some marine bivalves (e.g. Freeman & Byers 2006, Griffiths & Richardson 2006). Another interesting direction of investigation to pursue would be the assessment of predation rate differences and competition between male and female crayfishes, during different phases of their reproductive cycle and growth, as their aggressiveness and energy needs may vary. Future studies should also include more food sources in laboratory conditions and longer experiments to better understand crayfish feeding preferences and impacts on bivalve species and invaded ecosystems. Finally, and since crayfish metabolism is highly influenced by temperature it would be interesting to repeat these laboratorial experiments at different temperatures. Although speculative, we predict that much higher predation rates will be observed under higher temperatures than the ones tested in this study (i.e. 15 °C).

Nowadays, conservation in freshwater ecosystems is a hot topic since they are highly threatened and provide many services to humans. On the other hand, conservation of freshwater bivalves is gaining more attention as this faunal group have major roles in these ecosystems. However, studies reporting the decline of freshwater bivalves generally focus on habitat loss and fragmentation, pollution, host fish populations decline, commercial exploitation and the introduction

of invasive species (usually other bivalve species such as *C. fluminea* and *D. polymorpha*) that either compete with native bivalves or negatively affect host fish populations. Very little information relative to the direct impacts of invasive predators is available in the literature. Therefore, the results reported here are very important and demonstrate how the introduction of two invasive crayfish species may impair the conservation of native freshwater bivalves. To our knowledge this is the first study assembling laboratorial and field data demonstrating how the introduction of predators may affect freshwater bivalves, a basic information to conserve them through time. Finally, the information reported here has also management implications because any conservation measure devoted to the conservation of native freshwater bivalves in ecosystems invaded by *P. clarkii* and/or *P. leniusculus* (or even other crayfish species) should take into account the role of these animals as important predators.

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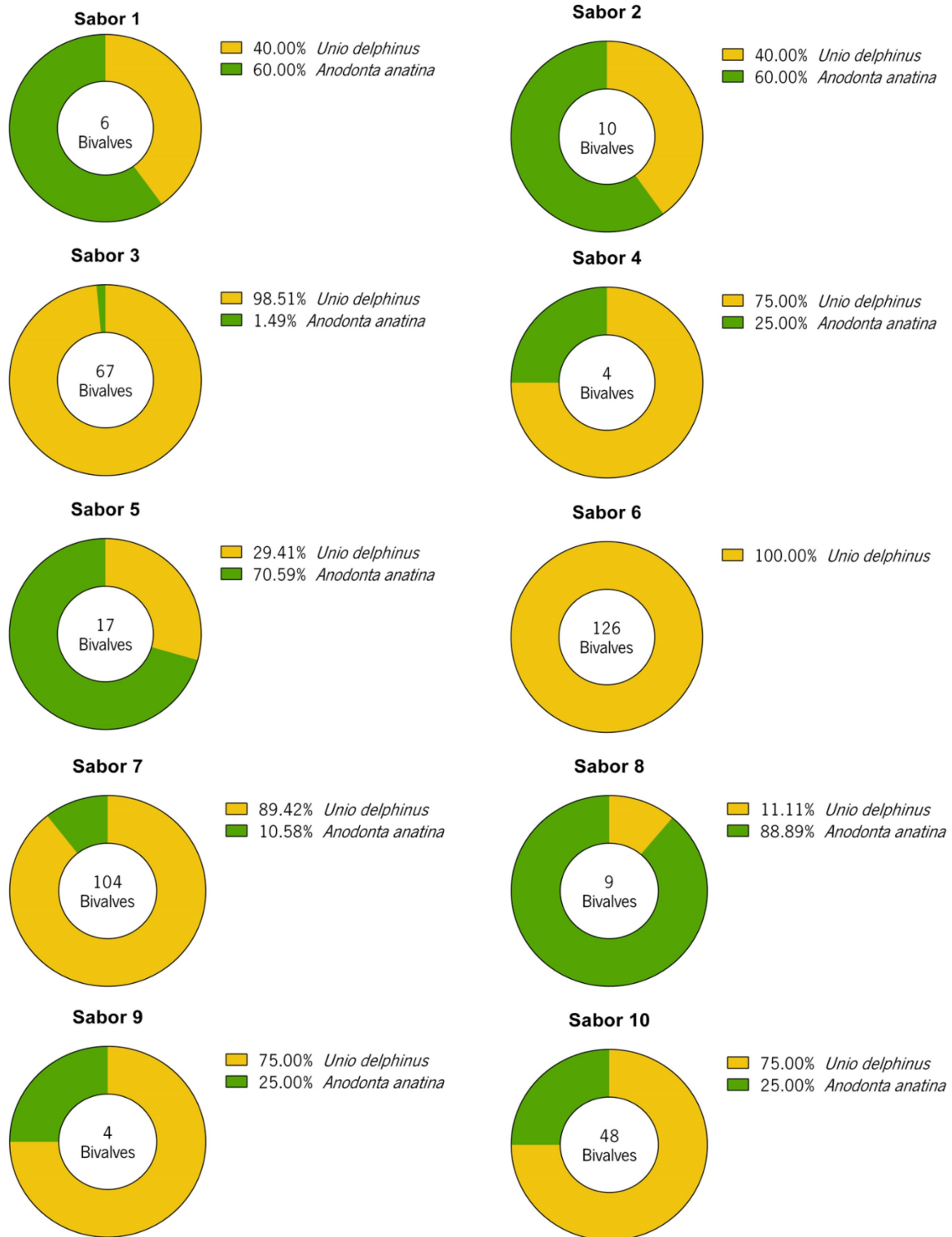
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Appendix

Appendix 1: Relative abundance of *Unio delphinus*, *Anodonta anatina*, *Potomida littoralis* and *Corbicula fluminea* within each sampling site in Sabor and Maças Rivers.



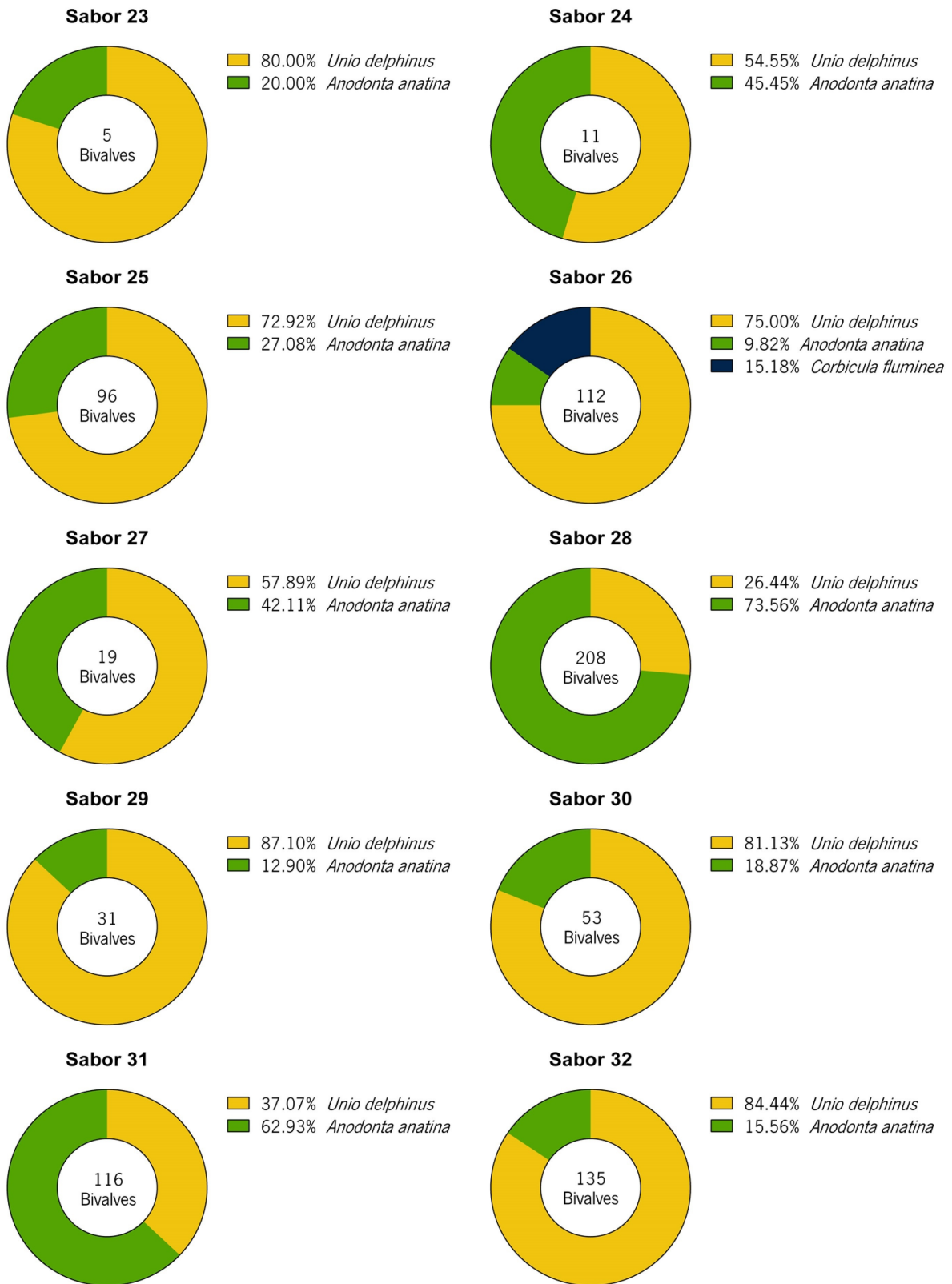
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Appendix 1: Continued.



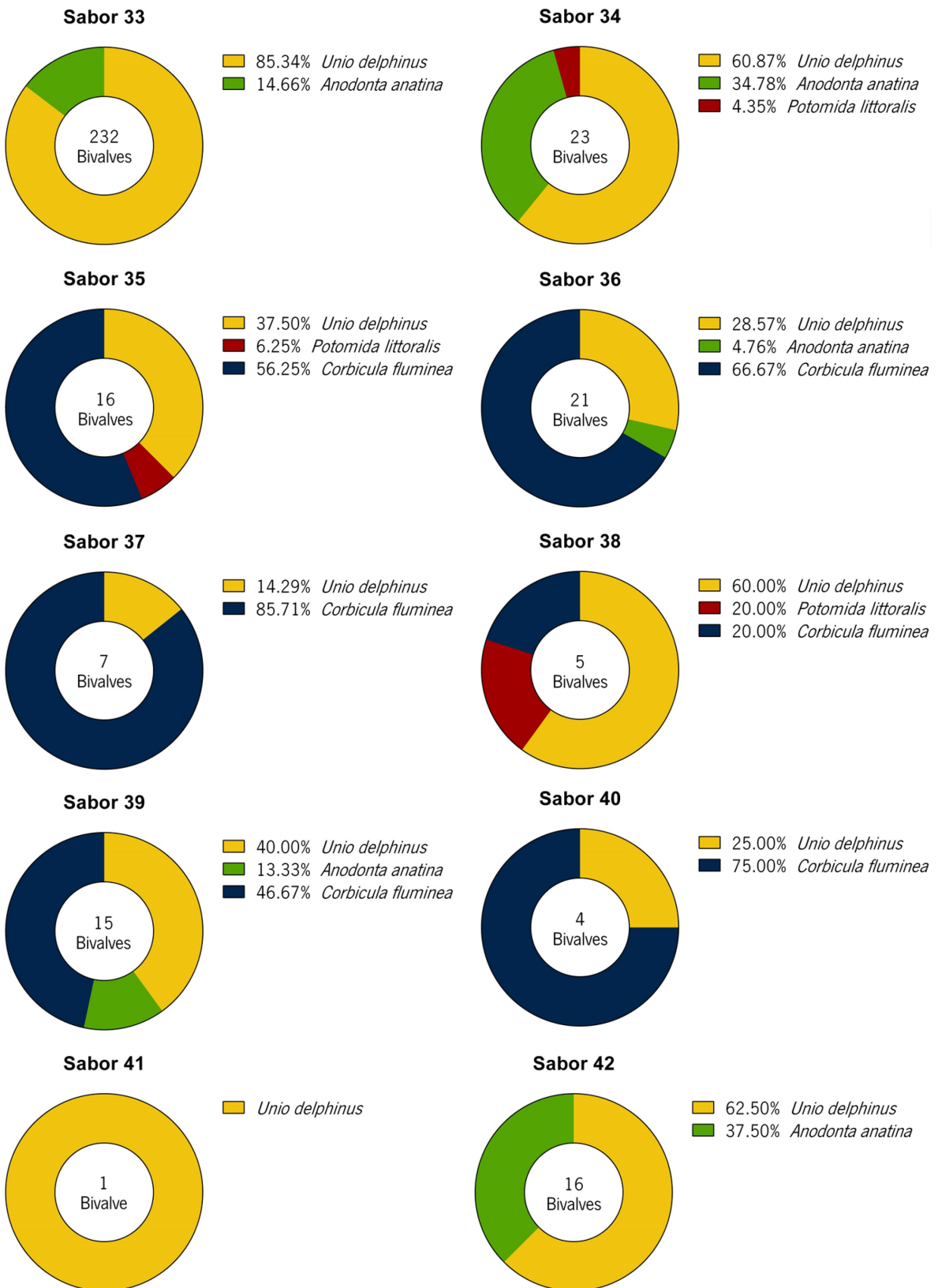
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Appendix 1: Continued.



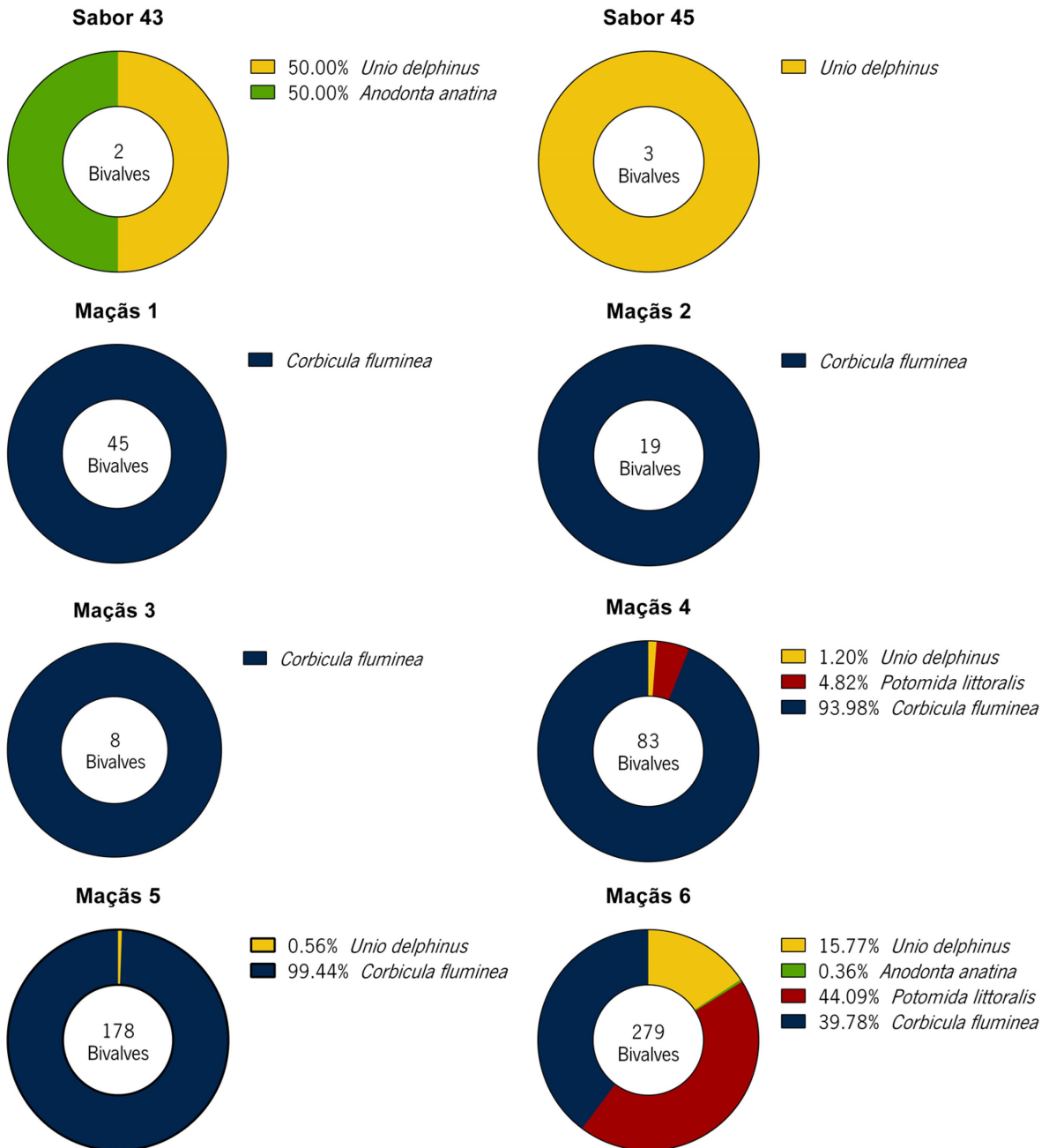
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Appendix 1: Continued.



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Appendix 1: Continued.



Appendix 2: Results of PERMANOVA pairwise comparisons of bivalve predation rate between *Unio delphinus* and *Anodonta anatina* within each site where both bivalve species were found.

River site	T	P
Sabor 2	1	0.34
Sabor 5	0.1544	0.869
Sabor 7	0.3424	0.733
Sabor 10	0.5732	0.581
Sabor 14	0.2675	0.806
Sabor 15	0.678	0.492
Sabor 16	4.0101	0.001
Sabor 17	2.4285	0.016
Sabor 18	0.0699	0.951
Sabor 19	1.994	0.048
Sabor 20	6.6686	0.001
Sabor 21	1.7087	0.095
Sabor 24	1.533	0.172
Sabor 25	1.3921	0.182
Sabor 26	1.4467	0.147
Sabor 27	1.3541	0.179
Sabor 28	4.6992	0.001
Sabor 29	0.0682	0.944
Sabor 30	0.3055	0.779
Sabor 31	0.7839	0.417
Sabor 32	1.67	0.092
Sabor 33	1.6332	0.09
Sabor 34	0.0084	0.09
Sabor 41	1.3258	0.205

Appendix 3: Results of PERMANOVA pairwise comparisons of *Anodonta anatina* predation rate among sites.

	T	P		T	P		T	P
Sabor 2 vs Sabor 5	1.1244	0.277	Sabor 2 vs Sabor 32	0.33606	0.72	Sabor 5 vs Sabor 29	1.9817	0.067
Sabor 2 vs Sabor 7	1.3933	0.195	Sabor 2 vs Sabor 33	0.6947	0.495	Sabor 5 vs Sabor 30	1.1389	0.27
Sabor 2 vs Sabor 8	1.711	0.244	Sabor 2 vs Sabor 34	1.1275	0.3	Sabor 5 vs Sabor 31	2.0373	0.039
Sabor 2 vs Sabor 10	1.4606	0.17	Sabor 2 vs Sabor 42	1.378	0.206	Sabor 5 vs Sabor 32	1.0959	0.296
Sabor 2 vs Sabor 14	0.8	0.454	Sabor 5 vs Sabor 7	1.4173	0.151	Sabor 5 vs Sabor 33	1.5197	0.132
Sabor 2 vs Sabor 15	1.2744	0.213	Sabor 5 vs Sabor 8	1.2	0.243	Sabor 5 vs Sabor 34	1.8786	0.076
Sabor 2 vs Sabor 16	0.40347	0.692	Sabor 5 vs Sabor 10	1.4832	0.152	Sabor 5 vs Sabor 42	2.1477	0.045
Sabor 2 vs Sabor 17	0.28819	0.773	Sabor 5 vs Sabor 14	0.83666	0.403	Sabor 7 vs Sabor 8	Denominator is 0	
Sabor 2 vs Sabor 18	3.0177	0.001	Sabor 5 vs Sabor 15	0.18519	0.88	Sabor 7 vs Sabor 10	Denominator is 0	
Sabor 2 vs Sabor 19	1.6475	0.115	Sabor 5 vs Sabor 16	0.60945	0.538	Sabor 7 vs Sabor 14	Denominator is 0	
Sabor 2 vs Sabor 20	1.249	0.25	Sabor 5 vs Sabor 17	0.81954	0.423	Sabor 7 vs Sabor 15	0.8516	0.41
Sabor 2 vs Sabor 21	0.71258	0.482	Sabor 5 vs Sabor 18	1.1328	0.259	Sabor 7 vs Sabor 16	2.1712	0.052
Sabor 2 vs Sabor 24	0.15283	0.904	Sabor 5 vs Sabor 19	1.6667	0.114	Sabor 7 vs Sabor 17	0.6566	0.389
Sabor 2 vs Sabor 25	0.23963	0.812	Sabor 5 vs Sabor 20	1.2762	0.224	Sabor 7 vs Sabor 18	Denominator is 0	
Sabor 2 vs Sabor 26	0.43797	0.696	Sabor 5 vs Sabor 21	0.24016	0.817	Sabor 7 vs Sabor 19	Denominator is 0	
Sabor 2 vs Sabor 27	1.9583	0.071	Sabor 5 vs Sabor 24	2.8204	0.016	Sabor 7 vs Sabor 20	0.5562	0.564
Sabor 2 vs Sabor 28	0.11472	0.909	Sabor 5 vs Sabor 25	0.76453	0.45	Sabor 7 vs Sabor 21	4.3665	0.001
Sabor 2 vs Sabor 29	0.76652	0.473	Sabor 5 vs Sabor 26	0.63988	0.543	Sabor 7 vs Sabor 24	1.0114	0.326
Sabor 2 vs Sabor 30	0.53144	0.612	Sabor 5 vs Sabor 27	3.1863	0.006	Sabor 7 vs Sabor 25	1	0.316
Sabor 2 vs Sabor 31	1.035	0.306	Sabor 5 vs Sabor 28	1.7925	0.081	Sabor 7 vs Sabor 26	3.1165	0.005

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Appendix 3: Continued.

	T	P		T	P		T	P
Sabor 7 vs Sabor 27	2.1109	0.041	Sabor 8 vs Sabor 26	0.846	0.42	Sabor 10 vs Sabor 26	1.0467	0.32
Sabor 7 vs Sabor 28	2.077	0.001	Sabor 8 vs Sabor 27	2.6283	0.019	Sabor 10 vs Sabor 27	3.2646	0.007
Sabor 7 vs Sabor 29	2.077	0.041	Sabor 8 vs Sabor 28	1.8	0.073	Sabor 10 vs Sabor 28	2.2048	0.029
Sabor 7 vs Sabor 30	1.1739	0.011	Sabor 8 vs Sabor 29	1.7369	0.134	Sabor 10 vs Sabor 29	2.1798	0.048
Sabor 7 vs Sabor 31	2.0859	0.001	Sabor 8 vs Sabor 30	0.9912	0.348	Sabor 10 vs Sabor 30	1.2294	0.224
Sabor 7 vs Sabor 32	1.1955	0.037	Sabor 8 vs Sabor 31	1.778	0.078	Sabor 10 vs Sabor 31	2.1789	0.042
Sabor 7 vs Sabor 33	1.5806	0.028	Sabor 8 vs Sabor 32	1.016	0.324	Sabor 10 vs Sabor 32	1.2499	0.225
Sabor 7 vs Sabor 34	1.8489	0.075	Sabor 8 vs Sabor 33	1.3457	0.198	Sabor 10 vs Sabor 33	1.6517	0.103
Sabor 7 vs Sabor 42	2.0793	0.062	Sabor 8 vs Sabor 34	1.5593	0.11	Sabor 10 vs Sabor 34	1.9368	0.082
Sabor 8 vs Sabor 10	Denominator is 0		Sabor 8 vs Sabor 42	1.7477	0.099	Sabor 10 vs Sabor 42	2.1798	0.036
Sabor 8 vs Sabor 14	Denominator is 0		Sabor 10 vs Sabor 14	Denominator is 0		Sabor 14 vs Sabor 15	0.5061	0.595
Sabor 8 vs Sabor 15	0.7223	0.488	Sabor 10 vs Sabor 15	0.8908	0.359	Sabor 14 vs Sabor 16	2.1952	0.291
Sabor 8 vs Sabor 16	1.8091	0.111	Sabor 10 vs Sabor 16	2.2804	0.036	Sabor 14 vs Sabor 17	0.5317	0.588
Sabor 8 vs Sabor 17	0.7526	0.463	Sabor 10 vs Sabor 17	0.9223	0.351	Sabor 14 vs Sabor 18	0.3954	0.699
Sabor 8 vs Sabor 18	0.5596	0.576	Sabor 10 vs Sabor 18	0.6859	0.489	Sabor 14 vs Sabor 19	Denominator is 0	
Sabor 8 vs Sabor 19	Denominator is 0		Sabor 10 vs Sabor 19	Denominator is 0		Sabor 14 vs Sabor 20	Denominator is 0	
Sabor 8 vs Sabor 20	Denominator is 0		Sabor 10 vs Sabor 20	Denominator is 0		Sabor 14 vs Sabor 21	0.334	0.757
Sabor 8 vs Sabor 21	0.4736	0.642	Sabor 10 vs Sabor 21	0.58131	0.568	Sabor 14 vs Sabor 24	2.4495	0.06
Sabor 8 vs Sabor 24	3.6515	0.006	Sabor 10 vs Sabor 24	4.5826	0.001	Sabor 14 vs Sabor 25	0.6058	0.538
Sabor 8 vs Sabor 25	0.8603	0.387	Sabor 10 vs Sabor 25	1.0572	0.301	Sabor 14 vs Sabor 26	0.5887	0.6

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Appendix 3: Continued.

	T	P		T	P		T	P
Sabor 14 vs Sabor 27	1.8137	0.09	Sabor 15 vs Sabor 29	2.2273	0.046	Sabor 16 vs Sabor 32	0.4678	0.66
Sabor 14 vs Sabor 28	1.2726	0.18	Sabor 15 vs Sabor 30	1.2976	0.202	Sabor 16 vs Sabor 33	0.6916	0.495
Sabor 14 vs Sabor 29	1.1651	0.292	Sabor 15 vs Sabor 31	2.3081	0.02	Sabor 16 vs Sabor 34	0.8694	0.413
Sabor 14 vs Sabor 30	0.6864	0.483	Sabor 15 vs Sabor 32	1.2578	0.231	Sabor 16 vs Sabor 42	0.9902	0.345
Sabor 14 vs Sabor 31	1.2564	0.232	Sabor 15 vs Sabor 33	1.7277	0.095	Sabor 17 vs Sabor 18	2.1342	0.033
Sabor 14 vs Sabor 32	0.7141	0.48	Sabor 15 vs Sabor 34	2.4282	0.032	Sabor 17 vs Sabor 19	1.0317	0.293
Sabor 14 vs Sabor 33	0.949	0.364	Sabor 15 vs Sabor 42	2.1253	0.037	Sabor 17 vs Sabor 20	0.7983	0.435
Sabor 14 vs Sabor 34	1.076	0.287	Sabor 16 vs Sabor 17	0.358	0.752	Sabor 17 vs Sabor 21	1.509	0.202
Sabor 14 vs Sabor 42	1.1939	0.307	Sabor 16 vs Sabor 18	1.5399	0.143	Sabor 17 vs Sabor 24	0.1771	0.852
Sabor 15 vs Sabor 16	0.603	0.549	Sabor 16 vs Sabor 19	2.582	0.027	Sabor 17 vs Sabor 25	0.78421	0.439
Sabor 15 vs Sabor 17	0.9391	0.352	Sabor 16 vs Sabor 20	1.9365	0.092	Sabor 17 vs Sabor 26	0.6121	0.548
Sabor 15 vs Sabor 18	0.7771	0.463	Sabor 16 vs Sabor 21	0.048	0.956	Sabor 17 vs Sabor 27	2.7363	0.011
Sabor 15 vs Sabor 19	0.7676	0.346	Sabor 16 vs Sabor 24	1.1952	0.282	Sabor 17 vs Sabor 28	1.2395	0.218
Sabor 15 vs Sabor 20	0.7676	0.467	Sabor 16 vs Sabor 25	0.2393	0.806	Sabor 17 vs Sabor 29	0.2631	0.787
Sabor 15 vs Sabor 21	0.3406	0.738	Sabor 16 vs Sabor 26	0.1332	0.896	Sabor 17 vs Sabor 30	0.4114	0.673
Sabor 15 vs Sabor 24	2.652	0.013	Sabor 16 vs Sabor 27	1.4861	0.153	Sabor 17 vs Sabor 31	1.3306	0.173
Sabor 15 vs Sabor 25	0.9109	0.378	Sabor 16 vs Sabor 28	0.6939	0.496	Sabor 17 vs Sabor 32	0.091	0.941
Sabor 15 vs Sabor 26	0.7706	0.441	Sabor 16 vs Sabor 29	0.8326	0.42	Sabor 17 vs Sabor 33	0.563	0.599
Sabor 15 vs Sabor 27	3.5963	0.002	Sabor 16 vs Sabor 30	0.5051	0.64	Sabor 17 vs Sabor 34	1.8344	0.074
Sabor 15 vs Sabor 28	2.0881	0.051	Sabor 16 vs Sabor 31	0.9475	0.346	Sabor 17 vs Sabor 42	3.2504	0.04

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Appendix 3: Continued.

	T	P		T	P		T	P
Sabor 18 vs Sabor 19	0.7672	0.449	Sabor 19 vs Sabor 27	3.6763	0.003	Sabor 20 vs Sabor 34	1.6608	0.11
Sabor 18 vs Sabor 20	0.5937	0.55	Sabor 19 vs Sabor 28	2.4653	0.018	Sabor 20 vs Sabor 42	1.864	0.095
Sabor 18 vs Sabor 21	1.0926	0.2777	Sabor 19 vs Sabor 29	2.4644	0.026	Sabor 21 vs Sabor 24	0.8267	0.441
Sabor 18 vs Sabor 24	1.5377	0.001	Sabor 19 vs Sabor 30	1.3835	0.18	Sabor 21 vs Sabor 25	0.6963	0.489
Sabor 18 vs Sabor 25	2.2697	0.018	Sabor 19 vs Sabor 31	2.4371	0.016	Sabor 21 vs Sabor 26	0.2959	0.775
Sabor 18 vs Sabor 26	2.21	0.047	Sabor 19 vs Sabor 32	1.4012	0.169	Sabor 21 vs Sabor 27	5.012	0.001
Sabor 18 vs Sabor 27	8.0303	0.001	Sabor 19 vs Sabor 33	1.8492	0.075	Sabor 21 vs Sabor 28	2.2409	0.021
Sabor 18 vs Sabor 28	4.9086	0.001	Sabor 19 vs Sabor 34	2.1811	0.036	Sabor 21 vs Sabor 29	1.9005	0.069
Sabor 18 vs Sabor 29	5.053	0.001	Sabor 19 vs Sabor 42	2.4588	0.019	Sabor 21 vs Sabor 30	1.6754	0.106
Sabor 18 vs Sabor 30	2.9591	0.005	Sabor 20 vs Sabor 21	0.5026	0.623	Sabor 21 vs Sabor 31	3.1931	0.002
Sabor 18 vs Sabor 31	5.1204	0.001	Sabor 20 vs Sabor 24	3.9027	0.003	Sabor 21 vs Sabor 32	1.4839	0.14
Sabor 18 vs Sabor 32	2.9007	0.003	Sabor 20 vs Sabor 25	0.9133	0.383	Sabor 21 vs Sabor 33	2.2912	0.026
Sabor 18 vs Sabor 33	3.8829	0.001	Sabor 20 vs Sabor 26	0.9	0.374	Sabor 21 vs Sabor 34	3.0469	0.008
Sabor 18 vs Sabor 34	4.7648	0.001	Sabor 20 vs Sabor 27	2.7994	0.015	Sabor 21 vs Sabor 42	3.661	0.002
Sabor 18 vs Sabor 42	5.4339	0.001	Sabor 20 vs Sabor 28	1.9092	0.056	Sabor 24 vs Sabor 25	0.37492	0.724
Sabor 19 vs Sabor 20	Denominator is 0		Sabor 20 vs Sabor 29	1.8564	0.086	Sabor 24 vs Sabor 26	0.67737	0.506
Sabor 19 vs Sabor 21	0.6507	0.534	Sabor 20 vs Sabor 30	1.0553	0.326	Sabor 24 vs Sabor 27	1.5684	0.146
Sabor 19 vs Sabor 24	5.1809	0.001	Sabor 20 vs Sabor 31	1.8862	0.074	Sabor 24 vs Sabor 28	0.13787	0.871
Sabor 19 vs Sabor 25	1.1844	0.246	Sabor 20 vs Sabor 32	1.079	0.296	Sabor 24 vs Sabor 29	0.6333	0.538

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Appendix 3: Continued.

	T	P		T	P		T	P
Sabor 24 vs Sabor 31	0.77051	0.43	Sabor 26 vs Sabor 32	0.77222	0.442	Sabor 29 vs Sabor 31	0.16157	0.87
Sabor 24 vs Sabor 32	0.19633	0.837	Sabor 26 vs Sabor 33	1.2199	0.231	Sabor 29 vs Sabor 32	0.41821	0.689
Sabor 24 vs Sabor 33	0.50009	0.61	Sabor 26 vs Sabor 34	1.6779	0.111	Sabor 29 vs Sabor 33	0.057347	0.963
Sabor 24 vs Sabor 34	0.8877	0.392	Sabor 26 vs Sabor 42	1.986	0.073	Sabor 29 vs Sabor 34	0.63653	0.543
Sabor 24 vs Sabor 42	1.091	0.309	Sabor 27 vs Sabor 28	8.3237	0.001	Sabor 29 vs Sabor 42	0.9512	0.39
Sabor 25 vs Sabor 26	0.23865	0.819	Sabor 27 vs Sabor 29	1.2115	0.221	Sabor 30 vs Sabor 31	0.16393	0.857
Sabor 25 vs Sabor 27	4.0846	0.001	Sabor 27 vs Sabor 30	1.4487	0.153	Sabor 30 vs Sabor 32	0.49963	0.609
Sabor 25 vs Sabor 28	0.89457	0.384	Sabor 27 vs Sabor 31	2.8735	0.011	Sabor 30 vs Sabor 33	0.11688	0.905
Sabor 25 vs Sabor 29	1.3379	0.211	Sabor 27 vs Sabor 32	2.8039	0.01	Sabor 30 vs Sabor 34	0.8161	0.406
Sabor 25 vs Sabor 30	1.2	0.241	Sabor 27 vs Sabor 33	2.6149	0.015	Sabor 30 vs Sabor 42	1.3658	0.195
Sabor 25 vs Sabor 31	2.3535	0.03	Sabor 27 vs Sabor 34	0.37532	0.695	Sabor 31 vs Sabor 32	1.2096	0.22
Sabor 25 vs Sabor 32	0.88737	0.379	Sabor 27 vs Sabor 42	0.55857	0.588	Sabor 31 vs Sabor 33	0.541	0.574
Sabor 25 vs Sabor 33	1.6125	0.114	Sabor 28 vs Sabor 29	1.7755	0.09	Sabor 31 vs Sabor 34	1.7	0.107
Sabor 25 vs Sabor 34	2.4474	0.017	Sabor 28 vs Sabor 30	2.0617	0.041	Sabor 31 vs Sabor 42	3.4012	0.001
Sabor 25 vs Sabor 42	3.0394	0.004	Sabor 28 vs Sabor 31	4.5662	0.001	Sabor 32 vs Sabor 33	0.63349	0.527
Sabor 26 vs Sabor 27	2.8573	0.013	Sabor 28 vs Sabor 32	1.109	0.257	Sabor 32 vs Sabor 34	1.6859	0.094
Sabor 26 vs Sabor 28	1.01	0.321	Sabor 28 vs Sabor 33	2.8369	0.005	Sabor 32 vs Sabor 42	2.4116	0.021
Sabor 26 vs Sabor 29	1.3497	0.204	Sabor 28 vs Sabor 34	5.1649	0.001	Sabor 33 vs Sabor 34	1.5608	0.121
Sabor 26 vs Sabor 30	0.91613	0.363	Sabor 28 vs Sabor 42	7.0111	0.001	Sabor 33 vs Sabor 42	2.6721	0.018
Sabor 26 vs Sabor 31	1.7037	0.086	Sabor 29 vs Sabor 30	0.023091	0.976	Sabor 34 vs Sabor 42	0.74502	0.481

Appendix 4: Results of PERMANOVA pairwise comparisons of *Unio delphinus* predation rate among sites.

	T	P		T	P		T	P
Sabor 1 vs Sabor 2	0.55709	1	Sabor 1 vs Sabor 25	2.5989	0.054	Sabor 2 vs Sabor 6	6.497	0.029
Sabor 1 vs Sabor 3	5.4505	0.049	Sabor 1 vs Sabor 26	3.1606	0.039	Sabor 2 vs Sabor 7	6.2764	0.045
Sabor 1 vs Sabor 4	1	1	Sabor 1 vs Sabor 27	1.0074	0.491	Sabor 2 vs Sabor 9	1	1
Sabor 1 vs Sabor 5	1.3352	0.368	Sabor 1 vs Sabor 28	2.4506	0.063	Sabor 2 vs Sabor 10	3.3902	0.061
Sabor 1 vs Sabor 6	7.5416	0.029	Sabor 1 vs Sabor 29	1.9781	0.091	Sabor 2 vs Sabor 11	2.596	0.139
Sabor 1 vs Sabor 7	6.6509	0.028	Sabor 1 vs Sabor 30	1.9229	0.137	Sabor 2 vs Sabor 14	4.9528	0.046
Sabor 1 vs Sabor 9	1	1	Sabor 1 vs Sabor 31	2.2817	0.085	Sabor 2 vs Sabor 15	7.2868	0.022
Sabor 1 vs Sabor 10	3.95	0.086	Sabor 1 vs Sabor 32	5.0925	0.024	Sabor 2 vs Sabor 16	4.0101	0.097
Sabor 1 vs Sabor 11	2.7146	0.146	Sabor 1 vs Sabor 33	3.953	0.021	Sabor 2 vs Sabor 17	5.4437	0.03
Sabor 1 vs Sabor 14	5.1063	0.052	Sabor 1 vs Sabor 34	0.412	0.853	Sabor 2 vs Sabor 18	5.2892	0.042
Sabor 1 vs Sabor 15	8.2243	0.026	Sabor 1 vs Sabor 35	0.412	0.853	Sabor 2 vs Sabor 19	0.50533	0.83
Sabor 1 vs Sabor 16	4.0101	0.073	Sabor 1 vs Sabor 36	1.2444	0.34	Sabor 2 vs Sabor 20	2.3256	0.152
Sabor 1 vs Sabor 17	6.4978	0.022	Sabor 1 vs Sabor 38	1.5275	0.327	Sabor 2 vs Sabor 21	0.62361	0.672
Sabor 1 vs Sabor 18	5.5368	0.065	Sabor 1 vs Sabor 39	1	1	Sabor 2 vs Sabor 22	1.3693	0.398
Sabor 1 vs Sabor 19	1.2629	0.271	Sabor 1 vs Sabor 42	0.93459	0.692	Sabor 2 vs Sabor 23	1.6739	0.224
Sabor 1 vs Sabor 20	0.78471	0.55	Sabor 1 vs Sabor 45	1	1	Sabor 2 vs Sabor 24	0.83333	0.513
Sabor 1 vs Sabor 21	1.1759	0.343	Sabor 1 vs Maças 6	2.667	0.07	Sabor 2 vs Sabor 25	0.75406	0.432
Sabor 1 vs Sabor 22	1.3693	0.329	Sabor 2 vs Sabor 3	4.7275	0.041	Sabor 2 vs Sabor 26	0.94038	0.338
Sabor 1 vs Sabor 23	1.1045	0.266	Sabor 2 vs Sabor 4	1	1	Sabor 2 vs Sabor 27	0.036415	1
Sabor 1 vs Sabor 24	7.6316E-09	1	Sabor 2 vs Sabor 5	1.2795	0.395	Sabor 2 vs Sabor 28	0.49459	0.68

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Appendix 4: Continued.

	T	P		T	P		T	P
Sabor 2 vs Sabor 29	0.45612	0.823	Sabor 3 vs Sabor 14	0.44053	1	Sabor 3 vs Sabor 34	5.0901	0.001
Sabor 2 vs Sabor 30	0.53252	0.629	Sabor 3 vs Sabor 15	0.2054	0.877	Sabor 3 vs Sabor 35	5.0919	0.006
Sabor 2 vs Sabor 31	0.60432	0.727	Sabor 3 vs Sabor 16	0.71597	1	Sabor 3 vs Sabor 36	0.29957	1
Sabor 2 vs Sabor 32	2.4642	0.078	Sabor 3 vs Sabor 17	0.19822	1	Sabor 3 vs Sabor 38	0.2117	1
Sabor 2 vs Sabor 33	1.3109	0.111	Sabor 3 vs Sabor 18	0.16769	1	Sabor 3 vs Sabor 39	4.1005	0.015
Sabor 2 vs Sabor 34	0.4287	0.789	Sabor 3 vs Sabor 19	3.6998	0.02	Sabor 3 vs Sabor 42	3.5558	0.015
Sabor 2 vs Sabor 35	0.80749	0.539	Sabor 3 vs Sabor 20	17.874	0.001	Sabor 3 vs Sabor 45	0.2117	1
Sabor 2 vs Sabor 36	1.5275	0.337	Sabor 3 vs Sabor 21	4.1005	0.015	Sabor 3 vs Maças 6	2.4651	0.007
Sabor 2 vs Sabor 38	1	1	Sabor 3 vs Sabor 22	0.27342	1	Sabor 4 vs Sabor 5	0.75	1
Sabor 2 vs Sabor 39	0.62361	0.645	Sabor 3 vs Sabor 23	10.843	0.001	Sabor 4 vs Sabor 6	0.50036	1
Sabor 2 vs Sabor 42	0.076923	1	Sabor 3 vs Sabor 24	7.5385	0.001	Sabor 4 vs Sabor 7	0.17869	1
Sabor 2 vs Sabor 45	1	1	Sabor 3 vs Sabor 25	3.4928	0.001	Sabor 4 vs Sabor 9	Denominator is 0	
Sabor 2 vs Maças 6	1.0346	0.181	Sabor 3 vs Sabor 26	4.6209	0.001	Sabor 4 vs Sabor 10	0.28516	1
Sabor 3 vs Sabor 4	0.2117	1	Sabor 3 vs Sabor 27	5.4544	0.001	Sabor 4 vs Sabor 11	0.41079	1
Sabor 3 vs Sabor 5	0.44129	0.136	Sabor 3 vs Sabor 28	5.8881	0.001	Sabor 4 vs Sabor 14	0.2316	1
Sabor 3 vs Sabor 6	0.66666	0.57	Sabor 3 vs Sabor 29	5.5795	0.001	Sabor 4 vs Sabor 15	0.2834	1
Sabor 3 vs Sabor 7	0.58488	0.735	Sabor 3 vs Sabor 30	2.7089	0.001	Sabor 4 vs Sabor 16	Denominator is 0	
Sabor 3 vs Sabor 9	0.2117	1	Sabor 3 vs Sabor 31	4.2972	0.001	Sabor 4 vs Sabor 17	0.27918	1
Sabor 3 vs Sabor 10	0.43561	1	Sabor 3 vs Sabor 32	1.7805	0.064	Sabor 4 vs Sabor 18	0.35294	1
Sabor 3 vs Sabor 11	0.26017	1	Sabor 3 vs Sabor 33	3.2525	0.003	Sabor 4 vs Sabor 19	0.84416	0.746

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Appendix 4: Continued.

	T	P		T	P		T	P
Sabor 4 vs Sabor 20	3.6015	0.018	Sabor 4 vs Sabor 45	Denominator is 0		Sabor 5 vs Sabor 27	1.435	0.174
Sabor 4 vs Sabor 21	1.0801	0.522	Sabor 4 vs Maças 6	0.56951	0.824	Sabor 5 vs Sabor 28	1.5543	0.098
Sabor 4 vs Sabor 22	Denominator is 0		Sabor 5 vs Sabor 6	0.36543	1	Sabor 5 vs Sabor 29	1.4829	0.134
Sabor 4 vs Sabor 23	2.0101	0.167	Sabor 5 vs Sabor 7	1.1493	0.087	Sabor 5 vs Sabor 30	0.68965	0.379
Sabor 4 vs Sabor 24	1.491	0.12	Sabor 5 vs Sabor 9	0.75	1	Sabor 5 vs Sabor 31	1.1173	0.299
Sabor 4 vs Sabor 25	0.78522	0.402	Sabor 5 vs Sabor 10	0.15959	1	Sabor 5 vs Sabor 32	0.35307	0.783
Sabor 4 vs Sabor 26	1.0446	0.325	Sabor 5 vs Sabor 11	0.33883	1	Sabor 5 vs Sabor 33	0.80092	0.408
Sabor 4 vs Sabor 27	1.1697	0.196	Sabor 5 vs Sabor 14	1.2271	0.175	Sabor 5 vs Sabor 34	1.3269	0.188
Sabor 4 vs Sabor 28	1.3146	0.156	Sabor 5 vs Sabor 15	0.80563	0.181	Sabor 5 vs Sabor 35	2.566	0.034
Sabor 4 vs Sabor 29	1.2536	0.191	Sabor 5 vs Sabor 16	2.8397	0.132	Sabor 5 vs Sabor 36	1.1078	0.469
Sabor 4 vs Sabor 30	0.60305	0.479	Sabor 5 vs Sabor 17	0.39566	1	Sabor 5 vs Sabor 38	0.75	1
Sabor 4 vs Sabor 31	0.96139	0.439	Sabor 5 vs Sabor 18	0.96101	0.253	Sabor 5 vs Sabor 39	1.2437	0.449
Sabor 4 vs Sabor 32	0.46172	0.696	Sabor 5 vs Sabor 19	0.986	0.489	Sabor 5 vs Sabor 42	0.9094	0.758
Sabor 4 vs Sabor 33	0.74564	0.497	Sabor 5 vs Sabor 20	4.7223	0.004	Sabor 5 vs Sabor 45	0.75	1
Sabor 4 vs Sabor 34	1.0581	0.384	Sabor 5 vs Sabor 21	1.2437	0.455	Sabor 5 vs Maças 6	0.6059	0.712
Sabor 4 vs Sabor 35	2.3932	0.109	Sabor 5 vs Sabor 22	1	1	Sabor 6 vs Sabor 7	1.7803	0.101
Sabor 4 vs Sabor 36	Denominator is 0		Sabor 5 vs Sabor 23	2.6837	0.01	Sabor 6 vs Sabor 9	0.50036	1
Sabor 4 vs Sabor 38	Denominator is 0		Sabor 5 vs Sabor 24	1.9251	0.088	Sabor 6 vs Sabor 10	0.11468	1
Sabor 4 vs Sabor 39	1.0801	0.537	Sabor 5 vs Sabor 25	0.89236	0.354	Sabor 6 vs Sabor 11	0.081482	1
Sabor 4 vs Sabor 42	0.74647	0.8	Sabor 5 vs Sabor 26	1.1908	0.228	Sabor 6 vs Sabor 14	1.4204	0.222

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Appendix 4: Continued.

	T	P		T	P		T	P
Sabor 6 vs Sabor 15	1.3249	0.23	Sabor 6 vs Sabor 35	6.9015	0.001	Sabor 7 vs Sabor 24	9.1883	0.001
Sabor 6 vs Sabor 16	1.687	0.133	Sabor 6 vs Sabor 36	0.70775	0.824	Sabor 7 vs Sabor 25	4.3001	0.001
Sabor 6 vs Sabor 17	0.46884	0.72	Sabor 6 vs Sabor 38	0.50036	1	Sabor 7 vs Sabor 26	5.7023	0.001
Sabor 6 vs Sabor 18	1.0807	0.343	Sabor 6 vs Sabor 39	5.55	0.003	Sabor 7 vs Sabor 27	6.78	0.001
Sabor 6 vs Sabor 19	5.0107	0.006	Sabor 6 vs Sabor 42	4.8593	0.007	Sabor 7 vs Sabor 28	7.2368	0.001
Sabor 6 vs Sabor 20	24.81	0.001	Sabor 6 vs Sabor 45	0.50036	1	Sabor 7 vs Sabor 29	6.9532	0.001
Sabor 6 vs Sabor 21	5.55	0.004	Sabor 6 vs Maças 6	3.2541	0.005	Sabor 7 vs Sabor 30	3.3304	0.001
Sabor 6 vs Sabor 22	0.64604	0.84	Sabor 7 vs Sabor 9	0.17869	1	Sabor 7 vs Sabor 31	5.3011	0.001
Sabor 6 vs Sabor 23	15.055	0.001	Sabor 7 vs Sabor 10	1.0623	0.281	Sabor 7 vs Sabor 32	2.3833	0.003
Sabor 6 vs Sabor 24	10.426	0.001	Sabor 7 vs Sabor 11	0.94521	0.293	Sabor 7 vs Sabor 33	4.0336	0.001
Sabor 6 vs Sabor 25	4.6833	0.001	Sabor 7 vs Sabor 14	0.081418	1	Sabor 7 vs Sabor 34	6.1524	0.001
Sabor 6 vs Sabor 26	6.2	0.001	Sabor 7 vs Sabor 15	0.62942	0.569	Sabor 7 vs Sabor 35	9.0428	0.001
Sabor 6 vs Sabor 27	7.4744	0.001	Sabor 7 vs Sabor 16	0.60311	1	Sabor 7 vs Sabor 36	0.25278	1
Sabor 6 vs Sabor 28	7.9817	0.001	Sabor 7 vs Sabor 17	0.9117	0.504	Sabor 7 vs Sabor 38	0.17869	1
Sabor 6 vs Sabor 29	7.5778	0.001	Sabor 7 vs Sabor 18	0.59086	0.651	Sabor 7 vs Sabor 39	5.8803	0.003
Sabor 6 vs Sabor 30	3.6411	0.001	Sabor 7 vs Sabor 19	4.8385	0.005	Sabor 7 vs Sabor 42	4.3788	0.011
Sabor 6 vs Sabor 31	5.8019	0.001	Sabor 7 vs Sabor 20	22.288	0.001	Sabor 7 vs Sabor 45	0.17869	1
Sabor 6 vs Sabor 32	2.1616	0.016	Sabor 7 vs Sabor 21	5.8803	0.005	Sabor 7 vs Maças 6	3.0982	0.002
Sabor 6 vs Sabor 33	4.2916	0.001	Sabor 7 vs Sabor 22	0.23074	1	Sabor 9 vs Sabor 10	0.28516	1
Sabor 6 vs Sabor 34	7.0094	0.001	Sabor 7 vs Sabor 23	13.036	0.001	Sabor 9 vs Sabor 11	0.41079	1

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Appendix 4: Continued.

	T	P		T	P		T	P
Sabor 9 vs Sabor 14	0.2316	1	Sabor 9 vs Sabor 34	1.0581	0.405	Sabor 10 vs Sabor 25	2.4727	0.018
Sabor 9 vs Sabor 15	0.2834	1	Sabor 9 vs Sabor 35	2.3932	0.112	Sabor 10 vs Sabor 26	3.2765	0.001
Sabor 9 vs Sabor 16	Denominator is 0		Sabor 9 vs Sabor 36	Denominator is 0		Sabor 10 vs Sabor 27	3.9216	0.001
Sabor 9 vs Sabor 17	0.27918	1	Sabor 9 vs Sabor 38	Denominator is 0		Sabor 10 vs Sabor 28	4.2191	0.001
Sabor 9 vs Sabor 18	0.35294	1	Sabor 9 vs Sabor 39	1.0801	0.505	Sabor 10 vs Sabor 29	3.9884	0.001
Sabor 9 vs Sabor 19	0.84416	0.756	Sabor 9 vs Sabor 42	0.74647	0.821	Sabor 10 vs Sabor 30	1.9184	0.015
Sabor 9 vs Sabor 20	3.6015	0.027	Sabor 9 vs Sabor 45	Denominator is 0		Sabor 10 vs Sabor 31	3.0595	0.004
Sabor 9 vs Sabor 21	1.0801	0.466	Sabor 9 vs Maças 6	0.56951	0.785	Sabor 10 vs Sabor 32	1.1144	0.291
Sabor 9 vs Sabor 22	Denominator is 0		Sabor 10 vs Sabor 11	0.097295	1	Sabor 10 vs Sabor 33	2.2654	0.018
Sabor 9 vs Sabor 23	2.0101	0.162	Sabor 10 vs Sabor 14	0.8491	0.777	Sabor 10 vs Sabor 34	3.689	0.001
Sabor 9 vs Sabor 24	1.491	0.143	Sabor 10 vs Sabor 15	0.83253	0.52	Sabor 10 vs Sabor 35	3.5758	0.009
Sabor 9 vs Sabor 25	0.78522	0.407	Sabor 10 vs Sabor 16	0.97143	1	Sabor 10 vs Sabor 36	0.40406	1
Sabor 9 vs Sabor 26	1.0446	0.333	Sabor 10 vs Sabor 17	0.34102	0.962	Sabor 10 vs Sabor 38	0.28516	1
Sabor 9 vs Sabor 27	1.1697	0.221	Sabor 10 vs Sabor 18	0.67567	0.721	Sabor 10 vs Sabor 39	2.8863	0.05
Sabor 9 vs Sabor 28	1.3146	0.169	Sabor 10 vs Sabor 19	2.6149	0.065	Sabor 10 vs Sabor 42	2.5476	0.055
Sabor 9 vs Sabor 29	1.2536	0.186	Sabor 10 vs Sabor 20	13.012	0.001	Sabor 10 vs Sabor 45	0.28516	1
Sabor 9 vs Sabor 30	0.60305	0.447	Sabor 10 vs Sabor 21	2.8863	0.04	Sabor 10 vs Maças 6	1.7068	0.092
Sabor 9 vs Sabor 31	0.96139	0.423	Sabor 10 vs Sabor 22	0.36863	1	Sabor 11 vs Sabor 14	0.88363	0.404
Sabor 9 vs Sabor 32	0.46172	0.71	Sabor 10 vs Sabor 23	7.8991	0.001	Sabor 11 vs Sabor 15	0.56356	0.807
Sabor 9 vs Sabor 33	0.74564	0.487	Sabor 10 vs Sabor 24	5.4702	0.001	Sabor 11 vs Sabor 16	1.4289	0.311

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Appendix 4: Continued.

	T	P		T	P		T	P
Sabor 11 vs Sabor 17	0.15226	1	Sabor 11 vs Sabor 38	0.41079	1	Sabor 14 vs Sabor 30	2.5537	0.001
Sabor 11 vs Sabor 18	0.59712	0.791	Sabor 11 vs Sabor 39	2.4543	0.08	Sabor 14 vs Sabor 31	4.0751	0.001
Sabor 11 vs Sabor 19	1.9595	0.094	Sabor 11 vs Sabor 42	1.7778	0.128	Sabor 14 vs Sabor 32	1.8261	0.059
Sabor 11 vs Sabor 20	9.2575	0.002	Sabor 11 vs Sabor 45	0.41079	1	Sabor 14 vs Sabor 33	3.0944	0.005
Sabor 11 vs Sabor 21	2.4543	0.061	Sabor 11 vs Maças 6	1.217	0.253	Sabor 14 vs Sabor 34	4.7139	0.001
Sabor 11 vs Sabor 22	0.533	1	Sabor 14 vs Sabor 15	0.45658	0.666	Sabor 14 vs Sabor 35	8.5164	0.001
Sabor 11 vs Sabor 23	5.3493	0.002	Sabor 14 vs Sabor 16	0.78453	1	Sabor 14 vs Sabor 36	0.32782	1
Sabor 11 vs Sabor 24	3.775	0.001	Sabor 14 vs Sabor 17	0.69782	0.749	Sabor 14 vs Sabor 38	0.2316	1
Sabor 11 vs Sabor 25	1.738	0.067	Sabor 14 vs Sabor 18	0.47972	0.746	Sabor 14 vs Sabor 39	4.7716	0.006
Sabor 11 vs Sabor 26	2.3131	0.023	Sabor 14 vs Sabor 19	3.7799	0.014	Sabor 14 vs Sabor 42	3.3632	0.027
Sabor 11 vs Sabor 27	2.7783	0.003	Sabor 14 vs Sabor 20	17.263	0.001	Sabor 14 vs Sabor 45	0.2316	1
Sabor 11 vs Sabor 28	2.9744	0.004	Sabor 14 vs Sabor 21	4.7716	0.011	Sabor 14 vs Maças 6	2.3786	0.007
Sabor 11 vs Sabor 29	2.8483	0.008	Sabor 14 vs Sabor 22	0.29918	1	Sabor 15 vs Sabor 16	0.95518	0.545
Sabor 11 vs Sabor 30	1.3434	0.191	Sabor 14 vs Sabor 23	9.9768	0.001	Sabor 15 vs Sabor 17	0.53518	0.702
Sabor 11 vs Sabor 31	2.1578	0.028	Sabor 14 vs Sabor 24	7.0565	0.001	Sabor 15 vs Sabor 18	0.001996	1
Sabor 11 vs Sabor 32	0.83255	0.443	Sabor 14 vs Sabor 25	3.3012	0.001	Sabor 15 vs Sabor 19	5.6843	0.004
Sabor 11 vs Sabor 33	1.5983	0.101	Sabor 14 vs Sabor 26	4.3839	0.001	Sabor 15 vs Sabor 20	27.108	0.001
Sabor 11 vs Sabor 34	2.5357	0.004	Sabor 14 vs Sabor 27	5.2294	0.001	Sabor 15 vs Sabor 21	6.4351	0.003
Sabor 11 vs Sabor 35	4.3828	0.002	Sabor 14 vs Sabor 28	5.5685	0.001	Sabor 15 vs Sabor 22	0.3659	1
Sabor 11 vs Sabor 36	0.58514	1	Sabor 14 vs Sabor 29	5.3648	0.001	Sabor 15 vs Sabor 23	16.293	0.001

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Appendix 4: Continued.

	T	P		T	P		T	P
Sabor 15 vs Sabor 24	11.367	0.001	Sabor 16 vs Sabor 19	3.0661	0.027	Sabor 16 vs Sabor 42	2.6691	0.045
Sabor 15 vs Sabor 25	5.2669	0.001	Sabor 16 vs Sabor 20	13.636	0.001	Sabor 16 vs Sabor 45	Denominator is 0	
Sabor 15 vs Sabor 26	6.9695	0.001	Sabor 16 vs Sabor 21	4.0187	0.021	Sabor 16 vs Maças 6	1.9341	0.057
Sabor 15 vs Sabor 27	8.2654	0.001	Sabor 16 vs Sabor 22	Denominator is 0		Sabor 17 vs Sabor 18	0.42109	0.801
Sabor 15 vs Sabor 28	8.8743	0.001	Sabor 16 vs Sabor 23	7.7932	0.001	Sabor 17 vs Sabor 19	4.2786	0.01
Sabor 15 vs Sabor 29	8.4519	0.001	Sabor 16 vs Sabor 24	5.5474	0.001	Sabor 17 vs Sabor 20	21.101	0.001
Sabor 15 vs Sabor 30	4.0872	0.001	Sabor 16 vs Sabor 25	2.6545	0.013	Sabor 17 vs Sabor 21	4.5917	0.009
Sabor 15 vs Sabor 31	6.4879	0.001	Sabor 16 vs Sabor 26	3.5273	0.002	Sabor 17 vs Sabor 22	0.3605	1
Sabor 15 vs Sabor 32	2.7572	0.001	Sabor 16 vs Sabor 27	4.1576	0.001	Sabor 17 vs Sabor 23	13.013	0.001
Sabor 15 vs Sabor 33	4.90097	0.001	Sabor 16 vs Sabor 28	4.453	0.001	Sabor 17 vs Sabor 24	8.9875	0.001
Sabor 15 vs Sabor 34	7.6526	0.001	Sabor 16 vs Sabor 29	4.2961	0.001	Sabor 17 vs Sabor 25	4.12397	0.001
Sabor 15 vs Sabor 35	8.3026	0.001	Sabor 16 vs Sabor 30	2.0487	0.005	Sabor 17 vs Sabor 26	5.456	0.001
Sabor 15 vs Sabor 36	0.40084	1	Sabor 16 vs Sabor 31	3.266	0.002	Sabor 17 vs Sabor 27	6.5533	0.001
Sabor 15 vs Sabor 38	0.2834	1	Sabor 16 vs Sabor 32	1.5572	0.092	Sabor 17 vs Sabor 28	6.9697	0.001
Sabor 15 vs Sabor 39	6.4351	0.002	Sabor 16 vs Sabor 33	2.5118	0.015	Sabor 17 vs Sabor 29	6.5726	0.001
Sabor 15 vs Sabor 42	5.3768	0.008	Sabor 16 vs Sabor 34	3.7123	0.001	Sabor 17 vs Sabor 30	3.2079	0.001
Sabor 15 vs Sabor 45	0.2834	1	Sabor 16 vs Sabor 35	8.904	0.001	Sabor 17 vs Sabor 31	5.0806	0.001
Sabor 15 vs Maças 6	3.7445	0.001	Sabor 16 vs Sabor 36	Denominator is 0		Sabor 17 vs Sabor 32	2.015	0.035
Sabor 16 vs Sabor 17	0.94222	0.655	Sabor 16 vs Sabor 38	Denominator is 0		Sabor 17 vs Sabor 33	3.8278	0.001
Sabor 16 vs Sabor 18	1.1938	0.476	Sabor 16 vs Sabor 39	4.0187	0.024	Sabor 17 vs Sabor 34	6.0864	0.001

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Appendix 4: Continued.

	T	P		T	P		T	P
Sabor 17 vs Sabor 35	5.4136	0.002	Sabor 18 vs Sabor 32	1.8715	0.05	Sabor 19 vs Sabor 30	0.070776	0.959
Sabor 17 vs Sabor 36	0.39495	1	Sabor 18 vs Sabor 33	3.2892	0.003	Sabor 19 vs Sabor 31	0.090475	0.981
Sabor 17 vs Sabor 38	0.27918	1	Sabor 18 vs Sabor 34	5.1071	0.001	Sabor 19 vs Sabor 32	1.8969	0.082
Sabor 17 vs Sabor 39	4.5917	0.013	Sabor 18 vs Sabor 35	8.3479	0.001	Sabor 19 vs Sabor 33	0.67644	0.519
Sabor 17 vs Sabor 42	4.2178	0.011	Sabor 18 vs Sabor 36	0.49945	1	Sabor 19 vs Sabor 34	1.1289	0.309
Sabor 17 vs Sabor 45	0.27918	1	Sabor 18 vs Sabor 38	0.35294	1	Sabor 19 vs Sabor 35	0.23773	0.892
Sabor 17 vs Maças 6	2.8896	0.004	Sabor 18 vs Sabor 39	4.9983	0.009	Sabor 19 vs Sabor 36	1.2219	0.469
Sabor 18 vs Sabor 19	4.0257	0.008	Sabor 18 vs Sabor 42	3.6248	0.014	Sabor 19 vs Sabor 38	0.84416	0.768
Sabor 18 vs Sabor 20	18.681	0.001	Sabor 18 vs Sabor 45	0.35294	1	Sabor 19 vs Sabor 39	0.11711	1
Sabor 18 vs Sabor 21	4.9983	0.012	Sabor 18 vs Maças 6	2.5245	0.008	Sabor 19 vs Sabor 42	0.42138	0.847
Sabor 18 vs Sabor 22	0.45584	1	Sabor 19 vs Sabor 20	4.318	0.005	Sabor 19 vs Sabor 45	0.84416	0.77
Sabor 18 vs Sabor 23	10.853	0.001	Sabor 19 vs Sabor 21	0.11711	1	Sabor 19 vs Maças 6	0.55474	0.775
Sabor 18 vs Sabor 24	7.6504	0.001	Sabor 19 vs Sabor 22	1.1083	0.477	Sabor 20 vs Sabor 21	4.2851	0.002
Sabor 18 vs Sabor 25	3.5322	0.001	Sabor 19 vs Sabor 23	3.13	0.008	Sabor 20 vs Sabor 22	4.802	0.007
Sabor 18 vs Sabor 26	4.6902	0.001	Sabor 19 vs Sabor 24	1.7923	0.109	Sabor 20 vs Sabor 23	1.0054	0.346
Sabor 18 vs Sabor 27	5.6342	0.001	Sabor 19 vs Sabor 25	0.14872	0.915	Sabor 20 vs Sabor 24	1.1392	0.308
Sabor 18 vs Sabor 28	5.9829	0.001	Sabor 19 vs Sabor 26	0.14947	0.91	Sabor 20 vs Sabor 25	6.8752	0.002
Sabor 18 vs Sabor 29	5.7579	0.001	Sabor 19 vs Sabor 27	0.75612	0.493	Sabor 20 vs Sabor 26	8.6244	0.001
Sabor 18 vs Sabor 30	2.7355	0.001	Sabor 19 vs Sabor 28	0.42017	0.696	Sabor 20 vs Sabor 27	3.5836	0.004
Sabor 18 vs Sabor 31	4.3701	0.001	Sabor 19 vs Sabor 29	0.36893	0.733	Sabor 20 vs Sabor 28	7.1958	0.001

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	T	P		T	P		T	P
Sabor 20 vs Sabor 29	6.2351	0.001	Sabor 21 vs Sabor 29	0.49819	0.67	Sabor 22 vs Sabor 30	0.77928	0.271
Sabor 20 vs Sabor 30	5.0738	0.001	Sabor 21 vs Sabor 30	0.037359	1	Sabor 22 vs Sabor 31	1.2423	0.261
Sabor 20 vs Sabor 31	6.4919	0.001	Sabor 21 vs Sabor 31	0.22015	0.883	Sabor 22 vs Sabor 32	0.591617	0.548
Sabor 20 vs Sabor 32	13.63	0.001	Sabor 21 vs Sabor 32	1.4985	0.109	Sabor 22 vs Sabor 33	0.96267	0.358
Sabor 20 vs Sabor 33	9.8049	0.001	Sabor 21 vs Sabor 33	0.41546	0.699	Sabor 22 vs Sabor 34	1.3755	0.167
Sabor 20 vs Sabor 34	1.8541	0.077	Sabor 21 vs Sabor 34	1.0543	0.345	Sabor 22 vs Sabor 35	3.1688	0.013
Sabor 20 vs Sabor 35	4.6747	0.003	Sabor 21 vs Sabor 35	0.14649	1	Sabor 22 vs Sabor 36	Denominator is 0	
Sabor 20 vs Sabor 36	5.3197	0.003	Sabor 21 vs Sabor 36	1.5811	0.453	Sabor 22 vs Sabor 38	Denominator is 0	
Sabor 20 vs Sabor 38	3.6015	0.015	Sabor 21 vs Sabor 38	1.0801	0.508	Sabor 22 vs Sabor 39	1.4302	0.457
Sabor 20 vs Sabor 39	4.2851	0.003	Sabor 21 vs Sabor 39	Denominator is 0		Sabor 22 vs Sabor 42	0.9753	0.741
Sabor 20 vs Sabor 42	3.0067	0.013	Sabor 21 vs Sabor 42	0.45883	0.869	Sabor 22 vs Sabor 45	Denominator is 0	
Sabor 20 vs Sabor 45	3.6015	0.019	Sabor 21 vs Sabor 45	1.0801	0.506	Sabor 22 vs Maças 6	0.7359	0.669
Sabor 20 vs Maças 6	7.2021	0.001	Sabor 21 vs Maças 6	0.36448	0.704	Sabor 23 vs Sabor 24	1.6422	0.143
Sabor 21 vs Sabor 22	1.4302	0.462	Sabor 22 vs Sabor 23	2.7079	0.012	Sabor 23 vs Sabor 25	7.8241	0.001
Sabor 21 vs Sabor 23	2.753	0.016	Sabor 22 vs Sabor 24	1.9742	0.075	Sabor 23 vs Sabor 26	9.173	0.001
Sabor 21 vs Sabor 24	1.6831	0.149	Sabor 22 vs Sabor 25	1.0141	0.224	Sabor 23 vs Sabor 27	3.2747	0.015
Sabor 21 vs Sabor 25	0.00311	1	Sabor 22 vs Sabor 26	1.3489	0.135	Sabor 23 vs Sabor 28	7.4453	0.002
Sabor 21 vs Sabor 26	0.040056	1	Sabor 22 vs Sabor 27	1.5257	0.125	Sabor 23 vs Sabor 29	5.5752	0.002
Sabor 21 vs Sabor 27	0.81528	0.447	Sabor 22 vs Sabor 28	1.6981	0.073	Sabor 23 vs Sabor 30	5.9123	0.002
Sabor 21 vs Sabor 28	0.5417	0.67	Sabor 22 vs Sabor 29	1.622	0.094	Sabor 23 vs Sabor 31	6.6516	0.001

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Appendix 4: Continued.

	T	P		T	P		T	P
Sabor 23 vs Sabor 32	12.33	0.001	Sabor 24 vs Sabor 35	1.7786	0.155	Sabor 25 vs Sabor 42	0.94978	0.399
Sabor 23 vs Sabor 33	12	0.001	Sabor 24 vs Sabor 36	2.1826	0.016	Sabor 25 vs Sabor 45	0.78522	0.431
Sabor 23 vs Sabor 34	2.6213	0.011	Sabor 24 vs Sabor 38	1.491	0.127	Sabor 25 vs Maçãs 6	0.77872	0.454
Sabor 23 vs Sabor 35	2.8076	0.012	Sabor 24 vs Sabor 39	1.6831	0.177	Sabor 26 vs Sabor 27	1.7352	0.069
Sabor 23 vs Sabor 36	3.0084	0.009	Sabor 24 vs Sabor 42	1.3276	0.24	Sabor 26 vs Sabor 28	1.3079	0.188
Sabor 23 vs Sabor 38	2.0101	0.164	Sabor 24 vs Sabor 45	1.491	0.118	Sabor 26 vs Sabor 29	1.0134	0.358
Sabor 23 vs Sabor 39	2.753	0.017	Sabor 24 vs Maçãs 6	3.736	0.005	Sabor 26 vs Sabor 30	0.043299	0.984
Sabor 23 vs Sabor 42	3.0052	0.021	Sabor 25 vs Sabor 26	0.081916	0.95	Sabor 26 vs Sabor 31	0.49236	0.628
Sabor 23 vs Sabor 45	2.0101	0.179	Sabor 25 vs Sabor 27	1.4273	0.148	Sabor 26 vs Sabor 32	3.686	0.001
Sabor 23 vs Maçãs 6	7.0704	0.003	Sabor 25 vs Sabor 28	1.1489	0.252	Sabor 26 vs Sabor 33	1.4424	0.172
Sabor 24 vs Sabor 25	3.6494	0.004	Sabor 25 vs Sabor 29	0.086962	0.405	Sabor 26 vs Sabor 34	3.251	0.005
Sabor 24 vs Sabor 26	4.4294	0.001	Sabor 25 vs Sabor 30	0.089417	0.944	Sabor 26 vs Sabor 35	0.18396	0.897
Sabor 24 vs Sabor 27	1.4235	0.201	Sabor 25 vs Sabor 31	0.46767	0.665	Sabor 26 vs Sabor 36	1.4778	0.105
Sabor 24 vs Sabor 28	3.4311	0.005	Sabor 25 vs Sabor 32	2.9015	0.002	Sabor 26 vs Sabor 38	1.0446	0.352
Sabor 24 vs Sabor 29	2.7653	0.021	Sabor 25 vs Sabor 33	1.1413	0.245	Sabor 26 vs Sabor 39	0.040056	1
Sabor 24 vs Sabor 30	2.704	0.025	Sabor 25 vs Sabor 34	2.7917	0.012	Sabor 26 vs Sabor 42	1.1253	0.286
Sabor 24 vs Sabor 31	3.1968	0.008	Sabor 25 vs Sabor 35	0.11362	0.967	Sabor 26 vs Sabor 45	1.0446	0.33
Sabor 24 vs Sabor 32	7.1118	0.001	Sabor 25 vs Sabor 36	1.1111	0.19	Sabor 26 vs Maçãs 6	1.0207	0.342
Sabor 24 vs Sabor 33	5.5609	0.001	Sabor 25 vs Sabor 38	0.78522	0.425	Sabor 27 vs Sabor 28	0.93653	0.375
Sabor 24 vs Sabor 34	0.58444	0.583	Sabor 25 vs Sabor 39	0.00311	1	Sabor 27 vs Sabor 29	0.7998	0.452

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	T	P		T	P		T	P
Sabor 27 vs Sabor 30	1.0207	0.333	Sabor 28 vs Sabor 38	1.3146	0.172	Sabor 30 vs Sabor 34	2.0816	0.027
Sabor 27 vs Sabor 31	1.111	0.278	Sabor 28 vs Sabor 39	0.5417	0.641	Sabor 30 vs Sabor 35	0.11925	0.944
Sabor 27 vs Sabor 32	4.1564	0.002	Sabor 28 vs Sabor 42	0.46728	0.687	Sabor 30 vs Sabor 36	0.85403	0.236
Sabor 27 vs Sabor 33	2.5125	0.024	Sabor 28 vs Sabor 45	1.3146	0.173	Sabor 30 vs Sabor 38	0.60305	0.476
Sabor 27 vs Sabor 34	0.73821	0.479	Sabor 28 vs Maçãs 6	1.8932	0.069	Sabor 30 vs Sabor 39	0.037359	1
Sabor 27 vs Sabor 35	0.93917	0.438	Sabor 29 vs Sabor 30	0.58269	0.645	Sabor 30 vs Sabor 42	0.67266	0.559
Sabor 27 vs Sabor 36	1.6783	0.073	Sabor 29 vs Sabor 31	0.43858	0.669	Sabor 30 vs Sabor 45	0.60305	0.442
Sabor 27 vs Sabor 38	1.1697	0.191	Sabor 29 vs Sabor 32	4.0576	0.001	Sabor 30 vs Maçãs 6	0.66693	0.575
Sabor 27 vs Sabor 39	0.81528	0.473	Sabor 29 vs Sabor 33	2.0372	0.042	Sabor 31 vs Sabor 32	3.4709	0.001
Sabor 27 vs Sabor 42	0.17695	0.888	Sabor 29 vs Sabor 34	1.725	0.084	Sabor 31 vs Sabor 33	1.6453	0.091
Sabor 27 vs Sabor 45	1.1697	0.202	Sabor 29 vs Sabor 35	0.64653	0.589	Sabor 31 vs Sabor 34	2.2277	0.027
Sabor 27 vs Maçãs 6	1.8282	0.069	Sabor 29 vs Sabor 36	1.7786	0.069	Sabor 31 vs Sabor 35	0.33864	0.782
Sabor 28 vs Sabor 29	0.015471	1	Sabor 29 vs Sabor 38	1.2536	0.221	Sabor 31 vs Sabor 36	1.3615	0.183
Sabor 28 vs Sabor 30	0.77825	0.52	Sabor 29 vs Sabor 39	0.49819	0.698	Sabor 31 vs Sabor 38	0.96139	0.411
Sabor 28 vs Sabor 31	0.56528	0.571	Sabor 29 vs Sabor 42	0.38579	0.738	Sabor 31 vs Sabor 39	0.22015	0.892
Sabor 28 vs Sabor 32	4.9509	0.001	Sabor 29 vs Sabor 45	1.2536	0.197	Sabor 31 vs Sabor 42	0.66206	0.534
Sabor 28 vs Sabor 33	10748	0.008	Sabor 29 vs Maçãs 6	1.5076	0.131	Sabor 31 vs Sabor 45	0.96139	0.422
Sabor 28 vs Sabor 34	2.2753	0.025	Sabor 30 vs Sabor 31	0.2747	0.824	Sabor 31 vs Maçãs 6	1.153	0.263
Sabor 28 vs Sabor 35	0.6853	0.52	Sabor 30 vs Sabor 32	2.3191	0.017	Sabor 32 vs Sabor 33	2.4181	0.012
Sabor 28 vs Sabor 36	1.8607	0.068	Sabor 30 vs Sabor 33	1.0079	0.319	Sabor 32 vs Sabor 34	5.4156	0.001

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Appendix 4: Continued.

	T	P		T	P
Sabor 32 vs Sabor 35	1.3316	0.106	Sabor 35 vs Sabor 38	2.3932	0.097
Sabor 32 vs Sabor 36	0.65311	0.484	Sabor 35 vs Sabor 39	0.14649	1
Sabor 32 vs Sabor 38	0.46172	0.706	Sabor 35 vs Sabor 42	0.53319	0.739
Sabor 32 vs Sabor 39	1.4985	0.114	Sabor 35 vs Sabor 45	2.3932	0.106
Sabor 32 vs Sabor 42	2.9884	0.017	Sabor 35 vs Maçãs 6	0.25667	0.905
Sabor 32 vs Sabor 45	0.46172	0.756	Sabor 36 vs Sabor 38	Denominator is 0	
Sabor 32 vs Maçãs 6	1.5273	0.139	Sabor 36 vs Sabor 39	1.5811	0.459
Sabor 33 vs Sabor 34	4.6622	0.003	Sabor 36 vs Sabor 42	1.0735	0.494
Sabor 33 vs Sabor 35	0.27208	0.803	Sabor 36 vs Sabor 45	Denominator is 0	
Sabor 33 vs Sabor 36	1.0546	0.257	Sabor 36 vs Maçãs 6	0.80648	0.626
Sabor 33 vs Sabor 38	0.74564	0.478	Sabor 38 vs Sabor 39	1.0801	0.495
Sabor 33 vs Sabor 39	0.41546	0.697	Sabor 38 vs Sabor 42	0.74647	0.815
Sabor 33 vs Sabor 42	1.8307	0.058	Sabor 38 vs Sabor 45	Denominator is 0	
Sabor 33 vs Sabor 45	0.74564	0.49	Sabor 38 vs Maçãs 6	0.56951	0.799
Sabor 33 vs Maçãs 6	0.1153	0.935	Sabor 39 vs Sabor 42	0.45883	0.886
Sabor 34 vs Sabor 35	1.1117	0.281	Sabor 39 vs Sabor 45	1.0801	0.488
Sabor 34 vs Sabor 36	1.5113	0.156	Sabor 39 vs Maçãs 6	0.36448	0.743
Sabor 34 vs Sabor 38	1.0581	0.396	Sabor 42 vs Sabor 45	0.74647	0.807
Sabor 34 vs Sabor 39	1.0543	0.37	Sabor 42 vs Maçãs 6	1.2694	0.13
Sabor 34 vs Sabor 42	0.79636	0.477	Sabor 45 vs Maçãs 6	0.56951	0.789
Sabor 34 vs Sabor 45	1.0581	0.397			
Sabor 34 vs Maçãs 6	2.8051	0.013			
Sabor 35 vs Sabor 36	3.5032	0.014			