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AGRESSÃO INTERESPECÍFICA EM LAGOSTINS: INVASÕES BIOLÓGICAS, DOMINÂNCIA E EXCLUSÃO COMPETITIVA (CRUSTACEA: ASTACIDEA)

TESE DE DOUTORADO

Marcelo Marchet Dalosto

Santa Maria, RS, Brasil
2016

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Tese apresentada ao Programa de Pós-Graduação em Biodiversidade Animal, da Universidade Federal de Santa Maria (UFSM, RS), comor equisito parcial para a obtenção do título de **Doutor em Biodiversidade Animal**

Orientador: Prof. Dr. Sandro Santos

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Aprovado em 29 de Janeiro de 2016:

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Santa Maria, RS
2016

Aos meus pais,
que nunca me disseram o que eu deveria ser,
e com isso me deram o mundo de presente.

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“Quisque est barbarus alio”
(“Todo mundo é um bárbaro para alguém”)

Provérbio latino

RESUMO

AGRESSÃO INTERESPECÍFICA EM LAGOSTINS: INVASÕES BIOLÓGICAS, DOMINÂNCIA E EXCLUSÃO COMPETITIVA (CRUSTACEA: ASTACIDEA)

AUTOR: Marcelo Marchet Dalosto
ORIENTADOR: Sandro Santos

A agressão interespecífica é um fenômeno biológico de ocorrência muito comum, mas que ainda é muito pouco compreendida, estando relacionada com a obtenção de recursos, padrões de distribuição geográfica e substituição de espécies, no caso de invasões biológicas. Sua melhor compreensão pode contribuir para o aumento do conhecimento em diferentes áreas, tais como ecologia, evolução, biogeografia e biologia da conservação. Um bom grupo modelo para se estudar a agressão interespecífica são os crustáceos decápodos, pois estes respondem bem à condições laboratoriais, e a agressão é um aspecto importante da ecologia de muitas espécies. O objetivo desta tese é utilizar decápodos como modelos para investigar a agressão interespecífica em três contextos diferentes: invasões biológicas, ingenuidade de competidor e desenho experimental. No primeiro capítulo, investigamos a relação do lagostim nativo *Parastacus brasiliensis* com o invasor *Procambarus clarkii*, para compreender as possíveis consequências da interação entre esses lagostins, visto que a espécie invasora ainda não entrou em contato com o nativo. Experimentos relizados com animais de tamanho semelhante demonstraram que a espécie invasora vence mais interações, é mais agressiva, alcança o recurso mais rápido e mantém a posse do recurso disputado por mais tempo do que a espécie nativa. Confrontos interespecíficos também escalonaram mais rapidamente do que confrontos intraespecíficos. Esses resultados significam que *P. clarkii* representa uma ameaça séria para espécies nativas, especialmente considerando que este invasor atinge maiores tamanhos e é mais fecundo do que as espécies nativas de nicho semelhante. No segundo capítulo, investigamos a agressão interespecífica entre *P. clarkii* e outras três espécies de lagostins invasores: *Orconectes limosus*, *Pacifastacus leniusculus* e *Astacus leptodactylus*. Essas interações foram comparadas com interações intraespecíficas de *P. clarkii*. Todas as interações foram repetidas ao longo de três dias consecutivos. Os resultados demonstraram que a duração do primeiro embate, duração média dos embates, tempo total em confronto, número de embates e maior nível agressivo diferem entre os dias apenas para as interações intraespecíficas, sendo maiores no primeiro dia, em relação ao segundo e terceiro dias. Em contraste, nos grupos interespecíficos apenas o maior nível agressivo diferiu significativamente, entre o primeiro e o segundo dia dos confrontos com *O. limosus*. Também encontramos diferenças para a latência e tempo até o maior nível agressivo, no primeiro dia de interação, indicando que confrontos envolvendo *P. leniusculus* tendem a escalonar mais rápido do que os envolvendo outras combinações de espécies. Nas combinações de espécies testadas, não parece haver formação de hierarquias de dominância entre espécies sem histórico de co-existência prévia, já que os níveis agressivos não diminuíram ao longo do tempo, indicando que a não-formação de ordens estáveis de dominância pode ser um fator impotante em invasões biológicas. No terceiro e último capítulo, investigamos interações agressivas entre o lagostim *P. brasiliensis* e um competidor nativo, o anomuro *Aegla longirostri*, com o objetivo de testar qual o critério mais parcimonioso para se delinear experimentos entre competidores com morfologias marcadamente distintas. Foram realizados confrontos entre pares interespecíficos com indivíduos de tamanho aleatório, para os quais determinamos a diferença de tamanho, diferença de peso e diferença na força do armamento. Realizamos uma série de modelos para testar se essas variáveis eram capazes de prever a espécie vencedora, o número de embates agressivos, a duração do primeiro embate, a duração média dos embates, o tempo total em confronto, o período de latência e o tempo até o maior nível agressivo. A diferença de força foi capaz de prever a espécie vencedora, sendo que a probabilidade de *A. longirostri* vencer um confronto é maior à medida que a diferença de força entre os animais diminui, indicando que este eglídeo está em vantagem quando os animais são equivalentes em força. Isso também indica que entre competidores de formas distintas, a força do armamento talvez seja o melhor preditor de sucesso em interações agressivas. A diferença de tamanho estava relacionada com o tempo até o escalonamento máximo, e confrontos escalonaram mais rápido a medida que a diferença de tamanho aumentava, o que pode se dever ao fato de animais maiores se perceberem como potenciais ganhadores de confrontos, embora essa hipótese necessite ser testada no futuro. No geral, os resultados indicam que a agressão interespecífica é um fenômeno marcente nos casos investigados nesta tese, e que necessita claramente de um *framework* que unifique a esparsa literatura acerca do tema e forneça claras direções futuras para pesquisadores interessados no tema.

Palavras-chave: Competição por interferência; Eficiência de armamento; Espécies invasoras; Ingenuidade de competidor

ABSTRACT

INTERSPECIFIC AGGRESSION IN CRAYFISH: BIOLOGICAL INVASIONS, DOMINANCE AND COMPETITIVE EXCLUSION (CRUSTACEA: ASTACIDEA)

AUTHOR: Marcelo Marchet Dalosto
ADVISOR: Sandro Santos

Interspecific aggression is a common ecological phenomenon, but is still very poorly understood. It is related to the obtention of resources, geographic distribution patterns and species replacement, in the case of biological invasions. A better understanding of this issue may contribute to different areas, such as ecology, evolution, biogeography and conservation biology. Decapod crustaceans are a good model group to study interspecific aggression: they respond well to laboratory conditions, and aggression is an important aspect of the ecology of many species. The goal of this thesis is to use decapods as models to investigate interspecific aggression in three different contexts: biological invasions, competitor naïveté and experimental designs. In the first chapter, we investigate the interaction of the native crayfish *Parastacus brasiliensis* with the invasive *Procambarus clarkii*, to better understand the consequences of a possible interaction of these species in nature, considering that the invasive species has not yet encountered the native. In experiments with size-matched animals, the invader won more interactions, was more aggressive, reached the resource first and kept possession of the resource for longer than the native species. Interspecific fights escalated faster than intraspecific fights. These results mean that *P. clarkii* is a serious threat to native species, especially considering that this invader reaches larger sizes and is more fecund than native species of similar niches. In the second chapter, we investigated the interspecific aggression between *P. clarkii* and three other invasive crayfish species: *Orconectes limosus*, *Pacifastacus leniusculus* and *Astacus leptodactylus*. These interactions were compared with *P. clarkii* intraspecific fights. All fights were repeated along three consecutive days. In intraspecific fights, the duration of the first bout, mean duration of bouts, total fighting time, number of bouts and highest aggressive level differed between days: they were higher in the first day in comparison to the second and third days. In contrast, in interspecific fights only the highest aggressive level differed, between the first and second days of the interactions with *O. limosus*. We also found differences between the experimental groups in the first day, regarding the latency and time to the highest aggression level, indicating that interactions with *P. leniusculus* tend to escalate faster than the others. In the species combinations we tested, there seems to be no dominance hyerarchies between species without previous co-existence, since aggressive levels did not decrease over time, suggesting that the inability to form stable dominance might be an important factor in biological invasions. In the third chapter we investigated aggressive interactions between the crayfish *P. brasiliensis* and a native competitor, the anomuran *Aegla longirostri*, aiming to test which criteria is the most parcimonious to outline laboratorial experiments between competitors with different morphologies. We observed fights of interspecific pairs with random-sized animals, for which we determined the size difference, weight difference and weapon strength difference. We used a series of models to test if these variables were able to predict the winning species, number of aggressive bouts, duration of the first bout, mean duration of bouts, total fighting time, latency period and time until the highest aggression. The strength difference was able to predict the winning species, with the probability of *A. longirostri* being the winner increasing as the difference in weapon strength decreased, meaning that this aeglid has an advantage when both animals are matched for strength. This also indicates that, between competitors with different body shapes, weapon strength may be the best predictor of success in aggressive interactions. The size difference was related to the time until the maximum aggression, and fights escalated faster as the size difference between contestants decreased. This could be due to the fact that large animals perceive themselves as potential winners of fights, even though such hypothesis should to be tested in the future. Overall, our results indicate that interspecific aggression is an important phenomenon to all investigated species, but this issue clearly requires an overall framework that unifies the scarce literature around this subject, to provide clear directions for future research on this topic.

Keywords: Competitor naïveté; Interference competition; Invasive species; Weapon performance

Sumário

Introdução geral

Agressão intraespecífica & agressão interespecífica

Agressão intraespecífica é um comportamento comum no reino animal, sendo empregada por organismos tão diversos quanto anêmonas, formigas, lagostas, peixes e veados (Cluton-Brock et al., 1979; Macom & Porter, 1996; Karavanich & Atema, 1998; Earley & Dugatkin, 2006; Rudin & Brifa, 2011). Essa agressão está associada com a aquisição de um status social elevado, aonde o animal dominante obteria um maior acesso a recursos (Maynard-Smith & Price, 1973; Fero & Moore, 2008). Por estar relacionado a formação de territórios, disputa por parceiros e outros recursos, o comportamento agressivo pode ser considerado um aspecto essencial da biologia e ecologia de diversos organismos (Atema & Steinbach, 2007; Herberholz et al., 2007; Fero & Moore, 2008; Ayres-Peres et al., 2011). Devido à frequência com que ocorre e a sua importância ecológica, o comportamento agressivo é tópico de um vasto corpo de literatura, englobando desde aspectos específicos de cada espécie até testes de modelos teóricos sobre comunicação animal (e.g. Grant et al., 2000; Earley & Dugatkin, 2006; Briffa, 2008; Dalosto et al., 2013; Palaoro et al., 2014).

Animais não interagem agressivamente apenas com seus conspecíficos, mas também com indivíduos de outras espécies (e.g. Höjesjö et al., 2005; Le Breton et al., 2007; Hudina et al., 2011). De maneira semelhante à agressão intraespecífica, essas interações interespecíficas ocorrem em um contexto de disputa por recursos. Espécies com nichos semelhantes disputam praticamente os mesmos recursos que indivíduos de uma mesma espécie, tais como alimentos, abrigos e territórios (Gherardi & Daniels, 2004; Gherardi & Cioni, 2004; Höjesjö et al., 2005). A única exceção seriam as disputas por parceiros sexuais, embora existam casos, nos quais a competição interespecífica por parceiros seja possível, como os lagostins do gênero *Orconectes,* no qual hibridização é comum em casos de translocação de espécies (PERRY et al., 2001).

Agressão interespecífica ocorre predominantemente na forma de competição por interferência, na qual uma das espécies restringe ou impede o acesso da outra a um recurso em comum (GHERARDI & CIONI, 2004). Esse tipo de competição pode explicar muitos padrões observados na natureza, quando consideramos espécies que competem entre si. Exemplos disso incluem trutas (*Onchorhyncus clarkii*) que obtém mais alimento do que juvenis de salmão (*Onchorhyncus kysuch*), que são menos agressivos (SABO & PAULEY, 1997); Raposas vermelhas (*Vulpes vulpes*) que excluem raposas do ártico (*Alopex lagopus*) de sítios de alta qualidade para nidificação (TANNERFELDT et al., 2002), lagostins (*Orconectes virilis*) que excluem uma espécie menos agressiva (*Orconectes immunis*) de um microhábitat preferido por ambas (BOVBJERG, 1970).

Além da agressão interespecífica poder ser considerada um fator importante nas relações ecológicas (PEIMAN & ROBINSON, 2010; GOODALE et al., 2010), ela também pode exercer um papel significativo na história evolutiva das espécies (GRETHER et al., 2009, 2013). Especificamente, competição e agressão entre espécies ecologicamente semelhantes pode levar à evolução de comunicação interespecífica se os custos do reconhecimento de competidor forem compensados por uma diminuição da agressão/competição através da comunicação interespecífica (CODY, 1969, 1973; GRETHER et al., 2009). Contudo, apesar da ocorrência de agressão interespecífica ser bastante comum e frequentemente tão intensa e custosa quanto a agressão intraespecífica, a quantidade de estudos investigando a primeira ainda é pequena (ORD & STAMPS, 2009; PEIMAN & ROBINSON, 2010; GRETHER et al., 2013).

Apesar da agressão/competição interespecífica estar recebendo um nível crescente de atenção nos últimos anos (veja PEIMAN & ROBINSON, 2010; GRETHER et al., 2013; CARTHEY et al., 2014), ela ainda é muito pouco compreendida dentro de um ponto de vista teórico. Um dos fatores que podem contribuir para tal ausência de um corpo teórico uniforme é a diversidade de termos utilizados, frequentemente com significados pouco claros e se sobrepondo entre si (PEIMAN & ROBINSON, 2007; GRETHER et al., 2009; TOBIAS & SEDDON, 2009). Outro fator que limita a compreensão é que a literatura já existente trata, em grande parte, de casos pontuais e cuja significância se restringe basicamente aos grupos taxonômicos investigados (e.g. WILLIAMS et al., 2006; FERRETTI, 2011; KNICKLE & ROSE, 2014), nos quais a contextualização dos resultados em um panorama mais geral é, no máximo, limitada. Agressão interespecífica pode ser relacionada a vários temas bastante amplos, como invasões biológicas, ingenuidade ecológica e evolução de sistemas de comunicação (ORD & STAMPS, 2009; GRETHER et al., 2009, 2013; CARTHEY et al., 2014). É dentro desse contexto que crustáceos decápodos surgem como bons organismos modelos para o estudo destes tópicos.

Crustáceos decápodos e agressão interespecífica

Crustáceos decápodos constituem um grupo bastante diverso de invertebrados aquáticos e semiterrestres, com cerca de 15.000 espécies (MARTIN & DAVIES, 2001). Embora apresentem uma grande diversidade morfológica e ecológica, a maioria das espécies desse grupo é generalista em termos de nicho, especialmente considerando-se as dietas e padrões de atividade (MARTIN & DAVIES, 2001; REYNOLDS & SOUTY-GROSSET, 2012; DUFFY & THIEL, 2007). Em adição à essa tendência de sobreposição de nicho, que implica na existência de uma competição interespecífica pronunciada e bem documentada (e.g. BOVBJERG, 1970; GHERARDI & CIONI, 2004; WILLIAMS et al., 2006; HUDINA & HOCK, 2012), há também o aspecto importante da agressão. Em crustáceos decápodos, a agressão é um aspecto conspícuo de sua ecologia (e.g. BARKI et al., 1991; GHERARDI, 2002; AMARAL et al., 2009). Essa agressão geralmente está relacionada com o acesso a recursos (WILLIAMS et al., 2006; HERBERHOLZ et al., 2007; FERO & MOORE, 2008), e está muito associada aos quelípodos, estruturas morfológicas adaptadas para várias funções, entre as quais o combate entre indivíduos (SNEDDON et al., 1997; MARIAPPAN et al., 2000; DENNENMOSER & CHRISTY, 2013).

Apesar da elevada conspicuidade e da reconhecida importância ecológica da agressão interespecífica em decápodos, a quantidade de investigações nesse tema é escassa se comparado com a agressão intraespecífica: decápodos são considerados modelos consagrados no estudo da agressão intraespecífica (ver DUFFY & THIEL, 2007), sendo frequentemente usados para o teste de teorias gerais sobre sistemas de avaliação/comunicação e de teoria dos jogos (e.g. BRIFFA & ELWOOD, 2001; HERBERHOLZ et al., 2007; FERO & MOORE, 2008; PALAORO et al., 2014). Em contraste, investigações de agressão interespecífica são pontuais e tendem apenas a investigar casos isolados, sendo a sua maioria interações entre espécies invasoras e espécies nativas realizadas após uma invasão biológica, que buscam apenas evidências que o declínio de espécies nativas talvez esteja relacionado à competição com espécies introduzidas (e.g. SÖDERBACK, 1991; GHERARDI & CIONI, 2004; GHERARDI & DANIELS, 2004; WILLIAMS et al., 2006; HAZLETT et al., 2007).

Invasões Biológicas

Invasões biológicas por crustáceos são comuns e comprovadamente geram grandes danos ambientais, econômicos e sociais, incluindo competição e predação de espécies nativas, incluindo vertebrados e invertebrados, destruição de arrozais, aumento da erosão nas margens de rios e canais, entre outros (GHERARDI, 2006; GHERARDI et al., 2011; FICETOLA et al., 2011). Considerando-se essa importância bem-documentada, pode-se considerar que a literatura acerca de agressão interespecífica e invasões biológicas nesses animais não corresponde a sua relevância no contexto atual. Conforme mencionado anteriormente, a maioria dos estudos apenas faz uma tentativa de relacionar um declínio observado em certas espécies com uma suposta exclusão competitiva por parte de espécies introduzidas. Existem alguns poucos estudos que investigam interações entre lagostins nativos e exóticos antes de um potencial contato na natureza, tendo em vista a obtenção de subsídios para estratégias de conservação, mas estes surgem como exceção à regra (e.g. VORBURGER & RIBI, 1999; JIMENEZ & FAULKES, 2010).

A realização de tais estudos preventivos se torna ainda mais pertinente quando levamos em conta a existência de muitos casos de invasões que estão ocorrendo atualmente e aonde a espécie introduzida está em pleno processo de expansão de sua distribuição. Exemplos de espécies com tal potencial incluem o lagostim vermelho da Louisiana, *Procambarus clakrii*, o lagostim da Califórnia, *Pacifastacus leniusculus* e o lagostim australiano *Cherax destructor*, entre outros (CAPINHA & ANASTÁCIO, 2011; CAPINHA et al. 2011). Um caso particularmente interessante é o da América do Sul, a qual possui populações de *P. clarkii* registradas desde 1999 (embora estime-se que esses animais foram introduzidos muitos anos antes, veja SILVA & BUENO, 2005). Mais recentemente, demonstrou-se que a América do Sul apresenta alta adequabilidade ambiental para o estabelecimento dessa espécie, e que a mesma vem aparentemente aumentando a sua distribuição (PALAORO et al., 2013a; LOUREIRO et al., 2015). Este cenário, aonde a invasão ainda pode ser considerada em stágios iniciais, é particularmente adequado para a realização de estudos preventivos, já que estes podem auxiliar na mitigação dos danos potencialmente causados por *P. clarkii* (SOUTY-GROSSET et al., 2006; PALAORO et al., 2013a).

Comunicação interespecífica, hierarquias de dominância e ingenuidade de competidor

Outro tópico para o qual crustáceos decápodos representam bons organismos modelos mas tem sido consideravelmente negligenciados é a comunicação interespecífica em um contexto de agressão e competição. Lagostins em particular seriam organismos interessantes para esses estudos, visto que já é sabido que todas as linhagens formam hierarquias estáveis de dominância (e.g. Astacidae: BERRY & BREITHAUPT, 2002; Cambaridae: HERBERHOLZ et al., 2007; Parastacidae: STEWART & TABAK, 2011); e que o status social dentro das hierarquias está relacionado a um maior acesso a recursos, tanto em ambientes laboratoriais (HERBERHOLZ et al., 2007) quanto naturais (FERO & MOORE, 2008).

De maneira semelhante ao descrito na sessão anterior, a maioria dos estudos relativos à agressão interespecífica usando crustáceos como modelos se limita a um desenho experimental simples aonde a espécie A interage com a espécie B em laboratório, e onde isso teria relação com a substituição de espécies nativas por espécies introduzidas (e.g. BLANK & FIGLER, 1996; WILLIAMS et al., 2006) ou com exclusão competitiva entre espécies nativas (e.g. BARBARESI & GHERARDI, 1997). Alguns destes estudos indicam que a agressão entre espécies filogeneticamente distantes, sem histórico de co-evolução, não diminui (GHERARDI & CIONI, 2004) ou não aparenta diminuir (DALOSTO et al., 2015) ao longo do tempo, e que apenas espécies filogeneticamente próximas (GHERARDI & DANIELS, 2004) ou espécies com histórico de co-evolução (GHERARDI & CIONI, 2004) possuem a capacidade de formar hierarquias de dominância interespecíficas estáveis. Contudo, esses estudos não se propõem a investigar comunicação interespecífica, e se limitam a abordar o assunto de maneira superficial. Essas evidências pontuais presentes em estudos possivelmente se enquadram em um fenômeno denominado ingenuidade ecológica, que consiste na resposta não-adaptativa de uma espécie em consequência do encontro entre espécies que não co-existiam previamente (CARTHEY & BANKS, 2014). Essa ingenuidade ecológica pode acontecer entre predador e presa, quando é nomeada ingenuidade de predador, ou entre competidores, sendo chamada de ingenuidade de competidor (PEIMAN & ROBINSON 2010; CARTHEY & BANKS 2014).

Dentro desse contexto, lagostins surgem como organismos interessantes para se investigar a comunicação interespecífica e a ingenuidade de competidor, já que: (1) a alta sobreposição de nichos entre esses crustáceos implica em uma elevada importância ecológica da agressão interespecífica (HUDINA & HOCK, 2012); e (2) a alta frequência de translocamento de espécies implica que muitas delas com diferentes graus de co-evolução e proximidade filogenética entram em contato (BREINHOLT, et al. 2009; GHERARDI et al., 2011), o que pode possibilitar que os efeitos dessas variáveis na capacidade de comunicação interespecífica seja estudado, bem como as possíveis implicações dessas interações nos cenários de invasões biológicas.

Variação morfológica e desenhos experimentais

Crustáceos decápodos possuem uma grande diversidade ecológica e ocupam uma variedade de nichos (MARTIN & DAVIES, 2001; DE GRAVE et al., 2009). Enquanto que essa diversidade de hábitos os torna bons organismos-modelo para uma quantidade de tópicos (revisões em DUFFY & THIEL, 2007; BREITHAUPT & THIEL, 2011), ela também pode ser um fator complicante em certos contextos. Um desses fatores seria que a diversidade morfológica limita a utilização dos desenhos experimentais mais comuns, os quais geralmente constituem de grupos de animais (geralmente pares) definidos por tamanhos corporais semelhantes. Essa medida de tamanho pode ser o comprimento de carapaça (e.g. BERGMAN et al., 2005), largura de carapaça (e.g. SNEDDON et al., 2000) ou massa corporal (e.g. DELGADO-MORALES et al., 2004). Como o tamanho do armamento (i.e. quelípodo) também pode influenciar no resultado das interações agressivas, o mais comum é estabelecer a equivalência dos animais com base no tamanho corporal e também no tamanho do quelípodo (SNEDDON et al., 1997; DALOSTO et al., 2013; PALAORO et al., 2013b).

Em interações interespecíficas, esse desenho "experimental tamanho + armamento" não pode ser utilizado, pois as diferenças na proporção quelípodos/corpo e na forma corporal dificulta a determinação de equivalência entre essas medidas. Em lagostins, por exemplo, animais tendem a ser pareados apenas pelo tamanho de carapaça (e.g. HUDINA & HOCK, 2012; DALOSTO et al., 2015), pois apesar de se saber da importância dos quelípodos nas interações agressivas (BYWATER et al., 2008), o tamanho proporcionalmente diferente e morfologia variada dos quelípodos impedem que o tamanho bruto do quelípodo seja utilizado como variável. Devido à essa inadequação dos métodos usados em estudos intraespecíficos, percebemos uma alta heterogeneidade nos métodos utilizados para delinear experimentos. O exemplo clássico seria dos estudos investigando interações entre lagostas (*Homarus americanus*) e caranguejos (*Carcinus maenas*): em um caso, os animais foram pareados por tamanho de carapaça (ROSSONG et al., 2006); em outro pela massa corporal (LYNCH & ROCHETTE, 2009); e em um terceiro o tamanho dos animais não foi considerado (WILLIAMS et al., 2006).

Outro problema pode ser detectado em estudos que verificam interações entre crustáceos com abdômens densenvolvidos, tais como lagostas e lagostins, com crustáceos carcinizados, como caranguejos. Neste caso, usa-se o comprimento da carapaça como medida de tamanho para os primeiros e largura da carapaça para os segundos (GHERARDI & CIONI, 2004; ROSSONG et al., 2006). O uso de tais medidas pode prejudicar a interpretação de dados, pois essas medidas podem não ser equivalentes exatos uma das outras, além de não serem homólogas. As limitações decorrentes dessas inadequações já foram constatadas por alguns autores (GHERARDI & CIONI, 2004; WILLIAMS et al., 2006). Apesar disso, a relação dessas diferentes morfologias com o possível resultado de estudos de agressão interespecíficas continua desconhecida, e a falta de padronização dos métodos provavelmente limita as conclusões dos estudos em agressão interespecífica.

Objetivos

Objetivo geral

O objetivo desta tese é estudar a agressão interespecífica utilizando crustáceos decápodos, sobretudo lagostins, como modelos para responder diferentes perguntas envolvendo a agressão interespecífica. A agressão interespecífica será investigada em um contexto de (1) invasões biológicas, (2) ingenuidade de competidor e comunicação interespecífica, e (3) delineamento de experimentos de agressão interespecífica e interações entre espécies nativas.

Objetivos específicos

Capítulo 1:

Investigar interações entre o lagostim invasor *Procambarus clarkii* e o nativo *Parastacus brasiliensis*. O lagostim norte-americano *P. clarkii* é uma espécie invasora bastante agressiva e introduzido no Brasil, nas cercanias da cidade de São Paulo. Embora sua área de ocorrência ainda seja pequena e esta espécie ainda não tenha entrado em contato com lagostins nativos, a literatura indica que ela seja uma séria ameaça às espécies nativas. Assim, nosso objetivo é investigar se esta espécie constitui uma ameaça real aos lagostins nativos, através de experimentos em laboratório utilizando animais de tamanho semelhante. Nossa hipótese é que devido à sua agressividade e histórico de invasões bem-sucedidas, *P. clarkii* será capaz de sobrepujar agressivamente o nativo *P. brasiliensis*. Especificamente, nossas predições são que *P. clarkii* vencerá mais confrontos e manterá a posse do recurso por mais tempo do que *P. brasiliensis*.

Capítulo 2:

Investigar interações entre o lagostim invasor *P. clarkii* e outras espécies invasoras: *Orconectes limosus*, *Pacifastacus leniusculus* e *Astacus leptodactylus*. Invasões biológicas por lagostins são bastante comuns, mas um aspecto dessas invasões, as hierarquias de dominância, permanece pouco investigado. Essas hierarquias são mantidas através de comunicação química e são comuns em interações intraespecíficas, mas a literatura indica que espécies sem histórico de co-existência não possuem essa capacidade, embora isso ainda não tenha sido efetivamente testado. Nossa hipótese é que devido ao fato de invasões biológicas frequentemente provocarem encontros de espécies que não evoluíram juntas, , estes lagostins invasores não irão formar ordens estáveis de dominância entre si, contrastando com interações intraespecíficas de *P. clarkii*. Nossas predições são de que comportamentos indicadores de agressividade irão diminuir ao longo de confrontos consecutivos entre pares da mesma espécie, mas que isso não irá ocorrer nas combinações interespecíficas.

Capítulo 3:

Investigar interações entre o lagostim *P. brasiliensis* e o anomuro *Aegla longirostri*, ambos nativos da América do Sul. Um problema frequente nos estudos de agressão interespecífica são as morfologias diferentes entre os competidores, o que dificulta o delineamento de experimentos e diminui a força das inferências que podem ser feitas. Nosso objetivo é usar duas espécies de decápodos neotropicais com nichos semelhantes, dos gêneros *Parastacus* e *Aegla*, para testar qual o método mais parcimonioso para a investigação da agressão interespecífica em crustáceos. Considerando que a força do quelípodo é uma medida menos subjetiva do que medidas de tamanho corporal e que o quelípodo é uma estrutura extremamente importante nas interações agressivas de crustáceos, nossa hipótese é que a força do quelípodo será mais importante nessas interações do que medidas mais comumente usadas, como o tamanho de carapaça e o peso. Nossa predição é de que a força do armamento será o melhor preditor da espécie que vencerá a interação, e que confrontos se tornarão mais agressivos à medida que a força do armamento dos contestantes se torna mais similar.

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ARTIGO 1: ONE STEP AHEAD OF THE ENEMY: INVESTIGATING AGGRESSIVE INTERACTIONS BETWEEN INVASIVE AND NATIVE CRAYFISH BEFORE THE CONTACT IN NATURE

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One step ahead of the enemy: investigating aggressive interactions between invasive and native crayfish before the contact in nature

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Abstract

Biological invasions are a major cause of biodiversity loss, and early action in these cases is more cost-effective than dealing with widespread invasions. Thus, understanding possible consequences of invasions is essential for control and management actions. Given the early stage of invasion of South America by *Procambarus clarkii*, a potentially harmful crayfish, we investigated aggressive interactions between this invasive crayfish and the native *Parastacus brasiliensis* to understand potential impacts of the invader on native species before they encounter each other in nature. We paired size-matched crayfish for two experiments: one with *Pr. clarkii* males and females against *Pa. brasiliensis*; and another with *Pr. clarkii* intraspecific interactions. We starved the crayfish then allowed to interact in the presence of food. In interspecific fights we compared the number of attacks, time with the resource, frequency of won interactions of each species and the first species to reach the resource. Regarding the interspecific fights, *Pr. clarkii* attacked more often, spent more time with the resource, won more interactions and reached the resource first more often than *Pa. brasiliensis*. Interspecific fights escalated faster than intraspecific fights. The invasive crayfish's ability to win might be enhanced due to ownership effects, and its impact is likely to be severe because of its life-history traits. We conclude that *Pr. clarkii* is definitely a threat for native crayfish, requiring that immediate actions be taken, such as dam construction and manual removal of *Pr. clarkii*.

Keywords: Aggressive behavior; Interspecific interactions; Invasion prevention; Invasive species; *Parastacus brasiliensis*; *Procambarus clarkii*

Introduction

Biological invasions are a pervasive global change, threatening many populations as well as the sustainability of many ecosystem services (Simberloff 2013; Simberloff et al. 2013). Many strategies have been proposed to deal with the negative impacts of invasive species, such as prevention of invasion and eradication of established invaders (Vander Zanden et al. 2010; Gherardi et al. 2011; Simberloff et al. 2013). Prevention of an invasion is often much more effective than dealing with established populations, since impacts may only become evident when palliative measures are already impossible or too costly (Rejmánek and Pitcairn 2002; Ficetola et al. 2011). The consequences of delayed action (or the lack of it) in this stage are well-documented. A practical example is the case of the Pacific alga *Caulerpa taxifolia*. Early detection of the invader in Californian waters quickly followed eradication efforts, effectively removing the invader. However, the failure to act quickly when it was detected on the Mediterranean led to its proliferation, and now it is considered ineradicable in that area (Meinesz 2001; Woodfield and Mooney 2002). Eradication techniques for non-established invaders have improved significantly (Genovesi 2011) and can be much more cost-effective than long-term management. For instance, the coypu's (*Myocastor coypu*s) control effort in Italy costs more than twice as much as the entire British eradication campaign (Panzacchi et al. 2007). These factors highlight the importance of prevention and early action in order to avoid the potentially irreversible costs of some invasive species.

Information concerning the invader and the invasion process are of vital importance for effective conservation measures (Palaoro et al. 2013a). One essential aspect of invasion biology is animal behavior, since it can provide invaluable information to prevent/contain the spread of invaders (Holway and Suarez 1999), such as: (1) direct competition with indigenous fauna over shared resources, which can lead to the displacement/local extinction of the weaker competitor (e.g. Gherardi and Cioni 2004; Pintor et al. 2008); (2) increase in the invader's population density due to the reduction of intraspecific aggression/competition in novel environments (e.g. Vandermeer et al. 1991; Macom and Porter 1996); and (3) higher dispersive abilities when compared to indigenous fauna (e.g. Bubb et al. 2006). Thus, understanding the behavior of invasive species and the nature of their interactions with native species can provide invaluable information for invasion prevention and/or management.

A situation requiring rapid response and accurate information can be encountered in South America: It is being invaded by a freshwater invertebrate, the red swamp crayfish, *Procambarus clarkii* (Palaoro et al. 2013a). This crayfish has high ecological plasticity, and is a fast-growing, resilient and aggressive species (Gherardi 2006; Souty-Grosset et al. 2006). It is an extremely successful invader known to cause severe damage on the ecosystems where it has been introduced, negatively affecting the biota of invaded areas, especially other crayfish (Gherardi 2006; Ficetola et al. 2011). The situation in South America is interesting because: (1) the invasion of *Pr. clarkii* in South America is in a much smaller scale than in other places (Silva and Bueno 2005; Loureiro et al. 2015a,b), such as Europe and China (Wang et al. 2005; Souty-Grosset and Reynolds 2012); and (2) so far, reports of occurrence of

Pr. clarkii do not yet include areas of distribution of native parastacid crayfishes. Furthermore, South America has a unique crayfish fauna, one of the oldest crayfish radiations with an unusually high predominance of burrowing habits and hermaphroditism (Rudolph and Almeida 2000; Breinholt et al. 2009), whose species may be excellent models to investigate the evolution of sociality and hermaphroditism (Rudolph and Almeida 2000; Dalosto et al. 2012, 2013; Almerão et al. 2014). If this is considered along the existing threats to this peculiar group of crustaceans, the establishment of conservation measures is of the utmost importance (Almerão et al. 2014).

Behavioral interactions between native and invasive crayfish are well-investigated (e.g. Gherardi and Cioni 2004; Gherardi and Daniels 2004; Hazlett et al. 2007). However, these studies were almost always performed after the contact between the invading and native species and aimed to link an already observed decline of native species to the invader, rarely being performed in a preventive manner. Studies that use behavioral traits as predictors of invader impact are rare (e.g. Alexander et al. 2014) and those that investigate aggressive behavior are exceptionally rare (notable exceptions include Vorburger and Ribi 1999 and Jimenez and Faulkes 2011). Hence, we are in an unusual position. We can investigate the potential consequences of this invasion before it reaches large scales and especially before the invader encounters the native crayfish in nature. This would allow us to mitigate the impacts caused by *Pr. clarkii*, opposed to what has happened in Europe (Gherardi 2006; Gherardi et al. 2011). Therefore, our objective is to investigate the outcome of a possible encounter of *Pr. clarkii* and a native South American species, *Parastacus brasiliensis*, in nature.

By doing so, we aim to obtain accurate information that could be used as a starting point for conservation measures to minimize the impacts of the invasion of *Pr. clarkii*. We used laboratorial experiments of size-matched interspecific and intraspecific pairs of crayfish to address this issue. We hypothesize that *Pr. clarkii* will dominate the interactions with *Pa. brasiliensis* due to a more pronounced aggressiveness, and predict that: (1) *Pr. clarkii* will win significantly more aggressive interactions than *Pa. brasiliensis*, and (2) it will also maintain the possession of the disputed resource for more time than the *Pa. brasiliensis*.

Materials and Methods

Studied species

Procambarus clarkii is a cambarid crayfish native from the southeastern United States and northern Mexico (Huner 2002). It is crayfish with a short, although variable life-cycle (Table 1, Huner 2002). It is highly fecund, and usually reproduces year-round, but reproduction may be centered in specific parts of the year (Souty-Grosset et al. 2006). It is remarkable for exhibiting high ecological plasticity and for its aggressiveness, which allows it to dominate native crayfish (e.g. Gherardi and Cioni 2004; Gherardi and Daniels 2004). It is considered an open-water species that occurs in basically all types of freshwater bodies, and is known to occasionally move through dry-land (Barbaresi et al. 2004). Despite not being a burrow specialist, it is able to burrow extensively, especially during droughts (Souty-Grosset et al. 2014).

The southern portion of South America has been shown to present high environmental suitability to this crayfish (Palaoro et al. 2013a). Although there are few published records of this crayfish in Brazil (Silva and Bueno 2005; Magalhães et al. 2005; Loureiro et al. 2015a,b), it is currently expanding its range of occurrence, with at least eleven additional populations confirmed in the city of São Paulo and its outskirts alone (Loureiro et al. 2015a). The native species, *Pa. brasiliensis*, is a burrowing parastacid commonly found on the banks of small streams and marshlands of Brazil's southernmost state (Buckup 2003). It is considerably smaller than *Pr. clarkii* (Tables 1 and 2). Despite being a burrow specialist, it shows considerable activity outside their burrows (Dalosto et al. 2013; Palaoro et al. 2013b). Thus, *Pa. brasiliensis* is the *Parastacus* species that has substantial niche similarity with *Pr. clarkii* (i.e. a crayfish that makes extensive use of burrows, but is also significantly active in the main water body), probably being the first to feel the negative impacts of the invader. A summary of the life-history traits of both species is shown in Table 1, and the general body morphology of each species can be seen in Fig. 1.

Crayfish sampling & experimental setup

We collected individuals of *Pr. clarkii* (n = 20 for each sex) using baited traps at the Alfredo Volpi Park, São Paulo, southeastern Brazil (23º35’16”S; 46º42’09”W) (See Silva and Bueno 2005 for a detailed description). We selected only intermolt adult crayfish with all appendages intact, also selecting only reproductively active when concerning the males (Form I; Moore 2007). We brought them to the laboratory at the Universidade de São Paulo, where they were acclimated in communal tanks separated by sex and fed daily with fish food pellets. After five days of acclimation, we accommodated the individuals in individual plastic containers (10 x 10 x 8 cm) filled with 1 cm of de-chlorinated water, and then placed in thermal boxes containing ice for transportation to the laboratory at the Universidade Federal de Santa Maria (UFSM), where the experiments were be performed. Despite the long distance travelled (approximately 1300 km), all crayfish were healthy and none perished during transportation. Upon arrival, we placed them in individual 2-L opaque plastic aquaria, containing de-chlorinated water, gravel, and stones/brick pieces as shelter. Water temperature averaged 16 °C (2 °C SD), and a natural (i.e. uncontrolled) photoperiod was adopted. We offered fish food pellets and senescent leaves twice a week. We removed any uneaten food in the following day, after which the water was changed.

We collected individuals of *Pa. brasiliensis* (n = 36) using a combination of baited traps and manual search at the municipality of Silveira Martins, southern Brazil (29°39’25.14”S; 53°37’33.53”W). We selected only intermolt adult crayfish with all appendages intact. We did not consider the sex of the individuals because this species possesses no evident sexual dimorphism and presents hermaphroditism (Almeida and Buckup 2000; Dalosto et al. 2013). We brought them to the laboratory at UFSM and acclimated them in the same conditions as described above. We measured all individuals for carapace length (CL; measured from the orbital sinus to the posterior edge of the carapace) and paired them according to their CL (i.e. maximum difference of 10%). Afterwards, we performed two experiments using size-matched crayfish. In the first experiment, we performed interspecific interactions between *Pa. brasiliensis* and male (n = 10 pairs) and female (n = 10 pairs) *Pr. clarkii*, to test for behavioral differences related to each species and the sex of the invader. The second experiment aimed to compare the dynamics of intraspecific fights with the interspecific fights of experiment one. To do so, intraspecific fights of male (n = 5 pairs) and female (n = 5 pairs) *Pr. clarkii* were performed, as well as intraspecific fights for *Pa. brasiliensis* (n = 8 pairs). Crayfish were never used more than once. Mean sizes (S.D.) are shown in Table 2. Size-matching the large *Pr. clarkii* with the smaller *Pa. brasiliensis* is difficult, and this is made more difficult because of the low densities of the native species (Table 1). Additionally, transporting live *Pr. clarkii* from São Paulo to Santa Maria requires a 1,228 km journey through road-transport, which is both logistically and economically costly. These factors restrained the number of crayfish of both species we could collect and use in the subsequent experiments.

We starved the individuals for 12 days prior to the experiments to standardize their fighting motivation (Moore, 2007). This acclimation time was determined from previous trials demonstrating that this is the time-window that is required to motivate *Pa. brasiliensis* to fight for a food resource. Despite being longer than the usual acclimation time adopted in crayfish experiments, our protocol did not result in excessive aggression or injuries for the crayfish. For the experiments, we placed the individuals in an aquarium (31 x 19 x 21 cm, henceforth "arena") containing three equal compartments separated by removable opaque dividers. We placed the individuals in opposite compartments of the arena, while the central compartment contained food (fish fillet). The food was placed inside a transparent and perforated plastic compartment, fixed in the center of the arena. We adopted this procedure to allow access to the resource, but not manipulation of it (Herberholz et al. 2007). After a 10 min acclimation period, we removed the dividers and the individuals were allowed to interact and/or access the resource for 30 min. We recorded the interaction with a SONY® HDR-CX560 camera positioned perpendicularly 30 cm above the arena. Experiments were performed at night, since *Pa. brasiliensis* is preferably nocturnal and *Pr. clarkii* is active at both daytime and nighttime, and thus any potential encounter between these crayfish would occur at night (Gherardi 2002; Palaoro et al. 2013b). Illumination was provided with red incandescent light bulbs (40 W) because crayfish have a low sensitivity to this wavelength (Dalosto et al. 2013). After the experiments, we returned the individuals to their respective aquaria, resumed feeding and monitored the individuals for one week for moltings/deaths. Since none died or molted in the week following experiments, we considered all data in the statistical analyses.

Determination of behavioral variables

Within each interaction, we quantified the time each individual spent in the possession of the resource, defined as the time which the individual spent at 1 cm or closer to the resource in the absence of the opponent. We quantified this variable to determine if species had different capacity to hold the resource. We noted if any individual was successfully evicted from the resource by its opponent. The number of evictions was too low for a proper statistical test (see Results), but it is a highly aggressive behavior that demonstrate dominance of one individual over the other, and thus should be considered for discussion. We annotated the first individual to access the resource. To determine if one species was more aggressive than the other, we also quantified the number of attacks performed by each individual (approaches, approaches with display, and lunges, following Dalosto et al. 2013). We also counted the number of agonistic bouts. A bout was deemed initiated when a crayfish performed an aggressive act that was responded by the opponent (Dalosto et al. 2013) and lasting at least 10 s. A bout was deemed finished when one individual either backed away, or assumed a submissive posture, with its abdomen curled beneath the cephalothorax (this individual will be referred as the loser; Dalosto et al. 2013). The crayfish who won more bouts was deemed the winner of the interaction, and its opponent the loser. For each interaction, we determined the latency period (i.e. time until the first bout), duration of first bout, mean duration of bouts, total fighting time, maximum intensity reached and time until maximum intensity. The latter two variables were determined according to the aggression levels adopted by Dalosto et al. (2013) (Table 3). We determined these interaction variables to test for possible differences in interaction dynamics among treatments.

Statistical analyses

In the first experiment, we compared *Pr. clarkii* and *Pa. brasiliensis* for two behavioral variables: time spent with resource and number of attacks. To do so, we performed a two-way factorial ANOVA with one within-subjects factor (species) and one between subjects factor (invader's sex) for each of the two variables. We *a priori* excluded two replicates of the "vs. males" group in the time with resource test, since one crayfish in the interaction did not show any interest on the resource.

We also tested which species reached the resource first, using the proportion of individuals to first reach the resource, and which species won frequently more interactions with G-tests. For the first species to reach the resource, we performed the analyses in two different ways: (1) we excluded two replicates *a priori*, since one crayfish of the dyad did not show interest on the resource; and (2) we performed the test with all crayfish dyads. For both tests, before testing the frequencies, we tested the difference between male *Pr. clarkii* and female *Pr. clarkii* for the time to reach the resource, which showed a non-significant difference between them (*t* test for independent samples; two replicates excluded: $t_{1,16} = -0.0912$; $p = 0.9285$; all replicates: $t_{1,17} = -0.1417$; $p = 0.8890$). Therefore, we pooled the data and discarded the effect of sex to increase statistical robustness for this test. The same was performed for the frequency of interactions won by each species: since aggressiveness (number of attacks) did not differ between the interspecific groups (the two-way factorial ANOVA mentioned above, see Results), we also pooled the data for this variable to increase statistical robustness.

To test for differences in the contest dynamics in the second experiment (latency, duration of first bout, mean duration of bouts, total fighting time, maximum intensity reached and time until maximum intensity), we chose two approaches: first, we performed a MANOVA to test if the experimental groups differed regarding the behavioral variables. Second, we did a PCA to see major trends in contest dynamics. For such comparisons, we considered two factors: Interspecific (with two levels – vs. males and vs. females) and Intraspecific (with two levels – *Pr. clarkii* and *Pa. brasiliensis*). For the Intraspecific fights of *Pr. clarkii*, we pooled the data from male and female replicates since they did not differ significantly (Table S1). We chose a PCA because we were not testing any hypothesis and had no *a priori* assumptions regarding these variables, and because this analysis can cope well with correlated variables, which corresponds to the current case. All data were previously checked for normality and homocedasticity with the Shapiro-Wilk and Levene tests, respectively. All statistical tests were performed in the R software (R development Core Team 2013), except for the MANOVA and the PCA, which were performed in PAST (Hammer et al. 2001).

Safety procedures & legal permits

We adopted safety measures because: (1) *Pr. clarkii* is an invasive species whose release in the wild is forbidden in Brazil (IBAMA 2008); and (2) to avoid contamination of indigenous crayfish populations and water bodies by the crayfish plague pathogen, *Aphanomyces astaci*, of which *Pr. clarkii* is a natural bearer (Souty-Grosset et al. 2006). The culture, maintenance and sale of *Pr. clarkii* in Brazil are forbidden since 2008 (IBAMA 2008). Thus, sampling, transport and maintenance of live *Pr. clarkii* were performed under special license nº 32673-1 provided by the ICMBio (Instituto Chico Mendes de Conservação da Biodiversidade). No crayfish were harmed during the collections, transportation, acclimation and execution of experiments. During acclimation and experimentation, all the water was sterilized before being discarded. We also sterilized the water used in the arena. All individuals had no contact with other animals. We sacrificed all *Pr. clarkii* immediately after the experiments. Individuals of *Pa. brasiliensis* were kept in observation for 4 months after the experiment, to check for possible pathogen-related mortality, after which they were also sacrificed. No crayfish escaped from the laboratory.

Results

Summary of the contests

Crayfish engaged each other in all replicates of the interspecific experiments and of the *Pr. clarkii* intraspecific experiment. In the *Pa. brasiliensis* intraspecific experiments, fights occurred in only five out of eight replicates. In the remaining three, crayfish did not exhibit aggressive behaviors.

Simultaneous feeding of both crayfish in the disputed resource was only observed in two replicates of the *Pa. brasiliensis* intraspecific group (these two being one of the three where no aggression occurred).

Species comparisons

The one-within one-between factorial ANOVA showed that *Pr. clarkii* possessed the resource for longer times than *Pa. brasiliensis* ($F_{1,16}$ = 14.869; p = 0.0014), and that crayfish spent more time with the resource in the vs. females group than in the vs. males group ($F_{1,16}$ = 6.522; p = 0.0212) (Fig. 2a, c). No significant differences were found for the interaction term ($F_{1,16}$ = 3.504; p = 0.0796). *Procambarus clarkii* performed more attacks than *Pa. brasiliensis* ($F_{1,18}$ = 8.906; p = 0.00795) (Fig 2b, d), but there were no differences between experimental groups ($F_{1,18}$ = 3.354; p = 0.0837) or for the interaction term ($F_{1,18}$ = 0.202; p = 0.6583). Regarding the proportion of interactions won by each species, *Pr. clarkii* was dominant in 17 interactions, while *Pa. brasiliensis* won only 3, which differed significantly from the expected proportion of equal dominance (G-test; $G_{1,1}$ = 10.554; p = 0.00116). Successful evictions were only performed by *Pr. clarkii* in one replicate of the vs. males group and one of the vs. females group. The proportion of species to access the resource first was statistically different from an even expected proportion in both tests performed, with *Pr. clarkii* being the first in 14 of 18 interactions when two replicates were removed (G-test; $G_{1,1}$ = 5.725; p = 0.017), and in 15 of 20 interactions when all replicates were considered (G-test; $G_{1,1}$ = 5.105; p = 0.024).

Group comparisons

The MANOVA showed that there was a significant difference in the behavioral variables between the groups ($F_{2,21}$ = 3.188; Pillai’s trace = 1.511; p = 0.0001722). Regarding the PCA, the first axis explained 69.557% of the variance (eigenvalue: 400336), and had a strong positive relation to the time to reach the highest intensity (loading: 0.81507) and with the latency (loading: 0.57852), while the second axis explained 25.397% of the variance (eigenvalue: 146172), and had a strong positive relation to the time to reach the highest intensity (loading: 0.55894) and a negative relation with the latency (eigenvalue: -

0.77641) (Fig. 3). A table with values of all PCA loadings is available as Supplementary Material (Table S2).

Post-experiment crayfish mortality

One *Pa. brasiliensis* perished one month after the experiment, and did not show any sign of melanization, tissue necrosis, autotomy of appendages and erratic activity, which are symptoms of the crayfish plague (Souty-Grosset et al. 2006). The remaining 19 individuals of this species remained alive for the following months until they were euthanized, and did not show any of the above-mentioned symptoms. Euthanized crayfish of both species were deposited on the scientific collection of the laboratory (voucher numbers UFSM-C 491-500).

Discussion

Procambarus clarkii dominated *Pa. brasiliensis* when fighting for a resource. *Procambarus clarkii* secured the resource for longer times than *Pa. brasiliensis*. It was also more aggressive, performing more attacks and winning more interactions than the native species. The PCA showed that fights in interspecific contests tend to initiate earlier than intraspecific fights. Lastly, *Pr. clarkii* also reached the resource first more frequently than the native species. Taken together, these results indicate that *Pr. clarkii* can severely impact native crayfish populations by restraining and/or preventing access to resources.

Dominance of successful invaders over native species have been reported for a variety of taxa (e.g. fish: Balshine et al. 2005; ants: Westermann et al. 2014; crustaceans: Lynch and Rochette 2009; MacNeil et al. 2011). Such dominance is also frequently associated with the decline of native species (Balshine et al. 2005; MacNeil et al. 2011), although direct aggression/predation between invaders and native species is not always the main driver of native species displacement (e.g. cane toads in Australia; Shine 2014). For crayfish, however, aggressive behavior is a very conspicuous aspect of their biology (reviewed by Gherardi 2002; Moore 2007). In these crustaceans, aggressive interactions can be directly related to differential access to resources, for example (Herberholz et al. 2007; Fero and Moore. 2008). They also readily attack both conspecifics and heterospecifics, and aggressive behavior is thought to play a major role in the displacement of native crayfish by invaders (Jimenez and Faulkes 2011).

Thus, despite a few exceptions (e.g. native crabs vs. invasive crayfish in Europe Gherardi & Cioni 2004 and native vs. invasive crayfish in Australia; Elvey et al. 1996), dominance in aggressive interactions seems an accurate proxy of the potential harm an invader crayfish can cause on native species. Our results thus indicate that *Pr. clarkii* is capable of outcompeting native crayfish through direct behavioral dominance and interference competition. Therefore, *Pr. clarkii* threatens *Pa. brasiliensis*, similarly to what has been reported for native European astacids (Gherardi and Cioni 2004; Gherardi and Daniels 2004). Although simple experiments of forced aggression performed in laboratory may not accurately represent interactions as they would be in natural environments, crayfish aggression in both laboratory and in nature exhibit very similar patterns (see Bergman & Moore 2003 and Fero & Moore 2008) and are considered a good indicator of how interactions might be in nature.

In addition to direct dominance, *Pr. clarkii* was more often the first to reach the resource. By doing so, *Pr. clarkii* might enhance its chance to win via the owner effect. Resource ownership is known to affect aggressive interactions (Maynard-Smith and Parker 1976), with resource ownership usually increasing the likelihood to win a dispute (e.g. decapods: Williams et al. 2006; insects: Chamorro-Florescano et al. 2011; Mohamad et al. 2012; lizards: López and Martín 2011). Thus, *Pr. clarkii*'s ability to reach the resource first suggests a competitive advantage against *Pa. brasiliensis* intensified by an increased likelihood to win aggressive interactions through ownership effects (Moore 2007).

Laboratorial studies are invaluable to understand aggressive interactions, but results must be interpreted with caution since animal interactions in nature are more complex than those in controlled experimental settings (Bergman and Moore 2003, Gherardi & Cioni 2004). However, if we consider: (1) the life-history traits of the studied species (Table 1), (2) a FI-ISK assessment indicating that this is high-risk invasive species (FI-ISK score of 41, Loureiro et al. 2015a) and (3) our results, we can safely conclude that *Pr. clarkii* constitutes a severe threat for *Pa. brasiliensis*. Taken together with our results, this means that *Pa. brasiliensis* would encounter *Pr. clarkii* more often than conspecifics (because of the different population densities), and that these *Pr. clarkii* individuals are much likely to be larger than the native crayfish (Tables 1 and 2). Thus, there remains no doubt that the large, aggressive *Pr. clarkii* will overcome the small, less aggressive *Pa. brasiliensis* (Gherardi and Cioni 2004; Dalosto et al. 2013).

Experimental groups were separated by the PCA. Although the separation was not complete, intraspecific fights vary mainly in PC1, whereas interspecific fights vary mainly in PC2 (Fig. 2, Table S2). Thus, their main difference is the latency to initiate fighting. While intraspecific fights tend to have a higher latency, interspecific fights initiate their fights earlier. This may indicate that individuals can face more opponents in the same amount of time. Interspecific aggression is much less studied than intraspecific aggression, so making inferences is usually difficult. However, it is known that fights between crustacean species with no previous co-existence experience are usually intense and do not de-escalate with time, unlike intraspecific fights (Gherardi and Cioni 2004). Although our data are not suitable to make statements regarding interspecific communication, it would be particularly interesting to check how intraspecific and interspecific crayfish aggression differ, and how these differences behave over time. Lastly, one may also consider the effect of the ongoing global warming on the possible interactions between *Pr. clarkii* and other species, since recent evidence demonstrates that increased temperatures may enhance *Pr. clarkii*'s success in aggressive interactions (Gherardi et al. 2013).

Another interesting result is the apparent lack of crayfish plague-related mortality in *Pa. brasiliensis*. *Procambarus clarkii* is a known bearer of this pathogen, to which it is immune (Diéguez-Uribeondo and Söderhäll 1993). South American crayfish have never been directly tested for this, but are assumed to be vulnerable like all non-North American crayfish tested so far (Unestam 1975; Souty-Grosset et al. 2006). Checking if Brazilian *Pr. clarkii* populations are plague-free (see Schrimpf. et al 2013) or if the South American species are plague-resistant arises as the next logical step.

The early stage of the invasion of *Pr. clarkii* in South America, with a few populations reported for São Paulo city and surrounding municipalities (Loureiro et al. 2015), suggests eradication as the first step. However, despite recent advances, these techniques have shown to be ineffective against crayfish

(Dana et al. 2011; Moorhouse and MacDonald 2011). Specifically, there is no currently known case of *Pr. clarkii* being eradicated once a viable population has been established, due to its burrowing ability that allows it to persist for months without a water body and its elevated fecundity (Table 1) (Correia and Ferreira 1995; Sousa et al. 2013). Since the endemic areas for South American crayfish are also the areas with the highest environmental suitability for *Pr. clarkii* (Palaoro et al. 2013a), and eradication is difficult at best, avoidance of further introductions combined with surveillance of both native and invasive crayfish populations seems the best alternative.

Special attention must be given to aquarium pet trade since it is a known introduction route for invasive crayfish (Mrugała et al. 2014). This link between pet trade and introduction of invasive aquatic species is well documented (Duggan et al. 2006, Chang et al. 2009, Chucholl 2013). Considering that live *Pr. clarkii* are readily available in pet shops, despite their illegality in Brazil (Loureiro et al. 2015a), it is of the utmost importance to enforce the prohibition in order to mitigate propagule pressure for this species. Thus, contention measures appear as a next logical step if introduction of the invader is not avoided. For areas already invaded by *Pr. clarkii*, dam construction seems a wise choice, since it has been demonstrated that it hampers upstream movements of this crayfish, even if we consider its ability to move overland (Kerby et al. 2005; Dana et al. 2011). Dam construction has also been effective to prevent the contact of this invader with the European native crayfish *Austropotamobius pallipes* in Spain, since the few remaining populations of this crayfish are located next to headwaters (Dana et al. 2011). The fact that the diversity of neotropical freshwater decapods is concentrated on headwaters further highlights the use of dams and similar obstacles as an effective method to mitigate the impacts of *Pr. clarkii* (Collins et al. 2011).

In summary, we have demonstrated the ability of *Pr. clarkii* to overcome South American crayfish in a competition scenario. *Procambarus clarkii*'s ability to aggressively overcome the native species and to maintain possession of a disputed resource for a longer time demonstrates its potential to overcome native crayfish through interference competition. It also tended to reach the resource first, which also suggest the potential to displace native crayfish through an increase in the likelihood to win contests through ownership effects. The larger size and population densities attained by *Pr. clarkii* mean that the dominance observed in size-matched laboratory contests is only likely to increase should the interactions occur in nature. Our study is one of the few aiming to anticipate the consequences of the contact of an invasive species with a native species of similar niche. Our results highlight the threat that this invasive crayfish pose to native crayfish and other native fauna. We suggest containment measures (e.g. dams) as means to mitigate its spread in invaded areas. Lastly, the most important action would be preventing new invasions, which could be achieved by a more thorough law enforcing and continuous surveillance of both native and invasive crayfish populations.

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Conflicts of Interest

We declare none

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Fig. 1 Representative specimens of crayfish used in the experiments: from the left to the right: Form I male *Procambarus clarkii*, female *Pr. clarkii* and adult *Parastacus brasiliensis*. In the latter, observe the smaller eyes, narrower abdomen and vertically-oriented chelipeds typical of specialized burrowers. All images taken in the same scale.

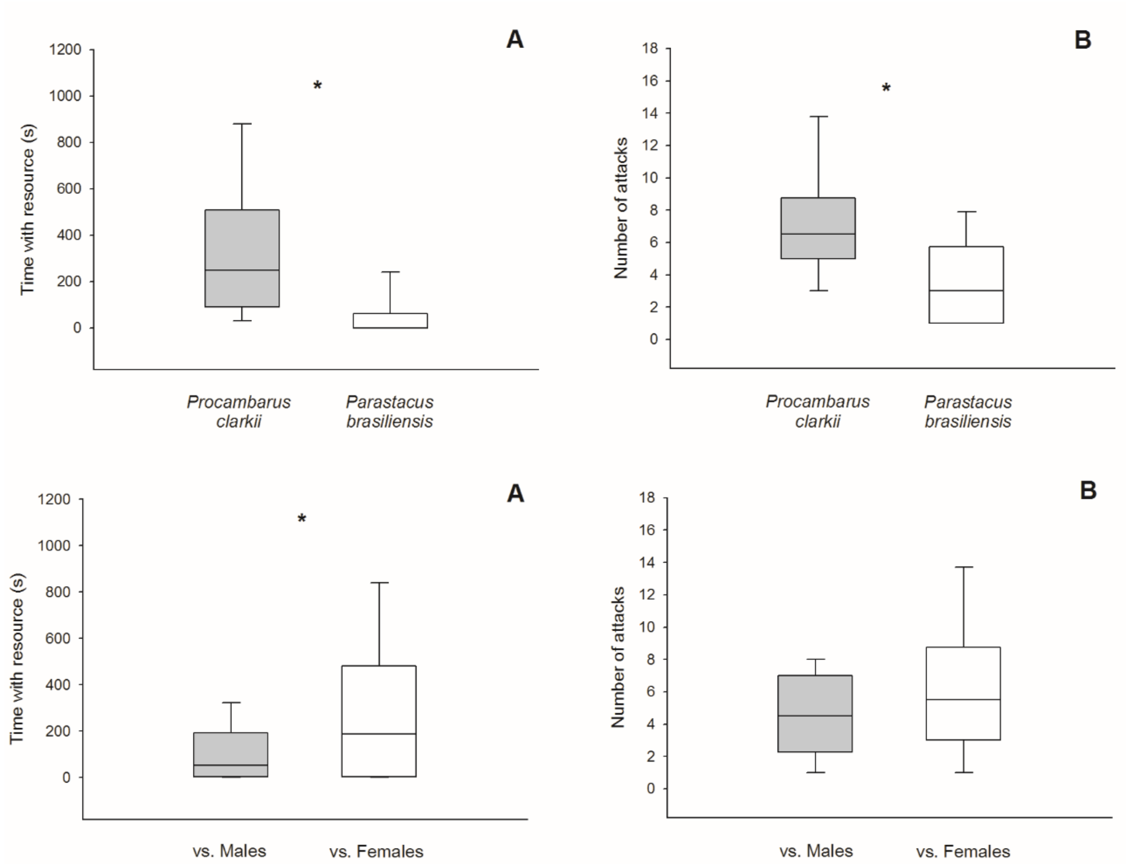

Fig. 2 Time spent with the resource (seconds) and number of attacks performed by *Procambarus clarkii* and *Parastacus brasiliensis* in interspecific interactions; A-B are comparisons between species, and C-D between experimental groups; bar height represent range between the first and third quartiles, whiskers represent maximum and minimum values, and horizontal bars represent medians; Asterisk (*) indicates statistically significant differences between the species/groups

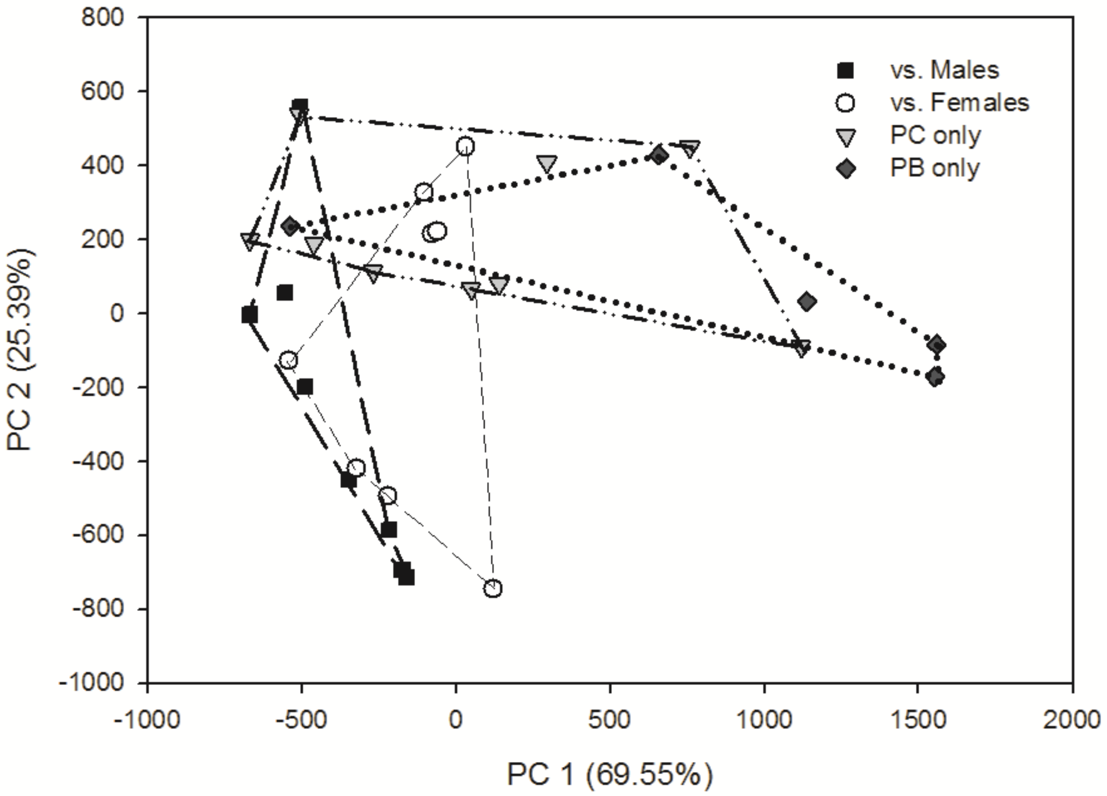

Fig. 3 Principal component analysis plot of the behavioral variables of interspecific and intraspecific interactions of crayfish, Black squares represent fights between *Parastacus brasiliensis* and male *Procambarus clarkii*; white circles represent *Pa. brasiliensis* vs. *Pr. clarkii* females; gray triangles represent intraspecific *Pr. clarkii* fights, and dark gray diamonds represent intraspecific *Pa. brasiliensis* fights

Table 1: Comparison of life-history traits of the native (*Parastacus brasiliensis*) and invasive (*Procambarus clarkii*) crayfish used on this study.

	Parastacus brasiliensis	***Procambarus clarkii***	**References**
Maximum size (total length - mm)	100	150	Fontoura and Buckup 1989a; Huner 2002
Lifespan (years)	4-5	1-9	Buckup 2003; Souty-Grosset et al. 2006; Scalici et al. 2010
Fecundity (number of eggs)	7-130	60-1017	Penn 1943; Oluoch 1990; Fontoura and Buckup 1989b
Age at onset of sexual maturity (months)	12-36	6-12	Penn 1943; Huner 2002; Dalosto et al. 2013
Population density for adults (ind.m^{-2})	~1	~3	Fontoura and Buckup 1989b, Oluoch 1990
Relation to crayfish plague	Unknown (presumed*)	Immune/bearer	Unestam 1975; Souty-Grosset et al. 2006

*All parastacids tested to date were vulnerable, while only North American crayfish were resistant, thus, we assume *Pa. brasiliensis* as vulnerable, despite the lack of direct evidence.

Table 2 Mean carapace length (SD) (mm) of each crayfish species on each treatment

Group	**Carapace length (SD) (mm)**
***Parastacus brasiliensis* (vs. males)**	31.14 (1.53)
***Parastacus brasiliensis* (vs. females)**	26.70 (2.29)
***Parastacus brasiliensis* (intraspecific)**	27.12 (4.81)
***Procambarus clarkii* (males)**	30.71 (1.86)
***Procambarus clarkii* (females)**	27.33 (2.61)
***Procambarus clarkii* (males - intraspecific)**	33.62 (2.01)
***Procambarus clarkii* (females - intraspecific)**	31.03 (1.60)

Table 3 Ethogram codes for crayfish agonistic behavior (modified from Dalosto et al. 2013).

Score	Behavior
-2	Retreat with a tail flip.
-1	Retreat by walking away from the opponent.
0	Ignore the opponent/non-aggressive behaviors.
1	Approach without agonistic display.
2	Approach with meral spread and/or antennal whip.
3	Aggression with closed chelae: touching, punching and pushing the opponent.
4	Active use of the chelae to grab the opponent’s appendages, or chela strike.
5	Intense combat: animals performing several agonistic acts simultaneously, trying to grab and pull the opponent’s body parts, or attempting to turn/carry the opponent.

Table S1 Comparison of behavioral variables between the male and female controls. No statistically significant differences were found (p > 0.05, in all cases)

	t_8	U_8	p	**Test**
Latency	0.5662	---	0.5868	*t*-test
Highest intensity	---	0.1044	0.9168	Mann-Whitney test
Time until higher intensity	-0.9002	---	0.3943	*t*-test
Number of bouts	---	0.3133	0.7540	Mann-Whitney test
Mean bout duration	0.7665	---	0.4654	*t*-test
First bout duration	-0.0719	---	0.9444	*t*-test
Total fighting time	0.9033	---	0.3927	*t*-test

Table S2 Loadings of the principal component analysis of the behavioral variables of crayfish interspecific and intraspecific interactions

Variable	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**	**Axis 5**	**Axis 6**	**Axis 7**
Latency	0.57852	-0.77641	0.24934	-0.01311	-0.01277	0.00146	-0.0006
Highest intensity	0.00052	0.00073	0.00208	-0.00102	0.00126	0.24820	0.96870
Time to highest intensity	0.81507	0.55894	-0.15098	-0.01758	0.01189	-0.00129	-0.00024
Number of bouts	-0.00063	0.00359	0.00556	-0.02467	0.00931	0.96834	-0.24816
First bout duration	0.010247	0.02396	0.12348	0.67051	0.73103	0.00856	-0.00273
Mean bout duration	0.015837	0.03325	0.07005	0.72770	-0.68089	0.02341	-0.00453
Total fighting time	-0.02490	0.28824	0.94596	-0.14062	-0.039799	-0.00968	0.00015

ARTIGO 2: BEWARE OF THY ENEMY: INTERSPECIFIC AGGRESSION AND COMPETITOR RECOGNITION AMONG INVASIVE CRAYFISH SPECIES (CRUSTACEA: DECAPODA)

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Beware of thy enemy: interspecific aggression and competitor recognition among invasive crayfish species (Crustacea: Decapoda)

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Abstract

While predator naïveté has been the subject of many studies, competitor naïveté is far less understood, despite competition being an important ecological driver in many communities. Competitor naïveté results in competitors being unable to communicate and/or recognize each other, maximizing the costs of aggression without offering additional benefits. We hypothesize that crayfish species that have not undergone co-evolution and have no close phylogenetic affinity will be ecologically naïve: in interspecific fights, they will not decrease their aggressiveness over time contrary to what is observed in intraspecific fights. To test this, we staged dyadic fights of size-matched crayfish of four invasive species, divided in four treatments: (1) *Procambarus clarkii* intraspecific fights; (2) *P. clarkii* against *Orconectes limosus*; (3) *P. clarkii* against *Pacifastacus leniusculus*; and (4) *P. clarkii* against *Astacus leptodactylus*. We performed repeated fights for each dyad 24 h and 48 h after the first interaction. We quantified the total time fighting, the duration of the first aggressive bout, mean duration of bouts, number of bouts and the highest aggressive level reached, and compared them among the days within each treatment. In intraspecific fights, all aggressive levels were higher in the first day compared to the second and third days. Regarding interspecific fights, only the highest aggressive level decreased significantly, between the first and second days of the *O. limosus* fights. Our results demonstrate that these crayfish are ecologically naïve, and that such competitor naïveté may play a role in the disruptive effects caused by introduced crayfish species, since the inability to recognize superior competitors may be assymetrically detrimental to submissive individuals.

Key-words: Agonistic behavior, biological invasions, competitor naïveté, interference competition, hierarchy, chemical communication.

Introduction

Biological invasions are considered a harmful phenomenon, often resulting in biodiversity loss and economic harm (Simberloff 2014; Simberloff et al. 2013). Despite the large amount of evidence regarding the negative impacts of biological invasions, they may also provide interesting research opportunities. One such case occurs when species without any previous coexistence experience come into contact as a consequence of biological invasions (Carthey & Banks 2014). Such novel encounters often result in negative outcomes for one or both species. Examples include prey species that are not able to recognize a novel predator, or a species that fails to recognize the threat posed by a novel competitor (Le Breton et al. 2007; Gérard et al. 2014). This non-adaptive response of such novel encounters is referred to as ecological naïveté, and may refer to any novel antagonistic interaction, such as a native predator and invasive prey (e.g. Webb et al. 2008); an invasive predator and a native prey (e.g. Anson & Dickman 2013; Gérard et al. 2014); and competition between an invasive and a native species (e.g. Le Breton et al. 2007; Heavener et al. 2014).

Naïveté has been classified in different levels according to the response of the naïve species, ranging from no response through inappropriate/insufficient response, to an exaggerated response (Banks & Dickman 2007; Carthey & Banks 2014). While the effect of naïveté in novel ecological interactions has been the subject of many studies, most of them focus mainly on predation, particularly in situations presenting an invasive predator against a naïve native prey (e.g. Verhoeven et al. 2009; Sih et al. 2010; Gérard et al. 2014). Conversely, investigations of ecological naïveté in a competition context have received far less attention (e.g. Le Breton et al. 2007; Heavener et al. 2014), despite the fact that the theoretical assumptions regarding naïveté and competition are largely the same of those related to predation (see Carthey & Banks 2014).

The main consequence of competitor naïveté is the inability to recognize and/or effectively communicate with a heterospecific competitor. Competitor recognition is expected to occur when species competing over a resource benefit from recognizing each other and avoiding unnecessary aggression (Cody 1969, 1973), in a similar way to intraspecific aggression/competition (Maynard-Smith 1982). However, when formerly allopatric species that are not phylogenetically close come into contact, they are unlikely to be able to recognize each other and effectively communicate, regardless of their niche overlap, simply because they evolved in isolation from each other (De Kort & Ten Kate 2001; Ghrether et al. 2009). The investigation of competitor naïveté is important because understanding its potential role in species replacement (a common consequence of biological invasions) can help us to better understand invasion processes and make more accurate predictions in ongoing and future cases. Also, since resources to deal with invasions are often limited (Vander Zanden et al. 2010), researchers, conservation biologists and government agencies would greatly benefit from any information that helps them to better allocate their resources. Lastly, investigating competitor naïveté may also raise interesting questions regarding the evolution of interspecific communication and the factors potentially related to it (see Grether et al. 2009).

Crayfish are frequently introduced species in many ecosystems. They are aggressive animals that readily attack each other (Gherardi 2002). They form linear dominance hierarchies, which are established and maintained by chemical communication via urine release (Breithaupt & Eger 2002; Berry & Breithaupt 2008, 2010). Such hierarchies manifest as a steady reduction in aggressive levels over time and are thought to minimize the cost of aggressive interactions, where a subordinate would avoid repeated encounters with a dominant individual which the subordinate is unlikely to win (Goessmann et al. 2000). The high social status obtained within this hierarchies is also thought to increase access to resources (Maynard Smith 1982), and there is evidence for this in crayfish (Herberholz et al. 2007; Fero & Moore 2008). When communication is impaired and/or crayfish are not able to form these hierarchies, aggression does not diminish over time, and may even escalate to unusually high levels, resulting in competitor naïveté (Gherardi & Cioni 2004; Horner et al. 2008).

Crayfish are considered ecological generalists, and thus many species have overlapping niches (Bovbjerg, 1970; Nyström 2002). Due to these niche overlappings, interspecific aggression is an important aspect of crayfish ecology almost as much as intraspecific aggression (Hudina & Hock 2012). Interspecific aggression is often considered a good proxy for the ecological patterns observed in nature, such as higher abundances of one species compared to another, or species replacement in cases of biological invasions (Bovbjerg 1970; Hudina & Hock 2012; Dalosto et al. 2015). Crayfish interspecific aggression can involve many different scenarios, including interactions between native species (Bovbjerg 1970; Barbaresi & Gherardi 1997) between invasive species (Hudina et al. 2011, Hudina & Hock 2012) and between invasive and native species (Gherardi & Daniels 2004; Gherardi & Cioni 2004). Crayfish are also invaders in many ecosystems due to their ecological plasticity, and encounters between formerly allopatric species are common (Gherardi 2006; Souty-Grosset et al. 2006; Gherardi et al. 2011). The importance of interspecific aggression and hierarchy formation in regulating access to resources and reducing the costs of aggression make crayfish good candidates for the study of ecological naïveté within the context of interspecific aggression/competition.

Considering the potential consequences of competitor naïveté, such as loss of competitor recognition resulting in excessive aggression levels, and the relative scarcity of studies, the goal of this study is to test the ability of certain ecologically similar species of invertebrates to communicate with each other by comparing intraspecific to interspecific aggression. Given that: (1) species that are ecologically similar but did not undergo co-evolution are unlikely to be able to effectively communicate; and (2) biological invasions often result in encounters of species that did not undergo such co-evolutioné; we hypothesize that invasive crayfish species that evolved in isolation from each other will not be able to effectively communicate in aggressive interactions, and will not form the stable dominance relationships typical of crayfish intraspecific aggression. Specifically, we predict that behavioral indicators of aggressiveness (latency, latency to escalate, first bout duration, mean bout duration, total fighting time and maximum aggressive level) of interspecific fights of crayfish will not show alterations over repeated interactions, unlike in intraspecific fights.

Material and Methods

Model species

We performed experiments using four species of crayfish: the red swamp crayfish (*Procambarus clarkii*), the spiny-cheek crayfish (*Orconectes limosus*), the signal crayfish (*Pacifastacus leniusculus*) and the narrow-clawed crayfish (*Astacus leptodactylus*). We chose these species because they do not co-occur in their native ranges, but have been introduced in many places, such as in France, where their distribution overlaps (Souty-Grosset et al. 2006; Gherardi et al. 2013). Additionally, these crayfish occupy similar niches, and thus are very likely to compete with each other (see Hudina et al. 2011; Hudina & Hock 2012). We chose *P. clarkii* as the reference species to compare intra vs interspecific aggression, because it is the most frequently introduced species of crayfish, the most abundant in the field, and also because its behavior is well documented in a number of publications (e.g. Issa et al. 1999; Aquiloni et al. 2012; Gherardi et al. 2013).

Crayfish sampling and acclimation

We captured the crayfish between July and November 2013. Only crayfish from allopatric populations were chosen (i.e. no species had any previous contact with each other), and we selected only intermolt adult males with all appendages intact. For cambarids (*P. clarkii* and *O. limosus*), we chose only crayfish in the reproductive form I, since this is known to affect their aggressive behavior (Moore 2007). We collected *P. clarkii* (N = 50) and *O. limosus* (N = 10) in ponds at the Parc Naturel Régional de la Brenne (46°46′61″N, 1°21′83″E and 46°72′85″N, 1°27′72″E for *P. clarkii* and *O. limosus*, respectively). Individuals of *P. leniusculus* (N = 10) were caught in the Le Miosson stream in Poitiers, France (46°33′04′′N, 0°21′49′′E) while *A. leptodactylus* (N = 10) were obtained from a commercial supplier, which imported them from Turkey. We placed the crayfish in thermal boxes and brought them to the laboratory of the University of Poitiers. We then acclimated the crayfish in communal aquaria (60x38x20 cm), each containing 5-7 crayfish of the same species. The aquaria were maintained at room temperature (18 ± 2 ºC), and food (carrots) was offered twice a week. After at least five days of acclimation in such conditions, we separated the crayfish in dyads of size-matched individuals, according to the carapace length (CL – measured in mm from the orbital sinus to the posterior edge of the carapace). We established these dyads with a maximum difference of 10% in CL to avoid biasing the results of the experiments (Ayres-Peres et al. 2011). Members of each dyad were always from separated experimental aquaria (i.e. never had previous contact with each other prior to the experiments). We then isolated the selected individuals of each dyad for one week in individual aquaria (54x37x20 cm), with food being offered only in the first and last days of acclimation, to standardize fighting motivation (Dalosto et al. 2013).

Experimental treatments

We distributed the crayfish in four experimental treatments that differ in the composition of the fighting dyads (N = 10 pairs per treatment), which were:

1) Control (CO): Two size-matched individuals from the same *P. clarkii* population;

2) *Orconectes* (OL): An interspecific size-matched pair of *P. clarkii* and *O. limosus*;

3) *Pacifastacus* (PL): An interspecific size-matched pair of *P. clarkii* and *P. leniusculus*;

4) *Astacus* (AL): An interspecific size-matched pair of *P. clarkii* and *A. leptodactylus*;

Experimental procedure

After individual acclimation, we transferred the crayfish to an experimental aquarium (39x21x25 cm) filled with de-chlorinated tap water, without substrate and any resource, and separated in two equal compartments by a removable plastic divider. We placed members of each pair in separate ends of the aquarium, and allowed them to acclimate for 10 min. After that, we removed the divider and allowed crayfish to interact for 30 min, and recorded them with a video camera (JVC – MG332) positioned 60 cm perpendicularly above the aquarium. Experiments were performed from 12:00 h to 18:00 h in a room with artificial dim light, since these crayfish are active in both day and night time (Gherardi 2002; Musil et al. 2010). After each trial, we placed each crayfish back in their individual acclimation aquaria. We then repeated the same procedure for the same pairs 24 h and 48 h after the first trial. Following that, we returned the crayfish to their individual aquaria and monitored them for five days. If any crayfish died or molted during this interval, their data would be discarded from analysis (this did not happen). Lastly, we returned the crayfish to their communal aquaria where they would remain until the end of the experiment, when they were euthanized through cryoanesthetizing, as French legislation forbids the release of invasive species in the wild (an exception was granted for this study allowing the transport and maintenance of live invasive crayfish, under the prefectoral order 2013_DDT_SEB N°262).

Behavioral quantifications

We analyzed interactions according to ethogram codes based on Moore (2007) and Dalosto et al. (2013) (Table 1). We defined an aggressive bout as a crayfish exhibiting an aggressive behavior responded by another aggressive behavior from its opponent. These behaviors included approaching the opponent with displays, antennal whipping, touching and/or grabbing with chelipeds, and lunging towards the opponent, and are summarized in Table 1. A bout had a minimal duration of 5 s, and was finished when one crayfish exhibited a submissive behavior (walk away, tail-flip or submissive posture, with chelipeds lowered and the abdomen curled beneath the cephalothorax) and did not engage the other for at least 10 s. We considered the crayfish that exhibited the submissive behavior as the loser of the bout, and its opponent as the winner. The crayfish that won more bouts was considered the winner of the interaction. For each dyadic interaction, we determined values of: (1) latency – the time until the first fight between crayfish; (2) the maximum aggressive level reached (0-5, Table 1); (3) the time until the maximum aggressive level; (4) number of bouts; (5) duration of the first bout; (6) mean duration of bouts; and (7) total time spent fighting.

Statistical analyses

Within each treatment, we tested if aggression would diminish at each day, which would indicate that crayfish were forming a stable dominance order. To do so, we tested how five behavioral variables (highest intensity, number of bouts, first bout duration, mean bout duration and total fighting time) varied among the three experimental days. We used a one-way Friedman’s Rank Sum Test for the highest intensity (since these were rank data), and a one-way repeated-measures ANOVA for the remaining variables. We used paired t-tests as a post-hoc for the repeated-measure ANOVAs, and a Wilcoxon signed-rank test for the Friedman's Rank Sum Test. We could not perform similar tests for the latency and time to reach the highest intensity because these values were missing for the second and third days of many crayfish pairs, which would result in very low statistical power. Instead, we used a one-way ANOVA followed by a Tukey HSD post-hoc test to analyze if the latency and time to the highest intensity differed between the experimental treatments when considering only the first day. We log-transformed the data for all ANOVAs in order to achieve normality and homoscedasticity. All statistical analyses were performed with the R software (R Development Core Team 2013).

Results

Summary of contests

Crayfish performed all the typical behaviors described for their aggressive interactions (see Moore 2007): often employing displays (e.g. meral spreads) when approaching the opponents, initiating contests with low-intensity behaviors (e.g. touching with chelae) and later escalating them towards more aggressive behaviors (e.g grasping with chelae). This escalation always occurred, but was faster in some interspecific treatments (see below). *Procambarus clarkii* was the dominant species in the OL and AL treatments, where it won all fights. In the PL treatment, the dominant species varied, *P. clarkii* won only 30% of the interactions on the first day, 50% of the interactions on the second day, and 60% of the interactions on the third day.

An unusual behavior happened in the OL treatment, where the *P. clarkii* individual grasped its *O. limosus* opponent, turned it backwards and eventually mounted it (Fig. S1). This happened in three replicates during the first day, and happened again for one of these dyads on the second day. This behavior largely resembles the pseudo-copulation reported by Issa & Edwards (2006) for pairs of *P. clarkii* males, except that: (1) *O. limosus* always kept its abdomen flexed, unlike female crayfish or subordinate male *P. clarkii*, which keep it extended; and (2) spermatophore deposition was not observed here, unlike in other reported cases.

Some contests escalated dangerously in the PL and AL treatments, which led us to suspend any further experiments in the AL treatment, but not in the PL treatment since this occurred in two replicates of the third day, the last day of experiments. We had to exclude three replicates of the AL treatment for the repeated-measures ANOVAs, since some fights escalated dangerously during the second day, meaning that some data for the third day fights were missing. Thus, the repeated-measures ANOVAs for the AL replicates had only seven instead of ten replicates.

Aggressive behaviors along the days

We found that the total fighting time, first bout duration, mean bout duration and number of bouts were higher in the first day of the control treatment than in the second and third days ($p < 0.05$ in all cases; see Table 2 for details) (Figs. 1-4). We also found that the highest aggressive level was higher in the first day than in the second and third days of the control treatment, and higher in the first day than in the second day of the OL treatment ($p < 0.05$; see Table 2) (Fig. 5). A summary of the statistical analyses between experimental treatments is shown on Table 2, and values of the post-hoc analyses are shown in Table 3.

Latency and escalation during the first day

We also found significant differences for the latency ($F_{1,3} = 3.487$; $p = 0.026$; Fig. 6) and time to reach the highest intensity ($F_{1,3} = 3.544$; $p = 0.022$; Fig. 7) between treatments during the first experimental day: latency was lower in the PL treatment than in the OL treatment (Tukey HSD test; $p = 0.016$); and time to the highest intensity was lower in the PL treatment than in the control treatment (Tukey HSD test; $p = 0.017$).

Discussion

Our data are in accordance with the hypothesis that competitor naïveté is expected when novel ecological interactions occur, since a consistent decrease in aggressive behaviors, a consequence of competitor recognition and subsequent communication, occurred only in intraspecific pairs of crayfish. In accordance with our hypothesis, interspecific pairs did not de-escalate their aggression over time, despite the investigated species having similar morphologies and exhibiting many shared behaviors (such as displays) in their aggressive interactions (Gherardi 2002; Moore 2007).

This apparent inability to recognize and effectively communicate with a heterospecific competitor could be directly related to the extinction of native crayfish species and its replacement by invaders. Such species replacement is thought to occur via an interplay of two factors: Pathogen transmission (Unestam 1975; Souty-Grosset et al. 2006) and competition/predation pressure (Gherardi et al. 2013; Dalosto et al. 2015). Competitor naïveté is very likely to intensify competition pressure in these situations, since crayfish would insist in repeated fights whose result is, in most cases, unlikely to change. Although such non-decreasing aggression is non-adaptive for both species involved, it would be much more harmful to the species that loses the interactions. The dominant species would spend energy and time fighting repeatedly but would very likely maintain possession of the disputed resource, whatever it may be. Conversely, the subordinate would repeatedly insist in fights that it is very unlikely to win and without obtaining any benefit to offset its expenditure of time and energy, unlike in intraspecific fights where subordinates quickly learn to avoid dominant crayfish (e.g. Issa et al. 1999; Sato & Nagayama 2012). Such disruptive effects arising from the incapacity of certain crayfish to recognize a threat have already been mentioned by some authors (Gherardi & Cioni 2004; Dalosto et al. 2015), but as far as the authors are concerned, this is the first time that the hypothesis that crayfish without co-existence cannot form stable dominance was directly tested.

In addition, fights of the PL treatment reached very high aggressive levels in all days (Fig. 5) and were quicker to start and to escalate (Figs. 6 and 7). Although interspecific aggression exhibits an overall trend to be more intense than intraspecific aggression (Lizards: Lailvaux et al. 2012; Crayfish: Hudina & Hock 2012; Ants: Liu et al. 2015), we must also consider the specific behavioral patterns of each species. In particular, *P. leniusculus* is known to be a very aggressive species, and its eagerness to initiate fights and to escalate them to high levels has been suggested to be a potentially adaptive trait when encountering novel opponents (Hudina & Hock 2012). While this seems likely at a short time-scale, since *P. leniusculus* won 70% interactions against *P. clarkii* in the first day, this pattern changed along time: in the end of the experiments, *P. clarkii* was the dominant species despite the initial successes of some *P. leniusculus* individuals. The strategy exhibited by *P. leniusculus* may be adaptive when there is a stable hierarchy to offset the energy expenditure, or when there is naïveté but its opponent is not a particularly aggressive species (such as *O. limosus*, Kozák et al. 2007; Chucholl et al. 2008). However, when there is competitor naïveté and its opponent is an equally aggressive species (such as *P. clarkii*, Alonso & Martínez 2006), this apparent eagerness to fight may not prove too effective. The dominance inversions seen in this treatment can also be a result of species-specific traits, such as energy reserves or levels of oxidative stress, which can influence how much an animal can/is willing to invest in aggressive behaviors (Briffa & Sneddon 2007; Metcalfe & Alonso-Alvarez 2010).

The level of naïveté observed in this study could be classified between level 1 (complete lack of response) and level 2 (inappropriate response) (Banks & Dickman 2007; Carthey & Banks 2014). Crayfish almost never avoided contact with dominant heterospecifics, but could eventually show some reduction in aggressiveness (as seen for the highest aggressive level in the OL treatment, Fig. 5). Although there is still some debate in this topic, dominance in crayfish is thought to be maintained by social status recognition via urine-mediated chemical communication, and likely to be reinforced by loser effects (Zulandt-Schneider et al. 2001; Aquiloni et al. 2012). When chemical communication is impaired, crayfish cannot form stable dominance relationships, and aggression levels and the number of fights do not decrease over time, as they would under normal conditions (Issa et al. 1999; Delgado-Morales et al. 2004; Horner et al. 2008). It is possible that in the interspecific fights, crayfish were not able to access the social status of their opponent (Horner et al. 2008; Aquiloni et al. 2012). This would in turn prevent them from being primed by their opponents' social status, and the loser effect and subsequent hierarchy formation would not occur. Our results are remarkably similar to what is reported to crayfish and lobsters when they are not allowed to use chemical communication, since aggressiveness did not decrease in interspecific fights (Karavanich & Atema 1998; Delgado-Morales et al. 2004; Horner et al. 2008) (Figs.1-5), which support our hypothesis that crayfish species that evolved in isolation are not able to effectively communicate and form stable dominance orders.

Another factor that must be taken into consideration are phylogenetic relationships. *Procambarus clarkii* is known to form stable dominance with *Procambarus acutus* (Gherardi & Daniels 2004). These species do not naturally co-occur, but their occurrence areas are close to each other, meaning that they might have co-occurred in the past, and they are more phylogenetically close that any of the species investigated in this study (Breinholt et al. 2009; Owen et al. 2015). Though our experimental design did not control for phylogeny, interactions between species with greater phylogenetic distance (*P. leniusculus* and *A. leptodactylus* are astacids, while *O. limosus* and *P. clarkii* are cambarids) were also those that reached the highest levels of aggressiveness: fights escalated dangerously in two replicates of the PL treatment in the third day and in three replicates of the AL treatment in the second day. The OL treatment, where the opponents belonged to the same family, was the only interspecific treatment where a significant difference in a behavioral variable was found between different days (the highest aggressive level reached, Fig. 5). It was also the only treatment where an unusual copulation-like behavior was displayed (Fig. S1). These findings mean that there could have been some sort of partial communication between *P. clarkii* and *O. limosus* with some inaccurate exchange of information, but not enough to form a stable hierarchy. Given the importance of interspecific aggression to crayfish biology (Hudina & Hock 2012; Dalosto et al. 2015), our results suggest that a study investigating interspecific aggression in crayfish with a control for phylogeny and co-evolution would be particularly interesting to determine the boundaries of interspecific communication and accurately predict cases where naïveté is expected to occur.

In conclusion, we have demonstrated that *P. clarkii* seems unable to recognize and form stable dominance with crayfish species with which it lacks co-evolution and close phylogenetic affinity, comprising a clear case of competitor naïveté. Our results also suggest that interactions between introduced and native crayfish (an unfortunately common scenario) might be more disruptive than previously thought, since naïveté in these cases potentially enhances the impact of interference competition. Since interspecific aggression has received far less attention than intraspecific aggression (Peiman & Robinson 2010), the limited availability of data for comparison may hinder our conclusions. However, a number of questions arise from our results: How do phylogeny and co-evolution relate to competitor naïveté? Can co-existence for a certain period change the observed pattern? Crayfish remain an interesting group to answer such questions, since their phylogeny is relatively well-known and stable (Breinholt et al. 2009; Owen et al. 2015) and many of the invasion cases by some species are well documented in both time and space (Souty-Grosset et al. 2006; Gherardi et al. 2011).

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Conflicts of interest

We declare none.

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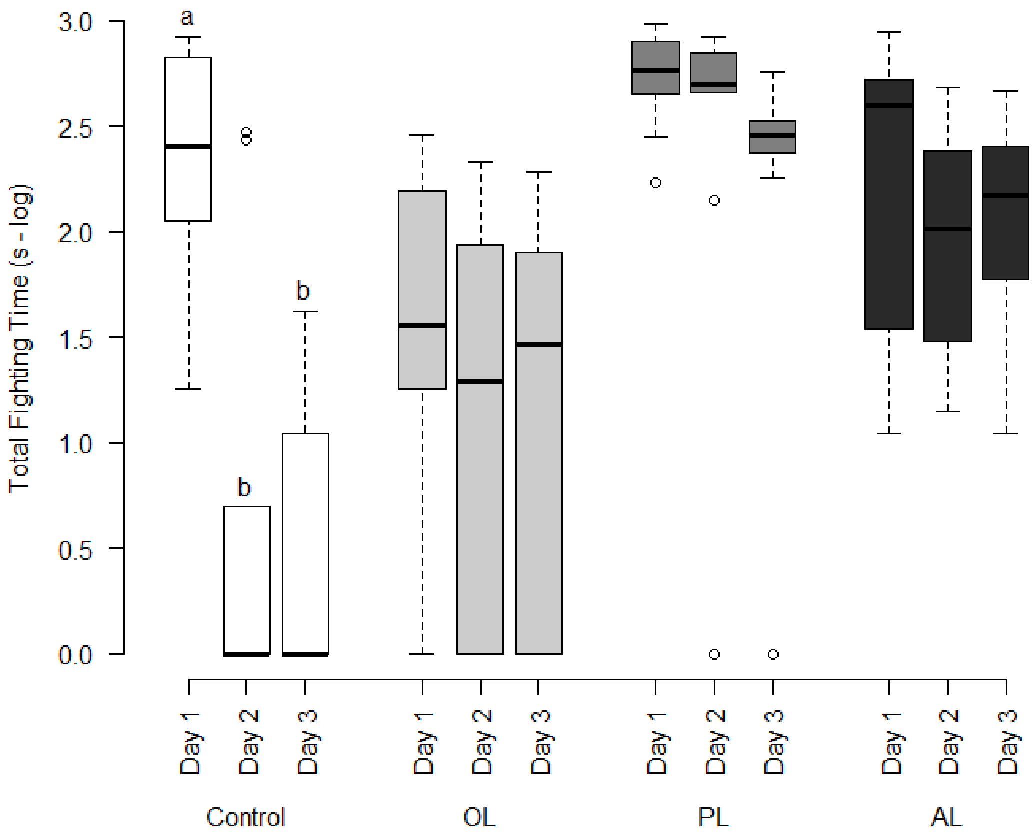

Fig. 1 Total fighting time (seconds – log transformed) of crayfish in intra- and interspecific fights along three consecutive days; white bars = Control treatment; light gray bars = *Orconectes* treatment (OL); gray bars = *Pacifastacus* treatment (PL); dark gray bars = *Astacus* treatment (AL); different letters indicate significant differences between days, within each treatment; bar height indicates range between 1st and 3rd quartiles, bold horizontal bar indicates the median, whiskers indicate data range, and white circles indicate outliers

Fig.

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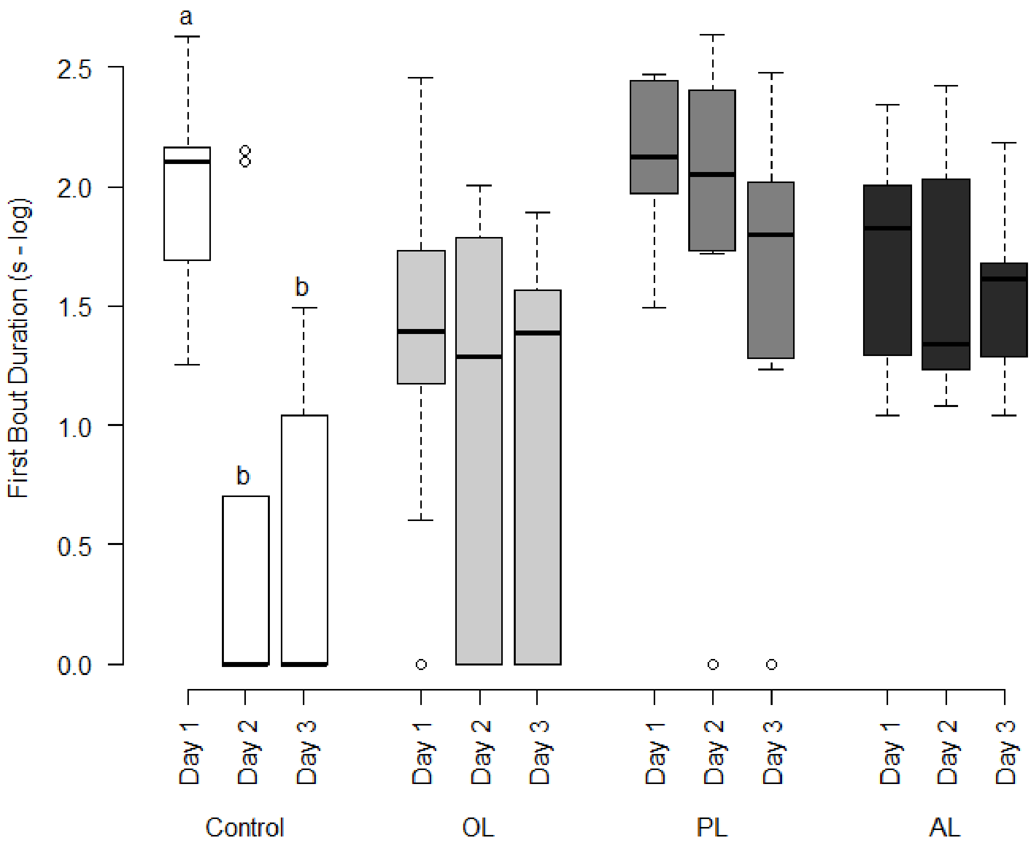

First bout duration (seconds – log transformed) of crayfish in intra- and interspecific fights along three consecutive days; white bars = Control treatment; light gray bars = *Orconectes* treatment (OL); gray bars = *Pacifastacus* treatment (PL); dark gray bars = *Astacus* treatment (AL); different letters indicate significant differences between days, within each treatment; bar height indicates range between 1st and 3rd quartiles, bold horizontal bar indicates the median, whiskers indicate data range, and white circles indicate outliers

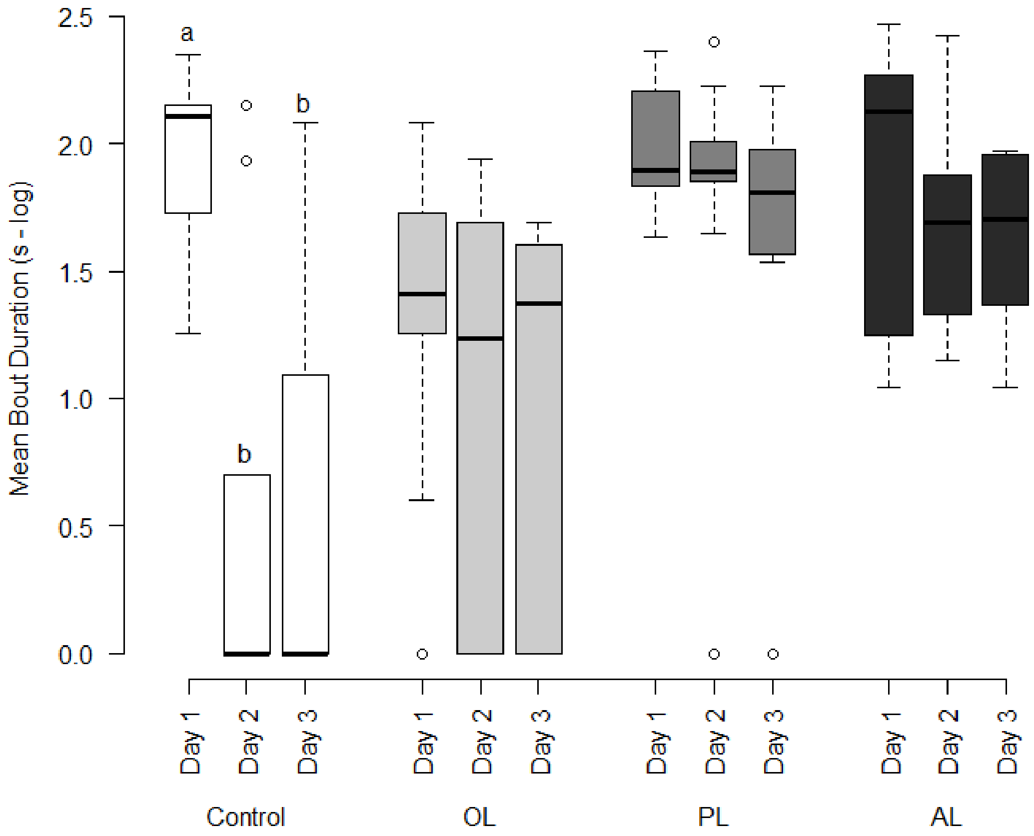

Fig. 3 Mean bout duration (seconds – log transformed) of crayfish in intra- and interspecific fights along three consecutive days; white bars = Control treatment; light gray bars = *Orconectes* treatment (OL); gray bars = *Pacifastacus* treatment (PL); dark gray bars = *Astacus* treatment (AL); different letters indicate significant differences between days, within each treatment; bar height indicates range between 1st and 3rd quartiles, bold horizontal bar indicates the median, whiskers indicate data range, and white circles indicate outliers

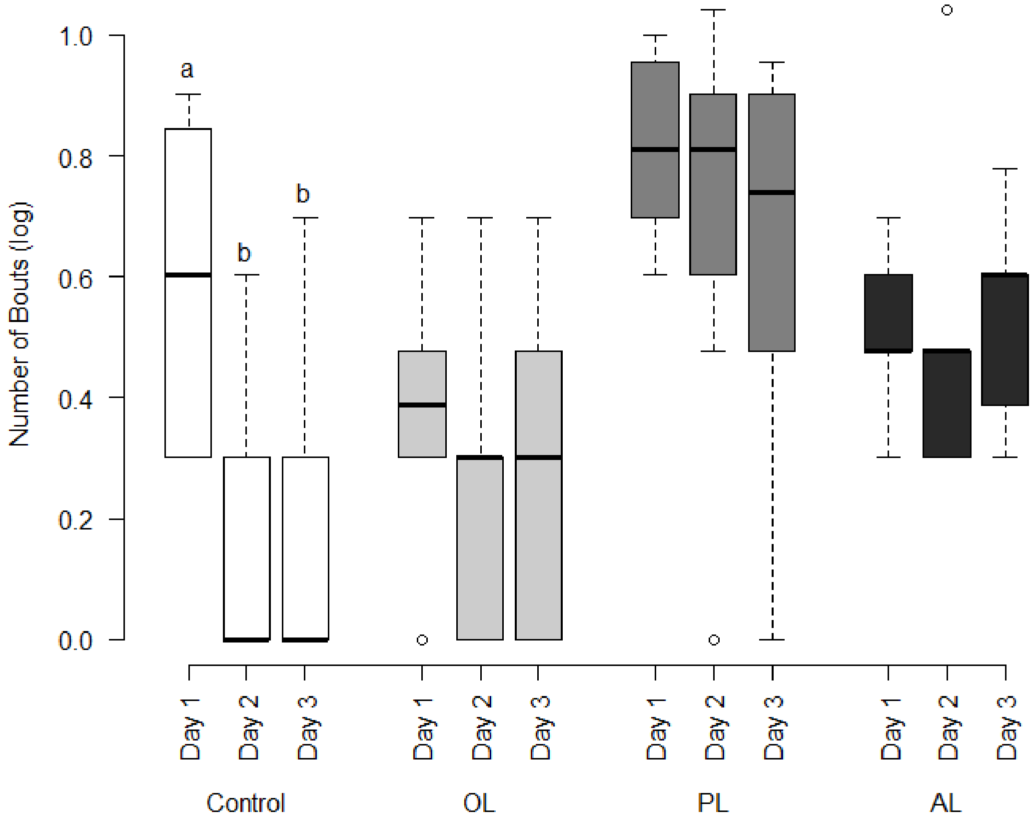

Fig. 4 Number of bouts (log transformed) of crayfish in intra- and interspecific fights along three consecutive days; white bars = Control treatment; light gray bars = *Orconectes* treatment (OL); gray bars = *Pacifastacus* treatment (PL); dark gray bars = *Astacus* treatment (AL); different letters indicate significant differences between days, within each treatment; bar height indicates range between 1st and 3rd quartiles, bold horizontal bar indicates the median, whiskers indicate data range, and white circles indicate outliers

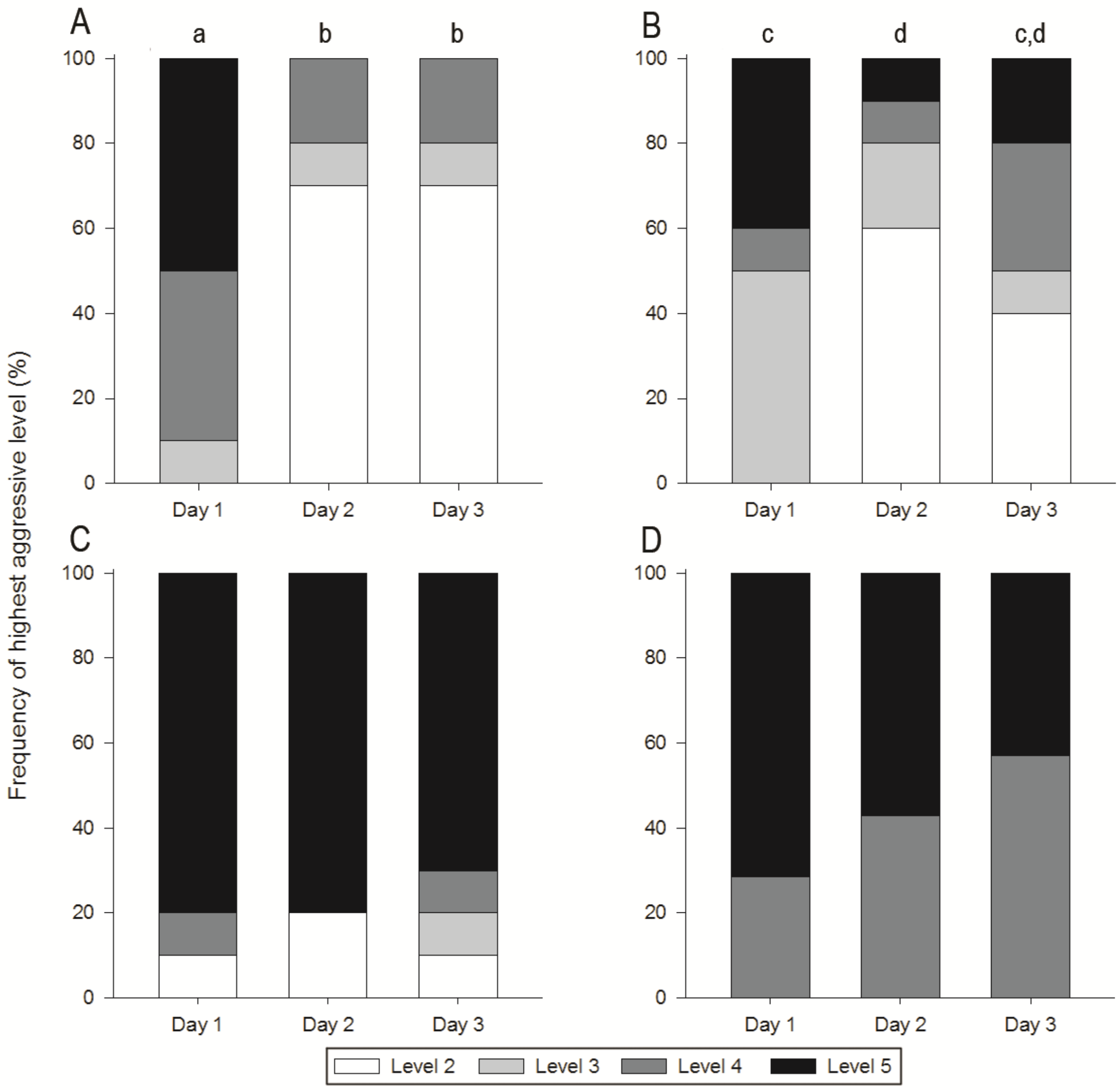

Fig. 5 Frequencies of the highest aggressive level reached for crayfish intra- and interspecific fights along three consecutive days; (A) Control treatment; (B) *Orconectes limosus* treatment; (C) *Pacifastacus leniusculus* treatment; (D) *Astacus leptodactylus* treatment; different letters indicate significant differences between the days, within each treatment

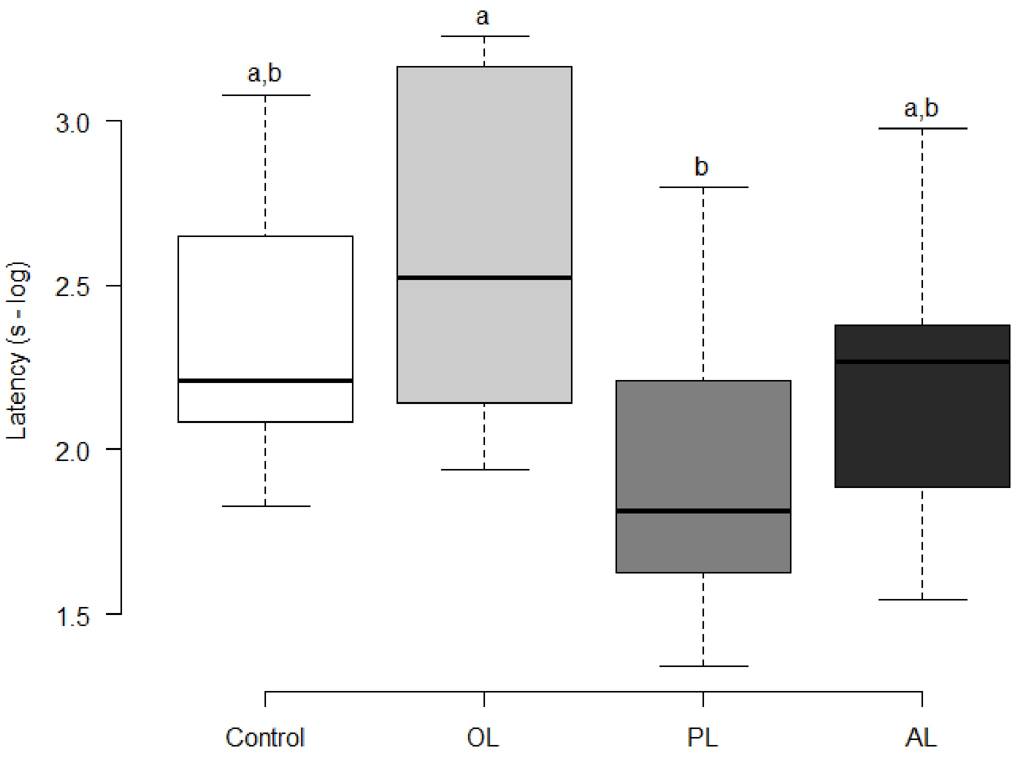

Fig. 6 Latency period (seconds – log transformed) of crayfish during the first day of intra- and interspecific fights; white bars = Control treatment; light gray bars = *Orconectes* treatment (OL); gray bars = *Pacifastacus* treatment (PL); dark gray bars = *Astacus* treatment (AL); different letters indicate significant differences between treatments; bar height indicates range between 1st and 3rd quartiles, bold horizontal bar indicates the median, whiskers indicate data range, and white circles indicate outliers

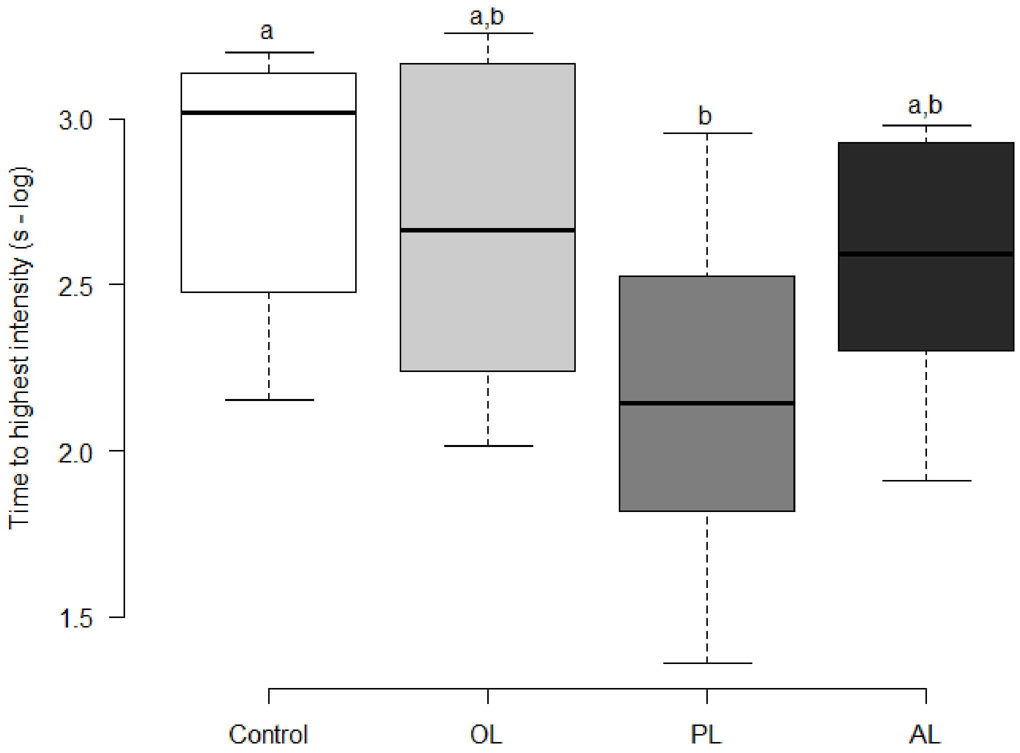

Fig. 7 Time until the highest aggressive level (seconds – log transformed) of crayfish during the first day of intra- and interspecific fights; white bars = Control treatment; light gray bars = *Orconectes* treatment (OL); gray bars = *Pacifastacus* treatment (PL); dark gray bars = *Astacus* treatment (AL); different letters indicate significant differences between treatments; bar height indicates range between 1st and 3rd quartiles, bold horizontal bar indicates the median, whiskers indicate data range, and white circles indicate outliers

Table 1 Ethogram codes for crayfish agonistic behavior (modified from Dalosto et al. 2013)

Score	Behavior
-2	Retreat with a tail flip.
-1	Retreat by walking away from the opponent.
0	Ignore the opponent/non-aggressive behaviors.
1	Approach without agonistic display.
2	Approach with meral spread and/or antennal whip.
3	Aggression with closed chelae: touching, punching and pushing the opponent.
4	Active use of the chelae to grab the opponent’s appendages, or chela strike.
5	Intense combat: animals performing several agonistic acts simultaneously, trying to grab and pull the opponent’s body parts, or attempting to turn/carry the opponent.

Table 2 Results of the within-treatment comparisons for each behavioral variable quantified along experimental days in crayfish intra- and interspecific fights; OL = interspecific fights vs. *Orconectes limosus*; PL = interspecific fights vs. *Pacifastacus leniusculus*; AL = interspecific fights vs. *Astacus leptodactylus*

Variable	Treatment	Test	DF	*p*	F	Friedman's χ^2
Total fighting time	Control	Repeated-measures ANOVA	1,9	<0.0001	44.89	-
	OL	Repeated-measures ANOVA	1,9	0.1060	3.218	-
	PL	Repeated-measures ANOVA	1,9	0.0847	3.753	-
	AL	Repeated-measures ANOVA	1,6	0.5510	0.399	-
First bout duration	Control	Repeated-measures ANOVA	1,9	0.0002	36.28	-
	OL	Repeated-measures ANOVA	1,9	0.0596	4.644	-
	PL	Repeated-measures ANOVA	1,9	0.0854	3.733	-
	AL	Repeated-measures ANOVA	1,6	0.3750	0.916	-
Mean bout duration	Control	Repeated-measures ANOVA	1,9	0.0001	43.29	-
	OL	Repeated-measures ANOVA	1,9	0.0538	4.914	-
	PL	Repeated-measures ANOVA	1,9	0.1790	2.121	-
	AL	Repeated-measures ANOVA	1,6	0.6220	0.270	-
Number of bouts	Control	Repeated-measures ANOVA	1,9	0.0021	18.23	-
	OL	Repeated-measures ANOVA	1,9	0.2060	1.858	-
	PL	Repeated-measures ANOVA	1,9	0.0691	4.258	-
	AL	Repeated-measures ANOVA	1,6	0.9330	0.008	-
Highest intensity	Control	Friedman's test	1,2	0.0027	-	11.793
	OL	Friedman's test	1,2	0.0063	-	10.138
	PL	Friedman's test	1,2	0.9048	-	0.200
	AL	Friedman's test	1,2	0.3679	-	2.000

Table 3 Results of the post-hoc pairwise comparisons of the behavioral variables at each day; OL = interspecific fights vs. *Orconectes limosus*

Variable	**Treatment**	**Test type**	**Paired comparison**	***p***
Highest intensity	Control	Wilcoxon Signed-rank	day1 x day2	0.014
Highest intensity	Control	Wilcoxon Signed-rank	day1 x day3	0.012
Highest intensity	OL	Wilcoxon Signed-rank	day1 x day2	0.012
Total fighting time	Control	Paired *t*-test	day1 x day2	0.012
Total fighting time	Control	Paired *t*-test	day1 x day3	<0.001
First bout duration	Control	Paired *t*-test	day1 x day2	0.012
First bout duration	Control	Paired *t*-test	day1 x day3	<0.001
Mean bout duration	Control	Paired *t*-test	day1 x day2	0.009
Mean bout duration	Control	Paired *t*-test	day1 x day3	<0.001
Number of bouts	Control	Paired *t*-test	day1 x day2	0.017
Number of bouts	Control	Paired *t*-test	day1 x day3	0.006

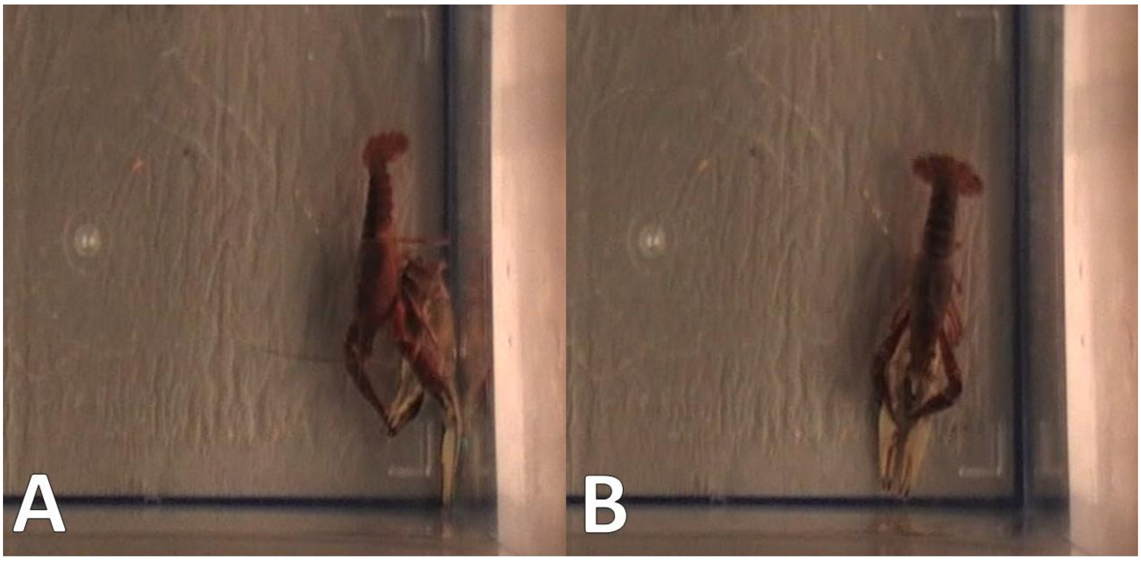

Fig. S1 Copulation-like behavior of *Procambarus clarkii* and *Orconectes limosus*; A) *P. clarkii* attacks *O. limosus* and grabs its chelipeds; B) *P. clarkii* turns *O. limosus* to a supine position and mounts it

ARTIGO 3: NOT THE BIGGEST, BUT THE STRONGEST: WEAPON STRENGTH, NOT BODY SIZE, PREDICTS INTERSPECIFIC DOMINANCE IN AGGRESSIVE INTERACTIONS BETWEEN TWO FRESHWATER DECAPODS (CRUSCATEA)

Artigo a ser submetido para a revista Ethology.

Not the biggest, but the strongest: weapon strength, not body size, predicts interspecific dominance in aggressive interactions between two freshwater decapods (Cruscatea)

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Running title. Weapon strength predicts winning species in interspecific aggression

Number of words. 5216

Abstract

Aggressive interactions between species is common in nature, but are still poorly understood. One factor related to it is the heterogeneity of experimental designs, which can be troublesome because the relationship between the ability to win contests and size of the contestants (the most commonly measured variable) is not identical between species, thus compromising potential conclusions. Following this, an approach that includes more objective variables, such as weapon performance, may comprise a more robust method to investigate interspecific aggression. We used the aeglid *Aegla longirostri* and the crayfish *Parastacus brasiliensis* to test if differences in body size, body weight and weapon strength accurately predict the outcome of interspecific fights. We hypothesize that (1) differences in claw strength will predict the winner of interspecific fights, and that (2) aggressiveness will increase as difference in weapon strength decreases. To do so, we performed fights of randomly-assigned interspecific pairs. We used size, weight and strength differences as predictor variables, and measures related to fight intensity and outcome as response variables. Weapon strength siginificantly predicted the winning species, with the likelihood of *A. longirostri* winning increasing as difference in strength decreased, corroborating our first hypothesis. However, no increase in aggressiveness was related to weapon strength. Instead, contests escalated faster when size difference was greatest, rejecting our second hypothesis. These results suggest that studies investigating aggression between species with similar weaponry should consider weapon strength as a factor. Our results agree with statements that aeglids hold a competitive advantage over crayfish, being more likely to win when weapon-matched, despite their smaller sizes. The faster-escalating fights of animals with large size differences might be a result of large animals seeing themselves as potential winners and thus escalating fights faster, though this requires further testing. Lastly, we highlight the need of a standardized methodology for laboratorial tests interspecific aggression, in order to be able to more accurately discuss the significance of this ecological interaction.

Keywords: *Aegla*; Experimental design; Interspecific aggression; Interference competition; *Parastacus*; Weapon performance.

Introduction

Aggressive behavior is widespread in many animal taxa, and can affect many aspects of an animal life history, such as reproductive success, resource acquisition and maintenance and growth (Corréa et al. 2003; Vøllestad & Quinn 2003; Lailvaux et al. 2012; Martin & Ghalambor 2014). Such behavior might occur not only between members of the same species, but also between individuals of species with considerable phylogenetic distance (e.g. Gherardi & Cioni 2004; Kindinger 2015). Interspecific aggression can result in one species obtaining a competitive advantage over limited resources such as territories (e.g. Tannerfeldt et al. 2002) or food (e.g. Dalosto et al. 2015), much like what happens in intraspecific aggression. However, despite interspecific aggression being very common (see Peiman & Robinson 2010) and having the potential to affect individual fitness, it has received far less attention than intraspecific aggression, and many of its aspects remain comparatively poorly understood (Peiman & Robinson 2010; Grether et al. 2013).

One of the possible reasons for this discrepancy between publications concerning intra- and interspecific aggresison is a lack of a theoretical framework, defined nomenclature and methods (Peiman & Robinson 2007; Grether et al. 2009). This is particularly clear when interspecific aggression is investigated in controlled experiments. When investigating intraspecific aggression, animals are often size-matched so that the effect of size can be controlled for (e.g. fish: Earley & Dugatkin 2006; beetles: Chamorro-Florescano et al. 2011; lizards: Martín & Lopéz 2011; crayfish: Dalosto et al. 2013). Meanwhile, interspecific aggression studies are usually limited to cases where the species have very similar morphologies (e.g. crayfish: Vorburger & Ribi 1999; lizards: Korner et al. 2000; fish: McCormick & Weaver 2012). When competitors have relatively different morphologies, the experimental approach may vary. Some studies adopt different measures of size for each species (e.g. carapace length for crayfish and carapace width for crabs, Gherardi & Cioni 2004), and others simply do not consider size (e.g. termites and ants: Kirschenbaum & Grace 2007; ants and beetles: Hawes et al. 2013). Such a heterogeneous approach may hamper the conclusions of certain studies, as it is not clear to what actually represents size and how it is related to the results (Gherardi & Cioni 2004; Williams et al. 2006).

An interesting alternative is to employ an approach based on weapon performance, such as bite force or claw force (Lailvaux & Irschick 2007; Dennenmoser & Christy 2012; Palaoro et al. 2014). Employing performance-based measures to outline experiments instead of size may be advantageous because of how animals fight and perceive each other. A considerable number of animals are known to be able to evaluate the size of their conspecific opponents and use this information in decisions during aggressive interactions (Briffa 2008; Rudin & Briffa 2011; Palaoro et al. 2014). However, when animals employ similar fighting styles, weapon performance could be an accurate predictor of success (McCollough et al. 2014) despite a singinficantly distinct overall body shape, which is a frequent scenario when considering interspecific aggression (e.g. Williams et al. 2006; Buczkowzki & Bennet 2008).

An interesting group to investigate the relationship between experimental designs and interspecific aggression are decapod crustaceans: these invertebrates exhibit great ecological and morphological variation (Martin & Davies 2001), yet niche overlap is very common, and interspecific aggression is thought to be an important factor in their ecology (Toscano et al. 2010; Dalosto et al. 2015). Interspecific aggression in decapods may include species as morphologically distinct as shrimps and crabs (Vannini, 1985), lobsters and crabs (Williams et al. 2006; Rossong et al. 2006; Lynch & Rochette 2009) and crayfish and crabs (Barbaresi & Gherardi 1997; Gherardi & Cioni 2004). They have specialized appendages (claws), which they use in aggressive behaviors, and for which we can obtain a performance indicator based on different metrics of the claw (Mariappan et al. 2000, Dennenmoser & Christy 2013; Palaoro et al. 2014).

Our goal is to use South American crayfish (*Parastacus*) and aeglids (*Aegla*) as models to test different approaches in the investigation of interspecific aggression through staged laboratorial encounters over a disputed resource. These crustaceans have overall similar niches and are often found in sympatry, and competition through interspecific aggression has been suggested by some authors as an explanation for both the higher abundance and diversity of aeglids observed in nature (Riek 1971; Dalosto & Santos 2011; Baumart et al. 2015). We hypothesize that, given the high morphological diversity in decapods and the subjectivity of size-measures within this context, a performance-based approach will be more effective in predicting the outcome of aggressive interactions than a size-based approach. Based on that, we predict that (1) weapon performance will be the best predictor of the species of the individual that wins aggressive interactions, and (2) that aggressiveness will be more intense as weapon strength between contestants becomes more similar.

Material and Methods

Model species

We chose the South American anomuran *Aegla longitostri* and the crayfish *Parastacus brasiliensis* because of their overall similar niches: both inhabit low-order streams, have similar diets and activity patterns, and their distribution is greatly overlapped (Morrone & Lopretto 1994; Sockolowicz et al. 2007; Palaoro et al. 2013; Cogo & Santos 2013).

Animal sampling and maintenance

We captured the aeglids (n = 23) at a first-order stream in the municipality of Santa Maria, Rio Grande do Sul state, Brazil (29º39’49”S, 53º44’34”W), and crayfish (n = 23) in a pond in the municipality of Silveira Martins, Rio Grande do Sul state, Brazil (29º39’25.14”S, 53º37’33.53”W). Both species were sampled from populations where crayfish and aeglids occur sympatrically and that belong to the same hydrographic basin (Vacacaí River), but we performed samplings where it was easiest for each species to be found. For aeglids, we selected only adult intermolt males (above 13.88 mm carapace length, following Colpo et al. 2005) with all appendages intact, and for crayfish we selected only intermolt adults (above 19 mm carapace length, following Dalosto et al. 2013) with all appendages intact. We did not consider the sex of crayfish because *P. brasiliensis* does not exhibit any sexual dimorphism and it is not possible to determine their sex without euthanizing them (Dalosto et al. 2015).

We brought the selected animals to the laboratory and acclimated them for five days. During acclimation, we kept each animal in an individual 2 L opaque plastic aquarium provided with shelter, constant aeration and food (fish pellets and decaying leaves from the collection sites). We maintained the temperature at 16±2 ºC, and chose a natural (i.e. uncontrolled) photoperiod. We measured all individuals for their carapace length, carapace width, claw length, claw height, dactyl height, and distance between the fulcrum and the tubercle, using a digital caliper (precision: 0.01 mm) (Fig. 1). For aeglids, only the left (major) claw was measured, since the smaller right claw is almost never used in aggressive interactions (Palaoro et al. 2014). Crayfish do not have claw dimorphism, and thus claw measures were identical for right/left claws. We also weighed the animals using a 0.001 g digital scale.

Determination of morphological variables

Using the biometric data gathered, we determined three morphological variables that were used as predicting variables in our analyses: size, weight, and weapon strength. For the size, we used a composite measure obtained by multiplying the carapace length with carapace width. We chose this approach because it accounts for the different carapace length/width proportions of the two species chosen, and it seems a more accurate measure than using just the carapace length (as most studies do, e.g. Gherardi & Cioni 2004; Jimenez & Faulkes 2010). Weight was considered the mass of the animal, as determined above, and was chosen because it is also employed in some studies (e.g. Lynch & Rochette 2009).

Finally, we determined the index of closing force of the claw, using an approach adapted from Dennenmoser & Christy (2013). To do so, we first determined the mechanical advantage of the claw by dividing the height of the dactyl by the distance between the fulcrum (i.e. the point where the dactyl flexes, Fig. 1f, i) and the tubercle in the dactyl. We then proceeded to determine the cross-sectional area of the muscle that closes the dactyl. This variable is normally determined by measuring the area of the apodeme, a flat cuticular projection where the muscle is attached. Doing so requires the animal to be euthanized, or at least having one of its claws removed. Instead, we chose a more parsimonious approach as described by Palaoro et al. (2014), using correlates of the muscle cross-sectional area that could be measured externally without euthanizing or harming the animals.

For *A. longirostri*, we directly followed Palaoro et al. (2014), who demonstrate that the apodeme area in this species is highly correlated to claw height. We used the regression equation provided by these authors (y = -0.3701 + 0.0804 * x) to calculate the apodeme area, where “x” is the claw height and “y” is the index of closing force. In a similar manner, for *P. brasiliensis* we used available data correlating the claw height with the apodeme area (obtained from Costa et al. 2015). We then used the regression equation (y = 0.232 + 0.0202 * x) provided by these authors, to calculate the apodeme area for the crayfish. By multiplying the mechanical advantage and the muscle cross-sectional area we obtain the index of closing force of each claw. Since the index of closing force is correlated to the force produced by the claw (Levinton et al., 1995), we henceforth refer to the index of closing force as weapon strength.

Lastly, we calculated the differences between the contestants for the size, weight and weapon strength. We calculated this difference by subtracting the value of the crayfish from the value of the aeglid ($\text{Variable}_{Aegla} - \text{Variable}_{Parastacus}$), in order to standardize the procedure. The results of these subtractions were used as predictor variables in a series of statistical models.

Experimental procedure

After the five day acclimation period, we randomly assigned the animals to interspecific fighting dyads. Members of these dyads were kept in isolation for 10 days in the same individual aquaria and conditions of the acclimation period, except that no food was offered, so that we could standardize their motivation to fight (Moore 2007; Dalosto et al. 2015). After that, we transferred the members of the dyad to an experimental aquarium (31 x 19 x 21 cm) divided in three equal compartments separated by removable opaque dividers. We placed the individuals in opposite compartments of the aquarium, while the central compartment contained food (fish fillet). The food was placed inside a transparent and perforated plastic compartment, fixed in the center of the aquarium. We adopted this procedure to allow access to the resource but not its manipulation, and the plastic container was small enough so that only one animal could access it at a given time (Herberholz et al. 2007). After a 10 min period of acclimation to the new environment, we lifted the dividers were lifted and allowed the animals to interact and/or to access the resource for 40 min, during which they were recorded with a SONY HDR-CX560 camera positioned 30 cm perpendicularly above the aquarium.

Experiments were performed at night, since it is the time of day when both species are most active and are more likely to encounter each other in nature (Sokolowicz et al. 2007; Palaoro et al. 2013). Illumination was provided by red incandescent light bulbs (40 W), because both aeglids and crayfish have low sensitivity to this wavelength (Dalosto et al. 2013; Palaoro et al. 2014). After the experiments, animals were placed back in their individual aquaria and monitored for one week for deaths/molts. Since no individual perished or molted within this period, all recordings were considered for further analysis. After this, all animals were released back where they were captured.

Behavioral variables

For each interaction, we determined how much time each animal spent in possession of the resource (in seconds), and also noted the species (aeglid/crayfish) and dominance status (winner/loser) of each individual as factors. Possession of the resource followed Dalosto et al. (2015), defined as the time spent at 1 cm or closer to the resource in the absence of the opponent. We quantified this variable in order to investigate relationship of the access to a disputed resource with species and behavioral status. We also quantified the number and duration of aggressive bouts. A bout was defined as an individual exhibiting aggressive behaviors towards the other, which also responded aggressively. These aggressive behaviors included approaching with their claws spread and/or antennal whipping, touching and punching with their claws, grabbing the opponent with the claw, and unrestrained aggressive behavior where animals actively tried to grab and pull their opponent's body parts (see Ayres-Peres et al. 2011; Dalosto et al. 2013). Bouts had a minimal duration of 10 s and were deemed finished when one animal exhibited any submissive behavior (walk away, tailflip or submissive posture, see Table 1) and did not interact with the other for at least 10 s. The animal that exhibited the submissive behavior was deemed the loser of the bout, and its opponent the winner. The animal that won most bouts was deemed the winner of the interaction, and its opponent the loser. For each interaction, we determined the latency time (i.e. time until the first bout), the duration of the first bout, mean duration of bouts, total fighting time, maximum aggressive level reached (see Table 1) and time until the maximum aggressive level.

Statistical analyses

For each dyad, we calculated the difference of the three morphological variables measured (size, weight and weapon strength) between the members of each pair. We then used the differences in these variables as predictor variables in a series of generalized linear models (GLMs), to see if they were able to explain the behavioral patterns observed. We tested if the difference in size, weight and weapon strength could predict: (1) the species that won the contest, using a GLM with a binomial error structure and logit link; (2-6) the latency time, time until the maximum aggressive level, duration of the first bout, mean duration of bouts and total fighting time using five GLMs with a gaussian error structure and identity link; and (7) the number of aggressive bouts, using a GLM with a poisson error structure and a log link. Models were compared according to the p values provided.

Additionally, in order to determine if winning an aggressive interaction results in increased access to resources, we performed a two-way ANOVA with the time spent with the resource as response variable, and an individual's species (aeglid/crayfish) and status (winner/loser) as predictor variables. Since data were not fully independent, we fitted our ANOVA model with an error structure considering the interaction of the fighting dyad with the two predicting variables, to account for data dependence. All analyses were performed in the R software (R development Core Team 2013).

Results

Summary of the contests

Crayfish approached the opponent employing antennal whips and meral spreads and proceeded to punch/push the opponent with closed chelae, eventually grasping the opponent and escalating to high aggression levels with various aggressive acts performed simultaneously. Aeglids approached the opponent either with its body lowered or performing antennal whips (*A. longirostri* does not perform meral spreads, Ayres-Peres et al. 2015), and then proceeded to touch/push the opponent with open chelae, but without grasping, and eventually went on to grab the opponent and possibly escalating to high aggression levels, also with various aggressive acts (grabing/pushing/pulling with the claws) performed simultaneously. These acts correspond to those already described for both *A. longirostri* and *P. brasiliensis* in experiments of intraspecific aggression (Ayres-Peres et al. 2011; Dalosto et al. 2013). After dominance was established, subordinates readily recognized and actively avoided the dominant, regardless of their species.

Only one behavioral act observed was not yet described: “Sneaky feeding”, where the subordinate would approach the dominant while it fed and try to grasp a portion of the food. The subordinate readily retreated if the dominant reacted, but would not retreat too far and would readily try to approach the food again, in a series of repeated quick attempts. Such behavior was exclusive of *A. longirostri* and never performed by *P. brasiliensis* (either in this study or in other interspecific interactions in similar settings, Dalosto et al. 2015).

Fight outcome, dynamics and resource possession

Of the 23 interactions, *A. longirostri* dominated in 7 and *P. brasiliensis* dominated 16 fights. Of the seven response variables tested with the GLMs, only two were significantly related to the predictor variables. First, the species that won was predicted by the difference in weapon strength, with the likelihood of *A. longirostri* being the winner increasing as the difference in weapon strength diminishes (GLM; $\chi^2_{1,19} = 4.732$; p = 0.029; Fig. 2). Second, the time to escalate to the highest aggressive intensity decreased as the size difference between contestants increased (GLM; $\chi^2_{1,20} = 0.547$; $p = 0.034$; Fig. 3). The number of aggressive bouts, duration of the first bout, mean duration of bouts, total fighting time and latency period were not significantly related to any of the predictor variables. The results of all GLMs are shown in Table 2.

The two-way ANOVA yielded significant results: status significantly affected the time with the resource, with winners possessing the resource for longer times ($F_{1,39} = 5.917$; $p = 0.02$), and there was no effect of species ($F_{1,39} = 0.138$; $p = 0.713$) or from the status*species interaction ($F_{1,39} = 0.576$; $p = 0.437$; Fig. 4).

Discussion

Our results demonstrate that, at least in the case of aggressive interactions between *Aegla* and *Parastacus*, weapon strength is an accurate predictor of the winning species, thus supporting one of our hypotheses. Specifically, it predicted the chance of *A. longirostri* winning the contest, which increased as the handicap in weapon performance decreased (Fig. 2). At first, it seems that the statements from previous authors (Riek 1971; Dalosto & Santos 2011; Baumart et al. 2015) that aeglids may outcompete crayfish via interference competition are mistaken, since the crayfish won the majority of the interactions (16 ouf of 23). However, since the pairs were randomly assigned and *P. brasiliensis* grows larger than *A. longirostri*, aeglids often faced a crayfish contestant that was both much heavier (t-test for independent samples: $t_{1,30} = -4.0359$; $p = 0.0003$) and had stronger claws (t-test for independent samples: $t_{1,30} = -5.4531$; $p < 0.0001$) than them.

In spite of this clear advantage of crayfish, aeglids still won a considerable number of interactions (7 out of 23). When the weapon strength difference nears -0.1 (i.e. *P. brasiliensis* still had a strength leverage over *A. longirostri*), crayfish no longer won any interaction. This means that in a contest where these species are matched for strength, the aeglids will have an advantage upon the crayfish. This advantage is likely to be more pronounced in the field, since aeglids can grow larger that the animals used in this study: the largest *A. longirostri* used had 26.09 mm carapace length, but individuals of the same population can reach up to 28.08 mm; and *A. longirostri* individuals from other populations can grow up to 30.27 mm (data from preserved specimens in scientific collections). This is also true for other species: *A. platensis* can reach 31.75 mm of carapace length, and *A. abtao* can reach 30.73 mm (Parra et al. 2011; Dalosto et al. 2014). Moreover, our results showed that winning a fight is associated with greater access to food, thus comprising a good example where overcoming an interspecific opponent grants a direct benefit, which is in line for what is known for crustaceans (Herberholz et al. 2007, Dalosto et al. 2015).

Though large *P. brasiliensis* could easily outcompete even large aeglids, it takes at least three years for this crayfish to grow significantly beyond the size range of aeglids, and their population density in this size range does not exceed 1 individual/m^2 (Fontoura & Buckup 1989; Buckup 2003). Until they reach such size, crayfish would be frequently encountering aeglids that are well matched to them in terms of weaponry, and which are capable to overcome them in agonistic interactions over disputed resources. This advantage of aeglids against young crayfish would be further enhanced by the greater population densities of aeglids (on average 4.24 ind/m^2, summarized in Dalosto et al. 2014). Thus, although we cannot assume it based solely on our results, our data corroborate the assumption that aeglids are capable of outcompeting crayfish, which could compensate by avoiding contacts by remaining in burrows (as suggested by Baumart et al. 2015), or moving to less-oxygenated waters where aeglids cannot survive for long (as suggested by Dalosto & Santos 2011). Moreover, small aeglids could compensate the loss of an interaction through alternate behaviors such as “sneak feeding”, a strategy known for other animals, such as salmonid fish (Höjesjö et al. 2005).

One of the few similar cases investigated was the aggression between several species of *Egernia* lizards (Langkilde & Shine 2004, 2007). Interestingly, although larger species were able to outcompete smaller ones (Langkilde & Shine 2004), further testing showed that species was more important than size, regarding the outcome of interspecific aggression (Langkilde & Shine 2007). Although these authors did not directly considered weapon strength (in this case, bite force) as a variable, they tested if bite force was related to size, and discovered that juveniles of a dominant species were able to overcome adults of a subordinate species event though these adults were much larger and had stronger jaws than their juvenile opponents. However, their case differ from ours in that *Egernia* lizards exhibit a social system in which the juveniles from the size range used in their study remain close to large adults, and an association with these large adults would trigger a flight response from the subordinate species (Langkilde & Shine 2005, 2007). For aeglids and crayfish, this is not the case: aeglids have very brief mother-offspring associations (López-Greco et al. 2004), and although *Parastacus* has a prolonged parental care, juveniles remain within burrows during this time and thus do not encounter any competitor (Dalosto et al. 2012).

More comparisons, however, can be made if we consider available information for intraspecific aggression. In lizards, for example, weapon performance is usually associated to success in aggressive interactions (e.g. Lappin & Husak 2005; Huyghe et al. 2009). In crustaceans the evidence is mixed: in the crayfish *Cherax dispar*, weapon strength is a reliable predictor of social dominance in female, but not in male crayfish (Wilson et al. 2007; Bywater et al. 2008); and seems to be an important factor, but not an accurate predictor of social dominance in *A. longirostri* (Palaoro et al. 2014). These differences could be explained by the nature of the weaponry involved: lizard jaws and muscles can easily and accurately be visually assessed by their size, especially in dimorphic species (Lappin et al. 2005; Lailvaux & Irschick 2007). Crustaceans, in contrast, have their musculature contained within their exoskeleton, which makes it impossible to be visually assessed: a claw may be large, but weak if it contains small muscles, which creates room for deception in species that employ visual communication (Wilson et al. 2007; Bywater et al. 2008; Dennenmoser & Christy 2013). Our data agree with the assumption that strength may be a more reliable indicator in cases where weapons are not used for displays, and hence do not suffer selection for use in visual communication (as suggested by Palaoro et al. 2014). Both our model species do not employ visual communication in aggressive interactions: as most aeglids investigated so far, *A. longirostri* does not perform visual displays (Ayres-Peres et al. 2015); and *P. brasiliensis*, although it does perform meral spreads, does so in a vestigial and erratic way, and does not seem to rely on visual communication either (Dalosto et al. 2013).

A context in which understanding the importance of weapon strength in interspecific interactions would be particularly important are biological invasions. These invasions often include aggressive interactions between an introduced species and an ecologically similar native species (e.g. Gherardi & Daniels 2004; Polo-Cavia et al. 2011) Thus, understanding the effects at play during these interactions is vital: invasions may result in severe ecological and economic damage (Gherardi 2006; Simberloff 2014; Shine 2014), and conservation biologists require an accurate idea of the threat posed by each invader, since their resources are often limited (Simberloff 2014; Dalosto et al. 2015). Though we have gathered data regarding only a pair of species, we believe that quantifying weapon strength and including it as a variable is useful not only for the study of interspecific aggression in crustaceans such as crayfish, crabs and lobsters (Gherardi & Cioni 2004; Lynch & Rochette 2009; Hudina et al. 2011), but could also be tested in other groups. Potential candidates include species that employ damage-delivering weaponry in their aggressive interactions and that are often invading species in ecosystems, such as ants (e.g. Rowles & O’Dowd 2006; Lai et al. 2015), turtles (e.g. Polo-Cavia et al. 2011), and fishes (e.g Nakano et al. 1998).

Despite the corroboration of our first hypothesis regarding strength as an accurate prediction of the species that would win the contests, our second hypothesis that contests would be more aggressive as differences in weapon strength decreased was rejected. None of the indicators of aggressiveness increased as animals became more closely-matched, either by size, weight or strength (see Results). Instead, the size difference between contestants was negatively related to time of escalation: the larger the size difference is, the quicker the fights escalated to the highest aggression level (Fig. 2). There are theoretical models that attempt to explain how animals make decisions in intraspecific contests (see Arnott & Elwood 2009), but so far no such theoretical framework exists for interspecific aggression. However, there are some possible explanations that can be deducted from information available for each species separately (Ayres-Peres et al. 2011; Dalosto et al. 2013).

Specifically, we know that *A. longirostri* seems to use a mixed sequential assessment + cumulative assessment strategy, while data for *P. brasiliensis* support a self-assessment model (Palaoro et al. 2014; Costa et al. 2015). Since crustaceans are able to change their fighting strategy when fighting a conspecific or a heterospecific opponent (Vannini 1985; Hudina & Hock 2012), and considering that *P. brasiliensis* does not seem to assess information regarding an opponent, aeglids may be adopting a cognitively simpler strategy similar to the crayfish. If this is true, then fights with large size discrepancies between the contestants could escalate faster simply because a large animal perceives himself as a potential winner and decides to escalate the contest quickly based on its perceived elevated fighting abilities (Mesterson-Gibbons et al. 1996). Nonetheless, our experimental design does not allow us to refute/corroborate such hypothesis, but this result suggests that it would be interesting to outline a study to investigate how much of the assessment models for intraspecific aggression (reviewed by Arnott & Elwood 2009) can be applied to interspecific aggression.

Overall, we have demonstrated that weapon strength is the most reliable predictor of winning in interspecific contests of *A. longirostri* and *P. brasiliensis*. We have also demonstrated that when these crustaceans are matched for weapon strength, *A. longirostri* holds an advantage upon *P. brasiliensis*, despite its overall smaller size, which agrees with the assumptions of previous studies that mention *A. longirostri* as capable of overcoming *P. brasiliensis* in aggressive interactions over disputed resources. Winners held the resource for longer, regardless of species, demonstrating that winning an interspecific interaction, at least in this case, results in a benefit to the winner. The time to escalation decreased as size discrepancy increased, which suggests that large animals may initiate contests earlier by perceiving themselves as potential winners, although this hypothesis require further testing. It would be particularly interesting to investigate if our results can be confirmed in animals other than crustaceans which also possess damage-delivering weaponry, especially in cases related to biological invasions. Another issue that should be investigated are the similarities/differences between intraspecific and interspecific aggression, perhaps leading to the creation of theoretical models about fighting and decision-making in aggression between species, similar to what exists for intraspecific aggression. This would certainly contribute to place the investigation of interspecific aggression in an evolutionary and ecological perspective.

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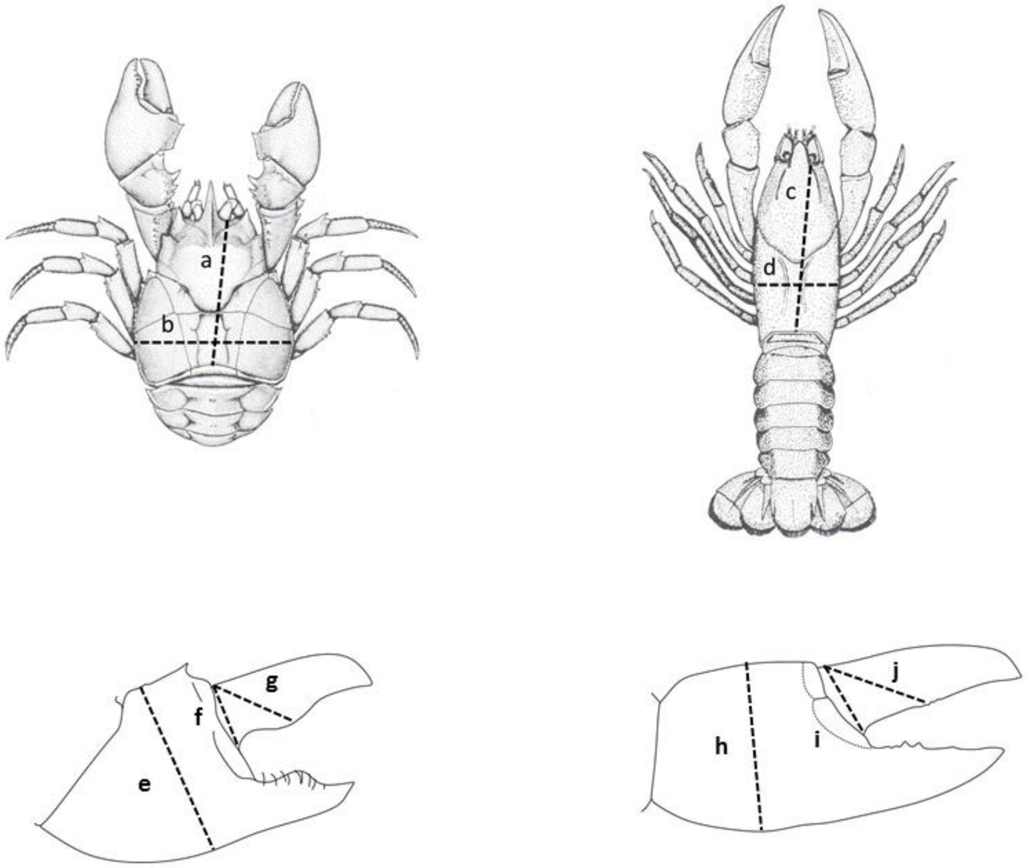

Fig. 1 Schematic representations of the body plans and chelipeds of *Aegla longirostri* and *Parastacus brasiliensis*; (a, c) carapace length, (b, d) carapace width, (e, h) claw height, (f, i) dactyl height, (g, j) distance from the fulcrum to the first tubercle; drawings adapted from Bond-Buckup (2003), and Buckup (2003)

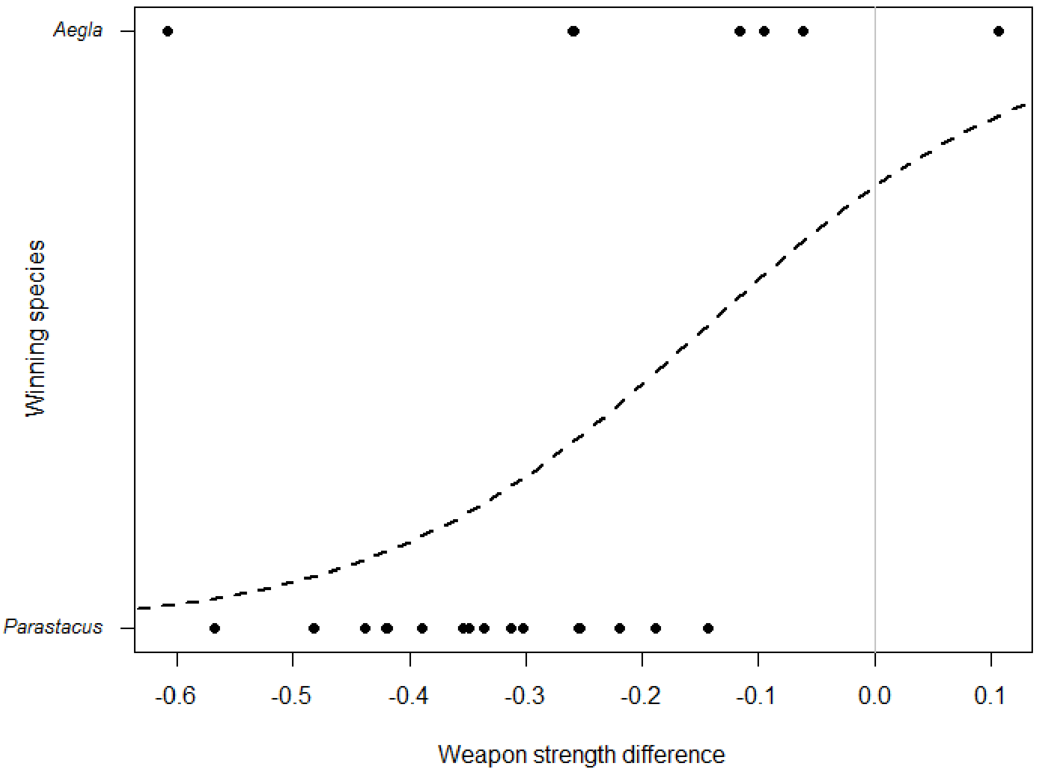

Fig. 2 The probability *Aegla longirostri* winning a contest against *P. brasiliensis* increases as the difference in weapon strength nears zero (Binomial GLM; $\chi^2_{1,19} = 0.5470$, $p = 0.0296$); gray line indicates the point where animals are matched for weapon strength; the x-axis is based on the weapon strength difference of the pair

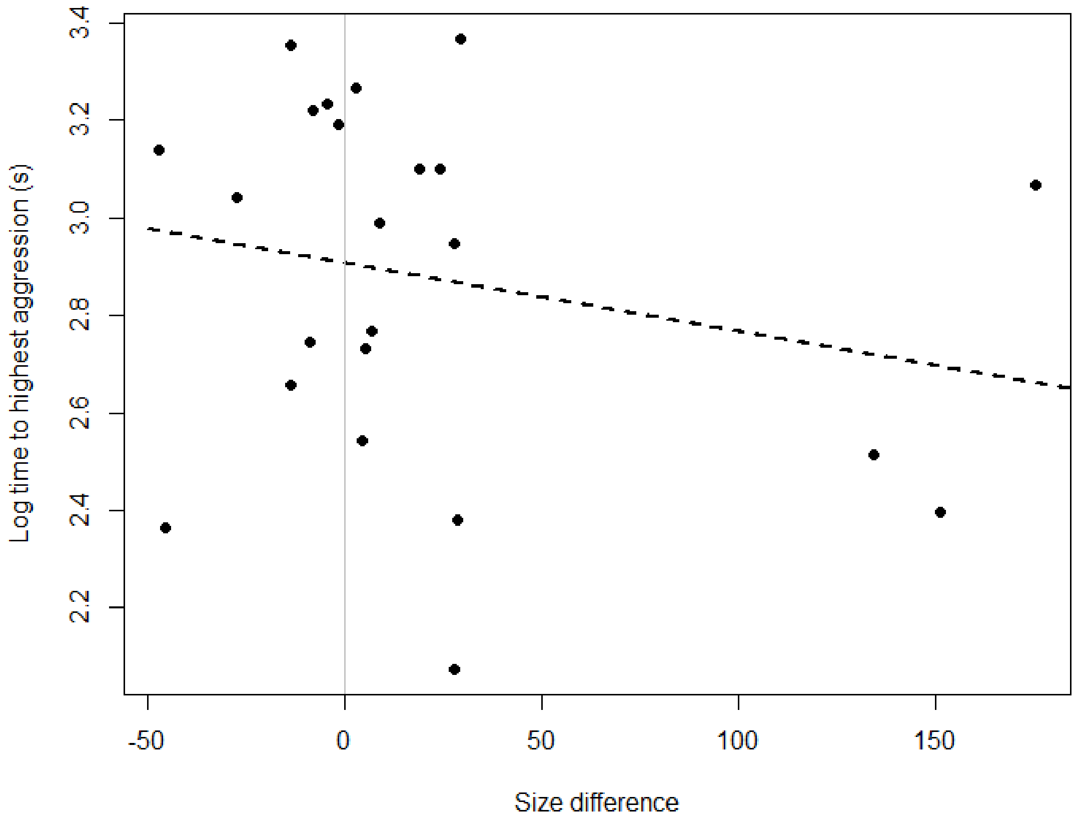

Fig. 3 Relation between the size difference of the pair and the time until the highest aggression level (Gaussian GLM; $\chi^2_{1,20} = 4.7318$, $p = 0.0337$); black line indicates the regression line; gray line indicates the point where animals are matched for size

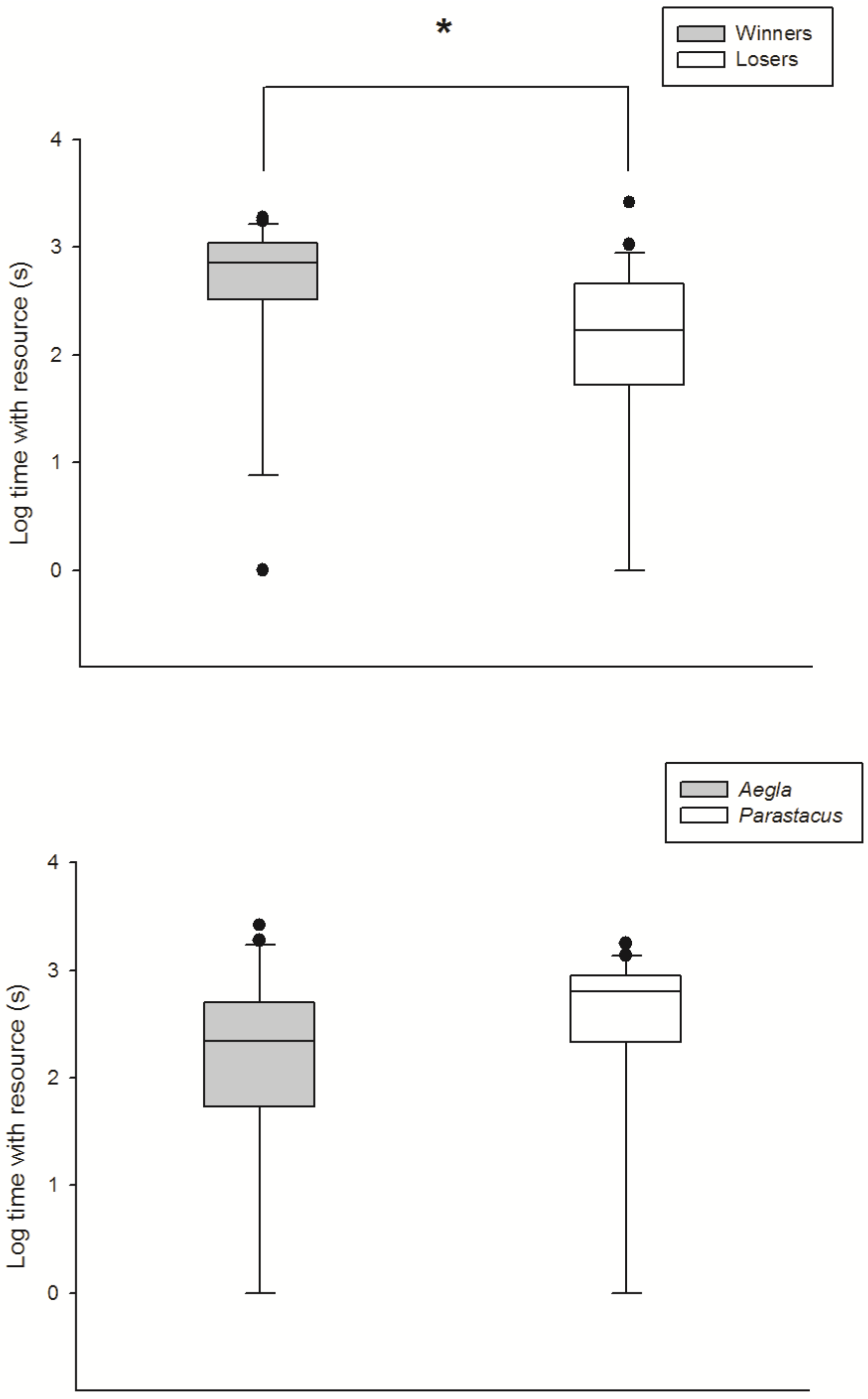

Fig. 4 Time spent in the possession of the resource; (a) plots winners versus losers, regardless of species; and (b) plots aeglids against crayfish, regardless of dominance status; (*) indicates significant differences; bar height represent range between the first and third quartiles, whiskers represent maximum and minimum values, and horizontal bars represent medians

Table 1 Ethogram codes for interspecific aggression between *Aegla* and *Parastacus*

Score	Behavior
-2	Retreat with a tail flip.
-1	Retreat by walking away from the opponent.
0	Ignore the opponent/non-aggressive behaviors.
1	Approach without agonistic display.
2	Approach with meral spread and/or antennal whip.
3	Aggression with closed claws: touching, punching and pushing the opponent.
4	Active use of the claws to grab the opponent’s appendages, or claw strike.
5	Intense combat: animals performing several agonistic acts simultaneously, trying to grab and pull the opponent’s body parts.

Table 2 Results of the GLMs of size, weight and weapon strength difference with behavioral variables of interspecific aggression of *Aegla longirostri* and *Parastacus brasiliensis*

Response variable	Weight difference			Size difference			Weapon strength difference		
	χ^2	df	p	χ^2	Df	p	χ^2	df	p
Dominant species	0.0450	21	0.8320	0.9333	20	0.3340	4.7318	19	0.0296
Number of bouts	0.0297	21	0.8849	0.2104	20	0.6464	0.1536	19	0.6951
First bout duration	0.0263	21	0.7822	0.2041	20	0.4416	0.6460	19	0.1710
Mean bout duration	0.0132	21	0.8292	0.0337	20	0.7107	0.4930	19	0.1871
Total fighting time	0.0001	21	0.9893	0.0597	20	0.7525	0.5858	19	0.3233
Latency	0.1660	21	0.3697	0.0851	20	0.5206	0.0267	19	0.7188
Time to highest aggression	0.0340	21	0.5966	0.5470	20	0.0337	0.0528	19	0.5092

Discussão geral & conclusões

Os resultados desta tese trazem algumas respostas para a compreensão da agressão interespecífica. Por exemplo, os capítulos 1 e 3 demonstram que vencer um oponente de outra espécie pode garantir o acesso a recursos disputados, e trazer benefícios à espécie vitoriosa. Experimentos em laboratório são frequentemente questionados quanto à sua aplicabilidade em contextos naturais mais complexos (VORBURGER & RIBI, 1999; ZULANDT-SCHNEIDER ET AL., 2001; DALOSTO ET AL., 2013). Nossos resultados para agressão interespecífica se somam às informações similares já reportadas para a agressão intraespecífica (HERBERHOLZ ET AL., 2007). Em particular, seriam interessantes estudos analisando a agressão interespecífica na natureza e comparando esses resultados aos obtidos em experimentos laboratoriais (como BERGMAN & MOORE, 2003 e FERO & MOORE, 2008, para agressão intraespecífica em lagostins).

Contudo, tão importantes quanto essas respostas são as perguntas que surgem a partir dos experimentos. Uma dessas perguntas e que está relacioada aos resultados dos três capítulos desta tese é: Como os animais de diferentes espécies percebem uns aos outros? Sabe-se que algumas espécies podem reconhecer alguns competidores heteroespecíficos, enquanto que outras não (GRETHER ET AL., 2009; CARTHEY & BANKS, 2014). Mas mesmo quando há reconhecimento e troca de informações, quais seriam as informações sendo transmitidas e/ou recebidas? Será que alguns animais percebem seus oponentes heteroespecíficos como apenas competidores (HÖJESJÖ ET AL., 2005), ou a agressão interespecífica seria, em certo grau, uma forma de tentativa de predação (LE BRETON ET AL., 2007)?

Alguns grupos de animais podem potencialmente auxiliar na construção deste proposto framework teórico. Esses grupos incluiriam animais para os quais a agressão é conspícua e para os quais há indícios de que a agressão interespecífica seja um fator determinante de suas relações ecológicas, tais como formigas (LE BRETON ET AL., 2007; LAI ET AL., 2015), lagostins (BOVBJERG, 1970; HUDINA & HOCK, 2012; DALOSTO ET AL., 2015) e eglídeos (PARRA ET AL.,

2011; PALAORO ET AL., 2013; AYRES-PERES ET AL., 2015). Essas questões relativas à percepção de competidores heterespecíficos poderiam ser testadas, por exemplo, através de estudos que quantifiquem os custos e benefícios da agressão interespecífica, potencialmente através de ensaios fisiológicos (tais como BRIFFA & ELWOOD, 2001, 2004, 2005; BRIFFA & SNEDDON, 2007).

Os resultados apresentados neste trabalho também contribuem para o eventual manejo de invasões biológicas, ressaltando a importancia não apenas de antecipar situações (e.g. DALOSTO ET AL., 2015), mas também de considerar fatores como comunicação animal (e.g. GÉRARD ET AL., 2014; HEAVENER ET AL., 2014) e determinantes do sucesso em agressão interespecífica (e.g. LANGKILDE & SHINE, 2007). Sempre que possível, esses fatores poderiam ser incluídos em estudos de potenciais impactos de espécies invasoras (considerando o aspecto de comportamento animal), para que se tenha uma avaliação mais precisa dos riscos e possíveis impactos de um determinado invasor (SIMBERLOFF, 2014).

Esta tese também contribui para o conhecimento específico dos grupos animais usados como modelos. Primeiro, ela ressalta a alta intensidade das interações entre duas espécies invsoras consideradas particularmente agressivas: *P. clarkii* e *P. leniusculus*, que apresentaram níveis agressivos distintamente altos (ver cap. 2), o que é bastante incomum em interações de lagostins (GHERARDI, 2002; MOORE, 2007). Outro aspecto interessante foi avaliar as interações entre essas espécies invasoras através de interações repetidas: isso demonstrou que, apesar de *P. leniusculus* vencer a maioria das interações no primeiro dia (contrariando em parte o documentado em outros trabalhos, e.g. GHERARDI ET AL., 2013), esse quadro se reverteu ao longo do tempo, e ao terceiro dia *P. clarkii* dominava na maioria dos pares. Isso não apenas demonstra a importância de se utilizar uma escala de tempo mais longa ao investigar interações ecológicas novas, mas também a importância de se considerar as estratégias de cada espécie

(HUDINA & HOCK, 2012) e de se evitarem generalizações quando se consideram "grupos-modelo", tal qual os lagostins (LUNDBERG, 2004; DALOSTO ET AL., 2013).

Além dessas informações a respeito de espécies invasoras, esses trabalhos trazem evidências relacionadas às constatações de que eglídeos possuem alguma vantagem competitiva sobre os lagostins parastacídeos, possivelmente excluindo estes de um nicho mais lótico (RIEK, 1971; CRANDALL ET AL., 2000; DALOSTO & SANTOS, 2011; BAUMART ET AL., 2015). Os dados levantados corroboram parcialmente essa proposição: lagostins venceram a maioria dos embates, mas eglídeos estão em vantagem quando os quelípodos possuem força semelhante ao de lagostins, sugerindo que podem exercer uma pressão competitiva nos lagostins mais jovens. Embora a proposição de que os eglídeos tenham "expulsado" os lagostins sul-americanos de seu nicho originalmente lótico (esse é o nicho basal dos lagostins, ver NYSTRÖM, 2002) e de que estes anomuros estariam relacionado a predominância usual de espécies escavadoras nos lagostins sul-americanos (um nicho derivado, ver RUDOLPH & CRANDALL, 2012) seja uma ideia difícil de se investigar, as evidências existentes apontam para isso como uma possibilidade bastante plausível.

Em síntese, essa tese trouxe informações inéditas acerca da agressão interespecífica, não apenas acerca de questões envolvendo crustáceos, mas também contribuindo para os temas de invasões biológicas e ecologia comportamental. A literatura sobre o tema desta tese ainda é bastante esparsa e eventualmente adota nomenclaturas e metodologias heterogêneas.

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