UNIVERSIDADE DE ÉVORA

ESCOLA DE CIÊNCIAS E TECNOLOGIA

DEPARTAMENTO DE PAISAGEM, AMBIENTE E ORDENAMENTO

Mecanismos de dispersão ativa de crustáceos invasivos aquáticos através de meio terrestre

Active dispersal mechanisms of aquatic invasive crustaceans through dry land

Mónica Alexandra da Silva Marques

Orientação: Professor Doutor Pedro Anastácio

Mestrado em Qualidade e Gestão do Ambiente

Área de especialização: *Ecologia e Gestão Ambiental*

Dissertação

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MECANISMOS DE DISPERSÃO ATIVA DE CRUSTÁCEOS INVASIVOS AQUÁTICOS ATRAVÉS DE MEIO TERRESTRE

Resumo

É sabido que os crustáceos de água doce se conseguem dispersar por terra, invadindo novos ecossistemas. Para analisar a influência de algumas variáveis (humidade, temperatura, iluminância, vegetação, inclinação) na direção do movimento de *Eriocheir sinensis*, *Pacifastacus leniusculus* e *Procambarus clarkii*, em terra, colocaram-se os animais no centro de uma calha de metal para que optassem por um de dois valores da mesma variável. A direção de *E. sinensis* foi afetada pela inclinação de 9°, revelando uma tendência de deslocamento no sentido descendente. *P. leniusculus* foi afetado pela temperatura, preferindo se deslocar para áreas mais frescas (preferiu a temperatura de 24ºC à de 28.6ºC). *P. clarkii* foi afetado pela temperatura (preferiu a temperatura de 18.8ºC à de 27.1ºC) e pelas inclinações de 9° e 14° (tendência de deslocamento no sentido descendente). Apesar da humidade não ter afetado as direções do movimento, foi possível determinar intervalos em que as espécies não respondem: 60% to 76% para *E. sinensis*; 55% to 85% para *P. leniusculus*, e 32% to 55% para *P. clarkii*. Para testar a capacidade de retornar à água posicionaram-se os animais numa arena circular junto à água e registaram-se as direções tomadas. Os resultados revelaram que ambas as espécies, *P. leniusculus* e *P. clarkii*, não se deslocaram em direção à água.

Palavras-chave: *Eriocheir sinensis*, *Pacifastacus leniusculus*, *Procambarus clarkii*, dispersão terrestre, variáveis ambientais, dessecação

ACTIVE DISPERSAL MECHANISMS OF AQUATIC INVASIVE CRUSTACEANS THROUGH DRY LAND

Abstract

Freshwater crustaceans are known to disperse overland and invade new ecosystems. To analyze the effect of some variables (humidity, temperature, illuminance, vegetation and land slope) on the movement direction of *Eriocheir sinensis*, *Pacifastacus leniusculus* and *Procambarus clarkii* on dry land, the animals were placed at the centre of a metal gutter and given a choice between two different values of each variable. *E. sinensis*‘ direction was affected by ground slope (9°), revealing a tendency to move downhill. *P. leniusculus* was affected by temperature since it preferred to move towards cooler areas (it preferred 24ºC rather than 28.6ºC). *P. clarkii* was affected by temperature (it preferred 18.8ºC rather than 27.1ºC) and ground slope (facing a 9º and a 14 º slope it moved downhill). Although humidity didn’t affect the animals’ movement direction, it was possible to determine some ranges that these species don’t respond to: 60% to 76% for *E. sinensis*; 55% to 85% for *P. leniusculus*, and 32% to 55% for *P. clarkii*. To test *P. leniusculus* and *P. clarkii* ability to return to the water they were placed near the water, in a circular arena, and the movement directions were registered. The results revealed that both species didn’t move towards the water.

Keywords: *Eriocheir sinensis*, *Pacifastacus leniusculus*, *Procambarus clarkii*, overland dispersal, environmental variables, desiccation

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1. Introduction

1.1 Biological invasions and freshwater ecosystems

The concern about biological invasions began in the late 1950s with Elton arguing that these '...are so frequent nowadays in every continent and island, and even in the oceans, that we need to understand what is causing them and try to arrive at some general viewpoint about the whole business' (Elton 1958). Biological invasions occur when non-indigenous species (NIS) are transported beyond their native range, spread from the introduction point and become abundant bringing economic, environmental, and/or human health impacts (Beisel 2001; Crooks 2002; Kolar and Lodge 2001). The establishment of NIS has been recognized for decades (Elton 1958; Simberloff and Stiling 1996) and has been increasing all over the world causing the extinction of indigenous species and a great loss of biodiversity (Assessment 2005; Townsend and Crowl 1991; Vitousek et al. 1996). The efforts to understand the ecology of biological invasions have also been rising (Kolar and Lodge 2001).

Considered to be one of the most worrying threats to global biodiversity (Genovesi and Shine 2004; Williamson and Fitter 1996) Invasive Alien Species (IAS) have become a relevant issue to the European Community due to its environmental, economic and social impacts (Genovesi and Shine 2004). In order to prevent and/or reduce their effects on native communities, understanding what affects their distribution and limits their spread is a major challenge to conservation (Kerby et al. 2005; Simberloff and Stiling 1996).

Invasions are one of the main drivers of environmental change in inland waters (Assessment 2005) and some studies suggest that biodiversity in fresh waters declines at far greater rates than in most of the terrestrial ecosystems (Ricciardi and Rasmussen 1999). Freshwater ecosystems are particularly vulnerable to NIS (Hobbs III et al. 1989; Hobbs and Mooney 1998) and its impacts have been documented by many studies (Carlton 1996). Such vulnerability is associated with the worldwide expansion in trade and tourism and the resulting human mediated introductions: intentional and accidental NIS transport into freshwater ecosystems through ships' ballast, fish bait trade, aquarium trade, aquaculture and fisheries management (Beisel 2001; Carlton 1996; Carlton and Geller 1993; Hobbs and Mooney 1998; Lodge et al. 1998). The release of species such as the Chinese mitten crab (*Eriocheir sinensis)*, a brachyuran crab that is often transported in ballast waters (Dittel and Epifanio 2009; Lowe et al. 2000; Veldhuizen 2001) and impacts both freshwater and marine ecosystems (Lowe et al. 2000; Veldhuizen 2001), has become more frequent since the end of the 20th century (Baldwin 1992; Carlton and Geller 1993). There are historical records indicating that on an annual basis 10000 different species are inadvertently carried between bio-geographic regions in ballast tanks (Carlton 1999). Since people began travelling in ships the rate of NIS establishment in ports worldwide has increased (Bax et al. 2003) and contributed to the current biodiversity crisis (Baillie 2004; Sakai et al. 2001).

1.2 Three invasive freshwater crustaceans (*Eriocheir sinensis*, *Pacifastacus leniusculus, Procambarus clarkii*)

Freshwater crustaceans are a well documented group, known for their ability to invade new ecosystems and their impact on native species worldwide (Guan and Wiles 1997; Lodge et al. 1998; Lodge et al. 2000; Westman et al. 2002). The processes that enable this group of organisms to spread and establish throughout the world has been an evergreen issue for scientists (Hastings 1996). The present work focuses on three invasive decapod crustaceans: *Eriocheir sinensis* (Chinese mitten crab), *Pacifastacus leniusculus* (Signal crayfish) and *Procambarus clarkii* (Red swamp crayfish).

1.2.1 The Chinese mitten crab, *Eriocheir sinensis*

The Chinese mitten crab, *Eriocheir sinensis*, is amongst the brachyuran crabs that are often transported in ballast waters (Lowe et al. 2000; Veldhuizen 2001). The introduction of this species and its widespread impacts makes *Eriocheir sinensis* an important subject for the study of biological invasions in aquatic ecosystems (Rudnick et al. 2003). *Eriocheir sinensis* is one of the few invasive species that impact both freshwater and marine ecosystems and it is listed as one of the 100 “World's Worst” alien invasive species by the International Union for the Conservation of Nature (IUCN) due to severe damage to the environment and economy (Lowe et al. 2000; Veldhuizen 2001).

The Chinese mitten crab is native to Asian waters (Panning 1938; Veilleux and De Lafontaine 2007), from the west coast of North Korea to south of Shanghai, China (Panning 1938). This species was first recorded outside its native range more than 100 years ago (Panning 1938) in 1912 in northern Europe; one of the earliest reports of invasive species in aquatic habitats (Panning 1938; Rudnick et al. 2000; Rudnick et al. 2003). During the last hundred years *Eriocheir sinensis* has colonized the coasts of Europe (Panning 1938) and it has become established, at varying levels of abundance, throughout most of western, central, and northern Europe (Panning 1938). The most likely cause of unintentional introduction of *Eriocheir sinensis* into the Iberian Peninsula was probably by its main dispersal vector; ballast waters (Dittel and Epifanio 2009).

More recently, this species has appeared on the east coast of the USA and became established on the west coast of North America (Cohen and Carlton 1997; Rudnick et al. 2000; Rudnick et al. 2003; Ruiz et al. 2006).

There are consequences of the Chinese mitten crab’s presence such as the displacement of native species, destruction of shores and coastal reinforcements (Rudnick et al. 2003). Documented impacts include declining of natural vegetation by feeding activities (Panning 1938; Veldhuizen and Stanish 1999) and weakening of levees and stream banks through burrowing activities (Panning 1938). This species also cause damage to fishing operations by consuming bait, entanglement in nets and injury to netted fish (Panning 1938; Veldhuizen and Stanish 1999).

1.2.2 The signal crayfish, *Pacifastacus leniusculus*

The signal crayfish *Pacifastacus leniusculus* is a major invasive species (Larson et al. 2010; Lodge et al. 2000). Possible introduction pathways include live fishing bait, stocking for harvest, and lake or river management (Lodge et al. 2000). It has commercial value and it has been exploited both in crayfish farms and naturalized populations (Guan and Wiles 1997).

Pacifastacus leniusculus is a native crayfish of western North America which has been introduced globally and most of the introduced populations derived from Lake Tahoe in California, (Hobbs III et al. 1989; Holdich et al. 2009). This species was first introduced into Sweden in the 1960s (Holdich et al. 2009; Holdich 2002) and into many other European countries by the 1980s (Guan and Wiles 1997). It has been introduced into over 20 countries in Europe since the 1960s (Holdich 2002).

The signal crayfish has been shown to have a dramatic effect as an invader (Lodge et al. 2000). Being highly polytrophic and feeding on plant material, detritus, zoobenthos, fish, and other crayfish (Guan and Wiles 1998; Momot 1995; Stenroth and Nyström 2003; Usio et al. 2009) it can have strong effects on species richness and the structure of food webs in lakes and ponds by feeding at several trophic levels (Nyström et al. 1996). It has a significant impact on native crayfish and benthic invertebrate fauna in many areas of the world (Lodge et al. 2000) as well as on a broad range of flora (Crawford et al. 2006). In Europe, signal crayfish are also vector of the crayfish plague (*Aphanomyces astaci* Shickola) to which it is resistant but which is lethal to European crayfish, causing local extinctions (Diéguez-Uribeondo et al. 1995). This disease is indigenous of North America and the higher resistance of North American species is a result of a coevolution of the pathogen and its host (Unestam and Weiss 1970). At high densities *Pacifastacus leniusculus* may affect the populations of native species and disrupt the river ecosystems (Guan and Wiles 1997; Statzner et al. 2003).

1.2.3 The red swamp crayfish, *Procambarus clarkii*

The Louisiana red swamp crayfish, *Procambarus clarkii*, is one of the world's most invasive and widely distributed crayfish (Capinha et al. 2011; Holdich et al. 2009). With a significant ecological plasticity *Procambarus clarkii* is able to live in a wide variety of harsh, abiotic conditions (Holdich 2002). Once introduced into a favorable habitat it is hardly eliminated (Hobbs III et al. 1989) representing a major threat to the biodiversity of freshwater ecosystems.

This species is native to the southeastern United States and Mexico but it has been spreading throughout the world: it has been successfully introduced in all continents except Antarctica and Australia (Hobbs III et al. 1989). Populations are most prevalent in Italy, Portugal, Spain and The Netherlands (Holdich et al. 2009). In many ecosystems this introduced crayfish became an ecosystem engineer (Gherardi et al. 2011) and a key element in controlling energy flow, producing important changes in ecosystem organization and functioning, and modulating the availability of resources to other species (Hobbs III et al. 1989). Yet, the majority of *Procambarus clarkii's* introductions have had negative impacts (Hobbs III et al. 1989; Lodge et al. 2000): just like other north American species, it is a plague vector (*Aphanomyces astaci* Shickola) responsible for the decrease on native crayfish species (Bernardo et al. 1997; Diéguez-Uribeondo et al. 1997); it is highly aggressive, antagonizing native crayfish (Hill and Lodge 1999); it is a predatory omnivore with impact on many trophic levels, for example on native aquatic plants (Creed Jr 1994); it leads to direct economic outcomes such as raising the cost of rice culture because of the burrowing into dikes and eating rice plants (Anastácio et al. 2005; Lodge et al. 2000). In addition to this, its short life-history, rapid growth and high population density, also contribute to a significant damage on the aquatic environment (Gherardi 2006; Gherardi and Acquistapace 2007). In the long term, this species will bring drastic habitat changes and cause the decline of several groups of organisms (Guan and Wiles 1997).

In light of the above mentioned, *Procambarus clarkii* became an invasive crayfish of increasing concern and has received considerable attention from biologists, policy makers and the general public (Lodge et al. 2000).

1.3 Invasive freshwater crustaceans - Overland dispersal

It is known that freshwater organisms can disperse across population boundaries (Bohonak and Jenkins 2003). Bilton defines dispersal as the movement of individuals or propagules between spatially (or temporally) discrete localities or populations, and he focused mainly on overland movement of freshwater invertebrates (Bilton et al. 2001). Dispersal mechanisms can be classified into two modes: passive (movements achieved using an external agent) and active (self-generated movements of individual organisms) (Bilton et al. 2001; Jenkins et al. 2007). Dispersal may be triggered by environmental conditions that are or will be changing in the near future (Velasco et al. 2012) and it's recognized to act in association with local factors even for organisms expected to be cosmopolitan (Bohonak and Jenkins 2003). The locomotor activity of most animals is strongly affected by a variety of biotic and abiotic factors such as ambient temperature, light, humidity, etc. (Ossenkopp et al. 1996). For instance, the effects of temperature on movements of crustaceans are well documented (Lagerspetz and Vainio 2006), however only a few studies have approached such effects on terrestrial locomotion (Claussen et al. 2000; Weinstein 1998).

As overland dispersal in most freshwater taxa is presumed to be frequent and widespread (Bohonak and Jenkins 2003), an analysis of the factors affecting the limits of locomotor performance could provide insight into the behavior and ecology of organisms such as freshwater crustaceans, known for their ability to invade new ecosystems (Guan and Wiles 1997; Lodge et al. 1998; Lodge et al. 2000; Westman et al. 2002). A thorough understanding of the patterns of overland movement and dispersal is relevant to understanding and predicting their colonization abilities.

1.3.1 *Eriocheir sinensis* overland dispersal

Transition from the marine to freshwater and terrestrial habitats is very common for the Chinese mitten crab (Anger 1991). Being a catadromous species, mitten crabs often live in inland water bodies several kilometers from the estuary where copulation occurs (Veilleux and De Lafontaine 2007). Adult crabs are capable of limited overland travel (Hanson and Sytsma 2005) e.g. when adults engage in a reproductive migration primarily in-channel but often crossing river dams (Panning 1938). Human infrastructures and natural barriers may represent physical limitations to the upstream migration but crabs can use locks and fish ladders to move upstream and they are frequently observed circumventing obstacles by walking over land (Panning 1938). In fact, further spread of this species worldwide is very likely, in part due to its ability to migrate over land (Bouma and Soes 2010).

1.3.2 *Pacifastacus leniusculus* overland dispersal

Freshwater crayfish are primarily aquatic, but can move effectively on land (Grote 1981). Such locomotion may be essential for the survival of certain species (Claussen et al. 2000). Crayfish species have high desiccation tolerances (Bentley and Hughes 2010) but agricultural and urban landscapes may introduce barriers to its movements. Although it is not known how much *Pacifastacus leniusculus* can move overland (Bondar et al. 2005), some studies confirm they can escape over land (Peay et al. 2006) and even travel several hundred meters over land in one night (Hiley 2003).

1.3.3 *Procambarus clarkii* overland dispersal

Red swamp crayfish have been known to exit bodies of water and travel overland (Chucholl 2011; Cruz and Rebelo 2007; Holdich 2002; Kerby et al. 2005; Penn 1943; Viosca 1939), and have been seen passing through highways in the swamps (Huner and Barr 1991; Penn 1943; Viosca 1939). *Procambarus clarkii* active dispersal capabilities, namely its overland dispersal, are a frequent and widespread phenomenon (Cruz and Rebelo 2007; Grote 1981; Penn 1943). Being highly resistant to adverse conditions and capable of withstanding drought periods, *Procambarus clarkii* have the ability to spread across dry land (the species is capable of climbing and traversing rough terrain) in search for suitable conditions and therefore colonizing isolated water bodies (Aquiloni et al. 2005; Claussen et al. 2000; Gherardi et al. 2002; Grote 1981; Huner and Lindqvist 1995; Kerby et al. 2005; Penn 1943; Ramalho 2012). In fact, Penn (1943) and Viosca (1939) mentioned a postreproductive migration of mature males and females in poor shape (often carrying eggs) to find a better habitat patch. Individuals may also exit the water to simply overcome obstacles to their natural movement (*e.g.* dams) (Kerby et al. 2005) and to feed on land (Grey and Jackson 2012). *Procambarus clarkii* may be capable of walking moderate distances (0.25-1.0 km) over land (Lutz and Wolters 1999). Most types of water bodies (small, ephemeral and anthropogenically-disturbed habitats) are likely to be invaded (Kerby et al. 2005). There is evidence that this occasional overland dispersal ability contributes to colonize new areas (Huner and Lindqvist 1995) and increases its range (Aquiloni et al. 2005; Cruz and Rebelo 2007). However, overland dispersal can also impose survival risks such as desiccation and predation (by birds and mammals for instance) (Ramalho 2012).

1.4 Objectives

This work focuses on mechanisms of detection, more precisely, on the detection of different environmental variables by three aquatic invasive crustaceans (*Eriocheir sinensis*, *Pacifastacus leniusculus*, and *Procambarus clarkii*). The main goal is, therefore, to analyze the effect (under laboratory conditions) of some variables (humidity, temperature, illuminance, vegetation and land slope) on the individual´s movement direction, once in dry land. A complementary goal is to test, in the field, the species' abilities to return from land to the water.

An analysis of the variables affecting the overland movement direction can provide insight into the behavior and ecology of the species and lead to a better understanding of what affects their distribution and mechanisms of spread overland. A thorough understanding of the patterns of movement and dispersal is relevant to understanding and predicting their colonization abilities.

2. Materials and methods

2.1 Capture locations

Between October of 2011 and February of 2012 *Procambarus clarkii* individuals were collected from irrigation ditches in Divor Dam, Alentejo, Portugal (38°42'03.77''N; 7°55'27.41''W) using a dip net. *Eriocheir sinensis* were collected in the Tagus Estuary, Vila Franca de Xira, Portugal (18°57''18.20N; 8°59''01.31''W) between April and May of 2012 using baited fyke net traps. During September of 2012 *Pacifastacus leniusculus* were collected from different locations in the Maçãs River (location 1: 41°40'28.47"N; 6°34'06.12"W/ location 2: 41°40'41.41"N; 6°33'44.61"W/ location 3: 41°45'16.55"N; 6°33'27.65"W/ location 4: 41°45'25.95"N; 6°33'21.05"W) in Bragança, Portugal using Swedish type baited traps

Once captured, the animals were transported to the Aquatic Ecology laboratory in the University of Évora and distributed by several plastic tanks (55 cm x 45 cm x 40 cm) containing tap water at room temperature (17° C). Each container housed up to 10 crayfish or 5 crabs, a filter pump and shelters (opaque PVC tubes). The animals were fed carrots and the water was changed regularly. To adjust to the new environment the animals were kept in these conditions for at least a 24h acclimation period, before running the experiments.

2.2 Laboratory and field experiments

Two sets of experiments were established. The first, under laboratory conditions, aimed to identify the environmental variables affecting the species' motion patterns and orientation in dry land, using animals from Divor dam, Vila Franca de Xira and Bragança. The second was on the field (using animals from Bragança) and aimed to test the two crayfish species' ability to actively return to the water. *Procambarus clarkii*, *Eriocheir sinensis* and *Pacifastacus leniusculus* laboratory experiments took place at different time periods: January/February, April/May and October of 2012 respectively. *Procambarus clarkii* and *Pacifastacus* leniusculus field experiments took place in Bragança on September of 2012 (*Eriocheir sinensis* wasn't tested since it didn't exist on the test site).

2.3 Laboratory experiments: the metal gutter apparatus

The testing apparatus consisted of a 2 meter long metal gutter (200 cm x 25 cm) covered with gravel (2 or 3 centimeters depth). Each animal, slightly dehydrated by air exposure during 30 minutes, was individually placed at the centre of the metal gutter and given a choice between two different values of the same variable, e.g. humidity (see figure 1). The animals were marked with a waterproof marker (Ramalho et al. 2010), measured (±0.1 mm precision) and weighed on an analytical balance (±0,5g precision) before the experiment. Before the experiments, the surrounding relative humidity was measured with a ±1% precision and the air temperature was measured with a ±0.01°C precision. Once positioned at the starting point, individuals were kept inside an opaque box during one minute. After that period the box was removed from a distance with the help of a nylon string and without visual contact between the animal and the observer. Movement preferences were registered and recorded using a webcam (Microsoft® Lifecam HD-3000) positioned 2 m above the metal gutter. For each variable (humidity, temperature, illuminance, vegetation and land slope) the experiment was, when possible, replicated at least 30 times always using a different individual (males and females alternately) and the metal gutter was randomly turned around. Statistical analysis was carried out with a chi-squared test on PASW® Statistics 18 (SPSS Inc.).

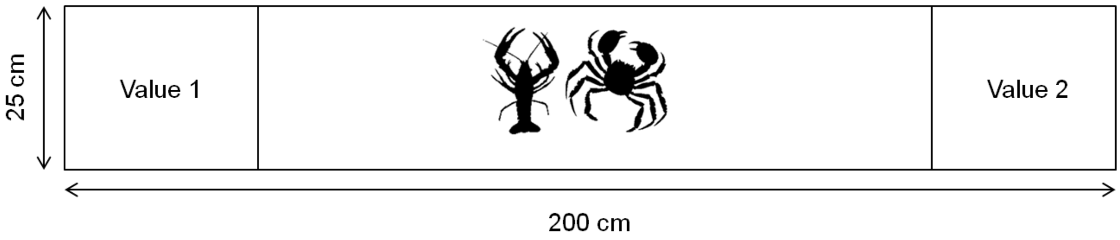


Figure 1: Schematic representation of the metal gutter

2.3.1 Humidity experiment apparatus

Different humidity values were achieved by using a nebulizer device (Artsana, model Project 2), which provided a high concentration of suspended water particles (aerosols) on one side of the apparatus. To avoid aerosols loss, the metal gutter was covered by a cellophane transparent sheet. The other side was kept under low humidity levels, with no aerosols supply. The species had to choose between the sides with higher or lower humidity.

Eriocheir sinensis (mean carapace length = 44.8 mm ± 7.2 S.D., mean carapace width = 51.02 mm ± 3.82 S.D., mean weight = 69.53 g ± 14.83 S.D.) relative humidity (R.H.) preferences were tested for 26 individuals (14 males and 12 females). One side of the apparatus had 76% of R.H. (and 20.4° C) and the other side had 60% R.H. (and 20.6° C), providing a 16% gradient between sides.

Pacifastacus leniusculus (mean cephalothorax length = 39.03 mm ± 7.51 S.D., mean weight = 19.1 g ± 11.56 S.D.) relative humidity preferences were tested for 30 individuals (15 males and 15 females). One side of the apparatus had 85% of R.H. (and 22.3° C) and the other side had 55% (and 22.7° C), providing a 30% gradient between sides.

Procambarus clarkii (mean cephalothorax length = 41.98 mm ± 4.03 S.D., mean weight = 15.3 g ± 4.81 S.D.) relative humidity preferences were tested for 29 individuals (14 males and 15 females). One side of the apparatus had 55% of R.H. (and 18.8° C), while the other side had 32% (and 19.2° C), providing a 23% gradient between sides.

2.3.2 Temperature experiment apparatus

During the temperature experiment the two sides of the apparatus were at different temperatures and individuals were allowed to choose among the two sides. To achieve high temperatures on one side of the metal gutter, hot water was placed in closed plastic boxes under the gravel and regularly replaced by new heated water. On the other side cold water was used instead.

Eriocheir sinensis (mean carapace length = 54.57 mm ± 6.57 S.D., mean carapace width = 50.10 mm ± 6.5 S.D., mean weight = 82.47 g ± 32.77 S.D.) temperature preferences were tested for the following values: 27° C (61% of R.H.) and 20.1° C (42% of R.H.), which correspond to a 6.9° C gradient. A total of 27 individuals were tested; 13 males and 14 females.

Pacifastacus leniusculus (mean cephalotorax length = 39.31 mm ± 6.57 S.D., mean weight = 18.27 g ± 11.11 S.D.) temperature preferences were tested for the following values: 28.6° C (82% of R.H.) and 24° C (63% of R.H.), which correspond to a 4.6° C gradient. A total of 30 individuals were tested, 15 males and 15 females.

Procambarus clarkii (mean cephalotorax length = 37.28 mm ± 4.49 S.D., mean weight = 11.45 g ± 3.95 S.D.) temperature preferences were tested for the following values: 27.1° C (37% of R.H.) and 18.8° C (46% of R.H.), which correspond to an 8.3° C gradient. A total of 30 individuals were tested, 15 males and 15 females.

2.3.3 Illuminance experiment apparatus

To create illuminance differences between the metal gutter sides, distinct plastics were used to cover the apparatus: a cellophane transparent sheet (which allowed the light to pass through) on one side, and a dark opaque plastic (which prevented the light from passing through) on the other. A luxmeter (AMPROBE, LM-80 model) was used to register illuminance values (in lux). The individuals had to choose between the darker and the lighter side.

For *Eriocheir sinensis* (mean carapace length = 58.37 mm ± 5.59 S.D., mean carapace width = 54.54 mm ± 6.04 S.D., mean weight = 103 g ± 31.53 S.D.) the following values were tested at 20.2° C and 48% of R.H.: 13 lux on the dark side and 211 lux on the other side – a difference of 198 lux was achieved. A total of 13 individuals were tested, 8 males and 5 females.

For *Pacifastacus leniusculus* (mean cephalotorax length = 38.5 mm ± 8.86 S.D., mean weight = 21.24 g ± 6.52 S.D.) the following values were tested at 22.4° C and 55% R.H.: 19 lux on the dark side and 229 lux on the other side – a difference of 210 lux was achieved. A total of 30 individuals were tested, 15 males and 15 females.

For *Procambarus clarkii* (mean cephalotorax length = 41.61 mm ± 5.85 S.D., mean weight = 16.78 g ± 10.24 S.D.) the following values were tested at 18.9° C and 50% of R.H.: 23 lux on the dark side, and 225 lux on the other side – a difference of 202 lux was achieved. A total of 30 individuals were tested, 15 males and 15 females.

2.3.4 Vegetation experiment apparatus

To test the movement preferences in respect to the presence or absence of vegetation, *Cyperus eragrostis* were placed on one side of the metal gutter. The density of the plant in the field (1 m^2) was replicated in the metal gutter (0.1 m^2), therefore 4 stalks were placed in the apparatus. During the experiment the plants were moved about ten times, from side to side. The individuals had to choose between sides with and without vegetation.

The fresh biomass (80 g) used for *Eriocheir sinensis* (mean carapace length = 56.07 mm ± 5.74 S.D., mean carapace width = 52.25 mm ± 5.12 S.D., mean weight = 84.73 g ± 21.18 S.D.) experiment weighted 12.5 g after a drying process. The mean height of the plants was 37.75 g ± 11.9 S.D.. The temperature and relative humidity on both sides of the apparatus were 24.7° C and 37%. A total of 16 individuals were tested (15 males and 1 female).

55 g of fresh plant biomass (16 g dry weight) were used for the experiment with *Pacifastacus leniusculus* (mean cephalotorax length = 38.55 mm ± 6.06 S.D., mean weight = 16.73 g ± 7 S.D.) and the mean height of the plants was 55.5 cm ± 8.43 S.D.. The temperature and relative humidity on both sides of the apparatus were 22.5° C and 59%. A total of 30 individuals were tested (15 males and 15 females).

37 g of fresh plant biomass (8 g dry weight) were used for the experiment with *Procambarus clarkii* (mean cephalotorax length = 44.04 mm ± 4.59 S.D., mean weight = 17.63 g ± 6.6 S.D.) and the mean height of the plants was 47.5 cm ± 5.76 S.D.. The temperature and relative humidity on both sides of the apparatus were 18.5° C and 27%. A total of 30 individuals were tested (15 males and 15 females).

2.3.5 Land slope experiment apparatus

For the land slope experiments, slopes of 9° and 14° were used. The species had to choose between moving down or up the metal gutter. The two slopes were tested alternately during the same experiment.

The *Eriocheir sinensis* 9° slope experiment (mean carapace length = 52.49 mm ± 5.83 S.D., mean carapace width = 47.55 mm ± 5.89 S.D., mean weight = 76.13 g ± 23.71 S.D.) and the 14° slope experiment (mean carapace length = 54.23 mm ± 5.69 S.D., mean carapace width = 50.43 mm ± 5.62 S.D., mean weight = 82.6 g ± 24.88 S.D.) happened, under the same conditions: 20.6° C and 41% of R.H.. For the 9° slope experiment 24 individuals were tested; 23 males and 1 female. For the 14° slope experiment 24 individuals were tested, all males.

The *Pacifastacus leniusculus* 9° slope experiment (mean cephalotorax length = 39.24 mm ± 5.75 S.D., mean weight = 20.2 g ± 8.87 S.D.) and the 14° slope experiment (mean cephalotorax length = 37.23 mm ± 8.64 S.D., mean weight = 19.68 g ± 11.04 S.D.) were performed at 20.9° C and 46% of R.H.. For each slope (9° and 14°), 30 individuals were tested (15 males and 15 females).

The *Procambarus clarkii* 9° slope experiment (mean cephalotorax length = 40.87 mm ± 4.69 S.D., mean weight = 14.67 g ± 4.91 S.D.) and the 14° slope experiment (mean cephalotorax length = 40.88 mm ± 5.24 S.D., mean weight = 15 g ± 6.53 S.D.) took place at 19.7° C and 42% of R.H.. For each slope (9° and 14°), 30 individuals were tested (15 males and 15 females).

2.4 Field experiments: the arena apparatus

The experiments took place at four different locations along the Maçãs River, referred as location 1-4 (previously described). Each location was chosen according to the flatness of the surface, preferably with an easy and short access to the water. The exact distance to the water was determined by the topography and was on average 5.13 meters (S.D.= 2.55).

The animals were captured on location and were tested at the nearest flat surface. They were marked with a waterproof marker, measured and weighed on a pocket balance (±0.01g precision scale) before being slightly dehydrated by air exposure during 30 minutes, and placed at the centre of the arena. The arena consisted of a 2 m diameter circular area, outlined by a thin string. This arena was further divided (with strings) into 4 sections (see figure 2). To reduce stress levels once positioned at the centre of the arena the animals were kept during 10 minutes inside a metal pail with holes. After that period the pail was removed with the help of a nylon string without visual contact between the animals and the observer. During every trial, movement directions in the arena were carefully registered. Variables such as relative humidity and temperature were registered only at the beginning of each species experiments because, in general, no more than 1 hour was spent testing the same species (see tables 1 and 2).

Pacifastacus leniusculus were tested individually (mean cephalothorax length = 42.92 mm ± 5.46 S.D., mean weight = 22.77 g ± 8.15 S.D.), in groups of 6 (mean cephalothorax length = 38.39 mm ± 7.55 S.D., mean weight = 16.68 g ± 11.18 S.D.), and in groups of 12 individuals (mean cephalothorax length = 37.98 mm ± 5.06 S.D., mean weight = 15.71 g ± 6.07 S.D.). *Procambarus clarkii* were tested individually (mean cephalothorax length = 41.53 mm ± 6.01 S.D., mean weight = 17.16 g ± 7.98

S.D.), in groups of 6 (mean cephalothorax length = 36.22 mm ± 5.48 S.D., mean weight = 10.20 g ± 5.73 S.D.), and in groups of 12 individuals (mean cephalothorax length = 37.11 mm ± 4.13 S.D., mean weight = 10.06 g ± 2.86 S.D.). P. *leniusculus* and *Procambarus clarkii* were tested separately and each group was tested at least 30 times always using different adult individuals. The replicates were randomly distributed by all four locations, according to the number of individuals captured on site. The experiments happened in 4 different days, one day per location. A distance of 4 m, in average, ensured different positions of the arena (a mobile structure) every time new animals were tested. Statistical analysis was carried out by performing a one sample chi-squared test for circular data in Oriana version 4.01.

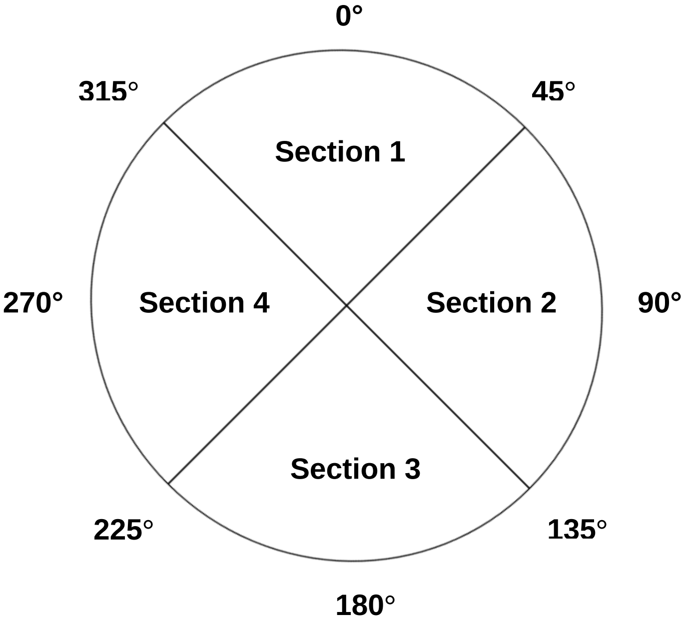


Figure 2: Schematic representation of the arena divided in 4 sections: section 1 (315°- 45°), section 2 (45° - 135°), section 3 (135° - 225°), section 4 (225° - 315°). The water is positioned at 0°, upstream is the left and downstream is the right. In locations 1, 2 and 3 the North is at 270°; in location 4 it is at 315°.

Table 1: Relative humidity (%) and temperature (°C) measured before the *P. leniusculus*' experiments in all 4 locations.

	P. leniusculus			
	Location 1	Location 2	Location 3	Location 4
Relative humidity (%)	30	58	57	29
Temperature (°C)	22.3	18.8	17.6	22.2

Table 2: Relative humidity (%) and temperature (°C) measured before the *P. clarkii*'s experiments in all 4 locations.

	P. clarkii			
	Location 1	Location 2	Location 3	Location 4
Relative humidity (%)	31	59	57	29
Temperature (°C)	20.6	18.8	17.6	22.2

3. Results

3.1 Laboratory experiments

3.1.1 Chi-squared test: testing the effect of environmental variables on the species' movement directions

During the experiments, *Eriocheir sinensis* tended to stand in a limb-extended position for several minutes. This implied that fewer animals were available for the experiments testing humidity (13% didn't move), temperature (10%), illuminance (57%), vegetation (47%), 9° slope (20%) and 14° slope (20%). For the active individuals, *Eriocheir sinensis* movement preferences were significantly affected by slope since 71% of the animals preferred to move downhill when the slope corresponded to 9º (Chi-squared test; X^2=4.167; df=1; p=0.041) (see figure 3). The chi-squared tests for humidity (X^2=0; df=1; p=1), temperature (X^2=0.926; df=1; p=0.336), illuminance (X^2=0.077; df=1; p=0.782), vegetation (X^2=2.25; df=1; p=0.134) and 14º slope (X^2=0; df=1; p=1) revealed non-significant differences. *Pacifastacus leniusculus* movement was affected by temperature (X^2=6.533; df=1; p=0.011). They had a significant preference to move towards cooler areas: 73% chose 18.8° C (see figure 4). The chi-squared tests for humidity (X^2=0.533; df=1; p=0.465), illuminance (X^2=0; df=1; p=1), vegetation (X^2=0.533; df=1; p=0.465), 9° slope (X^2=1.2; df=1; p=0.273) and 14° slope (X^2=1.2; df=1; p=0.273) revealed non-significant differences. *Procambarus clarkii* movement was affected by temperature (X^2=8.533; df=1; p=0.003), 9º slope (X^2=6.533; df=1; p=0.011) and 14º slope (X^2=6.533; df=1; p=0.001). Regarding the temperature, crayfish had a significant preference to move towards cooler areas: 77% of the crayfish chose the temperature of 18.8° C. Likewise, crayfish movement preferences were significantly affected by land slope since 73% preferred to move downhill when the slope corresponded to 9º. For a 14º slope 80% of the crayfish had the same behaviour (see figure 5). The chi-squared tests for humidity (X^2=0.862; df=1; p=0.353), illuminance (X^2=0.533; df=1; p=0.465) and vegetation (X^2=0.133; df=1; p=0.715) revealed non-significant differences.

3.1.2 Chi-squared test: testing the effect of gender on the species' movement directions

E. sinensis movement preferences weren't significantly affected by gender during the humidity, temperature and illuminance experiments. As for the land slope and vegetation experiments there weren't enough females to perform the test. *P. leniusculus* movement wasn't significantly affected by gender in any experiment (humidity, temperature, illuminance, vegetation, 9° slope and 14° slope). Likewise, *P. clarkii* movement wasn't significantly affected by gender (see table 3).

Table 3: Chi-square statistics for *Eriocheir sinensis'*, *Pacifastacus leniusculus'* and *Procambarus clarkii'*s experiments: chi-square value and asymptotic significance. The degrees of freedom are always 1.

		Chi-square value	Asymp. sig.
E. sinensis	*Humidity*	0.155	0.694
	Temperature	0.39	0.532
	Illuminance	0.048	0.826
P. leniusculus	*Humidity*	0.543	0.461
	Temperature	0.17	0.68
	Illuminance	0	1
	Land slope (9°)	0	1
	Land slope (14°)	0.139	0.709
	Vegetation	0	1
P. clarkii	*Humidity*	0.049	0.825
	Temperature	2.981	0.084
	Illuminance	0.543	0.461
	Land slope (9°)	0.17	0.68
	Land slope (14°)	0	1
	Vegetation	0	1

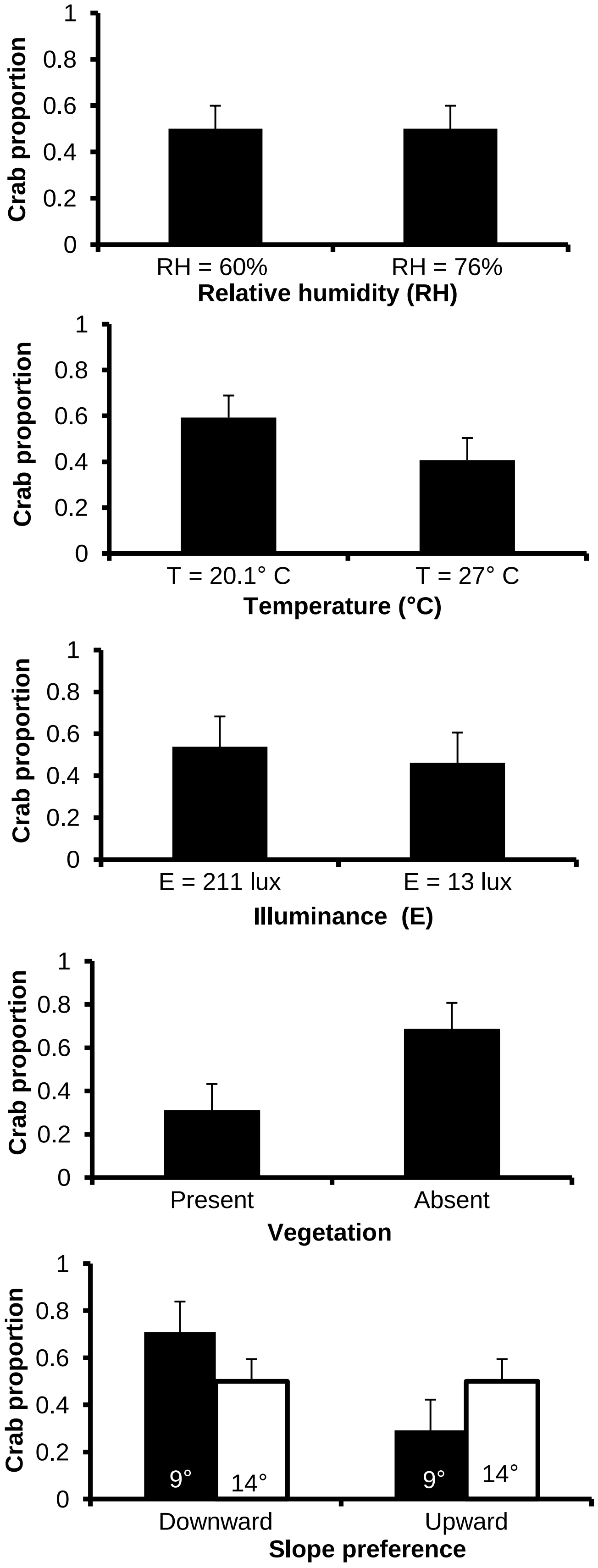


Figure 3: *Eriocheir sinensis* motion preferences depending on different variables: humidity (N=26), temperature (N=27), illuminance (N=13), vegetation (N=16), 9° slope (N=24), and 14° slope (N=24). The error bars represent the standard deviation.

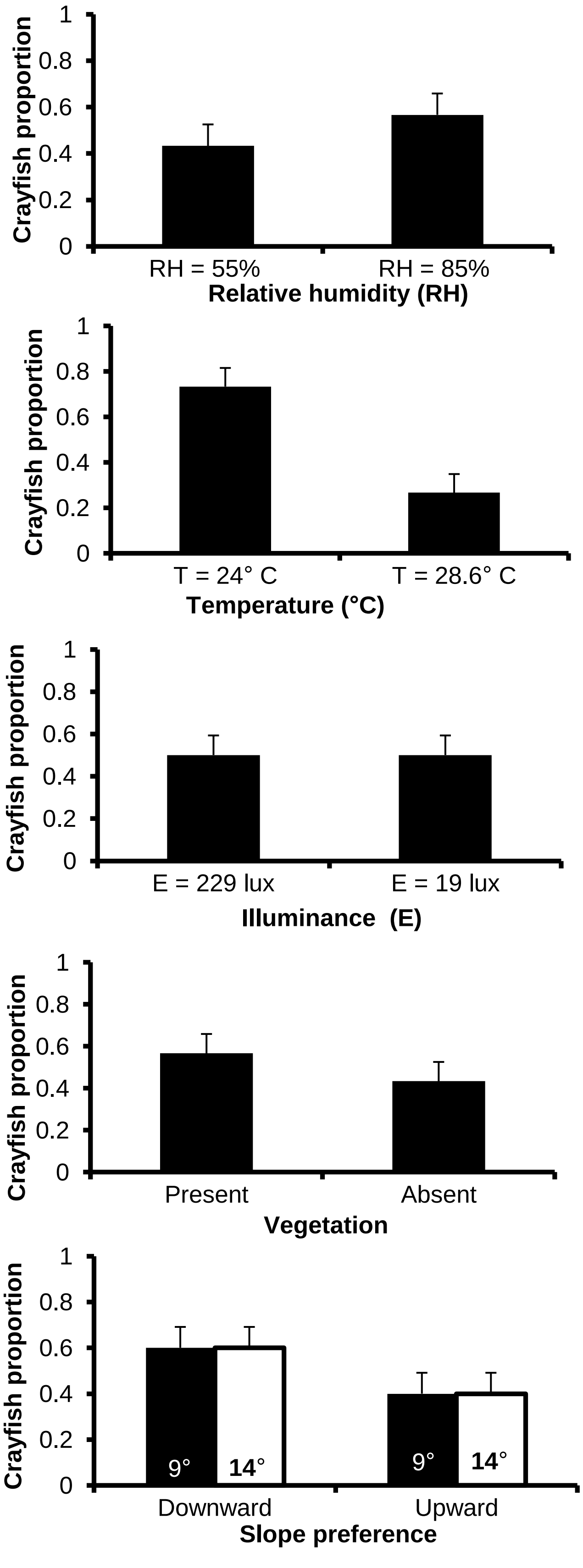


Figure 4: *Pacifastacus leniusculus* motion preferences depending on different variables: humidity (N=30), temperature (N=30), illuminance (N=30), vegetation (N=30), 9° slope (N=30), and 14° slope (N=30). The error bars represent the standard deviation.

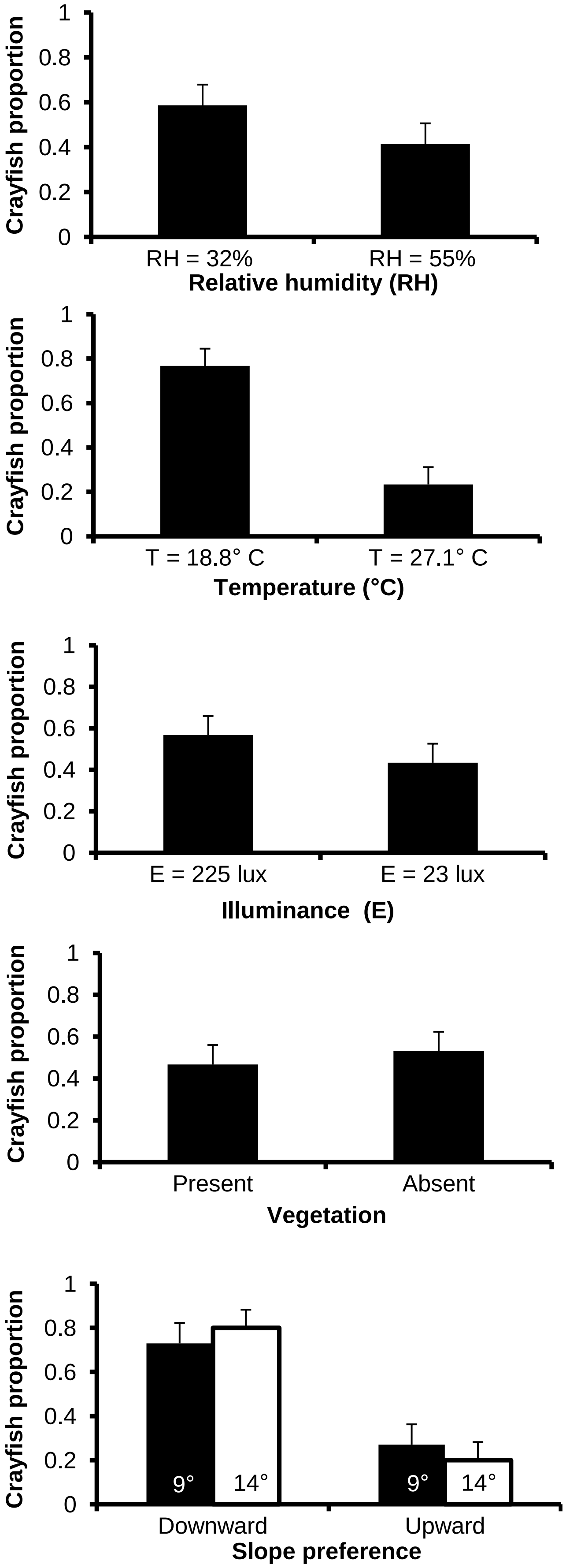


Figure 5: *Procambarus clarkii* motion preferences depending on different variables: humidity (N=29), temperature (N=30), illuminance (N=30), vegetation (N=30), 9° slope (N=30), and 14° slope (N=30). The error bars represent the standard deviation.

3.2 Field experiments

3.2.1 One sample chi-squared test: testing the uniform distribution of the movement direction data

The chi-squared test of uniformity performed for *Pacifastacus leniusculus* revealed that, when in groups of 12 individuals (X^2=1.556; df=3; p=0.67), the crayfish showed no significant preference for any particular direction; therefore the direction of the movement didn't differ significantly from a uniform distribution. For the other two crayfish densities, 1 (X^2=11.647; df=3; p=0.009) and 6 (X^2=10.267; df=3; p=0.016), the movement directions weren't uniformly distributed. When isolated, the individuals showed, on average, a significant tendency to move downstream but not directly to the water. The water was positioned at 0° but both the median and mean vectors were 90°). In groups of 6 individuals the tendency was also to move downstream and not directly to the water. As previously the water direction was at 0° but the median was 90° and the mean vector=101.31° (see figure 6).

Regarding *Procambarus clarkii*, no significant preference was obvious since in all three cases (1 individual with X^2=1.529; df=3; p=0.675/ 6 individuals with X^2=2.8; df=3; p=0.423/ 12 individuals with X^2=6; df=3; p=0.112) there was a uniform distribution of the movements (see figure 7).

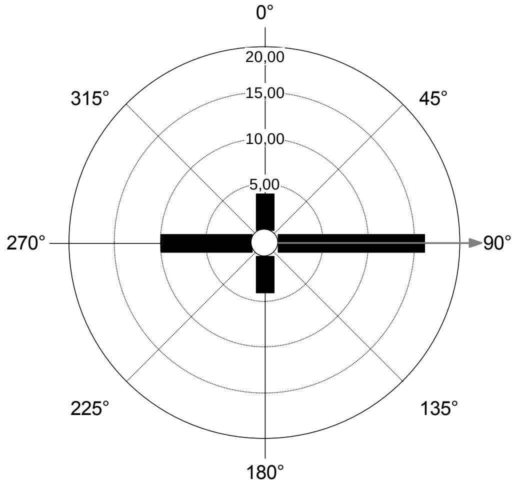
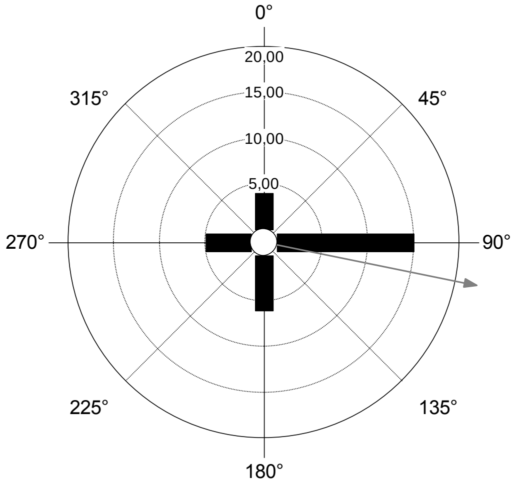
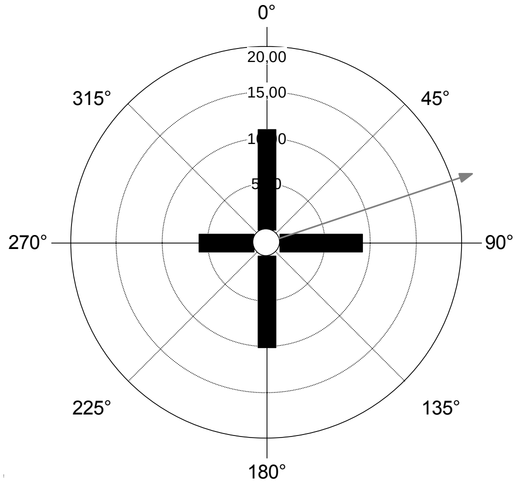
P. leniusculus distributions	*P. leniusculus* statistics
	**1 individual:** Mean vector = 90° Median = 90° Circular standard deviation = 103.437° Chi-squared test (Uniform, X^2) = 11.647 Chi-squared test (p) = 0.009
	**6 individuals:** Mean vector = 101.31° Median = 90° Circular standard deviation = 79.968° Chi-squared test (Uniform, X^2) = 10.267 Chi-squared test (p) = 0.016
	**12 individuals:** Mean vector = 71.565° Median = 90° Circular standard deviation = 123.611° Chi-squared test (Uniform, X^2) = 1.556 Chi-squared test (p) = 0.67

Figure 6: Circular graphics representing *P. leniusculus* movement directions for each group (1, 6 and 12 individuals) and corresponding circular statistics. Directions are represented in angles (0º for the water direction, upstream side is the left and downstream is the right), frequency values are represented in the scale from 0 to 20, and the mean vector is represented by a grey arrow.

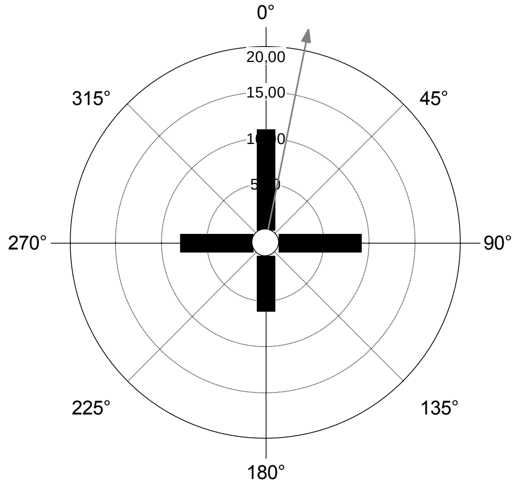
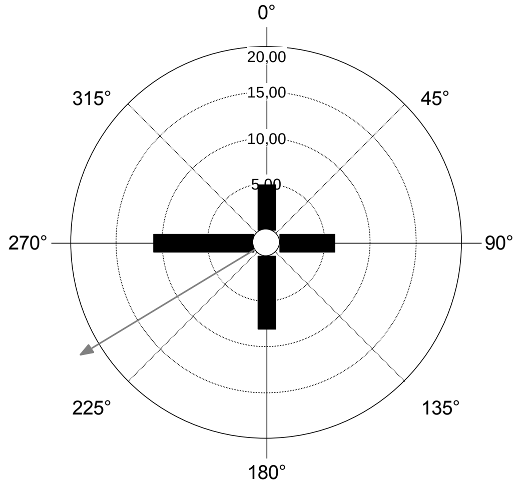
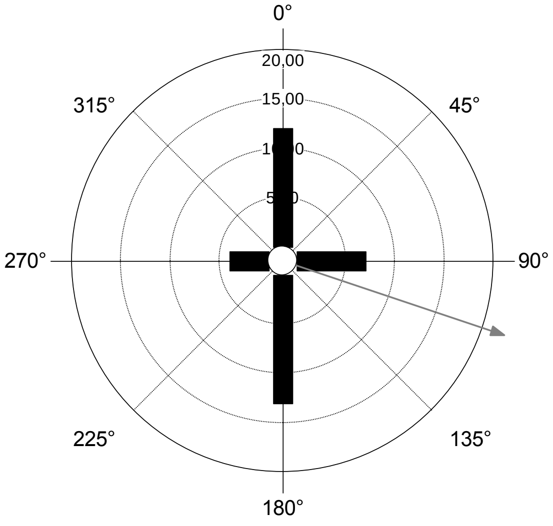
P. clarkii distributions	*P. clarkii* statistics
	**1 individual:** Mean vector = 11.31° Median = 0° Circular standard deviation = 108.478° Chi-squared test (Uniform, X^2) = 1.529 Chi-squared test (p) = 0.675
	**6 individuals:** Mean vector = 239.036° Median = 270° Circular standard deviation = 100.325° Chi-squared test (Uniform, X^2) = 2.8 Chi-squared test (p) = 0.423
	**12 individuals:** Mean vector = 108.435° Median = 90° Circular standard deviation = 123.611° Chi-squared test (Uniform, X^2) = 6 Chi-squared test (p) = 0.112

Figure 7: Circular graphics representing the P. clarkii movement directions for each group (1, 6 and 12 individuals) and corresponding circular statistics. Directions are represented in angles (0º for the water direction, upstream side is the left and downstream is the right, frequency values are represented in the scale from 0 to 20 and the mean vector is represented by a grey arrow.

4. Discussion

Decapod crustaceans are known for their burrowing behavior, in order to prevent thermal extremes and low humidity conditions (Eshky et al. 1995; Gherardi et al. 2002) therefore avoiding desiccation (Payette 2003). Some animal movements are related to the avoidance of dehydration stress (Barbaresi et al. 1997) and humidity gradients may represent a sensory clue for orientation in dry land. The ability to detect and avoid low humidity out of water has a survival value for species that inhabit temporary pools (Lagerspetz 1963). This kind of orientation would imply that the animals have some way of detecting the air humidity. Examples of other species with such capacity are: *Coenobita clypeatus*, a decapod crustacean that can detect and orient its movement to water vapour (Vannini and Ferretti 1997); *Porcellio scaber*, an isopod crustacean that displays receptors for humidity stimulus (Gunn 1937); *Asellus aquaticus*, an aquatic isopod crustacean that it is able to select high humidity values when out of water (Lagerspetz and Lehtonen 1961); or *Gammarus duebeni* and *Gammarus oceanicus* (two aquatic amphipods) able to avoid low relative humidity (Lagerspetz 1963).

In light of the above, the assumption regarding the experiments testing humidity was that the slightly dehydrated animals would prefer high humidity values and would choose the side of the apparatus with higher relative humidity. Yet, none of the three species (*Eriocheir sinensis*, *Pacifastacus leniusculus*, *Procambarus clarkii*) revealed significant preferences for high or low relative humidity values. One possible reason for such results is, as Lagerpetz and Lehtonen suggested, that the orientation towards water is guided through optic stimulus and not by air humidity gradients (Lagerspetz and Lehtonen 1961). Another possible reason is that the humidity gradient between sides (16%, 30% or 23% respectively) was not enough to generate an obvious reaction. However, it can be difficult to find the range that the animals respond to, since it is not a linear response. Actually, at different ranges, the same species can present different sensitivities to humidity gradients. For instance, *Ligia* (an isopod genus) can detect really small gradients (6%) between 35% and 60% but between 0% and 35 % it can only distinguish differences of 20% in humidity. Another example is *Porcellio* that, above 65%, cannot make any distinction between different values of relative humidity (Warburg 1968).

In this work it was possible to identify ranges for which the species couldn't detect differences in air humidity: 60%-76% for *Eriocheir sinensis*; 55%-85% for *Pacifastacus leniusculus*; and 32%-55% for *Procambarus clarkii*. However, it is worth underlining that this doesn't exclude the possibility that for other ranges these species might detect humidity differences. This would be similar to many other crustacean species, and is a matter of great interest for species known to disperse overland.

Temperature is an environmental variable that affects all organisms (Lagerspetz and Vainio 2006) and in extreme conditions it can cause deleterious effects on various physiological processes of decapods crustaceans (Payette 2003). Organisms capable of locomotion can achieve thermoregulation by avoiding temperature extremes. This behavior has been detected in many groups of Crustacea (Lagerspetz and Vainio 2006), being particularly important for ectothermic organisms whose internal temperature depends on the environment thermal conditions (Lagerspetz and Vainio 2006). Body temperature variations are a challenge that terrestrial and semi-terrestrial crustaceans face during locomotion on land (Weinstein 1998) and their response to increasing temperatures is more pronounced on air than in water (Payette 2003). Yet, there are few studies about behavioral thermoregulation in systems where they can move between water and air (Eshky et al. 1995). Thermal avoidance in crustaceans is considered to be klinokinetic rather than tactic, which means that the animals display an undirected locomotory reaction instead of moving straight towards or away from the source of stimulation (Fraenkel and Gunn 1961; Lagerspetz and Vainio 2006) and this has been observed in species such as *Orconectes immunis* (a decapod crustacean), *Asellus aquaticus* (an isopod crustacean) and *Saduria entomon (*an isopod crustacean) (Lagerspetz 2000; Lagerspetz 2003). In the present work, *Eriocheir sinensis* displayed the same klinokinetic kind of movement, moving in random directions. Contrarily to this outcome, *Pacifastacus leniusculus* and *Procambarus clarkii* revealed a tactic behavior, by moving straight away from the heat source. *Pacifastacus leniusculus* and *Procambarus clarkii* movement were, significantly affected by temperature and both species chose cooler areas, confirming that they avoid thermal extremes (high temperatures in this case) when out of the water, as result of a tactic reaction. This proves that temperature plays an important role on the animals' movement direction on dry land, and consequent invasion of new water bodies.

Illuminance and vegetation experiments didn't reveal any significant preferences. Regarding the experiments with *Eriocheir sinensis*, the results were affected by the crabs' immobility, since the animals tended to stand in a limb-extended position and this reduced the sample size on some experiments. The results are most likely related to the high immobility rates, 57% for illuminance and 47% for vegetation, than to a demonstrated absence of influence on the individual movement direction. The same behavior has been documented for another crab species, *Heterozius rotundifron (a* New Zealand endemic intertidal crab), and named as "catatonic posture". *Heterozius rotundifron* presented a defensive position with extreme lateral extension of the chelipeds, known to provide protection against fish predators (Hazlett and McLay 2000). Like *Heterozius rotundifron*, *Eriocheir sinensis* also displayed this behavior after tactile stimulus, as it would happen in the presence of a predator (Field 1990; Hazlett and McLay 2000). According to Tricarico *et al.* immobility reduces the probability of being detected by predators (Tricarico et al. 2011)*. Heterozius rotundifron* remained in this “catatonic posture” for several minutes after tactile input (Field 1990) and so did *Eriocheir sinensis*. This “frozen” position could also be part of the natural variability of crab activity. In fact, species such as the freshwater crab *Potamon fluviatile* display days of high activity followed by days of total immobility. This locomotor variability happens randomly, with no periodical pattern (Barbaresi et al. 1997).

As for the other two species, *Pacifastacus leniusculus* and *Procambarus clarkii*, it was expected that they would avoid the illuminated side, since a light source in nature is often a heat source. In fact, the animals should direct their movement to avoid extreme temperatures (Lagerspetz and Vainio 2006) and, as shown in the temperature experiments, these two species displayed a thermal avoidance behavior. A reason why they probably didn’t avoid the bright side is that the intensity of the light stimulus wasn’t enough to represent a critical temperature shift and to trigger the escaping behavior.

Pacifastacus leniusculus and *Procambarus clarkii* also didn’t show any preference regarding the vegetation experiments. Vegetation, as a key feature of any water body, can be important for the establishment of invasive species (Siesa 2011). It has been suggested that vegetation provides shelter during periods of vulnerability and protection against predation (Jones and Ruscoe 2001). However, when out of water during the experiments, there was no tendency for the species to choose the side with vegetation. A possible reason for such results is that *P. leniusculus* and *P. clarkii* might be able to discriminate between different types for shelter like *Cherax quadricarinatus* (Jones and Ruscoe 2001) and this type of vegetation (Cyperus eragrostris) didn’t represent their usual preference of shelter.

For the variable “slope”, the assumption made in these experiments was that the animals would be able to detect landscape characteristics such as ground slope, and move downwards in an attempt to find water. It has been shown that *Coenobita clypeatus*, a decapod crustacean, is able to detect inclinations of less than 5° by statocyst and proprioceptive inputs (Dunham and Schöne 1984). *Eriocheir sinensis* was significantly affected by 9° slope, *Procambarus clarkii* by 9° and 14°, both species preferring to move downwards as expected. However, *Pacifastacus leniusculus* didn’t show any preference to move downhill. One possibility, among others, could be that it is unable to detect the tested slope or any slope at all.

Finally, the arena experiments aimed to find if the crayfish could return to the water. The assumption that the animals would move towards the water couldn’t be confirmed. *Procambarus clarkii* didn’t display significant preferences for any particular direction. Thus, the movement away from the point of release (in this case three different densities were tested: 1, 6 and 12) should be described as a kinesis, i.e. random activity induced by a particular stimulus (Bovbjerg 1959).

The effects of density have been barely studied, and some of the few available interpretations can be conflicting. Nevertheless, according to Bovbjerg (1959), groups of crayfish (*Procambarus alleni*) seem to be stimulated by the need for space. It is possible that other crayfish species demonstrate dispersal patterns similar to these (Bovbjerg 1959). In fact, for *Pacifastacus leniusculus* (12 individuals) there are two possible reasons to explain the lack of a trend in movement: the animals’ movement was a kinetic type of behavior and the number of individuals promoted non-directional movements in search for free space. *Procambarus clarkii* didn´t move to any particular direction and *Pacifastacus leniusculus* moved to a direction that didn’t correspond to the water. This suggests that neither of the species could detect and return to the water. This finding might be related to the previous observations that there were some ranges of relative humidity gradients that these species couldn’t detect. Like in the humidity experiments this doesn’t exclude the possibility that for other humidity gradients the animals’ response could be different; there is still a chance that they could return to the water if different conditions were provided.

5. Conclusions

For many invasive species, eradication might prove difficult or even impossible. Thus, the best option is to limit their expansion. Water bodies free of invasive species and close to permanent invasive species sources are likely to be colonized, but it is still possible to limit such expansion. The analysis of the variables affecting the overland movement direction provided some insight into what affects the species’ distribution and mechanisms of spread overland.

None of the three species (*Eriocheir sinensis*, *Pacifastacus leniusculus* and *Procambarus clarkii*) revealed significant preferences regarding the humidity variable. Nevertheless, as suggested by some authors, humidity cues can play an important role in the overland dispersal. The conclusion from this work is that some ranges are not enough to trigger obvious responses. On the same basis, *Pacifastacus leniusculus* and

Procambarus clarkii did not move towards the water during field experiments. Future investigation should focus on finding which ranges of humidity gradients are enough to orient the animals in dry land.

Eriocheir sinensis didn't show any significant preference regarding the temperature variable, but *Pacifastacus leniusculus* and *Procambarus clarkii* chose cooler areas, confirming that they avoid high temperatures in dry land. This proves that temperature plays an important role on the animals' movement direction on dry land, and consequent invasion of new water bodies. As these species respond negatively to high temperatures, it was expected for them to avoid illuminated areas (usually heat sources). The reasonable assumption to make is, again, that the enabled illuminance wasn't enough to generate an avoidance response. However, it is possible that for more intense light stimulus gradients there may be an influence on the animals' overland dispersal.

For the vegetation experiments, no preferences were obvious and therefore it was concluded that *Cyperus eragrostris* doesn't play a role on the species orientation in dry land. Contrariwise, the ground slope revealed to be an important variable for the species overland dispersal, since *Eriocheir sinensis* and *Procambarus clarkii* showed a tendency to move downwards. This could effectively lead them down to a mass of water, usually confined between sloping borders.

Understanding the factors that influence dispersal direction is relevant to predict the species' colonization abilities. Since only few studies have addressed the effects of such factors on the terrestrial locomotion of freshwater crustaceans, this work contributes to a path towards new investigations and accomplishments.

6. References

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