Contributing factors for crayfish dispersion:

the influence of socio-environmental and serotonergic change to *Procambarus clarkii*

Daniel Fernando Araújo Oliveira
Mestrado em Ecologia e Ambiente
Departamento de Biologia
2023

Orientadora
Marta Sofia Candeias Soares, PhD, CIBIO-BIOPOLIS

Coorientador
Filipe Miguel Santos Banha, PhD, Centro de Ciências do Mar e Ambiente (MARE) da Universidade de Évora

U.PORTO

FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

Dedicado ao meu pai e padrinho

Acknowledgements

Gostava de começar por agradecer aos meus orientadores, Marta Soares e Filipe Banha. Á Marta, que apesar de só termos falado em pessoa umas poucas vezes, esteve sempre pronta para me ajudar à distância com a parte teórica e escrita. Ao Filipe, que me orientou por toda a parte prática, como capturar e tomar conta de lagostins ou criar e montar o desenho experimental, e claro, as revisões na escrita. Obrigado também ao Pedro Brandão que se tornou um dos meus coordenadores não-oficiais e orientou-me juntamente com o Filipe durante todo o caminho e esteve sempre disponível para me ajudar. Agradeço também à restante equipa do LifeInvasaqua que me acolheram e deram as boas-vindas a Évora. Obrigado pela oportunidade que me deram de participar no CrayfIT apesar da minha inexperiência. Foi uma experiência da qual não me irei esquecer. O contributo de todos vocês foi o que tornou esta dissertação possível.

Obrigado aos meus amigos do Porto que me acompanharam desde o secundário até agora. Obrigado ao Jorge que me ligava sempre para saber como eu estava e esteve sempre lá para eu desabafar depois de um dia cansativo com uma horinha de Minecraft antes de eu ir para a cama. Obrigada ao Pedro, ao irmão e aos seus pais que estão sempre prontos para me acolher e desfazer o cansaço. Vocês nunca deixaram de acreditar em mim, não sei como vos agradecer.

Quero agradecer à minha irmã, que da sua própria maneira e feitio, apoiou-me o tempo todo. Quero também dar beijinhos à minha mãe, avó e madrinha. Elas têm o seu próprio jeito de ser, mas eu sei que lá no fundo elas só querem o meu bem.

Acima de tudo e todos, quero agradecer ao meu pai e ao meu padrinho. Eu nunca fui o melhor filho ou afilhado, mas eles nunca, mais do que outra pessoa, deixaram de acreditar em mim. Tudo que sou ou virei a ser foi por causa deles. Não tenho maneira de repagar-lhes tudo o que fizeram por mim.

This study was financially supported by LIFE Invasaqua project (Aquatic Invasive Alien Species of Freshwater and Estuarine Systems: Awareness and Prevention in the Iberian Peninsula) (LIFE17 GIE/ES/000515) funded by the EU LIFE program and supported by the strategic plan of MARE – Marine and Environmental Sciences Centre (UID/MAR/04292/2019).

Resumo

As espécies invasoras estão entre as ameaças mais relevantes à biodiversidade a nível mundial, particularmente nos ecossistemas aquáticos, sendo estas responsáveis pela perda de espécies nativas e também causando impactos socioeconómicos consideráveis. Procambarus clarkii é uma espécie de lagostim dulçaquícola omnívora nativa do nordeste do México e do sul dos EUA. Esta espécie é um exemplo proeminente de uma invasora bem-sucedida devido ao seu ciclo de vida rápido, capacidade de dispersão, atividades de escavação, densidades populacionais altas e variações individuais significativas nas distâncias que dispersam. A capacidade de dispersão desta espécie é um fator importante na determinação do sucesso da invasão. No entanto, há ainda pouca informação sobre quais os fatores que podem contribuir de forma mais significativa para a dispersão dos lagostins, com isso o objetivo desta dissertação é aprofundar o conhecimento das capacidades de dispersão do Procambarus clarkii. Para isso, realizaram-se três experiências laboratoriais utilizando um set-up geral composto por uma calha de aço com dois metros de comprimento enchida com 7 cm de água e com 1 cm de substrato de areia. Na primeira destas experiências testou-se os efeitos de duas temperaturas (16°C e 22°C), luz e escuridão e a presença ou ausência de alimento. Na segunda experiência testou-se o efeito das interações sociais entre indivíduos do mesmo ou sexo opostos e de tamanhos diferentes e/ou semelhantes na dispersão. Na terceira experiência testou-se o papel da serotonina na dispersão através do uso do agonista da serotonina fluoxetina e do depletor de serotonina para-clorofenilalanina (PCPA) utilizando-se duas concentrações (1µg/L e 100µg/L). Os resultados demonstram que temperaturas mais altas e a exposição á luz aumentam a velocidade, enquanto a presença de alimento diminui-a. A interação de alimentos com a luz e de alimentos com a temperatura também aumentam a velocidade, no entanto, o tamanho e o sexo não aparentam ter efeito. Na experiência das interações sociais as fêmeas que estavam atrás do seu par tiveram velocidades mais altas do que os machos que estavam atrás do seu par, esta foi a única diferença relacionada com o sexo observada em qualquer uma das experiências. Não foram observadas outras diferenças significativas nas experiências das interações sociais. Ambas as concentrações de fluoxetina e PCPA não tiveram efeito na velocidade em comparação com o grupo de controlo. Fase às mudanças globais que observamos atualmente é importante expandir o nosso conhecimento sobre quais os fatores que contribuem para a capacidade dispersiva de P. clarkii, e os resultados desta dissertação podem vir a ter um impacto significativo neste tema, tal poderá ser fundamental para a formulação de novos planos e estratégias para limitar os impactos desta espécie.

Palavras-chave: Dispersão, *Procambarus clarkii*, Invasoras, Socio-ambiente, Serotonina

Abstract

Invasive species are amongst the most relevant threats to biodiversity worldwide, particularly in aquatic ecosystems, causing loss of native species and having major socio-economic consequences. *Procambarus clarkii* is an omnivore freshwater crayfish species native to northeastern Mexico and the southern USA and it is a prominent example of a successful invasive species due to its rapid life cycle, dispersal capacities, burrowing activities, high population densities and significant individual variations in dispersal distances. Crayfish's ability to disperse is known to be a key factor in determining the success of invasion. However, there is still limited information on which factors may contribute more significantly to crayfish dispersal. The goal of this dissertation is to deepen the knowledge of *Procambarus clarkii* dispersion capabilities. For this, three laboratorial experiments were performed using a general set-up consisting of a two-meter-long metal gutter filled with 7 cm of water and 1 cm of sand substrate where the crayfish could disperse under controlled conditions. The first experiment tested the effect of two temperatures (16°C and 22°C), light and dark and the presence or absence of food. The second experiment tested the effects of social interactions between individuals of the same and opposite sex and of different and similar sizes. The third tested the role of serotonin using the serotonin agonist fluoxetine and serotonin depletor para-chlorophenylalanine (PCPA) at two concentrations (1µg/L and 100µg/L). Results show that the higher temperature and exposure to light increase the velocity while the presence of food had the opposite effect. The interaction of food with light and food with temperature also increased speed. Size and sex don't seem to have an effect. In the social interactions experiment females that were behind they pair achieving faster speeds on average than males that were behind. This was the only difference observed related to sex on any of the experiments. There were no other significant differences observed for the social interactions' experiments. Both concentrations of fluoxetine and PCPA had no effect on speed compared to the control group. Faced with current global and habitat changes it is important to expand our understanding of which factors contribute to *P. clarkii* dispersion capabilities, and this dissertation's findings may have an impact in this subject. Doing so can prove instrumental in formulating new plans and strategies to limit its impacts.

Keywords: Dispersion, *Procambarus clarkii*, Invasive, Socio-environment, Serotonin

Table of Contents

List of Tables

List of Figures

Introduction

Invasive species are a global problem

Invasive species are one of the most important threats to biodiversity and ecosystem function after land use changes (Lodge et al., 2000a; Lodge, Taylor, Holdich, & Skurdal, 2000; Lodge, 2001; IPBES, 2023), particularly in aquatic ecosystems (Carpenter, Stanley, & Vander Zanden, 2011), The impact of non-native species in freshwater environments can be severe, altering ecosystems, causing the loss of native species and having major socio-economic consequences (Vitousek, D'Antonio, Loope, & Westbrooks, 1996; Pimentel, 2011).

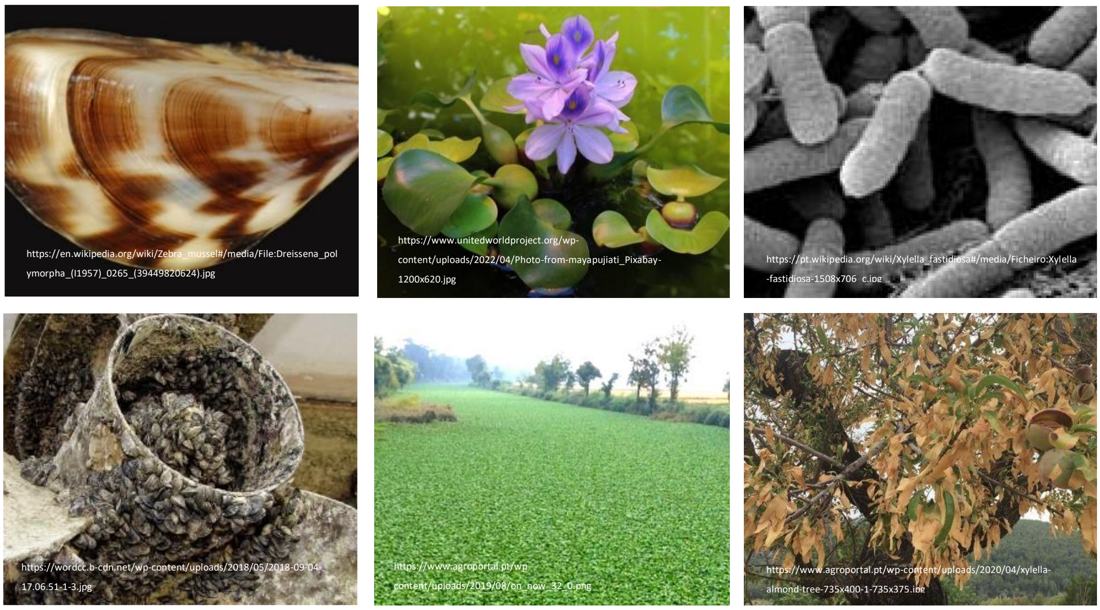

Figure 1 - Examples of invasive species present in Portugal and some associated impacts cause by them: zebra mussel (*Dreissena polymorpha*) - The former office of technology assessment of the USA reported in 1993 an approximate cost of 3.1 billion dollars over a 10 period due to *D. polymorpha* (Vitousek, D'Antonio, Loope, & Westbrooks, 1996); Water hyacinth (*Eichhornia crassipes*) - can alter water clarity and decrease phytoplankton production, dissolved oxygen, nitrogen, phosphorous among others (Villamagna, A. M., & Murphy, 2010); *Xylella fastidiosa* - a xylem-limited plant pathogenic bacterium native to the Americas that spread into countries in Europe, the Middle East and North Africa. This pathogen causes several devastating plant diseases characterized by yellowing of the leaves (Frem et al., 2020).

Crayfish are among the most widely translocated (willingly or accidentally) aquatic organisms worldwide, their global distribution been substantially altered by humans, both between and within continents (Holdich, 2002; Taylor, 2002). Crayfish include over 540 Decapoda species belonging to three families (Crandall & Buhay, 2008)—Astacidae (native to Western North America and Europe), Parastacidae (native to the Southern hemisphere), and Cambaridae (inhabiting the East of the Americas) (Shen, 2008). Found in freshwater environments (e.g., lakes, reservoirs and streams), crayfish tend to burrow or hide under rocks or other shelters to avoid their natural predators (Rhoades, 1962), such as fish, birds and mammals (Delibes & Adrián, 1987; Holdich, 1988). Non-native crayfish species can affect native species directly by preying on them (Dunn et al. 2009) or by out-competing them for limited resources such as food or shelter (Hill and Lodge 1994; Holdich and Domaniewski 1995; Hill and Lodge 1999). They can also affect indirectly native crayfish communities by altering water turbidity through burrowing activities and reducing aquatic macrophytes and macroinvertebrates (Hill and Lodge 1995; Gherardi and Acquistapace 2007) or by introducing new pathogens (Souty-Grosset et al., 2016).

Study model: a ubiquitous invader, the red swamp crayfish, *Procambarus clarkii*

Procambarus clarkii, also known as red swamp crayfish or Louisiana crayfish, is an omnivore species of the Cambaridae family native to northeastern Mexico and the southern USA. This species of crayfish is a polytrophic keystone species that can exert multiple pressures on ecosystems (Souty-Grosset et al., 2016) and is a prominent example of a successful invasive species with strong individual variations in dispersal distances (Anastácio et al., 2015).

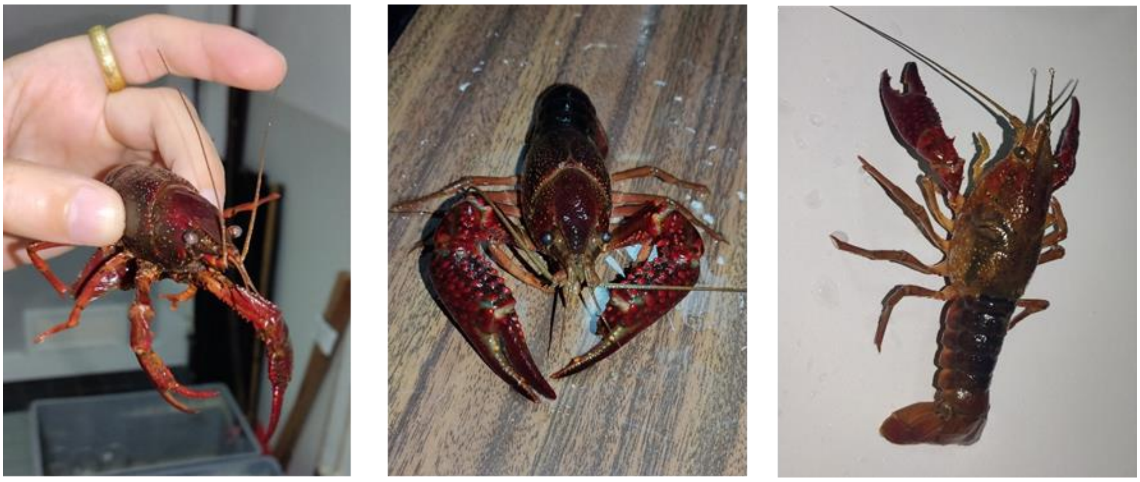

Figure 2 - Three examples of *Procambarus clarkii*. These three individuals displayed were part of the experiments done for this dissertation. Photographs taken by Daniel Oliveira.

It's success has an invasive species stems from characteristics such as early maturity, fast growth rate, high offspring number, rapid and adaptable life cycle, flexible feeding strategy, and a wide ability to disperse and to tolerate extreme environmental change (Alcorlo et al., 2004; de Abreu et al., 2020; Gherardi, 2007; Gonçalves Loureiro et al., 2015; Gutiérrez-Yurrita et al., 1999; Hobbs Iii et al., 1989; Souty-Grosset et al., 2006;

Souty-Grosset et al., 2016) such as verified in temporary streams (e.g., in southern Portugal; Gherardi et al. 2002) and polluted habitats (Gherardi and Barbaresi 2000). It is also a vector of crayfish plague, which is responsible for large-scale disappearance of native crayfish species (Souty-Grosset et al., 2016), exacerbating its potential as a invasive species. It has been introduced in all continents with the exception of Antarctica and Oceania and is currently recorded in 16 European territories (Souty-Grosset et al., 2016) and it is considered the most cosmopolitan freshwater crayfish species in the world (Chucholl, 2011; Gherardi, 2006; Gutiérrez-Yurrita et al., 1999; Hobbs, 1988; Lindqvist and Huner, 1999). The invasion of the Iberian Peninsula by this species, started with their introduction into Badajoz, Spain in 1974 for commercial purposes (Habsburgo-Lorena, 1978). The species dispersed, potentially aided by translocation by Spanish fishermen into Portugal where it was first recorded in 1979 (Gutiérrez-Yurrita et al., 1999). By the middle of the 1980s, *P.clarkii* was found throughout the entire hydrographic basin of the Guadiana River, including temporary streams of the area (Adão 1992). Today, *P. clarkii* has a wide distribution throughout the entire country, able to be found as far north in the river Minho (Sousa, Freitas, Mota, Nogueira, & Antunes, 2013).

Putative factors in the dispersion of an invasive species

The prediction of which species are probable invaders has been a long-standing dilemma and of increasing interest to ecologists (Elton, 1958; Forsyth et al., 2004; Kolar & Lodge, 2001) and their ability to disperse may be a key factor in determining the successes of invasion as well as the impact and rate of spread (Ehrlich, 1989; Lodge, 1993). Therefore, the study of which factors may contribute to higher rates of dispersion can prove instrumental in formulating conservation plans to stop *P. clarkii* spread. Many studies have approached this topic (e.g. (Aquiloni, Ilhéu, & Gherardi, 2005; Carvalho, Sousa, Cássio, & Pascoal, 2022; Galib, Sun, Twiss, & Lucas, 2022; Ramalho & Anastácio, 2015)), yielding a great deal of knowledge on the contributing factors for *P. clarkii* dispersion capabilities, such as the role of temperature, circadian rhythms and population densities, among others. For example, Francesca Gherardi, Barbaresi, & Salvi (2000) as shown that increases in water temperature between 5°C to 25°C as led to an increase of *P. clarkii* locomotive activity in irrigation ditches. Huner and Barr (1991) reported its preferred temperatures for activity in the field to be between 21°C and 27°C while (Anastácio et al., 1999) reports between 20°C to 28°C. *P.clarkii* has been shown to be more active during night periods by a number of laboratorial and field studies (Correia et al., 1998; Fernandez de Miguel & Arechiga, 1994; Miranda-Anaya, 2004; Ramaliro, 2012). According to Fernandez de Miguel & Arechiga (1994), *P. clarkii* will have outbursts of movement that can last for hours when food in presented to them. However, there are still much to uncover. The effect of some environmental factors on the locomotion of *P. clarkii* are known but there are others besides these, such as the social interactions between individuals, that too may influence crayfish dispersion. Factors, such as the differences in size, sexes or hierarchical positions could be determining elements of which individuals are more likely to spread into new territories. Through a combination of agonistic interactions, physical asymmetries and prior social experiences, *P. clarkii* individuals establish a hierarchical structure whether tangible resources are in dispute or not (Bovbjerg, 1956; Lowe, 1956; Copp, 1986; Issa et al., 1999; Momohara et al., 2013). Larger sizes, aggressive behaviours like attacking or approaching first other individuals and the prior experience of winning contributes to the formation and maintenance of a dominant hierarchical position (Bovbjerg, 1956; Lowe, 1956; Copp, 1986; Issa et al., 1999). Several past studies were done on *P. clarkii* and other crayfish species social interaction in regard to dominant/subordinate dyadic agonistic interactions (e.g. (Hayes, 1975; R. Huber & Delago, 1998; Issa, Adamson, & Edwards, 1999)). Some past studies suggest that crayfish social interactions may affect their mobility. For example, Issa, Adamson & Edwards (1999) have shown that larger crayfish tend to approach others more often and fight for longer while smaller crayfish tend to move away or quickly retreat, fleeing from fights or avoiding them entirely. Ranta & Lindström (1992) have shown that larger crayfish are able to evict smaller ones from their burrows. Another area that can prove important is the study on how exogenous chemical compounds may influence crayfish dispersion. The neuro-endocrine system modulates several functions in crustaceans, such as reproduction, growth, immune function, metabolism, and of particular note to this dissertation, behaviour (Diwan, 2005; Cheng et al, 2006; Diwan, 2005; Fingerman,1981, 1987, 1997a,b; Huber et al, 1997; Li et al, 2005; Sarojini et al., 1995a,b,c). One important biomolecule responsible for regulating many behavioural and physiological processes in crayfish is the neuromodulator serotonin or 5-hydroxytryptamine (5-HT). The central nervous system has an even distribution of serotonergic innervations, indicating that most brain regions receive serotonergic modulation (Chen & Condron, 2008; Sykes & Condron, 2005). The serotonergic system is evolutionarily conserved both in vertebrates and invertebrates, sharing many receptors and transporters with a common ancestor (Tierney et al., 2016). All but one of the serotonin receptors are G-protein-coupled receptors with the exception of the 5- HT3 ligand-gated ion channel(Daubert & Condron, 2010). In addition to its physiological role, serotonin is also suggested to regulate the connectivity of the brain by modulating developmental cellular migration and cytoarchitecture (Daubert &

Condron, 2010) and that it can auto regulate serotonergic branch morphology (Daubert & Condron, 2010). The study of monoamines and other such biomolecules has been of great interest to science but is becoming increasingly more important with the rise in the use of prescription drugs, in this case, fluoxetine is used as an anti-depressive drug (for instance, PROZAC). Many of these drugs that are excreted or discarded by humans pass unchanged through wastewater treatments and enter natural waterways (Tierney et al., 2016). The problem is known since circa 1970 (Hignite and Azarnoff, 1977; Richardson and Bowron, 1985) and grew in concern at the end of the 1990s with the rise in use of prescription drugs (Fong & Ford, 2014). Today there are several studies addressing the effects of many of these pharmaceuticals on aquatic organisms (Kolpin et al., 2002; Kummerer (2008); Daughton and Brooks (2011); Boxall et al. (2012); Brausch et al. (2012); Brooks and Huggett (2012); Fong & Ford, 2014; Silva, Pereira, Meisel, Lino, & Pena, 2015). The most widely prescribed antidepressants worldwide are selective serotonin reuptake inhibitors (SSRIs)(Fong & Ford, 2014). These work by binding and inhibiting pre-synaptic reuptake transport proteins, which are responsible for recycling neurotransmitters back into the pre-synaptic terminal (Fong & Ford, 2014). Inhibition of these transporters allows neurotransmitters to remain in the synaptic cleft for longer. Fluoxetine is a SSRI used to treat depression and other disorders. It is one of the most prescribed drugs in western countries and historically was the most commonly detected antidepressant in wastewater (Fong & Ford, 2014), with freshwater bodies reaching concentrations as high as 330 ng/L (Mole & Brooks, 2019). Like other SSRI it acts acutely to elevate serotonin levels in synapses and can elicits additional effects when chronically exposed to, such as causing changes in serotonin release, expression of serotonin receptor genes, and altering the activity of other transmitter systems (Andrews, Bharwani, Lee, Fox, & Thomson, 2015; Kroeze, Zhou, & Homberg, 2012). Ambient fluoxetine has been shown to be taken up and stored in animal tissues (Meredith-Williams et al., 2012; Paterson & Metcalfe, 2008) leading to behavioural or life history changes in many species including crustaceans (Bossus, Guler, Short, Morrison, & Ford, 2014; De Lange, Noordoven, Murk, Lürling, & Peeters, 2006; Mesquita et al., 2011). Many past studies have been done that show how serotonin influence crayfish behaviour, most of these on social interactions (e.g. ((Fossat, Bacqué-Cazenave, De Deurwaerdère, Delbecque, & Cattaert, 2014; Momohara, Kanai, & Nagayama, 2013)). Moreover, there are some studies on crayfish and other species of crustaceans regarding the effects of serotonin on aspects of locomotion. For instance, Tierney et al (2016) showed that juvenile *Orconectes rusticus* exposed for 7 days to concentrations of 2 and 500 µg/L fluoxetine dissolved in water have reduced locomotion compared to controls. Additionally, Mesquita et al (2011) exposing *Carcinus maenas* to concentrations greater than 120 µg/L of the same SSRI for 10 days showed that locomotion was significantly increased with animals moving for longer periods of time and walking further.

Objective and predictions for this dissertation

As previously stated, invasive species can become dangerous to the environment and a deep understanding of their capabilities is important to help mitigate their impacts. Past studies have given us insights on *P. clarkii* dispersion and locomotion, such as the ones referenced above. However, there is still much to uncover. The role of crayfish social interactions and how serotonin modulates *P. clarkii* dispersion and locomotion is still unclear. Even better understood factors, such as temperature and circadian rhythms, can vary between populations. As such, the aim of this dissertation is to deepen the understanding of what factors influence *Procambarus clarkii* dispersion capabilities so that in the future we can better predict their spread and formulate better mitigation plans. To achieve this goal, three laboratory experimental designs were used. The first aimed to test three environmental variables, namely temperature (16°C and 22°C), light levels (illuminated and dark) and food (presence or absence of food items). We also tested for the influence of size, sex and physical condition. The second experiment tested if social interactions between individuals of the same and opposite sex of different and similar sizes may lead to differences in their dispersion capabilities. The third experiment tested the role of water serotonin in crayfish' dispersal using exogeneous SSRI fluoxetine to raise its levels and para-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor that acts as a potent depletor of tissue serotonin in animals (Maximino et al., 2013), to reduce it. According to previous studies (ex.: Gherardi et al., 2000; Fernandez de Miguel & Arechiga, 1994; Miranda-Anaya, 2004), it is expected that higher temperatures, a dark environment, and the presence of food items will lead to higher values in distances travelled and speed. We also expect that exposure to fluoxetine will lead to decreased locomotion according to Tierney et al. (2016). Regarding the social interactions, smaller individuals have been shown to keep a distance from bigger ones (Issa et al., 1999), hence we expect that the smaller crayfish will disperse more to new areas to avoid the larger and more dominating crayfish.

Materials and Methods

Crayfish capture

Procambarus clarkii individuals were captured in a marateca stream (Figure 3) next to rice fields near the town of Cabrela, Portugal (38.58561 N, 8.528580 W). These were

Figure 3 – A) Location of crayfish capture in Google Maps; B) Photographs of the location of crayfish capture.

captured on three separate occasions on the 20th to 21st of November 2022, 11th to 12th of May 2023 and 13th to 14th of July 2023. The first of these catches was performed with the aid of dip nets and crayfish traps baited with sardines. The captured individuals consisted mostly of juveniles with nearly a third of the individuals not exceeding a cephalothorax length of 15 mm and had an even distribution of sexes. Regardless of the disparity in sizes of the catch, 88 individuals were of sufficient size to conduct experiments with a relative even size distribution. The 2nd catch was done only with crayfish traps and consisted almost entirely

Figure 4 - Crab traps used to capture crayfish.

of adults with few individuals below 20 mm of cephalothorax length with two thirds of these being female. The 3rd catch also was done solely with crayfish traps and yielded only adults, with no individual below 25 mm of cephalothorax length. This was most likely due to trap catches being biased towards capturing larger individuals (Dorn et al., 2005). After being captured, the crayfish were maintained in an acclimatised room at an average temperature of 20°C inside tanks filled with a depth of 5 to 7 cm of tap water kept

Figure 5 - Tanks used to acclimatize crayfish before selection.

oxygenated using an aquarium pump (Figure 5). Several PVC tubes sections were scattered on the bottom of the tanks to serve as shelter and individuals were fed with carrots. Every four to seven days the crayfish were removed temporarily from the containers, and these were cleaned, and the water and uneaten food was replaced anew. Crayfish that were selected had their wet weight and cephalothorax length measured. These measurements were used to help select individuals and form groups with similar size and weight distributions among them. The condition of the crayfish was calculated using Fulton's condition index with the formula K = W/L3, where W—wet weight (g) and L—Cephalothorax length (mm) (Ricker, 1975). The selected crayfish were them stored individually inside tagged containers for a minimum of 5 days before experimentation. These containers have an average size of 20x20x12 cm and were filed with dechlorinated tap water up to the top of the individual's cephalothorax (Figure 6). The crayfish were fed carrots as before. The containers were cleaned, and the water and uneaten food were replaced with fresh water and food every two days.

Figure 6 - Individual containers used to house selected individuals (the ones shown are awaiting to be measured and tagged).

General experimental design

The general set-up consists of a two-meter-long metal gutter filled with a depth of 7 to 8 cm of tap water that's replaced a day before every experiment (Figure 7 & 8). A substrate consisting of medium coarse sand with approximately 1cm of dept was used to cover the track's bottom. A removable translucent plastic plate acts as a divider that separates the initial 10 cm of the track from the rest of it (Figure 7). The crayfish would go through 3 days of fasting and would be acclimatised to the correct temperature for 24 hours before the day of the experiment. On the day of an experiment a few drops of water from each individual containers where the crayfish were kept in was mixed in with the water inside the track 10 minutes before the first replica starts. This was done to scent the water pre-emptively and prevent the possibility that crayfish tested last might behave differently because of scent from the previous individuals. The water's parameters, namely, pH, percentage and ppm of dissolved oxygen and temperature are noted before and after the experiments. For each treatment, an individual is placed on the first 10 cm of the track and is kept from roaming by the closed divider for 60 seconds to acclimatize to the new environment. Following 60 seconds, the divider was removed to allow the individual to roam the gutter freely for a total of 120 seconds or until it reaches the opposite end of the track, after which their position or the time they took to reach the end of the gutter was noted.

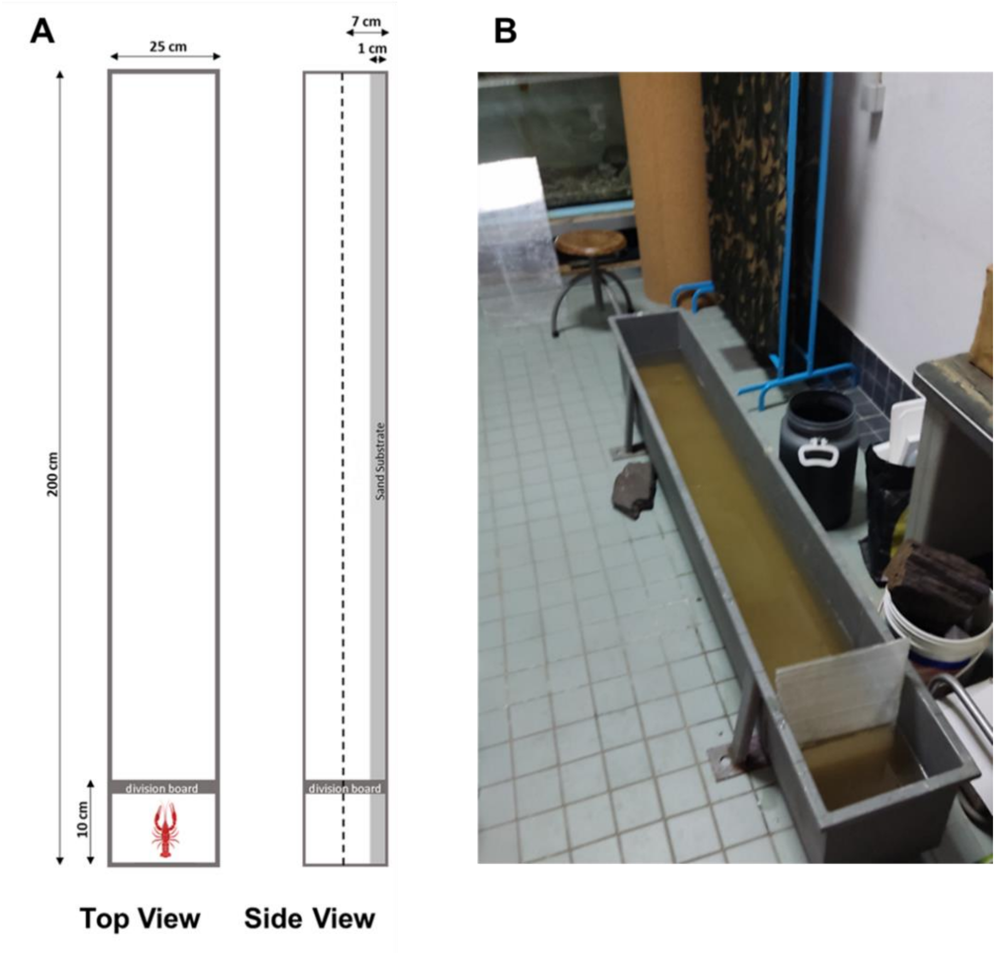

Figure 7 - A) Schematic top and lateral view of the general layout used for the experiments; B) Image of metal gutter use for the experimental tests with removable divider.

Environmental factors

A total of 216 individuals were selected from the three captures, 106 males and 110 females, with cephalothorax length mean of 34.27 mm (sd = 12.76 mm). These were distributed evenly by 8 treatments, with 27 individuals each (Table 1) with an even size distribution. Unfortunately, 4 of these individuals didn't survive to the experiment day, resulting in a slight imbalance on the number of crayfish per treatments. The experiment occurred over a period of 8 days. For the illumination in the light treatments, we used the

Table 1 - Sequence for the environmental factors experiment with treatment groups, number of individuals per treatment and respective factors these will be exposed to.

	1º Day *Acclimation*	2º Day *Experiment*		3º Day *Acclimation*	4º Day *Experiment*		5º Day *Acclimation*	6º Day *Experiment*		7º Day *Acclimation*	8º Day *Experiment*	
Temperature		16°C			22°C			16°C			22°C	
Food		No Food			No Food			With Food			With Food	
Luminosity		Light	Darkness		Light	Darkness		Light	Darkness		Light	Darkness
Group		A	B		C	D		E	F		G	H
Number of individuals		26	27		27	27		26	25		27	27

room’s fluorescent light bulbs. The room’s lights were turned off ensuring total darkness for the dark treatments and to identify when a crayfish reached the end of the track a commercial red laser pointer was used to illuminate for 1 to 2 seconds the end of the track every 15 seconds. Temperature control was achieved with an air conditioner unit installed on the experiment laboratory room. The temperatures used were 16°C and 22°C that corresponded to water temperatures registered in spring and summer, respectively, in rivers from the south of Portugal (Reis & Araujo, 2016). The target temperature was set the day prior the day of experimentation. For the food treatment a sardine cut in haft was placed on the end of the gutter 15 minutes before the start of the experimentation. The foodless treatments were performed first to ensure that there were no food particles or scent that could interfere with them. Both water and sand subtract were replaced and the track was cleaned between experiments.

Social context

164 individuals organized into 82 pairs were selected from the three captures and grouped into six treatments, males of the same size, females of the same size, males and females of the same size, different size males, different size females and different size males and females. Another 30 individuals, composing of only 9 male and 21 females, were used individually as a control group to compare to the results of the individual experiments (Table 2). Crayfish used in the paired treatments had cephalothorax length mean of 37.12 mm (sd = 11.84 mm). Those used in the control had mean of 29.92 (sd = 12.88 mm). Considering the 82 pairs only 79 yielded results as some individuals died before they could be tested, giving us a true total of 158 individuals plus the 30 in the control. These treatments were all conducted under the same light levels and in the absence of food but were divided between two temperatures, 16°C and 22°C. They occurred over a period of 4 days for each capture, adding up to 12 days in total. The procedure was same as the previous experiment, with 60 seconds of acclimatization at the start of the gutter, followed by 120 seconds of free roam through the entire gutter. The only difference is that it’s a pair of crayfish that are introduced

Table 2 - Sequence for the social interactions’ experiment with treatment groups, number of individuals per treatment and temperature these will be subjected to.

	1° Day *Acclimation*	2° Day *Experiment*		3° Day *Acclimation*	4° Day *Experiment*	
Temperature		16°C			22°C	
		Same size Males n=16	Different size males n=10		Same size Males n=18	Different size males n=10
		Same size Females n=14	Different size females n=10		Same size Females n=16	Different size females n=10
		Same size males and females n=16	Different size males and females n=12		Same size males and females n=20	Different size males and females n=6

instead of just a singular individual. The interactions of the crayfishes were recorded through a webcam placed above the gutter. Each individual in a pair was placed on either the right or left side of the track as a form of keeping track of which individual it was. Similar to the other procedures, the total distance that each individual reaches at the end of the 120 seconds was noted. If one of the two individuals reached the end of the track before the two minutes passed, the time it took for that crayfish to reach the end and the position of the other individual in that instance was noted.

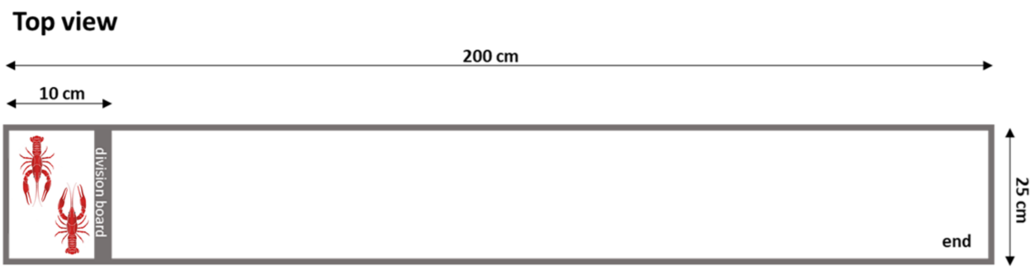

Figure 8 - Schematic top view of the layout used for the social interactions experiment.

Serotonergic effects

144 crayfish were used for this experiment. These individuals consisted entirely of adults with average cephalothorax length of 45.01 mm (sd = 3.67 mm). There were 85 males and 59 females, seven of which were carrying eggs. These crayfish were grouped into 5 treatments with an even size distribution. Four of these were exposed to solutions of fluoxetine and Para-chlorophenylalanine (PCPA), both with concentrations of 1µg/L and 100µg/L. The fifth group acted as a control and was exposed to regular tap water (Table 3). The lowest concentration used, which is higher than the maximum freshwater levels of fluoxetine detected (Mole & Brooks, 2019), was chosen in the expectation that an affect might be observed. The 100 µg/L was chosen as to give a noticeably distinct order of magnitude. The use of PCPA at the same concentrations was to test the effects of

Table 3 - Sequence for the Fluoxetine and PCPA experiment with treatment groups and number of individuals per treatment.

1º to 7º Day	8º Day	9º to 15º Day	16º Day
Exposure and Acclimation	*Experiment*	*Exposure and Acclimation*	*Experiment*
Fluoxetine 1µg n=28			
Fluoxetine 100µg n=29			
PCPA 1µg n=29			
PCPA 100µg n=29			
Control n=29			

serotonin disruption. For these experiments the crayfish were kept in small container with 300 ml of internal volume, which were filled with 200 ml of the appropriate solution (Figure 10). The top-covers of the containers are perforated, and an appropriate volume of open-air space is given to allow proper oxygenation of the water. The solutions were replaced with new solution every three days. The crayfish were exposed to the solutions for 16 days, with experiments conducted on the 8th and 16th days (Table 3). The procedure is the same as for the first experiment, the only difference being that no water from the individual's container was mixed with the water inside the gutter as to not cross-contaminate the treatments.

Figure 9 - Container used to house crayfish for fluoxetine and PCPA exposure.

Statistical analysis

All statistical analysis were done using RStudio (2023.06.1 Build 524, Posit Software, PBC) with the libraries tidyverse, car, lme4 and rstatix. Velocity was calculated by dividing the values of distance by the values of time. The data for the values of velocity from the environmental factors experiment did not fulfil the homogeneity assumption required for parametric statistical analysis (function leveneTest() from the car package). Because of this, a log(x+1) transformation was performed on the velocity values. An One-Way ANOVA (function aov()) was used to test for differences in the values of velocity between the different treatments for the environmental factors procedure and the social behaviour experiment, followed by post-hoc Tukey tests (function TukeyHSD()) to determine which treatment differed. For the fluoxetine and PCPA procedure a repeated measures ANOVA (function anova_test() from the rstatix package) was used instead, followed by a pairwise t-test (function pairwise_t_test() from the rstatix package) to determine if there were differences in the treatments between the two days of experimentation. The dataset of the social behaviour experiment was sub-divided into sub-datasets for the two temperatures, individuals that were ahead of their pair and another for those that were behind, and two more datasets for pairs with same size individuals and pairs with different size individuals. Additional ANOVAs and post-hoc Tukey tests were used to test for differences in the values of velocity between temperatures, size categories and the relative position of the individuals with their pair. Two sample t-test (function t.test()) were used to test velocity between the two temperatures and sexes for the environmental factor and the social behaviour experiments. An additional two sample t-test was used to test velocity between the two size categories in the social behaviour experiment. A Generalised Linear Model (function glm()) with the variable's contrasts set to sum-to-zero and gaussian family with link set as identity was used to test the effect of temperature, food and luminosity as well as the interaction of two and all three of these variables on velocity. The effect of sex, size (cephalothorax length) and condition (Fulton's index) were also tested. Other Generalised Linear Models, also with the variable's contrasts also set to sum-to-zero and gaussian family with link set to identity, were used to test the effects of the social behaviour treatments and size categories on velocity. These GLM were performed for the original data set, as well as the sub-sets for each temperature, size category and relative position of individuals. A repeated measures linear model (function lmer from the lme4 package) with contrasts set to treatment coding was used to analyse the effect of the treatments from the fluoxetine and PCPA experiment on the values of velocity.

Results

Environmental factors

After log10(x-1) transformation of the velocity values, Levene test resulted in a $p=0.023$, allowing parametric analyses. There are significant differences between treatments regarding the crayfish velocities (One-Way ANOVA: $F=4.881$; $p \leq 0.001$). Generalized linear model show that all variables tested alongside the size of the individuals have significant impact on the velocity (Table 4). Sex and the condition of the individuals given by the Fulton's Index don't show significant effects on the velocity (Table 4). Interaction between the variables also seem to have an effect. The interaction between food and temperature and food and luminosity influences the velocity, however, the interaction between temperature and luminosity and between temperature, food and luminosity don't seem to have an effect (Table 4). Considering the two temperatures tested, individuals introduced to 22°C scored the highest average speed, with means of 0.868 cm/s (sd = 0.998) (Figure 10). Individuals facing 16°C of temperature had average speeds of 0.645 cm/s (sd = 0.816) (Figure 10). Crayfish on average achieved higher speeds in the absence of food than with food items present. Mean values for velocity in the presence of food being 0.566 cm/s (sd = 0.620) (Figure 10). In the absence of food mean values for velocity were 0.948 cm/s (sd = 1.108) (Figure 10). The light treatments had the highest mean value for velocity, at 0.939 cm/s (sd = 1.140). In the absence of light, individuals moved on average 0.576 cm/s (sd = 0.573) (Figure 10). Velocity values are shown to be positively correlated with cephalothorax length, although the r2 values are verry low (Figure 9), indicating a small explanatory power.

Table 4 - GLM results for the environmental factors experiment on velocity. Statistic values correspond to t values, df to degrees of freedom. Significant p values are displayed in bold.

	Velocity (cm/s)				
Predictors	*Estimates*	*std. Error*	*Statistic*	*df*	*p*
(Intercept)	0.13	0.04	3.08	201.00	**0.002**
Sex	-0.01	0.01	-0.95	201.00	0.342
Cephalothorax Length (mm)	0.00	0.00	2.89	201.00	**0.004**
Fulton s Index	-80.86	133.33	-0.61	201.00	0.544
Temperature	-0.03	0.01	-2.21	201.00	**0.027**
Food	0.03	0.01	2.98	201.00	**0.003**
Luminosity	-0.03	0.01	-2.51	201.00	**0.012**
Temperature × Food	-0.02	0.01	-2.02	201.00	**0.043**
Temperature × Luminosity	0.01	0.01	1.11	201.00	0.267
Food × Luminosity	-0.03	0.01	-2.86	201.00	**0.004**
Temperature × Food × Luminosity	-0.00	0.01	-0.33	201.00	0.743
Observations	212				
R^2	0.180				

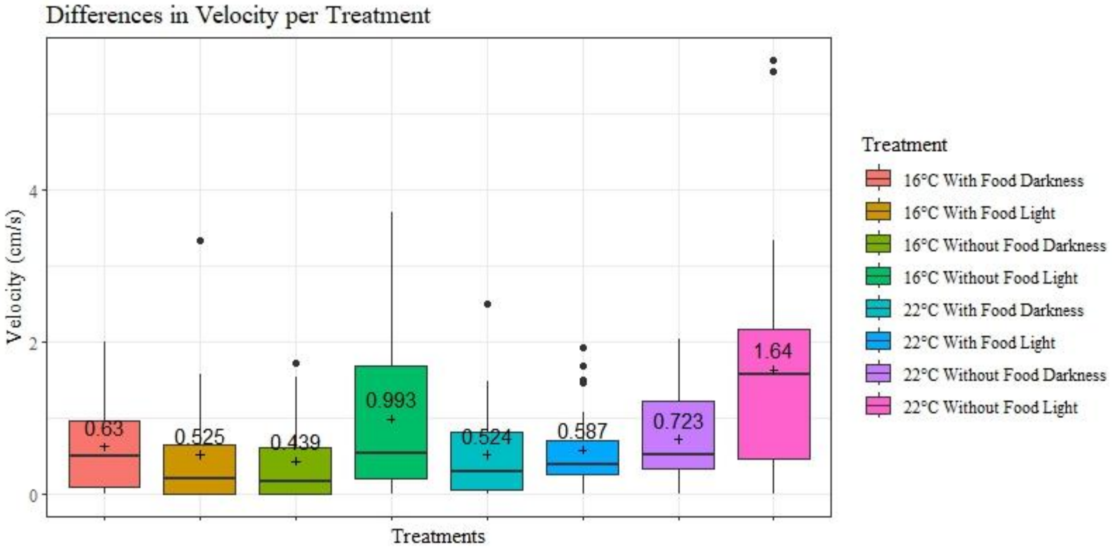

Figure 10 - Boxplots displaying variation of velocity between treatments from the environmental factors experiment. Plus sign indicates the mean value.

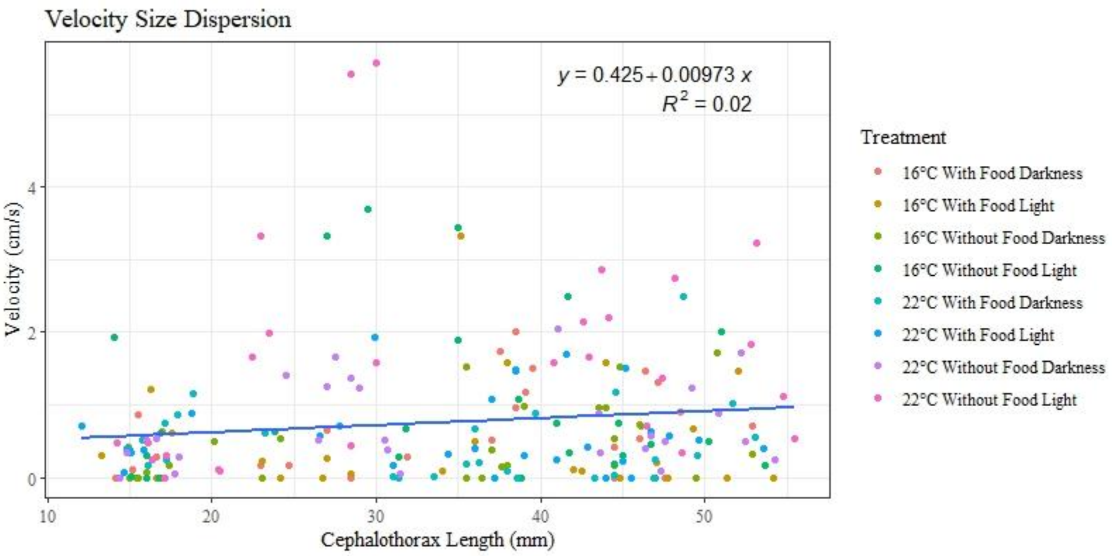

Figure 11 - Velocity in function to cephalothorax length with respective linear function adjust and r2 values are displayed. The values used are from the environmental factors experiment. Dots are colour coded for each treatment.

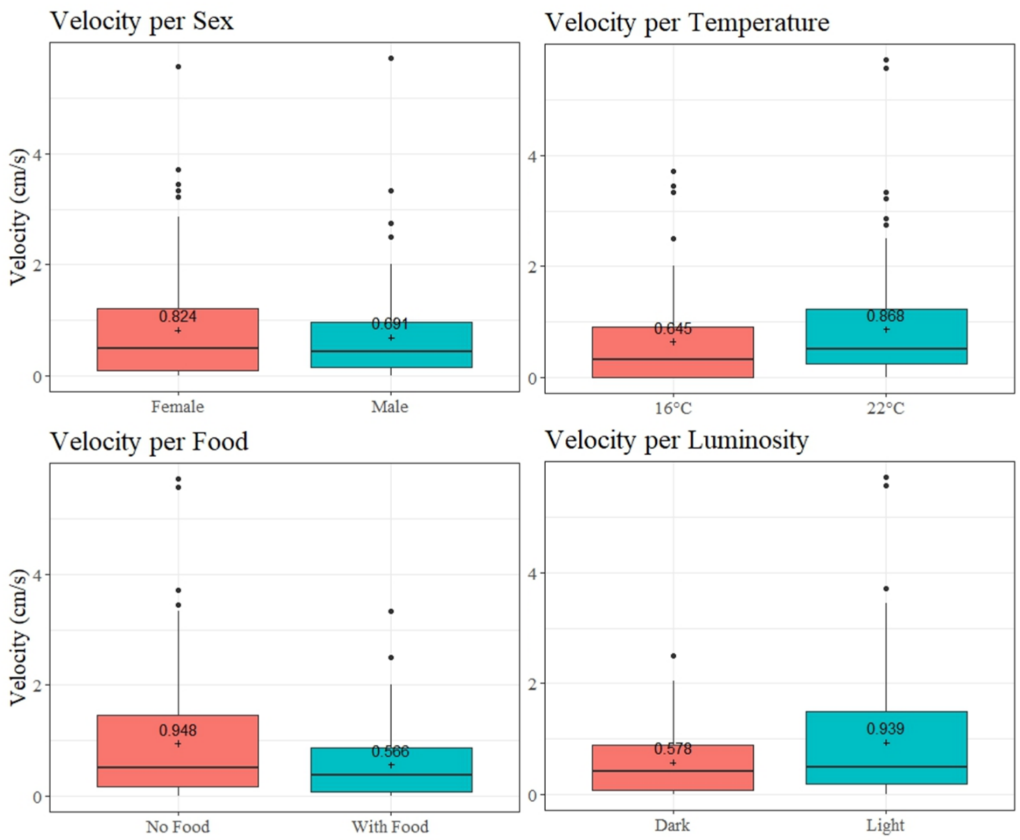

Figure 12 - Boxplots displaying the variation of velocity from the environmental experiment between sex, temperature, food, and luminosity. Plus sign indicates the mean value.

Social context

One-way ANOVA did not find differences between velocities from the social experiment's control and the light with no food treatments from the environmental factors experiment (Table A1). One-Way ANOVA performed on the data didn't show significant differences between any treatment on the velocity (One-Way ANOVA: F=0.299; p=0.913). No differences were observed either when comparing the velocities of different size individuals and same size ones (One-Way ANOVA: F=0.248; p=0.619). Generalized

Table 5 - GLM results using the treatments from the social interactions experiments to predict velocity. Treatment 1 – different size males; Treatment 2 – different size male and female; Treatment 3 - same size females; Treatment 4 – same size male and female; Treatment 5 – same size males. Statistic values correspond to t values, df to degrees of freedom.

	Velocity (cm/s)				
Predictors	*Estimates*	*std. Error*	*Statistic*	*df*	*p*
(Intercept)	1.38	0.15	9.24	152.00	**<0.001**
Treatment [1]	0.18	0.36	0.49	152.00	0.622
Treatment [2]	-0.29	0.36	-0.81	152.00	0.417
Treatment [3]	-0.09	0.38	-0.24	152.00	0.807
Treatment [4]	-0.07	0.31	-0.23	152.00	0.814
Treatment [5]	0.25	0.29	0.87	152.00	0.382

Table 6 - GLM results using size category from the social interactions experiments to predict velocity. Statistic values correspond to t values, df to degrees of freedom.

	Velocity (cm/s)				
Predictors	*Estimates*	*std. Error*	*Statistic*	*df*	*p*
(Intercept)	1.38	0.15	9.38	156.00	**<0.001**
Size Category	-0.07	0.15	-0.50	156.00	0.619

Linear Model yielded the same conclusions, with no significant effect of the treatments or size categories on velocity (Table 5 and Table 6). There were significant differences in velocity between the two temperatures (t-test: p-value = 0.006). When analysing subsets for each temperature with One-Way ANOVA there were no statistically significant differences in the values of velocity per treatment or size category (Table 7). There were significant differences between sexes among the individuals that were behind their pair inside the gutter (t-test for velocity: p = 0.020). On average females achieved faster speeds than males (Figure 11) but had no significant variation per treatment (One-Way ANOVA: F=1.953; p=0.140). No other significant differences were observed between sexes.

Table 7 - One-Way ANOVA results for the 16°C and 22°C data subsets from the social interactions experiment using velocity by treatment. df correspond to the degrees of freedom. Statistic values correspond to the F values.

	Velocity - 16°C					Velocity - 22°C				
Predictors	*df*	*Sum Sq*	*Mean Sq*	*Statistic*	*p*	*df*	*Sum Sq*	*Mean Sq*	*Statistic*	*p*
Treatment	5	8.26	1.652	1.03	0.407	5	16.6	3.318	0.736	0.599
Residuals	72	115.54	1.605			74	333.5	4.507		
Predictors	*df*	*Sum Sq*	*Mean Sq*	*Statistic*	*p*	*df*	*Sum Sq*	*Mean Sq*	*Statistic*	*p*
Size.Category	1	5.61	5.607	3.61	0.061	1	9.7	9.703	2.223	0.14
Residuals	76	118.19	1.55			78	340.4	4.364		

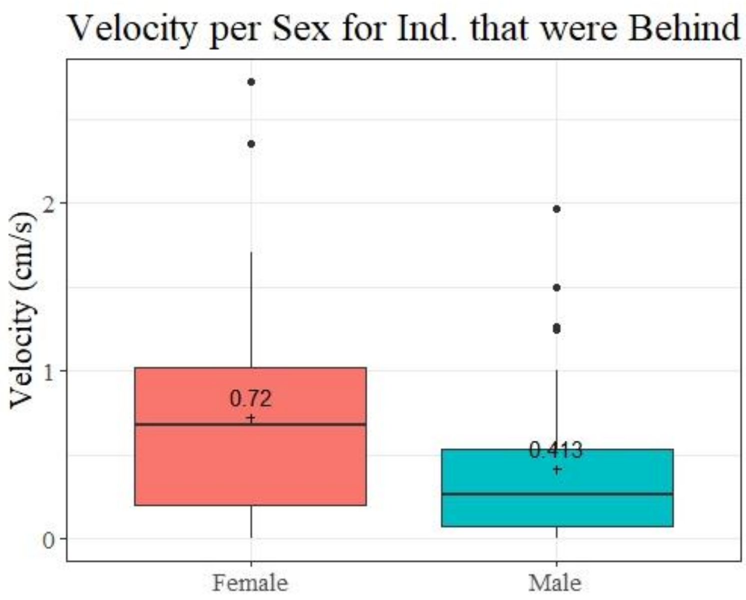

Figure 13 - Boxplot displaying the variation of velocity for individuals that were ahead or behind their inside the gutter pair in the social interactions experiment. Plus sign indicates the mean value.

Serotonergic effects

There were no significant differences between treatments regarding the crayfish velocities (Repeated measures ANOVA: F=0.693; p=0.597). There was also no difference between sexes in velocities (Repeated measures ANOVA: F=0.309; p=0.579).

Pairwise t-test showed no significant differences between the 8th and 16th day regarding the velocities achieved (Table 8). A repeated measures linear model also revealed no effect of each treatment on the velocity (Table 9).

Table 8 - Pairwise t-test results for velocity on the 8th and 16th day of exposure. n1 and n2 correspond to the sample counts, statistic values correspond to the values used to compute the p-value and df correspond to the degrees of freedom.

	Velocity							
Treatment	*group 1*	*group 2*	*n1*	*n2*	*statistic*	*df*	*p*	*p.adj.signif*
Control	Day 8	Day 16	29	29	-1.24	28	0.227	ns
Fluoxetine 1µg	Day 8	Day 16	28	28	1.18	27	0.25	ns
Fluoxetine 100µg	Day 8	Day 16	29	29	-1.87	28	0.072	ns
PCPA 1µg	Day 8	Day 16	29	29	-0.804	28	0.428	ns
PCPA 100µg	Day 8	Day 16	29	29	-1.22	28	0.231	ns

Table 9 - Results from the repeated measures linear model for the fluoxetine and PCPA experiments. Treatments were compared to the control group. Statistic values correspond to t values, df to degrees of freedom.

	Velocity (cm/s)				
Predictors	*Estimates*	*std. Error*	*Statistic*	*df*	*p*
(Intercept)	0.67	0.61	1.11	281.00	**<0.001**
Fluoxetine 1µg	0.07	0.20	0.37	281.00	0.688
Fluoxetine 100µg	-0.13	0.20	-0.64	281.00	0.539
PCPA 1µg	0.11	0.20	0.57	281.00	0.597
PCPA 100µg	0.19	0.20	0.95	281.00	0.346
Random Effects					
σ^2	1.11				
τ_{00} Day	0.01				
ICC	0.01				
N Day	2				

Discussion

The aim of this study is to test for several factors, socio-environmental and physiological in the crayfish *P. clarkii* dispersal capabilities. For that we exposed crayfish to differences in temperature, light and food, and different pairings of individuals in controlled laboratorial conditions. Similarly, to the environmental and social conditions, we exposed crayfish to 2 dosages of a serotonin facilitator and 2 dosages of a serotonin depletor for 16 days. Regarding our tested environmental candidates, our results suggest that temperature of 22°C and exposure to light lead to an increase in velocity while the presence of food seems to decrease these. The interaction of food with light and food with temperature also increase speed. Size and sex don't seem to have an effect. In the social interactions' procedure, there were significant differences in velocity between sexes among the individuals that were behind their pair inside the gutter, with females on average achieving faster speeds than males but there was no significant variation between treatments with other females or males of any size. No other significant differences were observed. Regarding the dosages tested in the SSRI exogenous manipulation experiments, no affect was detected in speed compared to the control group.

Environmental factors

It was expected that an increase in temperature would lead to an increase in the velocity of the crayfish giving *P. clarkii's* ectothermic nature. According to Huner and Barr (1991) the preferred temperatures are between 21°C and 27°C while Anastácio et al (1999) reports between 20°C and 28°C. In fact, temperature has been demonstrated to be a determining factor in both parameters. Here we show that the crayfish moved on average faster with 22°C than with 16°C. Similar increment in dispersal locomotion have been shown on previous studies, were increases in water temperature between 5°C to 25°C in laboratory lead to increased locomotion activity (Gherardi et al., 2000). We also expected that hunger could be a powerful incentive for any animal to move and seek new sources of nourishment. It has already been shown that *P. clarkii* will have outbursts of movement that can last for several hours when a single delivery of food in presented to them (Fernandez de Miguel & Arechiga, 1994). Faced with this, it was predicted that food would influence locomotion and that individuals would move faster with the presence of food than without it. Indeed, food did have a significant effect on speed according to GLM results. However, mean values for velocity were considerably higher in the absence of food than in presence of it, something that was unexpected. It's unknown at this moment why this is the case. Sardines are highly nutritive food items used as an affective bait to trap crayfish, and it was expected that individuals would be attracted to it in a laboratorial setting as well. It could be that the presence of food causes the individuals to feel secure that there's readily available sustenance nearby, therefor there's little need to move far in search of food, although further research is needed on the full effects of food on crayfish dispersion. Luminosity seems also to influence velocity. The highest mean registered for velocity occurred with the light treatment. Because *P. clarkii* is a mostly nocturnal species, it was expected that these would react more during the dark treatments. Some previous studies have shown that *P. clarkii* has higher activity during night periods (Correia et al., 1998; Fernandez de Miguel & Arechiga, 1994; Miranda-Anaya, 2004; Ramaliro, 2012) while others did not show conclusive evidence for an exclusive nocturnal activity (Aquiloni et al., 2005; Francesca Gherardi et al., 2000; Page & Larimer, 1972). This species is known to take shelter in borrows and such during the day. However, in this study higher speeds occur during light treatments which might be due to individuals necessity to look for potential shelter or maybe in trying to reduce the time they expose themselves to potential predators that largely depend on vision for hunting (Aquiloni et al., 2005; Flint, 1977; Cukerzis, 1988; Maitland and Campbell, 1992; Gherardi and Barbaresi 2000; Marques, M., Banha, F., Águas, M., & Anastácio, P., 2012). According to our results size may not contribute to speed. Although the line equation correlated size with velocity, the r^2 value was 0.02 (Figure 11). This value is very small, giving us practically no explanatory power. Previous studies have corelated size with speed. For example, according to F. Gherardi, Tricarico, & Ilhéu (2002), velocity is positively correlated with crayfish size with speeds ranging from 1 to 11 m/day on a section of a temporary stream. Our study recorded considerably higher speeds, with a mean of 0.76 cm/s, the equivalent of 656.64 m/day, and the highest speed of 5.7 cm/s is the equivalent of 4924.8 m/day. It's unlikely that these speeds can be maintained for long periods of time. They were most probably periodic bursts of locomotion as individuals look for shelter and try to avoid unnecessary exposure during light treatments. Other studies however seem to point otherwise. As an example, Ramaliro (2012) didn't find significant influence of crayfish weight on the average velocity using a linear channel. Sex didn't influence the dispersal velocity, something that's also corroborated in other studies (ex. (Gonçalves Loureiro et al., 2015)) but seems at odds with others that did find differences between sexes (ex.: Barbaresi, Santini, Tricarico, & Gherardi, 2004). This could be explained by local variation of the crayfish population, or it could be due to the experimental design.

Social context

Most previous studies done in the past scarcely touch on crayfish's dispersion as a result of social interactions, preferring instead to focus on the aggressive and social behaviours present during agonistic encounters or on dominant/subordinate relationships (Bovbjerg, 1956; Dingman, Hurlburt, & Otte, 2009; Issa et al., 1999; Moore, 2007; Sato & Nagayama, 2012; A. J. Tierney, Greenlaw, Dams-O'Connor, Aig, & Perna, 2004). This makes it difficult to discuss our results without similar examples to compare with, however it demonstrates the novelty of what we tested here. We expected that these experiments with crayfish dyads would give us insight on the importance of social context together with other factors such as life stage, size and sex are more likely to influence crayfish dispersal capabilities. It is known that size plays a major role in establishing dominance in crayfish (Figler, Finkelstein, Twum, & Peeke, 1995; Pavey & Fielder, 1996; Ranta & Lindström, 1992; Rutherford, Dunham, & Allison, 1995), with smaller individuals retreating without fighting or fleeing from a fight (Issa et al., 1999) and larger crayfish being able to evict smaller ones from burrows (Ranta & Lindström, 1992). Hence, it was expected that the smaller individual might achieve higher speeds, particularly when put together with larger individuals. Overall, our findings do not show any major effect on the velocity achieved by the individuals on any of the treatments, implying that there might not be any influence of size difference within or outside of the same sex. Surprisingly, we observe that, on average females that were behind their pair in the gutter had greater speeds than males that were behind. The reason why is unclear. In the other experiments, sex didn't seem to affect the velocity. Even in the other statistical analysis of the social interactions data, sex doesn't seem to influence locomotion. This result could be a statistical anomaly due to low sample size or it could be an error done during the experiments and/or statistical analysis. It's also important to note that the crayfish used in the experiments were kept in individual containers and didn't socialise with other crayfish before the experiments. According to Drozdz et al. (2006) crayfish behaviour seems to depend on prior socialization. Crayfish that have been housed individually don't show differences in social behaviours like rearing, turning, cornering or backing away from other crayfish while individuals that are housed in pairs do (Drozdz et al., 2006). But for instance, unfamiliar pairs of crayfish through dyadic agonistic interactions may form a hierarchical structure (Issa, Adamson, & Edwards, 1999; Hayes, 1975; Huber & Delago, 1998) where dominant individuals display behaviours like attacks and approaches more often than less dominant individuals that will retreat and try to escape more often (Issa et al., 1999). Since the individuals used haven't socialise for a few weeks before the experiments, the single minute the dyads had before the water gate opened may have not been enough to establish a hierarchical structure between the two individuals that might lead to potential changes in their locomotion. However, as we saw, it was enough to show some antagonistic behaviour. Future studies should perform prior socialisation between individuals before experimentation to account behaviour changes brought about by crayfishes' hierarchical structures.

Serotonergic effects

Several studies have catalogued a variety of effects of serotonin and dopamine on crayfish, although very little of these talk of their role on dispersion, focusing instead on the social behaviours, giving us little to compare our results to. Serotonin or serotonin facilitators have been shown to reduced locomotion in crayfish injected with serotonin (Fossat et al., 2014; A. Tierney & Mangiamele, 2001) and lobsters (Peeke, Blank, Figler, & Chang, 2000), hence we were expecting fluoxetine treatments to have reduced speeds compared to the other treatments. Because we wanted to simulate crayfish exposure to these monoamines in natural conditions, we introduced them to 16 days of exposure. Nevertheless, our results did not find significant between any of the treatments with the control on either the 8th or 16th day of exposure. This does not mean however that fluoxetine and PCPA don't influence crayfish dispersion entirely. Numerous previous studies have shown that serotonin plays an important role in crayfish social interactions. According to Tierney et al. (2016) juvenile crayfish exposed for 10 days to solutions of 2 and 500 µg/L fluoxetine displayed significantly reduced locomotion in open field tests compared to controls, however, fluoxetine exposed adults walking behaviour did not differ significantly from the controls. This according to the author suggests that adult crayfish are more resistant to the behavioural effects of fluoxetine than juveniles are. This could explain the lack of differences between our treatments since all individuals tested were fully mature adults. Dosage may have also been an issue. Mesquita et al (2011) observed an increased locomotion in *Carcinus maenas* after 7 days of exposure to solutions of fluoxetine at concentrations equal or greater than 120 µg/L. Tierney et al (2016) reported a decrease in motion in *Orconectes rusticus* after 10 days exposure to solutions of 2 and 500 µg/L of fluoxetine, but not at 200 µg/L. While these two studies have opposite effects, they both used bigger dosages than what we tested here (1 and 100 µg/L), leading us to believe that the concentrations used may have been too low to have an effect or that the exposure time was not enough. However, Tierney et al (2016) did see a change in locomotion at 2 µg/L. This could be because *Orconectes rusticus* has a different response to fluoxetine compared to *P. clarkii* or variations in populations. It may also be possible that pharmaceuticals, such as fluoxetine and PCPA, have specific concentration in which it acts. The individuals were also tested singularly. Serotonin is well known to affect the behavioural patterns of crayfish (Huber, R., Orzeszyna, M., Pokorny, N., & Kravitz, E. A.,1997; Robert Huber, Panksepp, Yue, Delago, & Moore, 2001; Momohara et al., 2013; Sneddon, Taylor, Huntingford, & Watson, 2000). But, in this case, if instead of singular individuals we had used dyads or groups of crayfish we might have seen different outcomes than those we report.

Experimental design limitations and future work

Although the results of these experiments may demonstrate relevancy to better understand *P. clarkii* dispersion capabilities, the experimental design used may hold limitations that could have skewed the results. The use of the metal gutter allowed us to easily determine the distances, and consequently, velocities of the individuals but poses several limitations. For one it only allows the crayfish to take one possible direction through a narrow path, limiting their movements. There was also no water flow, meaning we didn't have a way to test if crayfish preferred to travel up or down stream under the different treatments. The metric used to determine the velocity of each individual was the final distance in relation to the start of the steel track. However, several crayfish would turn around during their travel, sometimes more than once, meaning they would move a greater distance than that which was recorded. The behaviour of the crayfish may also be a point of contention. Individuals had to be manually picked up and placed at the start of the track. This means that each crayfish had only one minute between them being picked up and the 2 minutes of free movement. This could have not been enough time for the crayfish to recover from a potentially stressing situation and lead to erroneous results. Also, 60 seconds may not be enough time for two individuals to properly establish a hierarchical structure during the social interactions' experiments. Two minutes may also be too little time to properly observe changes in individuals' locomotion. A revised set-up that could possibly assess better crayfish dispersion would be a circular tank, instead of a straight track, as the one use by Mesquita et al. (2011). This would allow crayfish a greater range of possible directions in which to move. Another possibility is a variation of the set-up used for this dissertation, where crayfish are placed in the centre of the gutter instead of in one of its ends. This second option would allow us to create a current of water, allowing us to test if crayfish movements are affected by water flow. The metal gutter can also be elevated on one end to simulate inclination and test that as well. The lack of differences between the social interactions treatments may have been because of lack of prior socialisation by the crayfish, as it was shown by Drozdz et al. (2006). If social interactions are to be tested further, then the periods of time a given pair of individuals must interact should be longer, allowing them to socialise and form a hierarchy to properly evaluate its effects on dispersion. Tests on the role of serotonin needs to be revised somewhat. The periods of exposure don't seem to be a problem as what can be seen from other studies, but the concentrations might have been. In this dissertation was used 1 and 100 µg/L and no effect was observed, but Tierney et al (2016) saw differences at 2 and 500 µg/L and Mesquita, Guilhermino, & Guimarães (2011) only saw effects at concentrations equal or greater than 120 µg/. An idea for future studies would be to use a greater number of treatments with concentrations for 1 to 500 µg/L or possibly higher.

Closing remarks

The experiments done for this dissertation have shown that higher temperatures and increased light exposure lead to a potential increase in *P. clarkii* dispersion capabilities while the presence of food items seems to decrease it. The combine effects of the availability of food items with light levels or with temperature also seem to influence the dispersion abilities of the crayfish while the combination of temperature with illumination and the combination of all three factors don't seem to have an effect. It was also noticed that females that were behind their pair inside the gutter achieved higher speeds than males. The dosages of serotonin used seems to not influence the dispersion of adult *P. clarkii* at all. Although it was expected to observe greater differences between treatments in the social experiments and fluoxetine and PCPA experiments, these results still give us a greater insight into *P. clarkii* dispersion capabilities. However, further analysis is required into the role of social interactions and serotonin utilizing an improved experimental design. *P. clarkii* and other invasive crayfish species pose several environmental and social economic dangers. Faced with current global changes and habitat alterations it's important to continuously expand our knowledge and understanding of which factors contribute to a higher rate of dispersion. Doing so can prove instrumental in formulating new plans and strategies to limit the impacts of *P. clarkii*.

References

Adão H. 1992. *Procambarus clarkii* (Girard 1852) (Decapoda, Cambaridae) espécie exotica em Portugal. Considerações sobre a ecobiologia e aspectos sobre sua biologia populational na barragem de Monte Novo (Alentejo, Portugal). Available from: Universidade de Évora, Provas de Aptidao Pedagogica e Capacidade Cientifica (Trabalho de síntese)

Alcorlo, P., Geiger, W., Otero, M., Alcorlo, P., Geiger, W., & Otero, M. (2004). Feeding Preferences and Food Selection of the Red Swamp Crayfish , *Procambarus clarkii* , in Habitats Differing in Food Item Diversity Stable URL : http://www.jstor.org/stable/20105729 Your use of the JSTOR archive indicates your acceptance of the Terms &, 77(4), 435–453.

Anastácio, P. M., Banha, F., Capinha, C., Bernardo, J. M., Costa, A. M., Teixeira, A., & Bruxelas, S. (2015). Indicators of movement and space use for two co-occurring invasive crayfish species. *Ecological Indicators*, 53, 171–181. Retrieved from https://doi.org/10.1016/j.ecolind.2015.01.019

Anastácio, P. M., Nielsen, S. N., & Marques, J. C. (1999). CRISP (crayfish and rice integrated system of production): 2. Modelling crayfish (*Procambarus clarkii*) population dynamics. *Ecological Modelling*, 123(1), 5–16. Retrieved from https://doi.org/10.1016/S0304-3800(99)00164-7

Andrews, P. W., Bharwani, A., Lee, K. R., Fox, M., & Thomson, J. A. (2015). Is serotonin an upper or a downer? The evolution of the serotonergic system and its role in depression and the antidepressant response. *Neuroscience and Biobehavioral Reviews*, 51, 164–188. Retrieved from https://doi.org/10.1016/j.neubiorev.2015.01.018

Aquiloni, L., Ilhéu, M., & Gherardi, F. (2005). Habitat use and dispersal of the invasive crayfish *Procambarus clarkii* in ephemeral water bodies of Portugal. *Marine and Freshwater Behaviour and Physiology*, 38(4), 225–236. Retrieved from https://doi.org/10.1080/10236240500310195

Barbaresi, S., Santini, G., Tricarico, E., & Gherardi, F. (2004). Ranging behaviour of the invasive crayfish, *Procambarus clarkii* (Girard). *Journal of Natural History*, 38(22), 2821–2832. Retrieved from https://doi.org/10.1080/00222930410001663308

Bossus, M. C., Guler, Y. Z., Short, S. J., Morrison, E. R., & Ford, A. T. (2014). Behavioural and transcriptional changes in the amphipod Echinogammarus marinus exposed to two antidepressants, fluoxetine and sertraline. *Aquatic Toxicology*, 151, 46–56. Retrieved from https://doi.org/10.1016/j.aquatox.2013.11.025

Bovbjerg, R. V. (1956). Some factors affecting aggressive behavior in crayfish. *Physiological Zoology*, *29*(2), 127-136

Bunin, M. A., & Wightman, R. M. (1998). Quantitative evaluation of 5-hydroxytryptamine (serotonin) neuronal release and uptake: An investigation of extrasynaptic transmission. *Journal of Neuroscience*, 18(13), 4854–4860. Retrieved from https://doi.org/10.1523/jneurosci.18-13-04854.1998

Carpenter, S. R., Stanley, E. H., & Vander Zanden, M. J. (2011). State of the world's freshwater ecosystems: Physical, chemical, and biological changes. *Annual Review of Environment and Resources*, 36, 75–99. Retrieved from https://doi.org/10.1146/annurev-environ-021810-094524

Carvalho, F., Sousa, R., Cássio, F., & Pascoal, C. (2022). Temperature and interspecific competition alter the impacts of two invasive crayfish species on a key ecosystem process. *Biological Invasions*, 24(12), 3757–3768. Retrieved from https://doi.org/10.1007/s10530-022-02875-7

Chen, J., & Condron, B. G. (2008). Branch architecture of the fly larval abdominal serotonergic neurons. *Developmental Biology*, 320(1), 30–38. Retrieved from https://doi.org/10.1016/j.ydbio.2008.03.038

Cheng, W., Chieu, H. T., Ho, M. C., & Chen, J. C. (2006). Noradrenaline modulates the immunity of white shrimp Litopenaeus vannamei. *Fish and Shellfish Immunology*, 21(1), 11–19. Retrieved from https://doi.org/10.1016/j.fsi.2005.09.003

Chucholl, C. (2011). Écologie des populations d'écrevisses non indigènes 'd'eau chaude' (*Procambarus clarkii*) dans un nouvel habitat froid. *Knowledge and Management of Aquatic Ecosystems*, (401), 1–21. Retrieved from https://doi.org/10.1051/kmae/2011053

Correia, A. M., Crustaceana, S., Mar, N., & Correia, A. M. (1998). Seasonal and Circadian Foraging Activity of *Procambarus clarkii* (Decapoda , Cambaridae) in Portugal Stable URL : http://www.jstor.org/stable/20105970 Your use of the JSTOR archive indicates your acceptance of the Terms & Conditions of Use , available at, 71(2), 158–166.

Crandall, K. A., & Buhay, J. E. (2008). Global diversity of crayfish (Astacidae, Cambaridae, and Parastacidae - Decapoda) in freshwater. *Hydrobiologia*, 595(1), 295–301. Retrieved from https://doi.org/10.1007/s10750-007-9120-3

Cukerzis JM, 1988. Astacus astacus in Europe. In: Holdich DM, Lowery RS, editors. Freshwater Crayfish: Biology, Management and Exploitation. Cambridge: Cambridge University Press. pp 309–340

Daubert, E. A., & Condron, B. G. (2010). Serotonin: A regulator of neuronal morphology and circuitry. *Trends in Neurosciences*, 33(9), 424–434. Retrieved from https://doi.org/10.1016/j.tins.2010.05.005

de Abreu, M. S., Maximino, C., Banha, F., Anastácio, P. M., Demin, K. A., Kalueff, A. V., & Soares, M. C. (2020). Emotional behavior in aquatic organisms? Lessons from crayfish and zebrafish. *Journal of Neuroscience Research*, 98(5), 764–779. Retrieved from https://doi.org/10.1002/jnr.24550

De Lange, H. J., Noordoven, W., Murk, A. J., Lürling, M., & Peeters, E. T. H. M. (2006). Behavioural responses of Gammarus pulex (Crustacea, Amphipoda) to low concentrations of pharmaceuticals. *Aquatic Toxicology*, 78(3), 209–216. Retrieved from https://doi.org/10.1016/j.aquatox.2006.03.002

Delibes, M., & Adrián, I. (1987). Effects of crayfish introduction on Otter Lutra lutra food in the Doñana National Park, SW Spain. *Biological Conservation*, 42(2), 153–159. Retrieved from https://doi.org/10.1016/0006-3207(87)90021-8

Dingman, S., Hurlburt, L., & Otte, M. (2009). Exploring new compounds for functional imaging using a crayfish (*Procambarus clarkia*) aggression paradigm. *Perceptual and Motor Skills*, 109(2), 487–499. Retrieved from https://doi.org/10.2466/PMS.109.2.487-499

Diwan, A. D. (2005). Current progress in shrimp endocrinology - A review. *Indian Journal of Experimental Biology*, 43(3), 209–223.

Drozdz, J. K., Viscek, J., Brudzynski, S. M., Mercier, A. J., Catharines, S., & Ls, O.

(2006). BEHAVIORAL RESPONSES OF CRAYFISH TO A REFLECTIVE ENVIRONMENT displays or aggression , in such diverse animal species such 1965a , b). Such observations indicate that the ability of animals to perceive their own mirror image as a conspecific is widely dis. *Animals*, 26(4), 463–473.

Ehrlich P.R. (1989) Attributes of invaders and the invading process, vertebrates. In: Biological Invasions, A Global Perspective (Eds J.A. Drake, H.A. Mooney, F. di Castri, H.A. Groves, F.A. Kruger, M. Rejma´nek & M. Williamson), pp. 315–328. John Wiley & Sons, Chichester

Elton C.S. (1958) The Ecology of Invasions by Animals and Plants. Chapman & Hall, London

Fernandez de Miguel, F., & Arechiga, H. (1994). Circadian locomotor activity and its entrainment by food in the crayfish *Procambarus clarkii*. *Journal of Experimental Biology*, 190, 9–21. Retrieved from https://doi.org/10.1242/jeb.190.1.9

Figler, M. H., Finkelstein, J. E., Twum, M., & Peeke, H. V. S. (1995). Intruding male red swamp crayfish, *Procambarus clarkii*, immediately dominate members of established communities of smaller, mixed-sex conspecifics. *Aggressive Behavior*, 21(3), 225–236. Retrieved from https://doi.org/10.1002/1098-2337(1995)21:3<225::AID-AB2480210305>3.0.CO;2-#

Fingerman, M. (1987). The Endocrine Mechanisms of Crustaceans Author (s): Milton Fingerman Source : Journal of Crustacean Biology , Vol . 7 , No . 1 (Feb ., 1987), pp . 1-24 Published by : The Crustacean Society Stable URL : http://www.jstor.org/stable/1548622 THE ENDOCRIN. *Society*, 7(1), 1–24.

Fingerman, M. (1997). Crustacean endocrinology: A retrospective, prospective, and introspective analysis. *Physiological Zoology*, 70(3), 257–269. Retrieved from https://doi.org/10.1086/639593

Fingerman, M. (1997b). Roles of neurotransmitters in regulating reproductive hormone release and gonadal maturation in decapod crustaceans. *Invertebrate Reproduction & Development*, *31*(1-3), 47-54

Fingerman, M., Hanumante, M. M., Fingerman, S. W., & Reinschmidt, D. C. (1981). Effects of norepinephrine and norepinephrine agonists and antagonists on the melanophores of the fiddler crab Uca pugilator. *Journal of Crustacean Biology*, 16-27

Flint RW. 1977. Seasonal activity, migration and distribution of the crayfish, Pacifastacus leniusculus, in Lake Tahoe. Amer. Midl. Natur. 97:280–292

Fong, P. P., & Ford, A. T. (2014). The biological effects of antidepressants on the molluscs and crustaceans: A review. *Aquatic Toxicology*, 151, 4–13. Retrieved from https://doi.org/10.1016/j.aquatox.2013.12.003

Forsyth, D. M., Duncan, R. P., Bomford, M., & Moore, G. (2004). Climatic suitability, life-history traits, introduction effort, and the establishment and spread of introduced mammals in Australia. *Conservation Biology*, 18(2), 557–569. Retrieved from https://doi.org/10.1111/j.1523-1739.2004.00423.x

Fossat, P., Bacqué-Cazenave, J., De Deurwaerdère, P., Delbecque, J. P., & Cattaert, D. (2014). Anxiety-like behavior in crayfish is controlled by serotonin. *Science*, 344(6189), 1293–1297. Retrieved from https://doi.org/10.1126/science.1248811

Frem, M., Chapman, D., Fucilli, V., Choueiri, E., Moujabber, M. El, Notte, P. La, & Nigro, F. (2020). NeoBiota Xylella fastidiosa invasion of new countries in Europe , the

Middle East and North Africa : Ranking the potential exposure scenarios, 97, 77–97. Retrieved from https://doi.org/10.3897/neobiota.59.53208

Galib, S. M., Sun, J., Twiss, S. D., & Lucas, M. C. (2022). Personality, density and habitat drive the dispersal of invasive crayfish. *Scientific Reports*, 12(1), 1–12. Retrieved from https://doi.org/10.1038/s41598-021-04228-1

Gherardi, F. (2006). Crayfish invading Europe: The case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology*, 39(3), 175–191. Retrieved from https://doi.org/10.1080/10236240600869702

Gherardi, F. (2007). Understanding the impact of invasive crayfish. *Biological Invaders in Inland Waters: Profiles, Distribution, and Threats*, 507–542. Retrieved from https://doi.org/10.1007/978-1-4020-6029-8_28

Gherardi, F., & Barbaresi, S. (2000). Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). *Archiv für Hydrobiologie*, 153-168

Gherardi, F., Barbaresi, S., & Salvi, G. (2000). Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences*, 62(2), 179–193. Retrieved from https://doi.org/10.1007/s000270050006

Gherardi, F., Tricarico, E., & Ilhéu, M. (2002). Movement patterns of an invasive crayfish, *Procambarus clarkii*, in a temporary stream of southern portugal. *Ethology Ecology and Evolution*, 14(3), 183–197. Retrieved from https://doi.org/10.1080/08927014.2002.9522739

Gonçalves Loureiro, T., Silva Gentil Anastácio, P. M., Souty-Grosset, C., Araujo, P. B., & Pereira Almerão, M. (2015). Red swamp crayfish: biology, ecology and invasion - an overview. *Nauplius*, 23(1), 1–19.

Gutiérrez-Yurrita, P.J.; Martínez, J.M.; Bravo-Utrera, M.Á.; Montes, C.; Ilhéu, M. and Bernardo, J.M. 1999. The status of crayfish populations in Spain and Portugal. p. 161-192. In: F. Gherardi and D.M. Holdich (eds), Crayfish in Europe as Alien Species: How to Make the Best of a Bad Situation? Rotterdam, A.A. Balkem

Habsburgo-Lorena, A.S., 1978. Present situation of exotic species of crayfish introduced into Spanish continental waters. Freshw. Crayfish 4, 175–184.

Hayes, W. (1975). Behavioral components of social interactions in the crayfish Procambarus gracilis (Bundy)(Decapoda, Cambaridae). *Proceeding of the Oklahoma Academy of Science*, 5(2), 1–5. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:BEHAVIORAL+COMPONENTS+OF+SOCIAL+INTERACTIONS+in+the+crayfish+procambarus+gracilis+(bundy)+(decapoda,+cambaridae)#0

Hobbs, H.H. 1988. Crayfish distribution, adaptive radiation, and evolution. p. 52-82. In: D.M. Holdich and R.S. Lowery (eds), Freshwater Crayfish: biology, management and exploitation. London, Croom Helm

Hobbs Iii, H. H., Jass, J. P., Huner, J. V, & Iii, H. H. H. (1989). A Review of Global Crayfish Introductions with Particular Emphasis on Two North American Species (Decapoda, Cambaridae). *Source: Crustaceana*, 56(3), 299–316.

Holdich, D. M. (1988). The dangers of introducing alien animals with particular references to crayfish. *Freshwater crayfish*, *77*, XV-XXX

Holdich, D. M. (2002). Distribution of crayfish in Europe and some adjoining countries.

BFPP - Bulletin Francais de La Peche et de La Protection Des Milieux Aquatiques, (367), 611–650. Retrieved from https://doi.org/10.1051/kmae:2002055

Huber, R., & Delago, A. (1998). Serotonin alters decisions to withdraw in fighting crayfish, Astacus astacus: The motivational concept revisited. *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, 182(5), 573–583. Retrieved from https://doi.org/10.1007/s003590050204

Huber, R., Panksepp, J. B., Yue, Z., Delago, A., & Moore, P. (2001). Dynamic interactions of behavior and amine neurochemistry in acquisition and maintenance of social rank in crayfish. *Brain, Behavior and Evolution*, 57(5), 271–282. Retrieved from https://doi.org/10.1159/000047245

Huber, R., Orzeszyna, M., Pokorny, N., & Kravitz, E. A. (1997). Biogenic amines and aggression: experimental approaches in crustaceans. *Brain, behavior and evolution*, *50*(Suppl. 1), 60-68

Huber, R., Smith, K., Delago, A., Isaksson, K., & Kravitz, E. A. (1997). Serotonin and aggressive motivation in crustaceans: Altering the decision to retreat. *Proceedings of the National Academy of Sciences of the United States of America*, 94(11), 5939–5942. Retrieved from https://doi.org/10.1073/pnas.94.11.5939

Huner, J.V., Barr, L.E., 1991. Red Swamp Crawfish: Biology, Culture, and Exploitation. Louisiana State University Sea Grant College System, Louisiana State University, Baton Rouge, Louisiana, p. 128

IPBES. (2023). *Summary for Policymakers of the Thematic Assessment Report on Invasive Alien Species and their Control of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services*. *Environmental Indicators*.

Issa, F. A., Adamson, D. J., & Edwards, D. H. (1999). Dominance hierarchy formation in juvenile crayfish *Procambarus clarkii*. *Journal of Experimental Biology*, 202(24), 3497–3506. Retrieved from https://doi.org/10.1242/jeb.202.24.3497

Kolar, C. S., & Lodge, D. M. (2001). Progress in invasion biology: Predicting invaders. *Trends in Ecology and Evolution*, 16(4), 199–204. Retrieved from https://doi.org/10.1016/S0169-5347(01)02101-2

Kroeze, Y., Zhou, H., & Homberg, J. R. (2012). The genetics of selective serotonin reuptake inhibitors. *Pharmacology and Therapeutics*, 136(3), 375–400. Retrieved from https://doi.org/10.1016/j.pharmthera.2012.08.015

Li, J. T., Lee, P. P., Chen, O. C., Cheng, W., & Kuo, C. M. (2005). Dopamine depresses the immune ability and increases susceptibility to Lactococcus garvieae in the freshwater giant prawn, Macrobrachium rosenbergii. *Fish and Shellfish Immunology*, 19(3), 269–280. Retrieved from https://doi.org/10.1016/j.fsi.2005.01.003

Lindqvist, O.V. and Huner, J.V. 1999. Life history characteristics of crayfish: What makes some of them good colonizers? p. 23-30. In: F. Gherardi and D.M. Holdich (eds), Crayfish in Europe as alien species: How to make the best of a bad situation? Rotterdam, A.A. Balkema

Lodge D.M. (1993) Biological Invasions, lessons for ecology. Trends in Ecology and Evolution, 8, 133–137

Lodge, D.M., 2001. Lakes. In: Chapin III, F.S., Sala, O.E., Huber-Sannwald, E. (Eds.),Global Biodiversity in a Changing Environment: Scenarios for the 21st Century. Springer— Verlag, New York, pp. 277–313

Lodge D.M., Taylor C.A., Holdich D.M. & Skurdal J. (2000a) Nonindigenous crayfishes threaten American freshwater biodiversity. Fisheries, 25, 7–20

Lodge, D. M., Taylor, C. A., Holdich, D. M., & Skurdal, J. (2000). Nonindigenous Crayfishes Threaten North American Freshwater Biodiversity: Lessons from Europe. *Fisheries*, 25(8), 7–20. Retrieved from https://doi.org/10.1577/1548-8446(2000)025<0007:nctnaf>2.0.co;2

Maitland PS, Campbell RN. 1992. Freshwater Fishes. London: Harper Collins Publishers. Merkle EL. 1969. Home range of crayfish Orconectes juvenalis. Am. Midl. Natur. 81:228–235

MARQUES, M., BANHA, F., ÁGUAS, M., & ANASTÁCIO, P. Environmental variables affecting dispersion patterns and orientation in dry land of two invasive crustaceans: *Procambarus clarkii* and Eriocheir sinensis. *NEOBIOTA 2012*, 334

Maximino, C., Puty, B., Benzecry, R., Araújo, J., Lima, M. G., De Jesus Oliveira Batista, E., … Herculano, A. M. (2013). Role of serotonin in zebrafish (Danio rerio) anxiety: Relationship with serotonin levels and effect of buspirone, WAY 100635, SB 224289, fluoxetine and para-chlorophenylalanine (pCPA) in two behavioral models. *Neuropharmacology*, 71, 83–97. Retrieved from https://doi.org/10.1016/j.neuropharm.2013.03.006

Meredith-Williams, M., Carter, L. J., Fussell, R., Raffaelli, D., Ashauer, R., & Boxall, A. B. A. (2012). Uptake and depuration of pharmaceuticals in aquatic invertebrates. *Environmental Pollution*, 165, 250–258. Retrieved from https://doi.org/10.1016/j.envpol.2011.11.029

Mesquita, S. R., Guilhermino, L., & Guimarães, L. (2011). Biochemical and locomotor responses of *Carcinus maenas* exposed to the serotonin reuptake inhibitor fluoxetine. *Chemosphere*, 85(6), 967–976. Retrieved from https://doi.org/10.1016/j.chemosphere.2011.06.067

Miranda-Anaya, M. (2004). Circadian locomotor activity in freshwater decapods: An ecological approach. *Biological Rhythm Research*, 35(1–2), 69–78. Retrieved from https://doi.org/10.1080/09291010412331313241

Mole, R. A., & Brooks, B. W. (2019). Global scanning of selective serotonin reuptake inhibitors: occurrence, wastewater treatment and hazards in aquatic systems. *Environmental Pollution*, 250, 1019–1031. Retrieved from https://doi.org/10.1016/j.envpol.2019.04.118

Momohara, Y., Kanai, A., & Nagayama, T. (2013). Aminergic Control of Social Status in Crayfish Agonistic Encounters. *PLoS ONE*, 8(9). Retrieved from https://doi.org/10.1371/journal.pone.0074489

Moore, P. A. (2007). Agonistic Behavior in Freshwater Crayfish The Influence of Intrinsic and Extrinsic Factors on Aggressive Encounters and Dominance. In E. J. Duffy, & M. Thiel (Eds.), Evolutionary ecology of social and sexual systems: Crustaceans as model organisms (pp. 90–114). New York, NY: Oxford University Press

Page, T. L., & Larimer, J. L. (1972). Entrainment of the circadian locomotor activity rhythm in crayfish - The role of the eyes and caudal photoreceptor. *Journal of Comparative Physiology*, 78(2), 107–120. Retrieved from https://doi.org/10.1007/BF00693608

Paterson, G., & Metcalfe, C. D. (2008). Uptake and depuration of the anti-depressant fluoxetine by the Japanese medaka (Oryzias latipes). *Chemosphere*, 74(1), 125–130. Retrieved from https://doi.org/10.1016/j.chemosphere.2008.08.022

Pavey, C. R., & Fielder, D. R. (1996). The influence of size differential on agonistic behaviour in the freshwater crayfish, Cherax cuspidatus (Decapoda: Parastacidae). *Journal of Zoology*, 238(3), 445–457. Retrieved from https://doi.org/10.1111/j.1469-7998.1996.tb05405.x

Peeke, H. V. S., Blank, G. S., Figler, M. H., & Chang, E. S. (2000). Effects of exogenous serotonin on a motor behavior and shelter competition in juvenile lobsters (Homarus americanus). *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, 186(6), 575–582. Retrieved from https://doi.org/10.1007/s003590000113

Pimentel, D. (2011). *Biological invasions: economic and environmental costs of alien plant, animal, and microbe species*. USA: CRC Press; ISBN 978-1-4398-2007-0

Ramalho, R. O., & Anastácio, P. M. (2015). Factors inducing overland movement of invasive crayfish (*Procambarus clarkii*) in a ricefield habitat. *Hydrobiologia*, 746(1), 135–146. Retrieved from https://doi.org/10.1007/s10750-014-2052-9

Ramaliro, R. M. O. (2012). *Dispersal and population regulation of the red swamp crayfish (Procambarus clarkii)* (Doctoral dissertation, Universidade de Evora (Portugal))

Ranta, E., & Lindström, K. (1992). Power to Hold Sheltering Burrows by Juveniles of the Signal Crayfish, Pasifastacus leniusculus. *Ethology*, 92(3), 217–226. Retrieved from https://doi.org/10.1111/j.1439-0310.1992.tb00961.x

Reis, J., & Araujo, R. (2016). Life history of the freshwater mussel Unio tumidiformis (Bivalvia: Unionidae) in a temporary Mediterranean-type stream. *Invertebrate Biology*, 135(1), 31–45. Retrieved from https://doi.org/10.1111/ivb.12114

Rhoades, R. (1962). Further studies on Ohio crayfishes: Cases of sym- patry of stream species in southern Ohio. Ohio Journal of Science, 62, 27–33

Ricker, W. E., 1975. Computation and interpertation of biological statistics of fish populations. Bulletin of the Fisheries Research Board of Canada 191: 1–382

Rutherford, P. L., Dunham, D. W., & Allison, V. (1995). Winning agonistic encounters by male crayfish *Orconectes rusticus* (Girard) (Decapoda, cambaridae): Chela size matters but chela symmetry does not. *Crustaceana*, 68(8), 526–529. Retrieved from https://doi.org/10.1163/156854095X01709

Sarojini, R., Nagabhushanam, R., Fingerman, M., 1995a. Dopaminergic and enkephalinergic involvement in the regulation of blood glucose in the red swampcrayfish, *Procambarus clarkii*. General and Comparative Endocrinology 97 (1),160–170

Sarojini, R., Nagabhushanam, R., Fingerman, M., 1995b. Mode of action of the neurotransmitter 5-hydroxytryptamine in stimulating ovarian maturation in the redswamp crayfish, *Procambarus clarkii*: an in vivo and in vitro study. Journal ofExperimental Zoology 271 (5), 395–400

Sarojini, R., Nagabhushanam, R., Devi, M., Fingerman, M., 1995c. Dopaminergic inhibition of 5-hydroxytryptamine-stimulated testicular maturation in the fiddlercrab, Uca pugilator. Comparative Biochemistry and Physiology Part C: Pharma-cology, Toxicology and Endocrinology 111 (2), 287–292

Sato, D., & Nagayama, T. (2012). Development of agonistic encounters in dominance hierarchy formation in juvenile crayfish. *Journal of Experimental Biology*, 215(7), 1210–1217. Retrieved from https://doi.org/10.1242/jeb.066191

Shen, Y.-B. (2008). Shrimps. In M.-M. Chang (Ed.), The Jehol fossils (pp. 52–57). San

Diego, CA: Academic Press

Silva, L. J. G., Pereira, A. M. P. T., Meisel, L. M., Lino, C. M., & Pena, A. (2015). Reviewing the serotonin reuptake inhibitors (SSRIs) footprint in the aquatic biota: Uptake, bioaccumulation and ecotoxicology. *Environmental Pollution*, 197, 127–143. Retrieved from https://doi.org/10.1016/j.envpol.2014.12.002

Sneddon, L. U., Taylor, A. C., Huntingford, F. A., & Watson, D. G. (2000). Agonistic behaviour and biogenic amines in shore crabs *Carcinus maenas*. *Journal of Experimental Biology*, 203(3), 537–545. Retrieved from https://doi.org/10.1242/jeb.203.3.537

Sousa, R., Freitas, F. E. P., Mota, M., Nogueira, A. J. A., & Antunes, C. (2013). Invasive dynamics of the crayfish *Procambarus clarkii* (Girard, 1852) in the international section of the River Minho (NW of the Iberian Peninsula). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 23(5), 656–666. Retrieved from https://doi.org/10.1002/aqc.2323

Souty-Grosset, C., Anastácio, P. M., Aquiloni, L., Banha, F., Choquer, J., Chucholl, C., & Tricarico, E. (2016). The red swamp crayfish *Procambarus clarkii* in Europe: Impacts on aquatic ecosystems and human well-being. *Limnologica*, 58, 78–93. Retrieved from https://doi.org/10.1016/j.limno.2016.03.003

Souty-Grosset, C.; Holdich, D.M.; Noel, P.Y.; Reynolds, J.D. and Haffner, P. 2006. Atlas of crayfish in Europe. Paris, Publications Scientifiques du Muséum national d'Histoire naturelle, Patrimoines Naturels 64, 188p

Sykes, P. A., & Condron, B. G. (2005). Development and sensitivity to serotonin of Drosophila serotonergic varicosities in the central nervous system. *Developmental Biology*, 286(1), 207–216. Retrieved from https://doi.org/10.1016/j.ydbio.2005.07.025

Taylor C.A. (2002) Taxonomy and conservation of native crayfish stocks. In: Biology of Freshwater Crayfish (Ed. D.M. Holdich), pp. 236–256. Blackwell Scientific, Oxford

Tierney, A. J., Greenlaw, M. A., Dams-O'Connor, K., Aig, S. D., & Perna, A. M. (2004). Behavioral effects of serotonin and serotonin agonists in two crayfish species, *Procambarus clarkii* and *Orconectes rusticus*. *Comparative Biochemistry and Physiology - A Molecular and Integrative Physiology*, 139(4), 495–502. Retrieved from https://doi.org/10.1016/j.cbpb.2004.10.010

Tierney, A. J., Hanzlik, K. N., Hathaway, R. M., Powers, C., & Roy, M. (2016). Effects of fluoxetine on growth and behavior in the crayfish *Orconectes rusticus*. *Marine and Freshwater Behaviour and Physiology*, 49(2), 133–145. Retrieved from https://doi.org/10.1080/10236244.2015.1119974

Tierney, A., & Mangiamele, L. (2001). Effects of serotonin and serotonin analogs on posture and agonistic behavior in crayfish. *Journal of Comparative Physiology - A Sensory, Neural, and Behavioral Physiology*, 187(10), 757–767. Retrieved from https://doi.org/10.1007/s00359-001-0246-x

Villamagna, A. M., & Murphy, B. R. (2010). Ecological and socio-economic impacts of invasive water hyacinth (Eichhornia crassipes): a review, 282–298. Retrieved from https://doi.org/10.1111/j.1365-2427.2009.02294.x

Vitousek, P. M., D'Antonio, C. M., Loope, L. L., & Westbrooks, R. (1996). Biological invasions as global environmental change. *American Scientist*, 84(5), 468–478.

Attachments

Table A1 - One-way ANOVA results for velocity and distances between the social experiment's control and the light with no food treatments from the environmental factors experiment. df correspond to the degrees of freedom. Statistic values correspond to the F values.

	Velocity (cm/s) - 16°C					**Distance (cm) - 16°C**				
Predictors	*df*	*Sum Sq*	*Mean Sq*	*Statistic*	*p*	*df*	*Sum Sq*	*Mean Sq*	*Statistic*	*p*
Treatment	1	0.11	0.109	0.089	0.767	1	265	265	0.045	0.833
Residuals	39	47.61	1.221			39	230470	5909		
Observations	41					41				
R^2	0.002 / -0.023					0.001 / -0.024				
	Velocity (cm/s) - 22°C					**Distance (cm) - 22°C**				
Predictors	*df*	*Sum Sq*	*Mean Sq*	*Statistic*	*p*	*df*	*Sum Sq*	*Mean Sq*	*Statistic*	*p*
(Intercept)	1	1.11	1.109	0.402	0.529	1	0	0	0	0.997
Size Category	40	110.22	2.756			40	240398	6010		
Observations	42					42				
R^2	0.010 / -0.015					0.000 / -0.025				