PH.D. THESIS

PH.D. COURSE IN ENVIRONMENTAL SCIENCES

DISPERSAL AND POPULATION REGULATION OF THE RED SWAMP CRAYFISH (*Procambarus clarkii*)

RICARDO MANUEL OLIVEIRA RAMALHO

Supervisor: Prof. Doutor Pedro Manuel Gentil Anastácio

University of Évora

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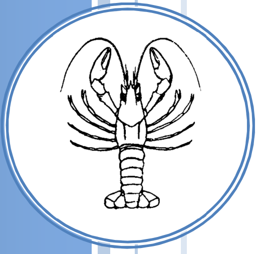
IMAR – Institute of Marine Research



2012

INSTITUTO DE INVESTIGAÇÃO E FORMAÇÃO AVANÇADA
INSTITUTE FOR ADVANCED STUDIES AND RESEARCH IIFA

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“It is not the strongest of the species that survives, nor the most intelligent that survives. It is the one that is the most adaptable to change.”

Charles Darwin

Ao Zé… que já não é.

"Since the Age of Exploration began, there has been a drastic breaching of biogeographic barriers that previously had isolated the continental biota for millions of years. We are now developing a whole new cosmopolitan assemblage of organisms across the surface of the Earth with large consequences not only for the functioning of ecosystems but also for the future evolutionary trajectory of life."

Harold Mooney and Elsa Cleland (2001)

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ABSTRACT

The main objective of the present thesis was to contribute to the improvement of the knowledge about red swamp crayfish (*Procambarus clarkii*), an exotic invasive species in Iberian Peninsula.

The main conclusions of this thesis were:

- Population density had a significant negative effect on juvenile crayfish growth;
- A learning coefficient was determined that can provide an useful tool to evaluate and compare the learning capabilities of different freshwater predators;
- *M. salmoides* revealed prey switching towards *P. clarkii* and this may be an indication of its potential for population regulation in crayfish recently invaded areas;
- Population density significantly affected underwater *P. clarkii* dispersal. Other factors involved were the water temperature and period of the day;
- One of the main factors inducing *P. clarkii* overland dispersal was the drainage of the study area. Other variables that significantly influenced the overland dispersal of *P. clarkii* were the temperature, relative humidity and the period of the day.

"Each invasion has a certain degree of specificity. Yet, generalities are emerging yielding encouraging insights into how invasions operate and how they may be best addressed by conservation managers and policymakers. Although complicated by economic, social, and political concerns, IAS policy decisions must also be based on clear, scientific reasoning."

Jeb Byers et al. (2002)

RESUMO

DISPERSÃO E REGULAÇÃO POPULACIONAL DO LAGOSTIM-VERMELHO-DA-LOUISIANA (*PROCAMBARUS CLARKII*)

O objetivo principal da presente tese foi contribuir para o aumento do conhecimento acerca do lagostim-vermelho-da-Louisiana (*Procambarus clarkii*), uma espécie alóctone invasora na Península Ibérica.

As principais conclusões da presente tese foram:

- A densidade populacional teve um efeito negativo significativo sobre o crescimento de lagostins juvenis;
- Foi proposto um coeficiente de aprendizagem que pode constituir uma ferramenta útil para avaliar e comparar as capacidades de aprendizagem de diferentes predadores aquáticos;
- O achigã demonstrou apresentar *prey-switching* sobre o *P. clarkii*, e este facto pode ser indicador do seu potencial para regular populações de lagostim em áreas recentemente invadidas;
- A densidade populacional teve um efeito significativo sobre a dispersão do *P. clarkii* dentro de água;
- A drenagem da área de estudo constituiu um dos principais despoletadores da dispersão terrestre do lagostim. A temperatura, a humidade relativa e o período do dia foram outras variáveis ambientais que influenciaram significativamente a dispersão terrestre do *P. clarkii*.

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> *"Learning to identify invaders in advance would tell us a great deal about how life history traits evolve and how biotic communities are assembled. In practical terms, it could reveal the most effective means to prevent future invasions."*
>
> Richard Mack et al. (2000)

CHAPTER 1

General Introduction

> *"Nowadays we live in a very explosive world, and while we may not know where or when the next outburst will be, we might hope to find ways of stopping it or at any rate damping down its force."*
>
> Charles Elton (1958)

1. Introduction

The invasion of habitats by exotic species poses one of the major threats to biodiversity (Vitousek *et al.* 1997). Human activities are amongst the main reasons for the alterations of the natural range of species by the artificial translocation of many species from their natural distribution ranges, where they may establish and have substantial ecological effects on the native species communities (Mack *et al.* 2000; Clavero and García-Berthou 2005; Stigall 2010). In the past decades, it has become clear to scientists and policy-makers that the deliberated or accidental introduction of species (microbes, fungi, plants and animals, including genetically modified organisms) into new areas outside their native geographic distribution is one of the main drivers of biodiversity change (Sala *et al.* 2000). This is particularly true when the effect of the breakdown of isolating barriers between biogeographical provinces is cumulative with the shrinking of the natural areas that accommodate wild species (Rosenzweig 2001). The native distribution of the species changed, and the world changed accordingly (Mooney and Cleland 2001). Changes in the natural distribution of species should not, in general, be viewed as abnormal events since they may be considered a commonplace in nature, often occurring over the course of geological times in association with climate change (Graumlich and Davis 1993; Lodge 1993). Only stochastic events (*e.g.* associated with unusual climatic conditions such as storms) may induce the natural dispersal of species to habitats previously beyond their natural dispersal capabilities (MacIsaac *et al.* 2001). Nevertheless, human actions are more frequent and can have wider impacts. Those impacts have greatly increased the spatial and temporal rate at which species disperse and the distances they travel. By these means exotic species are able to accomplish in a few decades something that could have never been accomplished by the means of natural events alone (Lodge 1993). The World is global and an increasingly higher number of people are, nowadays, traveling faster and farther, and more goods and materials are being traded among nations and continents (Pimentel *et al.* 2002), creating what could be called as a *New Pangaea* for some species (McKinney 2005). All these factors combined have facilitated the introduction and expansion of non-indigenous species (NIS) in several ecosystems (Vitousek *et al.* 1997) and it is estimated that over 480,000 NIS have been introduced (Pimentel *et al.* 2002).

Invasive NIS are a global concern, sometimes raising health issues and frequently having irreversible consequences on natural ecosystems. NIS can alter ecosystems by changing the roles of indigenous species (IS), disrupting evolutionary processes and causing significant changes in species abundance (Sala *et al.* 2000; Williams 2001; Lodge and Shrader-Frechette 2003). Ultimately IS will be less capable to resist and will decline, while invasive NIS will thrive (McKinney 2005). Their impact may be continuously increasing over time, even when their introduction ceases. Impacts of NIS species are diverse and, in fact, several introduced NIS can be beneficial to humans. Species such as corn, wheat, rice, plantation forests, domestic chicken, cattle, among others, provide now more than 98% of the world's food supply (Ewel *et al.* 1999, Pimentel *et al.* 2002). Many NIS cause minimal environmental impact, as predicted by the often cited tens rule. It states that approximately 10% of the imported species will escape or be introduced in the wild, 10% of which will establish themselves and 10% of the established NIS will become damaging pests (Williamson and Fitter 1996). Not all NIS have deleterious effects and the same species may have significant effects in some areas and negligible ones in others (Byers *et al.* 2002). For example, it is currently accepted that climate change can exacerbate the establishment and range expansion of many invasive NIS (Thomas *et al.* 2003; Hellmann *et al.* 2008). The proportion of the introduced NIS that can cause problems can be rather small, but their impact can be very serious. These species spread from the point of introduction and are often able to dominate IS populations and communities (Kolar and Lodge 2001). They may profoundly and adversely affect indigenous species, ecosystem processes, economic interests, and public health (Ricciardi *et al.* 1998). In one word, they may turn out to be *invasive* (Lockwood *et al.* 2007). The costs they inflict form a hidden but onerous tax on many goods and services and the damages they cause are often irrevocable. Biological invaders act as biological pollutants that, unlike chemicals, reproduce and spread autonomously, over great distances, and can adapt to changing conditions (Gherardi 2007).

Crayfish are a group of invaders outside their native geographic distribution with particularly important ecological effects (Holdich 2002). Freshwater crayfish are an important component of the aquatic fauna, frequently being the largest invertebrate predator in their habitats. They are considered both key-species and ecosystem engineers (Statzner *et al.* 2003; Creed and Reed 2004). Crayfish have been introduced worldwide by deliberated translocations and stocking for diverse reasons such as economic interests, aquaculture production, biological control, reduction of aquatic vegetation and aquarium hobbyists (Holdich and Lowery 1988; Henttonen and Hunner 1999; Holdich 2002). Invasive crayfish largest sphere of action are the European inland waters, where a considerable reduction of native crayfish population occurred in the 19th century mostly due to crayfish plague, an infection caused by the oomycete *Aphanomyces astaci* (Holdich and Lowery 1988; Holdich 2002). There are several other negative factors affecting native crayfish populations – *e.g.* habitat alterations, water pollution, habitat and shelter losses, genetic pollution, introduction of non-indigenous crayfish and subsequent competition with non-native species, predation, overfishing (Gutiérrez-Yurrita *et al.* 1999; Aquiloni *et al.* 2010). In addition, climate alterations also negatively affect native crayfish populations (Diéguez-Uribeondo 2006). One reason for the introduction of non-indigenous crayfish in Europe was the crayfish plague resistant substitutes for indigenous crayfish that should repopulate depleted benthic habitats. Unfortunately, the risks and threats associated with such non-native crayfish species introductions into European freshwater ecosystems were generally not previously accessed and, in most cases, are now beyond control (Holdich *et al.* 1999a).

Once established, invasive crayfish species can eradicate indigenous crayfish and reduce populations of food items such as fish, gastropods, algae, and macrophytes, which can have cascading trophic effects elsewhere in the ecosystem (Holdich 2002). Furthermore, the indigenous crayfish species can be displaced through direct competition for the resources available and other interactions with non-invasive crayfish species (Gherardi 2006). Sympatric crayfish species compete for limited resources such as food, shelter and space and larger crayfish routinely win competitive interactions with smaller crayfish species (Momot 1984). Invasive non-indigenous crayfish species are very well equipped for competition with indigenous crayfish species due to several intrinsic characteristics (*e.g.* early maturation, high fecundity, high growth rate and higher levels of activity and aggressiveness) (Lindqvist and Huner 1999). They show high tolerance towards extreme environmental conditions (including chemical pollution, high temperatures, and drought) and resistance to parasites and diseases (Scalici and Gherardi 2007). In addition to the ecological effects of invasive non-indigenous crayfish introduced into Europe, some species (*e.g. Orconectes limosus,* Rafinesque 1817; *Pacifastacus leniusculus*, Dana 1852; *Procambarus clarkii*, Girard 1852) are known to carry the crayfish plague pathogen (*Aphanomyces astaci* Schikora) and function as vectors of the disease to the indigenous crayfish population (Vogt 1999; Holdich 2003).

The spread of non-indigenous crayfish species are therefore amongst the most important threats for indigenous crayfish species in Europe (Füreder *et al.* 2006) and the Iberian Peninsula is no exception. It is also important to notice that the Iberian Peninsula is included in one of the two main centres of biodiversity in the Mediterranean region hotspot of biodiversity (Médail and Quézel 1999). For this reason, improving the quality of the information about invasive non-indigenous crayfish species (*e.g.* distribution, life cycle, ecology) is of extreme importance to properly access their invasive potential and develop effective management strategies to control and/or supress their continuously spreading, ultimately contributing to the conservation of the native crayfish species (Aquiloni *et al.* 2010).

2. Meet the red swamp crayfish (*Procambarus clarkii,* Girard 1852)

The red swamp crayfish, *Procambarus clarkii*, is a relatively large – mean total length averaging 12-13 cm (Anastácio *et al.* 2009) and up to a maximum of 19 cm (Correia 1995) in Portuguese populations – burrowing, temperate freshwater crayfish species. Its native distribution is comprised between north-eastern Mexico and south-central USA (Hobbs 1989).

From its natural range, *P. clarkii* was successfully introduced into the western and eastern USA (Hobbs *et al.* 1989; McClain *et al.* 2006). This species has also been widely introduced in other countries and its current distribution comprises all continents, except Australia and the Antarctic (Hobbs 1989; Campos and Rodríguez-Almaraz 1992; Holdich *et al.* 1999b; Rodríguez and Suárez 2001; Campos 2005; Gherardi 2006; Harlioğlu and Harlioğlu 2006; Wizen *et al.* 2008). Its translocation has been mainly motivated for aquaculture purposes because this species is a popular dining delicacy (*e.g.* in the USA) (Gherardi 2006; McClain *et al.* 2006). Additionally, attempts have been made to use *P. clarkii* as a biological control organism in Africa since it has been experimentally demonstrated that this crayfish species is an active predator of the schistosome transmitting snails (Hofkin *et al.* 1991; Mkoji *et al.* 1999a,b).

The success of *P. clarkii* introductions is attributable, as mentioned before to many other invasive non-indigenous crayfish species, to factors such as its resistance to the crayfish plague, its *R*- reproductive strategy that comprises a short life-history, high plasticity and high fecundity rate, tolerance to extreme environmental conditions (*e.g.* brackish water conditions, high temperatures, dry periods, and low dissolved oxygen environments) and its ability to use a wide spectrum of food (Paglianti and Gherardi 2004; Campos 2005; Gherardi 2006; ISSG 2007; Scalici *et al.* 2009). Taking into account the previously mentioned ecological characteristics, *P. clarkii* is considered one of the most plastic species of the order Decapoda (Lodge *et al.* 1998; Campos 2005; ISSG 2007). Likewise, it is also looked upon as a keystone species that might modify the nature of native plants and animal communities (Correia 2001; ISSG 2007; Correia and Anastácio 2008).

The first record of *P. clarkii* in Europe was registered in the Iberian Peninsula in 1973, in the Guadiana river basin – province of Badajoz, and in 1974, in the Guadalquivir river basin – near Sevilla, where it was introduced for aquaculture purposes (Habsburgo-Lorena 1978). In less than 20 years from this first introduction, new populations of *P. clarkii* have been reported in at least 13 European countries (Figure 1) (Souty-Grosset *et al.* 2006).

In Portugal, the red swamp crayfish was reported for the first time by Ramos and Pereira (1981) in the Guadiana River basin (southern Portugal) and resulted from the natural expansion of Spanish populations (Correia 1993b). Since then, the Portuguese populations of *P. clarkii* have increased very quickly due to the abundance of warm, shallow wetlands and agricultural areas (Correia 1995) combined with the ecological plasticity of the species (Gherardi 2006). *P. clarkii* severe impacts on ecosystem structure and biodiversity and on rice cultures have been extensively documented (*e.g.* Anastácio and Marques 1997; Gutiérrez-Yurrita *et al.* 1998; Anastácio *et al.* 2000; Anastácio *et al.* 2005a,b; Cruz *et al.* 2006; Cruz and Rebelo 2007). Nowadays it has a wide distribution in the country, where it seems to be well adapted to water reservoirs and wetlands such as rice fields (Correia 1993a,b; Ilhéu and

Bernardo 1993a,b). In both countries (Spain and Portugal), soon after introduction, crayfish populations increased without control (Habsburgo-Lorena 1978; Correia, 1993b). Although it is considered an invasive species in Europe, *P. clarkii* is already widespread and dominant – a stage V invasive species according to Colautti and MacIsaac (2004) – and should be considered as being already naturalized, at least in the Iberian Peninsula (García-Berthou *et al.* 2007). Unfortunately the Mediterranean region, housing many endemic species, has been especially susceptible to species invasions and Portuguese freshwater ecosystems have suffered numerous successful introductions (Cruz and Rebelo 2007; Ribeiro and Collares-Pereira 2010; Rebelo *et al.* 2010). Although the ecological impacts of these introductions are still poorly documented, they have been implicated in the decline of native freshwater fauna (Cabral *et al.* 2006).

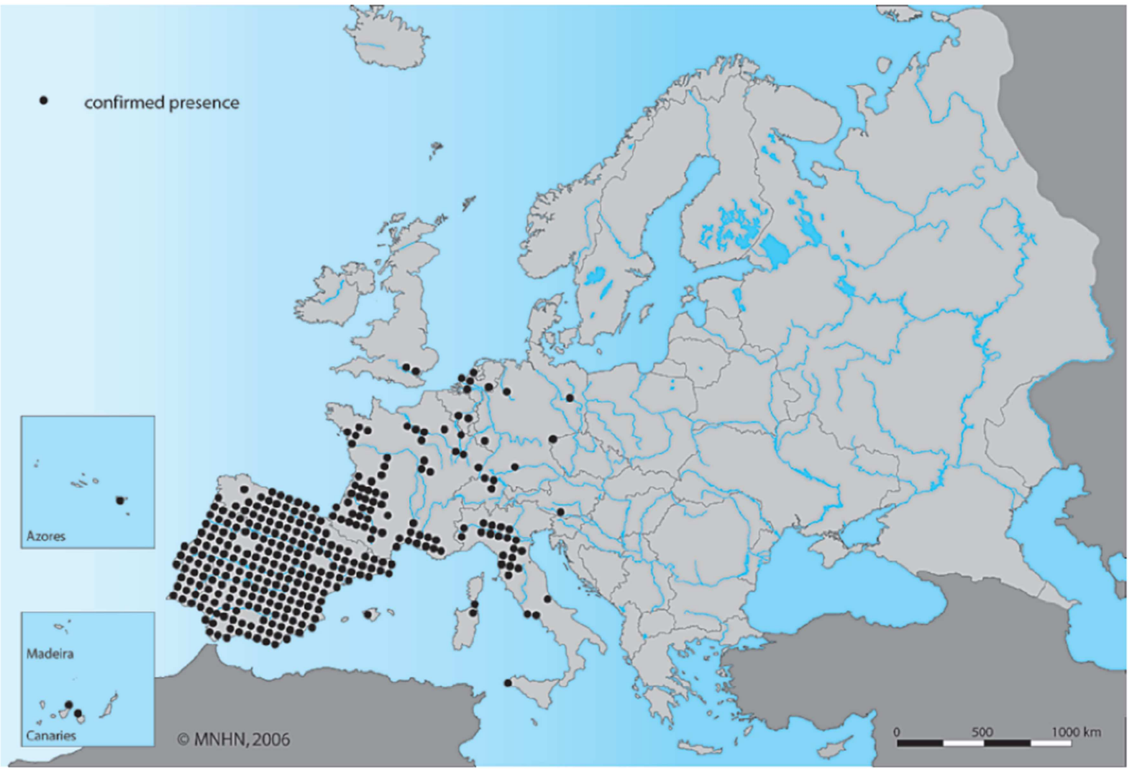

Figure 1. Current distribution of *Procambarus clarkii* in Europe. Portugal, Spain, France and Italy have the majority of the populations (adapted from Souty-Grosset *et al.* 2006).

Its intense burrowing inflicts structural damages to the banks of rivers and lakes (Barbaresi *et al.* 2004b) and causes water bioturbation leading to a reduction in primary productivity (Gherardi 2007). *P. clarkii* is an important pest in rice fields because it destroys levees and consumes young rice plants (Sommer and Goldman 1983; Grigarick 1984; Sommer 1984; Correia 1993b; Anastácio and Marques 1997; Anastácio *et al.* 2000). It is well known that, just after its introduction, when populations are rapidly growing, *P. clarkii* has dramatic effects on the community by depleting all food sources available because of its voracity and ability to shift its diet (Huner and Barr 1991; Ilhéu and Bernardo 1993a,b; Gutiérrez-Yurrita *et* *al.* 1998). Nevertheless, it is possible to increase the profits of rice-farmers by double cropping rice (*Oryza sativa*) and red swamp crayfish as it has been done intensively in the USA (Chien and Avault 1980; McClain *et al.* 2006). Like in other places *P. clarkii* introduction in Portugal became a problem for rice farmers (Anastácio and Marques 1997; Anastácio *et al.* 2005a,b). To prevent damage to rice crops, farmers tried to eliminate crayfish by means of xenobiotic chemicals. This had a strong negative effect in the environment and did not solve the problem. In fact, once introduced into favourable habitats, *P. clarkii* is difficult to eliminate (Holdich and Lowery 1988). A better solution could be the use of crayfish as a food resource, which would control the size of crayfish populations, with a simultaneous socio economical profit (Chien and Avault 1980; McClain *et al.* 2006). Nevertheless, caution should be taken since it can accumulate heavy metals (Gherardi *at al.* 2002a) and toxins from microalgae (Tricarico *et al.* 2008).

P. clarkii outcompetes indigenous crayfish species (*e.g. Astropotamobius pallipes*) being dominant in aggressive interactions (Gherardi and Cioni 2004) and transmitting the oomycete *Aphanomyces astaci* (Diéguez-Uribeondo and Söderhäl 1993). Due to its voracious feeding habits and high densities achieved, *P. clarkii* is today recognized to be a cause of biodiversity loss in the invaded water bodies (Correia and Anastácio 2008). It causes the local extinction of various species of molluscs, fish, amphibians and hydrophytes (Cruz *et al.* 2006; Gherardi *et al.* 2001; Renai and Gherardi 2004; Gherardi and Acquistapace 2007). There is a negative relationship between *P. clarkii* abundance and the distribution and abundance of floating leaved and submerged aquatic plants (Harper *et al.* 2002) as well as a so-called *special* relationship with water hyacinth (*Eichornia crassipes*) being associated with the significant reduction of this macrophyte in the water bodies (Foster and Harper 2006). The consequences of the introduction of *P. clarkii* on Portuguese riverine and aquatic communities are still poorly understood, although some studies have documented its role as a prey of diverse avian, mammal and fish (see Godinho and Ferreira 1994; Beja 1996a,b; Correia 2001) and also its trophic preferences (Ilhéu and Bernardo 1993b; Foster and Slater 1995; Ilhéu and Bernardo 1995; Gutiérrez-Yurrita *et al.* 1999; Anastácio *et al.* 2011). It is important to mention that *P. clarkii* is an important component of the diet of endangered and emblematic species in Portugal, such as the otter (*Luttra luttra*) and the white stork (*Ciconia ciconia*) (Beja 1996a,b; Correia 2001).

3. In this thesis

Since Charles Elton (1958) seminal book, ecologists have tried to predict and control the distribution and spread of non-indigenous species. It seems that a key factor regulating species invasion is opportunity: the more frequently and persistently a foreign plant or animal is exposed to a new environment, the better are its odds of invading it (Davis 2009). Several studies modelled the distribution and the deleterious effects of non-indigenous crayfish species on ecosystems, however such studies often requires that a large amount of quantitative information is available. Understanding the spatial and temporal distribution of species, and invasive species in particular, requires profound knowledge of the limiting factors (biotic and abiotic) regulating species distribution (Davis 2009).

Understanding the environmental factors determining the establishment and colonization of new areas by the invasive populations is a crucial issue in the study of biological invasions. Knowledge of these variables and the factors determining them can be used to elaborate risk assessment maps for other invasive species, in order to define high-risk areas susceptible to invasion. In fact, an important approach to prevent further invasions is predicting the potential outcome of introductions on the basis of the knowledge of ecological requirements of potential invaders. Because of the severity of the impacts of biological invasions and the difficulty of eradicating an exotic species once it has established, it is pivotal to develop prospective work allowing the detection of invasions in their initial stages (Zalba *et al.* 2000).

Crayfish growth is affected by several variables such as: water temperature, water quality, food availability, light intensity, photoperiod, crayfish length and density, among others (*e.g.* McClain *et al.* 1992; Nyström 1994; Gutiérrez-Yurrita and Del Olmo 2004; Paglianti and Gherardi 2004). The negative effect of crowding or, in other terms, population density, on growth has been previously reported (Lutz and Wolters 1986; Jarboe and Romaire 1995). However, in these studies the highest densities tested were rarely above 20 individuals m^{-2}. Thus, there is a lack of knowledge on the response of red swamp crayfish to population density at higher densities and further research is needed in order to quantify and determine the extent of such effects, in spite of food availability. This knowledge is especially relevant for juvenile production facilities because it helps to understand the reactions of crayfish to overcrowding. The determination of growth parameters is also relevant for the management of wild population, especially in invaded areas. The density regulation of growth is a form of intra-specific population regulation. There is a lack of knowledge on the response of red swamp crayfish, particularly of the early stages of development, to population density under controlled laboratory conditions at high densities and a laboratory experiment was conducted in order to determine and quantify such effects (Chapter 2).

Learning abilities are fundamental for survival and, in any ecosystem, prey animals are required to learn to recognize certain predatory cues of potential predators. This has been demonstrated, for example, in fish (Karplus *et al.* 2006; Siebeck *et al.* 2009), damselflies (Chivers *et al.* 1996; Wisenden *et al.* 1997) and crayfish (Hazlett and Schoolmaster 1998). Invasive crayfish exhibit a high degree of plasticity in learning to reduce predation risk (Hazlett 2000), and some species (e.g. *Orconectes virilis* and *P. clarkii*) seem to learn to avoid predation quicker than native species (Gherardi *et al.* 2002b; Hazlett *et al.* 2002). This characteristic constitutes an important adaptive advantage when invading a new habitat (Acquistapace *et al.* 2003). Learning to quickly recognize new prey items will also substantially increase the invader predator fitness. Crustaceans are important predators in many ecosystems and their learning plasticity has been demonstrated both on crabs (Roudez *et al.* 2008) and invasive crayfish. In fact, invasive crayfish have the ability to readily switch to new prey items in contrast to native crayfish species (Gherardi *et al.* 2001). The plasticity of crayfish for learning to recognize new prey may provide important insights about the potential success of a species when invading new habitats. Several studies have reported evidence of learning in a variety of arthropod species (Papaj and Prokopy 1989; Dukas 2008; Ishii and Yamada 2010), however, the application of equations and mathematical modelling to the learning processes is not common. A study was conducted to determine whether prior experience influences the success rate of prey capture and to estimate and mathematically explain the learning curve of *P. clarkii* as a naïve predator (Chapter 3).

When an area is recently invaded, the invader often encounters other invasive species already established and the invasion process is the result of the inter-specific interaction between both native and non-native species. As an attempt to manage invasive species studies focusing on invasive species in both natural and humanized ecosystems have been carried out (Simon and Townsend 2003). There are some studies about the inter-specific consumption and influence of *P. clarkii* on aquatic biocenoses (Correia 2002, 2003; Correia *et al.* 2005; Anastácio *et al.* 2011) but not much information is available about the effects of other predators (*e.g.* fish) over *P. clarkii* populations. As stated above for *P. clarkii* learning also plays an important role when considering fish predators (Karplus *et al.* 2006; Siebeck *et al.* 2009). An experiment was conducted (Apendix 1) with the objective of determine the effect of learning time on the predation of *P. clarkii* by the largemouth bass (*Micropterus salmoides*), a non-native invasive fish predator in the Iberian Peninsula inland waters (Godinho and Ferreira 1998).

Prey-switching in predators, which attack several prey species, can potentially stabilize prey populations numbers (Murdoch 1975; Nilsson 2001; Palomino-Bean *et al.* 2006). When switching occurs, the number of attacks upon a species is disproportionally large when the species is abundant relative to other prey and disproportionally small when the species is relatively rare. Although several other factor may be involved in prey population regulation (*e.g.* prey and predator size, environmental conditions). This process may be important when considering the regulation of invasive crayfish species. Detailed information and mathematical approaches to the interspecific relation between *P. clarkii* and other invasive species are scarce, particularly regarding prey switching among its fish predators. Understanding of switching in natural systems will requires detailed research of the behavioural mechanisms in the response of predator preference to changes in prey density (Abrams and Matsuda 2004). This is important to provide new insights on the processes and mechanisms of invasion and its effects on invaded aquatic biocenoses (Marco *et al.* 2002). Attempts were conducted to access the occurrence of prey switching in a system with three invasive species, involving *P. clarkii* and two fish species, *Micropterus salmoides* and mosquitofish (*Gambusia holbrooki*) (Apendix 2).

There are several different marking methods successfully used in mark-recapture experiments, each of them with its advantages and disadvantages (*e.g.* visible implant elastomers, visible implant alphanumeric tags, radio-telemetry, uropod clipping among others) (Abrahamsson 1965; Guan 1997; Guan and Wiles 1999; Gherardi and Barbaresi 2000; Robinson *et al.* 2000; Bubb *et al.* 2002; Gherardi *et al.* 2004; Bubb *et al.* 2006; Clark and Kershner 2006; Mazlum 2007; Kuhlmann *et al.* 2008). The choice of the technique to be used will depend on the theoretic and management question, the behaviour of the species, the habitat it inhabits and the available resources (economic and logistic constraints). During the present research a simple, cheap and effective (for our objectives) marking method was developed (Chapter 4). This method made it easier to proceed with several field and laboratory work programmes described in this thesis.

The identification of the initial locus of invasive non-indigenous species and their route of invasion is of great theoretical and practical importance (Wilson *et al.* 1999; Kreiser *et al.* 2000). Migration and dispersion[1] may be critical, not only for the continuity of the propagule pressure, but also as an important source of genetic variation necessary for the adaptative evolution (Etterson and Shaw 2001) of the invasive population. Crustacean dispersal behaviour evolved for the displacement of the animal in space (Herrnkind 1983). Although the world-wide spread of *P. clarkii* is mainly attributed to human introductions (Gherardi *et al.* 2006), the rapid and widespread expansion of the species, following its establishment, is the result of its active dispersal capabilities. The ability to disperse overland enables a freshwater species to colonize new water bodies in the neighbourhood and it is known that *P. clarkii* has the ability to exit the water and move overland (Penn 1943; Holdich 2002; Kerby *et al.* 2005; Cruz and Rebelo 2007; Chucholl 2011). *P. clarkii* can resist drought by burrowing until the next rainy season (Huner and Barr 1991) or use overland dispersion to move to a larger pool or one with more favourable conditions (Penn 1943; Aquiloni *et al.* 2005). *P. clarkii* population dynamics is well documented in the Iberian Peninsula (Correia 1995a,b; Gutiérrez-Yurrita *et al.* 1999; Ligas 2007; Gherardi 2006; Alcorlo *et al.* 2008; Anastácio *et al.* 2009) and some studies have explored its underwater dispersal abilities (Gherardi and Barbaresi 2000; Gherardi *et al.* 2000; Aquiloni *et al.* 2005) Nevertheless, little is known about *P. clarkii* ability to disperse overland

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[1] In the context of this thesis dispersal is considered as the active or passive spreading of individuals away from others (*e.g.* from an existing populations or form their inicial location). Migration, on the other hand, is an active and directional movement of large numbers of individuals from one location to another (Townsend 2008).

and colonize new freshwater areas and the factors involved in its overland dispersal remains unclear and not quantified. Some studies were conducted in order to characterize *P. clarkii* overland dispersion and determine the main environmental triggers involved (Chapter 5, Chapter 6 and Apendix 3).

Considering *P. clarkii* plasticity and invasive potential, particularly its high resistance to adverse conditions and behavioural adaptations, this species provides an excellent opportunity to analyse how behaviour contributes to its invasive potential in European ecosystems. One of the objectives of this thesis was to identify the factors involved in *P. clarkii*'s seasonal overland dispersion, so that in the future, more efficient predictions of the spread in invaded areas can be obtained and more effective management or exploitation is possible. It has also been reported that crayfish behaviour changes with population density (Bovbjerg and Stephen 1975; Holdich 2002) and some studies (*e.g.* Bovbjerg 1959) support the hypothesis that crayfish underwater dispersion increases with population density. An experiment was set to determine whether density influences *P. clarkii* underwater movement (Apendix 5).

The crayfish aquaculture industry of the USA is located primarily in Louisiana where over 1,100 producers cultivate procambarid crayfish on over 70,000 ha, producing in excess of 44,000 metric tons worth over $100 million USD annually (LCES 2009). The sole method used for harvest of procambarid crayfish from aquaculture ponds in the Southern USA is the baited wire-mesh trap. Baited trap efficiency is dependent on a number of variables, such as crayfish density, bait type, trap soak interval, and environmental factors. However, the efficiency of the standard trapping protocol to remove harvest size crayfish from a population has not been thoroughly examined. Baited traps are often used to determine crayfish population parameters and the trap efficiency may significantly influence the results os such assessments (Acosta and Perry 2000). On the course of the present thesis some attemps were conducted to determine the capture rate and efficacy of baited traps in Louisiana production systems (Chapter 5, Apendix 3 and Apendix 4).

Detailed data regarding the inter- and intra-specific population regulatory mechanisms, migratory ability, seasonal migrations and dispersal, learning abilities and interaction with sympatric predators are missing for *P. clarkii*. The increase of the knowledge about invasive species is crucial to develop detailed and more accurate models in order to manage the existing populations and provide tools that may contribute to a future predictor of the impacts of these species in nature. In the present thesis detailed information about *P. clarkii* was collected in order to contribute to increase the knowledge regarding this invasive species. Ultimately, this information can be integrated in mathematical models that would contribute significantly to the understanding of the invasiveness of crayfish species. These could also be used to manage the existing naturalized populations, to control or predict future suitable areas and to minimize the impacts in non-indigenous species in the ecosystem and in the economy.

The main objectives of the research within this thesis were:

- Evaluate intra-specific population regulation, namely the importance of population density on growth and survival of young of the year *P. clarkii*, and to understand the effects of density on both the period between moults and the length increment between moults without food limitation;
- Determine crayfish learning abilities, in terms of their capacity to learn how to prey on new preys;
- Evaluate the possibility of inter-specific population regulation, namely the influence of learning on *P.clarkii* capture rate by the largemouth bass (*Micropterus salmoides*) and the occurrence of prey-switching in a three invasive species system.
- Study and characterize *P. clarkii* dispersion, particularly the post-reproductive dispersion and determine the environmental triggers involved in *P. clarkii* overland dispersal;
- Determine the effects of population density on *P. clarkii* underwater activity;

4. References

Abrahamsson S.A.A. (1965). A method of marking crayfish *Astacus astacus* Linné in population studies. *Oikos* **16**, 228 – 231.

Abrams P.A. and Matsuda H. (2004). Consequences of behavioural dynamics for the population dynamics of predator-prey systems with switching. *Population Ecology* **46**, 13 – 25.

Acosta C. and Perry S.A. (2000). Effective sampling area: a quantitative method for sampling crayfish populations in freshwater marshes. *Crustaceana* **73**(4), 425 – 431.

Acquistapace P., Hazlett B.A. and Gherardi F. (2003). Unsuccessful predation and learning of predator cues by crayfish. *Journal of Crustacean Biology* **23**, 364 – 370.

Alcorlo P., Geiger W. and Otero M. (2008). Reproductive biology and life cycle of the invasive crayfish *Procambarus clarkii* (Crustacea: Decapoda) in diverse aquatic habitats of South-Western Spain: Implications for population control. *Archiv für Hydrobiologie* **173**, 197 – 212.

Anastácio PM., Leite E.P., Ferreira M., Vicente L. and Correia A.M. (2011). Ontogenic shifts in predatory roles involving two invasive species. *Limnologica* Online early, doi:10.1016/j.limno.2010.12.002

Anastácio P.M., Leitão A.S., Boavida M.J. and Marçal A.M. (2009). Population dynamics of the invasive crayfish (*Procambarus clarkii* Girard, 1852) at two marshes with differing hydroperiods. *Annales de Limnology - International Journal of Limnology* **45**(4), 247 – 256.

Anastácio P.M., Correia A.M. and Menino J.P. (2005)a. Processes and patterns of plant destruction by crayfish: effects of crayfish size and developmental stages of rice. *Archive für Hydrobiologie* **162**, 37 – 51.

Anastácio P.M., Parente V.S. and Correia A.M. (2005)b. Crayfish effects on seeds and seedlings: identification and quantification of damage. *Freshwater Biology* **50**, 697 – 704.

Anastácio P.M., Frias A.F. and Marques J.C. (2000). Impact of crayfish densities on wet seeded rice and the inefficiency of a non-ionic surfactant as an Ecotechnological solution. *Ecological Engineering* **15**, 17 – 25.

Anastácio P.M. and Marques J.C. (1997). Crayfish, *Procambarus clarkii*, effects on initial stages of rice growth in the lower Mondego River valley (Portugal). *Freshwater Crayfish* **11**, 608 – 617.

Aquiloni L., Tricarico E. and Gherardi F. (2010). Crayfish in Italy: distribution, threats and management. *International Aquatic Research* **2**, 1 – 14.

Aquiloni L., Ilhéu M. and 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**, 225 – 236.

Barbaresi S., Tricarico E., Santini G. and Gherardi F. (2004)a. Ranging behaviour of the invasive crayfish, *Procambarus clarkii*. *Journal of Natural History* **38**, 2821 – 2832.

Barbaresi S., Tricarico E., Santini G. and Gherardi F. (2004)b. Factors inducing the intense burrowing activity by the red swamp crayfish, *Procambarus clarkii*, an invasive species. *Naturwissenschaften* **91**, 342 – 345.

Beja P.R. (1996)a. An analysis of otter *Lutra lutra* predation on introduced American crayfish *Procambarus clarkii* in Iberian streams. *Journal of Applied Ecology* **33**(5), 1156 – 1170.

Beja P.R. (1996)b. Seasonal breeding and food resources of otters, *Lutra lutra* (Carnivora, Mustelidae), in south-west Portugal: a comparison between coastal and inland habitats. *Mammalia* **60**(1), 27 – 34.

Bovbjerg R.V. and Stephen S.L. (1975). Behavioral changes with increased density in the crayfish *Orconectes virilis*. *Freshwater crayfish* **2**, 429 – 442.

Bovbjerg R. V. (1959). Density and dispersal in laboratory crayfish populations. *Ecology* **40**(3), 504 – 506.

Bubb D.H., Thom T.J. and Lucas M.C. (2006). Movement patterns of the invasive signal crayfish determined by PIT telemetry. *Canadian Journal of Zoology* **84**(8), 1202 – 1209.

Byers J.E., Reichard S., Randall J.M., Parker I.M., Smith C.S., Lonsdale W.M., Atkinson I.A.E., Seastedt T.R., Williamson M., Chornesky E. and Hayes D. (2002). Directing research to reduce the impacts of nonindigenous species. *Conservation Biology* **16**, 630 – 640.

Cabral M.J. (coord.), Almeida J., Almeida P.R., Dellinger T., Ferrand de Almeida N., Oliveira M.E., Palmeirim J.M., Queiroz A.I., Rogado L. and Santos-Reis M. (eds.) (2006). *Livro Vermelho dos Vertebrados de Portugal.* 2ª ed. Instituto da Conservação da Natureza/Assírio & Alvim. Lisboa 660 pp.

Campos M.R. (2005). *Procambarus (Scapulicambarus) clarkii* (Girard, 1852), (Crustacea: Decapoda: Cambaridae). Una langostilla no nativa en Colombia. *Academia Colombiana de Ciencias exactas Físicas y Naturales* **111**, 295 – 302.

Campos E. and Rodríguez-Almaraz G. (1992). Distribution of the red swamp crayfish *Procambarus clarkii* (Girard 182) (Decapoda; Cambaridae) in Mexico: an update. *Journal of Crustacean Biology* **12**(4), 627 – 630.

Chien Y.H. and Avault J.W., Jr. (1980). Production of crayfish in rice fields. *Progressive Fish-Culturist* **42**, 67 – 71.

Chivers D.P., Wisenden B.D. and Smith R.J. (1996). Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. *Animal Behaviour* **52**, 315 – 320.

Chucholl C. (2011). Disjunct distribution pattern of *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in an artificial lake system in Southwestern Germany. *Aquatic invasions* **6**(1), 109 – 113.

Clark J.M. and Kershner M.W. (2006). Size-dependent effects of visible implant elastomer marking on crayfish (*Orconectes obscurus*) growth, mortality, and tag retention. *Crustaceana* **79**(3), 275 – 284.

Clavero M. and García-Berthou E. (2005). Invasive species are a leading cause of animal extinctions. *Trends in Ecology and Evolution* **20**, 110.

Colautti R. I. and MacIsaac H.J. (2004). A neutral terminology to define 'invasive' species. *Diversity and Distributions* **10**, 135 – 141.

Correia A.M. and Anastácio P.M. (2008). Shifts in aquatic macroinvertebrate biodiversity associated with the presence and size of an alien crayfish. *Ecological Research* **23**, 729 – 734

Correia A. M., Bandeira N. and Anastácio P. M. (2005). Predator-prey interactions of *Procambarus clarkii* with aquatic macroinvertebrates in single and multiple prey systems. *Acta Oecologica* **28**(3), 337 – 343.

Correia A. M. (2003). Food choice by the introduced crayfish *Procambarus clarkii*. *Annales Zoologici Fennici* **40**, 517 – 528.

Correia A.M. (2002). Niche breadth and trophic diversity: feeding behaviour of the red swamp crayfish (*Procambarus clarkii*) towards environmental availability of aquatic macroinvertebrates in a rice field (Portugal). *Acta Oecologica* **23**, 421 – 429.

Correia A.M. (2001). Seasonal and interspecific evaluation of predation by mammals and birds on the introduced red swamp crayfish *Procambarus clarkii* (Crustacea, Cambaridae) in a freshwater marsh (Portugal). *Journal of Zoology* **255**, 533 – 541.

Correia A.M. (1995)a. *Biologia populacional e interacções tróficas de Procambarus clarkii (Crustacea, Decapoda, Cambaridae) no sistema hidrográfico do rio Tejo (Ribatejo, Portugal).* Doctoral Dissertation, Faculdade de Ciências, Universidade de Lisboa, Lisboa, 255 pp.

Correia A. M. (1995)b. Population dynamics of *Procambarus clarkii* (Crustacea: Decapoda) in Portugal. *Freshwater Crayfish* **8**, 276 – 290.

Correia A.M. (1993)a. Length-weight relationships for two populations of red swamp crayfish, *Procambarus clarkii* (Decapoda, Cambaridae) from Portugal. *Freshwater Crayfish* **9**, 442 – 450.

Correia A.M. (1993)b. Situation de l’acclimatation de l’écrevisse rouge des marais *Procambarus clarkii* au Portugal. *L’Asticulteur de France* **35**, 2 – 9.

Creed R.P. and Reed J.M. (2004). Ecosystem engineering by crayfish in a headwater stream community. *Journal of the North American Benthological Society* **23**, 224 – 236.

Cruz M. and Rebelo R. (2007). Colonization of freshwater habitats by an introduced crayfish, *Procambarus clarkii*, in southwest Iberian Peninsula. *Hydrobiologia* **575**(1), 191 – 201.

Cruz M.J., Rebelo R. and Crespo E.G. (2006). Effects of an introduced crayfish, *Procambarus clarkii*, on the distribution of south-western Iberian amphibians in their breeding habitats. *Ecography* **20**, 329 – 338.

Davis M.A. (2009). *Invasion Biology*. Blackwell, Malden MA. 264 pp.

Diéguez-Uribeondo J. (2006). The dispersion of the *Aphanomyces astaci*-carrier *Pacifastacus leniusculus* by humans represents the main cause of disappearance of the indigenous crayfish *Astropotamobius pallipes* in Navarra. *Bullettin Français de la Pêche et de la Pisciculture* **381**, 1303 – 1312.

Diéguez-Uribeondo J. and Söderhäll K. (1993). *Procambarus clarkii* Girard as a vector for the crayfish plague fungus, *Aphanomyces astaci* Schikora. *Aquaculture Research* **24**, 761 – 765.

Dukas R. (2008). Evolutionary biology of insect learning. *Annual Review of Entomology* **53**, 145 – 160.

Elton C.S. (1958). *The ecology of invasions by animals and plants*. University of Chicago Press, Chicago, IL.

Etterson J.R. and Shaw R.G. (2001). Constraint to Adaptive Evolution in Response to Global Warming. *Science* **294**, 151 – 154.

Ewel J.J., O'Dowd D.J., Bergelson J., Daehler C.C., D'Antonio C.M., Gomez D., Gordon D.R., Hobbs R.J., Holt A., Hopper K.R., Hughes C.E., Lahart M., Leakey R.R.B., Lee W.G., Loope L.L., Lorence D.H., Louda S.M., Lugo A.E., Mcevoy P.B., Richardson D.M, and Vitousek P.M. (1999). Deliberate introductions of species: research needs. *BioScience* **49**, 619 – 630.

Foster J. and Harper D.M. (2006). The Alien Louisiana Red Swamp Crayfish *Procambarus clarkii* Girard in Lake Naivasha, Kenya 1999–2003. *Freshwater Crayfish* **15**, 195 – 202.

Foster J. and Slater F.M. (1995). A global review of crayfish predation with observations on the possible loss of *Austropotamobius pallipes* in the Welsh Wye due to crayfish plague. *Freshwater Crayfish* **8**, 589 – 613.

García-Berthou E.D., Boix D. and Clavero M. (2007). *Non-indigenous animal species naturalized in Iberian inland waters.* In: Biological invaders in inland waters: profiles, distribution, and threats. (ed. Gherardi F), pp. 123-140. Invading Nature: Springer Series in Invasion Ecology, Springer, Dordrecht, The Netherlands.

Gherardi F. (2007). *Understanding the impact of invasive crayfish*. In: Biological invaders in inland waters: profiles, distribution, and threats. (ed. Gherardi F), 507-542 pp. Invading Nature: Springer Series in Invasion Ecology, Springer, Dordrecht, The Netherlands.

Gherardi F. and Acquistapace P. (2007). Invasive crayfish in Europe: the impact of *Procambarus clarkii* on the littoral community of a Mediterranean lake. *Freshwater Biol*ogy **52**, 1249 – 1259.

Gherardi F. and Barbaresi S. (2007). Feeding preferences of the invasive crayfish *Procambarus clarkii*. *Bullettin Français de la Pêche et de la Pisciculture* **385**, 7 – 20.

Gherardi F. (2006). Crayfish invading Europe: the case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology* **39**, 175 – 191.

Gherardi F. and Cioni A. (2004). Agonism and interference competition in freshwater decapods. *Behaviour* **141**, 1297 – 1324.

Gherardi F., Acquistapace P., Hazlett B. and Whisson G. (2002)b. Behavioural responses to alarm odours in indigenous and non-indigenous crayfish species: a case study from Western Australia. *Marine and Freshwater Research* **53**, 93 – 98.

Gherardi F., Barbaresi S., Vaselli O. And Bencini A. (2002)a. A comparison of trace metal accumulation in indigenous and alien freshwater macro-decapods. *Marine and Freshwater Behaviour and Physiology* **35**, 179 – 188.

Gherardi F., Renai B. and Corti C. (2001). Crayfish predation on tadpoles: a comparison between a native (*Austropotamobius pallipes*) and an alien species (*Procambarus clarkii*). *Bullettin Français de la Pêche et de la Pisciculture* **361**, 659 – 668.

Gherardi F. and Barbaresi S. (2000). Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). *Archiv für Hydrobiologie* **150**(1), 153 – 168.

Gherardi F., Barbaresi S. and Salvi G. (2000). Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences* **62**, 179 – 193.

Godinho F.N. and Ferreira M.T. (1998). Spatial variation in diet composition of pumpkinseed sunfish, *Lepomis gibbosus*, and largemouth bass, *Micropterus salmoides*, from a Portuguese stream. *Folia Zoologica* **47**, 205 – 213.

Godinho F.N. and Ferreira M.T. (1994) Diet composition of largemouth bass *Micropterus salmoides* (Lacepède) in southern Portuguese reservoirs: its relation to habitat characteristics. *Fisheries Management and Ecology* **1**(2), 163 – 179.

Graumlich L.J. and Davis M.B. (1993). Holocene variation in spatial scales of vegetation pattern in the upper Great Lakes. *Ecology* **74**, 826 – 839.

Guan R.Z. and Wiles P.R. (1999). Growth and reproduction of the introduced crayfish *Pacifastacus leniusculus* in a British lowland river. *Fisheries Research* **42**(3), 245 – 259.

Guan R.Z. (1997). An improved method for marking crayfish. *Crustaceana* **70**(6), 641 – 652.

Gutiérrez-Yurrita P.J. and Del Olmo C.M. (2004). Population dynamics of juveniles of red swamp crayfish (*Procambarus clarkii*) under controlled conditions. *Freshwater Crayfish* **14**, 180 – 189.

Gutierrez-Yurrita P.J., Martínez J.M., Bravo-Utrera M.A., Montes C., Ilhéu M.A. and Bernardo J.M. (1999). The status of crayfish populations in Spain and Portugal. In: Gherardi F. and Holdich D.M. (eds.), *Crayfish in Europe as alien species – How to make the best of a bad situation?* Balkema, 161 – 192 pp.

Gutiérrez-Yurrita P.J., Gorka S., Bravo-Ultrera M.Á., Baltanás Á. and Montes C. (1998). Diet of the red swamp crayfish (*Procambarus clarkii*) in natural ecosystems of the Doñana National Park temporary fresh-water marsh (Spain). *Journal of Crustacean Biology* **18**, 120 – 127.

Habsburgo-Lorena A.S. (1986). The status of the *Procambarus clarkii* population in Spain. *Freshwater Crayfish* **6**, 131–136.

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

Harlioğlu M.M. and Harlioğlu A.G. (2006). Threat of non-native crayfish introductions into Turkey; global lessons. *Reviews in Fish Biology and Fisheries* **16**, 171 – 181.

Harper D., Smart A.C., Coley S., Schmitz S., North R., Adams C., Obade P. and Mbogo K. (2002). Distribution and abundance of the Louisiana red swamp crayfish *Procambarus clarkii* Girard at Lake Naivasha, Kenya between 1987 and 1999. *Hydrobiologia* **488**, 57 – 71.

Hazlett B.A., Acquistapace P. and Gherardi F. (2002). Differences in Memory Capabilities in Invasive and Native Crayfish. *Journal of Crustacean Biology* **22**, 439 – 448.

Hazlett B.A. (2000). Information use by an invading species: do invaders respond more o alarm odours than native species? *Biological Invasions* **2**, 289 – 294.

Hazlett B.A. and Schoolmaster D.R. (1998). Responses of cambarid crayfish to predator odour. *Journal of Chemical Ecology* **24**, 1757 – 1770.

Hellmann J.J., Byers J. E., Bierwagen B.G and Dukes J.S. (2008). Five Potential Consequences of Climate Change for Invasive Species. *Conservation Biology* **22**, 534 – 543.

Henttonen P. and Huner J.V. (1999). The introduction of alien species of crayfish in Europe: A historical introduction. In: Gherardi, F. and Holdich, D.M. (eds.) *Crustacean Issues 11: Crayfish in Europe as Alien Species (How to make the best of a bad situation?)* A.A. Balkema, Rotterdam, Netherlands: 13-22.

Herrnkind W. F. (1983). Movement Patterns and Orientation. In: *Behavior and Ecology*. Eds: F. J. Vernberg and W. B. Vernberg. New York, Academic Press: 41 – 106 pp.

Hobbs H.H., Jass J.P. and Huner J.V. (1989). A review of global crayfish introductions with particular emphasis on two North American species (Decapoda: Cambaridae). *Crustaceana* **56**, 229 – 316.

Hobbs H.H. (1989). An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). *Smithsonian Contributions to Zoology* **480**, 1 – 236.

Hofkin P.V., Hofinger D.M., Koech D.K. and Loker E.S. (1991). Predation of Biomphalaria and non-target mollusks by the crayfish *Procambarus clarkii*: implications for the biological control of schistosomiasis. *Annals of Tropical Medicine and Parasitology* **86**, 663 – 670.

Holdich D. M. (2002). *Biology of freshwater crayfish*. Oxford: Blackwell Science. 702 pp.

Holdich D.M, Ackefors H. and Gherardi F. (1999)b. *Native and Alien crayfish in Europe: Some conclusions.* pp. 281 – 292. In: Gherardi F. and Holdich D.M (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield.

Holdich D.M., Gydemo R. and Rogers W.D. (1999)a. A review of possible methods for controlling alien crayfish populations. *Crustacean Issues* **11**, 245 – 270.

Holdich D.M. and Lowery R.S. (1988). Freshwater crayfish biology, management and exploitation. Timber Press, Portland, Oregon, 498 pp.

Huner V. and Barr J. E. (1991). *Red Swamp Crayfish: Biology and Exploitation*. Louisiana Sea Grant College Program. Baton Rouge, Louisiana, 128 pp.

Huner J.V. (1984). Growth responses of juvenile male crayfish, *Procambarus clarkii,* fed artificial diets supplemented with *Egeria densa*, a vascular aquatic plant. *Journal of the World Mariculture Society* **15**, 129 – 131.

Huner J.V. and Avault J. W., Jr. (1979). Introductions of *Procambarus spp. Freshwater Crayfish* **4**, 191–194.

Huner J.V. (1977). Introductions of the Louisiana red swamp crayfish, *Procambarus clarkii* (Girard): an update. *Freshwater Crayfish* **3**, 193–202.

ILHÉU M. AND BERNARDO J.M. (1995). Trophic ecology of red swamp crayfish *Procambarus clarkii* (Girard) - preferences and digestibility of plant foods. *Freshwater Crayfish* **10**, 132 – 139.

ILHÉU M. AND BERNARDO J.M. (1993)a. Experimental evaluation of food preference of red swamp crayfish, *Procambarus clarkii*, vegetal vs animal. *Freshwater Crayfish* **9**, 359 – 364.

ILHÉU M. AND BERNARDO J.M. (1993)b. Aspects of trophic ecology of red swamp crayfish (*Procambarus clarkii* Girard) in Alentejo, South of Portugal. In: *Actas VI Congresso Español de Limnologia* (ed. by L.C. Pizarro, R.M. Baquero, P.S. Castillo and P. Carrillo), Instituto de Ciencias de la Educación **13**, 417 – 423. University of Granada, Spain.

ISHII Y. AND SHIMADA M. (2010). The effect of learning and search images on predator-prey interactions. *Population Ecology* **52**, 27 – 35.

ISSG (2007). *Global invassive species database. Procambarus clarkii*. Available from: http://www.issg.org/database/species/distribution.asp?si=608&fr=1&sts=sss&lang=EN. Accessed 13 December 2010.

JARBOE H.H. AND ROMAIRE R.P. (1995). Effects of density reduction and supplemental feeding on stunted crayfish *Procambarus clarkii* populations in earthen ponds. *Journal of the World Aquaculture Society* **26**, 29 – 37.

KARPLUS I., KATZENSTEIN R. AND GOREN M. (2006). Predator recognition and social facilitation of predator avoidance in coral reef fish *Dascyllus marginatus* juveniles. *Marine Ecology Progress Series* **319**, 215 – 223.

KERBY J. L, RILEY. S. P. D., KATS L. B. AND WILSON P. (2005). Barriers and flow as limiting factors in the spread of an invasive crayfish (*Procambarus clarkii*) in southern California streams. *Biological Conservation* **126**, 402 – 409.

KOLAR C.S. AND LODGE D.M. (2001). Progress in invasion biology: predicting invaders. *Trends in Ecology and Evolution* **16**, 199 – 204.

KREISER B.R., MITTON J.B. AND WOODLING J.D. (2000). Single versus multiple sources of introduced populations identified with molecular markers: a case study of a freshwater fish. *Biological Invasions* **2**, 295 – 304.

KUHLMANN M.L., BADYLAK S.M. AND CARVIN E.L. (2008). Testing the differential predation hypothesis for the invasion of rusty crayfish in a stream community: laboratory and field experiments. *Freshwater Biology* **53**(1), 113 – 128.

LCES (LOUISIANA COOPERATIVE EXTENSION SERVICE) (2009). Louisiana Summary of Agriculture and Natural Resources. Louisiana State University Agricultural Center, Baton Rouge, Louisiana. 323 pp.

LIGAS A. (2007). Population dynamics of *Procambarus clarkii* (Girard, 1852) (Decapoda, Astacidea, Cambaridae) from southern Tuscany (Italy). *Crustaceana* **81**(5), 601 – 609.

Lindqvist O.V. and Huner J:V. (1999). *Life history characteristics of crayfish: what makes some of them good colonizers?* pp. 281 – 292. In: Gherardi F. and Holdich D.M (eds.): Crayfish in Europe as alien species: How to make the best of the bad situation? A.A. Balkema, Rotterdam, Brookfield.

Lockwood J., Hoopes M. and Marchetti M. (2007). *Invasion Ecology*. Blackwell Publishing. Oxford, UK. 313pp.

Lodge D.M. and Shrader-Frechette K. (2003). Nonindigenous Species: Ecological Explanation, Environmental Ethics, and Public Policy. *Conservation Biology* **17**, 31 – 37.

Lodge D.M., Stein R.A., Brown K.N., Covich A.P., Broenmark C., Garvey J.E. and Klosiewski S.P. (1998). Predicting impact of freshwater species on native biodiversity: Challenges in spatial scaling. *Australian Journal of Ecology* **23**, 53 – 67.

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

Lutz C.G. and Wolters W.R. (1986). The effect of five stocking densities on growth and yield of red swamp crayfish *Procambarus clarkii. Journal of the World Aquaculture Society* **17**, 33 – 36.

MacIsaac H.J., Grigorovich I.A., and Ricciardi A. (2001). Reassessment of species invasion concepts: the Great Lakes basin as a model. *Biological Invasions* **3**, 405 – 416.

Mack R.N., Simberloff D., Lonsdale W.M., Evans H., Clout M., and Bazzaz F.A. (2000). Biotic invasions: causes, epidemiology, global consequences, and control. *Ecological Applications* **10**, 689 – 710.

Marco D. E., Páez S.A. and Cannas S.A. (2002). Species invasiveness in biological invasions: a modeling approach. *Biological Invasions* **4**, 193 – 205.

Mazlum Y. (2007). Influence of visible implant fluorescent elastomer (VIE) tagging on growth, molting and survival of the eastern white river crayfish, *Procambarus acutus acutus* (Girard, 1852). *Turkish Journal of Zoology* **31**, 209 – 212.

McClain W.R., Romaire R.P., Lutz G.C. and Shirley G.M. (2006). *Louisiana Crawfish Production Manual*, LSU AgCenter Research & Extension. Pub 2637. 58 pp.

McClain W.R., Neil W.H. and Gatlin III D.M. (1992). Partitioning the contributions of forage-based production system components to weight gain of juvenile crayfish (*Procambarus clarkii*). *Aquaculture* **101**, 267 – 281.

McKinney M. (2005). New Pangea: homogenizing the future biosphere. *Proceedings of the California Academy of Sciences* **56**(11), 119 – 129.

Médail F. and Quézel P. (1999). Biodiversity hotspots in the Mediterranean basin: setting global conservation priorities. *Conservation Biology* **13**, 1510 – 1513.

Mkoji G.M., Hofkin B.V., Kuris A.M., Stewart-Oaten A., Mungai B.N., Kihara J.H., Mungai F., Yundu J., Mbui J., Rashid J.R., Kariuki C.H., Ouma J.H., Koech D.K. and Loker E.S.

(1999)a. Impact of the crayfish *Procambarus clarkii* on Schistosoma haematobium transmission in Kenya. *American Journal of Tropical Medicine and Hygiene* **61**(5), 751 – 759.

Mkoji G.M., Boyce T.G., Mungai B.N., Copeland R.S., Hofkin B.V. and Lokeret E.S. (1999)b. Predation of aquatic stages of *Anopheles gambiae* by the Louisiana red swamp crayfish (*Procambarus clarkii*). *Journal of the American Mosquito Control Association* **60**(1), 22 – 27.

Mkoji G.M., Hofkin B.V., Mungai B.N., Kihara J.H., Mungai F.K., Ouma, J.H., Koech, D.K. and Loker E.S. (1992). Control of natural, populations of Schistosome-transmitting snails by crayfish, *Procambarus clarkii* in temporary man-made ponds in Kenya. *American Journal of Tropical Medicine and Hygiene* 264 – 271.

Mommot W.T. (1984). Crayfish production: a reflection of community energetics. *Journal of Crustacean Biology* **4**, 35 – 54.

Mooney H.A. and Cleland E.E. (2001). The evolutionary impact of invasive species. *Proceedings of the National Academy of Sciences of the United States of America* **98**(10), 5446 – 5451.

Murdoch W.W., Avery A. and Smyth M.B. (1975). Switching in predatory fish. *Ecology* **56**(5), 1094 – 1105.

Nilsson P.A. (2001). Predator behaviour and prey density: evaluating density-dependent intraspecific interactions on predator functional responses. *Journal of Animal Ecology* **70**: 14 – 19.

Nyström P. (1994). Survival of juvenile signal crayfish (*Pacifastacus leniusculus*) in relation to light intensity and density. *Nordic Journal of Freshwater Research* **69**, 162 – 166.

Paglianti A. and Gherardi F. (2004). Combined effects of temperature and diet on growth and survival of YOY crayfish: a comparison between indigenous and invasive species. *Journal of Crustacean Biology* **24**, 140 – 148.

Palomino-Bean S., Vilcarromero A.C.S. and Bonato O. (2006). Co-existência de espécies em sistemas presa-predador com switching. *Tendências em Matemática Aplicada e Computacional* **7**(2), 317 – 326.

Papaj D.R. and Prokopy R.J. (1989). Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Review of Entomology* **34**, 315 – 350.

Penn G.H. (1943). A study of the life history of the luisiana red-crayfish, *Cambarus clarkii* Girard. *Ecology* **24**(1), 1 – 18.

Pimentel D., McNair S., Janecka J., Wightman J., Simmonds C., O'Connell C., Wong E., Russel L., Zern J., Aquino T., and Tsomondo T. (2002). *Economic and environmental threats of alien plant, animal, and microbe invasions.* pp. 307–329 in D. Pimentel (ed). Biological invasions: economic and environmental costs of alien plant, animal, and microbe species. CRC Press, Boca Raton, Florida.

Ramos M.A. and Pereira T.M (1981). Um novo astacidae para a fauna portuguesa: *Procambarus clarkii* (Girard, 1952). *Boletim do Instituto Nacional de Investigação das Pescas* **6**, 37 – 47.

Rebelo R., Amaral P., Bernardes M., Oliveira J., Pinheiro P and Leitão D. (2010). *Xenopus laevis* (Daudin, 1802), a new exotic amphibian in Portugal. *Biological Invasions* **12**(10), 3383–3387.

Renai B. and Gherardi F. (2004). Predatory efficiency of crayfish: comparison between indigenous and non-indigenous species. *Biological Invasions* **6**, 89 – 99.

Ribeiro F.and Collares-Pereira M.J. (2010). Life history variability of non native centrarchids in regulated river systems in the lower Guadiana drainage (Southwest Iberian Peninsula). *Journal of Fish Biology* **76**, 522 - 537.

Ricciardi A., Neves R.J., and Rasmussen J.B. (1998). Impending extinctions of North American freshwater mussels (Unionida) following the zebra mussel (*Dreissena polymorpha*) invasion. *Journal of Animal Ecology* **67**, 613 – 619.

Roudez R.J., Glover T. and Weiss J.S. (2008). Learning in an invasive and a native predatory crab. *Biological Invasions* **10**, 1191 – 1196.

Rosenzweig M. (2001). The four questions: what does the introduction of exotic species do to diversity? *Evolutionary Ecology Research* **3**, 361 – 367.

Robinson C.A., Thom T.J. and Lucas M.C. (2000). Ranging behavior of a large freshwater invertebrate, the white-clawed crayfish *Astropotamobius pallipes*. *Freshwater Biology* **44**(3), 509 – 521.

Rodríguez G. and Suárez H. (2001). Anthropogenic dispersal of decapod crustaceans in aquatic environment. *Interciencia* **26**, 282 – 288.

Rosenzweig M. (2001). The four questions: what does the introduction of exotic species do to diversity? *Evolutionary Ecology Research* **3**, 361 – 367.

Sala O.E., Chapin F.S., Armesto J.J., Berlow E., Bloomfield J., Dirzo R., Huber-Sannwald E., Huenneke L., Jackson R., Kinzig A., Leemans R., Lodge D.M., Mooney H.A., Oesterheld M., Poff N.L., Sykes M.T., Walker B.H., Walker M. and Wall D.H. (2000). Biodiversity scenarios for the year 2100. *Science* **287**, 1770 – 1774.

Scalici M., Chiesa S., Scuderi S., Celauro D. and Gibertini G. (2009). Population structure and dynamics of *Procambarus clarkii* (Girard, 1852) in a Mediterranean brackish wetland (Central Italy). *Biological Invasions,* doi: 10.1007/s10530-009-9557-6.

Scalici M. and Gherardi F. (2007). Structure and dynamics of an invasive population of the red swamp crayfish (*Procambarus clarkii*) in a Mediterranean wetland. *Hydrobiologia* **583**, 309 – 319.

Siebeck U.E., Litherland L. and Wallis G.M. (2009). Shape learning and discrimination in reef fish. *Journal of Experimental Biology* **212**, 2113 – 2119.

Simon K.S. and Townsend C.R. (2003).Impacts of freshwater invaders at different levels of ecological organization with emphasis on salmonids and ecosystem consequences. *Freshwater Biology* **48**, 982 – 994.

Sommer T.R. and Goldman C.R. (1983). The crayfish *Procambarus clarkii* from California rice fields: ecology, problems and potential harvest. *Freshwater Crayfish* **5**, 418 – 428.

Sommer T.R. (1984). The biological response of the crayfish *Procambarus clarkii* to transplantation into California rice fields. *Aquaculture* **41**, 373 – 384.

Souty-Grosset C., Holdich D.M., Nöel P.Y., Reynolds J.D. and Haffner P. (2006). *Atlas of Crayfish in Europe*. Muséum National d'Histoire Naturelle, Collection Patrimoines Naturels, Paris, France.

Statzner B., Peltret O. and Tomanova S. (2003). Crayfish as geomorphic agents and ecosystem engineers: effect of a biomass gradient on baseflow and flood-induced transport of gravel and sand in experimental streams. *Freshwater Biology* **48**(1), 147 – 163.

Stigall A.L. (2010). Invasive Species and Biodiversity Crises: Testing the Link in the Late Devonian. *PLoS ONE* **5**(12), e15584. doi:10.1371/journal.pone.0015584

Thomas C.D., Cameron A., Green R.E, Bakkenes M., Beaumont L.J., Collingham Y. C., Erasmus B. F. N., de Siqueira M.F., Grainger A., Hannah L., Hughes L., Huntley B., van Jaarsveld A.S., Guy F. Midgley A.S., Miles L., Ortega-Huerta M.A., Peterson A.T, Phillips O.L. and Williams S.E. (2003). Extinction risk from climate change. *Nature* **427**, 145 – 148.

Thorp J.H. and Wineriter S.A. (1981). Stress and growth response of juvenile crayfish to rhythmic and arrhythmic temperature fluctuations. *Archives of Environmental Contamination Toxicology* **10**, 69 – 77.

Townsend C.R. (2008). *Ecological applications: toward a sustainable world*. Blackwell Publishing, Oxford, UK. 346pp.

Tricarico E., Bertocchi S., Brusconi S., Casalone E., Gherardi F., Giorgi G., Mastromei G. and Parisi G. (2008). Depuration of a cyanobacterial toxin from red swamp crayfish *Procambarus clarkii* and assessment of its food quality. *Aquaculture* **285**, 90 – 95.

Vitousek P.M., Mooney , Lubchenco J. and Melillo J.M. (1997). Human domination of Earth's ecosystems. *Science* **277**, 494 – 499.

Williamson M. and Fitter A. (1996). The varying success of invaders. *Ecology* **77**, 1666 – 1670.

Wilson A.B., Naish K.A. and Boulding E.G. (1999). Multiple dispersal strategies of the invasive quagga mussel (*Dreissena bugensis*) as revealed by microsatellite analysis. *Canadian Journal of Fisheries and Aquatic Sciences* **56**, 2248 – 2261.

Wizen G., Galil B.S., Shlagman A. and Gasith A. (2008). First record of red swamp crayfish, *Procambarus clarkii* (Girard, 1852) (Crustacea: Decapoda: Cambaridae) in Israel – too late to eradicate? *Aquatic Invasions* **3**, 181 – 185.

Wisenden B.D., Chivers D.P. and Smith R.J. (1997). Learned recognition of predation risk by Enallagma damselfly larvae (Odonata, Zygoptera) on the basis of chemical cues. *Journal of Chemical Ecology* **23**, 137 – 151.

ZALBA S.M., SONAGLIONI M. I. AND BELENGUER C. J. (2000). Using a habitat model to assess the risk of invasion by an exotic plant. *Biological Conservation* **93**, 203 – 208.

ZAR J.H. (1996). *Biostatistical Analysis*, 3rd ed. Prentice Hall, New Jersey, 718pp.

CHAPTER 2

Effects of density on growth and survival of juvenile red swamp crayfish, Procambarus clarkii (Girard), reared under laboratory conditions

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EFFECTS OF DENSITY ON GROWTH AND SURVIVAL OF JUVENILE RED SWAMP CRAYFISH, *PROCAMBARUS CLARKII* (GIRARD), REARED UNDER LABORATORY CONDITIONS

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Abstract

The aim of this study was to quantify the importance of population density on the growth and survival of juvenile red swamp crayfish, *Procambarus clarkii* (Girard), and to understand the effects of density on both the period between moults and the increment per moult at high densities without food limitation. A laboratory experiment was performed to determine growth and survival of juvenile crayfish at five densities (20, 40, 60, 80 and100 crayfish m^{-2}). Density clearly affected crayfish growth. The impact of density on final weight was higher than on total length increase, growth rate and final length (FL) (by decreasing order of effect). When comparing minimum and maximum densities, we observed that FL suffered a 34% reduction. Number of moults, mean intermoult period (IP) and survival were not significantly affected by density. Our results indicated that the IP and the percentage of increment per moult are affected by the size of crayfish before moult and we provide equations for these relations. Our findings are relevant both for crayfish aquaculture management and for the management of wild or harvested rice-field crayfish populations.

Keywords: crayfish; Procambarus clarkii; density; growth; survival; juvenile

Introduction

The red swamp crayfish (*Procambarus clarkii*, Girard 1852) was first reported in southern Portugal by Ramos and Pereira (1981) in the Guadiana River basin. Nowadays it has a wide distribution in the centre and south of the country, where it seems to be well adapted to reservoirs and wetlands such as rice fields (Correia 1993a,b; Ilhéu and Bernardo 1993a,b). The red swamp crayfish is an important pest in rice fields of California and Portugal (Sommer and Goldman 1983; Grigarick 1984; Sommer 1984; Correia 1993b; Anastácio and Marques 1997; Anastácio *et al.* 2000). Nevertheless, it is possible to increase the profits of rice-farmers by double cropping rice (*Oryza sativa*) and red swamp crayfish (Chien and Avault 1980). To exploit the red swamp crayfish in a sustainable manner and also to control their population it is essential to understand the ecophysiology of the juveniles.

Crayfish growth is affected by several variables such as water temperature, water quality, food availability, light intensity, photoperiod, crayfish length and density, among others (*e.g.* McClain *et al.* 1992; Nyström 1994; Gutiérrez-Yurrita and Del Olmo 2004; Paglianti and Gherardi 2004). Huner *et al.* (1974) reported the negative effect of crowding on growth in spite of food availability. Several outdoor studies showed that red swamp crayfish exhibit density-dependent growth (Huner *et al.* 1974; Lutz and Wolters 1986; Jarboe and Romaire 1995; McClain 1995a,b,c). However, in these studies the highest densities tested were rarely above 20 individuals m^{-2}. Thus, there is a lack of knowledge on the response of red swamp crayfish to population density under controlled laboratory conditions at higher densities. This knowledge is especially relevant for juvenile production facilities because it helps to understand the reactions of crayfish to overcrowding. Normal field densities average from 0.13 to 3.8 crayfish m^{-2} (Correia and Bandeira 2004). Romaire *et al.* (1978) reported field stunted populations for densities above 12 crayfish m^{-2} and some authors indicate stocking densities of approximately 0.13 to 0.6 crayfish m^{-2} for optimal crayfish production in ponds (de la Bretonne and Romaire 1990; McClain *et al.* 2006) and rice fields (Chien and Avault 1980).

Growth in crustaceans, including freshwater crayfish (Astacoidea), is confined by the exoskeleton. In order to increase in size, freshwater crayfish must moult (Hammond *et al.* 2006). Moulting is a growth process in crustaceans in which the old exoskeleton is shed, allowing stretching and increase in size of the newly secreted one (Bauer 2002). Some authors have reported that the reduced growth of crayfish under the effect of high densities and small size containers is due to increased intermoult periods and decreased increment per moult (Aiken and Waddy 1978 in Goyert and Avault 1979). However little is known about these effects on crayfish growth.

The aims of this study were to evaluate the importance of population density on growth and survival of juvenile red swamp crayfish, and to understand the effects of density on both the period between moults and the length increment between moults without food limitation.

Methods

A 129 days laboratory experiment was performed at an average (± Standard Deviation = SD) temperature of 19.8 ±1.99 ºC and a photoperiod of 12/12 (L/D) with non-limiting food. We used 25 aerated polyethylene reservoirs with a bottom area of 0.05 m^2 (22.5x22.5x10cm), filled with 2.5 l of dechlorinated tap water, replaced weekly. The calcium concentration during the experimental trial averaged 153±1.88 p.p.m, which is adequate for the survival of crayfish during the moult (*e.g.* Oliveira and Fabião 1998). Dissolved oxygen and pH were recorded every three days. The NO_3–N concentration in the water was evaluated every three weeks by the 4500-NO_3^- E. Cadmium Reduction Method (Clesceri *et al.* 1998).

Juvenile red swamp crayfish were caught on a rice drainage channel near Salvaterra de Magos (Portugal) using a dip net (1mm mesh size). Crayfish were fed carrots (Anastácio *et al.* 2005) and pelleted food (40% proteins, 2% fibbers, 5% fat, 10% ashes and 7% humidity) during a two week acclimatization period in a large container. The protein and lipid contents of this diet were adequate for crayfish requirements (Oliveira and Fabião 1998; Jover *et al*. 1999; Gutiérrez-Yurrita and Del Olmo 2004). After the acclimatization period, 75 juvenile red swamp crayfish with an average carapace length of 8.54±1.17 mm and a mean fresh weight of 0.037±0.006 g, were selected for the experiment and placed in the polyethylene reservoirs. The experimental design consisted of five treatment levels (densities) with five replicates each. Densities were: one, two, three, four and five crayfish per reservoir or the equivalent to 20, 40, 60, 80 and 100 crayfish m^{-2}, respectively. Each crayfish was marked with a different colour, using nail enamel (O'Neill *et al.* 1993), in order to unequivocally identify it. There were no statistically significant length differences between density levels at the beginning of the experiment (One-way Anova: F=1.596; $P>0.05$). Crayfish were fed daily *ad libitum* with carrots and pellet food on a 50:50 ratio. Remains and faeces were removed on a daily basis. *Exuviae* were always left in the aquariums after *ecdysis* so that crayfish could ingest them. Dead individuals were removed immediately from the system and replaced with new ones of similar size (± 1 mm) in order to maintain density levels constant. Replacement crayfishes were kept in 0.25 m^2 containers at a density higher than 100 ind m^{-2}. No shelters were provided so that we could take conclusions based solely on the effect of density. Every time a moult occurred and after the carapace became hard, we measured carapace length (length from the tip of rostrum to the posterior margin at the dorsal midline, hereafter referred simply as CL) to the nearest 0.01

mm using a digital calliper. The animal was marked again and released in the original reservoir as soon as possible to minimize disturbance. Juveniles were observed daily, during the 129 days trial, in order to register the occurrence of moults, number of days between each moult (intermoult period – IP), growth and mortality. The first record of IP was not included in the statistical analysis. There were no significant differences between males and females for any of the variables and therefore data from both sexes were pooled and analyzed together. Growth rates were expressed as the increase in crayfish length (Carmona-Osalde *et al.* 2004a,b; Mazlum and Eversole 2005).

We calculated survival, total length increase (TLI), growth rate (GR), increment per moult (IM) and percentage of increment per moult (%IM) as follows:

*Survival (%) = 100 * ((initial number of crayfish – number of deaths)/initial number of crayfish)*

Total Length Increase (mm) = (final length – initial length)

Growth Rate (mm day^{-1}*) = ((final length – initial length)/time)*

Increment per Moult (mm) = length after moult – length before moult

*% of Increment per Moult = 100 * ((length after moult – length before moult)/length before moult)*

All calculations of length increase were based on CL. Final length (FL) was determined by converting CL at the end of the experimental trial to total length (length from the tip of rostrum to the end of telson) using the following equation (Adão 1991):

*Total length (mm) = 0.310912 + 1.89845 * CL (mm)* (r=0.98; *P*<0.001)

We determined fresh weight from carapace length (in mm) using the following equation (Paglianti and Gherardi 2004):

*Fresh weight (g) = (112.7 * e* $^{(-0.13 * CL)}$*)/1000*

This fresh weight was only used for comparison with results from other authors.

Several functional relations were estimated by adjusting a non-linear regression line to the data using the Quasi-Newton estimation method. In the case of the final length a power non-linear regression was adjusted. For growth rate and % of increment per moult the equations were modified from Edwards *et al.* (1989). In the case of IP the equation was modified from Brewer (1994).

Statistical analysis was performed with SPSS version 12. Survival results were compared applying a G-test for goodness-of-fit. Estimated FL and final fresh weight (FW) results were analysed using a ONE-WAY ANOVA and the remaining variables were analysed using a multivariate analysis of variance (MANOVA). A TWO-WAY ANOVA was performed in order to analyse the effects of density and carapace length (independent variables) on IM, %IM and IP. In this analysis data were divided into six CL classes of 4mm each, from <9.5mm to >25.5mm. The differences between groups were determined with the POST-HOC TUKEY TEST (Zar 1996). In order to meet ANOVA and MANOVA assumptions FW, IM, %IM and IP were submitted to a logarithmic transformation.

Results

During the experimental trial, dissolved oxygen averaged (±SD) 6.41±1.35 mg l^{-1} (65-75% of saturation), pH averaged 7.80±0.31 and NO_3–N averaged 2.05±0.26 mg l^{-1}. There were no statistical differences between treatments for dissolved oxygen (ONE-WAY ANOVA: F=0.854; df=4; $P>0.05$), pH (ONE-WAY ANOVA: F=1.021; df=4; $P>0.05$) and NO_3–N (ONE-WAY ANOVA: F=0.956; df=4; $P>0.05$).

At the end of the experiment the highest average estimated Final Length (FL), 58.37±5.13 mm, was obtained for the group with the lowest density, 20 ind m^{-2}, and the lowest FL was 38.69±2.11 mm at 80 ind m^{-2} (a 33.71% FL reduction). From 20 to 40 ind m^{-2} we observed a 20.78% reduction and from 20 ind m^{-2} to 100 ind m^{-2} the reduction in FL was 29.52%. The estimated final length decreased with the increase of density (Figure 1). ONE-WAY ANOVA showed significant differences in FL between density groups (F=5.246; df=4; $P<0.001$) and the post-hoc Tukey test showed significant differences in FL between the lowest densities, 20 and 40 ind m^{-2}, and 60, 80 and 100 ind m^{-2} (Figure 1).

Figure 1 shows individual values of FL and the following equation was fitted to the data:

$$\textit{Final Length (mm)} = 107.23 * \textit{Density}^{-0.2214}$$

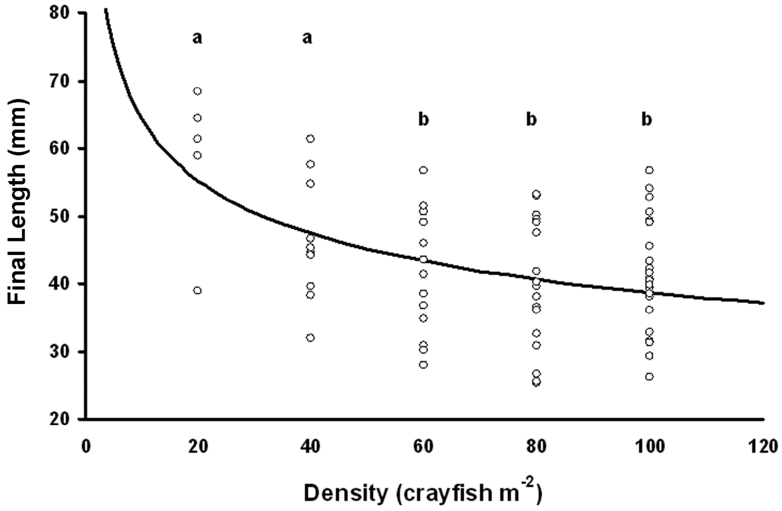

Figure 1. Individual values of final length in relation to density. An equation was fitted to the data resulting on the line shown on the figure. Final length is based on total length. Tukey's non-significantly different groups have the same superscript (P>0.05).

The r value obtained for this non-linear regression was 0.44 (n=74; p<0.001). The curve shows a rapid decrease of FL from 20 to 40 ind m^{-2} and a slow but constant decrease with densities higher than 40 ind m^{-2}.

The highest average estimated final fresh weight (FW) (7.23±1.72 g) was also obtained with the group at the lowest density, (20 ind m^{-2}) and the lowest FW (1.89±0.27 g) was at 80 ind m^{-2} which corresponds to a 73.86% reduction (Table 1). From 20 ind m^{-2} to 40 ind m^{-2} we observed a 56.29% FW reduction and from 20 to 100 ind m^{-2} the reduction in FW was 70.26%. The increase on density caused a decrease on estimated FW. A one-way Anova showed significant differences in estimated FW between groups (F=5.445; df=4; P<0.001) and the post-hoc Tukey test showed significant differences in FW between the lowest densities, (20 and 40 ind m^{-2}) and the highest densities (60 ind m^{-2}, 80 ind m^{-2} and 100 ind.m^{-2} (Table 1).

Significant differences were obtained for the three variables analyzed (Manova: Wilk's Lambda=0.624; F=2.906; df=1.12; P<0.001) and individual Anovas showed that density significantly affected TLI (F=5.906; df=1.4; P<0.001), GR (F=4.808; df=1.4; P<0.01) and NM (F=3.208; df=1.4; P<0.05). The post-hoc Tukey test showed differences between some groups. As density increased mean TLI declined and significant differences in TLI were found between the groups 20/40 ind m^{-2} and 60/80/100 ind m^{-2} (Figure 2A). The highest mean TLI was 27.17±5.20 mm and the minimum was 11.53±4.61 mm at 20 and 80 ind m^{-2} respectively, which means a 57.56% TLI reduction. For the average GR (Figure 3) we found a reduction from the lowest to highest density, with a maximum of 0.19±0.02 mm day^{-1} and a minimum of 0.12±0.01 mm day^{-1} at 20 and 100 ind m^{-2} respectively (a 36.84% GR reduction). Average NM (Figure 2B) decreased with density with a maximum of 7.8±1.09 moults and a minimum of 6.2±1.40

moults at 20 and 80 ind m^{-2}. NM decreased from the lowest density to 80 ind m^{-2}, but increased again at the highest density, 100 ind m^{-2}, and the relation between NM and density was not statistically significant.

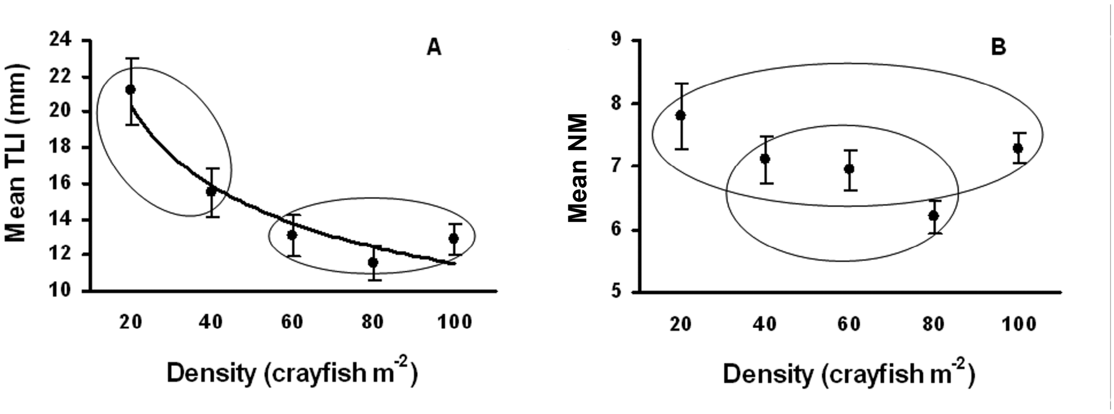

Figure 2. Mean values of: **A** – Total length increase (TLI), **B** – Number of moults (NM). Vertical bars represent standard error. Tukey's not significantly different groups are encircled ($P>0.05$). Values of TLI are based on measured carapace length.

Figure 3 shows individual values of GR plotted against density and the following equation was fitted to the data:

$$GR\ (mm/day) = 0.195 * Exp\ (-0.0455 * Density) + 0.1178$$

The r value obtained for this non-linear regression was 0.48 (n=74; $P<0.001$).

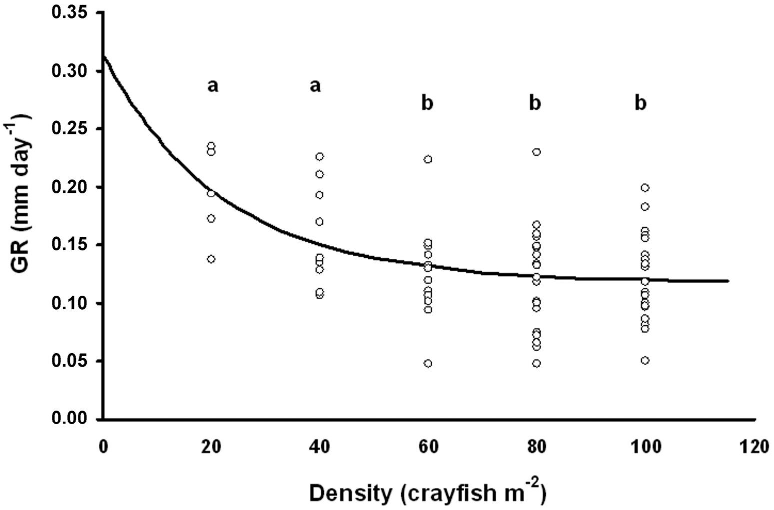

Figure 3. Individual values of growth rates (GR) in relation to density. An equation was fitted to the data resulting on the line shown on the figure. Tukey's non-significantly different groups have the same superscript ($P>0.05$).

Table 1 summarizes the mean individual values for increment per moult (IM), % of increment per moult (%IM), intermoult period (IP) and survival. Survival did not differ significantly between groups (G-TEST: G=7.314; df =4; n=75; *P*=0.120). Nevertheless, there was an apparent reduction in crayfish survival from the lowest to the highest density, except for the density of 60 ind m^{-2} which presented the lowest value (26.67%) (Table 1). The highest survival was at 20 ind m^{-2} (80%) and at this density only one individual died. This was probably due to an unsuccessful moult. The majority of the deaths were due to cannibalism and many individuals were found with chelipeds missing prior to death. At least 24.4% of the mortality occurred during or on the 3 days after moulting and 46.7% of the dead crayfish were eaten.

Table 1. Mean values for individual final fresh weight (FW), increment per moult (IM), percentage of increment per moult (%IM), intermoult period (IP) and survival at the end of the experiment. IM and %IM results are based on measured carapace length. Tukey's not significantly different groups have the same superscript (*P*>0.05). IM and %IM results are based on measured Carapace Length. Tukey's not significantly different groups have the same superscript (*P*>0.05). SE, standard error. D, number of dead individuals at the end of the experiment.

Density		**FW (g)**		**IM (mm)**		**%IM**		**IP (days)**		**Survival**	
Individuals m^{-2}	**Ind. per container**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**	**Mean**	**SE**		
20	**1**	7.23^{a}	1.72	2.71^{a}	0.18	15.53	1.09	13.33	0.87	80	1
40	**2**	3.16^{a}	0.66	2.19^{b}	0.11	13.95	0.79	13.46	0.74	50	5
60	**3**	2.20^{b}	0.35	1.77^{c}	0.07	12.74	0.56	15.52	0.67	27	11
80	**4**	1.89^{b}	0.27	1.84^{c}	0.08	14.12	0.69	15.79	0.75	50	10
100	**5**	2.15^{b}	0.25	1.74^{c}	0.06	13.06	0.51	15.02	0.54	28	18

Average IM and %IM decreased with density from 20 to 60 ind m^{-2}, increased at 80 ind m^{-2} and decreased at 100 ind m^{-2} (Table 1). Mean IP increased from the lowest density to 80 ind m^{-2}, and decreased slightly at 100 ind m^{-2} (Table 1). The total average IP was 14.96±0.32 days. A TWO-WAY MANOVA (on the effect of density and carapace length at the moment of moult over IM, % IM and IP) showed that these factors had some effect over the variables (Table 2). ANOVA showed that density significantly affected IM and %IM but not IP and that CL at the moment of moult affected significantly IM, %IM and IP (Table 2). No significant effects were found regarding the interaction of the two factors on the three dependent variables (Table 2). A Tukey post-hoc test showed differences between density 20 ind m^{-2} and 40, 60, 80 and 100 ind m^{-2} ($p<0.001$) and between density 40 and 100 ($p<0.05$) for the effect of density on IM but did not show any differences between density groups for %IM and IP.

Regarding the effect of CL at the moment of moult, the post-hoc Tukey test showed differences between class <9.5 mm and 25.5 mm and >25.5 mm ($p<0.05$), 13.5 mm and 21.5, 25.5 mm and <25.5 mm ($p<0.001$) and between class 17.5 mm and 25.5 mm and >25.5 mm ($p<0.01$) for IM, %IM (Figure 4) and IP (Figure 5). In what concerns %IM and IP the differences were found essentially between the lowest density and the densities above, in the case of the factor *density*, and between the lowest classes of carapace length (9.5 and 13.5 mm) and classes above (>17.5 mm) in the case of the *carapace length*.

Table 2. Statistical tests (TWO-WAY MANOVA and TWO-WAY ANOVA’S) for the effects of density and carapace length (CL) at the moment of moult over the increment per moult (IM), % increment per moult (%IM) and intermoult period (IP). Bold characters indicate statistical significance. α= 0.05. MS – mean squares.

	Effects	**Wilk’s Lambda**	**df**	**F**	***P***
MANOVA	**Density**	0.924	12	2.271	**0.008**
	CL	0.100	15	81.699	**<0.001**
	Density vs CL	0.880	54	0.824	0.813
ANOVA	**Density vs IM**		4	3.34	**0.011**
	Density vs %IM		4	2.69	**0.031**
	Density vs IP		4	2.05	0.087
	CL vs IM		5	3.54	**<0.001**
	CL vs %IM		5	4.87	**<0.001**
	CL vs IP		5	3.54	**0.004**

Figure 4 shows %IM plotted against carapace length at the moment of moult and the following equation was fitted to the data:

$$\%IM = 45 * Exp\,(-0.1728 * CL) + 8.734$$

The r value obtained for this non-linear regression was 0.48 ($n=483$; $P<0.001$).

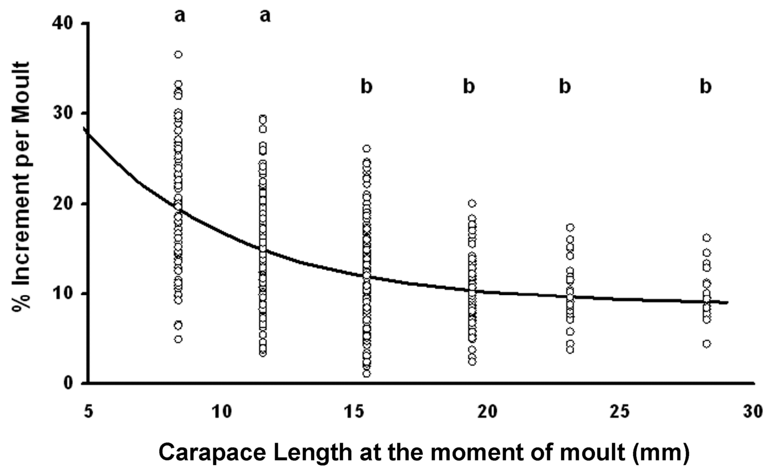

Figure 4. Individual values of percentage of increment per moult in relation to carapace length at the moment of moult. An equation was fitted to the data resulting on the line shown on the figure. Tukey's non-significantly different groups have the same superscript ($P>0.05$).

Figure 5 shows IP plotted against carapace length at the moment of moult and the following equation was fitted to the data:

$$IP\ (days) = 14.736/(1 + e^{(3.281 - 0.357 * CL)}) + 2.243$$

The r value obtained for this non-linear regression was 0.36 (n=373; $P<0.001$).

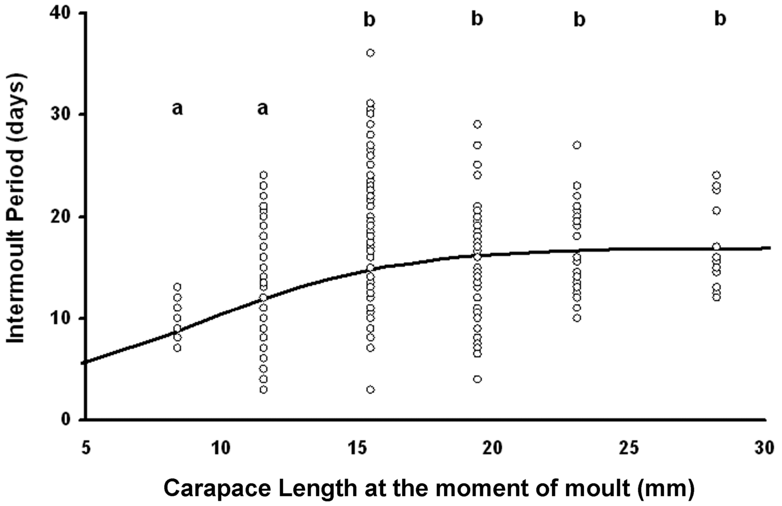

Figure 5. Individual values of intermoult period in relation to carapace length at the moment of moult. An equation was fitted to the data resulting on the line shown on the figure. Tukey's non-significantly different groups have the same superscript ($P>0.05$).

Discussion

Density had a profound effect on crayfish growth. With the exception of survival and intermoult period all the variables analysed were significantly affected by density. As density increased mean estimated final length, estimated final weight, total length increase and growth rate decreased. Growth suffered a reduction ranging from about 34%, in the case of estimated final length, to 74% in the case of the calculated final weight. We can say that the impact of stocking density is likely to be higher on estimated final weight than it is on total length increase, growth rate and estimated final length (by decreasing order of effect). Some authors (Lutz and Wolters 1986) reported a similar reduction on mean final length (31.7%) when the density was increased from 1 to 16 ind m^{-2} in a field study. Clark *et al.* (1975) reported a reduction of 14.3% on crayfish mean final length when density doubled from 3.1 to 6.2 ind m^{-2} and McClain (1995b) reported a 62% reduction of final weight when density increased from 2 to 18 ind m^{-2}. However, these references have reduced comparability since in the present study we used very small juveniles (0.037g) to cover an early stage of crayfish growth before maturity was achieved. In the studies mentioned above the initial weights ranged from 0.1g to 0.23g (Clark *et al.* 1975 and Lutz and Wolters 1986, respectively). The final weights attained at the lower densities were as higher as 21g and 20.7g in Clark *et al.* (1975) and Lutz and Wolters (1986) respectively indicating that in these studies the period of growth studied covered a period of crayfish maturity. Avault *et al.* (1975) suggested overpopulation as a cause of stunting (growth cessation at a small market size) and these findings are corroborated by others for red swamp crayfish (*e.g.* Romaire *et al.* 1978; McClain 1995a,b,c). Other crayfish species, such as *Cherax quadricarinatus* (Barki and Karplus 2000; Jones and Ruscoe 2000; Rodgers *et al.* 2006), *Cherax destructor* (Mills and McCloud 1983; Naranjo-Páramo *et al.* 2004), *Pacifastacus leniusculus* (Nyström 1994; Savolainen *et al.* 2004), *Procambarus llamasi* (Carmona-Osalde *et al.* 2004a) also exhibit density-dependent growth. In fact for *C. destructor* some authors reported a 28.5% reduction in growth when density was increased from 10 to 20 ind m^{-2} and 33% when it was increased from 10 to 30 ind m^{-2} (Mills and McCloud 1983). With *C. quadricarinatus* Jones and Ruscoe (2000) reported a reduction of 37.6% in growth when density was increased from 3 to 15 ind m^{-2}, and growth rate was also significantly affected by density. These findings, supported by the results obtained in the present study, demonstrate that density clearly affects crayfish growth. However, above a certain density the effect seems to loose strength or to stabilize, and in the present study this occurred above 40 crayfish m^{-2}. The lack of significant differences between densities higher than 40 ind m^{-2} in mean final length, final weight, growth rate and total length increase suggests that this density sets a limit. Nevertheless, it is possible that at even much higher densities new curve behaviours may arise driven by factors such as *e.g.* pure space limitation.

The decline in growth with increasing stocking densities can be attributed to behavioural factors (increased social interactions and antagonism) food availability, territorial restrictions and possibly deteriorated water conditions due to increased organic wastes (Goyert and Avault 1979; Chien and Avault 1983; Jones and Ruscoe 2000; Savolainen *et al.* 2003; Karplus and Barki 2004). Social interactions may reduce growth (Karplus and Barki 2004), and this can be explained because individuals probably spend more energy in agonistic behaviors (Gutiérrez-Yurrita *et al.* 1998; Gutiérrez-Yurrita and Montes 2001). Goyert and Avault (1978) in an experiment with crayfish stocked at 10 and 40 ind m^{-2} found higher growth and lower mortality at high densities when shelters were provided. The authors hypothesized that these results are caused by a shift from antagonistic to passive behaviours at higher densities, and this also occurs with the crayfish *Orconectes virilis* (Bovbjerg and Stephen 1975) and with lobsters (Squires 1970).

In this study food was supplied *ad libitum* and protein contents were considered enough to supply crayfish requirements (Oliveira and Fabião1998; Gutiérrez-Yurrita and Montes 2001; Gutiérrez-Yurrita and Del Olmo 2004) and decrease cannibalism. Moreover, water conditions were uniform for all treatments, pH and NO_3–N mean values were between acceptable values for freshwater crayfish (de la Bretonne and Romaire 1990; Camargo and Ward 1995; Jensen 1996; Oliveira and Fabião 1998; Camargo *et al.* 2005). We recorded the highest growth in the 20 ind m^{-2} treatment. In this treatment crayfish were deprived from social interaction, because each aquarium was occupied only by one crayfish. This reinforces the idea that behavioural factors were determinant for the decreased crayfish growth at high densities. Other crayfish species described as less aggressive, such as redclaw crayfish (*C. quadricarinatus*), also exhibit density dependent growth and survival (Geddes *et al.* 1993; Jones and Ruscoe 2000). Besides aggression, other non-aggressive interactions, *e.g.* chemical and visual signs, dominant status (Karplus and Barki 2004; Bergman and Moore 2005; Barki *et al.* 2006), may still involve significant expenditure of energy and interruption of feeding. However, no specific research has been made about the effects of behavioural interactions on crayfish growth.

The mean period between moults observed in this study (ca. 15 days) was in accordance with intermoult periods obtained by other authors, from 10 to 14 days for a median CL ranging from 20 to 30 mm (Huner *et al.* 1974; Huner and Avault 1976; Huner 1984) and 18 days for a median CL of 14.0 mm (Bauer 2002). Reduced growth among crayfish may be due to a longer intermoult period and/or smaller increments in growth per moult (Aiken and Waddy in Goyert and Avault 1979). In the present study the intermoult period wasn't significantly affected by density, but we observed an apparent increase in mean intermoult period from around 13 to 16 days when density was increased from 20 to 100 ind.m^{-2}. Nevertheless, both increment per moult and % of increment per moult were significantly affected by density. Mean intermoult period appears to be a direct non-linear function of body size (Thorp and Wineriter 1981) and some authors also found positive correlations between intermoult period and crayfish size (Bauer 2002). The results of our work revealed that both intermoult period and % of increment per moult are affected by the size of crayfish prior to moult. The lack of significant differences between treatments above 17.5 mm in carapace length demonstrates the fact that there is a stronger modification of these variables in the early stages of crayfish growth. Our results indicate that besides density, the increase of intermoult period and reduced growth with increase of crayfish size are important factors concerning the evaluation of overall crayfish growth and that it should be considered in similar studies. However further research is needed in this area.

Survival was not significantly affected by density, but there was a notorious decrease from 80 to 28% at 20 and 100 ind m^{-2} respectively. This high survival recorded for the lowest density treatment is in accordance with survival results (70 to 100%) obtained by other authors at densities varying from 1 to 20 ind m^{-2} (Huner *et al.* 1974; Lutz and Wolters 1986; McClain 1995b,c). Crayfish vulnerability during the moulting period (Thorp and Wineriter 1981; Gutiérrez-Yurrita and Montes 2001) becomes more relevant with juvenile crayfish which have a higher moulting frequency. During this period crayfish are more vulnerable to the strong intraspecific pressure to occupy a refuge. In fact, some authors observed that, under high-density production systems, *i.e.* conditions similar to our experiment, intermoult conspecifics that touched recent postmoult animals attempted to cannibalize them (O'Neill *et al.* 1993; O' Neill *et al.* 1995; Hartman and O'Neill 1999).

It is possible to maintain juvenile crayfish of both genders at densities of 40 ind m^{-2}, using proper food supply, with mortalities below 50% and reasonable mean final length and weight. If the goal is the production of marketable size crayfishes, higher densities will reduce survival, final length and weight but probably will increase crayfish yield per area. The usual solution is to furnish a proper food supply and to provide proper shelter to decrease density effects on these variables.

The conclusions of this study are relevant for the management of both crayfish production and wild crayfish populations. We provided a set of equations relating density to several growth variables. These equations are an important contribution for the available mathematical models of crayfish growth and population dynamics (Anastácio *et al.* 1999a,b,c; Nielsen *et al.* 1999). With these improved models, crayfish (or rice-crayfish) farmers may be able to optimise their production.

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References

Adão M.H.S.M. (1991). *Procambarus clarkii* (Girard, 1852) (Decapoda, Cambaridae) espécie exótica em Portugal. Considerações sobre a ecobiologia e aspectos da sua biologia populacional na barragem de Monte Novo (Alentejo, Portugal). Master Thesis, Ecology Department, University of Évora, 129 pp.

Anastácio P.M. and Marques J.C. (1997). Crayfish, *Procambarus clarkii*, effects on initial stages of rice growth in the lower Mondego River valley (Portugal). *Freshwater Crayfish* **11**, 608 – 617.

Anastácio P.M., Frias A.F. and Marques J.C. (1999)a. CRISP (crayfish and rice integrated system of production): 1. Modelling rice (*Oryza sativa*) growth and production. *Ecological Modelling* **123**, 17 – 28.

Anastácio P.M., Nielsen S. N., Frias A.F. and Marques J.C. (1999)b. CRISP (crayfish and rice integrated system of production): 4. Modelling water, algae and oxygen dynamics. *Ecological. Modelling* **123**, 29 – 40.

Anastácio P.M., Nielsen S.N. and Marques J.C. (1999)c. CRISP (crayfish and rice integrated system of production): 2. Modelling crayfish (*Procambarus clarkii*) population dynamics. *Ecological Modelling* **123**, 5 – 16.

Anastácio P.M., Frias A.F. and Marques J.C. (2000). Impact of crayfish densities on wet seeded rice and the inefficiency of a non-ionic surfactant as an Ecotechnological solution. *Ecological Engineering* **15**, 17 – 25.

Anastácio P.M., Parente V.S. and Correia A.M. (2005). Crayfish effects on seeds and seedlings: identification and quantification of damage. *Freshwater Biology* **50**, 697 – 704.

Avault J.W. Jr., de la Bretonne L.W. and Huner J.V. (1975). Two major problems in culturing crayfish in ponds: oxygen depletion and overcrowding. *Freshwater Crayfish* **2**, 138 – 144.

Barki A. and Karplus I. (2000). Crowding female red claw crayfish, *Cherax quadricarinatus*, under small tanks hatchery conditions: what is the limit? *Aquaculture* **181**, 235 – 240.

Barki A., Karplus I., Manor R., Parnes S., Aflalo E.D. and Sagi A. (2006). Growth of redclaw crayfish (*Cherax quadricarinatus*) in a three-dimensional compartments system: Does a neighbour matter? *Aquaculture* **252**, 348 – 355.

Bauer R.T. (2002). The ineffectiveness of grooming in prevention of body fouling in the red swamp crayfish, *Procambarus clarkii*. *Aquaculture* **208**, 39-49.

Bergman D.A. and Moore P.A. (2005). Prolonged exposure to social odours alters subsequent social interactions in crayfish (*Orconectes rusticus*). *Animal Behaviour* **70**, 311 – 318.

Brewer R. (1994). *The science of Ecology*. Saunders College Publishing, Ft. Worth. 773pp.

Bovbjerg R.V. and Stephen S.L. (1975). Behavioral changes with increased density in crayfish *Orconectes virilis*. *Freshwater Crayfish* **2**, 429 – 442.

Camargo J.A. and Ward J.V. (1995). Nitrate (NO_3–N) toxicity to aquatic life: a proposal of safe concentrations for two species of neartic freshwater invertebrates. *Chemosphere* **31**, 3211 – 3216.

Camargo J.A., Alonso A. and Salamanca A. (2005). Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. *Chemosphere* **58**, 1255 – 1267.

Carmona-Osalde C., Rodríguez-Serna M., Olvera-Novoa M.A. and Gutiérrez-Yurrita P.J. (2004)a. Effect of density and sex ratio on gonad development and spawning in the crayfish *Procambarus llamasi*. *Aquaculture* **236**, 331 – 339.

Carmona-Osalde C., Rodríguez-Serna M., Olvera-Novoa M.A. and Gutiérrez-Yurrita P.J. (2004)b. Gonadal development, spawning, growth and survival of the crayfish *Procambarus llamasi* at three different water temperatures. *Aquaculture* **232**, 305 – 316.

Chien Y.H. and Avault J.W., Jr. (1980). Production of crayfish in rice fields. *Progressive Fish-Culturist* **42**, 67 – 71.

Chien Y.H. and Avault J.W., Jr. (1983). Effects of flooding dates and type of disposal of rice straw on the initial survival and growth of caged juvenile crayfish, *Procambarus clarkii*, in ponds. *Aquaculture* **31**, 339 – 349.

Clark D.F., Avault, J.W. and Meyers S.P. (1975). Effects of feeding, fertilization and vegetation on production of red swamp crayfish, *Procambarus clarkii*. *Freshwater Crayfish* **2**, 125 – 138.

Clesceri L.S., Greenberg A.E. and Eaton A.D. (1998). *Standard methods for the examination of water and wastewater*. 20th Edition, United Book Press, Inc., Baltimore, Maryland.

Correia A.M. (1993)a. Length-weight relationships for two populations of red swamp crayfish, *Procambarus clarkii* (Decapoda, Cambaridae) from Portugal. *Freshwater Crayfish* **9**, 442 – 450.

Correia A.M. (1993)b. Situation de l'acclimatation de l'écrevisse rouge des marais *Procambarus clarkii* au Portugal. *L'Asticulteur de France* **35**, 2 – 9.

Correia A.M. and Bandeira N. (2004). Seasonal availability of *Procambarus clarkii* in the Tejo river basin, Portugal. *Freshwater Crayfish* **14**, 1 –7.

De La Bretonne L.W., Jr. and Romaire R.P. (1990). *Crayfish culture: site selection, pond construction and water quality*. Southern Regional Aquaculture Center **240**, 4 pp.

Edwards D., Hamson M. and Towers D.A. (1989). *Guide to mathematical modelling*. MacMillan, London, 277 pp.

Geddes M.C., Smallridge M. and Clark S. (1993). The effect of stocking density, food type and shelters on survival and growth of the Australian freshwater crayfish, *Cherax destructor*, in experimental ponds. *Freshwater Crayfish* **9**, 57 – 69.

Goyert J.C. and Avault J.W., Jr. (1978). Effects of stocking density and substrate on growth and survival of crayfish (*Procambarus clarkii*) grown in a recirculating culture system. *Proceedings of the 9th Annual Meeting of the World Mariculture Society*, 731 – 735.

Goyert J.C. and Avault J.W., Jr. (1979). Effects of container size on growth of crayfish (*Procambarus clarkii*) in a recirculating culture system. *Freshwater Crayfish* **4**, 278 – 286.

Grigarick A.A. (1984). General problems with rice invertebrate pests and their control in the United States. *Protection Ecology* **7**, 105 – 114.

Gutiérrez-Yurrita P.J. and Del Olmo C.M. (2004). Population dynamics of juveniles of red swamp crayfish (*Procambarus clarkii*) under controlled conditions. *Freshwater Crayfish* **14**, 180 – 189.

Gutiérrez-Yurrita P.J. and Montes C. (2001). Bioenergetics of juveniles of red swamp crayfish (*Procambarus clarkii*). *Comparative Biochemistry Physiology* **130A**, 29 – 38.

Gutiérrez-Yurrita P.J., Gorka S., Bravo-Ultrera M.Á., Baltanás Á. and Montes C. (1998). Diet of the red swamp crayfish (*Procambarus clarkii*) in natural ecosystems of the Doñana National Park temporary fresh-water marsh (Spain). *Journal of Crustacean Biology* **18**, 120 – 127.

Hammond K.S., Hollows J.W, Townsend C.R. and Lockman P.M. (2006). Effects of temperature and water calcium concentration on growth, survival and moulting of freshwater crayfish, *Paranephrops zealandicus*. *Aquaculture* **251**, 271 – 279.

Hartman A. and O'Neill D. (1999). Fish predators and conspecifics influence moult location choice by red swamp crayfish, *Procambarus clarkii* (Girard). *Freshwater Crayfish* **12**, 244 – 251.

Huner J.V. (1984). Growth responses of juvenile male crayfish, *Procambarus clarkii*, fed artificial diets supplemented with *Egeria densa*, a vascular aquatic plant. *Journal of the World Mariculture Society* **15**, 129 – 131.

Huner J.V. and Avault J.W., Jr. (1976). The moult cycle of subadult red swamp crayfish, *Procambarus clarkii* Girard. *Proceedings of the World Mariculture Society* **7**, 267 – 273.

Huner J.V., Meyers S.P. and Avault J.W., Jr. (1974). Response and growth of freshwater crayfish to an extruded, water diet. In: *Proceedings of The Second International Crayfish Symposium* (ed. by J.W. Avault, Jr.), pp. 149 – 157. Baton Rouge, Louisiana, U.S.A.

Ilhéu M. and Bernardo J.M. (1993)a. Experimental evaluation of food preference of red swamp crayfish, *Procambarus clarkii*, vegetal vs animal. *Freshwater Crayfish* **9**, 359 – 364.

Ilhéu M. and Bernardo J.M. (1993)b. Aspects of trophic ecology of red swamp crayfish (*Procambarus clarkii* Girard) in Alentejo, South of Portugal. In: *Actas VI Congresso Español de Limnologia* (ed. by L.C. Pizarro, R.M. Baquero, P.S. Castillo and P. Carrillo), Instituto de Ciencias de la Educación **13**, 417 – 423. University of Granada, Spain.

Jarboe H.H. and Romaire R.P. (1995). Effects of density reduction and supplemental feeding on stunted crayfish *Procambarus clarkii* populations in earthen ponds. *Journal of the World Aquaculture Society* **26**, 29 – 37.

Jensen F.B. (1996). Uptake, elimination and effects of nitrite and nitrate in freshwater crayfish (*Astacus astacus*). *Aquatic Toxicology* **34**, 95 – 104.

Jones C.M. and Ruscoe I.M. (2000). Assessment of stocking size and density in the production of redclaw crayfish, *Cherax quadricarinatus* (von Martens) (Decapoda: Parastacidae), cultured under earthen pond conditions. *Aquaculture* **189**, 63 – 71.

Jover M., Férnandez-Carmona J., Del Río M.C. and Soler M. (1999). Effect of feeding cooked-extruded diets, containing different levels of protein, lipid and carbohydrate on growth of red swamp crayfish *Procambarus clarkii*. *Aquaculture* **178**, 127–137

Karplus I. and Barki A (2004). Social control of growth in the redclaw crayfish, *Cherax quadricarinatus*: testing the sensory modalities involved. *Aquaculture* **242**, 321 – 333.

Lutz C.G. and Wolters W.R. (1986). The effect of five stocking densities on growth and yield of red swamp crayfish *Procambarus clarkii. Journal of the World Aquaculture Society* **17**, 33 – 36.

Mazlum Y. and Eversole A.G. (2005). Growth and survival of *Procambarus acutus acutus* (Girard, 1852) and *Procambarus clarkii* (Girard, 1852) in competitive settings. *Aquaculture Research*, **36**, 537 – 545.

McClain W.R., Romaire R.P., Lutz C.G. and Shirley M.G. (2006). *Louisiana crawfish production manual*. Louisiana State University Agricultural Center, Louisiana Crawfish Promotion & Research Board, Louisiana Cooperative Extension Service, Publication **2637**, 57 pp.

McClain W.R. (1995)a. Effects of population density and feeding rate on growth and feed consumption of red swamp crayfish *Procambarus clarkii*. *Journal of the World Aquaculture Society* **26**, 14 – 23.

McClain W.R. (1995)b. Growth of *Procambarus clarkii* as a function of density and food resources. *Journal of the World Aquaculture Society* **26**, 24 – 28.

McClain W.R. (1995)c. Investigations of crayfish density and supplemental feeding as influencing growth and production of *Procambarus clarkii*. *Freshwater Crayfish* **10**, 512 – 520.

McClain W.R., Neil W.H. and Gatlin III D.M. (1992). Partitioning the contributions of forage-based production system components to weight gain of juvenile crayfish (*Procambarus clarkii*). *Aquaculture* **101**, 267 – 281.

Mills B.J. and McCloud P.I. (1983). Effects of stocking and feeding rates on experimental pond production of the crayfish *Cherax destructor* (Clark) (Decapoda: Parastacidae). *Aquaculture* **34**, 51 – 72.

Naranjo-Páramo J., Hernadez-Llamas A. and Villarreal H. (2004). Effect of stocking density on growth, survival and yield of juvenile redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) in gravel-lined commercial nursery ponds. *Aquaculture* **242**, 197 – 206.

Nielsen S.N., Anastácio P.M., Frias A.F. and Marques J.C. (1999). CRISP (crayfish and rice integrated system of production): 5. Simulation of nitrogen dinamics. *Ecological Modelling* **123**, 41 – 52.

Nyström P. (1994). Survival of juvenile signal crayfish (*Pacifastacus leniusculus*) in relation to light intensity and density. *Nordic Journal of Freshwater Research* **69**, 162 – 166.

Oliveira J. and Fabião A. (1998). Growth responses of juvenile red swamp crayfish, *Procambarus clarkii* Girard, to several diets under controlled conditions. *Aquaculture Research* **29**, 123 – 129.

O'Neill D.J., French D.P., Rebach S. and Handwerker T.S. (1993). Chelae removal alters attainment of sexual maturity in male *Procambarus clarkii* (Girard) and mortality in groups. *Freshwater Crayfish* **9**, 38 – 49.

O'Neill D.J., Handwerker T. and Rebach S. (1995). Location of moulting and distribution of postmoult crayfish (*Procambarus clarkii*) in high-density culture. *Freshwater Crayfish* **8**, 635 – 647.

Paglianti A. and Gherardi F. (2004). Combined effects of temperature and diet on growth and survival of young-of-year crayfish: a comparison between indigenous and invasive species. *Journal of Crustacean Biology* **24**, 140 – 148.

Ramos M.A. and Pereira T.M.G. (1981). Um Novo Astacidae para a fauna Portuguesa: *Procambarus clarkii* (Girard, 1952). *Boletim do Instituto Nacional de Investigação e das Pescas* **6**, 37 – 47.

Rodgers L.J., Saoud, P.I. and Rouse, D.B. (2006). The effects of monosex culture and stocking density on survival, growth and yield of redclaw crayfish (*Cherax quadricarinatus*) in earthen ponds. *Aquaculture* **259**, 164–168.

Romaire R.P., Forester J.S. and Avault J.W., Jr., (1978). Growth and survival of stunted red swamp crayfish (*Procambarus clarkii*) in a feeding-stocking density experiment in pools. *Freshwater Crayfish* **4**, 331 – 336.

Savolainen R., Ruohonen K. and Railo E. (2004). Effect of stocking density on growth, survival and cheliped injuries of stage 2 juvenile signal crayfish *Pacifastacus leniusculus* Dana. *Aquaculture* **231**, 237 – 248.

Savolainen R., Ruohonen K. and Tulonen J. (2003). Effects of bottom substrate and presence of shelter in experimental tanks on growth and survival of signal crayfish, *Pacifastacus leniusculus* (Dana) juveniles. *Aquaculture Research* **34**, 289 – 297.

Sommer T.R. (1984). The biological response of the crayfish *Procambarus clarkii* to transplantation into California rice fields. *Aquaculture* **41**, 373 – 384.

SOMMER T.R. AND GOLDMAN C.R. (1983). The crayfish *Procambarus clarkii* from California rice fields: ecology, problems and potential harvest. *Freshwater Crayfish* **5**, 418 – 428.

SQUIRES H.J. (1970). Lobster (*Homarus americanus*) fishery and ecology in Port au Port Bay, Newfoundland, 1960-65. *Proceedings of the National Shellfisheries Association* **60**, 23 – 29.

THORP J.H. AND WINERITER S.A. (1981). Stress and growth response of juvenile crayfish to rhythmic and arrhythmic temperature fluctuations. *Archives of Environmental Contamination Toxicology* **10**, 69 – 77.

ZAR J.H. (1996). *Biostatistical Analysis*, 3rd ed. Prentice Hall, New Jersey, 718 pp.

CHAPTER 3

Crayfish learning abilities: how does familiarization period affects the capture rate of a new prey item?

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CRAYFISH LEARNING ABILITIES: HOW DOES FAMILIARIZATION PERIOD AFFECTS THE CAPTURE RATE OF A NEW PREY ITEM?

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Abstract

This study explores the effect of the length of learning period on capture rate of a previously unfamiliar prey by an invasive freshwater crayfish (*Procambarus clarkii*). Juvenile crayfish were subjected to different periods of contact (learning period) with a larvae prey (*Chaoborus* sp.). The length of the learning period significantly affected the number of prey consumed by the predator. Our results indicate that the naïve crayfish require less than 12 hours to learn to maximize capture rate of this prey. The learning coefficient, adopted in the present study, may be useful in exploring predation capabilities of alien species in newly invaded habitats.

Keywords: learning in crustaceans; Chaoborus sp. larvae; Procambarus clarkii; learning coefficient; biological invasions

Introduction

Learning abilities are fundamental for survival and, in any ecosystem, prey animals are required to learn to recognize certain predatory cues of potential predators. This has been demonstrated, for example, in fish (Karplus *et al.* 2006; Siebeck *et al.* 2009), damselflies (Chivers *et al.* 1996; Wisenden *et al.* 1997) and crayfish (Hazlett and Schoolmaster 1998). Invasive crayfish exhibit a high degree of plasticity in learning to reduce predation risk (Hazlett 2000), and some species (*e.g. Orconectes virilis* and *P. clarkii*) seem to learn to avoid predation quicker than native species that are not expanding their range (Gherardi *et al.* 2002; Hazlett *et al.* 2002). This characteristic constitutes an adaptive advantage when invading a new habitat (Acquistapace *et al.* 2003). Crustaceans are important predators in many ecosystems and their learning plasticity has been demonstrated both on crabs (Roudez *et al.* 2008) and invasive crayfish. In fact, invasive crayfish have the ability to readily switch to new prey items in contrast to native crayfish species (Gherardi *et al.* 2001). The plasticity of crayfish for learning to recognize new prey may provide important insights about the potential success of a species when invading new habitats.

The red swamp crayfish, (*Procambarus clarkii* Girard, 1852) (Crustacea: Decapoda), is a successful invader worldwide and its current distribution comprises all continents, except Australia and the Antarctic (Hobbs *et al.* 1989; Campos 2005; Gherardi 2006). Invasive species are in fact a global concern, sometimes raising health issues and frequently having irreversible consequences on natural ecosystems. Invaders can disrupt ecosystem processes by altering their structure and function, can reduce biological diversity of native species, and are one of the most important contributors of extinction (Sala *et al.* 2000; Williams 2001; Lodge and Shrader-Frechette 2003). Crayfish invasions may have a simultaneous impact on different trophic levels by interfering with species from distinct functional groups, such as plants, fishes and invertebrates (Cruz *et al.* 2006; Gherardi and Acquistapace 2007; Correia and Anastácio 2008). Plant material, particularly vegetal detritus and associated microbiota, is the major component of adult crayfish diet with aquatic macroinvertebrates representing a lower contribution to its level of dietary intake (Avault and Brunson 1990; Ilhéu *et al.* 2002). In contrast, juveniles are more carnivorous than adults, preying mostly on insect larvae with reduced or no swimming behaviour (Correia 2002; Correia 2003).

Chaoborus larvae, a natural prey item of crayfish are the aquatic life stage of the terrestrial phantom midge, a non-biting fly similar to a mosquito. They are important members of freshwater food webs worldwide and are a key primary carnivore in lakes ranging from the tropics to high temperate latitudes, being often the major invertebrate predators of zooplankton in lakes and ponds (Bezerra-Neto and Pinto-Coelho 2002). These larvae can cause shifts in the size structure of zooplankton communities and eliminate some prey species (Lencioni and

Wilhlem 2006; Sell 2006). *Chaoborus* species and *P. clarkii* are sympatric in both the crayfish's native habitat and invaded habitats (Borkent 1981; Berendonk 2002), therefore, *Chaoborus* may provide an appropriate test organism for observing learned predatory response in *P. clarkii*, a noted invader.

Learning is common in invertebrates, but is also likely to be frequent in invertebrate species with relatively simple nervous systems. There are several references in literature reporting evidence of learning in a variety of arthropod species (Papaj and Prokopy 1989; Dukas 2008; Ishii and Yamada 2010), however, the application of equations and mathematical modelling to the learning processes is not common. Previous studies on the crayfish learning related to predation activity report to the *search-image* formation and explored the learning of predation cues (as a prey) (Hazlett 2000; Renai and Gherardi 2004). The originality of the present study relies on the approach to the learning ability of crayfish as a naïve predator and the attempt to explain mathematically its learning curve. The objective of this study was therefore to (1) determine whether prior experience in individual *P. clarkii* influences the success rate of prey capture and (2) determine a mathematical expression of the learning ability of *P. clarkii* that could be used in future comparative studies. Crayfish are able to form a recognizable *search image* (Renai and Gherardi 2004) of a new prey item over time, as a result of either visual and/or chemical perceptual changes. This recognizable search image allows for a faster detection and an increased capacity to capture the prey over items encountered for the first time. Therefore we hypothesized that an increased time of contact by crayfish with a new prey (learning time) will significantly increase the subsequent prey capture rate. This hypothesis was tested under laboratory conditions using a *natural* predator-prey association but with completely *naïve* predatory individuals.

Methods

Ovigerous *Procambarus clarkii* females were collected from rice fields near Salvaterra de Magos, Portugal (39º2'N, 8º44'W). These females were reared individually in the laboratory and early detached juveniles were removed from their mothers and reared in tanks (0.25 m^2 floor area). *P. clarkii* juveniles were fed on a daily basis with carrots (Anastácio *et al.* 2005) and pelleted food (40% proteins, 2% fibres, 5% fat, 10% ashes and 7% water) and were kept under a photoperiod of 12/12 (Light/Dark). The protein and lipid contents of this diet met the nutrient requirements of crayfish (Jover *et al.* 1999). Forty juveniles, 18.80 mm (±1.80 s.d.) mean carapace length, were randomly selected for the experimental trial.

Forty five aquaria (22.5x22.5x10.0 cm), each with 3 L of dechlorinated tap water (6 cm of water depth) and covered with a wire cloth (1 mm mesh size) were stocked with crayfish and maintained at 18.0 ºC (±1.8 s.d.). A photoperiod of 24 hours of daylight was used, which would maximize the *time of contact between the predator and prey* (from now on simply referred as *learning period*). No shelters were provided. Experimental individuals were acclimated for 15 days under the feeding regime described above, and were starved for 24 hours prior the beginning of the experiment. The experimental design consisted of seven learning periods, in which individual crayfish were exposed to their prey (*Chaoborus* larvae) for the first time for a period of 72, 48, 24, 12, 3, 2 hours or 1 hour. Each treatment was replicated five times. Two controls with five replicates each were also used: one containing crayfish with no previous contact with the prey (0 hours of learning period) and another containing prey but no crayfish. The control with only larvae was important to determine if missing larvae at the end of the trials was due to crayfish consumption or to other external factors. At the beginning of the experiment, fifty *Chaoborus* sp. larvae (amtra®, premium live, AL-003), 3.5 mm (±1.5 s.d.) total length, were added to each aquarium. This number was determined from preliminary observations, under similar experimental conditions, that indicated a maximum number of captures and consumption of about 45 per crayfish in one hour. For the 72 and 48 h treatment all larvae were removed at the end of each 24 h period and replaced with 50 new larvae to maintain a more constant density of larvae. At the end of each learning period, remaining larvae were removed and, after a resting period of 3 h, 50 new larvae were added and the larvae consumed by individual crayfish during a 1 h test period were recorded. All treatments were initiated simultaneously and were randomly assigned to the tanks.

Statistical analysis was performed with SPSS version 14 (SPSS Inc. Chicago, Illinois, USA). An ANOVA was used to test for differences among the number of prey captured by crayfish subjected to each learning period. The post-hoc Tukey test (Zar 1996) was applied to determine which pairs of treatment levels differed ($\alpha=0.05$).

The non-linear Mitscherlich equation (Haefner 1996) (equation 1) was adjusted to the data using the Quasi-Newton method. This is a rectangular hyperbola in which the maximum is approached gradually and it was widely used both in nutrient dynamics and agriculture production modelling (Mitscherlich 1909, Harmsen *et al.* 2001; Nijland *et al.* 2008) and also in the study of fish feeding rates (Gosh *et al.* 1984) but wasn't used in past predation experiments.

(1) The Mitscherlich equation: $C = k(1 - e^{-bT})$

In this particular case, C is the number of consumed *Chaoborus larvae,* T is the learning period (hours), parameter k is the theoretical maximum number of *Chaoborus* larvae that crayfish can consume and b was considered a learning coefficient.

The time required by an individual crayfish to attain half of the theoretical maximum prey consumption (half of the learning period - T_{50}), was determined by solving the linear form of equation 1 (equation 2) in order to *T*, given that C equals 50% of the value of *k* (equation 3). The learning coefficient (*b*) could then be determined by equation 4 which is simply a rearranged form of equation 3.

(2) Linear form of equation 1: $ln(k - C) = ln(k) - bT$

(3) $T_{50} = - ln(0.5)/b$

(4) $b = ln(0.5/T_{50})$

Results

During both the learning period and the test period, crayfish remained relatively inactive prior to the introduction of the larvae in the aquaria. When larvae were placed into the aquaria with *P. clarkii*, the crayfish almost immediately started to actively search for the prey. Crayfish were observed raising their body, trying to get closer to the water surface where larvae stayed most of the time. Some crayfish adopted a sit-and-wait predation strategy, while others were often observed actively searching for the larvae. The capture process was very quick, taking only a few seconds for the crayfish to detect and capture the larvae, but often the phantom midge larvae reacted with a rapid response and were able to escape before consumption.

During the test period there was no mortality of the *Chaoborus* sp. larvae in control aquaria without crayfish and larvae did not metamorphose to the adult stage. The red swamp crayfish consumed 33.38 larvae (±2.48 s.e.), on average, during the 1 hour experimental period. Only three individuals (one from a 12 h, one from a 24 h and one from the 48 h learning period treatments) consumed all the larvae within the 1h test period. The highest average prey consumption (46.20 larvae/crayfish ±1.82 s.e.) was recorded for the 24 h treatment and the minimum average prey consumption (1.80 larvae/crayfish ±0.96 s.e.) occurred in the control or 0 h treatment (Table 1). ONE-WAY ANOVA showed that learning period significantly affected successful prey capture (F=25.902; df=7; $P<0.001$) and the post-hoc Tukey test showed no significant differences ($P<0.05$) between the control (0 h) and up to 2 h of learning period treatment (Figure 1). The post-hoc Tukey test showed significant differences between the shorter learning periods (0 to 3 h) and revealed no significant differences ($P>0.05$) between the learning treatments above 3 h (Figure 1). It is also worthy to note the lack of significant differences in larvae consumption between crayfish with no learning period (control) and those with a 1 h learning period (Figure 1).

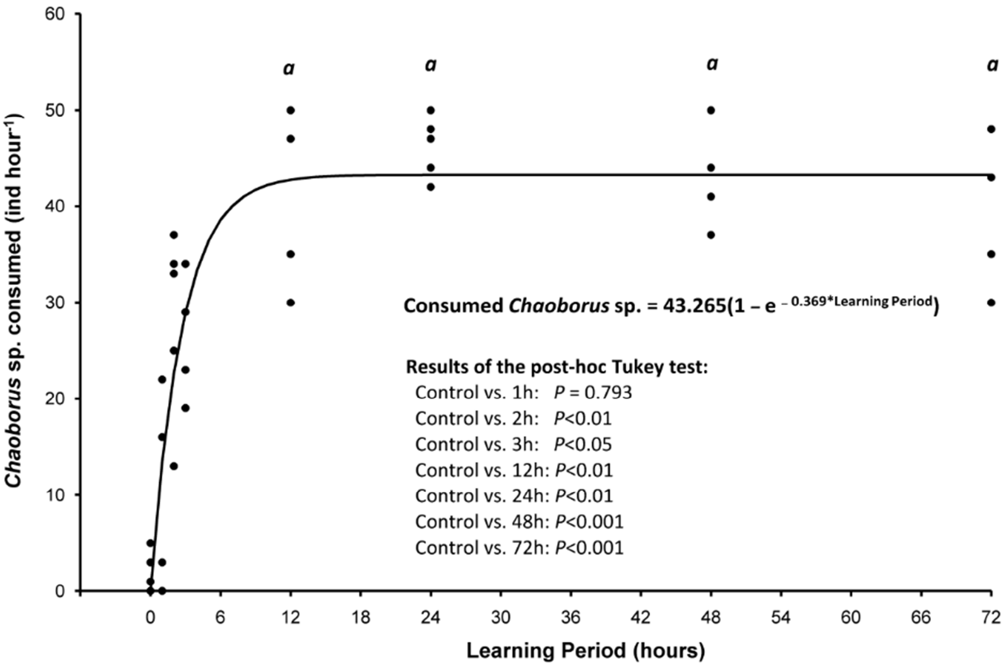

Figure 1. Number of *Chaoborus* sp. larvae consumed by crayfish submitted to different learning period treatments. The line represents the equation that was fitted to the data. Results refer to one hour of prey consumption 3 hours after crayfish were submitted to seven different learning periods (1, 2 3, 12, 24, 48 and 72 hours in contact with *Chaoborus* prey). Control: crayfish without previous contact with *Chaoborus* prey, *i.e.* 0 hours of learning. Results of the Tukey post-hoc multiple comparisons test for the control treatment group are presented. a) no significantly ($P<0.05$) different groups. Other non-significantly different groups are learning period 2h vs. 3h and 2h vs. 12h.

Figure 1 shows consumption data for each treatment level together with the parameterized equation (1) for the fitted curve. This curve represents an r^2 value of 0.82 (n=39; $P<0.001$) and the theoretical maximum prey consumption (k = 43.265) was reached at the 48 h learning period treatment, although the observed maximum prey consumption was reached at the 12 h of learning period treatment (Figure 1). The number of successful prey captured per hour increased rapidly from 1 to 12 hours of learning period and the learning coefficient (b) was 0.369. During the experimental trial, crayfish from 12, 24, 48 and 72 hours of learning period treatments consumed, on average, 42.20 larvae (±1.92 s.e.) within the 1 h test period. This represents an average consumption rate of 0.72 larvae per minute (±0.03 s.e.), with a maximum of 0.83 larvae min-1 and a minimum of 0.50 larvae min-1. The learning time required for crayfish to attain half of the theoretical maximum prey consumption (T50) was 1.9 hours.

Discussion

This study demonstrates that red swamp crayfish need a very short period (less than 12 hours) to learn to identify a new food item and maximize their efficiency at capturing and utilizing a new and previously not encountered prey item. When dealing with spatial and temporal variability of prey species, predators may optimize their capture rates if they are flexible and have behavioural plasticity (Stephens *et al.* 2007 *in* Ishii and Yamada 2010). The learning plasticity and memory skills of *P. clarkii* have been reported as an adaptive advantage of this invasive crayfish species when invading a new habitat (Gherardi *et al.* 2002; Hazlett *et al.* 2003). We consider that our findings reinforce the idea that *P. clarkii* is capable of readily utilizing new and unknown prey items (Renai and Gherardi 2004) which may partially account for the worldwide success of this invader.

In our experiments crayfish quickly identified *Chaoborus* sp. as a potential prey and started to actively search for it. This behaviour was similar to that observed in other crustaceans (Collins 1998) and in fact, the effects of *Procambarus clarkii* over soft bodied metazoan (such as *Chaoborus* sp. larvae) communities were demonstrated in previous studies (Mikoji *et al.* 1999; Correia *et al.* 2008). In our study, *P. clarkii* soon combined the active search of prey with a sit-and-wait strategy such as described during predation of amphibian larvae (Gherardi. *et al.* 2001). Feeding behaviour in crustaceans is triggered by chemical, mechanical and visual prey cues (Derby *et al.* 2001). The quick predatory response of *P. clarkii* to the new prey item in this experiment may be explained by the visual (Tuthill and Johnsen 2006; Correia *et al.* 2007) and/or mechanical response to the *Chaoborus* larvae movements (Renai and Gherardi 2004), or to a chemical cue given off by the larvae (Hazlett 1994; Hazlett 2000). Although other mechanisms or cues may have been involved in crayfish food search response, it is possible that the quick response of crayfish towards the new prey was mostly due to chemical cues. Crustaceans are likely to respond to the strongest stimuli received from the prey (Hughes and Seed 1995) and it is probable that the water containing the prey used in the experimental trials had high concentration of *Chaoborus* olfactory cues. It is important to notice that experimental crayfish had no previous contact with living macroscopic prey. For this reason, one of the hypotheses to explain the prompt predatory response by crayfish may be the fact that *P. clarkii* and *Chaoborus* sp. are sympatric species and such associations are already imprinted as a result of co-evolution. This could indicate that *P. clarkii* is not *genetically* naïve to *Chaoborus* larvae, but further studies should be addressed to clarify this question.

Since the predatory response of *P. clarkii* to the new prey item was almost instantaneous, the differences in the amount captured during the test period could be due to differences in learned attack and/or prey handling time before consumption. The experience gained by a predator in approaching its prey can be decisive on whether that predator will proceed or abort an attack and whether that attack will be successful (Mascaró and Seed 2001). An increase in attack efficiency or effectiveness has been suggested to be related to the length of the learning period in arthropods (Lawton *et al.* 1974). The results of the present study indicate that 1 hour learning period is not enough for crayfish to significantly improve capture success, but in contrast, a 2 h learning period produced a significant association (Figure 1) with increased consumption of prey by naïve *P. clarkii*. These results are in accordance with Hazlett *et al.* (2002), which concluded that two hours of training produced significant evidence of memory of the learned association, when tested just one day later. *Procambarus clarkii* was also reported as being able to retain new learned associations for up to 3 weeks after 24 h periods of training (Hazlett *et al.* 2002). Similar retention time for food associations (up to three weeks) have been reported for other crayfish species such as *Orconectes virilis*, *O. rusticus* and *Cambarus rubustus* (Hazlett 1994), whereas lobsters (*Homarus americanus*) remembered social interactions for at least two weeks (Karavanich and Atema 1998). Covich *et al.* (1981) also reported on the benefits of a familiarization process with new prey (*Corbicula*) by crayfish. In the present study, crayfish apparently remembered the learned association for at least three hours (the resting period) but no further efforts were made to determine crayfish duration of memory retention.

Prey behaviour, size and mobility seem to determine the impact crayfish can have on prey populations (Stenroth and Nyström 2003). For instance, active macroinvertebrate predators and sediment-living prey may be more difficult to capture for crayfish than large slow-moving herbivores (Nyström *et al*. 1999; Stenroth *et al.* 2003). This appears to be the case with *Chaoborus* larvae, which are active zooplankton predators and an important food item for fish (Bezerra-Neto and Pinto-Coelho 2002; Lencioni and Wilhlem 2006; Garcia and Mittelbach 2008). *P. clarkii* had some difficulty capturing them in the beginning of this study, but soon learned effective strategies for doing so. Nevertheless, it is important to point out that laboratory conditions cannot completely reflect the complex interactions between predators and predatory behavior of *P. clarkii* in the wild. The size of the experimental container may change predator-prey interactions (McCarthy and Dickey 2002) although Fernandes *et al.* (1999) argues that these interactions are not necessarily affected. Under field conditions, the effects of crayfish over their prey may be substantially different due to the existence of a large number of inter- and intra-specific interactions and also due to environmental complexities caused by factors such as water turbidity, presence of substrate, vegetation and/or refuges (Anastácio *et al.* 2005; Correia *et al.* 2005).

This experiment did not attempt to uncover the underlying mechanisms for the ability of crayfish to learn. Questions remain as to whether the learning period affected the crayfish's success rate of capture or finding the prey, or even their eagerness to look for prey. Further research should be addressed to clarify these emergent questions. New experimental studies are required in order to fully evaluate the crayfish's learning ability for predation under different environmental conditions and influences of learning period required for successful predation on other new prey items. We propose that the learning coefficient, as used in this study, is a good indicator for assessing the ability of a predator to learn to utilize new prey items in its diet. This coefficient should be useful for future comparisons between the learning abilities of predators and the interaction with different types of prey (sympatric and allopatric), especially when dealing with invasive species.

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References

Acquistapace P., Hazlett B.A. and Gherardi F. (2003). Unsuccessful predation and learning of predator cues by crayfish. *Journal of Crustacean Biology* **23**, 364 – 370.

Anastácio P.M., Parente V.S. and Correia A.M. (2005). Crayfish effects on seeds and seedlings: identification and quantification of damage. *Freshwater Biology* **50**, 697 – 704.

Avault J.W. and Brunson M.W. (1990). Crawfish forage and feeding systems. *Reviews in Aquatic Science* **3**, 1 – 10.

Berendonk T.U. (2002). Distribution and ecology of phantom midge larvae in Southern France and Italy (Diptera: Chaoboridae). *Studia Dipterologica* **9**, 1 – 4.

Bezerra-Neto J.F. and Pinto-Coelho R.M. (2002). The influence of the *Chaoborus brasiliensis* (Theobald, 1901) (Diptera, Chaoboridae) larvae in the zooplankton vertical distribution at Nado Lagoon, Belo Horizonte, state of Minas Gerais, Brazil. *Acta Scientiarum - Biological Sciences* **24**, 337 – 344.

Borkent A. (1981). The distribution and habitat preferences of the Chaoboridae (Culicomorpha: Diptera) of the Holarctic Region. *Canadian Journal of Zoology* **59**, 122 – 133.

Campos M.R. (2005). *Procambarus (Scapulicambarus) clarkii* (Girard, 1852), (Crustacea: Decapoda: Cambaridae). Una langostilla no nativa en Colombia. *Academia Colombiana de Ciencias exactas Físicas y Naturales* **111**, 295 – 302.

Chivers D.P., Wisenden B.D. and Smith R.J. (1996). Damselfly larvae learn to recognize predators from chemical cues in the predator's diet. *Animal Behaviour* **52**, 315 – 320.

Collins H.P. (1998). Laboratory evaluation of the freshwater prawn, *Macrobrachium borellii*, as a predator of mosquito larvae. *Aquatic Sciences* **60**, 22 – 27.

Correia A.M (2002). Niche breadth and trophic diversity: feeding behaviour of the red swamp crayfish (*Procambarus clarkii*) towards environmental availability of aquatic macroinvertebrates in a rice field (Portugal). *Acta Oecologica* **23**, 421 – 429.

Correia A.M. (2003). Food choice by the introduced crayfish *Procambarus clarkii*. *Annales Zoologici Fennici* **40**, 517 – 528.

Correia A.M., Bandeira N. and Anastácio P.M. (2005). Predator-prey interactions of *Procambarus clarkii* with aquatic macroinvertebrates in single and multiple prey systems. *Acta Oecologica* **28**, 337 – 343.

Correia A.M., Bandeira N. and Anastácio P.M. (2007). Influence of chemical and visual stimuli in food-search behaviour of Procambarus clarkii under clear conditions. *Marine and Freshwater Behaviour and Physiology* **40**, 189 – 194.

Correia A.M. and Anastácio P.M. (2008.) Shifts in aquatic macroinvertebrate biodiversity associated with the presence and size of an alien crayfish. *Ecological Resesearch* **23**, 729 – 734.

Covich A.P., Dye L.L. and Mattice J.S. (1981). Crayfish predation on corbicula under laboratory conditions. *The American Midland Naturalist Journal* **105**, 181 – 188.

Cruz M.J., Rebelo R. and Crespo E.G. (2006). Effects of an introduced crayfish, *Procambarus clarkii*, on the distribution of south-western Iberian amphibians in their breeding habitats. *Ecography* **20**, 329 – 338.

Derby C.D., Steullet P., Horner A.J. and Cate H.S. (2001). The sensory basis of feeding behaviour in the Caribbean spiny lobster, *Panulirus argus*. *Marine and Freshwater Behaviour and Physiology* **52**, 1339 – 1350.

Dukas R. (2008). Evolutionary biology of insect learning. *Annual Review of Entomology* 53, 145 – 160.

Fernandes T.F., Huxham M. and Piperb S.R. (1999). Predator caging experiments: a test of the importance of scale. *Journal of Experimental Marine Biology and Ecology* **241**, 137 – 154.

Garcia E.A. and Mittelbach G.G. (2008). Regional coexistence and local dominance in *Chaoborus*: species sorting along a predation gradient. *Ecology* **89**, 1703 – 1713.

Gherardi F., Renai B. and Corti C. (2001). Crayfish predation on tadpoles: A comparison between a native (*Austropotamobius pallipes*) and an alien species (*Procambarus clarkii*). *Bulletin Française de la Peche et Pisciculture* **361**, 659 – 668.

Gherardi F., Acquistapace P., Hazlett B. and Whisson G. (2002). Behavioural responses to alarm odours in indigenous and non-indigenous crayfish species: a case study from Western Australia. *Marine and Freshwater Research* **53**, 93 – 98.

Gherardi F. (2006). Crayfish invading Europe: the case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology* **39**, 175 – 191.

Gherardi F. and Acquistapace P. (2007). Invasive crayfish in Europe: the impact of *Procambarus clarkii* on the littoral community of a Mediterranean lake. *Freshwater Biology* **52**, 1249 – 1259.

Ghosh S.K., Mandal B.K. and Borthakur D.N. (1984). Effects of feeding rates on production of common carp and water quality in paddycum fish culture. *Aquaculture* **40**, 97 – 101.

Haefner J.W. (1996). *Modeling Biological Systems*. Principles and Aplications. ITP - Chapman & Hall, New York.

Harmsen K., Matar A.E., Saxena M.C. and Silim S.N. (2001). Yield response to phosphorus fertilizer in a wheat-lentil rotation in a Mediterranean environment. *Netherlands Journal of Agricultural Science* **49**, 385 – 404.

Hazlett B.A. (1994). Crayfish feeding responses to Zebra Mussels depend on microorganisms and learning. *Journal of Chemical Ecology* **20**, 2623 – 2630.

Hazlett B.A. and Schoolmaster D.R. (1998). Responses of cambarid crayfish to predator odour. *Journal of Chemical Ecology* **24**, 1757 – 1770.

Hazlett B.A. (2000). Information use by an invading species: do invaders respond more o alarm odours than native species? *Biological Invasions* **2**, 289 – 294.

Hazlett B.A., Acquistapace P. and Gherardi F. (2002). Differences in Memory Capabilities in Invasive and Native Crayfish. *Journal of Crustacean Biology* **22**, 439 – 448.

Hazlett B.A., Burba A., Gherardi F. and Acquistapace P. (2003). Invasive species use broader range of predation-risk cues than native species. *Biological Invasions* **5**, 223 – 228.

Hobbs H.H., Jass .JP. and Huner J.V. (1989). A review of global crayfish introductions with particular emphasis on two North American species (Decapoda: Cambaridae). *Crustaceana* **56**, 229 – 316.

Hughes R.N. and Seed R. (1995). Behavioural mechanisms of prey selection in crabs. *Journal of Experimental Marine Biology and Ecology* **193**, 225 – 238.

Ilhéu M., Guilherme P. and Bernardo J.M. (2002). Impact of the red swamp crayfish (*Procambarus clarkii*) on aquatic invertebrate and macrophyte assemblages: a case study in the south of Portugal. *Verhandlungen Internationale Vereinigung Limnologie* **28**, 144 – 147.

Ishii Y. and Shimada M. (2010). The effect of learning and search images on predator-prey interactions. *Population Ecology* **52**, 27 – 35.

Jover M., Férnandez-Carmona J., Del Río M.C. and Soler M. (1999). Effect of feeding cooked-extruded diets, containing different levels of protein, lipid and carbohydrate on growth of red swamp crayfish *Procambarus clarkii*. *Aquaculture* **178**, 127 – 137.

Karavanich C. and Atema J. (1998). Individual recognition and memory in lobster dominance. *Animal Behaviour* **56**, 1553 – 1560.

Karplus I., Katzenstein R. and Goren M. (2006). Predator recognition and social facilitation of predator avoidance in coral reef fish *Dascyllus marginatus* juveniles. *Marine Ecology Progress Series* **319**, 215 – 223.

Lawton J.H., Beddington J.R. and Bonser R. (1974). *Switching in invertebrate predators*. In: Usher, M.B.,Williamson, M.H. (Eds.) Ecological Stability. Chapman and Hall, London, pp. 141 – 158.

Lencioni M. and Wilhlem D.F.M. (2006). Prey Selection by *Chaoborus* in the field and laboratory. *The Journal of Young Investigators* **15**(4): http://www.jyi.org/research/re.php?id=777

Lodge D.M. and Shrader-Frechette K. (2003). Nonindigenous Species: Ecological Explanation, Environmental Ethics, and Public Policy. *Conservation Biology* **17**, 31 – 37.

McCarthy T.M. and Dickey B.F. (2002). Chemically mediated effects of injured prey on behavior of both prey and predators. *Behaviour* **139**, 585 – 602.

Mascaró M. and Seed R. (2001). Choice of prey size and species in *Carcinus maenas* (L.) feeding on four bivalves of contrasting shell morphology. *Hydrobiologia* **449**, 159 – 170.

Mikoji G.M , Boyce T.G., Mungai B.N., Copeland R.S., Hofkin B.V. and Loker E.S. (1999). Predation of aquatic stages of *Anopheles gambiae* by the Louisiana red swamp crayfish (*Procambarus clarkii*). *Journal* of the American *Mosquito Control Association* **15**, 69 – 71.

Mitscherlich E.A. (1909). Des Gesetz des Minimums und das Gesetz des abnehmended Bodenertrages. *Landwirsch Jahrb* **3**, 537 – 552.

Nijlandl G.O., Schouls J. and Goudriaan J. (2008). Integrating the production functions of Liebig, Michaelis-Menten, Mitscherlich and Liebscher into one system dynamics model. *NJAS - Wageningen Journal of Life Sciences* **55**, 199 – 224.

Nyström P., Brönmark C. and Granéli W. (1999). Influence of an exotic and a native crayfish species on a littoral benthic community. *Oikos* **85**, 545 – 553.

Papaj D.R. and Prokopy R.J. (1989). Ecological and evolutionary aspects of learning in phytophagous insects. *Annual Review of Entomology* **34**, 315 – 350.

Renai B. and Gherardi F. (2004). Predatory efficiency of crayfish: comparison between indigenous and non-indigenous species. *Biological Invasions* **6**, 89 – 99.

Roudez R.J., Glover T. and Weiss J.S. (2008). Learning in an invasive and a native predatory crab. *Biological Invasions* **10**, 1191 – 1196.

Sala O.E., Chapin F.S., Armesto J.J., Berlow E., Bloomfield J., Dirzo R., Huber-Sannwald E., Huenneke L., Jackson R., Kinzig A., Leemans R., Lodge D.M., Mooney H.A., Oesterheld M., Poff N.L., Sykes M.T., Walker B.H., Walker M. and Wall D.H. (2000). Biodiversity scenarios for the year 2100. *Science* **287**, 1770 – 1774.

Sell A.F. (2006). A trophic cascade with *Chaoborus:* population dynamics of ex-ephippial generations of Daphnia. *Archiv für Hydrobiologie* **167**, 115 – 134.

SIEBECK U.E., LITHERLAND L. AND WALLIS G.M. (2009). Shape learning and discrimination in reef fish. *Journal of Experimental Biology* **212,** 2113 – 2119.

STENROTH P. AND NYSTRÖM P. (2003). Exotic crayfish in a brown water stream: effects on juvenile trout, invertebrates and algae. *Freshwater Biology* **48,** 466 – 475.

TUTHILL J.C. AND JOHNSEN S. (2006). Polarization sensitivity in the red swamp crayfish *Procambarus clarkii* enhances the detection of moving transparent objects. *Journal of Experimental Biology* **209,** 1612 – 1616.

WILLIAMS C.E. (2001). Review: Biological Invasions and Global Change: What Might the Future Bring? *Ecology* **82**, 1498 – 1499.

WISENDEN B.D., CHIVERS D.P. AND SMITH R.J. (1997). Learned recognition of predation risk by Enallagma damselfly larvae (Odonata, Zygoptera) on the basis of chemical cues. *Journal of Chemical Ecology* **23**, 137 – 151.

ZAR J.H. (1996). *Biostatistical Analysis*. 3rd edition. Prentice Hall, NewJersey, USA.

CHAPTER 4

An effective and simple method of marking crayfish

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AN EFFECTIVE AND SIMPLE METHOD OF MARKING CRAYFISH

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Abstract

An experiment was conducted to evaluate the effectiveness and applicability of an external, non-invasive crayfish marking technique for population or production studies. A general-use permanent marker (Dykem®, ITW Dymon, Olathe, Kansas, USA) suitable for under water use and with an oil based ink, was tested. The marker was applied on the wet carapace of red swamp crayfish (*Procambarus clarkii*), which were kept for 14 days in open- and closed-top cages in simulated crayfish production ponds.

There was no significant degradation of the tested marks at the end of the 14-day experimental trial, except from natural fouling and dirt accumulation. Marked and non-marked crayfish averaged less than 4.5% and 7.0% mortality, respectively, suggesting that this marking technique is not directly harmful to crayfish. In open-top cages an increase (38%) in missing marked crayfish was observed, possibly caused by predation. In closed-top cages less than 3% crayfish were missing but it was inconclusive whether the bright markings contributed to the predation in the open-top trial.

This technique appears to be an excellent tool for conveniently marking crayfish for indoor or outdoor studies where moulting is not expected. It produces a durable mark, can be applied directly on wet or dry surfaces, dries in 25-35 seconds and there are several different colour and mark combinations possible. This technique also seems promising for marking other aquatic organisms.

Keywords: crustacean; marking technique; permanent marker; red swamp crayfish; Procambarus clarkii

Introduction

Research on crayfish growth, density, production, population dynamics, migration and dispersal often requires marking of the animals to ensure traceability (Hästein *et al.* 2001; Nowicki *et al.* 2008). Several marking methods have been employed in crayfish research. These include external visual marks applied directly to the exoskeleton such as external plastic tags (Gherardi *et al.* 2000), painting with fluorescent paint (Brandt and Schreck 1975) or with nail enamel (O'Neill *et al.* 1993; Ramalho *et al.* 2008), clipping or punching holes in the telson or uropods (Guan 1997; Guan and Wiles 1999; Toyota *et al.* 2003; Nowicki *et al.* 2008) and branding with a soldering iron (Abrahamsson 1965; Buřič *et al.* 2008; Kuhlmann *et al.* 2008). More invasive techniques have also been used such as injecting ink (Black 1963) and injecting visible implant elastomers (Jerry *et al.* 2001; Arce *et al.* 2003; Brown *et al.* 2003; Clark and Kershner 2006; Mazlum 2007). Advanced marking techniques require the use of additional equipment to detect the tag's position and/or read the tag's information. Some examples of these are the use of microchips – Passive Integrated Transponders – (Wiles and Guan 1993; Bubb *et al.* 2002a), coded wire tags (Isely and Eversole 1998; Kneib and Huggler 2001; Graaf 2007) and radio transmitters (Gherardi and Barbaresi 2000; Robinson *et al.* 2000; Bubb *et al.* 2002b; Bubb *et al.* 2004; Aquiloni *et al.* 2005).

For effective tagging, tags should be easily recognizable, should not disrupt crayfish behaviour, and should have a minimum duration of the experiment. One of the major problems encountered when tagging or using external markers on crayfish is the loss of the mark or tags after moulting (Brandt and Schreck 1975; Guan 1997; Gherardi *et al.* 2000; Jerry *et al.* 2001; Bubb *et al.* 2006; Frisch and Hobbs 2006). Some marking techniques may interfere with the organism's behaviour (Guan 1997) or movement, and may also influence growth rates (Guan 1997; Brown *et al.* 2003) or mortality (Brandt and Schreck 1975; Guan 1997; Brown *et al.* 2003).

The objective of this study was to evaluate the effectiveness and applicability of an external, non-invasive marking technique on crayfish for future use in field and laboratory experiments. A general-use permanent waterproof marker (Dykem®, BRITE-MARK®, ITW Dymon, Olathe, Kansas, USA) with an oil based ink was tested.

Material and Methods

In an experimental crayfish production pond in Crowley, Louisiana, two sets of 12 cylindrical metal wire cages, 0.5 m^2 bottom surface (McClain 1995), were placed in a line, each separated by approximately 1 meter. Each cage had a wire net bottom and a 10 cm wide metal band on the inside upper portion of the cage to prevent crayfish escape. One set of cages was maintained with open-tops (open-top trial) and the other set consisted of cages that were covered with 0.5 cm square mesh (closed-top trial). During the two experimental trial periods water depth was held at approximately 30 cm, pH was 8.32, average water temperature was 21.5±0.65 (±SE) ºC and early morning dissolved oxygen averaged 0.84±0.07 mg l^{-1}.

For each trial, 72 mature red swamp crayfish (*Procambarus clarkii*, Girard) (35.93±1.58 grams) were captured from adjacent production ponds and used as experimental individuals. Crayfish were randomly assigned to one of three marking treatments, with each treatment equally represented in every cage, and crayfish were placed in the cages at one of three densities (6, 12 or 18 crayfish m^{-2}). Sex ratio in each cage was approximately 1:1 and each marker by density factor was replicated with four cages per trial. One third of the crayfish in each cage was not marked (control group), one third was marked on the carapace with a general use permanent marker (Dykem®, BRITE-MARK® FAMILY, ITW Dymon, Olathe, Kansas, USA), from now on simply referred as Dykem marker, and one third was marked on the carapace with common fingernail paint. In this last case, the carapace had to be cleaned and dried prior to marking and the required five to ten minutes to thoroughly dry, whereas with the Dykem marker it was applied to the wet carapace without further preparation. The Dykem marker is xylene free, is inexpensive, produces a durable mark, can be applied directly on wet or dry surfaces, dries in 25-35 seconds and there are many different colour and mark combinations available. All marks were applied to the dorsal and/or lateral sides of the carapace in various colours and configurations.

The cages were examined after 1, 4, 7, 10 and 14 days and condition of crayfish and the condition/erosion of the mark was noted. Individuals were considered DEAD only when remains were found, otherwise absent crayfish were considered as being MISSING. Practical survival was determined as follows:

$$\textit{Survival (\%)} = ((\textit{total crayfish} - \textit{DEAD crayfish} - \textit{MISSING crayfish})/\textit{total crayfish})*100$$

Statistical analysis was performed using SPSS version 14.0 software (SPSS 2005). Because data did not fulfil the assumptions of most parametric statistics, the non-parametric MANN-WHITNEY and KRUSKAL WALLIS tests were used. Data from both trials were pooled and analysed for the main effects of mark type.

Results and Discussion

Throughout the experiment all marked crayfish appeared to be in good health and exhibited no signs of sickness or abnormal behaviour. Both marking techniques (Dykem marker and fingernail paint) proved to be suitable for use in studies with crayfish, or other crustaceans, that involve population dynamics, aquaculture and wild population management, survival, ecology, or migration and dispersal of individuals, because these methods did not appear to harm the animal. Both the Dykem marker and the fingernail marks showed no significant signs of erosion during the study. The marks remained in good condition, and only algae and dirt accumulation contributed to reduced brightness by the study end.

By the end of the experiment (14 days), five (6.94%) crayfish where found dead in the closed-top trial, and only one died (1.39%) in the open-top trial. Mortality averaged less than 7% for all treatments and no significant differences were found between marking techniques for either trial (Mann-Whitney: U=574.50; n=6; *P*>0.05) (Figure 1).

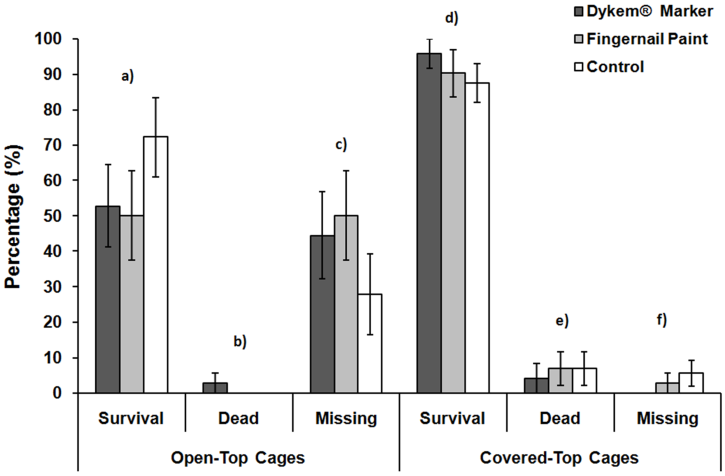

Figure 1. Average percentages for practical survival, dead and missing crayfish at the end of the 14 day experimental trials for both the open- and covered-top cages with each marking treatment. Vertical bars represent standard error. a) Kruskal-Wallis; χ^2=2.152; df=2; *P*>0.05. b) Kruskal-Wallis; χ^2=2.000; df=2; *P*>0.05. c) Kruskal-Wallis; χ^2=1.886; df=2; *P*>0.05. d) Kruskal-Wallis; χ^2=1.825; df=2; *P*>0.05. e) Kruskal-Wallis; χ^2=0.377; df=2; *P*>0.05. f) Kruskal-Wallis; χ^2=2.121; df=2; *P*>0.05.

The open-top trial had a significant lower average practical survival of 58.33±6.88% while the closed-top trial ended with an average survival of 91.20±3.15% (Mann-Whitney: U=356.50; n=111; *P*<0.001). No indications of cannibalism were detected during the experiments but this cannot be ruled out completely. *Survival* in this study was quantified using the original number of crayfish minus the observed dead and missing individuals. For this reason and due to the lack of differences between the two trials in the number of deaths, there was an inverse relationship between the number of missing crayfish and the resulting survival.

There were significant differences observed for missing individuals between the closed- and open-top trials (MANN-WHITNEY: U=316.50; n=29; *P*<0.001). While bright colour markings may improve recognition by researchers, in some situations the use of highly visible marks may distort research results. Though not significant, crayfish with paint markings (40.74±6.98%; 26 individuals) were recorded as missing in higher numbers than non-marked individuals (2.78±1.56%; 2 individuals) in the open-top trial (Figure 1). The increase of approximately 38% in missing marked crayfish from the open-top trial (over those from the closed-top trial) could be a consequence of predation by birds or other predators. During the experiment some crayfish predatory birds were observed at the cages but there was no way to determine the extent, if any, they had on the caged individuals. Another possible explanation for the increased percentage of missing crayfish in the open-top trial is that crayfish may have found a way to escape from the open-top cages. Two experimental crayfish (marked with Dykem paint) were captured outside the cages in adjacent experimental ponds.

While the cause for the missing crayfish is not clearly determinable, it is plausible that enhanced visual detection by predators due to the colour and/or size/location of the marks may have contributed to increased predation in the confinement of the cages. In this experiment the crayfish were marked with a large round mark on the dorsal surface of the carapace. An alternative approach could be to mark the crayfish in a less visible site, such as on the ventral side. This may only be a concern in ponds with clear water and/or scarce vegetation (*i.e.* high visibility locations). We believe the location of the mark would be irrelevant in locations where predators are absent, such as under laboratory conditions and of little concern in locations where dense vegetation (*e.g.* rice fields and marshes) and turbid waters with low visibility are the norm.

The Dykem markers were used in other parallel mark-recapture studies with excellent results and several marked crayfish were recaptured up to three months after release without noticeable marking degradation. Two marked females were recaptured in that trial after 131 and 150 days and were carrying young crayfish (unpublished results). This reinforces the presumption that the Dykem marking technique allows individuals to continue their normal life cycle without being affected. In the case of the female recaptured 131 days after release, the mark had an estimated degradation of less than 15%. In the case of the female recaptured after 150 days the mark was identifiable but was more than 70% eroded. However, these crayfish subjected their marks to extreme conditions of pond culture and subsequent burrowing, as they were excavated from burrows during the dry period following the production season.

This practical marking technique seems to be most suitable for short term mark-recapture studies, and also seems promising for mid/long term studies if the intermoult period is expected to be long. Furthermore, it may be well suited when there is the need for different marks. The markers are available in 14 different colours and several combinations can be easily created (*e.g.* by using dots, stripes, letters, symbols, etc.), applied to wet carapaces (in contrast to fingernail paint) and the animals immediately released in water without ill effects to the mark. This technique clearly has the potential to be used on other crustacean species and other macro invertebrates with calcified exoskeletons.

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References

ABRAHAMSSON S.A.A. (1965). A method of marking crayfish *Astacus astacus* Linné in population studies. *Oikos* **16**, 228 – 231.

AQUILONI L., ILHÉU M. AND 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.

ARCE S.M., ARGUE B. J., THOMPSON D.A. AND MOSS S.M. (2003). Evaluation of a fluorescent, alphanumeric tagging system for penaeid shrimp and its application in selective breeding programs. *Aquaculture* **228**, 267 – 278.

BLACK J.B. (1963). Observations on the home range of stream-dwelling crayfishes. *Ecology* **44**(3), 592 – 595.

BRANDT T.M. AND SCHRECK C.B. (1975). Crayfish marking with fluorescent pigment. *American Midland Naturalist* **94**(2), 496 – 499.

BROWN J.H., MCCAULEY B.R., TAYLOR A. AND HUNTINGFORD F. (2003). A test of two methods of marking larvae and postlarvae of the giant freshwater prawn, *Macrobrachium rosenbergii*. *Aquaculture Research* **34**(1), 49 – 54.

BUBB D.H., LUCAS M.C. AND THOM T.J. (2002)b. Winter movements and activity of signal crayfish *Pacifastacus leniusculus* in a upland river, determined by radiotelemetry. *Hydrobiologia* **483**, 111 – 119.

Bubb D.H., Lucas M.C., Thom T.J. and Rycroft P. (2002)a. The potential use of PIT telemetry for identifying and tracking crayfish in their natural environment. *Hydrobiologia* **483**, 225 – 230.

Bubb D.H., Thom T.J. and Lucas M.C. (2004). Movement and dispersal of the invasive freshwater crayfish *Pacifastacus leniusculus* in upland rivers. *Freshwater Biology* **49**(3), 357 – 368.

Bubb D.H., Thom T.J. and Lucas M.C. (2006). Movement patterns of the invasive signal crayfish determined by PIT telemetry. *Canadian Journal of Zoology* **84**(8), 1202 – 1209.

Buřič M.P., Kozák P. and Vích P. (2008). Evaluation of different marking methods for spiny-cheek crayfish *(Orconectes limosus)*. *Knowledge and Management of Aquatic Ecosystems* **389**(2), 1 – 8.

Clark J.M. and Kershner M.W. (2006). Size-dependent effects of visible implant elastomer marking on crayfish (*Orconectes obscurus*) growth, mortality, and tag retention. *Crustaceana* **79**(3), 275 – 284.

Frisch A.J. and Hobbs J.P. (2006). Long-term retention of internal elastomer tags in a wild population of painted crayfish (*Panulirus versicolor* [Latreille]) on the Great Barrier Reef. *Journal of Experimental Marine Biology and Ecology* **339**(1), 104 – 110.

Gherardi F. and Barbaresi S. (2000). Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir. *Archiv für Hydrobiologie* **150**(1), 153 – 168.

Gherardi F., Barbaresi S. and Salvi G. (2000). Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences* **62**, 179 – 193.

Graaf M. (2007). Tag retention, survival and growth of marron *Cherax tenuimanus* (Crustacea: Decapoda) marked with coded micro wire tags. *Marine and Freshwater Research* **58**, 1044 – 1047.

Guan R.Z. (1997). An improved method for marking crayfish. *Crustaceana* **70**(6), 641 – 652.

Guan R.Z. and Wiles P.R. (1999). Growth and reproduction of the introduced crayfish *Pacifastacus leniusculus* in a British lowland river. *Fisheries Research* **42**(3), 245 – 259.

Håstein T., Hill B.J., Berth F. and Lightner D.V. (2001). Traceability of aquatic animals. *Revue Scientifique et Technique (International Office of Epizootics)* **20**(2), 564 – 583.

Isely J.J. and Eversole A.G. (1998). Tag retention, growth and survival of red swamp crayfish marked with coded wire tags. *Transactions of the American Fisheries Society* **127**, 658 – 660.

Jerry D.R., Stewart T., Purvis I.W. and Piper L.R. (2001). Evaluation of visual implant elastomer and alphanumeric internal tags as a method to identify juveniles of the freshwater crayfish, *Cherax destructor*. *Aquaculture* **193**(1-2), 149 – 154.

Kneib R.T. and Huggler M.C. (2001). Tag placement, mark retention, survival and growthof juvenile white shrimp (*Litopenaeus setiferus* Pérez Farfante, 1969) injected with coded wire tags. *Journal of Experimental Marine Biology and Ecology* **266**(1), 109 – 220.

Kuhlmann M.L., Badylak S.M. and Carvin E.L. (2008). Testing the differential predation hypothesis for the invasion of rusty crayfish in a stream community: laboratory and field experiments. *Freshwater Biology* **53**(1), 113 – 128.

McClain W.R. (1995). Effects of population density and feeding rate on growth and feed consumption of red swamp crawfish *Procambarus clarkii*. *Journal of the World Aquaculture Society* **26**(1), 14 – 23.

Mazlum Y. (2007). Influence of visible implant fluorescent elastomer (VIE) tagging on growth, molting and survival of the eastern white river crayfish, *Procambarus acutus acutus* (Girard, 1852). *Turkish Journal of Zoology* **31**, 209 – 212.

Nowicki P., Tirelli T., Sartor R.M., Bona F. and Pessani D. (2008). Monitoring crayfish using a mark-recapture method: potentials, recommendations, and limitations. *Biodiversity Conservation* **17**(14), 3513 – 3530.

O'Neill D.J., French D.P., Rebach S. and Handwerker T.S. (1993). Chelae removal alters attainment of sexual maturity in male *Procambarus clarkii* (Girard) and mortality in groups. *Freshwater Crayfish* **9**, 38 – 49.

Ramalho R.O., Anastácio P.M. and Marçal A.M. (2008). Effects of density on growth and survival of juvenile red swamp crayfish, *Procambarus clarkii* (Girard), reared under laboratory conditions. *Aquaculture Research* **39**, 577 – 586.

Robinson C.A., Thom T.J. and Lucas M.C. (2000). Ranging behavior of a large freshwater invertebrate, the white-clawed crayfish *Astropotamobius pallipes*. *Freshwater Biology* **44**(3), 509 – 521.

SPSS Inc. (2005). *SPSS for Windows*. Rel. 14.0.1. Chicago. USA.

Toyota K., Yamauchi T. and Miyajima T. (2003). A marking method of cutting uropods using malformed regeneration for kuruma prawn *Marsupenaeus japonicus*. *Fisheries Science* **69**, 161 – 169.

Wiles P.R. and Guan R.Z. (1993). Studies on a new method for permanently tagging crayfish with microchip implants. *Freshwater Crayfish* **9**, 419 – 425.

CHAPTER 5

Capture rate and crayfish movements among experimental crayfish production ponds

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CAPTURE RATE AND CRAYFISH MOVEMENTS AMONG EXPERIMENTAL CRAYFISH PRODUCTION PONDS

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Abstract

Crayfish cultured in the Southern USA are harvested solely by baited wire mesh traps at trap densities ranging from 16 to 50 traps ha^{-1}. Few data exist regarding the efficiency with which these traps remove crayfish from the harvestable population. This information is key to accurately implementing several management strategies in crayfish aquaculture. Therefore this study was undertaken to ascertain capture rate using a mark/recapture technique with mature, non-molting, individuals in small experimental crayfish ponds. Only about 50% of the marked population of crayfish was captured over time with baited traps, and only about 40% were captured in the pond of release. Most of the marked crayfish (39.7%), or 0.8% per trap lift, were caught within the first week following release, and captures of marked crayfish rarely occurred after 3 weeks from the release date. Crayfish movement into adjacent ponds (likely by moving over land) occurred, but no marked crayfish were found at a distance of more than two ponds from their release site. In conclusion, the efficiency of baited wire mesh traps at capturing the population of market-size individuals may not be as great as many culturists assume. Further research is need in this area.

Key words: Crayfish; crayfish capture; harvest; movement; Procambarus clarkii; trapping efficiency

Introduction

The crayfish aquaculture industry of the USA is located primarily in Louisiana where over 1,100 producers cultivate procambarid crayfish on over 70,000 ha, producing in excess of 44,000 metric tons worth over $100 million USD annually (LCES 2009). Crayfish are cultivated in mono-cropping systems, where crayfish is the sole crop harvested, and in multi-crop rotational systems where rice is produced in addition to crayfish (McClain 2005).

The sole method used for harvest of procambarid crayfish from aquaculture ponds in the Southern USA is the baited wire-mesh trap. The standard trap currently in use today is the 3-funnel, pyramid-shape trap constructed of PVC-coated 19 mm (0.75 inch) or 22 mm (0.875 inch) square mesh welded wire. In general, the most efficient trap density in forage-based production systems is from 25 to 49 traps ha^{-1}, utilizing a 3- or 4-day per week harvest schedule. Optimum trap density is primarily based on crayfish population – with the higher trap densities recommended for the higher population densities (McClain *et al.* 2007).

Efficacy of the baited trap is dependent on a number of variables, such as crayfish density, bait type, trap soak interval, and environmental factors. However, the efficiency for which the standard trapping protocol can remove harvest size crayfish from a population has not been thoroughly examined. In one limited study (Ramalho *et al.* 2008), capture rate of marked crayfish over a short (6-day) interval in a commercial crayfish pond was examined and, while conditions were representative of commercial operations in the region, those findings were considered preliminary and limited.

Movement patterns of *Procambarus clarkii* Girard are well documented (Gherardi *et al.* 2000, 2002; Barbaresi *et al.* 2004), but the extent to which individuals move in and out of production ponds, which can confound harvesting efficiency studies, is largely unknown. Therefore, the objectives of this study were to provide additional data regarding the harvest efficiency of marked crayfish in small experimental ponds over an extended period of time and to examine crayfish movement patterns among contiguous, earthen levee ponds.

Material and Methods

Mark and recapture trials, using the Louisiana red swamp crayfish (*P. clarkii*), were conducted at the Rice Research Station, Louisiana State University Agricultural Center, Crowley, Louisiana, USA. A system of earthen ponds was managed to simulate a typical commercial rice-crayfish field rotational cropping strategy and consisted of two sets of six contiguous 0.4 ha (surface area) experimental ponds separated by a water supply channel or lateral (0.36 ha) that provided water by gravity flow to individual ponds (Figure 1). The ponds had a levee height of approximately 0.41 m and base width of 3.7 m. Water depth in the ponds was 0.3 m. Each pond was fitted with inflow and outflow pipes of 30 cm diameter and all interior ends of inflow pipes were held off the pond bottom and near the water surface with a tension line to minimize free movement of crayfish between ponds and the water supply lateral. Rice was planted in April of 2006, harvested in September 2006, and the rice stubble was subsequently reflooded in October 2006 and managed for a crayfish crop until drained in June 2007.

The experimental ponds were also used to examine the effects of supplemental stocking of hatchlings (during autumn) on annual yield in a separate study. Low correlations (r = 0.26) were observed for supplemental stocking and annual yield (mean yield = 218 kg ha-1) in that study, and it was assumed that the supplemental stocking study had little, if any, impact on the mark/recapture study. Data were separately maintained for each respective study, and this article constitutes the report for the mark/recapture study only.

A total of 489 crayfish (246 mature males and 243 mature females) were marked and released on 10 occasions from 2 April to 14 June 2007. For each event, crayfish were captured from the research ponds, marked, and released within 2 to 3 h of their capture. All crayfish released were judged to be sexually mature based on Form I ischial hooks of males and various secondary sexual characteristics for females (Holdich and Lowery 1988). Crayfish were marked with a long lasting waterproof marker (Dykem®, BRITE-MARK®, ITW Dymon, Olathe, Kansas, USA) using a combination of marker colours (*e.g.* yellow, red, white, and blue) and identifying marks (*e.g.* dots, circles, and stripes) placed on the dorsal and lateral surfaces of the carapace (Ramalho *et al.* 2010). The colour and graphic symbols used allowed easy identification as to date and location of release. Releases occurred within the interior of three random ponds (A-2, A-5, and B-1; Figure 1).

Harvesting method employed was consistent with commercial crayfish operations and consisted of baited wire-mesh traps emptied by boat 2 to 4 days per week after a 24- or 48-h baited soak duration (Romaire 1995). Harvests were conducted both in all ponds and in the central water supply lateral from 3 April until 26 June 2007. For the purpose of this study, the water supply lateral was treated as an adjacent pond to each and all other 0.4 ha ponds because of its contiguous nature to each pond (Figure 1). Trap density generally consisted of 40 traps ha^{-1}, with the exception that 55 traps ha^{-1} were used in the water supply lateral and 9 additional traps were placed in the vicinity of the release location in respective release ponds, but only for the first 24 h. All other harvest efforts were consistent across all ponds on each harvest day and all trap-lifts were accounted for in the cumulative harvest results. Bait used was one of the commercially available formulated crayfish baits (McClain *et al.* 2007), and bait type was consistent across all ponds each day.

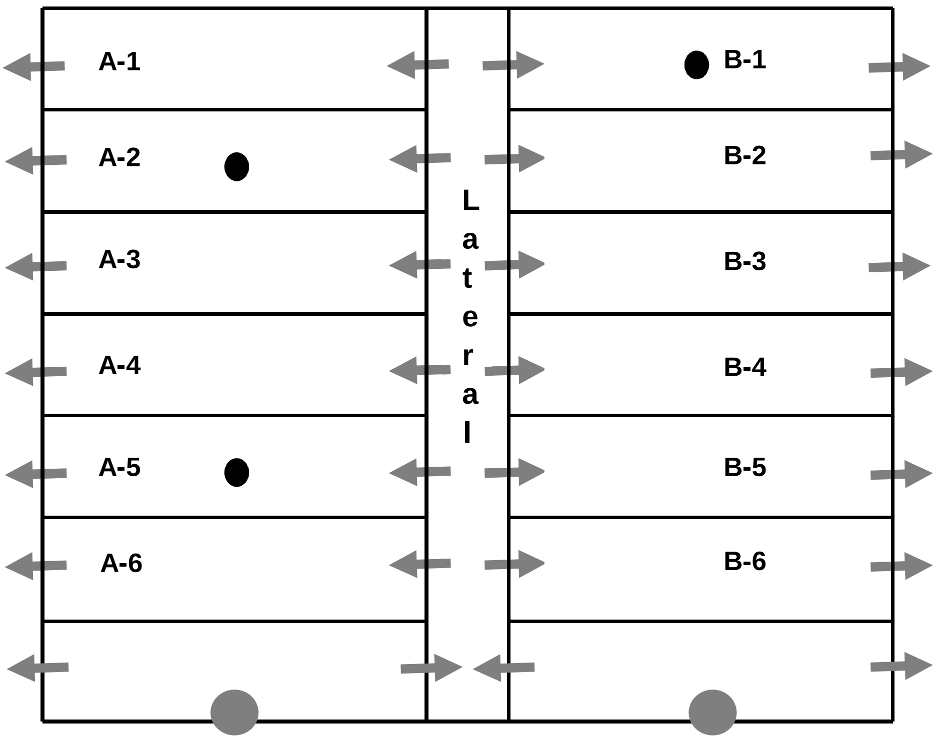

Figure 1. Schematic representation of the pond system where this study was conducted. All ponds (A-1 to A-6 and B-1 to B-6) were 0.4 ha in size (56 x 72 m), and the central lateral area consisted on 0.36 ha (357 x 10 m). The two large circles represent fresh water inlets and arrows represent water flow direction into and out of ponds. Release locations are represented by the small circles in ponds B-1, A-2, and A-5.

For each marked crayfish retrieved in a baited trap, the mark identifier and colour were noted and associated with date and pond of release. Captured marked crayfish were removed from the population. Data parameters included recapture rates, locations, lapsed time after release, crayfish gender, and cumulative harvest efforts (*i.e.* total trap lifts) from release to recapture. Data were analysed using SPSS version 14.0 software (SPSS 2005) and means were considered to be significantly different at α=0.05. Non-normal data were subjected to non-parametric tests (Tanner 1970; Zar 1996).

Results and Discussion

Results of the mark/recapture efforts are summarized by release date and release pond in Table 1. By the end of the crayfish harvesting season, a total of 240 marked crayfish, out of the 489 released, or 49.1%, were recaptured. Recapture rates for individual release events ranged from 21 to 71% (Table 1). Others have reported recapture rates of marked crayfish below 10% (Robinson *et al.* 2000, Byron and Wilson 2001). The reason for the large range in recapture rate in this study is unknown. Since release events occurred from early April to mid-June and all harvesting was suspended on 26 June, differential cumulative harvest efforts (*i.e.*

total trap-lifts) for the various release events resulted. Nonetheless, there was no apparent trend in capture rate as related to cumulative harvest effort following release. Seemingly, recapture rate was independent of the intensity of harvest effort – at least within the ranges of harvest effort examined in this study.

Regardless of the total harvest effort, on average, over half of the marked crayfish released were never captured during the study period. Few marked crayfish (n=13) were observed at pond draining and two (including one female with hatchlings) were found from a sample of burrows excavated several months later. The cause for the low capture rate was not determinable. A number of possible causes could have contributed to the low returns, including natural mortality, mortality from predators, escape or migration from the ponds, attrition as a result of burrow occupations, or the low capture rate was simply due to the inefficiencies of the passive system of trap harvesting.

While natural and predator-induced mortalities can be substantial in open ponds (Huner 1994), Ramalho *et al.* (2010) found no evidence that this marking technique was directly harmful. Nevertheless, it cannot be ruled out though, that depredation rate may have been higher for the population of marked individuals.

Escape of marked crawfish from ponds via the drain pipes is possible, but little water outflow occurred during the study period and no marked crayfish were observed in the shallow perimeter ditch surrounding the pond complex. Some evidence was provided by this study for possible migrations out of the pond. While 82.5% of those crayfish recaptured (or 39.9% of those released) were captured within the same pond that they were released into, 17.5% were captured outside of the release pond. Fifteen percent of those ultimately captured were caught in a pond adjacent to the release pond, and 2.5% were captured two ponds over from the ponds in which they were released. However, no marked crayfish were retrieved further than two ponds away. The most plausible explanation for crayfish captures outside of the release pond was that individuals simply exited the water and crossed over one or two levees to enter other ponds.

Although crayfish could have moved from one pond to another via the central water supply lateral by navigating through the water inflow pipes (Figure 1), this seems less likely because the pipe ends were raised and maintained at, or near the water surface, such that crayfish would have had to leave the pond bottom and enter the pipe near the water/air surface. If this was the most plausible means of crayfish movements, the majority of crayfish found outside of the pond of their release would not likely have been concentrated in adjacent ponds. Once in the water supply lateral, crayfish would have been free to move into any pond some distance from their release location, and no crayfish were captured during this study more than two ponds over from where they were released. Therefore, overland travel is the most likely means of crayfish migration from one pond to another and this suggests that some could have exited the pond system via the perimeter levees as well. Exiting the water is a behaviour that increases the risk of predation in crayfish, but crayfish have been known to exit bodies of water and travel overland (Penn 1943; Cruz and Rebelo 2007; Chucholl 2011; Ramalho 2011, unpublished data), and this is a common occurrence for adult *P. clarkii* during the spring or early summer in the southern USA (Penn 1943).

Table 1. Summary of release and captures of marked crayfish, and associated harvest effort (cumulative number of trap-lifts per pond) by release event. N.º = number of individuals; s.e. = standard error. [1] Total harvesting effort represents the cumulative trap-lifts within the pond of release following each release event. [a] Represents mean percentage of crayfish recaptured by pond category and in total, regardless of category. [b] Represents percentage of total crayfish recaptured by pond category.

Pond	**Release Date**	**Total Crayfish Released N.º**	**Total Harvest Effort[1] Trap-lifts per Pond**	**Total Crayfish Recaptured**		**Crayfish Recaptured in Release Pond**		**Crayfish Recaptured in Adjacent Pond**		**Crayfish Recaptured Two Ponds Over**	
				N.º	**% by N.º**	**N.º**	**% by N.º**	**N.º**	**% by N.º**	**N.º**	**% by N.º**
B-1	02 Apr	53	656	19	35.9	16	30.2	2	3.8	1	1.9
A-5	19 Apr	60	605	32	53.3	15	25.0	16	26.7	1	1.7
A-2	18 May	60	372	37	61.7	36	60.0	1	1.7	0	0
A-5	18 May	60	356	29	48.3	23	38.3	5	8.3	1	1.7
A-2	06 June	80	203	29	36.3	27	33.8	1	1.3	1	1.3
A-5	06 June	80	203	46	57.5	43	53.8	3	3.8	0	0
A-2	07 June	24	187	17	70.8	15	62.5	2	8.3	0	0
A-5	07 June	24	242	5	20.8	5	20.8	0	0	0	0
A-2	14 June	24	105	13	54.2	9	37.5	4	16.7	0	0
A-5	14 June	24	105	13	54.2	9	37.5	2	8.3	2	8.3
Total (Mean)		**489**	**---**	**240**	**(49.3[a])**	**198**	**(39.9[a])**	**36**	**(7.9[a])**	**6**	**(1.5[a])**
% of Total Recaptured		---	---	---	---	---	**82.5[b]**	---	**15.0[b]**	---	**2.5[b]**
s.e.		---	---	---	4.61	---	4.52	---	2.60	---	0.80

While little evidence exists for a large attrition of marked crayfish in the harvestable population as a result of burrowing by marked individuals, burrowing could account for some degree of reduction in retrieval rates in this study. It has been observed that females occupy the majority of burrows constructed around commercial crayfish ponds (Gonul 1995; McClain 2010). The overall proportion of males and females in recaptured crayfish in this study was statistically significant (Chi-Square: χ^2=7.135; df=1; p=0.008) with 55.3% of the total recaptures being male and 42.8% being female. The proportion of males to females by relative location of recapture was not significantly different (Figure 2). Though burrow occupation was probably not the major cause for overall low recovery rates it could be one of the explanations for the lower recapture rate of females.

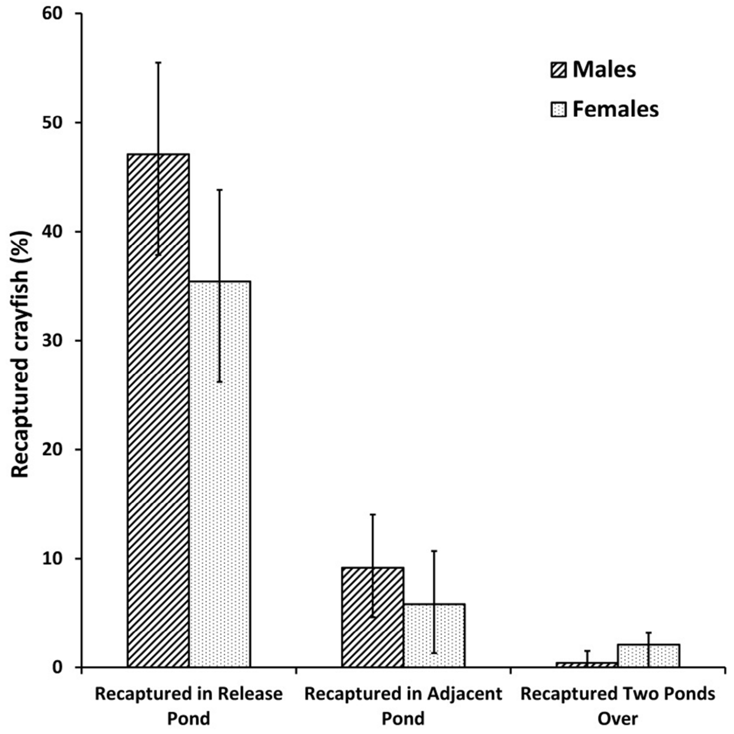

Figure 2. Proportions of recaptured crayfish, by gender, by relative location of capture. Vertical bars represent the confidence limits (±95%).

Most of the marked crayfish were caught within the first week following release (39.7%, n=194, Table 2) and these were captured with an average cumulative harvest effort of 51 trap-lifts per pond (trap density = 40-55 ha^{-1}). Recapture rates were drastically diminished after one week post-release and recapture rarely occurred after 3 weeks from the release event (Table 2). Because all marked crayfish in this study were judged to be sexually mature upon release, and it is generally assumed that moulting probabilities for sexually mature individuals during the latter part of the season in aquaculture ponds is low (Suko 1970; Huner 1994), moulting is not suspected as the primary reason for the low rate of capture after release. However, the assumption of a seasonal terminal moult at sexual maturity in *P. clarkii* under pond culture conditions may need to be examined more closely.

At peak density of marked crayfish in this study (*i.e.* within one week following release) the capture rate of marked individuals was 39.7% with a mean of 51 trap lifts (Table 2) or 0.8% per trap lift. This was substantially greater than that observed in a preliminary mark/recapture trial conducted in a commercial pond where the recapture rate of mature marked crayfish within a week following release was only 0.02% per trap lift (Ramalho *et al.* 2008). However, in that study the trap density (16 traps ha^{-1}) was much lower than the mean trap density of 42 traps ha^{-1} in this study. Relative density of marked crayfish was also substantially different between the two studies, but Dorn *et al.* (2005) observed that a passive system of sampling (*i.e.* baited traps) captured increasingly smaller proportions of crayfish as crayfish density increased.

Table 2. Capture rates of marked crayfish in relation to time passed after release (in weeks). Average harvesting efforts (*i.e.* number of trap-lifts per pond) were derived by averaging the total trapping effort respective of the number of weeks observed for each release event. N.º = number of crayfish; Cum = cumulative percentage. [1] Mean harvesting effort represents the average number of trap-lifts across all fields by week after release.

Weeks after Release	**Applicable Number of Release Events**	**Mean Harvesting Effort[1] (Trap-lifts per Pond)**	**Crayfish Recaptured in Release Pond**			**Crayfish Recaptured in Adjacent Pond**			**Crayfish Recaptured Two Ponds Over**		
			N.º	**%**	**Cum %**	**N.º**	**%**	**Cum %**	**N.º**	**%**	**Cum %**
1	10	51	167	34.2	34.2	25	5.1	5.1	2	0.4	0.4
2	10	99	28	5.7	39.9	9	1.8	7.0	1	0.2	0.6
3	8	151	2	0.4	40.3	1	0.2	7.2	1	0.2	0.8
4	4	179	1	0.2	40.5	0	0.0	7.2	1	0.2	1.0
5	4	237	0	0	40.5	0	0.0	7.2	0	0	1.0
6	4	284	0	0	40.5	1	0.2	7.4	0	0	1.0
7	2	323	0	0	40.5	0	0	7.4	0	0	1.0
8	2	378	0	0	40.5	0	0	7.4	0	0	1.0
9	2	434	0	0	40.5	0	0	7.4	1	0.2	1.2
10	2	498	0	0	40.5	0	0	7.4	0	0	1.2
11	1	554	0	0	40.5	0	0	7.4	0	0	1.2
12	1	618	0	0	40.5	0	0	7.4	0	0	1.2
Total	---	---	**198**	**40.5**	---	**36**	**7.4**	---	**6**	**1.2**	---

In conclusion, this study, although preliminary, has revealed some interesting and possibly important aspects of crayfish harvesting not revealed to date. First, it has shown that the current harvesting technique of baited wire-mesh traps may not be as effective or efficient as some have assumed. Under the conditions of this study, which were generally typical of commercial culture in Louisiana, the mark/recapture technique indicated that less than 50% of the population of harvestable crayfish was captured over time. Moreover, when considering crayfish captured solely within the confines of the earthen pond of release (albeit small ponds of 0.4 ha), only about 40% of the harvestable population was taken with baited traps. The implication of these observations is not good news for those crayfish producers that routinely release the smaller, less desirable, crayfish from the harvest with intentions of recapturing them later at a larger, more valuable, size for market. The low return or recapture rate may not be conducive for maximum profits in some cases, especially with declining prices as the season progresses (McClain *et al.* 2007). Another important insight gleaned from these observations deals with the intrinsic dynamics associated with crayfish movements in, and around, crayfish ponds. These findings document to some extent the propensity for crayfish to move out of and into habitats, likely as a result of some overland travel. This emphasizes the potential for the red swamp crayfish to colonize suitable neighbouring habitats, whether they are nearby crayfish ponds, flood-irrigated agricultural land, or sensitive ecological habitats. Additional research is warranted for the use of mark/recapture techniques in objectives dealing with crayfish aquaculture, as well as aspects relating to crayfish ecology.

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References

Barbaresi S., Santini G., Tricarico E. and Gherardi F. (2004). Ranging behaviour of the invasive crayfish, *Procambarus clarkii* (Girard). *Journal of Natural History* **38**, 2821 – 2832.

Byron C.J. and Wilson K.A. (2001). Rusty crayfish (*Orconectes rusticus*) movement within and between habitats in Trout Lake, Vilas County, Wisconsin. *Journal of the North American Benthological Society* **20**(4), 606 – 614.

Chucholl C. (2011). Disjunct distribution pattern of *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in an artificial lake system in Southwestern Germany. *Aquatic invasions* **6**(1), 109 – 113.

Cruz M. and Rebelo R. (2007). Colonization of freshwater habitats by an introduced crayfish, *Procambarus clarkii*, in Southwest Iberian Peninsula. *Hydrobiologia* **575**(1), 191 – 201.

Dorn N.J., Urgelles R. and Trexler J.C. (2005). Evaluating active and passive sampling methods to quantify crayfish density in a freshwater wetland. *Journal of the North American Benthological Society* **24**(2), 346 – 356.

Gerardi F. and Barbaresi S. (2000). Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). *Archives of Hydrobiology* **150**, 153 – 168.

Gherardi F., Barbaresi S. and Salvi G. (2000). Spatial and temporal patterns in the movement of the red swamp crayfish, *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences* **62**, 179 – 193.

Gherardi F., Tricarico E. and Ilhéu M. (2002). Movement patterns of the invasive crayfish, *Procambarus clarkii*, in a temporary stream of southern Portugal. *Ethology, Ecology & Evolution* **14**, 183 – 197.

Gonul M. (1995). Reproductive biology and ecology of red swamp crawfish and white river crawfish in ponds with emphasis on burrowing ecology. MS Thesis. Louisiana State University, Baton Rouge, LA.

Holdich D.M. and Lowery R.S. (1988). *Freshwater crayfish biology, management and exploitation.* Timber Press, Portland, Oregon, 498 pp.

Huner J.V. (1994). Cultivation of freshwater crayfishes in North America. Section I. Freshwater Crayfish Culture. *In:* F*reshwater Crayfish Aquaculture in North America, Europe, and Australia*. Families Astacidae, Cambaridae, and Parastacidae. Huner JV (ed.), 5 – 89 pp. Haworth Press, Binghamton, New York.

LCES (Louisiana Cooperative Extension Service) (2009). Louisiana Summary of Agriculture and Natural Resources. Louisiana State University Agricultural Center, Baton Rouge, Louisiana. 323 pp.

McClain W. (2005). Crawfish culture in forage-based production systems. In: *Aquaculture in the 21st Century*. Kelly A and Silverstein J (eds.). 151 – 169 pp. American Fisheries Society, Symposium 46, Bethesda, Maryland.

McClain W.R., Romaire R.P., Lutz C.G. and Shirley M.G. (2007). *Louisiana crawfish production manual.* Louisiana State University Agricultural Center, Pub. 2637, Baton Rouge, Louisiana.

McClain W.R. (2010). Spawning date and potential contribution to yield from winter-burrowing *Procambarus clarkii* in crawfish aquaculture ponds. *Journal of Shellfish Research* **29**(1), 7 – 12.

Penn G.H. (1943). A study of the life history of the Louisiana red-crayfish, *Cambarus clarkii* Girard. *Ecology* **24**(1), 1 – 18.

Ramalho R.O., McClain W.R. and Anastácio P.M. (2008). Crayfish dispersal in a production pond: a preliminary mark-recapture approach. Program and Abstract, 17th International Symposium of Astacology. Kuopio, Finland. August 2008. p. 52.

Ramalho R.O., McClain W.R. and Anastácio P.M. (2010). An effective and simple method of marking crayfish. *Freshwater Crayfish* **17**, 57 – 60.

Robinson C.A., Thom T.J. and Lucas M.C. (2000). Ranging behaviour of a large freshwater invertebrate, the white-clawed crayfish *Austropotamobius pallipes*. *Freshwater Biology* **44**(3), 509 – 521.

Romaire R.P. (1995). Harvesting methods and strategies used in commercial procambarid crawfish aquaculture. *Journal of Shellfish Research* **14**, 545 – 551.

SPSS Inc. (2005). *SPSS for Windows*. Rel. 14.0.1. Chicago. USA.

Suko T. (1953). Studies on the development of the crayfish. 1. The development of secondary sex characters in appendages. *Science Reports of the Saitama University* **1B**, 77 – 96.

Tanner J.T. (1970). *Guide to the study of animal populations*. University of Tenesse Press, Knoxville.

ZAR J.H. (1996). *Biostatistical Analysis*, 3rd edn. Prentice-Hall, New Jersey

CHAPTER 6

Factors inducing invasive crayfish (Procambarus clarkii) overland dispersion

This chapter is currently under submission to a journal.

FACTORS INDUCING INVASIVE CRAYFISH (*PROCAMBARUS CLARKII*) OVERLAND DISPERSION

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Abstract

The ability for overland dispersion in some freshwater invertebrates can provide an important advantage over other species when colonizing new water bodies. The red swamp crayfish (*Procambarus clarkii*) is a widespread freshwater invader with known ecological and economic impacts which has been observed to disperse overland. The major aim of this study was to collect detailed information about the factors involved on the overland dispersion of *P. clarkii*. During a 12 month period the numbers of *P. clarkii* out of water in a rice cultivation system in Portugal were monitored. Data was gathered regarding the preferential time period for overland dispersal, the population structure in land and in the water, the direction of the movement, and the relation between environmental variables and the number of crayfish out of water. One of the main factors inducing crayfish overland dispersal was the drainage of the study area and the number of crayfish dispersing overland was inversely correlated with the water level in the rice pads. Overland dispersion was only observed immediately after the drainage of the study area and occurred until the area was reflooded due to heavy rain events. 77% of the individuals dispersing overland were observed during a maximum of 35 days after the drainage of the study area. During the post-drainage period, the number of crayfish dispersing overland was positively correlated with precipitation. Other variables significantly affecting the overland dispersal of crayfish were the temperature, relative humidity and the period of the day. *P. clarkii* overland dispersal was only observed when the average relative humidity was above 70% and 99% of the total crayfish dispersing overland were observed when the rice pads water level was below 6 cm. Overland dispersion was observed for a range of water temperatures between 16.3 and 24.2ºC. Approximately 50% of the overland dispersing individuals were recorded during the dusk/night sampling period, 30% were recorded during the sunrise period and less than 20% were recorded during the afternoon. Crayfish were observed moving overland at a maximum speed of 90 $m.h^{-1}$, although not significantly moving in a particular direction. We found significant differences in the structure of the population in the water and out of the water, with a higher proportion of mature individuals dispersing overland. Approximately 82% of the crayfish registered overland were mature, with 4.5% of the mature females carrying eggs and 8.1% transporting juveniles. The results of the present study can be of high importance considering the urge to properly manage the invasive crayfish populations in Europe, and considering that new legislation is being formulated on this matter.

Keywords: Iberian Peninsula; invasive species; overland dispersion; red swamp crayfish; Procambarus clarkii

Introduction

Many crayfish species have become established outside their natural range through various mechanisms, as by natural dispersion, accidental escape from holding facilities and by deliberate introductions (Ligas 2007). Native to north-eastern Mexico and south-central USA, red swamp crayfish (*Procambarus clarkii* Girard, 1852) is nowadays one of the world's most successful invasive species (Huner and Avault 1979; Gherardi and Holdich 1999; Holdich *et al.* 2009; DAISIE 2010). Its spread throughout the world is mainly attributed to human introductions (Gherardi and Holdich 1999; Gherardi 2006). Following its establishment, the rapid and widespread expansion of this species, is the result of its active dispersal capabilities. Several traits of its life history (*e.g.* polytrophism, rapid growth, high fecundity and disease resistance) make its commercial cultivation (or exploitation) feasible but also improve the success rate of its invasions (Huner and Lindqvist 1995; Lindqvist and Huner 1999; Holdich 2002). In Portugal, *Procambarus clarkii* was introduced in the late 1970's (Ramos and Pereira 1981) and since then it has invaded all river basins. However, its world distribution has been continuously increasing and extensive areas are still available for invasion (Capinha and Anastácio 2011; Capinha *et al.* 2010). *P. clarkii* is primarily adapted to life in habitats that have alternating dry and wet periods (Huner and Barr 1991). This species spends most of its life in open water and burrowing activity may occur in response to environmental extremes, such as to avoid desiccation (Huner and Barr 1991; McClain 2010) but may also occur during the late stages of reproduction (Hobbs 1981; Blakewood *et al.* 1993; McClain and Romaire 2004; Gherardi 2006).

As invasive species are often confronted with adverse environmental conditions in the invaded areas, the key to their success involves how they are able to cope with them. Overland dispersal in freshwater crayfish species can, in fact, be considered a successful behavioural adaptation to face adverse environmental conditions. It can provide an important advantage when invading new areas, or when the freshwater habitat is unpredictable with alternating dry and wet periods. However, this behavioural adaptation also seems to be energetically expensive and can impose severe risks to the survival of overland dispersing individuals, such as increased risk of desiccation and predation (*e.g.* by birds and mammals).

Even though some studies have investigated *P. clarkii* underwater dispersal abilities (Gherardi and Barbaresi 2000; Gherardi *et al.* 2000; Aquiloni *et al.* 2005), little is known about its overland dispersal capabilities. *P. clarkii* has the ability to exit the water and move overland (Penn 1943; Holdich 2002; Kerby *et al.* 2005; Cruz and Rebelo 2007; Chucholl 2011) but the factors involved in this behaviour remain unclear and not quantified. Cruz and Rebelo (2007) found that overland dispersion was a common phenomenon contributing to the colonization of temporary or permanent ponds. These authors also found that the distance to a source of crayfish was the main predictor for the probability of crayfish presence in these habitats. In spite of this, the causes and the conditions for overland dispersal were never studied.

The ability to move overland and the conditions for the occurrence of the phenomenon may in fact be extremely relevant for *P. clarkii* invasiveness, especially in what concerns the speed of the invasion front. Some general hypotheses about crayfish overland dispersal have been placed although these were never tested. Penn (1943) and Viosca (1939) mentioned a post-reproductive migration of mature males and females in poor shape but often carrying eggs. Females carrying eggs or juveniles may benefit from overland dispersion particularly if they can find a better habitat patch. Huner and Barr (1991) suggested that when heavy rains follow dry periods, the cement-hard burrow plugs are softened, making it easier for buried crayfish to escape. As the rain water recedes, crayfish move with it and great numbers may be observed crossing fields or even roads. A third explanation was that in several lentic waters inhabited by this species, where water stagnation is not unusual, the organic content of swamp and marsh soils is very high and microbial decomposition creates a high biological oxygen demand. When water flow is restricted this demand increases and crayfish move overland, possibly seeking more acceptable living conditions (Gherardi 2002). A final, and simpler, explanation was that individuals may exit the water to overcome obstacles to their natural movement (*e.g.* dams) (Kerby *et al.* 2005).

The main objective of this study was to determine the factors involved in red swamp crayfish seasonal overland dispersion. This is a crucial topic in the study of biological invasions and should allow both a timely control and an adequate management of the natural ecosystem. Moreover, this issue is particularly relevant in the studied area, since new legislation regarding invasive species is being prepared. This legislation will facilitate state managed programs that aim to identify and prioritise exotic invasive species pathways and implement control or eradication programs for priority species under the European Union biodiversity strategy to 2020 (EU 2011).

Methods

This study was conducted in the Tagus river Basin, at an experimental rice research station (COTArroz – Centro Operativo e Tecnológico do Arroz), within Paul de Magos, Portugal (38º 58'W, 8º 45'W). Paul de Magos is a rice (*Oryza sativa*) production area of 700 ha (Figure 1). In this region, rice production is characterized by a continuously flooded spring – summer crop which development and growth is strongly influenced by the water level (Correia 1995a). Water was supplied to the rice fields from ribeira de Magos by a pumping station and the main function of the adjacent drainage channels was to capture overflow and drainage water from the rice fields. Fields are prepared for cultivation in March–April, sown in April–May and harvested in September–October. Water level in the rice pads is maintained between 20 and 30 cm during most of the rice growth period to control aquatic weeds and animal pests. In the adjacent areas of this marsh there are pine and eucalyptus production areas, among agriculture and urban areas. The hydrological cycle of the study area is almost completely dependent on the rice culture practices. With the exception of a small period in winter, when occasional flood events may occur due to intense precipitation, the presence/absence of water in the drainage channels is artificially regulated according to the rice cultivation needs.

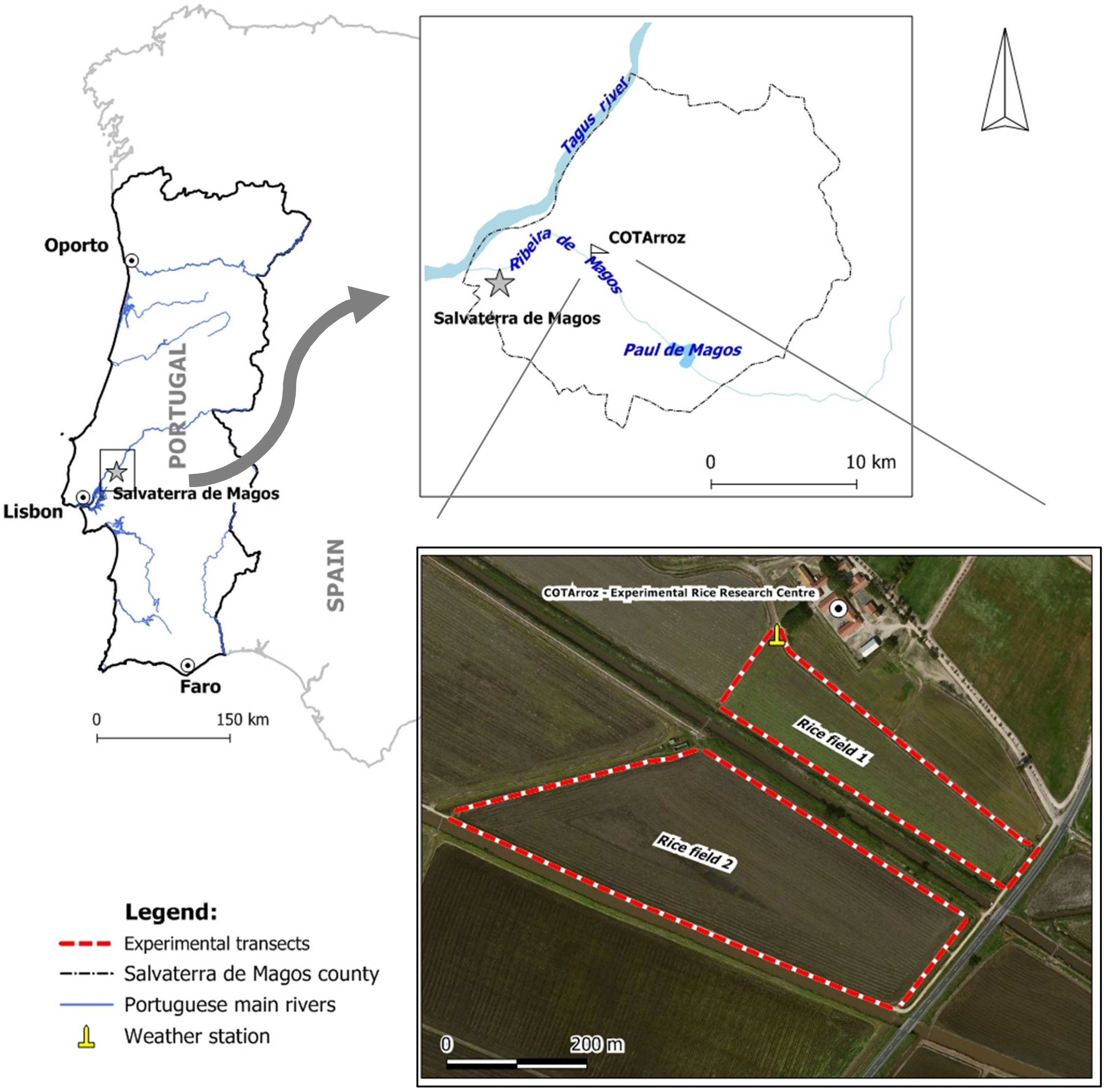

Figure 1. The study area at the national, regional and local context.

Overland dispersion (movements outside of the water) of *P. clarkii* was monitored over the course of one year, from March 2006 until April 2007. During this period, 46 visits to the study area were conducted with a minimum frequency of every two weeks, during periods with and without precipitation. An effort was conducted in order to visit the experimental area whenever precipitation occurred.

Overland Dispersal Experimental Protocol

We set up two sets of four transects each (Figure 1). Each set corresponded to non-paved roads around the rice pad (Figure 1). A total of 1225 meters were monitored (510 meters surrounding one rice pad and 715 m surrounding the other rice pad). On each visit, all transects were checked by one observer at three different periods of the day, early morning (7 – 9 a.m.), afternoon (1 – 3 p.m.) and at dusk/night (7 – 9 p.m.). On rainy days, sampling started just after the rain stopped or was substantially reduced. The observer walked on the centre of the road, covering a width of 1 meter (0.5 meters to each side of the observer) registering all the crayfish observed (moving or standing outside of water). Dead crayfish or exhibiting evident signs of predation were not recorded.

For each individual, we recorded the following data: gender, state of maturation (form I male, mature female, form II male and immature female), fresh weight (using a portable balance; ±0.01g) and carapace length (CL) – distance from the tip of the rostrum to the end of the carapace – (using a digital calliper; ±0.01 mm). All females carrying eggs or hatchlings attached to the abdomen were registered. Monitored transects were classified according to the type of surface cover in terms of the resistance to free movement by individual crayfish. The 3 categories were: (1) open transect; (2) transects with uniform and sparse vegetation; and (3) transects with a dense line (25-30 cm wide) of vegetation in the middle only, due to utilization by agriculture vehicles. The general direction of the crayfish movement was also recorded as moving *along* the transect or *crossing* the transect.

Whenever a group of crayfish were observed exiting the water, on a spot, at frequencies above 5 ind.min^{-1}, we named this an *Overland Dispersal Event* (ODE). The ODE location was registered with the help of a handheld GPS (Garmin® GPSMAP 60) and the following parameters were recorded: number of crayfish exiting the water during a period of 5 minutes, composition of the dispersing population (gender, state of maturation), direction of the movement, and general characteristics of the site of exit.

Between 22th – 24th September 2006 an attempt was made to track the movement of individual *P. clarkii* dispersing overland. From 9 a.m. to 4 p.m., 50 individuals, exiting the water drainage channel, were marked on the dorsal carapace with Dykem® markers (Ramalho *et al.* 2009). The marking process was executed as quickly as possible, fresh weight and CL immediately determined and the individuals were released at the same position and location where they were collected. The path of each individual was followed by one observer. The position, distance and direction of the movement of each individual were recorded every 5 minutes during a maximum of one hour, from a location that did not disturb the crayfish. The exact location was recorded every 5 minutes using a small coloured flag and after the terminus of the experimental period (generally after the individual returned to the water channel or the observer lost visual contact) the distance and direction of travel at each 5 min interval was registered and the average speed of crayfish movement overland was determined. During the experimental period we also occasionally recorded noteworthy observations in relation to crayfish behaviour.

Characterization of the resident crayfish population

To access the characteristics of the *P. clarkii* resident population, relative density (individuals.trap.hour^{-1}) in the water drainage channels was determined on a fortnight basis using crayfish traps baited with fresh sardines (Beecher and Romaire 2010). Crayfish density in the rice pads was not evaluated due to rice cultivation restrictions. Regular sampling of the drainage ditches was also performed with a handheld dip net (65 cm x 40 cm frame; 3 mm mesh size) to monitor the presence/absence of juveniles in the population. However, juvenile crayfish densities were not determined since crayfish overland dispersal was observed only on adults. In the laboratory, each adult specimen was weighted to the nearest 0.001 g, and the CL was measured to the nearest 0.01 mm, using a digital calliper. For individuals with a CL longer than 13 mm, gender and state of maturation was verified by the presence of developed gonopodia (Suko 1953; Guerra and Niño 1996).

Physical characterization of the Study Site

Environmental variables were recorded in a permanent meteorological station located in the study area (Figure 1). The meteorological station recorded continuously the following parameters: air temperature (±0.01 ºC), leaf wetness (0-10), air relative humidity (%) and precipitation (±0.1 mm). Two ONSET® Hobo water temp pro data logger (±0.2 ºC) one located in the main water channel and the other buried 20 cm in the soil of a rice pad, recorded the water and soil temperature, respectively, every 30 minutes. Water dissolved oxygen, conductivity and pH were recorded, with a portable multi-parametric probe (WTW® multiline F-set P4), in both rice pads and drainage channel in each visit to the field. The water depth (rice pads and drainage channels) was also registered by the observer during every visit to the field.

Data analysis

Data were compiled and analysed using the SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). Parametric and non-parametric statistics were used (McDonald 2009; Zar 1996). Because crayfish overland dispersal was observed only between 21/09/2006 and 16/11/2006, and the study area was completely flooded on 29/11/2006, the relation between the number of crayfish moving overland and the environmental variables: daily precipitation, relative air humidity and days after drainage of the study area, were determined for the period 14/09 to 29/11/2006, except when otherwise stated.

Values of the environmental variables (DO_2, pH and water conductivity) collected in the rice pads and drainage channels were compared by means of a Wilcoxon signed ranks test in which the pairs consisted of the values of the variable at both rice pads and drainage channels at each date. The maturation state/gender proportions of crayfish observed moving overland were compared for significant differences with the proportions of the crayfish resident population captured in the drainage channels using a Chi-square test of independence, and a post-hoc analysis based on the standard residuals (converted to a z-score) was used to determine which groups were significantly different. The size of the standardized residuals (converted to a z-score) were compared to the critical values (+/-1.96) that corresponded to an α of 0.05. To compare the number of crayfish moving overland versus the period of the day a Friedman test was used followed by a post-hoc analysis with Wilcoxon signed-rank tests, with a Bonferroni correction applied resulting in a significance level set at $P<0.017$. Differences between overland dispersing crayfish gender/state of maturation were accessed using the non-parametric Kruskal-Wallis test followed by the Tamhane’s T2 post-hoc test. When analysing the average speed of crayfish moving overland *vs* transect type the non-parametric Kruskal-Wallis test was used followed by the Tamhane’s T2 post-hoc test. Differences between number of crayfish moving *along* or *crossing* each transect type were determined by means of a binomial test.

To determine the influence of the variables analysed on the crayfish overland dispersal, the number of crayfish moving overland was organized by classes of each variables. Variables were divided in the following classes: water level in rice pads and water level in drainage channels – 10 cm classes; days after drainage of study area – 20 days classes; relative air humidity – 5% classes; water temperature and soil temperature – 2ºC classes. The median and the 25% and 75% percentiles of each variable class were determined and the results plotted. For each variable, a median test was applied to determine if there were significant differences in the proportions of the classes (α =0.05).

Results

Environmental Variables

We found no statistical differences between rice pads and drainage channels water regarding the following variables: dissolved oxygen (DO_2) ($Z= -0.674$, $n=41$, $P>0.05$), pH ($Z= -0.474$, $n=41$, $P>0.05$) and conductivity ($Z= -0.497$, $n=41$, $P>0.05$). Average values of DO_2 were 4.92 mg.l^{-1} (±1.18 s.e., min: 1.53 mg.l^{-1}, max: 7.05 mg.l^{-1}) – approx. 55±14 % – average pH was 6.98 (±0.46, min: 6.17, max: 7.54) and average conductivity was 736.24 µ. cm^{-1} (±197.65 s.e., min: 521.33 µs cm^{-1}, max: 1063.86 µs.cm^{-1}).

Total precipitation varied on a monthly basis. The most rainy months where December, November and October of 2006 and the driest months were July-06, August-06 and March-07 (Figure 2). January of 2007 was the coldest month with an average air temperature of 8.5 ºC (min: –1.0 ºC; max: 15.1 ºC) (Figure 2). The lowest air temperature was registered during December 2006 (–1.7 ºC) and the highest was registered in July 2006 (31.6 ºC) (Figure 2).

The hydrological cycle of the study area was almost exclusively regulated by the rice culture practices. However, high rain events had a significant influence on the water level recorded both in the rice pads and drainage channels, including the seasonal floods of the whole study area (Table 1, Figure 3A). The hydrology of the study area was divided in two phases: **1** – Constant water level, artificially regulated during the rice culture (April – September) with an average water depth of roughly 50 cm and 29 cm, in drainage channels and rice pads, respectively (Table 1); **2** – Irregular water level, characterized by drainage of the area and consequent abrupt water level reduction. This is characterized by a progressive hydrological stress interrupted by the high sporadic precipitation events that constituted an important input of water, especially during the hot and dry summer months (Table 1). During this period the average depth of the drainage channels was reduced to 27.43 cm (±3.99 s.e; min: 0 cm; max: 75 cm, when all the experimental area was completely flooded) and reduced to 7.74 cm (±2.30 s.e.) in the rice pads. However, if the rainiest months are not taken into account, the mean water depth is reduced to approximately 11 cm and 1.45 cm in drainage channel and rice pads, respectively (Figure 3B).

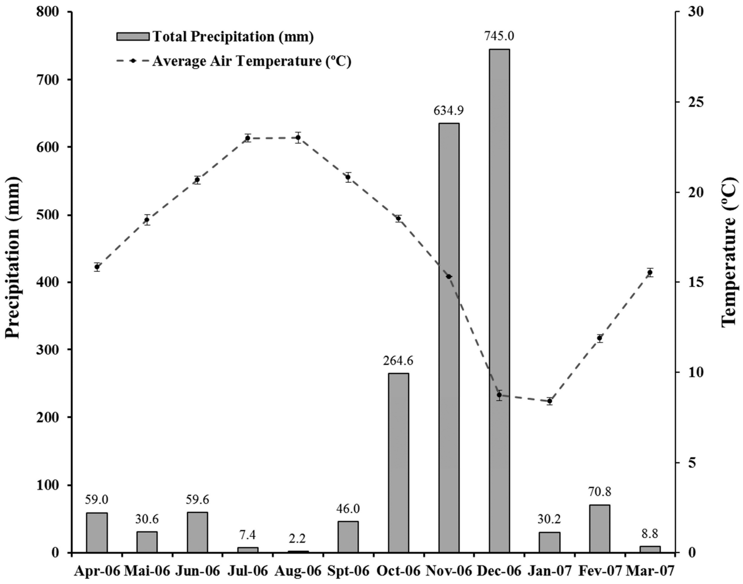

Figure 2. Total monthly precipitation and monthly average air temperature during the study period. Vertical bars represent standard errors.

Water and soil temperature were strongly correlated with air temperature (water temperature: r=0.94, *P*<0.001 / soil temperature: r=0.96, *P*<0.001). Overall average water temperature was 18.64 ºC (±0.27 s.e.), varying from a maximum of 25.71 ºC (±0.27 s.e.) in July, to a minimum of 10.64 ºC (±0.24 s.e.) in January (Table 1). The maximum water temperature recorded was 29.24 ºC in August and the minimum was 4.69 ºC in January. Overall average soil temperature was 17.79 ºC (±0.30 s.e.), varying from a maximum average temperature of 25.68 ºC (±0.17 s.e.), in August, and a minimum of 9.17 ºC (±0.26 s.e.) in January (Table 1). The maximum soil temperature recorded was 33.03 ºC in July and the minimum was 5.64 ºC in January. It is worth to be noted that during the period comprised between September and November (*i.e.* when overland crayfish dispersal was recorded) the difference between the maximum and minimum daily temperature of both water and soil increased (Figure 3C and Figure 3D). This phenomenon started immediately after the drainage of the study area and finished after the seasonal flooding, (Figure 3C and Figure 3D).

Overall average air relative humidity (RH) was 69.99 % (±1.04 s.e.), ranging from a maximum average RH of 87.40 % (±4.21 s.e.), in November, and a minimum of 22.75 % (±19.48 s.e.) in December (Table 1). With the exception of December, average RH varied between 65% and 87% and a strong variability was recorded on a daily basis. The leaf wetness index (LW) recorded varied on a daily basis from a minimum of 0, during daytime, to a maximum of 10, especially during the night.

During the period from 13/09/2006 to 16/11/2006 daily photoperiod decreased steadily, from 752.8 minutes (12.5h) to 600.5 min. (10.0h), respectively.

Table 1. Water depth, water and soil temperature and relative humidity recorded in the study area. The rice culture stages are shown. An asterisk marks the months when crayfish overland dispersal was observed. s.e. – standard error.

Rice Culture Stage	Month/ year	Water Level (cm) Drainage channels		Water Level (cm) Rice Pads		Water Temperature (ºC)		Soil Temperature (ºC)		Air Relative Humidity (%)	
		Min	Max	Min	Max	Mean	s.e.	Mean	s.e.	Mean	s.e.
• Rice sown	Apr. 06	50	51	30	31	18.92	0.25	17.03	0.28	76.28	1.39
• Fields flooded	May 06	49	52	29	31	21.18	0.31	20.45	0.48	65.06	1.41
• Fields flooded	Jun. 06	49	51	29	30	23.99	0.21	22.60	0.27	70.52	1.73
	Jul. 06	49	48	28	29	25.71	0.27	25.00	0.38	68.60	0.99
	Aug. 06	48	48	27	28	24.03	0.21	25.68	0.17	65.98	1.45
• Field drainage started 13/09/06	Sep. 06*	0	27	0	27	22.28	0.27	22.50	0.53	74.67	1.51
• Rice harvesting	Oct. 06*	2	50	0	15	19.83	0.18	18.62	0.13	82.15	1.28
• Drainage channels “open”. Fields and drainage channels “dry” except when precipitation occurred.	Nov. 06*	2	75	0	55	17.39	0.26	16.20	0.32	87.40	0.77
	Dec. 06	60	75	35	55	11.72	0.37	10.34	0.48	22.75	3.50
	Jan. 07	17	60	0	35	10.64	0.24	9.79	0.26	70.05	5.04
	Feb. 07	14	14	0	1	12.74	0.21	11.84	0.32	86.07	0.90
• Soil preparation	Mar. 07	14	20	0	0	14.94	0.21	13.03	0.12	72.86	1.64
Total Average		---		---		18.64±0.27		17.79±0.30		69.99±1.04	
Average during artificial flooding: April – September		49.58±0.36		28.67±0.45							
Average after fields drainage: September – March		27.43±3.99		7.74±2.30							

Characterization of the resident crayfish population

During the period between September and December of 2006 the first record of juvenile crayfish individuals was in 03/11/2006 with an average of 4.37 juv.m^{-2}, a mean CL of 8.59 mm (±0.69 s.e.) and a mean fresh weight of 0.17 g (±0.37 s.e.). During this period no other captures of juvenile crayfish occurred in the regular dip-net samplings. We estimate that only one recruitment event occurred during the period comprised between mid-October and November.

The average *P. clarkii* relative density varied from 0.64 ind.trap.hour^{-1} (April 2006) to 10.03 ind.trap^{-1}.hour^{-1} (July 2006), the maximum recorded during the experimental period. From July to the end of August crayfish relative density decreased steadily to 3.3 ind.trap^{-1}.hour^{-1}. From the last week of August to the first week of September the density increased again to 7.5 ind.trap.hour^{-1}. Between September to December the relative density of the crayfish population in the drainage ditches decreased from 7.5 to 1.3 ind.trap^{-1}.hour^{-1} (Figure 3E). During the same period, the crayfish population in the drainage ditches was dominated by Form I males (50.5 % of the captured crayfish), followed by mature females (22.2 %). The form II males and immature females represented only between 12.1 % and 15.2 %, respectively, of the crayfish captured with the baited traps (Figure 4).

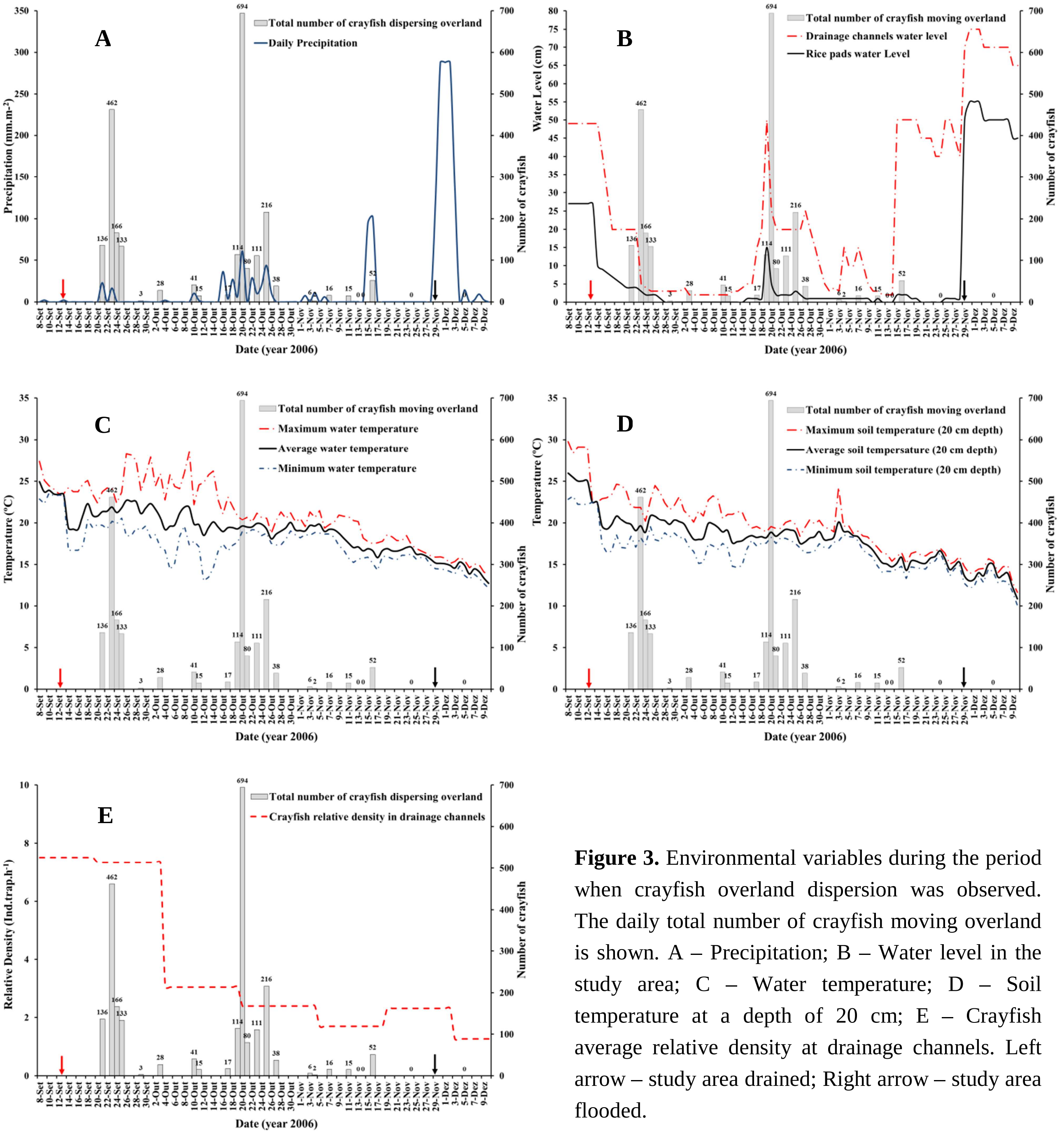

Figure 3. Environmental variables during the period when crayfish overland dispersion was observed. The daily total number of crayfish moving overland is shown. A – Precipitation; B – Water level in the study area; C – Water temperature; D – Soil temperature at a depth of 20 cm; E – Crayfish average relative density at drainage channels. Left arrow – study area drained; Right arrow – study area flooded.

It's important to mention that, during the period following the drainage of the study area, between September and November, due to the drastical decrease of the water levels in drainage channels and rice pads, crayfish were observed to gather at high densities in the shallower areas that preserved water for a longer period. These areas were mainly depressions on the drainage channels and cement boxes within the irrigation system. Frequently, these locations did not allow for crayfish escape and it was usual to observe very high densities (*e.g.* hundreds of ind.m^{-2}) trapped inside.

Crayfish overland dispersion

During the whole experimental period, crayfish were only observed dispersing overland after the rice fields were drained, between 21/09/2006 and 16/11/2006. Local farmers confirmed the first observation of crayfish overland dispersal in the 13/09/2006. This was the first report of overland movements for the season at the studied area. It was concomitant with the drainage of the study area and with a precipitation event that started at the end of the afternoon (5 p.m.). The first events of crayfish overland dispersion occurred after a period of 21 days without precipitation. During the field work, the longest period without precipitation (43 days), occurred from 03/07/2006 to 13/08/2006 and despite some precipitation (2.2 mm.m^{-2}) between 14 and 16/08/2006 no crayfish were recorded dispersing overland. The last event of crayfish overland dispersion was coincident with the first peak of high precipitation (102.2 mm.m^{-2} in one day – 16/11/2006) which resulted on the reestablishment of the normal water height on the drainage channels.

A total of 2345 individuals were registered dispersing overland between 21/09/2006 and 16/11/2006. Approximately 82% of the crayfish registered dispersing overland were mature individuals while 18% were immature crayfish (Figure 4). The maturation state/gender proportions observed dispersing overland were different from the proportions observed in the drainage channels (χ^2=114.426; df=3; P<0.001). The standard residuals of the Chi-square test (converted to a z-score) were smaller than the critical value (-1.96) for all the maturation state/gender groups, from which we may state that all the group proportions moving overland differ from the ones in the drainage channels. Based on the analysis of the standard residuals we may state that the proportion of Form I males, Form II males and immature females involved in overland dispersion are significantly smaller than the correspondent proportion present in the drainage channels, while the proportion of mature females registered dispersing overland were significantly higher than the correspondent proportion present in the drainage channels (Figure 4). Of the total number of mature females observed dispersing overland, 32 females (4.55%) were carrying eggs and 57 (8.11%) were transporting juveniles. Most of the eggs carried by the dispersing females presented a dark brown colour. Of the crayfish dispersing overland, Form I males presented the highest average fresh weight (22.47 g ±0.42 s.e.) and immature females the lowest average fresh weight (11.59 g ±0.62 s.e.) (Table 2).

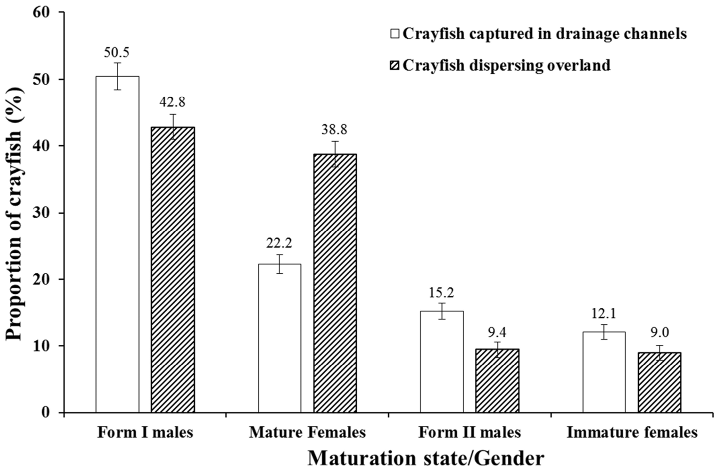

Figure 4. Proportion of crayfish captured in the drainage channels (resident population sampled with baited traps) and the proportion of crayfish registered dispersing overland, ordered by gender and state of maturation. Vertical bars represent 95% confidence limits.

Table 2. Average crayfish fresh weight recorded for the crayfish resident in the drainage channels and for the crayfish observed dispersing overland. Tukey non significantly different groups have the same letter superscript. FW – fresh weight. s.e. – standard error.

maturation/Gender	Drainage channels		Moving overland	
	Average FW (g)	s.e.	Average FW (g)	s.e.
Form I males	27.24	0.37	22.47	0.42
Mature females	23.58	0.52	18.89	0.38
Form II males	15.03	0.58	11.61[a]	0.75
Immature females	19.92	0.57	11.59[a]	0.62

Figure 5 shows the average number of crayfish observed dispersing overland in relation to the period of the day. There was a statistically significant difference in the number of crayfish dispersing overland among periods of the day (Friedman test: χ^2=19.520; df=2; *P*<0.001). There was no significant differences between the number of overland dispersing individuals recorded during the sunrise and the afternoon period (Wilcoxon signed rank test: Z=-1.374; df=1; *P*=0.169). Half (50.33%) of the overland dispersing individuals were recorded during the dusk/night period while only 19.18% were recorded during the afternoon and these two groups were statistically different (Wilcoxon signed rank test: Z=-3.582; df=1; *P*<0.001). 30.49% of the overland dispersing individuals were registered during the sunrise period and this group was significantly different from the dusk/night period (Wilcoxon signed rank test: Z=-2.839; df=1; *P*<0.01). Regarding the afternoon period, 91.9% out of the total crayfish observed in overland dispersion were registered in days when some precipitation occurred during the afternoon (at least 0.4 mm of precipitation).

It's important to mention that some crayfish dispersing overland were registered in 6 days without precipitation. From the total crayfish registered moving overland in these six days without precipitation (n=233), 82.8% were during the dusk/night sampling period, 12.0% during the sunrise period and only 5.2% during the afternoon sampling period. It is worth to be noted that, in these six days, the crayfish were observed dispersing overland during the afternoon period on a unique event (07/11/2006). During this day, the RH was always above 70% (while in the other five days the minimum RH was never above 53%) and the average LW was 6.3, opposed to the other 5 days when the average LW was always 0 during the afternoon sampling period. In sum, the 07/11/2006 sampling day was a foggy and moist day, with lower air temperatures, in comparison to the other five days without precipitation, with a maximum air temperature during the afternoon sampling period of 21.6ºC.

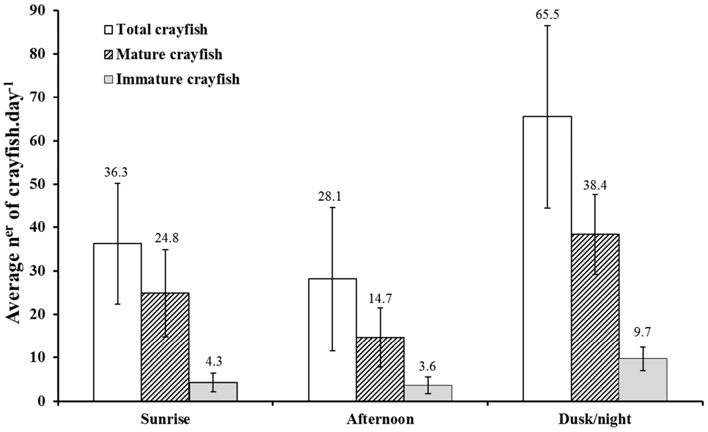

Figure 5. Average number of crayfish observed dispersing overland versus the sampling period and crayfish state of maturation. Vertical bars represent the standard error of the mean.

In all sampling periods (sunrise, afternoon and dusk/night), the average number of mature individuals was higher than the average number of immature individuals (Figure 5). With the exception of the afternoon period, the average number of form I males was higher than the average number of females. The number of crayfish dispersing overland differed among gender/state of maturation (Kruskal Wallis: χ^2=18.303; df=3; P<0.01), and the Tamhane's T2 post-hoc test showed no statistically significant differences between the number of mature crayfish (males vs females) but revealed statistically significant differences between form I males and both Form II (P<0.01) males and immature females (P<0.01). Tamhane's T2 post-hoc test showed statistically significant differences between mature females and form II males (P<0.01) and immature females (P<0.01).

The number of crayfish registered dispersing overland was inversely related with the water level in the rice pads (Pearson Correlation Index= -0.303; n=46; $P<0.05$). In fact 94.6% of the total crayfish dispersing overland were observed when the rice pads water lever was below 6 cm. The remaining 5.4% of the dispersing overland crayfish were observed on a single event when the rice pads water level was 15 cm (Figure 6A). 37.1% of the total dispersing overland crayfish were observed when the drainage ditches water level was below 6 cm. 54.2% of the crayfish dispersing overland were observed when the water level was comprised between 5 and 25 cm) (Figure 6B). The medians of the number of individual crayfish dispersing overland were not the same across the water levels in the rice pads (Median Test: $\chi^2=21.057$; df=5; $P<0.01$) and were also not the same across the water levels in the drainage ditches (Median Test: $\chi^2=20.691$; df=6; $P<0.01$). Crayfish overland dispersion was related with the drainage of the rice pads since we only observed crayfish dispersing overland after this event took place. Most of the crayfish dispersing overland (77.1%) were observed during a maximum of 35 days after the fields have been drained, and 41.3% were observed up to a maximum of 25 days after this event (Figure 6C). The medians of the number of crayfish dispersing overland were not the same across the number of days after the drainage of the study area (Median Test: $\chi^2=22.000$; df=9; $P<0.01$). Crayfish were only observed dispersing overland when the average RH was above 70%. 99% of the total crayfish were recorded dispersing overland when the average RH was between 75 and 90 % (Figure 6D). The medians of the number of crayfish dispersing overland were not the same across the RH classes (Median Test: $\chi^2=24.438$; df=11; $P<0.05$). In respect to water temperature, crayfish were observed dispersing overland only when this variable was comprised between 16.34 and 24.17 ºC. 66.2 % of the total crayfish were observed dispersing overland when the average water temperature was comprised between 20 and 22 ºC (Figure 6E) and the medians of the number of crayfish moving out of the water were not the same across the average water temperature classes (Median Test: $\chi^2=51.374$; df=8; $P<0.001$). Crayfish overland dispersion was recorded only when the minimum soil temperature was higher than 15.1ºC. 94.5% of the total individuals recorded were observed when the minimum soil temperature was comprised between 18 and 22ºC (Figure 6F) and the medians of the number of crayfish moving out of the water were not the same across the average soil temperature classes (Median Test: $\chi^2=50.736$; df=11; $P<0.001$).

During the experimental period we observed two peaks of crayfish overland dispersal and the phenomenon seems to be highly related with rain events (Figure 2A). In fact, there is a significant relation between the number of crayfish registered moving overland and daily precipitation ($r^2=0.65$; $P<0.001$) and the linear regression line is shown in Figure 7.

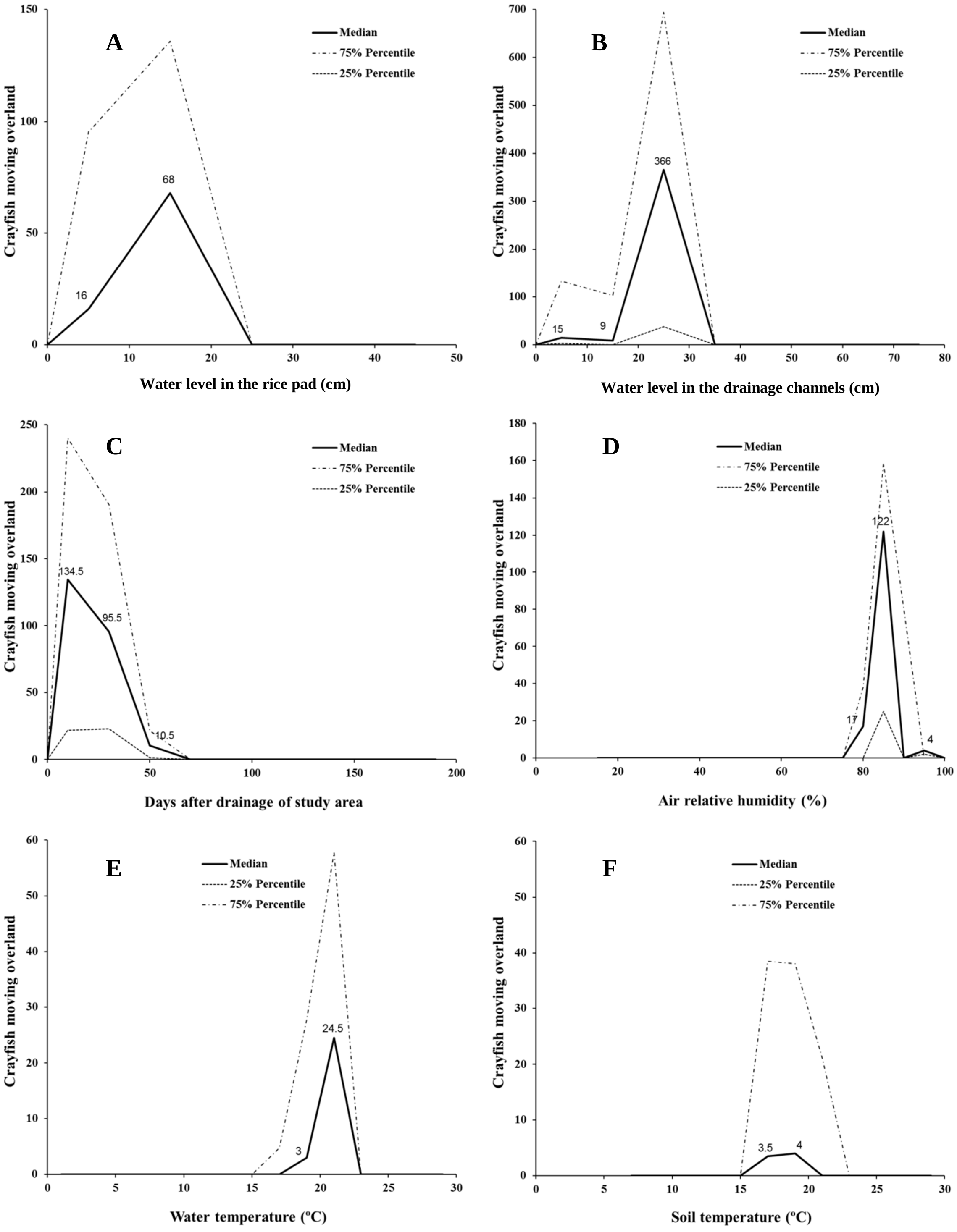

Figure 6. Number of crayfish observed dispersing overland in relation with: A – water level in the rice pads; B – water level in the drainage ditches; C – number of days after the beginning of the drainage of the study area; D – air relative humidity; E – water temperature and F – soil temperature (20 cm depth).

After the fields were drained, and during some period after the heavy rains, large numbers of crayfish were observed moving upflow. During these events, water was frequently observed flowing out of the rice pads trough small drainage openings and crayfish were observed exiting the water at the location where water was flowing with more intensity. Even when no previous heavy rains were observed, it was frequent to observe crayfish moving in the opposite direction of the water flow. In some cases, crayfish were observed using crayfish burrows to *detour* around the strong water flow, moving in large numbers upstream, lining up to use the burrow channel. It was also frequent to observe crayfish following path lines, using the paths others had used to detour obstacles or vegetation.

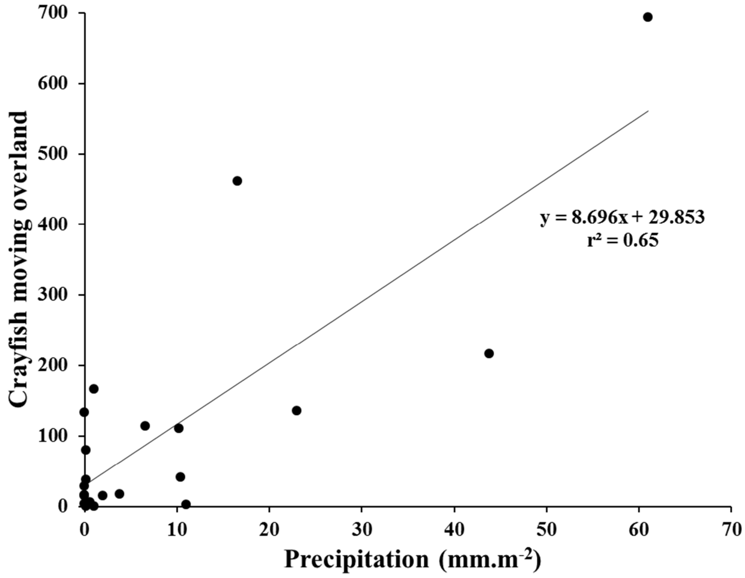

Figure 7. Number of crayfish observed dispersing overland in relation with daily precipitation. The value corresponding to day 16/11/2006 was considered an outlier (precipitation higher than 102 mm.m^{-2}). Only data referring to the period between 13/09/2006 and 29/11/2006 are included.

Between 21/09 and 16/11/2006 four ODE locations were registered and a total of six ODE events were observed and recorded. A maximum of 123 crayfish were registered in a single ODE event (ODE 1), with a total of 24.6 ind.min^{-1} and the densities of the population dispersing overland were up to 25.7 ind.m^{-2} (Table 4). The recorded ODE events were coincident with the days when the largest number of crayfish was observed dispersing overland (23/09/2006, 10/10/2006 and 25/10/2006). ODE 1 and 4 locations presented similar characteristics, considering that they were the upstream end of a drainage channel. At these locations crayfish were observed climbing the dirt walls of the channel, near the water entry points. The ODE 2 and 3 were located at a corner of a rice pad, with the only difference that ODE 3 consisted of an exit point of the water from the rice pad and ODE 2 consisted of an upper corner of the rice pad. No significant differences were observed between the ODE crayfish population structure and crayfish moving overland. All these events occurred in rainy days, with total precipitation comprised from 16.6 up to 61 mm.m^{-2} (Table 4).

Table 4. Overland dispersal events (ODEs) observed and recorded from 13/09 to 16/11/2006.

ODE id	Date	Precipitation ($mm.m^{-2}$)	Frequency of exits from water ($ind.min^{-1}$)	Density of overland moving population ($ind.m^{-2}$)
ODE 1	23-09-2006	16.6	18.5	17.6
	20-10-2006	61	24.6	25.7
ODE 2	20-10-2006	61	5.0	6.2
ODE 3	20-10-2006	61	6.1	5.4
ODE 4	20-10-2006	61	16.4	17.1
	25-10-2006	43.8	10.3	11.6

When analysing crayfish movement in relation to the transect characteristics we observed that there were no significant differences between crayfish moving along or crossing the clean transects (Binomial test: N=140; $P<0.05$) (Figure 8). Results indicated that the proportions of crayfish moving along the transect and crossing the transects were not equal either for both transects with sparse or uniform vegetation (Binomial test: N=550; $P<0.001$) and transects with obstacles in the middle (Binomial test: N=906; $P<0.001$) (Figure 8). At transects with obstacles in the middle the majority of the crayfish (72.69 %) were observed moving along the obstacle. We observed that crayfish don't have a preferential movement direction, but rather move in a random direction, changing it when finding an obstacle in the way.

Regarding the sample of 50 crayfish followed by the marking procedure, individuals appeared to maintain the general direction they had before being marked, in general moving away from the point of origin. Crayfish were observed moving at a maximum speed of 1.48 $m.min^{-1}$, (*i.e.* 90 $m.h^{-1}$). Form I males presented the highest average speed (0.77 $m.min^{-1}$ ±0.12 s.e.) and differences between Form I males, mature females and immature crayfish were significant (Kruskal-Wallis test: $\chi^2=10.593$; df=2; $P<0.01$). Two experimental crayfish were observed during 50 min, and a total of 11 crayfish were observed during a minimum of 20 minutes. A total of 38 crayfish were observed returning to the drainage channel (place of origin), after moving overland, on average, 8.5 meters. The maximum distance a crayfish was observed moving overland was 21.4 meters. One individual was observed struggling during 25 minutes with the dense vegetation located at the centre of the study area, and it was the only individual to be observed to completely overcome the obstacle. All the other individuals that were observed in the same situation changed the movement direction. One mature female was observed burying in the adjacent rice pad, after crossing the dirt road.

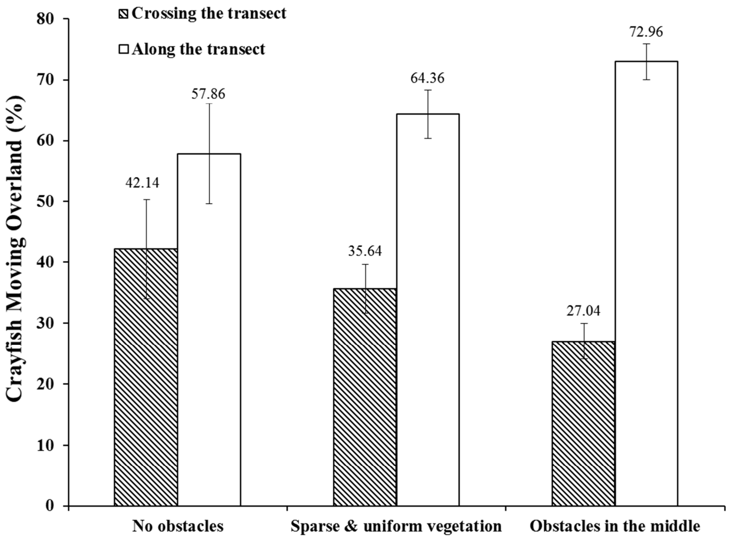

Figure 8. Proportion of crayfish moving overland across or along the transect for 3 degrees of *resistance* to crayfish movement (N=1596). Vertical bars represent 95% confidence limits.

Table 5. Data obtained from monitoring 50 crayfish moving overland. Avg. = Average; CL = Carapace length; FW = Fresh weight; s.e. = Standard error.

	N	Avg. CL (mm) ±s.e.	Avg. FW (g) ±s.e.	Avg. Speed ($m.min^{-1}$) ±s.e.		
				Total	"Clear Path"	"Path with obstacles"
Form I Males	21	42.72±1.73	22.59±2.15	0.77±0.12	0.97±0.07	0.28±0.06
Mature Females	19	45.24±1.27	19.37±2.15	0.34±0.07	0.62±0.09	0.09±0.03
Immature	10	38.49±1.10	13.24±0.75	0.25±0.05	0.42±0.10	0.15±0.04

Discussion

In the present study, *P. clarkii* dispersed overland only within specific environmental conditions and overland dispersal occurred after the drainage of the rice fields. It is important to notice that the drainage of the study area took place during the summer season, right after the air temperature reached the annual maximum, after a prolonged period without precipitation and when the crayfish densities in the drainage channels were the highest recorded. In this area, *P. clarkii* shows several spawning/recruitment events during the year (Correia 1995a,b; Anastácio *et al.* 2009). In fact, we found that overland dispersal was coincident with one of the annual spawning peaks since the first record of juveniles occurred in early November and some of the overland dispersing females were carrying eggs and juveniles. There was also a significantly higher proportion of mature individuals overland than in the water and this could be an indication that overland dispersal is related with the reproductive cycle. In fact, there are indications that the bulk of crayfish recruitment in Portuguese rice fields and freshwater marshes generally occurs during the autumn (Anastácio and Marques 1995; Correia 1995a,b; Correia and Bandeira 2004) after the first periods of rain. Similar results have been referred by other authors for Spain and Italy (Gutiérrez-Yurrita *et al.* 1999; Ligas 2007; Gherardi 2006; Alcorlo *et al.* 2008). However, several factors indicate that, although a post-reproductive dispersal event could partially explain this phenomenon, reproduction may not be the principal factor inducing crayfish to disperse overland. *P. clarkii* has several recruitment events in Paul de Magos during an annual cycle (Anastácio *et al.* 2009) but no crayfish overland dispersion was observed except during the period between September and November. At this location, previous studies by Anastácio *et al.* (2009) showed that this period follows a peak of the female maturation index and presents the highest proportions of Form I males. The dispersal of large numbers of Form I males (almost 43% of the population) could be explained as an opportunity to find new mates and increase the reproductive success. Nevertheless, the presence of immature individuals, representing 18% of the individuals dispersing overland, makes less sense from a reproductive point of view. The fact that almost 20% of the overland dispersing populations was composed of immature individuals is intriguing and must be an indication of another strong reason, besides reproduction, for crayfish to exit the water. Moreover, in Louisiana crayfish production ponds, the major pulse of recruitment occur in autumn after the ponds are reflooded (McClain 2010), but overland dispersal of *P. clarkii,* is not frequently observed in these areas (McClain W.R., personal communication). The overland dispersion was coincident with the reproductive onset of *P. clarkii* in the study area. Between September and October, there is a reduction in the proportion of females in the population (Anastácio *et al.* 2009), preceded by a peak of the female maturation index (Guerra and Niño 1996; Alcorlo *et al.* 2008; Anastácio *et al.* 2009). This is compatible with a tendency for mature females to spend most of the time in burrows (Anastácio *et al.* 2009) and may be particularly relevant for the survival of females carrying eggs and juveniles by reducing the risks of desiccation and intra- and inter-specific predation (Holdich 2002). Moreover, in this area, the maximum proportion of form I males in the population is high from September to January (Anastácio *et al.* 2009). Only 13% of the females recorded dispersing overland were carrying eggs or juveniles, but the remaining mature females could be previously fecundated and ready to spawn in burrows. *P. clarkii* females that disperse overland may therefore enhance the species invasibility since they may eventualy release their offspring in a more favourable habitat patch and/or at a new uncolonized suitable area.

Our results provided insights about the environmental conditions favourable to *P. clarkii* overland dispersal. This phenomenon is highly related with the drainage of the rice fields, considering that almost 80% of the overland dispersing individuals were recorded during the first 35 days after the drainage of the study area. Consequently, overland dispersion is inversely related with the water level. The reduction of water levels to nearly zero should increase individuals stress and induce burrowing behaviour or should alternatively lead to overland dispersion. Other authors have reported that crayfish activity (underwater) increases with the variation of the water level. Hazlett *et al.* (1979) reported that drops in water level of 5-26 cm caused increased activity, and levels raised 6-29 cm caused decreased activity of the stream-dwelling *Orconectes virilis*, although dispersion was not induced. The hydrological and intra-specific stress in the study area may be the trigger for the crayfish overland dispersal, although crayfish are expected to burrow when water levels drop. The drainage of the rice fields should constitute an extreme alteration in the habitat conditions of the resident population, just as in a temporary freshwater marsh (Correia 1995a), or a Mediterranean temporary stream (Aquiloni *et al*. 2005). *P. clarkii* population density in the drainage channels, at the time of the drainage of the study area, was the maximum recorded during the study period. With the reduction of the water level it is expected that the intra-specific stress increases greatly because crayfish are confined to small areas, with high levels of intra-specific interactions at poor water quality conditions (Gherardi 2002). Although population densities decreased gradually from September to December we hypothesize that the water availability was a limiting factor and the resident crayfish population must have been submitted to a gradually increasing stress, aggravated by the input of individuals coming from rice pads to the drainage channels.Since the conditions were gradually becoming aggravated (*e.g.* reduction of water availability, increasing populations densities and intra-specific stress and decreasing of water quality), exiting the water could constitute a strategy to increase survival chances, assuming that overland dispersing individuals could find an area with more suitable conditions.

An overland dispersal strategy may be adaptive for survival in seasonal wetlands, although it could carry an increased risk in terms of survival. Survival in burrows during the dry season can be an effective strategy for surviving the adverse conditions in disturbed habitats with long hydro-periods. On other hand, survival in burrows can be dramatically reduced in disturbed habitats with short hydro-periods, where the ground water level may fall more than 1 meter (Acosta and Perry 2001). Considering that individual crayfish have no way to predict the duration of the dry period, the overland dispersal strategy could increase greatly the survival chances of a burrowing species when facing an abrupt change in the habitat conditions. Based on these assumptions we suggest that overland dispersing crayfish are responding to an abrupt change in the habitat conditions, posing serious threats to its survival, and a proportion of the resident population exit the water searching for an area with more suitable habitat (*e.g.* higher water levels, lower population densities, more suitable areas for burrowing). This is valid also from the reproduction point of view, considering that females carrying their brood could increase the survival of their progeny if they release them in a more suitable habitat.

Approximately half of the crayfish dispersing overland were registered during the night sampling period. Crayfish activity is higher during the night although it may also be active during the daytime (Gherardi and Barbaresi 2000; Gherardi *et al.* 2000; Aquiloni *et al.* 2005). Higher nocturnal underwater decapod activity is usually considered adaptive, being associated with lower predation risk from diurnal predators (*e.g.* birds and fishes) (Aquiloni *et al.* 2005) although in Portugal there are also some nocturnal crayfish predators (*e.g.* mammals) (Beja 1996a,b). While predation may also play an important role in overland dispersal (it was frequent to observe heavy bird predation over dispersing individuals), it is our opinion that the major factors involved in the nocturnal overland dispersion are related to the favourable values of the environmental variables (higher dew, fog and moist) that enhance crayfish survival. This assumption is supported by the high percentage of individuals dispersing overland in the sunrise/morning period, that also generally gathers the more favourable environmental conditions for the overland dispersion and by the fact that the majority of the individuals dispersing during the afternoon period were observed when precipitation events occurred.

Overland dispersal of *P. clarkii* occurred only when the temperature was comprised between 16 and 24ºC. Approximately 65% of the total crayfish dispersing overland were observed when the water temperature was comprised between 20 and 22 ºC. *P. clarkii* underwater activity is positively related with water temperature (Gherardi *et al.* 2000; Gherardi and Barbaresi 2000; Barbaresi and Gherardi 2001; Aquiloni *et al.* 2005). On the other hand higher air temperatures can also reduce significantly crayfish survival out of water (Anastácio *et al.* 2010) because high temperatures increase crayfish desiccation rates. Survival time in dry conditions depends on relative humidity, temperature and individual size (Anastácio *et al.* 2010). Laboratory experiments have shown that *P. clarkii* can survive from 150 minutes (juveniles) to 990 minutes (adults) under dry conditions ranging from 16 to 24 ºC and 44 to 53% of relative humidity. The results of the present study indicate that *P. clarkii* overland dispersion is influenced by the relative humidity and that it is not expected to occur when this variable is below 50%. In fact, crayfish overland dispersal is more likely to occur when relative humidity exceeds 75%. The only observation of *P. clarkii* overland dispersal during the afternoon occurred when the relative humidity was higher than 70% (*e.g.* heavy fog) and the leaf wetness index was relatively high (considering that it is normally zero during the afternoon). Overland crayfish dispersal during days without precipitation, occurred mainly during the night and during the sunrise period, when the relative humidity or the leaf wetness index are usually high. Leaf wetness may be a good indicator of the crayfish overland dispersal occurrence and it measures the wetness of the plants leafs due to precipitation, dew and fog (Sivertsen 2005). Crayfish may function as a *leaf*, staying wet and surviving out of water with the proper environmental conditions. Crayfish may also stay wet when crossing wet vegetation. If the environmental conditions are adverse (*e.g.* strong and direct sun light, incidence of dry wind, high air temperatures and absence of precipitation, fog or dew) crayfish would dry out very quickly as soon as they exit the water. We suspect that crayfish are equipped with a mechanism to evaluate the environmental conditions out of the water and have adapted its behaviour in order to use the opportunity to disperse overland to colonize new areas or to find more suitable areas to increase their survival chances. This very well defined environmental window could be the key for the successful *P. clarkii* overland dispersal.

As a freshwater crustacean, *P. clarkii* respiratory system is highly dependent on water to function properly. Rain events and air humidity may allow crayfish to exit the water and keep the gills properly wet, at levels that allow the respiratory exchanges (Holdich *et al.* 2002). Crayfish have their gills protected by the exoesqueleton which provides extra protection during the periods of overland dispersion as long as they have the necessary humidity. Although most data in the literature is merely anecdotal, Penn (1943) reported that *P. clarkii* overland dispersal occurred during or after heavy rain storms. In fact, its overland movements seem to be associated with heavy rains after dry periods, spring flooding and low dissolved oxygen in the water (Holdich 2002). Our study clearly showed an association between the number of crayfish out of water and the amount of precipitation.

Overland dispersal events (ODEs) were associated with the terminal ends of drainage channels and the intersection of drainage channels were the water was confluent, creating rifles. These locations generally constituted an obstacle to the crayfish movement, especially when water within drainage channels was falling into deeper channels or when the water flow was too strong. At these locations *P. clarkii* was observed climbing the dirt walls or even using burrows to overcome obstacles, such as a small dirt wall or larger anthropogenic structures (*e.g.* damns) (Acosta and Perry 2001).In rice field areas *P. clarkii* was found up to 200 meters away from the water (Ramalho R.O., personal observation) but we believe that the species is able to cross much larger distances overland. This may lead us to think that *P. clarkii* have an objective of finding new suitable areas to colonize and not just simply to overcome obstacles to water flow. Nevertheless, by dispersing in the opposite way of the water flow they may increase the chances of finding new areas to colonize (Bruxelas *et al*. 2005) and reaching areas with more suitable environmental conditions for crayfish survival, particularly, more water availability.

Concluding remarks

We determined the main factors that induce crayfish to exit the water and the environmental conditions that restrict the crayfish overland dispersal. Crustacean migration and dispersal can be viewed as a specific adaptation to areas in which changes in habitat quality in different locations occur asynchronously so that movement allows the exploitation of temporary resources as they arise (Dingle and Drake 2007). Decapods dispersal under variable environmental conditions cannot be explained by simple mechanisms (Herrnkind 1983) and this paper intended to determine which variables where involved in *P. clarkii* overland dispersion. Overland dispersal may be an ancestral reminiscence of Decapods evolutive history (Bliss 1968) being intrinsically related with reproduction (*e.g.* search for new mates, juvenile recruitment). Decapods have evolved to adapt to inland freshwater environments and also to the terrestrial environment, but the overland dispersal only takes place under restricted environmental conditions, generally during the night and in days with precipitation and high relative humidity (Herrnkind 1983). These environmental conditions were clarified in the present study for red swamp crayfish, *Procambarus clarkii.* In our opinion the overland dispersion of *P. clarkii* is the result of several factors that *push* individuals to exit the water. Reproduction is, as stated above, one of the factors. Mature males may take the opportunity to find new mating females and increase their reproductive success. Reproductive females, especially ovigerous individuals or females carrying their brood, may release their offspring in a not colonized watershed, or simply, in a habitat with more suitable environmental conditions than the ones they inhabited previously. This is reinforced by the fact that hydrology is fundamental for the timing of release of the young and recruitment frequently occurs after water inputs into the system (*i.e.* after dry periods) (Gutierrez-Yurrita *et al.* 1999; Alcorlo *et al.* 2008; Anastácio *et al.* 2009). Because water is limiting the system, crayfish can only reproduce and grow during a certain period and therefore the population adapts to these conditions. The capacity to leave the water and conduct overland dispersion events will definitely contribute to the success of *P. clarkii* when invading new areas and may give this invasive species an incredible advantage over other freshwater species competing for the invasion of the same water bodies.

It is our opinion that the major triggering factors for *P. clarkii* overland dispersion are: hydric stress and population density stress (*e.g.* driven from the increase of individual interactions). In spite of this, overland dispersion of *P. clarkii* only occurs when certain environmental conditions are met. From the results of the present study we concluded that the required environmental conditions are precipitation, high air humidity (high leaf wetness associated with the occurrence of fog and dew) and water temperature between 16 and 24ºC. There are also strong indications that *P. clarkii* overland dispersion is related with the crayfish reproduction cycle, however, further studies are necessary to confirm it. Although there is a generally more nocturnal pattern of activity of the species, we believe that the preference for the nocturnal overland dispersal of *P. clarkii* may be intrinsically related with the gathering of the necessary environmental conditions for this phenomenon to occur.

The results of the present study could be of high importance considering the urge to properly manage the invasive crayfish populations in Europe (Holdich 2002; Gherardi 2006), and considering that new legislation is being formulated on this matter (Genovesi 2007; EU 2011). Any mechanism for containing the spread of the species by isolating water bodies, must take into account this species strong capability to disperse overland. It was frequent to observe females carrying eggs or juveniles moving overland and these can easily be the founders of new invading populations. Our results may be especially relevant considering the modelling techniques that are being used to access the effects of invasive crayfish species and to predict their invasion patterns (Marco *et al.* 2002; Capinha *et al.* 2010).

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References

Acosta C.A. and Perry S.A. (2001). Impact of hydropattern disturbance on crayfish population dynamics, in the seasonal wetlands of Everglades National Park, USA *Aquatic Conservation: Marine and Freshwater Ecosystems* **11**, 45 – 57.

Alcorlo P. Geiger W. and Otero M. (2008). Reproductive biology and life cycle of the invasive crayfish *Procambarus clarkii* (Crustacea: Decapoda) in diverse aquatic habitats of South-Western Spain: Implications for population control. *Archiv für Hydrobiologie* **173**, 197 – 212.

Anastácio P.M, Correia C. and Gonçalves P. (2010). *Crayfish (Procambarus clarkii) survival time out of water and its implications for overland dispersion.* Poster Presentation. European Crayfish: food, flagships and ecosystem services, 26-29 October, Poitiers, France.

Anastácio P.M., Leitão A. S., Boavida M.J. and Correia A.M. (2009). Population dynamics of the invasive crayfish (*Procambarus clarkii* Girard, 1852) at two marshes with differing hydroperiods. *Annales de Limnology - International Journal of Limnology* **45**(4), 247 – 256.

Anastácio P.M., Correia A.M., Menino J.P. and Martins da Silva L. (2005). Are rice seedlings affected by changes in water quality caused by crayfish? *Annales of Limnology* **41**(1), 1 – 6.

Anastácio P.M. and Marques J. C. (1995). Population biology and production of the red swamp crayfish *Procambarus clarkii* (Girard) in the lower Mondego river valley, Portugal. *Journal of Crustacean Biology* **15**(1), 156 – 168.

Aquiloni L., Ilhéu M. and Gherardi F. (2005). Habitat use and dispersal of the invasive crayfish *Procambarus clarkii* in ephemeral water bodies of Portugal. *Marine and Freshwater Behavior and Physiology* **38**, 225 – 236.

Barbaresi S. and Gherardi F. (2001). Daily activity of the white-clawed crayfish, *Austropotamobius pallipes* (Lereboullet): a comparison between field and laboratory studies. *Journal of Natural History* **35**(12), 1861 – 1871.

Beecher L.E. and Romaire R.P. (2010). Evaluation of baits for harvesting procambarid crawfishes with emphasis on bait type and bait quantity. *Journal of Shellfish Research* **29**(1), 13 – 18.

Beja P.R. (1996)a. An analysis of otter *Lutra lutra* predation on introduced American crayfish *Procambarus clarkii* in Iberian streams. *Journal of Applied Ecology* **33**, 1156 – 1170.

Beja P.R. (1996)b. Seasonal breeding and food resources of otters, *Lutra lutra* (Carnivora, Mustelidae), in south-west Portugal: a comparison between coastal and inland habitats. *Mammalia* **60**(1), 27 – 34.

Blakewood E.G. Burras C.L. and Huner J.V. (1993). *The effect of hydrological parameters on the burrowing ability of the red swamp crawfish*. Crawfish Research Center, University of Southwestern Louisiana, Lafayette, Louisiana. (Mimeograph, p. 2).

Bliss D.E. (1968). Transition from Water to Land in Decapod Crustaceans. *American Zoologist* **8**, 355 – 392.

Bruxelas S. Costa A.M., Bernardo J.M., Teixeira A. and Ilhéu M. (2005). Progressão de dois lagostins exóticos no nordeste de Portugal: *Pacifastacus leniusculus* e *Procambarus clarkii*. *V Congresso Ibérico de Limnologia*. XIII Congresso de la Asociación Espanõla de Limnologia. Barcelona, Spain.

Capinha C. and Anastácio P. (2011). Assessing the environmental requirements of invaders using ensembles of distribution models. *Diversity and Distributions* **17**, 13 – 24.

Capinha C., Leung B. and Anastácio P. (2010). Predicting worldwide invasiveness for four major problematic decapods: an evaluation of using different calibration sets. *Ecography* **34**(3), 448 – 459.

Chucholl C. (2011). Disjunct distribution pattern of *Procambarus clarkii* (Crustacea, Decapoda, Astacida, Cambaridae) in an artificial lake system in Southwestern Germany. *Aquatic invasions* **6**(1), 109 – 113.

Correia A.M., Bandeira N. and Anastácio P.M. (2005). Predator-prey interactions of *Procambarus clarkii* with aquatic macroinvertebrates in single and multiple prey systems. *Acta Oecologica* **28**(3), 337 – 343.

Correia A.M. and Bandeira N. (2004). Seasonal availability of *Procambarus clarkii* in the Tejo River basin, Portugal. *Freshwater Crayfish* **14**, 190 – 196.

Correia A.M. (1995)a. Biologia populacional e interacções tróficas de *Procambarus clarkii* (Crustacea, Decapoda, Cambaridae) no sistema hidrográfico do rio Tejo (Ribatejo, Portugal). *Doctoral Dissertation*, Faculdade de Ciências, Universidade de Lisboa, Lisboa, 255 pp.

Correia A.M. (1995)b. Population dynamics of *Procambarus clarkii* (Crustacea: Decapoda) in Portugal. *Freshwater Crayfish* **8**, 276 – 290.

Cruz M. and Rebelo R. (2007). Colonization of freshwater habitats by an introduced crayfish, *Procambarus clarkii*, in southwest Iberian Peninsula. *Hydrobiologia* **575**(1), 191 – 201.

Cruz M.J., Rebelo R. and Crespo E.G. (2006). Effects of an introduced crayfish, *Procambarus clarkii*, on the distribution of south-western Iberian amphibians in their breeding habitats. *Ecography* **29**, 329 – 338.

DAISIE – European Invasive Alien Species Gateway (2010). *One hundred of the worst*. Available from: http://www.europe-aliens.org/speciesTheWorst.do (Accessed 18th June 2011).

Dingle H. and Drake V. A. (2007). What Is Migration? *BioScience* **57**(2), 113 – 121.

EU – European Union (2011). *Our life insurance, our natural capital: an EU biodiversity strategy to 2020*. Communication from the Commission to the European Parliament, the Council, the Economical and Social Commitee and the Commitee of the Regions. European Commission. Brussels.

Ferreira M.P. (2010). *Interacções entre espécies indígenas e não indígenas: dispersão passiva de Procambarus clarkii por aves.* Master Thesis on Conservation Biology. Évora University. Évora, Portugal.

Ferreira M.P., Capinha C., Rabaça, J. and Anastácio, P.M. (2009). *Waterbird-Mediated Crayfish Procambarus clarkii Dispersal.* Poster Presentation. BIOLIEF – World Conference on Biological Invasions and Ecosystem Functioning. 27-30 October. Porto, Portugal

Genovesi P. (2007). Towards a European strategy to halt biological invasions in inland waters. In: *Biological invaders in inland waters: Profiles, distribution, and threats.* Ed: Francesca Gherardi. A. A. Dordrecht, The Netherlands. Springer. 627 – 637 pp.

Gherardi F. (2006). Crayfish invading Europe: the case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology* **39**, 175 – 191.

Gherardi F. (2002). Behaviour. In: Holdich D. M. (Ed). *Biology of freshwater crayfish*. Oxford: Blackwell Science. 257 – 290 pp.

Gherardi F. and Barbaresi S. (2000). Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). *Archiv für Hydrobiologie* **150**(1), 153 – 168.

Gherardi F., Barbaresi S. and Salvi G. (2000). Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences* **62**, 179 – 193.

GHERARDI F. AND HOLDICH D.M. (1999). *Crayfish in Europe as Alien Species. How to Make the Best of a Bad Situation?* Balkema, 161–192.

GUERRA J.L. AND NIÑO A.E. (1996). Ecology of red swam crayfish (*Procambarus clarkii*) in the Central Meseta of Spain. *Freshwater Crayfish* **13**, 179 – 200.

GUTIÉRREZ-YURRITA P.J., MARTINEZ J.M., BRAVO-UTRERA M.A., MONTES C., ILHÉU M. AND BERNARDO J.M. (1999). *The status of crayfish populations in Spain and Portugal*. In: Gherardi F. and Holdich D. M. (Eds). Crayfish in Europe as alien species. How to make the best of a bad situation? Rotterdam: A.A. Balkema. pp. 161–192.

HAZLETT B., RITTSCHOF D. AND AMEYAW-AKUMFI (1979). Factors affecting the daily movements of the crayfish *Orconectes virilis* (Hagen, 1870) (Decapoda, Cambaridae). *Crustaceana* **5**, 121 – 130.

HERRNKIND W.F. (1983). Movement Patterns and Orientation. In: *Behavior and Ecology*. Eds: F. J. Vernberg and W. B. Vernberg. New York, Academic Press: 41 – 106 pp.

HOBBS H.H. JR. (1981). The crayfishes of Georgia. *Smithsonian Contributions to Zoology* **318**, 1 – 549.

HOLDICH D.M., REYNOLDS J.D., SOUTY-GROSSET C. AND SIBLEY P.J. (2009). A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* **11**, 394 – 395.

HOLDICH D.M. (2002). *Biology of freshwater crayfish*. Oxford: Blackwell Science. 702 pp.

HUNER J.V. AND LINDQVIST O.V. (1995). Physiological adaptations of freshwater crayfish that permit successful aquacultural enterprises. *American Zoologist* **35**, 12 – 19.

HUNER V. AND BARR J.E. (1991). *Red Swamp Crayfish: Biology and Exploitation*. Louisiana Sea Grant College Program. Baton Rouge, Louisiana, 128 pp.

HUNER J.V. AND AVAULT, J.V. JR (1979). Introductions of *Procambarus* spp, *Freshwater Crayfish* **4**, 191 – 194.

KERBY J.L, RILEY. S.P.D., KATS L.B. AND WILSON P. (2005). Barriers and flow as limiting factors in the spread of an invasive crayfish (*Procambarus clarkii*) in southern California streams. *Biological Conservation* **126**, 402 – 409.

LIGAS A. (2007). Population dynamics of *Procambarus clarkii* (Girard, 1852) (Decapoda, Astacidea, Cambaridae) from southern Tuscany (Italy). *Crustaceana* **81**(5), 601 – 609.

LINDQVIST O.V. AND HUNER J.V. (1999). Life history characteristics of crayfish: what makes some of them good colonizers? 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), pp. 23–30.

MCCLAIN W.R. (2010). Spawning date and potential contribution to yield from winter-burrowing *Procambarus clarkii* in crawfish aquaculture ponds. *Journal of Shellfish Research* **29**(1), 7 – 12.

MCCLAIN W.R. AND ROMAIRE R.P. (2004). Effects of simulated drought on crayfish survival and reproduction in experimental burrows: a preliminary study. *Freshwater Crayfish* **14**, 106 – 115.

Marco D.E., Páez S.A. and Cannas S.A. (2002). Species invasiveness in biological invasions: a modeling approach. *Biological Invasions* **4**, 193 – 205.

McDonald J.H. (2009). *Handbook of Biological Statistics*, 2nd ed. Sparky House Publishing, Baltimore, Maryland.

Penn G.H. (1943). A study of the life history of the Louisiana red-crayfish, *Cambarus clarkii* Girard. *Ecology* **24**(1), 1 – 18.

Ramalho R.O., McClain W.R. and Anastácio P.M (2009) An Effective and Simple Method of Temporarily Marking Crayfish. *Freswater Crayfish* **17**, 57 – 60.

Ramos M.A. and Pereira T.M (1981). Um novo astacidae para a fauna portuguesa: *Procambarus clarkii* (Girard, 1952). *Boletim do Instituto Nacional de Investigação das Pescas* **6**, 37 – 47.

Sivertsen T.H. (2005). The Concept of Leaf Wetness used in Agro Meteorology, in '*Leaf Wetness Duration: Analysis of the Agrometeorological requirements and Evaluation of New Estimation Methods*', COST ACTIONS 718 'Meteorological Applications for Agriculture', Edited by G. Maracchi, L. Kajfez Bogataj, S. Orlandini, A. Dalla Marta and F. Rossi.

Suko T. (1953). Studies on the development of the crayfish. 1. The development of secondary sex characters in appendages. *Science Reports of the Saitama University* **1B**, 77 – 96.

Viosca P., Jr. (1939). Where to fish in Louisiana for crawfish. *Louisiana Conservation Review* **8**(1), 17 – 19.

Zar J.H. (1996) *Biostatistical Analysis*, 3rd edn. Prentice-Hall, New Jersey.

CHAPTER 7

General Discussion

"The need for more research should not be casually invoked as an excuse for inaction."

Daniel Simberloff (2003)

1. General Discussion

The red swamp crayfish (*Procambarus clarkii*) has been extensively studied both in its native and invaded areas (Huner and Barr 1991; Gutiérrez-Yurrita *et al.* 1999; Gherardi 2006; Gherardi 2007). However, detailed and quantitative data regarding the inter- and intra-specific population regulatory mechanisms, migration and dispersal, learning abilities and interaction with sympatric predators are missing for *P. clarkii*. In this thesis detailed information about *P. clarkii* was collected in order to increase the knowledge of this invasive species. The research focused on some aspects of *P. clarkii* intra- and inter-specific population regulation and on clarifying some uncertainties in its ability to colonize new areas, namely: density dependent growth, the learning abilities of this crayfish when confronted with new prey, its predators learning abilities and the dispersal capabilities of the species, especially regarding overland dispersion.

Growth in crustaceans is confined by the exoskeleton and, in order to increase in size, freshwater crayfish must moult. *P. clarkii* growth is affected by several environmental variables (*e.g.* water temperature, water quality, food availability, light intensity, photoperiod, among others – McClain *et al.* 1992; Nyström 1994; Gutiérrez-Yurrita and Del Olmo 2004; Paglianti and Gherardi 2004). The reduced growth of crayfish under the effect of high densities and small size containers was reported (Huner *et al.* 1974) and several outdoor studies demonstrated that *P. clarkii* exhibit density-dependent growth (Lutz and Wolters 1986; Jarboe and Romaire 1995; McClain 1995a,b,c). Some authors have reported that the reduced growth of crayfish under the effect of high densities and small size containers is characterized by larger intermoult periods and decreased increment per moult (Goyert and Avault 1979). A laboratory study (Chapter 2) was conducted in order to quantify the effects of population density on *P. clarkii* early stages of growth and covering a wide range of population density, gathering results on a maximum relative density of 100 ind.m^{-2}. During a 129 days laboratory trial this study demonstrated that density had a profound effect on young of the year *P. clarkii* growth. With the exception of survival and intermoult period all the variables analysed were significantly affected by population density. As density increased mean estimated final length, estimated final weight, total length increase and growth rate decreased. Growth suffered a reduction ranging from about 34%, in the case of estimated final length, to 74% in the case of the estimated final weight. This study showed that the impact of stocking density is likely to be higher on estimated final weight than it is on total length increase, growth rate and estimated final length (by decreasing order of effect). The results obtained were in accordance with other studies (Clark *et al.* 1975; Lutz and Wolters 1986; McClain *et al.* 1995a,b,c) in which the influence of population density over the *P. clarkii* growth were also observed. However, these references have reduced comparability since in the present study we used very high densities (comparing to the densities used on other studies, with a maximum of 20 ind.m^{-2}) and very small juveniles (0.037 g). The results obtained are also with accordance with studies conducted on other crayfish species (*e.g.* Carmona-Osalde *et al.* 2004a,b; Naranjo-Páramo *et al.* 2004; Rodgers *et al.* 2006). The inverse relation between *P. clarkii* population density and juvenile growth may also constitute an important factor in its population regulation. Although this relation was not explored in this study, its conclusions indicate a possible density-dependent population regulation mechanism of *P. clarkii*. Crayfish under high population densities may take more time to reach maturity or reach maturity at lower individual sizes. This could have implications with other life history characteristics such as the reproductive success (Celada *et al.* 2006; Carmona-Osalde *et al.* 2004a). For instance, it is known that larger crayfish females have more and bigger eggs (Barki and Karplus 2000) which may lead to an increased reproductive success. These factors should be taken into account when accessing newly invaded areas or when managing crayfish populations. The conclusions of this study are relevant for the management of both crayfish production and wild crayfish populations and a set of equations relating density to several growth variables were determined. These equations could be an important contribution for the available mathematical models of crayfish growth and population dynamics (Anastácio *et al.* 1999a,b,c; Nielsen *et al.* 1999) but can also be useful for models of crayfish population dispersion.

It was demonstrated that *Procambarus clarkii* need a very short period (less than 12 hours) to learn to identify a new food item (phantom midge larvae – *Chaoborus* sp.) and maximize its efficiency at capturing and utilizing it (Chapter 3). When dealing with spatial and temporal variability of prey species, predators may optimize their capture rates if they have behavioural plasticity (Stephens *et al.* 2007). The learning plasticity and memory skills of *P. clarkii* have been reported as an adaptive advantage of this invasive crayfish species when invading a new habitat (Gherardi *et al.* 2002; Hazlett *et al.* 2003). We consider that our findings reinforce the idea that *P. clarkii* is capable of readily utilizing new and unknown prey items (Renai and Gherardi 2004) which may partially account for the worldwide success of this invader. However, under field conditions, the effects of crayfish over their prey may be substantially different due to the existence of a large number of inter- and intra-specific interactions and also due to environmental complexities caused by factors such as water turbidity, presence of substrate, vegetation and/or refuges (Anastácio *et al.* 2005; Correia *et al.* 2005). This experiment did not attempt to uncover the underlying mechanisms for the ability of crayfish to learn. Several questions remain, such as whether the learning period affected crayfish success rate of capturing or of finding the prey and further research should be addressed to clarify these emergent questions. An equation and a learning coefficient were determined. This coefficient should be useful for future comparisons between the learning abilities of predators and the interaction with different types of prey (sympatric and allopatric), especially when dealing with invasive species.

A similar experiment (Appendix 1) to the previous one was set up to determine the effects of learning time on the predation of *P. clarkii* by a non-native invasive fish predator in the Iberian Peninsula inland waters, the largemouth bass (*Micropterus salmoides*) (Godinho and Ferreira 1998). The results indicated that naïve *M. salmoides* needed at least 96 hours to maximize *P. clarkii* capture rate revealing that it is a flexible predator. In recently invaded areas *M. salmoides* can quickly learn to prey on new and unfamiliar prey items.

An attempt to determine whether *M. salmoides* could have a role in *P. clarkii* population regulation was conducted (Appendix 2). In the three preys system set up *M. salmoides* revealed a preference for mosquito fish (*Gambusia holbrooki*) consumption when this was the most abundant prey. However, when *P. clarkii* proportion in the microcosmos was greater than 60% *M. salmoides* switched its preference from *G. holbrooki* to crayfish. Prey-switching in predators, which attack several prey species, can potentially stabilize the numbers in prey populations (Murdoch 1975; Nilsson 2001; Palomino-Bean *et al.* 2006). When switching occurs, the number of attacks upon a species is disproportionally large when the species is abundant relative to other prey and disproportionally small when the species is relatively rare. Our results demonstrated that *M. salmoides* prey consumption is dependent on prey availability. Although several other factors may be involved in prey population regulation (*e.g.* prey and predator size, environmental conditions, prey handling times, predator attack rates, prey behaviour and space occupancy) (Savino and Stein 1989; Elliot 2006) our results indicate that *M. salmoides* may play an important role in *P. clarkii* population regulation.

During the progress of the studies included into the present thesis, the need for a marking method that could be cheap, easy to execute and effective for the forthcoming experiments, emerged. Therefore, commercial, permanent, water resistant markers were tested in the crayfish hard shell (Chapter 4). At the end of the 14 days experimental trial no negative effects of the marking technique on the test crayfish was observed. The technique tested appears to be an excellent tool for conveniently marking crayfish for indoor or outdoor studies when moulting is not expected. It produces a durable mark, can be applied directly on wet or dry surfaces, dries in 25-35 seconds and there are several different colour and mark combinations possible. This technique was applied with success in our own mark-recapture experiments on crayfish dispersal and seemed very promising for marking other aquatic organisms.

Mark-recapture techniques have been extensively used to obtain information regarding biological organisms, namely on their ecology, areas of distribution and movement patterns. However, these techniques are strongly affected by the catch efficiency. It has been shown that the currently most used crayfish harvesting technique, consisting of baited wire-mesh traps, may not be as effective or efficient as some crayfish farmers assume (Chapter 5; Appendix 3). Under the conditions of this study, which were generally typical of commercial crayfish culture in Louisiana, the mark/recapture technique indicated that less than 50% of the population of harvestable crayfish was captured over time. This experiment pointed out some important insights related with the intrinsic dynamics of *P. clarkii* populations in typical production ponds not revealed up to date. After the release on the centre of the pond crayfish spread through the area of the pond and were recaptured each day farther from the release point. On the sixth day *P. clarkii* were recaptured at a maximum distance of approximately 270 m from the release point. An average of 53.4±5.5 m.day^{-1} of underwater travel speed was recorded. These experiments showed that, even under farming conditions crayfish disperse overland, which could partially explain the reduced recapture rate. This emphasizes the potential for the red swamp crayfish to colonize suitable neighbouring habitats, whether these are nearby crayfish ponds, flood-irrigated agricultural land, or sensitive ecological habitats. These results provided also important insights to *P. clarkii* commercial production. The implications of these observations are not good news for those crayfish producers that routinely release the smaller, less desirable, crayfish from the harvest with the intention of recapturing them later at a larger, more valuable, size for market (Appendix 4). The low return or recapture rate may not be conducive for maximum profits in some cases, especially with declining prices as the season progresses (McClain *et al.* 2007). Another important insight gleaned from these observations deals with the intrinsic dynamics associated with crayfish movements in, and around, crayfish production ponds. These findings document to some extent the propensity for crayfish to move out of and into habitats, likely as a result of some overland travel. This emphasizes the potential for the red swamp crayfish to colonize suitable neighbouring habitats, whether they are nearby crayfish ponds, flood-irrigated agricultural land, or sensitive ecological habitats.

An experiment was conducted to determine the effects of *P. clarkii* population density on crayfish underwater dispersal (Appendix 5). The results of this experiment demonstrated that population density affected both mean total distance and mean individual velocity attained by crayfish at the end of the experiment. Crayfish kept at the highest densities achieved higher velocities (2.89±1.11 m.s^{-1}) and moved further (114.12±42.67 m) than those kept at the lowest densities (2.38±1.09 m.s^{-1} vs 77.55±31.79 m). Crayfish gender, water temperature and period of the day significantly affected crayfish average velocity. The results were in accordance with other obtained under natural conditions (*e.g.* Gherardi and Barbaresi 2000; Gherardi *et al.* 2000). The most significant conclusion of this experiment was the determination of the relation between *P. clarkii* population density and its locomotory speed.

The study of overland dispersal behaviour in *P. clarkii* is of interest to the ever growing discipline of biological invasions, especially when considering the use of computer models to predict, manage and prevent the spread of invasive species. The new modelling techniques are of great use but often require detailed and integrated information about the complex biological systems they aim to predict, revealing the need for more and more detailed information. Our results (Chapter 6) provided important insights about the main factors involved in *P. clarkii* overland dispersion and are, as far as we know, the first detailed quantification of this phenomenon.

Crustacean migration and dispersal can be viewed as a specific adaptation to areas in which changes in habitat quality in different locations occur asynchronously so that movement allows a succession of temporary resources to be exploited as they arise (Dingle and Drake 2007). Decapods dispersal under variable environmental conditions cannot be explained by simple mechanisms (Herrnkind 1983) and we determined the main factors that induce crayfish to exit the water and the environmental conditions that restrict *P. clarkii* overland dispersal in Portugal (Chapter 6). Decapods have evolved to adapt to inland freshwater environments and also to the terrestrial environment, but the overland dispersal only takes place under restricted environmental conditions, generally during the night and in days with precipitation and high humidity levels (Bliss 1968). Although overland dispersal may be an ancestral reminiscence of the Crustaceans evolutive history (Herrnkind 1983) being intrinsically related with reproduction (*e.g.* search for new mates, juvenile recruitment), in our opinion overland dispersion of *P. clarkii* is the result of several factors that *push* individuals to exit the water. Reproduction is one of the factors for overland dispersal. Mature males may take the opportunity to find new mating females and increase their reproductive success. Reproductive females, especially ovigerous individuals or females carrying their brood, may release their offspring in a watershed not colonized, or simply, in a habitat with more suitable environmental conditions than the ones inhabited previously (*e.g.* lower densities, higher water height or stable water levels). This is reinforced by the fact that hydrology is fundamental for the timing of release of the young and recruitment frequently occurs after water inputs into the system (*i.e.* after dry periods) (Gutierrez-Yurrita *et al.* 1999; Alcorlo *et al.* 2008). Because water is limiting the system, crayfish can only reproduce and grow during a certain period and therefore the population adapts to these conditions. The capacity to leave the water and conduct overland dispersion events will definitely contribute to the success of *P. clarkii* when invading new areas and gives this invasive species an incredible advantage over freshwater species competing for the invasion of the same water bodies.

It is our opinion that the major triggering factors of *P. clarkii* overland dispersion are: 1) hydric stress – the alteration of the hydrological cycle, either by natural (*e.g.* temporary freshwater habitats) or anthropogenic factors (*e.g.* rice cultivated fields) and 2) individual crayfish stress – driven from the increase of individual interactions. Overland migration of *P. clarkii* only occurs when certain environmental conditions are met. From the results of the present study (Chapter 6) we concluded that the main environmental factors involved are: 1) precipitation; 2) high air humidity (high leaf wetness associated with the occurrence of fog and dew) and 3) water temperature between 16 and 24ºC. Although there are strong indications that *P. clarkii* overland dispersion is related with the reproductive cycle, further studies are necessary to confirm whether reproduction is determinant or not for the overland dispersion to take place. We believe that the preference for the more intense nocturnal activity of *P. clarkii* dispersing overland may be more intrinsically related with the gathering of the necessary environmental conditions to this phenomenon occur rather than a strategy to reduce the risk of predation.

As an active disperser, *P. clarkii* act as a biological pollutant that, unlike chemicals, reproduce and spread autonomously over great distances, and can adapt to changing conditions (Gherardi 2007). The ability of *P. clarkii* to autonomously spread overland increases greatly its invasive potential of new suitable areas that the species could only reach with external help, such as human transportation and release (Gutiérrez-Yurrita *et al.* 1999; Gherardi 2006) or by other animal vectors, such as birds (Ferreira *et al.* 2009; Ferreira 2010). The behavioural adaptations to avoid desiccating conditions and/or other adverse environmental conditions that may constitute a threat to the crayfish survival could be more important than physiological mechanisms to reduce water loss (Morritt and Spicer 1998) and the present study is in agreement with this point of view.

Understanding the environmental factors determining the establishment of invasive populations is a crucial issue in the study of biological invasions (Capinha and Anastácio 2011) and the present study provided an important insight about *P. clarkii* dispersion. The results of the present study can also be of high importance considering the urge to properly manage the invasive crayfish populations in Europe (Holdich 2002; Gherardi 2006), and considering that new legislation is being formulated on this matter (Genovesi 2007; EU 2011). Our results may be especially relevant considering the modelling techniques that are being used to access the effects of invasive crayfish species and to predict their invasion patterns (Marco *et al.* 2002; Capinha *et al.* 2010).

Natural areas and environment managers are currently confronted with a bewildering array of potential sources of information on invading non-indigenous species. At the same time, environmental managers often lack sufficiently comprehensive tools to assess current and likely impacts of these (Simberloff 2003; Simberloff *et al.* 2010) as well as the probabilities and dates of invasion at each site, in order to prioritize control or mitigation measures. Priorities need to be set at all scales, from management of local reserves to national and international policy decisions. Ultimately, the availability of quantitative data will make scientists able to generalize, and even predict, which species and/or which areas will most likely be invasive and/or invaded (Parker *et al.* 1999).

2. Major Conclusions

As the major conclusions of this thesis I would like to list:

- Density has a significant effect on crayfish growth. Juvenile *P. clarkii* submitted to the higher densities treatments experience higher mortality and grow less than those maintained at lower densities (a growth reduction of up to 74% on total weight and 34% on total lenght was registered).
- Crayfish (*Procambarus clarkii*) need a very short period (less than 12 hours) to learn to identify a new food item (phantom midge larvae – *Chaoborus* sp.) and maximize their efficiency at capturing and utilizing a new and previously not encountered prey item.
- A mathematical expression, including a *learning coefficient* was determined and should provide a useful tool to evaluate and compare the learning capabilities of different freshwater predators with special emphasis to invasive species.
- Largemouth bass (*Micropterus salmoides*), an invasive exotic fish in Iberian Peninsula, has a quick learning curve when dealing with new preys, in this case, *P. clarkii.*
- *M. salmoides* revealed prey switching towards *P. clarkii* and this may be an indication of its potential for population regulation in areas recently invaded by crayfish.
- The harvesting technique of baited wire-mesh traps may not be as effective or efficient as have been assumed. Under the conditions of the present study, which were generally typical of commercial culture in Louisiana, the mark/recapture technique indicated that less than 50% of the population of harvestable crayfish was captured over time.
- Population density has a significant effect over crayfish underwater dispersal. Mature *P. clarkii* females moved faster than mature males. *P. clarkii* underwater dispersal is dependent also from environmental variables such as water temperature and period of the day.

- One of the main factors inducing *P. clarkii* overland dispersion was the drainage of the study area, and consequent stress increase of the resident individuals.
- The main environmental factors involved in the overland dispersion were precipitation, temperature, relative humidity and the period of the day.

3. References

Alcorlo P. Geiger W. and Otero M. (2008). Reproductive biology and life cycle of the invasive crayfish *Procambarus clarkii* (Crustacea: Decapoda) in diverse aquatic habitats of South-Western Spain: Implications for population control. *Archiv für Hydrobiologie* **173**, 197 – 212.

Anastácio P.M., Parente V.S. and Correia A.M. (2005). Crayfish effects on seeds and seedlings: identification and quantification of damage. *Freshwater Biology* **50**, 697 – 704.

Anastácio P.M., Frias A.F. and Marques J.C. (1999)a. CRISP (crayfish and rice integrated system of production): 1. Modelling rice (*Oryza sativa*) growth and production. *Ecological Modelling* **123**, 17 – 28.

Anastácio P.M., Nielsen S. N., Frias A.F. and Marques J.C. (1999)b. CRISP (crayfish and rice integrated system of production): 4. Modelling water, algae and oxygen dynamics. *Ecological. Modelling* **123**, 29 – 40.

Anastácio P.M., Nielsen S.N. and Marques J.C. (1999)c. CRISP (crayfish and rice integrated system of production): 2. Modelling crayfish (*Procambarus clarkii*) population dynamics. *Ecological Modelling* **123**, 5 – 16.

Barki A. and Karplus I. (2000). Crowding female red claw crayfish, *Cherax quadricarinatus*, under small-tanks hatchery conditions: what is the limit? *Aquaculture* **181**, 235 – 240.

Bliss D.E. (1968). Transition from water to land in Decapod Crustaceans. *American Zoologist* **8**, 355 – 392.

Capinha C. and Anastácio P. (2011). Assessing the environmental requirements of invaders using ensembles of distribution models. *Diversity and Distributions* **17**, 13 – 24.

Capinha C., Leung B. and Anastácio P. (2010). Predicting worldwide invasiveness for four major problematic decapods: an evaluation of using different calibration sets. *Ecography* **33**, online early: doi:10.1111/j.1600-0587.2010.06369.x

Carmona-Osalde C., Rodríguez-Serna M., Olvera-Novoa M.A. and Gutiérrez-Yurrita P.J. (2004)a. Effect of density and sex ratio on gonad development and spawning in the crayfish *Procambarus llamasi*. *Aquaculture* **236**, 331 – 339.

Carmona-Osalde C., Rodríguez-Serna M., Olvera-Novoa M.A. and Gutiérrez-Yurrita P.J. (2004)b. Gonadal development, spawning, growth and survival of the crayfish *Procambarus llamasi* at three different water temperatures. *Aquaculture* **232**, 305 – 316.

Celada J.D., Antolín J.I., Carral J.M., Pérez J.R. and Sáez-Royuela M. (2006). Reproductive efficiency of the signal crayfish (*Pacifastacus leniusculus* Dana. Astacidae) at different densities under both culture and laboratory conditions. *Aquaculture* **252**, 298 – 304.

Clark D.F., Avault, J.W. and Meyers S.P. (1975). Effects of feeding, fertilization and vegetation on production of red swamp crayfish, *Procambarus clarkii*. *Freshwater Crayfish* **2**, 125 – 138.

Correia A.M., Bandeira N. and Anastácio P.M. (2005). Predator-prey interactions of *Procambarus clarkii* with aquatic macroinvertebrates in single and multiple prey systems. *Acta Oecologica* **28**, 337 – 343.

Dingle H. and Drake V. A. (2007). What Is Migration? *BioScience* **57**(2), 113 – 121.

Diéguez-uribeondo j. and Söderhäll k. (1993). *Procambarus clarkii* Girard as a vector for the crayfish plague fungus, *Aphanomyces astaci* Schikora. *Aquaculture Research* **24**(6), 761 – 765.

Elliot J.M. (2006). Prey switching in *Rhyacophila dorsalis* (Trichoptera) alters with larval instar. *Freshwater Biology* **51**, 913 – 924.

EU – European Union (2011). *Our life insurance, our natural capital: an EU biodiversity strategy to 2020*. Communication from the Commission to the European Parliament, the Council, the Economical and Social Commitee and the Commitee of the Regions. European Commission. Brussels.

Ferreira M.P. (2010). *Interacções entre espécies indígenas e não indígenas: dispersão passiva de Procambarus clarkii por aves.* Master Thesis on Conservation Biology. Évora University. Évora, Portugal.

Ferreira M.P., Capinha C., Rabaça, J. and Anastácio, P.M. (2009). *Waterbird-Mediated Crayfish Procambarus clarkii Dispersal.* Poster Presentation. BIOLIEF – World Conference on Biological Invasions and Ecosystem Functioning. 27-30 October. Porto, Portugal

Genovesi P. (2007). Towards a European strategy to halt biological invasions in inland waters. In: *Biological invaders in inland waters: Profiles, distribution, and threats.* Ed: Francesca Gherardi. A. A. Dordrecht, The Netherlands. Springer. pp. 627 – 637.

Gherardi F. (2007). *Understanding the impact of invasive crayfish*. In: Biological invaders in inland waters: profiles, distribution, and threats. (ed. Gherardi F), 507-542 pp. Invading Nature: Springer Series in Invasion Ecology, Springer, Dordrecht, The Netherlands.

Gherardi F. (2006). Crayfish invading Europe: the case study of Procambarus clarkii. *Marine and Freshwater Behaviour and Physiology* **39**, 175 – 191.

Gherardi F., Acquistapace P., Hazlett B. and Whisson G. (2002). Behavioural responses to alarm odours in indigenous and non-indigenous crayfish species: a case study from Western Australia. *Marine and Freshwater Research* **53**, 93 – 98.

Gherardi F. and Barbaresi S. (2000). Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). *Archiv für Hydrobiologie* **150**(1), 153 – 168.

Gherardi F., Barbaresi S. and Salvi G. (2000). Spatial and temporal patterns in the movement of *Procambarus clarkii*, an invasive crayfish. *Aquatic Sciences* **62**, 179 – 193.

Godinho F.N. and Ferreira M.T. (1998). Spatial variation in diet composition of pumpkinseed sunfish, *Lepomis gibbosus*, and largemouth bass, *Micropterus salmoides*, from a Portuguese stream. *Folia Zoologica* **47**, 205 – 213.

Goyert J.C. and Avault J.W., Jr. (1979). Effects of container size on growth of crayfish (*Procambarus clarkii*) in a recirculating culture system. *Freshwater Crayfish* **4**, 278 – 286.

Gutiérrez-Yurrita P.J. and Del Olmo C.M. (2004). Population dynamics of juveniles of red swamp crayfish (*Procambarus clarkii*) under controlled conditions. *Freshwater Crayfish* **14**, 180 – 189.

Gutierrez-Yurrita P.J., Martínez J.M., Bravo-Utrera M.A., Montes C., Ilhéu M.A. and Bernardo J.M. (1999). The status of crayfish populations in Spain and Portugal. In: Gherardi F. and Holdich D.M. (eds.), *Crayfish in Europe as alien species – How to make the best of a bad situation?*, Balkema, 161 – 192.

Hazlett B.A., Burba A., Gherardi F. and Acquistapace P. (2003). Invasive species use broader range of predation-risk cues than native species. *Biological Invasions* **5**, 223 – 228.

Herrnkind W.F. (1983). Movement Patterns and Orientation. In: *Behavior and Ecology*. Eds: F. J. Vernberg and W. B. Vernberg. New York, Academic Press: 41 – 106 pp.

Holdich D.M. (2002). *Biology of freshwater crayfish*. Oxford: Blackwell Science. 702 pp.

Huner V. and Barr J. E. (1991). *Red Swamp Crayfish: Biology and Exploitation*. Louisiana Sea Grant College Program. Baton Rouge, Louisiana, 128 pp.

Huner J.V., Meyers S.P. and Avault J.W., Jr. (1974). Response and growth of freshwater crayfish to an extruded, water diet. In: *Proceedings of The Second International Crayfish Symposium* (ed. by J.W. Avault, Jr.), pp. 149 – 157. Baton Rouge, Louisiana, U.S.A.

Jarboe H.H. and Romaire R.P. (1995). Effects of density reduction and supplemental feeding on stunted crayfish *Procambarus clarkii* populations in earthen ponds. *Journal of the World Aquaculture Society* **26**, 29 – 37.

Lutz C.G. and Wolters W.R. (1986). The effect of five stocking densities on growth and yield of red swamp crayfish *Procambarus clarkii. Journal of the World Aquaculture Society* **17**, 33 – 36.

Marco D.E., Páez S.A. and Cannas S.A. (2002). Species invasiveness in biological invasions: a modeling approach. *Biological Invasions* **4**, 193 – 205.

McClain W.R., Romaire R.P., Lutz C.G. and Shirley M.G. (2007). *Louisiana crawfish production manual.* Louisiana State University Agricultural Center, Pub. 2637, Baton Rouge, Louisiana.

McClain W.R. (1995)a. Effects of population density and feeding rate on growth and feed consumption of red swamp crayfish *Procambarus clarkii. Journal of the World Aquaculture Society* **26**, 14 – 23.

McClain W.R. (1995)b. Growth of *Procambarus clarkii* as a function of density and food resources. *Journal of the World Aquaculture Society* **26**, 24 – 28.

McClain W.R. (1995)c. Investigations of crayfish density and supplemental feeding as influencing growth and production of *Procambarus clarkii*. *Freshwater Crayfish* **10**, 512 – 520.

McClain W.R., Neil W.H. and Gatlin III D.M. (1992). Partitioning the contributions of forage-based production system components to weight gain of juvenile crayfish (*Procambarus clarkii*). *Aquaculture* **101**, 267 – 281.

Morritt D. and Spicer J.I. (1998). The physiological ecology of talitrid amphipods: an update. *Canadian Journal of Zoology* **76**, 1965 – 1982.

Murdoch W.W., Avery A and Smyth M.B. (1975). Switching in predatory fish. *Ecology* **56**(5), 1094 – 1105.

Naranjo-Páramo J., Hernadez-Llamas A. and Villarreal H. (2004). Effect of stocking density on growth, survival and yield of juvenile redclaw crayfish *Cherax quadricarinatus* (Decapoda: Parastacidae) in gravel-lined commercial nursery ponds. *Aquaculture* **242**, 197 – 206.

Nielsen S.N., Anastácio P.M., Frias A.F. and Marques J.C. (1999). CRISP (crayfish and rice integrated system of production): 5. Simulation of nitrogen dynamics. *Ecological Modelling* **123**, 41 – 52.

Nilsson P.A. (2001). Predator behaviour and prey density: evaluating density-dependent intraspecific interactions on predator functional responses. *Journal of Animal Ecology* **70**: 14 – 19.

Nyström P. (1994). Survival of juvenile signal crayfish (*Pacifastacus leniusculus*) in relation to light intensity and density. *Nordic Journal of Freshwater Research* **69**, 162 – 166.

Paglianti A. and Gherardi F. (2004). Combined effects of temperature and diet on growth and survival of young-of-year crayfish: a comparison between indigenous and invasive species. *Journal of Crustacean Biology* **24**, 140 – 148.

Palomino-Bean S., Vilcarromero A.C.S. and Bonato O. (2006). Co-existência de Espécies em Sistemas Presa-predador com Switching. *Tendências em Matemática Aplicada e Computacional* **7**(2), 317 – 326.

Parker I.M., Simberloff D., Lonsdale W.M., Goodell K., Wonham M., Kareiva P.M., Williamson M.H., Von Holle B., Moyle P.B., Byers J.E. and Goldwasser L. (1999). Impact: Toward a framework for understanding the ecological effects of invaders. *Biological Invasions* **1**(1), 3 – 19.

Renai B. and Gherardi F. (2004). Predatory efficiency of crayfish: comparison between indigenous and non-indigenous species. *Biological Invasions* **6**, 89 – 99.

Rodgers L.J., Saoud, P.I. and Rouse, D.B. (2006). The effects of monosex culture and stocking density on survival, growth and yield of redclaw crayfish (*Cherax quadricarinatus*) in earthen ponds. *Aquaculture* **259**, 164–168.

SAVINO J.F. AND STEIN R.A. (1989). Behavioural interactions between fish predators and their prey: effects of plant density. *Animal Behaviour* **37**(2), 311 – 321.

SIMBERLOFF D., GORDON D., HIEBERT R., LONSDALE M. AND DRAHEIM R. (2010). A global database: the key to weighing on-the-ground invasive species impacts. *CiteSeerX - Scientific Literature Digital Library and Search Engine* 33 pp.

SIMBERLOFF D. (2003). How much information on population biology is needed to manage introduced species? *Conservation Biology* **17**, 83 – 92.

STEPHENS D.W., BROWN J.S., YDENBERG R.C. (2007). *Foraging: behaviour and ecology*. University of Chicago Press, Chicago.

APPENDIX

APPENDIX 1

Naïve fish learning abilities: how does learning period affects the capture rate of a new prey item?

This appendix is consisted of unpublished results presented at the following international meeting:

Ramalho R.O. and Anastácio P.M. (2010). Naïve fish learning abilities: how does learning period affects the capture rate of a new prey item? European Crayfish: Food, Flagships and Ecosystem Services. Poster Communication. Poitiers, France. 26th – 29th of October.

NAÏVE FISH LEARNING ABILITIES: HOW DOES LEARNING PERIOD AFFECTS THE CAPTURE RATE OF A NEW PREY ITEM?

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Abstract

The learning abilities of an invasive fish (largemouth black bass: *Micropterus salmoides*, Lacepède 1802) when dealing with a new crustacean prey item (red swamp crayfish: *Procambarus clarkii*, Girard 1852) were studied. Both predator and prey are invasive species in Iberian Peninsula and were naïve to one another at the beginning of the experiment. Largemouth Black Bass (LBB) were submitted to four different periods of contact with juvenile crayfishes and prey consumption was recorded.

We found significant differences between learning periods both for consumed crayfish fresh weight and for number of individuals. LBB submitted to four days of learning period preyed more crayfish than the control group (5.75±2.50 vs 0.40±0.54 crayfishes respectively). Our results indicate that LBB have a four days learning period when switching to new food items. These results showed that naïve predacious fishes, such as largemouth black bass, have a fast learning curve having the potential to easily adapt to new preys in a recently invaded habitat. We consider the results are relevant both for management and modelling of LBB and crayfish populations.

Keywords: learning; Micropterus salmoides; Procambarus clarkii; naïve fish; predation success

NAÏVE FISH LEARNING ABILITIES:
how does learning period affects the capture rate of a new prey item?

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READ THE POSTER IN 1 MINUTE!

1. The purpose of the present study was to determine the effect of the learning time on prey capture success by the introduced Largemouth Bass (*Micropterus salmoides*) (LMB);

2. Twenty Largemouth Bass were submitted to four different periods of contact with Red-Swamp Crayfish (*Procambarus clarkii*), namely: 0, 24, 48, and 96 hours;

3. During the experimental trial 10 juvenile crayfish were left during 8 hours in the aquarium together with LMB submitted to previously referred learning periods;

4. There were significant differences between learning periods both for Crayfish fresh weight consumed and for the number of individuals consumed by Largemouth Bass;

5. Our results indicate that LMB have a four days learning period when switching to new food items.

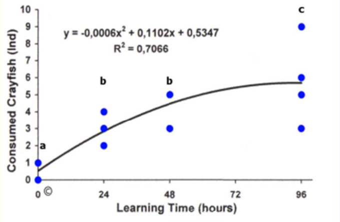

Figure 1. Individual LargeMouth Black Bass consumption of Red-Swamp Crayfish after different learning periods. Ind = Individuals. © = Control. Groups with the same superscript are non statistically different (Post-hoc Tukey test: $p>0.05$).

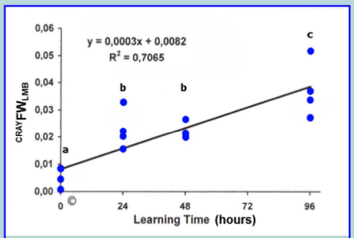

Figure 2. Mean Red-Swamp Crayfish Fresh Weight consumption per gram of LargeMouth Black Bass ($^{CRAY}FW_{LMB}$) after different learning periods. © = Control. Groups with the same superscript are non statistically different (Post-hoc Tukey test: $p>0.05$).

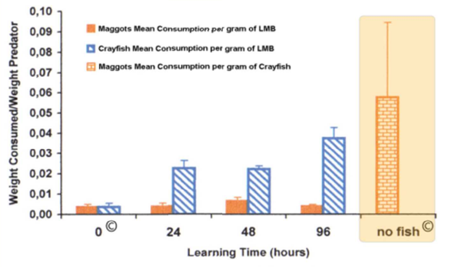

Figure 3. Mean Red-Swamp Crayfish Fresh Weight consumption per gram of LargeMouth Black Bass and Mean Maggots Fresh Weight Consumption per gram of LMB. Shaded area represents the non-fish control, corresponding to the Maggots consumption per gram of Crayfish in the absence of the LMB and it's only here for visual comparison purposes. Vertical bars represents standard error. © = Control. Groups with the same superscript are non statistically different (Post-hoc Tukey test: $p>0.05$).

RESULTS and DISCUSSION

A one-way ANOVA showed that learning period significantly affects successful prey capture ($F=10.564$; $df=3$; $p<0.01$). The number of crayfish preyed seems to increase rapidly with the learning time. A non linear regression ($p<0.001$) indicates that the maximum efficiency was attained after 96 hours of contact with the prey (Figure 1). Number of maggots consumed was, as expected, NOT significantly affected by the four different treatments (ANOVA: $F=1.758$; $df=3$; $p>0.05$).

Learning period significantly affected crayfish Fresh Weight consumed per gram of LMB ($^{CRAY}FW_{LMB}$) (MANOVA: $F=15.244$; $df=3$; $p<0.001$) and a post-hoc Tukey test found significant differences between treatments (Figure 2). As expected learning period with crayfish prey did not affect the fresh weight consumption of maggots per gram of LMB ($^{M}FW_{LMB}$) (MANOVA: $F=0.910$; $df=3$; $p>0.05$).

In the no fish controls NO crayfish died or where predated by their conspecifics. In this control 65% of the Larvae where found with signs of crayfish predation, but in the presence of LMB only 2% of all the larvae showed the same signs. This fact seems to be the result of differences of behaviour and/or space occupation by crayfish in the presence of a potential predator. An interesting result was the fact that in the no-fish tanks the consumption of Maggots per gram of Crayfish was relatively high comparing with the $^{M}FW_{LMB}$ and even comparing with the total fresh weigh consumed per gram of fish ($^{CRAY}FW_{LMB} + ^{M}FW_{LMB}$) (Figure 3).

These results suggest that as a new invader LMB has a very plastic behaviour and a quick learning pattern when preying on new food items.

Acknowledgements - This work was funded by FCT by a PhD Grant (SFRH/BD/19373/2004), project POCTI/BSE/46862/2002 and by FEDER.

METHODS

We used 25 plastic aquariums (55x45x40cm), each with 62 litres of de-chlorinated tap water, at 19.15±0.45 ºC, pH was 8.08±0.16, dissolved oxygen was 8.25±0.50 mg/l and photoperiod was 12h light/12h dark. Artificial vegetation (20cm high and 30cm wide) was placed inside each aquarium. Twenty LargeMouth Black Bass (*Micropterus salmoides*) (LMB), with an average initial fresh weigh of 21.60±7.31 g and average total length of 12.67±1.62 mm, were caught in a private dam devoid of Red-Swamp Crayfish (*Procambarus clarkii*). LMB individuals were fed maggots (domestic-fly larvae) during a three weeks acclimatization period and were starved 24 hours prior to the experiment.

The experimental design consisted of four treatment levels (learning time), namely: 96, 48, 24 and 0 (control) hours of previous fish contact with crayfish and one additional control without fish to account for crayfish predation on maggots. All treatment levels had five replicates.

At the start of the experiment, ten maggots (0.79 ± 0.06 g of fresh weight) and ten crayfish (10.72±1.68 mm of carapace length and 2.19 ± 0.58 g of fresh weight) where added to each aquarium. The experiment lasted eight hours, during daylight. At the end of the experiment the number and weight of the remaining prey were quantified in order to calculate consumption. A correction index was applied in order to obtain predation on the maggots exclusively due to the LMB.

At the end we obtained data on Crayfish Fresh Weight consumption per gram of LMB ($^{CRAY}FW_{LMB}$), Maggots Fresh Weight consumption per gram of LMB ($^{M}FW_{LMB}$) and in the non-fish control Maggots Fresh Weight consumption per gram of Crayfish ($^{M}FW_{CRAY}$). Data were analysed using an ANOVA to test for differences among the number of prey captured by LMB at each treatment level and a MANOVA was used to compare the $^{CRAY}FW_{LMB}$ and $^{M}FW_{LMB}$. A post-hoc Tukey test was applied to determine which pairs of treatment levels differed.

APPENDIX 2

Predatory relations between three invasive species in Iberian Peninsula: does prey-switching occur?

This appendix is consisted of unpublished results presented at the following international meeting:

> *Ramalho R.O. and Anastácio P.M. (2010). Predatory relations between three invasive species in Iberian Peninsula: does prey-switching occur? BIOLIEF – World Conference on Biological Invasions and Ecosystem Functioning. Porter Communication. Porto, Portugal. 27th – 30th of October.*

PREDATORY RELATIONS BETWEEN THREE INVASIVE SPECIES IN IBERIAN PENINSULA: DOES PREY-SWITCHING OCCUR?

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Abstract

Prey switching in predators which attack several prey species can potentially stabilize prey populations. This study was an attempt to access predator-prey relationships between an invasive generalist predator, the largemouth black bass (*Micropterus salmoides*, Lacepède 1802) and two invasive preys, the red swamp crayfish (*Procambarus clarkii*, Girard 1852) and the mosquitofish (*Gambusia holbrooki*, Girard 1859). The question we attempted to answer was: given that largemouth bass consume both preys when presented alone, does it switch from a prey that becomes rare to another which becomes more abundant?

Assuming the preference of largemouth black bass for mosquitofish and also assuming a null hypothesis that - in case of no switching the proportion of prey in diet should not differ from the expected consumption - the results indicate that largemouth black bass *switched* its preference to crayfish when it became the most abundant prey. It is therefore our opinion that largemouth black bass has a potential for limiting crayfish populations.

Keywords: Micropterus salmoides; Procambarus clarkii; Gambusia holbrooki; predation; prey switching

PREDATORY RELATIONS BETWEEN THREE INVASIVE SPECIES IN IBERIAN PENINSULA: DOES PREY-SWITCHING OCCUR?

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THE POSTER IN A SNAP!

1. "Switching" in predators which attack several prey species potentially can stabilize the numbers in prey populations. When switching occurs, the number of attacks upon a species is disproportionally large when the species is abundant relative to other prey, and disproportionally small when the species is relatively rare. In the simplest case, the two prey would be attacked at the same rate and the expected ratio in the diet should be the ratio in the environment (given) and the expected consumption curve has a slope 1 (c=1).
2. Largemouth black bass (*Micropterus salmoides*) was exposed to different proportions of two usual prey items in its diet, red swamp crayfish (*Procambarus clarkii*) and mosquitofish (*Gambusia hoolbroki*).
3. Five different prey density combinations were created: 100% crayfish, 70% crayfish/30% mosquitofish, 50/50%, 30% crayfish/70% mosquitofish, 100% mosquitofish.
4. Largemouth black bass presented a stronger preference for mosquitofish than for crayfish. However the results indicated that largemouth black bass "switched" its preference to crayfish when it became the most abundant prey. It is therefore our opinion that largemouth black bass has a potential for limiting crayfish populations.

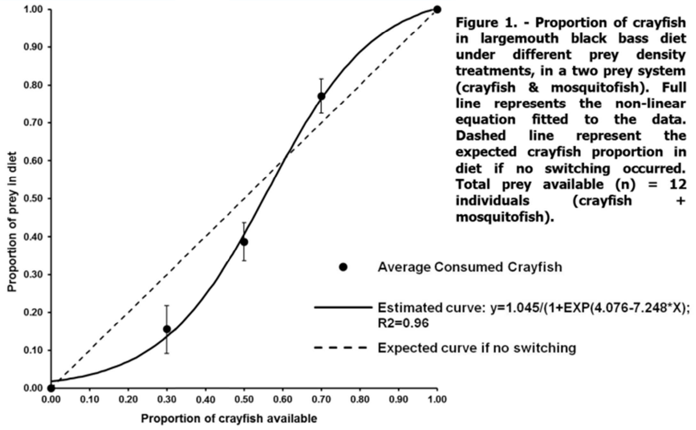

Figure 1. - Proportion of crayfish in largemouth black bass diet under different prey density treatments, in a two prey system (crayfish & mosquitofish). Full line represents the non-linear equation fitted to the data. Dashed line represent the expected crayfish proportion in diet if no switching occurred. Total prey available (n) = 12 individuals (crayfish + mosquitofish).

METHODS

We used 25 plastic tanks (50x40x40cm), each with 10 litres of de-chlorinated tap water, at 20.15±0.02 ºC (±SE), pH was 8.34±0.01, dissolved oxygen was 8.62±0.02 $mg.l^{-1}$ and photoperiod was 12h light/ 12h dark. Two sets of artificial vegetation (20cm high and 30cm wide) were placed inside each aquarium attached to the bottom. Twenty Largemouth Black Bass (*Micropterus salmoides*) (LMB), with an average initial fresh weigh of 22.96±1.36 g (±SE) and average total length of 128.08±3.60 mm, were caught in a private dam in south-center of Portugal near Évora. LMB individuals were fed juvenile red swamp crayfish (*Procambarus clarkii*) and mosquitofish (*Gambusia holbrooki*) during a two weeks acclimatization period and were starved 24 hours prior to the experiment. Juvenile red swamp crayfish (average fresh weight: 0.098±0.004 g; average total length: 16.18±0.09 mm) were caught in rice fields irrigation ditches and mosquitofish (average fresh weight: 0.109±0.003 g; average total length: 18.04±0.10mm) were captured in a small pond near the University Campus. Both prey were submitted to the same two weeks acclimatization period.

The experimental design consisted of five treatment levels (proportion of prey items available), namely: 100% crayfish, 70/30% (9 crayfish/ 3 mosquitofish), 50/50%, 30/70% (3 crayfish/ 9 mosquitofish), 100% fish. Each treatment consisted of one LMB and 12 prey individuals at the respective prey proportions. An additional control group, with 12 crayfish, 12 mosquitofish and no LMB, was created to account for the crayfish/mosquitofish predation dynamics. All treatment levels had four replicates. The experiment started one hour after lights on and lasted 1 hour. Prey items were weighted prior to the experiment and the remaining prey items were collected at the end of the experiment trial. The consumed prey fresh weight was determined. No mortality was registered at the control group (12 crayfish, 12 mosquitofish and no LMB) and no evidences of predation were seen between crayfish and mosquitofish. We assumed that all the predation results could be attributed to LMB. Relative Prey Consumption (RPC) was determined dividing total fresh weight of prey consumed by LMB fresh weight.

Proportion of crayfish in diet (PD_C) was determined as follows: *PDc = Number of consumed crayfish/total number of prey consumed*

ANOVA was used to compare differences between treatments. When switching occurs, the number of successful attacks upon a species is disproportionally large when the species is abundant relative to other prey and disproportionally small when the species is relatively rare. Prey switching is also known as frequency-dependent selection by predators or as apostatic selection (Greenwood & Elton, 1979). The relationship is typically described by an S-shaped curve showing that the most abundant prey is preferred and suffers the greatest percentage of mortality. The null hypothesis can be written as $P_1/P_2=cN_1/N_2$, where P_1/P_2 is the ratio of the two prey expected in diet, N_1/N_2 is the ratio of the prey in the environment (given) and c is a proportionality constant (Murdoch, 1989; Oaten & Murdoch, 1975). The value of c could be used as an indicator of the preference of a predator for each prey species, and can be determined based on the average consumption of each prey at the treatment where both preys are equally represented (50/50%) (Oaten & Murdoch, 1975). A non linear equation *(y=a/(1+Exp(b-c*X)),* adapted from Holling (1959), was fitted to the data.

RESULTS and DISCUSSION

Average relative prey consumption (RPC) was 0.039±0.003 (g of prey/g of LMB), average total prey consumption was 8.33±0.31 individuals.h^{-1} (crayfish + mosquitofish) and no significant differences were found between treatment groups for either variable ($p>0.05$) (Table I). Each largemouth black bass consumed, on average, 0.81±0.06 grams of prey fresh weight (crayfish + mosquitofish).

Table I – Average total prey consumption and average relative prey consumption at each treatment. Average (±SE) total prey consumption refers to total number of individuals consumed (crayfish+mosquitofish) per largemouth black bass.

Treatment	Average Total Prey Consumption ($Ind.h^{-1}$)	Average Relative Prey Consumption
100% Crayfish	9.33±0.67	0.031±0.001
70%crayfis/ 30% mosquitofish	8.00±0.70	0.030±0.003
50/50%	7.67±0.67	0.043±0.005
30% crayfish/ 70% mosquitofish	7.75±0.75	0.047±0.009
100% mosquitofish	9.00±0.58	0.047±0.005
Total	**8.33±0.31**	**0.039±0.003**

The estimated c values in our experiment were 0.78 for crayfish and 1.22 for mosquitofish, indicating a preference of LMB to consume mosquitofish. When crayfish was less available, LMB consumed more mosquitofish than expected, but when crayfish was 70% of the available prey it was consumed more than expected. This relationship is well described by a sigmoid (S-shaped) curve (Figure 1). The non-linear equation applied to the data had a r^2 of 0.96 which is a better fit than a linear regression ($r^2=0.86$). We estimate prey switching from mosquitofish to crayfish to occur only when crayfish proportion is greater than 0.6 (Figure 1).

It seems that consumption of prey items by LMB is dependent on prey availability. We expected that crayfish would be more difficult and costly to catch. In fact visual observations during the experiment revealed that crayfish were able to find shelters and seemed successfully hidden under the artificial vegetation provided, while mosquitofish remained in the water column, swimming around, trying to avoid the LMB.

Even under these laboratory experimental conditions, on small microcosms, largemouth black bass apparently presented a shift in its predatory behavior, capturing hidden crayfish when confronted with low densities of mosquitofish. These conclusions should be taken with special attention because there are several factors, not taken into account in this analysis, such as prey handling times, predator attack rates (Elliott, 2004; 2006) and prey behavior and space occupancy (Savino & Stein, 1989).

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References:
Elliott, J.M. (2004). Prey switching in four species of carnivorous stone flies. *Freshwater Biology*. **49**: 709 – 720.
Elliott, J.M. (2006). Prey switching in *Rhyacophila dorsalis* (Trichoptera) alters with larval instar. *Freshwater Biology*. **51**: 913 – 924.
Greenwood, J.J.D. and Elton, R.A. (1979). Analyzing experiments on frequency-dependent selection by predators. *Journal of Animal Ecology*. **48**: 721 – 737.
Holling, C.S. (1959). Some characteristics of simple types of predation and parasitism. *Canadian Entomologist*. **91**: 385 – 398.
Murdoch, W.W. (1989). Switching in general predators: experiments on predator specificity and stability of prey populations. *Ecological Monographs*. **39**: 335 – 354.
Oaten, A. and Murdoch, W.W. (1975). Functional response and stability in Predator-Prey systems. *The American Naturalist*. **109**: 289 – 298.
Savino, J.F. and Stein, R.A. (1988). Behavioural interactions between fish predators and their prey: effect of plant density. *Animal Behaviour*. **37**: 311 – 321.

APPENDIX 3

Preliminary assessments of capture rate and crawfish movement in a commercial crawfish pond

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Ramalho R.O., McClain W.R. and Sonnier J.J. (2009). Preliminary assessments of capture rate and crawfish movement in a commercial crawfish pond. 101st Annual Research Report. Louisiana State University. Rice Research Center. Crowley. Louisiana. pp. 214 – 219

PRELIMINARY ASSESSMENTS OF CAPTURE RATE AND CRAWFISH MOVEMENT IN A COMMERCIAL CRAWFISH POND

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Introduction

Crawfish farmers rely solely on baited wire-mesh traps to harvest crawfish. Unlike seining with nets, which is a more effective and efficient means of harvesting foodfishes and baitfishes, crawfish farmers are relegated to the use of baited traps for several reasons: (1) thick stands of vegetation (*i.e.* cultivated food resource) in ponds impede seine harvesting; (2) harvestable crawfish are recruited in variable numbers into the trappable population over 4 to 6 months; (3) only large, hard-shelled crawfish are targeted for capture and traps are designed to select for size and prevent harvest of recently molted animals and (4) baited traps are currently the only practical option in large and irregular shaped ponds that are dominant in the industry. The efficiency of baited traps to remove market size animals from the population is unknown however. The standard square-mesh pyramid trap currently used in the crawfish aquaculture industry is the most efficient trap adopted to date, and research has shown that a trap density of 10 to 20 traps per acre, depending on crawfish population density, is usually the most cost efficient. Yet the efficiency of which this type of system can capture marketable crawfish from a population of market size individuals has been poorly documented.

Notwithstanding, one management strategy to increase the average size of crawfish sold relies on the release of smaller crawfish back to the pond with hopes of recapturing them later at a larger, more valuable size. This theory assumes released crawfish will continue to grow and their recapture rate later, at a larger size, will justify the effort and expense. This strategy has increased in popularity in recent years with the increased demand by buyers for larger crawfish and fewer opportunities for markets willing to accept the smaller individuals.

Therefore, this study was initiated to gain some preliminary information regarding capture efficiency of baited traps using crawfish that were marked and released within a single pond, with their recapture rate documented over a short interval. A secondary objective under these efforts was to examine the dispersal and movement patterns of marked crawfish from the time they were released until their recapture.

Location: A commercial crawfish pond in Acadia Parish, southeast of Crowley, Louisiana.

Date: Last week of May 2007.

Pond Description: The field was employed in a typical rice-crawfish field rotational strategy where a rice crop was realized in 2006, followed by a crawfish crop that was being harvested during the spring of 2007. The field was roughly U-shaped and approximately 28 acres in size (Figure 1).

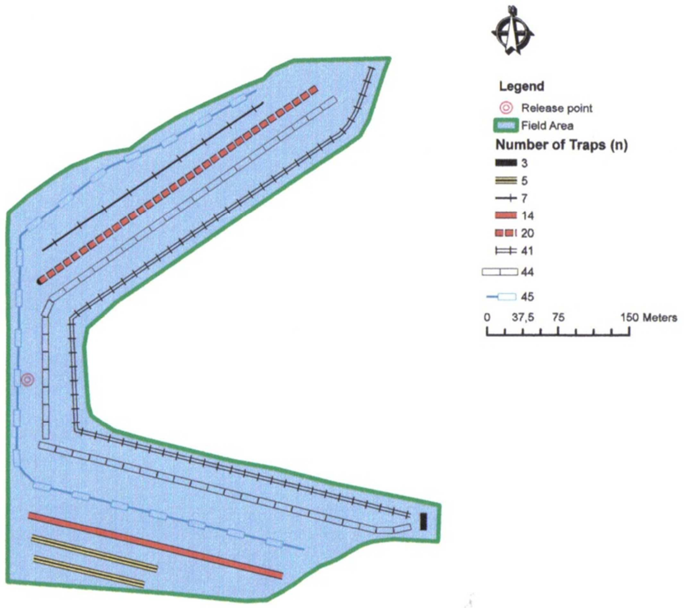

Figure 1. Representation of the shape and orientation of the commercial pond used for the mark/recapture study, and depiction of the number and placement of crawfish traps. The release site for marked crawfish is also depicted.

Crawfish: A random sample of crawfish (470) captured on the day of release was marked and released (within 2 hours) as a group at the designated release site. Crawfish were released in the same pond they were captured in. All crawfish were red swamp crawfish (*Procambarus clarkii*) and consisted of 73 mature males (avg. wt. 21.6 g), 127 mature females (avg. wt. 22.4 g), 149 immature females (avg. wt. 15.6 g), and 121 immature males (avg. wt. 17.4 g).

Marking Technique: A general-use permanent waterproof marker (Dykem®, BRITE-MARK®, ITW Dymon, Olathe, Kansas, USA) was used to mark crawfish on the dorsal and lateral surfaces of the carapace with a large, bold white line. The mark started on one side of the carapace and ran across the top and down the other side such that marked animals could be easily spotted in a group of crawfish when captured no matter their orientation. The Dykem marker did not require a dry carapace and was quickly applied to each individual. This marker was previously determined to work well for such use in research.

Release Point: All marked crawfish were released at a designated point in the field, which was somewhat central to the two long axis of the pond (Figure 1).

Harvesting Protocol: Harvesting method employed was that typically used in commercial ponds. Traps were accessed via a hydraulically powered boat traveling down each lane of traps and all traps were check, emptied, and rebaited on days 1, 2, 3, 4 and 6 following the release of marked crawfish.

Trap Density: 184 (3/4-inch square mesh) pyramid traps (about 6 traps/A) randomly distributed in parallel trapping lanes covering most of the field area (Figure 1).

Bait: Manufactured bait: Cajun World (Purina Mills, Inc., St. Louis, MO)

Data: Two persons examined the emptied catch from each trap and marked crawfish were collected and location of the catch was flagged. Crawfish gender, state of maturation, weight, and cephalothorax length was recorded. Distance and direction travelled from the release point to the capture location was noted and nominal rate of travel (meters/day) was calculated based on days since release.

Statistical Analysis: Data was subjected to ANOVA or MANOVA for determination of significant differences between sex/maturity groups, and rate of travel data was transformed and subjected to a logarithmic regression.

Comments: When marked crawfish (200 mature and 270 immature) were released at a central location in the 28-acre pond and all traps within the pond were subsequently run almost daily for one week, the results provided us some important data regarding the dispersal and capture rate of marketable size crawfish under commercial aquaculture conditions. Within the first three days of harvest after release of the marked crawfish, the recapture rate averaged 3.2% per day; after which it decreased to only 1.2% per day (Table 1). Some percentage of the immature crawfish could be expected to molt after release and their recapture could go unnoticed. Indeed, the recapture percentage for immature crawfish over the 6-day period was substantially less than with matures (Table 2). However, at best, the rate of capture for mature (non-molting) crawfish totaled 20% (on average) over the course of 5 harvest days or 4% per day. When all marked crawfish are considered, the total recapture rate was only 11.5% (*i.e.* 54 marked crawfish recaptured) or 2.3% per day. Although natural mortality was not measured in this study, it was not expected to be great due to the care and considerations in handling/marking and short duration of the study. Therefore, based on this study, the preliminary data suggests that the capture rate for a population of market size crawfish will range between 2.3 and 4.0% per day, given the commercial conditions of culture (*i.e.* pond type, trap density, season, forage biomass, etc.) in this study.

Table 1. Number (N) and percentage (%) of marked crawfish recaptured during the 6-day experimental period. Daily percentage of recaptures is based on adjusted numbers of marked crawfish remaining. [mM = mature males; mF = mature females; iM = immature males; iF = immature females]

Day	**Adjusted Number of Marked Crawfish**	**Total Recaptured**		**mM**		**mF**		**iM**		**iF**	
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
1	470	15	3.19	4	5.48	9	7.09	1	0.83	1	0.67
2	455	14	3.08	3	4.35	8	6.78	3	2.50	0	0.00
3	441	15	3.40	3	4.55	7	6.36	1	0.85	4	2.70
4	426	6	1.41	1	1.59	4	3.88	1	0.86	0	0.00
6	420	4	0.95	1	1.61	0	0.00	3	2.61	0	0.00
Average (Total)	**---**	**(54)**	**11.5**	**(12)**	**22.2**	**(28)**	**51.9**	**(9)**	**17.7**	**(5)**	**9.3**

Table 2. Average weight (Wt.) and number (N) of released and recaptured crawfish, and percentage (%) recaptured by maturity/gender. [mM = mature males; mF = mature females; iM = immature males; iF = immature females]

Maturity/Gender	**Released Individuals**		**Recaptured Individuals**		**Recapture Rate**
	Wt. (g)	**N**	**Wt. (g)**	**N**	**%**
mM	21.64	73	24.29	12	16.44
mF	22.44	127	25.34	28	22.05
iM	17.35	121	21.33	9	7.44
iF	15.64	149	15.72	5	3.36
Average (Total)	**18.9**	**(470)**	**23.5**	**(54)**	**11.5**

Released crawfish dispersed in all directions based on the location of recaptured individuals. Marked crawfish were captured in all areas of the pond and within 4 days, several individuals had been captured at the extreme most points of the pond. On average, those captured on day 4 were found in traps 272 meters (or 297 yards) from the release point (Table 3). Average dispersion speed was 53.4 meters/day (minimum 3.2 meters/day; maximum 167.4 meters/day), and average nominal distance travelled until recapture was 134.8 meters (minimum 14.6; maximum 538.6). Figure 3 shows the great variability in distance captured from the release point, and also depicts a general trend line for nominal rate of travel.

In conclusion, these results indicate that crawfish are very mobile and can spread quickly, utilizing large areas of suitable habitat. Also, based on the observed rate of capture in this study, it appears that baited traps are not as efficient as is often thought. Many farmers view baited traps as *magnets*, attracting any crawfish that wonder within a certain radius of the trap, but these results do not bear this out. With a capture rate of between 2.3 and 4% of the population of marked crawfish per day in this study, and the fact that some crawfish obviously passed baited traps before being captured, one can only conclude that trap harvesting is far less efficient than perceived by many.

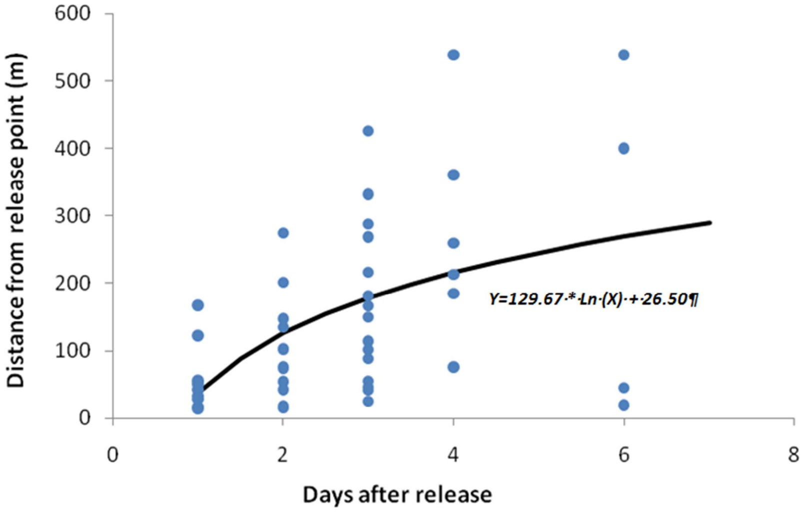

Figure 2. Individual values of nominal distance travelled until captured by day after release, with a line best fit to the data to depict the general trend.

Table 3. Average distance travelled in meters (m) and nominal rate of travel (m/day) based on location of captured marked crawfish in relation to release site. Data is arranged by days following release and maturity/gender of captured crawfish. [mM = mature males; mF = mature females; iM = immature males; iF = immature females; SE = standard error, a measure of variance]. Values with the same superscript in reference to distance travelled by day represents significant differences according to Tukey post-hoc test (*P*<0.05).

		Distance Travelled		**Nominal Rate of Travel**	
		m	**SE**	**m/day**	**SE**
Day	**1**	53.71 ^{a,b}	12.01	53.71	12.01
	2	95.43	19.86	47.71	9.93
	3	166.84 ^{a}	30.74	55.62	10.25
	4	272.19 ^{b}	65.50	68.05	16.38
	6	250.65	129.47	41.77	21.78
Average		**134.8**	**18.1**	**53.4**	**5.5**
Gender	**mM**	81.15	25.34	33.98	7.68
	mF	127.41	24.52	54.30	6.83
	iM	227.12	59.51	73.32	18.13
	iF	128.01	52.87	59.01	23,71
Average		**140.9**		**55.2**	

APPENDIX 4

Assessing the efficacy of releasing crawfish back to the pond for further growth

This appendix was published in:

McClain W.R., Sonnier J.J. and Ramalho R.O. (2011). Assessing the efficacy of releasing crawfish back to the pond for further growth. Louisiana Agriculture Magazine. Louisiana Agriculture Experimental Station. Louisiana State University. Baton Rouge, Louisiana. Summer 2011 Issue.

ASSESSING THE EFFICACY OF RELEASING CRAWFISH BACK TO THE POND FOR FURTHER GROWTH

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The United States crawfish aquaculture industry is largely located in Louisiana, where some 1,200 farmers produce in excess of 110 million pounds of procambarid crawfish annually from about 184,000 acres of ponds. Less than 20,000 additional acres is devoted to crawfish farming in a handful of other southern states, but Louisiana crawfish farmers often have to compete with a native crawfish fishery, mainly from the Atchafalaya River basin. A large majority of the annual crawfish production is marketed as live or whole-boiled crawfish, with less than 20% processed for the tail meat. Large volumes of live crawfish are delivered to metropolitan areas within and outside of Louisiana, such as Baton Rouge, New Orleans, Shreveport, Little Rock, Dallas, Houston, Mobile, Jackson, Memphis, and Atlanta. Marketing issues for live crawfish include significant variation in sizes, with the larger crawfish being the most valuable.

Although size grading of crawfish is initially accomplished by the mesh size of the wire-mesh traps, further grading is sometimes accomplished by large volume dealers at dock-side, but there are logistical and cost issues with this additional step in handling. Some crawfish farmers have begun employing a culling (grading) operation on the boat at the time crawfish are emptied from traps.

Harvesting boats are fitted with a bar grader that will effectively cull smaller crawfish from the catch and allow those to immediately re-enter the pond. The retained crawfish are more uniform in size (Figure 1) and are easier to sell because of the greater appeal with buyers. The common assumption is that the culled crawfish will resume growth and will be recaptured later at a larger, more valuable size.

Figure 1. Representative example of one of several types of on-board graders designed to cull smaller sizes of crawfish from the catch in commercial aquaculture ponds. Photo by Ray McClain.

Figure 2. Photo showing the culled or graded small crawfish being returned immediately to the pond for further growth. Photo by Ray McClain.

This practice of using on-board graders (Figure 2) has increased dramatically in recent years, and often renders further grading at dock-size unnecessary. However, the efficacy of this practice is largely unknown because the rate of recapture has not been determined. Frankly, little is known about the efficiency of trap harvesting in general as a means of removing crawfish from the population once they reach market size. Therefore, a series of studies was conducted at the LSU AgCenter's Rice Research Station to estimate the percentage of recapture and to better understand aspects of trap harvesting as well as crawfish movements within the pond.

A mark/recapture technique was used in a small commercial crawfish pond, and subsequently in the experimental pond complex of the Rice Research Station, to ascertain capture efficiency rates using baited traps. Captured crawfish were marked with a long lasting water-proof marker (Dykem®, BRITE-MARK® FAMILY, ITW Dymon, Olathe, Kansas) and released back into the pond within two hours of their capture. Bold marks were made on the carapace of crawfish that could be quickly and easily observed from a dorsal angle or from either side of the individual as they were emptied from traps (Figure 3). Various colors and/or markings distinguished the date and/or site of release. Subsequent trap catches were observed for marked individuals, which were noted for date and location of capture.

The initial trial was conducted in a 28-acre commercial crawfish pond where 200 mature and 270 immature individuals were marked and released at a central location in the pond. The subsequent harvest was monitored for one week, and only 54 marked individuals (11.5%) were recovered. While there was a possibility that molting occurred in some immature individuals (*i.e.* lost marks), the rate of recapture for mature (non-molting) individuals was noted at 20% – still a low recovery. Some crawfish traveled up to 589 yards, surely passing baited traps, before being recaptured, although the average distance traveled before recapture was 147 yards. Trap spacing typically ranges between 50 and 70 feet between traps.

Trials 2 and 3 were conducted in a contiguous arrangement of small (1 acre) experimental ponds where *mature-only* crawfish (489 crawfish for trial 2 and 200 crawfish for trial 3) were released in selected ponds, and recaptures were monitored from the entire pond complex. Those trials lasted for 9 - 12 weeks with an average total recapture rate of 49 and 45%, respectively. Of those crawfish recaptured, 83 and 97% (for trials 2 and 3, respectively) were caught from the same pond they were released into. Most of the others were captured in adjacent ponds, indicating some movement across a levee.

Figure 3. Typical mark placed on crawfish using a long-lasting waterproof marker prior to its release back to the pond in the mark-recapture study. Photo by John Sonnier.

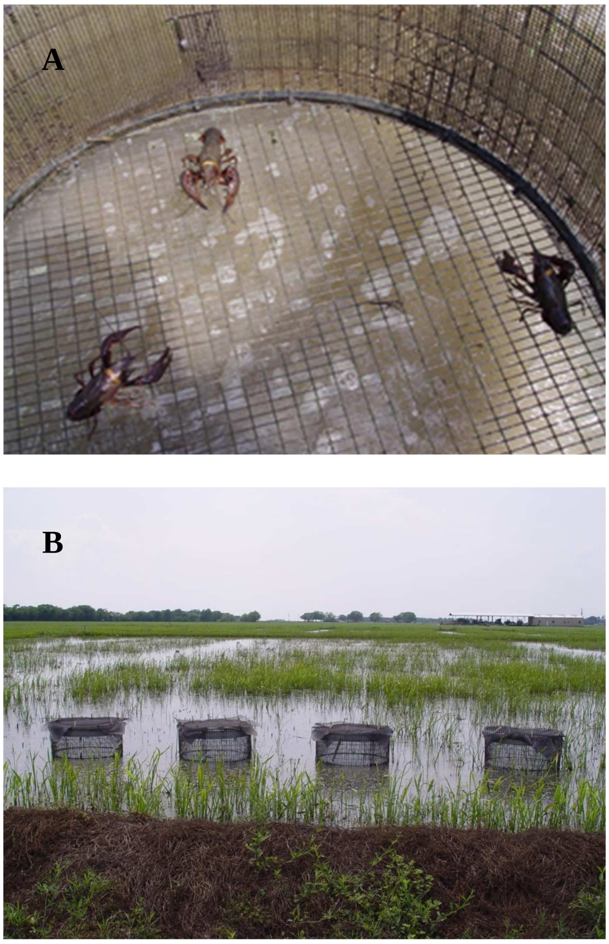

Figure 4. Random marked crawfish were maintained in cages (A) under the same environmental conditions as in trial 3 (B) to ascertain natural mortality over the duration of the trial. Photo by John Sonnier.

Recapture rates were greatest within the first 2 weeks of capture, with very few crawfish recaptures occurring after 4 weeks.

For trial 3, recaptured crawfish were marked with a different identifier and released after each capture. Also for that trial, additional marked crawfish were held in cages within the ponds from the beginning of the trial to estimate natural mortality in the marked population during the experiment (Figure 4A and 4B). While 45% of the crawfish released in trial 3 were captured only once, 17% were captured twice, and 5% were captured 3 times. Adjusted for natural mortality, the recovery rate for first time recaptures remained below 50% (at 49.5%).

Precise causes for the consistent low recovery (less than 50%) in these trials are unclear, but such factors as natural and predator-induced mortality, attrition from the pond population due to burrowing, and individuals exiting the pond are likely contributing factors. However, these results also seemingly illustrate the inefficiency with which baited crawfish traps remove market-size individuals from the pond population.

In conclusion, based on the results of this study, it appears that only half (or less) of the crawfish released back to the pond are ever recaptured. These findings do not bode well for the practice of releasing less-desired sizes of crawfish back to the pond with the expectation of recapturing them later at a larger, more valuable size. With recovery rates below 50%, one has to closely examine the economics of such a practice – thus, it may be far better to sell all crawfish at the point of first capture, even if a lower price is received for the smaller size grades.

In addition to market-economic considerations, there may be a biological reason to avoid releasing crawfish back to the pond once captured. Those crawfish would only compete with other, yet un-harvested crawfish for food and space. That would only exacerbate the problem of overcrowding if pond populations were high and food resources were scarce – contributing to reduced growth and lower production.

APPENDIX 5

Invasive crayfish dispersal: the effect of population density

This appendix is consisted of unpublished results presented at the following international meeting:

Ramalho R.O., Capinha C. and Anastácio P.M. (2010). Invasive crayfish dispersal: the effect of population density. NEOBIOTA– Biological Invasions in a Changing World. Poster Communication. Copenhagen. Denmark. 14th – 17th of September.

INVASIVE CRAYFISH DISPERSAL: THE EFFECT OF POPULATION DENSITY

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Abstract

The spreading of the invasive crayfish *Procambarus clarkii* throughout the world is attributed to human introductions, however, the rapid and widespread diffusion of the species following its establishment is the result of its dispersal capabilities. Introduced in Portugal in the late 1970s, the red swamp crayfish has invaded many water bodies, giving rise to breeding populations that now altered freshwater ecosystems. An understanding of the spatial behaviour of this crayfish could be the baseline for future research aimed at control and management. This study aimed to provide further information for the comprehension of the invasive potential of this crayfish by underlining some factors that could affect crayfish dispersal ability such as population density and other external co-variables.

Crayfish (54.45±3.80 mm mean cephalotorax length, sex ratio approximately 1:1) were submitted to an outdoor experiment in an experimental irrigation ditch. Five densities, 1, 5, 10, 15 and 20 individuals, previously confined for five days in 0.25 m^2 tanks, were released in the centre of the ditch at five different periods of the day and the position of each crayfish was recorded every five minutes. Each density was replicated five times.

Density affected both mean total distance and mean individual velocity attained by crayfish at the end of the experiment (ANCOVA; $P<0.001$). Crayfish kept at the higher density achieved higher velocities (2.89±1.11 m.s^{-1}) and went further (114.12±42.67 m) than those kept at the lowest density (2.38±1.09 m.s^{-1} vs 77.55±31.79 m) (Figure 1). Crayfish gender, water temperature and period of the day significantly affected crayfish average velocity (ANCOVA; $P<0.05$).

These results are relevant both for aquaculture and for modeling crayfish population dynamics.

INVASIVE CRAYFISH DISPERSAL:
THE EFFECT OF POPULATION DENSITY

READ THE POSTER IN 1 MINUTE!

1. This study aimed to provide further information for the comprehension of the invasive potential of *Procambarus clarkii* by underlining some factors that could affect crayfish dispersal ability such as population density and other external co-variables;

2. Adult crayfish (*Procambarus clarkii*) were acclimatized to 5 population densities (4, 20, 40, 60 and 80 ind./m^2). Acclimatized crayfish were released in a linear channel and individual speed and total distance were recorded;

3. Population density affected both average individual velocity and average total distance attained by crayfish at the end of the experiment;

4. Other variables that significantly affected crayfish average velocity: gender, water temperature and period of the day.

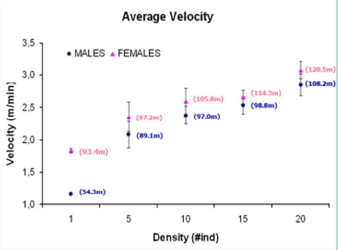


Figure 1. – Average Velocity versus different density treatments. Vertical bars represent standard errors. Superscript values stands for average Total Distance in meters.

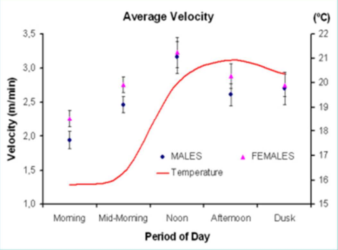
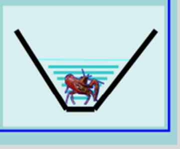


Figure 2. – Average Velocity versus different Period of Day treatments. Vertical bars represent standard errors. Average temperature is represented.

Parameter	Type III Sum of Squares	df	Mean Square	F	Sig.
Period of Day (Covariate)	0.077	1	0.077	4.643	<0.05
Water Temperature (Covariate)	0.636	1	0.636	38.255	<0.001
Fresh Weight (Covariate)	0.023	1	0.023	1.414	0.236
Density	0.314	4	0.079	4.725	<0.01
Gender	0.092	1	0.092	5.524	<0.05
Density*Gender	0.037	4	0.009	0.561	0.691

Table 1. – ANCOVA (Analysis of Covariance) results for the Average Velocity. Null hypothesis rejected at α<0.05.



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RESULTS and DISCUSSION

The ANCOVA showed that average velocity was significantly affected by crayfish density (Table 1). Crayfish submitted to higher densities moved faster, and traveled longer distances than crayfish kept at lower densities (Figure 1).

Crayfish gender also influenced the average velocity, this result could be due to weight differences between males and females (T-test; t=-2.503; df=253; p<0.05), however ANCOVA didn't show significant influence of crayfish weight, as a covariate, on average velocity.

External covariates analyzed (Period of the Day and Water Temperature) significantly affected crayfish average velocity under water (Table 1). Females also moved faster than males at different periods of the day and the maximum velocity attained at Noon (Figure 2). Average velocity seems to follow the daily water temperature variation (Pearson Correlation=0.441; p<0.001) (Figure 2).

There are other external factors involved such as: light intensity and light direction[2].

Other author also reported high peaks of locomotory activity during daylight in the absence of shelters[1,3].

Results are within the range of other research[1]: e.g. 0.1 – 4.25 km per day

The results of the present study confirms the high invasive potential of *Procambarus clarkii*, and provides insights about the importance of population density on the dispersal of this invasive species.

An understanding of the spatial behaviour of this crayfish could be the baseline for future research aimed at more effective population control and management.

References:
[1] Gherardi, F. & Barbaresi, S. (2000). Invasive crayfish: activity patterns of *Procambarus clarkii* in the rice fields of the Lower Guadalquivir (Spain). Arch. Hydrobiol. 150: 153 – 168.
[2] Lozán, J. L. (2000). On the threat of the European crayfish: a contribution with the study of activity behaviour of four crayfish species (Decapoda: Astacidae). Limnologica. 30: 156 – 161.
[3] Kozák, P., Kajtman, J., Kouril, J., Policar, T. (2000). The effect of crayfish density and shelter number on the daily activity of signal crayfish. Freshwater Crayfish 13: 457 – 462.

METHODS

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DENSITY ACCLIMATIZATION:
- Adult, intermolt and mature red-swamp-crayfish (*Procambarus clarkii*), average carapace length 54.45±0.24 mm (±SE) & average fresh weight (FW) 47.68±0.68g: Males (43.76±0.98g) & Females (47.32±1.03g);
- Were kept well feed for 5 days in 0.25 m^2 polyethylene tanks at five different densities: 1, 5, 10, 15 and 20 individuals per tank, corresponding to theoric densities of 4, 20, 40, 60 and 80 ind./m^2 respectively and a sex ratio of approximately 1:1 (male:female);
- Plastic aquariums filled 70 litres of aged tap water, artificially aerated;
- Light regime 14h Light/10h Dark similar to natural light regime and no shelters were provided;
- At least five replicates of each density were conducted.

PHYSICO-CHEMICAL CONDITIONS:
- Average water PH =8.20±0.13 (ANOVA: F=0.554; df=4; p>0.05);
- Dissolved O_2 was >85% at all treatments due to algae production;
- Water temperature varied from a minimum of 15.8 ºC at the morning treatment and a maximum of 21.9 ºC at the afternoon treatment (ANOVA: F=122.316; df=4; p<0.001).

EXPERIMENTAL DESIGN:
- Experiment conducted at experimental irrigation channel with 130 m length and a static "crystal clear" water column, 35cm high and 30 cm wide. No shelters were provided. The channel was marked every 20 cm;
- Each crayfish was individually marked with a permanent waterproof paint;
- After a period of 15 min of acclimatization and relax, in the center of the channel, the density acclimatized crayfish were released;
- The positions of each crayfish, in the channel, were recorded every 5 min during the experimental period (1hour);
- Crayfish released at five different periods of the day (Sunrise; Morning; Mid-Day (Solar); Afternoon and Dusk) with 5 replicates each;
- Water temperature, PH and Dissolved Oxygen were recorded;
- Average Speed and Total Distance were determined.