Abstract

The introduction of exotic species is a problem all over the world, causing great damages in the economy and ecology of a particular territory. Exotic species are known to affect native aquatic systems at various levels, often leading to the loss of biodiversity by a decrease in both the number and abundance of native species or even causing their elimination. In a conservationist perspective, there are two important purposes: to evaluate the occurrence of dense populations and the rapid expansion of exotic species in their new habitat. In general, invasive species show a high capacity to adapt to new habitat and a wide range of tolerance to environmental conditions. An example of this plasticity is shown by the red swamp crayfish, *Procambarus clarkii* (Girard, 1852), a native species from Northeastern Mexico and South Central USA that has become the most cosmopolitan crayfish. It is now found in all continents except Australia and Antarctica. *P. clarkii* is a successful species with invasive characteristics such as being an r-selected species, with high growth rates, highly tolerant and adaptable to extreme environments. Furthermore, *P. clarkii* behaves as an omnivore and is known to consume various types of food resources while acting as detrivore, herbivore and predator. Since its introduction in the Iberian Peninsula, there has been a rapid expansion, and its presence was already detected in most of freshwater ecosystems, especially in the South. In Portugal, *P. clarkii* has also shown the ability to rapidly increase its populations because of its large niche breath, which enabled it to use a wide variety of resources, and to expand its range to new areas. The first reference of *P. clarkii* in Portugal was around 1979 and since then populations have increased very fast. Its growth rate, associated to a strategy of reproductive success, is responsible for the development of extremely dense populations that may have a major negative impact on the cultivated areas in wetlands. The study of the population biology of this species
is essential to understand its relation with the environment and to predict the evolution of its geographic distribution. The intensification of scientific research on the population dynamics of *P. clarkii* will lead to a broader understanding of its invasion mechanisms and processes, providing quantitative data to predict future crayfish invaders. To better understand the biology of *P. clarkii*, two populations from Paul de Cadaval (PCD) and Paul de Magos (PMG) were studied for 13 months. In the context of conservation biology, this kind of studies is extremely important to get a better knowledge on this species and its invasion strategies that may lead to the extinction of native species. The first part of this work refers to the dynamics of the populations of *P. clarkii*, so several aspects of the population dynamics, such as population structure, density, sex ratio, biomass, production, productivity, mortality rate, average age or mean lifetime were analysed. The results of the modal analysis, using first the Bhattacharya’s method and after the Normsep’s method, distinguished the presence of 4 cohorts for the crayfish population from PCD and 6 cohorts for the population from PMG. In fact, *P. clarkii*’s age cohorts may vary in their number from one population to another. In addition, the presence of several cohorts at PCD and at PMG indicates that both populations are productive. These results also show that the crayfish population from PCD is composed by large animals in spring and summer and juveniles at the end of autumn and winter. Therefore, our results indicate the entrance of newly hatched crayfish throughout the year in the population of PMG. Large individuals were more abundant in late spring, summer and early autumn. In this study, the relative density of crayfish was much higher in PMG (10.98 ind/m²) than in PCD (1.69 ind/m²). During this study, 682 crayfish were caught at PCD and 6272 were sampled at PMG from May 2005 to May 2006. At PCD 208 were captured with traps and 474 animals with the dip net. At PMG 704 animals were collected with the traps and 5568 with dip net. The
mean relative density estimated for traps at PCD was 0.57 ind/m² and 0.99 ind/m² for the dip net. At PMG, the mean relative density was 1.29 ind/m² and 9.66 g/m² for traps and the dip net respectively. Analysing the results from these two methods, the dip net was considered the best method, because this device is more selective towards juveniles than the traps. Both marshes with high densities had previously been classified as suitable habitat to the rapid development of *P. clarkii* populations. The ratio between sexes is a good indicator of species regulation, i.e. the proportion of males to females is a good indicator of the population stability. The sex ratio results obtained for both marshes were favourable to females. The proportion of females was higher in the population from PCD probably in response to the drought of the pond during 4 months. In both marshes, the highest values of relative biomass were observed in spring and summer, and the lowest in winter. Productivity is also related with environmental alterations and depends on density processes that will determine the future maintenance of crayfish populations. The lower productivity of the population from PMG in relation to that from PCD may be explained by the higher densities observed in PMG, which probably inhibited the increment in body size and consequently, the observable productivity of this population. However, high values of production and productivity indicate that both populations were, in fact, productive. The mortality rate and the mean lifetime of both populations were almost identical. The second part of this study refers to the reproduction and growth of the same populations and was based on the analyses of reproductive traits and growth parameters, such as maturation status, maturation index, population recruitment, growth rate and growth curve, and on the analyses of the cohort’s growth of each population. The analyses of the maturation index and the evolution of the maturation state of both sexes at PCD indicated as reproductive periods the spring 2005, the autumn 2005 and the beginning of spring 2006. These seasons
coincide with the best availability periods in terms of food resources and refuge. During this period, it was also found that large females and its future offspring have a greater probability of developing and surviving. The abundance of mature males is also a good indicator of the reproduction state of a population. There were few FI males in winter and greater abundance in spring 2005/2006 and summer 2005 at PCD. The same happened in autumn 2005 and spring 2006 at PMG. At PCD recruitment occurred in spring and in autumn. At PMG, recruitment occurred all year round, this extension of the reproduction period being mainly caused by more favourable climatic conditions. Finally, the asymptotic length \((L_{\infty})\) and the curvature parameter \((K)\) revealed a faster growth of individuals from PCD than those from PMG. The growth rate was also faster in PCD than in PMG. From the present results, it can be concluded that both populations were stable and productive. This kind of knowledge is useful for the management of crayfish populations and prevention of future invasions.

**Keywords:** Exotic species, Freshwater marshes, Population dynamics, Reproduction, Growth.
**Resumo**

A introdução de espécies é um problema a nível mundial que provoca prejuízos sérios na economia e ecologia de um determinado território. As espécies exóticas são conhecidas por afectarem os sistemas aquáticos a vários níveis, por causarem a perda da biodiversidade tanto pelo decréscimo em número como em abundância de espécies nativas ou mesmo a sua eliminação. Numa perspectiva conservacionista, existem dois importantes objectivos: avaliar a ocorrência de populações densas e a rápida expansão de espécies exóticas no seu novo *habitat*. Em geral, as espécies invasivas mostram uma elevada capacidade de adaptação a novos *habitats* e uma grande tolerância a variações diversas das condições ambientais. Um exemplo desta plasticidade é observável no lagostim vermelho, *Procambarus clarkii*, uma espécie nativa do Nordeste do México e da região Central Sul dos USA. Este tornou-se no lagostim mais cosmopolita, sendo actualmente encontrado em todos os continentes, com exceção da Austrália e da Antártica. *P. clarkii* é uma espécie de sucesso com características invasivas, tais como, o facto de ter uma estratégia de selecção r, com elevadas taxas de crescimento, elevada tolerância e capacidade de adaptação a ambientes extremos. Para além disso, *P. clarkii* é omnívoro e conhecido por consumir vários tipos de recursos alimentares, agindo também como detrítivoro, herbívoro e predador. Desde a sua introdução na Península Ibérica, que se tem verificado uma rápida expansão e a sua presença tem sido detectada na maioria dos ecossistemas de água doce, especialmente no Sul. Em Portugal, *P. clarkii* também mostrou uma grande capacidade de aumentar rapidamente as suas populações devido ao seu nicho abrangente, que possibilitou o uso de uma grande variedade de recursos e a sua expansão para novas áreas. A primeira referência de *P. clarkii* em Portugal remonta a 1979 e desde então as populações cresceram rapidamente. A sua taxa de crescimento, associada a uma estratégia reprodutiva de sucesso, tem sido
responsável por um desenvolvimento de populações extremamente densas que causam um grande impacto em áreas húmidas cultivadas. O estudo da biologia populacional desta espécie é essencial para compreender a sua relação com o ambiente e para prever a sua evolução e a distribuição geográfica. A intensificação da investigação científica na dinâmica populacional de *P. clarkii* levará a um melhor conhecimento dos mecanismos e processos da sua invasão, providenciando dados quantitativos para prever o futuro dos lagostins invasores. Com intuito de conhecer melhor a biologia de *P. clarkii*, estudaram-se, durante 13 meses, duas populações provenientes de duas zonas húmidas: o Paul de Cadaval (PCD) e o Paul de Magos (PMG). No contexto da biologia da conservação, este tipo de estudos é muito importante para aprofundar o conhecimento sobre esta espécie, em particular das suas estratégias de invasão. A primeira parte deste trabalho refere-se ao estudo da dinâmica das referidas populações de *P. clarkii*. Nesta fase do estudo analisaram-se vários aspectos da dinâmica populacional, tais como, a estrutura da população, a densidade, o *sex ratio*, a biomassa, a produção e a productividade, a taxa de mortalidade e a longevidade média. Os resultados da análise modal usando primeiro o método Bhattacharya e depois o de Normsep, distinguiram a presença de 4 coortes de uma população de lagostim de PCD e 6 coortes da população de PMG. De facto, as coortes em *P. clarkii* variam em número de uma população para outra. Para além disso, a presença de várias coortes no PCD e no PMG indica que ambas as populações são produtivas. Estes resultados também mostram que a população de lagostim de PCD é composta maioritariamente por animais grandes na primavera e no verão, e por juvenis no final do outono e inverno. Os nossos resultados indicam também que houve entrada de lagostins recém ecolididos na população de PMG ao longo do ano. Os indivíduos grandes são muito abundantes no fim da primavera, no verão e no início do outono. Neste estudo, a densidade relativa do lagostim foi muito maior no PMG.
Durante este estudo capturaram-se 682 lagostins no PCD e 6272 no PMG, entre Maio de 2005 e Maio de 2006. No PCD, 208 animais foram capturados com armadilhas e 474 com camaroeiro. No PMG, a amostragem com armadilhas foi de 704 animais e com camaroeiros, 5568. A densidade relativa média estimada com armadilhas em PCD foi de 0.57 ind/m² e com camaroeiros, 0.99 ind/m². No PMG a densidade relativa média estimada com armadilhas foi de 1.29 ind/m² e com camaroeiros, 9.66 g/m². Analisando os resultados obtidos com estes dois métodos, o camaroeiro foi considerado o melhor método, o que pode ser explicado por ser esta a ferramenta mais selectiva em relação aos juvenis. Ambos os pauis revelaram elevadas densidades, tendo sido já no passado classificados como habitat adequados ao rápido desenvolvimento das populações de *P. clarkii*. A proporção entre os sexos é um bom indicador da regulação da espécie, ou seja, o equilíbrio na população entre a proporção dos machos e das fêmeas é um bom indicador da estabilidade populacional. Os resultados obtidos do sex ratio em ambos os pauis foram favoráveis às fêmeas. A proporção das fêmeas foi maior na população de PCD, provavelmente em resposta à secura do paul durante 4 meses. Em ambos os pauis, os valores mais elevados da biomassa relativa foram observados na primavera e no verão e os mais baixos, no inverno. A produtividade também está relacionada com as alterações das condições ambientais e depende dos processos da densidade que determinarão a manutenção futura das populações de lagostim. A baixa produtividade da população do PMG em relação à do PCD pode ser explicada pelas densidades elevadas observadas no primeiro, que podem ter inibido o incremento do tamanho corporal e a produtividade desta população. Contudo, valores elevados de produção e de produtividade indicam que ambas as populações são produtivas. Os valores da taxa de mortalidade e da longevidade de ambas as populações foram muito semelhantes. A segunda parte deste
trabalho refere-se ao estudo da reprodução e do crescimento das mesmas populações e foi baseada na análise de parâmetros de reprodução e de crescimento, tais como, o estado de maturação, o índice de maturação, o recrutamento da população, a taxa de crescimento, a curva de crescimento e a análise de crescimento das coortes de cada população. A análise dos índices de maturação e da evolução do estado de maturação de ambos os sexos no PCD, indica que a primavera de 2005, o outono de 2005 e o início da primavera de 2006 foram os principais períodos reprodutivos. Estas estações coincidem com os períodos com uma maior disponibilidade de recursos alimentares e de refúgios. O que também se verifica é que existe uma maior probabilidade de desenvolvimento e de sobrevivência tanto para as fêmeas grandes como para a sua futura prole. A abundância dos adultos maduros é também um bom indicador do estado reprodutivo da população. Em ambos os pâris houve poucos machos FI no inverno e uma grande abundância na primavera de 2005/2006 e no verão de 2005 no PCD. O mesmo aconteceu no outono de 2005 e na primavera de 2006 no PMG. O recrutamento no PCD ocorreu na primavera e outono. No PMG, o recrutamento ocorreu durante todo o ano, devendo-se principalmente esta extensão do período reprodutivo a condições climáticas favoráveis. Finalmente, o crescimento assintótico ($L_\infty$) e o parâmetro da curvatura (K) revelaram um crescimento rápido, tanto dos indivíduos do PCD, como do PMG. A taxa de crescimento foi também mais rápida no PCD do que no PMG. Os resultados deste trabalho mostraram que ambas populações estão estáveis e produtivas. Este tipo de conhecimento poderá ser muito útil na gestão das populações de lagostim e na prevenção de futuras invasões.

**Palavras-chave:** Crescimento, Dinâmica da população, Espécies exóticas, Reprodução, Zonas húmidas.
General introduction

The study of introduced species is essential to identify potential invasive species and to detect and predict the changes in ecosystems caused by them (Simon & Townsend, 2003). The impact of introduced species on native ones, on communities and ecosystems has been studied for decades (Sakai et al., 2001). Overall, invasive species have negative impacts on the structure and functioning of ecosystems (Correia, 2003), affecting both the economy and the ecology of a particular territory (Sakai et al., 2001). Exotic species are known to affect native aquatic systems at various levels, often leading to the loss of biodiversity by a decrease in both the number and abundance of native species or even causing their elimination (Hobbs et al., 1989). At the population level, exotic species often cause changes in the behaviour of native ones, e.g. in the way they use the habitats, in communities, by inducing changes in the abundance and distribution of autochthonous species and at the ecosystem level by changing the flow of energy and nutrients (Simon & Townsed, 2003). From a conservationist point of view, there are two important issues to evaluate, the occurrence of dense populations and the rapid expansion of exotic species in their new habitat. These aspects become increasingly important with climate change since they may lead to homogenization of biota worldwide (Sakai et al., 2001).

In general, invasive species show a high capacity to adapt to new habitats and a wide range of tolerance to environmental conditions (Hobbs et al., 1989, Anastácio & Marques, 1995). An example of this plasticity is shown by the red swamp crayfish, *Procambarus clarkii* Girard, 1852, a native species from Northeastern Mexico and South Central USA (Hobbs, 1972) that has become the most cosmopolitan crayfish as result of its massive translocation for aquaculture purposes all over the world, being found in all continents except Australia and Antarctica (Huner, 2002). Gherardi (2006)
reviewed several works that confirm the invasive characteristics of *P. clarkii*. For example, *P. clarkii* is an *r*-selected species (Huner & Lindquist, 1991), with high growth rates (Holdich, 1988), highly tolerant and adaptable to extreme environments such as temporary streams in Southern Portugal (Gherardi *et al*., 2002) and polluted habitats (Gherardi *et al*., 2000). Several strategies of *P. clarkii* make it possible. Among those, it has been observed that the individuals did not aestivate, they hide inside burrows, which are fundamental for keeping crayfish body temperature below lethal limits (Huner & Barr, 1991; Correia, 1995; Correia & Ferreira, 1995). In other cases *P. clarkii* probably switches to habitats with silt where they can burrow, spawn and grow (Correia & Ferreira, 1995). These movements mainly occur at night to avoid predators (mostly fish and birds) and are dependent on water depth (Flint, 1977; Cukerzis, 1988; Maintland & Campbell, 1992). Furthermore, *P. clarkii* behaves as an omnivore and is known to consume various types of resources while acting as detrivore, herbivore and predator (Ilhêu & Bernardo, 1993).

This species is also responsible for the decline of autochthonous crayfish by means of competitive exclusion and also acting as a vector of the crayfish fungus plague, *Aphanomyces astaci*. It is also associated with the virus *Vibriosis* in crayfish farms and is a host of helming parasites of vertebrates (Hobbs III *et al*., 1989; Rodriguez *et al*., 2005). Several studies reported the effects of *P. clarkii* on macrophytes: the burrowing activity increased the turbidity of water, thus reducing the light conditions needed for the development of the submerged vegetation (Olsen *et al*., 1991; Nystrom, 1999; Correia, 2002; Rodriguez *et al*., 2005).

However, this species became an important resource for both invertebrates and vertebrates (Hobbs *et al*., 1989). In Portugal, Correia (2001) found that *P. clarkii* has become a new food source for some species of carnivore mammals and ciconiiform
birds, being fundamental to the survival of these birds when native prey are scarce or unavailable.

For all these reasons Gherardi (2006) in her revision of several studies about P. clarkii, referred that this species should be added to the ever-lengthening list of species that invaded European freshwaters.

Since its introduction in the Iberian Peninsula, there has been a rapid expansion and its presence has already been detected in most of freshwater ecosystems, especially in the South (Gutiérrez-Yurrita et al., 1996). In the Iberian Peninsula P. clarkii increased its effective population without control, invading many rice fields and wetlands, causing serious damage to drainage systems because of their burrowing activity (Correia, 1993; Correia & Ferreira, 1995; Cano & Ocete, 1997; Correia, 2001; Correia, 2002; Anastácio et al., 2005). In Mediterranean wetlands, P. clarkii is able to colonize most water bodies, even the small and shallow ones, because the conditions found in this region are similar to the conditions in its native area (Cruz & Rebelo, 2007).

In Portugal, P. clarkii has also shown the ability to rapidly increase its populations because of its large niche, which enabled it to use a wide variety of resources and to expand its range to new areas (Correia, 1993; 1995; 2002).

The first reference of P. clarkii in Portugal appeared by 1979 (Ramos & Pereira, 1981) and since then populations have increased rapidly (Correia, 2003). Its growth rate, associated to a strategy of reproductive success, is responsible for the development of extremely dense populations that may have a major negative impact on the cultivated areas in wetlands (Anastácio & Marques, 1995; Correia & Ferreira, 1995). This crayfish causes great damages on the rice production. Anastácio et al. (2005) observed that all early rice developmental stages were affected (usually the 3 - 6 day rice seedlings).
According to Sakai et al. (2001) studies on the population dynamics of invasive species help to define more effective control measures. For instance, the enormous difficulty to eradicate *P. clarkii* is well known. Farmers have repeatedly tried to eradicate its populations by means of xenobiotic chemicals in the rice fields. However, this kind of methods was ineffective on the crayfish and caused a great damage on useful species (Anastácio & Marques, 1995).

Several models have been created to analyse the expansion and management of invasive species. These models can be used to predict with great accuracy the rapid expansion of *P. clarkii* and to improve the management of this species, through the identification of biotic factors sensitive to control (Sakai et al., 2001).

This study aimed to provide data that can be integrated in ecological models used for the management of this exotic species. The main objective of this research was to study the biology of two populations of *P. clarkii* in two freshwater marshes, Paul de Cadaval (PCD) and Paul de Magos (PMG), from May 2005 to May of 2006. Correia (1995) has also studied these populations and after ten years, the present work was very important to gather more information about its dynamics and evolution. The population dynamics of *P. clarkii* is important to understand the differences between the two populations and their evolution since the last work in 1995 in the same areas.

This work had two main purposes to accomplish the above mentioned objective: to analyse the population dynamics of *P. clarkii* and to determine its reproduction and growth in two freshwater marshes, a pond (PCD) and a rice field (PMG) located in the Tejo river basin from May of 2005 to May of 2006.

Within the first purpose of the dissertation, the structure, density, production and mortality were determined for both populations.
Within the second purpose, the mean size at sexual maturity for both sexes, the maturation index, maturation state, recruitment, growth parameters and growth curve, were also determined for both populations.

References


Population dynamics of the invasive crayfish (*Procambarus clarkii* Girard, 1852) from two freshwater marshes

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**Abstract**

The population dynamics of two populations of *Procambarus clarkii* (Girard, 1852) in two freshwater marshes, a pond (PCD) and a rice field (PMG) located in the Tejo river basin, Portugal, were studied from May 2005 to May 2006. Using the Bhattacharya’s methods 4 and 6 cohorts were detected at PCD and PMG respectively. At both marshes, large crayfish were abundant in spring, summer and early autumn and juveniles, were abundant in autumn (especially at PCD). Crayfish density at PCD, of 1.69 ind/m$^2$ was lower than at PMG, of 10.98 ind/m$^2$. The sex ratio (M/F) was higher 0.91 at PCD against 0.78 at PMG. Mean biomass was lower at PCD: 26.63 g/m$^2$ against 27.15 g/m$^2$ at PMG. Production was estimated to be 2.7 greater at PCD, 460 kg/ha (166 Kg/ha for PMG). Productivity was also higher in the first marsh (1.75 for PCD, 0.61 for PMG). Mortality rate was 2.39 at PCD and 2.47 at PMG. The average age or mean lifetime of the crayfish populations was 0.42 years, approximately 153 days, at PCD and 0.41 years, or approximately 148 days at PMG. Overall, the dynamics of both *P. clarkii* populations revealed stability and spreading potential.

**Keywords** – Bhattacharya, Biological traits, Environmental parameters, Modal analysis.
Introduction

Nowadays, the introduction and invasion of new species is one of the most important issues in conservation biology. Recent analyses suggest that biodiversity in freshwater ecosystems tends to decline faster than in most terrestrial ecosystems (Ricciardi & Rasmussen, 1999; Gherardi, 2006). Freshwater habitats are invaded by new species and if this process of homogenization continues, in less than 100 years most of these habitats will be dominated by an array of cosmopolitan species that will replace most of the native ones (Gherardi, 2006). In this context, the invasion by crayfish species has been held responsible for the biological homogenization of many freshwater systems since they cause significant alterations on community structure especially on aquatic food webs (Lodge et al., 1998).

The red swamp crayfish, *Procambarus clarkii* Girard, 1852, has been introduced in many countries of Europe, Asia and Africa for aquaculture purposes (Hobbs & Huner, 1989; Huner & Avault, 1979). This exotic species caused great damage to local bentic fauna and flora (Gutiérrez-Yurrita et al., 1997) all over the world and thus the majority of *P. clarkii* introductions had negative consequences (Hobbs & Huner, 1989; Anastácio & Marques, 1995; Lodge et al., 2000; Cruz & Rebelo, 2007). Its life cycle is characterized by a considerable plasticity, invading diversified environments where it adapts to extreme conditions such as temporary streams and polluted habitats (Gutiérrez-Yurrita & Montes, 1999; Gherardi et al., 2000). In Portugal this species was introduced from Spain in the 1970’s (Ramos & Pereira, 1981) and its populations increased in number without control throughout the country. It is considered a pest because of its burrowing activity which causes serial damage in dams and levees and consequently in rice cultures (Correia, 1993; Anastácio & Marques, 1995).
In a general way, the population dynamics of the red swamp crayfish is highly dependent on the interaction between environmental and biological factors. If environmental conditions are favourable, the population acts as a r-strategist, with a fast growth rate and high fertility (Ilhéu & Bernardo, 1996).

The study of the population dynamics of this species is essential to understand its relation with the environment and to predict the evolution of its geographic distribution (Cruz & Rebeiro, 2007; Scalici & Gherardi, 2007).

The objective of this study was to examine several aspects of the population dynamics of *P. clarkii* such as population structure, density, sex ratio, biomass, production, productivity, mortality rate, average age or mean lifetime.

**Material and Methodology**

**Study area**

This study took place from May 2005 to May 2006 in two freshwater marshes, Paul de Cadaval (39º N, 8º 30´ W) and Paul de Magos (38º 58´ W, 8º 45´ W), located at the Salvaterra de Magos County in the Tejo river basin.

Paul de Cadaval (PCD) is a freshwater marsh of about 14 km² where the landscape is widely variable, with agricultural fields including ponds and reservoirs (Correia, 2001). This freshwater marsh is also surrounded by riparian vegetation and is linked to the Lamarosa stream. The peripheral land is occupied by oaks, reeds and eucalyptus (Correia, 1995).

Paul de Magos (PMG) is a rice (*Oriza sativa*) field area of 7 km², characterized by a continuous irrigated spring-summer culture whose development and growth is strongly influenced by the water level (Correia, 1995). The preparation of the fields cultivation begins in March-April with the plough by leveling the ground and then with the
flooding of beds. The sowing is done in April-May and harvesting in September-October, then the fields are left to dry until March-April. The water level is controlled through a system of irrigation and drainage boxes, also useful to control aquatic weeds and animal pests. In the adjacent areas there are oaks and pinewoods (Correia, 1995; 2003).

**Crayfish sampling and laboratory procedures**

Crayfish were sampled once a month at the two freshwater marshes, from May 2005 to May 2006, with a dip net (65 cm x 40 cm frame; 3 mm mesh size) and traps. Collecting of crayfish was conducted in a pond at PCD and in irrigations channels at PMG. Sampling was replicated three times in each marsh. Water environmental parameters were recorded: depth, temperature, dissolved oxygen, conductivity, ammonium ion ($\text{NH}_4^+$) and pH. In each replicate, 3 traps with bait (canned sardine) were set out (with a distance of 5 m between them) at sunset covering a total area of 15 m$^2$. After 12 h crayfish were collected from the traps and in the same area of 15 m$^2$ crayfish were also sampled with the dip net, using a CPUE (catch for unity effort) of 15 min. Samples obtained by both methods were separately kept in bags, previously identified, and preserved in 70 % alcohol for further analysis.

Paul de Cadaval dried from July to October 2005, therefore during these months the sampling and the measurement of environmental variables did not take place.

In the laboratory, each specimen was weighted to nearest 0.001 g, and the carapace length (from the tip of rostrum to the carapace end) was measured to the nearest 0.01 mm.
Sex was differentiated in individuals with a carapace length longer than 1.3 cm. The sex of the individuals was distinguished by the presence of gonopodia in males that results from the modification of the first pair of pleopods (Huner, 1978).

**Analytical procedures**

*Population structure*

The modal progression analysis was used to study the population structure of each marsh (Gayanilo *et al.*, 2005). This analysis is based on the body size data and polymodal frequency distribution histograms are created and submitted to the Bhattacharya’s method routine of the FITSAT (FAO-ICLARM Stock Assessment Tools) computer program. Size-frequency distributions (cm) of the two study populations were constructed using a 0.5 cm class interval. The results of the Bhattacharya’s method routine were afterwards refined by other FITSAT routine, the Normsep’s method. For each cohort identified by the Normsep’s method the mean length (L), the standard deviation (SD) and the total number of individuals of each cohort (NC) were estimated. The mean carapace length (CL) of each population was also calculated monthly.

*Density*

Relative density (RD) was calculated by the ratio between the total number of individuals (N) and the total area sampled (15 m$^2$) at each study area. Trap density was determined by the ratio between the number of individuals caught by the traps and the total area sampled (15 m$^2$) at each marsh. Dip net density was assessed by the ratio between the number of individuals caught by the dip net and the total area sampled (15 m$^2$) at each study area. Density (D) was also estimated for each cohort identified by the
Normsep’s method, by calculating the ratio between the total number of individuals of each cohort (NC) and the total area sampled (15 m²).

Sex ratio

Sex ratio (SR) was calculated through the ratio between the males and females (M/F) for each month at each marsh.

Biomass, Production and Productivity

The relative biomass (RB) was calculated monthly through the equation RD x MW for each marsh. The Mean MW is the mean total weight of each population and it was monthly determined. The biomass (CB) for each cohort was also calculated, based on Density (D) and the wet weight of each one. The wet weight (WW) was estimated by the equation: W = a L^b. The coefficients a e b were estimated by the relation L∞ = -a/b; and the CB was calculated by the equation: CB = D x WW. Mean Biomass (B) was estimated by the equation: \[ \int CB, \] for each marsh.

Production (P) of each population was calculated by the equation: W x B, where W is the instantaneous growth rate (Allen, 1971) and was given by the equation: W = 10^a (L) (Allen, 1971).

Productivity (PR) of each marsh was given by the ratio (P/B) (Winberg, 1971).

Mortality

The mortality index (Z) was calculated using the Powell-Wetherall Plot equation (Powell, 1979) using the length-frequency data, the asymptotic length (L∞) and the ratio between the mortality and the curvature parameter (Z/K) in the option length-converted catch curve analysis provided by the FITSAT program (Gayanilo et al., 2005).
Based on $Z$, the mean lifetime of each population ($t_{1/2}$) was given by the ratio $1/Z$, considering that $Z$ is constant and that both populations did not undergo migration (Allen, 1971).

**Statistical analysis**

The statistical analysis was performed using the software program Statistic version 7.0 and all results were grouped in seasons. Several dependent variables such as length, density, sex ratio and biomass were compared between marshes and seasons. Only the variance of the sex ratio was homoscedastic, therefore a Two Way ANOVA was used for this case, considering site and season, the two independent variables. Afterwards, Fisher LSD post-hoc test was used when the differences were significant. Since the other variables showed heterogeneous variances, after several unsuccessful transformations, two nonparametric methods were used. The Kolmogorov-Smirnov test and the Kruskal-Wallis test to analyse length, density and biomass. Differences between the two marshes were analysed by the Kolmogorov-Smirnov test (Sokal & Rohlf, 1981). To analyze the differences between seasons at each marsh the Kruskal-Wallis test was applied. When the null hypothesis was rejected, a nonparametric multiple comparison test was applied to better identify the differences between seasons (Siegel & Castellan, 1988).

**Results**

*Physical and chemical variables*

The mean water temperature (TEMP) was 14.68 °C at PCD and 16.73 °C at PMG. (Tables 1 and 2). At PCD the minimum TEMP, 6.43 °C, was recorded in December 2005 and the maximum TEMP, 23.07 °C, in June 2005. At PMG the minimum water
TEMP, 8.3 °C, was recorded in December 2005 and the maximum TEMP, 29.53 °C, in May 2005. The mean dissolved oxygen (OXY) in water was 5.17 mg/l at PCD and 6.80 mg/l at PMG (Tables 1 and 2). At PCD the minimum OXY was 2.19 mg/l, in March 2006, and the maximum was 22 mg/l, in January 2006. At PMG the minimum OXY was 0.405 mg/l, in September 2005, while the maximum was 32.76 mg/l, reporting to May 2006. The mean pH was 6.46 at PCD and 6.26 at PMG (Tables 1 and 2). At PCD the highest pH, 6.76, was recorded in June 2005 and the lowest, 6.07, in March 2006. At PMG the highest pH, 7.19, was recorded in May 2006 and the lowest, 3.49, in August 2005. PCD presented a lower mean conductivity (COND), 323.85 μs/cm, than PMG, 848.4 μs/cm (Tables 1 and 2). At PCD the maximum COND, 432.33 μs/cm, was recorded in May 2006 and the minimum, 231.33 μs/cm, in March 2006. In PMG the maximum COND, 1268.67 μs/cm, was recorded in May 2005 and the minimum, 432.33 μs/cm, in June 2005. The mean concentration of ammonium ion (NH₄⁺) in the water was 0.92 mg/l at PCD and 0.74 mg/l at PMG (Tables 1 and 2). At PCD, the maximum NH₄⁺ ion was 2.07 mg/l in June 2005. At the PMG, NH₄⁺ varied between 0.6 mg/l in July 2005 and 1.2 mg/l in May 2005. The mean water depth was 0.50 m at PCD and 0.44 m at PMG (Tables 1 and 2). At PCD, the maximum water depth was 0.64 m in March 2006 and the minimum 0.423 m in February 2006. At PMG, the maximum water depth was 0.69 m, in March 2006, and the minimum 0.2 m, in September 2005.
Table 1. Values of the water physicochemical parameters measured at PCD. TEMP – mean water temperature; OXY – mean dissolved oxygen; COND – conductivity; NH$_4^+$ – ammonium ion; DEPH – profundity.

<table>
<thead>
<tr>
<th></th>
<th>TEMP (ºC)</th>
<th>OXY (mg/l)</th>
<th>OXY (%)</th>
<th>pH</th>
<th>COND (µS/cm)</th>
<th>NH$_4^+$ (mg/l)</th>
<th>DEPH (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May-05</td>
<td>21.9</td>
<td>4.13</td>
<td>48.1</td>
<td>6.72</td>
<td>255</td>
<td>0.8</td>
<td>0.49</td>
</tr>
<tr>
<td>Jun.</td>
<td>23.07</td>
<td>3.74</td>
<td>63.4</td>
<td>6.76</td>
<td>388.33</td>
<td>2.07</td>
<td>0.45</td>
</tr>
<tr>
<td>Nov.</td>
<td>10.27</td>
<td>4.55</td>
<td>39.67</td>
<td>6.73</td>
<td>342.33</td>
<td>0.6</td>
<td>0.49</td>
</tr>
<tr>
<td>Dec.</td>
<td>6.43</td>
<td>9.68</td>
<td>86.3</td>
<td>6.52</td>
<td>343</td>
<td>0.6</td>
<td>0.49</td>
</tr>
<tr>
<td>Jan-06</td>
<td>8.7</td>
<td>10.22</td>
<td>87.57</td>
<td>6.61</td>
<td>325.67</td>
<td>0.6</td>
<td>0.49</td>
</tr>
<tr>
<td>Feb.</td>
<td>9.8</td>
<td>5.57</td>
<td>57.4</td>
<td>6.24</td>
<td>318</td>
<td>0.6</td>
<td>0.42</td>
</tr>
<tr>
<td>Mar.</td>
<td>14.63</td>
<td>2.19</td>
<td>21.57</td>
<td>6.07</td>
<td>231.33</td>
<td>1.2</td>
<td>0.64</td>
</tr>
<tr>
<td>Apr.</td>
<td>16.53</td>
<td>3.18</td>
<td>34.8</td>
<td>6.18</td>
<td>278.67</td>
<td>1.2</td>
<td>0.51</td>
</tr>
<tr>
<td>May</td>
<td>20.83</td>
<td>3.29</td>
<td>39.57</td>
<td>6.31</td>
<td>432.33</td>
<td>0.6</td>
<td>0.53</td>
</tr>
<tr>
<td>Mean</td>
<td>14.68</td>
<td>5.17</td>
<td>53.15</td>
<td>6.46</td>
<td>323.85</td>
<td>0.92</td>
<td>0.50</td>
</tr>
</tbody>
</table>
Table 2. Values of the water physicochemical parameters measured at PMG. TEMP – mean water temperature; OXY- mean dissolved oxygen; COND – conductivity; NH$_4^+$ – ammonium ion; DEPH- profundity.

<table>
<thead>
<tr>
<th></th>
<th>TEMP (ºC)</th>
<th>OXY (mg/l)</th>
<th>OXY (%)</th>
<th>pH</th>
<th>COND (µs/cm)</th>
<th>NH$_4^+$ (mg/l)</th>
<th>DEPH (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May-05</td>
<td>29.53</td>
<td>2.63</td>
<td>0.55</td>
<td>6.89</td>
<td>1268.67</td>
<td>1.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Jun.</td>
<td>20.83</td>
<td>3.29</td>
<td>53.83</td>
<td>6.31</td>
<td>432.33</td>
<td>0.6</td>
<td>0.53</td>
</tr>
<tr>
<td>Jul.</td>
<td>23.7</td>
<td>4.07</td>
<td>50.07</td>
<td>5.78</td>
<td>1001</td>
<td>0.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Aug.</td>
<td>21.97</td>
<td>6.12</td>
<td>40.3</td>
<td>7.19</td>
<td>845</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>Sep.</td>
<td>8.8</td>
<td>0.405</td>
<td>13.97</td>
<td>3.49</td>
<td>459.5</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>Oct.</td>
<td>11.3</td>
<td>2.15</td>
<td>21.43</td>
<td>4.54</td>
<td>434.83</td>
<td>0.6</td>
<td>0.82</td>
</tr>
<tr>
<td>Nov.</td>
<td>12.57</td>
<td>3.89</td>
<td>36.27</td>
<td>6.31</td>
<td>783.81</td>
<td>1</td>
<td>0.48</td>
</tr>
<tr>
<td>Dec.</td>
<td>8.3</td>
<td>10.9</td>
<td>93.2</td>
<td>6.86</td>
<td>995</td>
<td>0.6</td>
<td>0.39</td>
</tr>
<tr>
<td>Jan-06</td>
<td>10.6</td>
<td>9.71</td>
<td>86.4</td>
<td>6.75</td>
<td>1195</td>
<td>0.6</td>
<td>0.39</td>
</tr>
<tr>
<td>Feb.</td>
<td>7.53</td>
<td>7.36</td>
<td>60.8</td>
<td>7.02</td>
<td>1095</td>
<td>0.8</td>
<td>0.41</td>
</tr>
<tr>
<td>Mar.</td>
<td>17.43</td>
<td>2.74</td>
<td>29.53</td>
<td>6.53</td>
<td>800.67</td>
<td>0.6</td>
<td>0.69</td>
</tr>
<tr>
<td>Apr.</td>
<td>21.1</td>
<td>2.33</td>
<td>25.7</td>
<td>6.61</td>
<td>577.33</td>
<td>0.6</td>
<td>0.22</td>
</tr>
<tr>
<td>May</td>
<td>23.83</td>
<td>32.76</td>
<td>7.75</td>
<td>7.07</td>
<td>1141.33</td>
<td>1.2</td>
<td>0.37</td>
</tr>
<tr>
<td>Mean</td>
<td>16.73</td>
<td>6.80</td>
<td>39.99</td>
<td>6.26</td>
<td>848.42</td>
<td>0.74</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Population structure

The population structure of crayfish from PCD is presented in figure 1. Four cohorts were identified at this marsh. The first two, observed as early as May 2005 and the third cohort, in June 2005. These tree cohorts could not be followed because PCD dried from July to October 2005. The first cohort was only represented in May 2005 and it was composed of 37 individuals with a mean carapace length (CL) of 4.47 cm. The second cohort could be followed in May and June 2005 and it was composed of 75 individuals.
The mean CL varied from 2.74 cm (May 2005) to 2.83 cm (June 2005). A third cohort was detected in June 2005 and it was composed of 29 crayfishes with a mean CL of 3.83 cm. The fourth cohort, with the largest number of individuals (239), was detected in November 2005 with the flooding of PCD and was possible to follow it until May 2006. Individuals from this cohort exhibited a mean total CL of 0.41 cm and were considered as offspring. Mean CL of this cohort varied from 0.41 cm (November 2005) to 3.95 cm (May 2006).

The population structure of crayfish from PMG is shown in figure 2. Six cohorts were observed at this marsh. The first two were identified in May 2005. The first was composed of 56 individuals with a mean CL of 4.35 cm; the second cohort was composed of 1065 crayfish with a mean CL of 1.5 cm; it was followed until August 2005 and exhibited a mean CL of 3.5 cm. The third cohort was detected in June 2005 and it was composed of 1545 crayfish with a mean CL of 1.5 cm; it was followed until November 2005 and exhibited then a mean CL of 4.21 cm. In November 2005, a fourth cohort was detected and it was composed of 2379 individuals with a mean CL of 0.99 cm. This cohort was followed until April 2006 and exhibited a mean CL of 3.0 cm. The fifth cohort was detected in April 2005 and it was composed of 909 individuals with a mean CL of 1.5 cm. This cohort was still observed in May 2005 and exhibited a mean CL of 2.85 cm. In May 2006, the sixth cohort was identified and it was composed of 217 small sized individuals with a mean CL of 1.18 cm.
Figure 1. Population structure of crayfish from PCD. Identification of cohorts through modal analysis by using the Normsep’s method (FITSAT). From July to November no crayfish were captured at this study site. C – cohort.
Figure 2. Population structure of crayfish from PMG. Identification of cohorts through modal analysis by using the Normsep’s method (FITSAT). C – Cohort.
The population from PCD was composed by individuals with a mean CL of 2.56 cm, varying from 1.07 cm (November 2005) to 4.2 cm (May 2006). The highest number of small individuals (CL<1.5 cm) was observed in November 2005, 103. In fact, the population was composed mainly of small crayfish from November 2005 to February 2006 and large individuals in April 2005, June 2005 and May 2006.

The population of crayfish from PMG was composed by individuals with a mean CL of 1.8 cm, ranging from 1.18 cm (October 2005) to 4.03 cm (February 2006). The highest number of small individuals (CL<1.5 cm) was observed in May 2005, 622, and the highest number of large individuals was observed in October 2005, 135. In September and October 2005, no small crayfish were observed. On the other hand, few large animals were observed from December 2005 to February 2006.

The mean crayfish size varied significantly between the two populations (Kolmogorov-Smirnov, p < 0.001). It also varied significantly among seasons both at PCD (df = 5, z = 0.0001) and at PMG (Siegel & Castelan (z), df = 5, p = 0.0001). At PCD, the mean crayfish size in spring 2005 was significantly different from the one in autumn 2005, winter 2005 and winter 2006 (Siegel & Castelan (z), df = 5, p = 0.00001) the mean crayfish size in summer 2005 differed from the one in autumn 2005, winter 2005 and winter 2006 (Siegel & Castelan (z), df = 5, p = 0.00001). The mean crayfish size in spring 2006 differed from the one in autumn 2005, winter 2005 and winter 2006 (Siegel & Castelan (z), df = 5, p = 0.00001).

At PMG, the mean crayfish size in spring 2006 was significantly different of all seasons (Siegel & Castelan (z), df = 5, p < 0.0001). Only spring 2005 did not present differences (Siegel & Castelan (z), df = 5, p = 0.02). Spring 2005 and winter 2005 mean sizes similar (Siegel & Castelan (z), df = 5, p = 0.09) but differed from summer 2005, autumn 2005, winter 2006 and spring 2006 ones (Siegel & Castelan (z), df = 5, p < 0.0001).
Again, there were similarities between summer 2005 and autumn 2005 (Siegel & Castelan (z), df = 5, p = 1), although the Summer result showed differences in comparison to other seasons (Siegel & Castelan (z), df = 5, z < 0.0001). The results for winter 2006 was similar in winter 2005 (Siegel & Castelan (z), df = 5; p = 1) and significantly different in the other seasons (Siegel & Castelan (z), df = 5; z < 0.0001).

Density

During this study, 682 crayfish were caught at PCD and 6272 were sampled at PMG from May 2005 to May 2006. At PCD, 208 animals were captured with the traps and 474 with the dip net. At PMG, 704 animals were collected with the traps and 5568 with the dip net. The Catch per Unity Effort (CPUE) estimated for both methods through the sampling period can be observed in table 3.

The mean relative density estimated from traps at PCD was 0.57 ind/m$^2$ and from the dip net, 0.99 ind/m$^2$. At PMG the mean relative density was 1.29 ind/m$^2$ and 9.66 g/m$^2$ for traps and the dip net respectively. At PCD comparing the density results from traps with the population structure (Fig. 1), it seems that the density was highest in months with the highest number of large individuals (April 2006 and May 2006) and lowest in those months with fewest large crayfishes (November 2005 to February 2006) (Fig. 3). On the other hand, the density determined for the dip net showed that this device is more selective towards juveniles than the traps, because it captured more juveniles (November 2005 to February 2005). This was also revealed by the mean size of crayfishes caught by both capture methods. At PCD the mean size of crayfishes caught by traps was 38.37 cm and with the dip net, 22.27 cm.

At PMG comparing the results of density with the population structure (Fig. 2), it seems that the density obtained by traps was highest in months with the highest number of
large individuals (October 2005) and lowest in those months with fewest large crayfish (November 2005 to February 2006). The number of crayfish caught with traps was always inferior to the number of the individuals caught with the dip net throughout the year. On the other hand, the density determined for the dip net allowed one to see that this device is more selective towards juveniles than the traps, because it captured more juveniles in months with small individuals (May 2005) (Fig. 4). This was also demonstrated by the mean size of crayfishes caught by both capture methods. At PMG the mean size of crayfishes caught by traps was 34.64 cm and with the dip net, 17.39 cm.

At PCD the mean relative density (RD) was 1.69 ind/m² and it ranged from 0.89 ind/m² (November 2005) to 3.53 ind/m² (December 2005) (Fig. 3). At PMG the mean RD was 10.98 ind/m² and it ranged from 1.53 ind/m² (September 2005) to 24.49 ind/m² (July 2005) (Fig. 4). At PCD the mean CPUE was 9.65 ind/h and 3.97 ind/h for traps and dip net respectively (Table 3). At PMG the mean CPUE was 19.27 ind/h and 45.15 ind/h for traps and dip net respectively (Table 3).
Figure 3. Relative Density analysis of PCD. No crayfish were captured at PCD from July to November.

Figure 4. Relative Density analysis of PMG.

The RD was significantly different between the two areas (Kolmogorov-Smirnov test, p < 0.005). The RD at PCD did not vary significantly between seasons (Kruskall-Wallis,
p = 0.84). In contrast RD at PMG was significantly different among seasons (Siegel & Castelan (z), df = 5, p = 0.0019). At PMG the RD in winter 2006 was statistically different from the one in spring 2005 and summer 2005 (Siegel & Castelan (z), df = 5, p < 0.0019).

Table 3. Variation of the Catch per Unity Effort (CPUE) at PCD (a) and PMG (b) obtained with two different sample methods: the dip net and traps. From July to November no crayfish were captured at PCD.

<table>
<thead>
<tr>
<th>CPUE</th>
<th>Local May</th>
<th>Jun</th>
<th>Jul</th>
<th>Aug</th>
<th>Sep</th>
<th>Oct</th>
<th>Nov</th>
<th>Dec</th>
<th>Jan</th>
<th>Feb</th>
<th>Mar</th>
<th>Apr</th>
<th>May</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dip net (ind/h)</td>
<td>a</td>
<td>0.64</td>
<td>0.36</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13.76</td>
<td>3.48</td>
<td>7.12</td>
<td>5.68</td>
<td>4.08</td>
<td>0.16</td>
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<tr>
<td></td>
<td>b</td>
<td>78.76</td>
<td>41.88</td>
<td>82.84</td>
<td>74.56</td>
<td>0.52</td>
<td>98.24</td>
<td>27.36</td>
<td>7.84</td>
<td>7.72</td>
<td>10.4</td>
<td>37.76</td>
<td>83.2</td>
</tr>
<tr>
<td>Traps (ind/h)</td>
<td>a</td>
<td>0.64</td>
<td>17.76</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.76</td>
<td>0.44</td>
<td>2.24</td>
<td>0.88</td>
<td>19.56</td>
<td>27.12</td>
</tr>
<tr>
<td></td>
<td>b</td>
<td>16</td>
<td>20.88</td>
<td>74.68</td>
<td>0</td>
<td>28</td>
<td>13.6</td>
<td>16</td>
<td>11.12</td>
<td>0.88</td>
<td>5.32</td>
<td>16.44</td>
<td>33.32</td>
</tr>
</tbody>
</table>

**Sex ratio**

The mean sex ratio (SR) was 0.91 and 0.78 at PCD and PMG respectively. The variation of the SR throughout the sampling period, at both marshes (Fig. 5 and 6).

At PCD, the mean SR ranged from 0.40 to 1.93, in November 2005 and February 2006 respectively. The SR was mostly favourable to the females (SR < 1), with the exception of November 2005, April 2006 and May 2006. At PMG, the mean SR ranged from 0.39 to 1.32, in May 2005 and June 2005 respectively. The mean SR was mostly favourable to females (SR < 1) with the exception of June 2005, July 2005, September 2005 and October 2005.
The Two Way ANOVA showed that the SR varied significantly between the two study areas (p = 0.023) and among seasons (p = 0.025). The statistical results obtained by the Fisher LSD for sites and seasons are shown in table 4. Namely, the Fisher LSD post-hoc test showed that SR varied significantly between summer 2005 at PCD and all other seasons at PMG (p < 0.05); and the SR in spring 2005 at PCD was significantly different from the SR in spring 2005 at PMG; the SR in spring 2005/2006 at PCD was significantly different the SR in winter 2006 at PMG.
Figure 5. Variation of sex ratio (SR) through the sampling period at PCD. From July to November no crayfish were captured at PCD.

Figure 6. Variation of sex ratio (SR) through the sampling period at PMG.
Table 4. LSD test post-hoc comparisons between the sex ratio of both study areas and seasons. Numbers listed are P values. PCD – Paul de Cadaval; PMG – Paul de Magos; Sp – Spring; Su – Summer; Au – Autumn; Wi – Winter.

<table>
<thead>
<tr>
<th>Local x season</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 PCD x Sp 05</td>
<td>0.295</td>
<td>0.259</td>
<td>0.182</td>
<td>0.021</td>
<td>0.493</td>
<td>0.034</td>
<td>0.120</td>
<td>0.343</td>
<td>0.216</td>
<td>0.020</td>
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</tr>
<tr>
<td>2 PCD x Su 05</td>
<td>0.032</td>
<td>0.020</td>
<td>0.001</td>
<td>0.052</td>
<td>0.002</td>
<td>0.006</td>
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<td>0.025</td>
<td>0.001</td>
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</tr>
<tr>
<td>3 PCD x Au 05</td>
<td>0.834</td>
<td>0.293</td>
<td>0.483</td>
<td>0.304</td>
<td>0.853</td>
<td>0.663</td>
<td>0.911</td>
<td>0.288</td>
<td>0.896</td>
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</tr>
<tr>
<td>4 PCD x Wi 05</td>
<td>0.417</td>
<td>0.340</td>
<td>0.412</td>
<td>0.942</td>
<td>0.489</td>
<td>0.921</td>
<td>0.410</td>
<td>0.899</td>
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<tr>
<td>5 PCD x Wi 06</td>
<td>0.024</td>
<td>0.891</td>
<td>0.240</td>
<td>0.053</td>
<td>0.355</td>
<td>0.988</td>
<td>0.214</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 PCD x Sp 06</td>
<td>0.053</td>
<td>0.213</td>
<td>0.707</td>
<td>0.403</td>
<td>0.023</td>
<td>0.242</td>
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<tr>
<td>7 PMG xSp 05</td>
<td>0.283</td>
<td>0.093</td>
<td>0.359</td>
<td>0.901</td>
<td>0.259</td>
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<tr>
<td>8 PMG x Su 05</td>
<td>0.381</td>
<td>0.961</td>
<td>0.233</td>
<td>0.939</td>
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<tr>
<td>9 PMG x Au 05</td>
<td>0.567</td>
<td>0.051</td>
<td>0.424</td>
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<tr>
<td>10 PMG x Wi 05</td>
<td>0.349</td>
<td>0.995</td>
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<tr>
<td>11 PMG x Wi 06</td>
<td>0.208</td>
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<td>12 PMG x Sp 06</td>
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Biomass, Production and Productivity

The mean relative biomass (RB) obtained for PCD and for PMG was 9.21 g/m² and 26.39 g/m² (Table 5) respectively. At PCD RB ranged from 0.33 to 19.98, in May 2005 and December 2005, respectively. At PMG RB varied from 1.27 to 78.89, in June 2005 and October 2005, respectively. RB was significantly different between the two areas (Kolmogorov-Smirnov test, p < 0.005). At PCD RB changed significantly over the several seasons (Siegel & Castelan (z), df = 5; p = 0.095). At PMG there were also
significant differences among seasons (Siegel & Castelan (z), df = 5, p = 0.0007). At PMG RB in winter 2006 was different from the one in spring and summer 2005 (Siegel & Castelan (z), df = 5, p < 0.0007).

Table 5. Variation of relative biomass at both marshes: a – PCD; b – PMG.

<table>
<thead>
<tr>
<th></th>
<th>Local May 05</th>
<th>Local Jun 05</th>
<th>Local Jul 05</th>
<th>Local Aug 05</th>
<th>Local Sep 05</th>
<th>Local Oct 05</th>
<th>Local Nov 05</th>
<th>Local Dec 05</th>
<th>Local Jan 06</th>
<th>Local Feb 06</th>
<th>Local Mar 06</th>
<th>Local Apr 06</th>
<th>Local May 06</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Biomass (g/m²) a</td>
<td>19.98</td>
<td>11.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2.19</td>
<td>0.33</td>
<td>3.46</td>
<td>0.75</td>
<td>11.98</td>
<td>17.01</td>
<td>15.38</td>
<td></td>
</tr>
<tr>
<td>Relative Biomass (g/m²) b</td>
<td>37.91</td>
<td>1.27</td>
<td>70.78</td>
<td>48.08</td>
<td>15.10</td>
<td>78.89</td>
<td>11.13</td>
<td>5.50</td>
<td>1.17</td>
<td>1.82</td>
<td>18.02</td>
<td>36.43</td>
<td>16.99</td>
</tr>
</tbody>
</table>

The mean biomass (B), production (P), productivity (PR), mortality rate (Z) and the mean lifetime (t½) of crayfish populations from both marshes are shown in table 6. The population from PMG exhibited a slightly higher B but a lower P and PR than these from PCD. Z and t½ results for both populations were almost identical.

Table 6. Analysis of several parameters such as the total Mean Biomass, Production, Productivity, Mortality Rate and Mean Lifetime of crayfish populations from both marshes, PCD and PMG.

<table>
<thead>
<tr>
<th></th>
<th>Mean Biomass (g/m²)</th>
<th>Production (Kg/ha/year)</th>
<th>Productivity</th>
<th>Mortality rate (years) – Z</th>
<th>Mean lifetime (years) - t½</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCD</td>
<td>26.63</td>
<td>460</td>
<td>1.75</td>
<td>2.386</td>
<td>0.42</td>
</tr>
<tr>
<td>PMG</td>
<td>27.15</td>
<td>166.61</td>
<td>0.67</td>
<td>2.47</td>
<td>0.41</td>
</tr>
</tbody>
</table>
Discussion

The results of the modal analysis using first the Bhattacharya’s method and the Normsep’s method distinguished 4 cohorts for the crayfish population from Paul de Cadaval (PCD) and 6 from Paul de Magos (PMG). Correia (1995) obtained similar results for these two marshes. In fact, *P. clarkii* age classes or cohorts may vary in their number from one population to another. Anastácio & Marques (1995) distinguished 7 cohorts, Lozano-Guerra & Escamilla-Niño (1995) and Chiesa *et al.* (2006) distinguished 3 cohorts while Scalici & Gherardi (2007) found 5 cohorts. In addition, the presence of several cohorts at PCD and PMG indicates that both populations are productive.

At PCD the first 3 cohorts observed in spring 2005 and summer 2005 were composed by large crayfish. From July 2005 to October 2005, this marsh was dry but a new cohort composed by newly-hatched juveniles was observed right after the pond was flooded in autumn 2005. These results show that the crayfish population from PCD is composed by large animals in spring and summer and juveniles at the end of autumn and winter.

At PMG the first 2 cohorts, detected in spring, were composed by large individuals as well as juveniles. However the 3rd cohort identified in the summer 2005 was composed by large animals. The 4th cohort was observed in the autumn and it was composed by juveniles, which also formed the bulk of this population in winter. Finally, both 5th and 6th cohorts were detected in spring 2005 and were composed by juveniles. Therefore, our results indicate the entrance of newly hatched crayfish throughout the year in the population of PMG. Large individuals were more abundant in late spring, summer and early autumn. Lowery & Mendes (1977) and Oluoich (1990) reported the same occurrence in Kenya, of newly-hatched juveniles in free-swimming population throughout the year which indicates an increment of the productivity in the population.
The presence of juveniles, especially in winter, and the abundance of adults in spring, summer and autumn seem to be related to the temperature, which limits the endogenous activities of the species (Correia, 1995). In fact, it seems that the minimum water temperatures in winter (< 10 °C) inhibit adult activities (Huner & Barr, 1991) whereas the activities of the juveniles are stimulated in this same period (Sommer, 1984). According to Gutiérrez-Yurrita & Montes (1999), temperature is one of the most critical factors influencing biological processes in ectotherm organisms, as it modifies their metabolism rates and activities.

*Procambarus clarkii* is a density-regulated species (McClain, 1995). In this study the relative density of crayfish was much higher in PMG (10.98 ind/m$^2$) than in PCD (1.69 ind/m$^2$). Correia (1993) classified these two marshes as suitable habitats to the rapid development of *P. clarkii* populations. Our results are in accordance with Correia (1995) for high densities were related to the abundance of juveniles and density decreased as crayfish enlarged. In fact, the population of PMG had the highest number of juveniles with a regular distribution throughout the year.

According to Correia (1995) the ratio between sexes is a good indicator of species regulation, i.e. the balance between the proportion of males and females in the population is a good indicator of the population stability. The sex ratio results obtained for both marshes were favourable to females, which is similar to the ones recorded by several authors (e.g. Huner, 1978; Oluoch, 1990; Correia, 1995; Anastácio, 1993; Adão & Marques, 1993; Anastácio & Marques, 1995; Fidalgo *et al*., 2001; Scalici & Gherardi, 2007; Ligas, 2008). Nevertheless, the proportion of females was higher in the population from PCD probably in response to the drought of the pond during 4 months. According to Anastácio & Marques (1995) an increase in the number of females is an adaptation strategy to unfavourable conditions, which may cause high mortality rates.
In both marshes, the highest values of relative biomass were observed in spring and summer and the lowest in winter. This happened because the population structure was composed in spring and summer by larger individuals and in winter by smaller ones. Correia (1995) obtained the same results for both marshes.

The mean biomass was also higher in PMG than in PCD. Anastácio & Marques (1995) obtained lower results of mean biomass (5.41 g/m²) than these now presented (PMG - 27.15 g/m²; PCD - 26.63 g/m²) in the Mondego region, but Correia (1995) reported values slightly higher than ours (PMG - 29.29 g/m²; PCD - 31.68 g/m²) in the same marshes.

The productivity results obtained for the two populations (PCD - 1.75; PMG - 0.67) are in accordance with the values referred by Momot (1984), who indicates productivities ranging 0.3 - 7.3 for crayfish populations. However, the major P/B ratios recorded for this species were approximately 1 (Momot et al., 1978; Roell & Orth, 1992). Nevertheless, Correia (1995) obtained higher results for productivity (PCD - 2.4; PMG - 2.5) just like other authors in Portugal, e.g. Anastácio (1993) and Anastácio & Marques (1995). According to Momot et al. (1978) and Roell & Orth (1992) productivity varies between populations living in different habitats. Momot & Gowing (1977) stated that biomass and productivity variations are related with environmental alterations and depend on density processes that will determine the future maintenance of crayfish populations. The lower productivity of the population from PMG in relation to that from PCD may be explained by the higher population densities observed in the first, that probably inhibited the increment in body size and the productivity.

The mortality rate and the mean lifetime of both populations were almost identical. The mortality results from this study were lowest than those obtained by Scalici & Gerardi
(2007), but in both works the mean lifetime did not overreach 12 months, which is a lower time than the limit of 18 months proposed by Huner (2002).

The knowledge on the population dynamics provided by this study may be used in specific models to predict the evolution of crayfish populations.

To conclude, it seems that both crayfish populations were stable and their structure and dynamics seem to reveal both stability and spreading potential, confirming the invasiveness characteristics of this species.

**Acknowledgements**

The authors are very grateful to D. Carvalho for her field assistance, A. Cartaxana for her help in growth analysis, H. Cabral for help in the FITSAT program and other statistical analyses, H. Pereira and P. Garcia Pereira for help in the statistical analyses. This work was funded by the Fundação para a Ciência e a Tecnologia project POCT/BSE/46862/2002, by FEDER ("Fundo Europeu de Desenvolvimento Regional" – European Fund for Regional Development) and by the Universidade de Lisboa MNHN, Museu Bocage.

**References**


Ana Sofia Leitão, Maria José Boavida e Alexandra Marçal Correia

**Abstract**

Reproduction and growth of *Procambarus clarkii* was studied for 13 months in two freshwater marshes, a pond - Paul de Cadaval (PCD) and a rice field - Paul de Magos (PMG) located in the Tejo river basin. The analyses of the maturation index and the evolution of the maturation state of both sexes indicated that the reproductive periods at PCD were spring, autumn and at PMG were continuous with peaks. At PCD, the main recruitment period occurred in autumn and at PMG, there was an extended recruitment period with two peaks in autumn and spring. The asymptotic length (L∞) and the curvature parameter (K) revealed a faster growth of crayfish at PCD than the one at PMG. Average growth rate was also higher at PCD, 0.123 than at PMG, 0.119. For both populations recruitment was high and growth rates fast, supporting the stability and spreading potential of *P. clarkii* in Portugal.

**Keywords:** Growth patterns, Reproductive traits, Recruitment, Von Bertalanffy’s equation.
Introduction

The invasion of species leading to the extinction or range contraction of indigenous species is a continual process of homogenization of the ecosystems (Gerardi, 2006). Freshwater crustaceans easily invade new ecosystems producing several abiotic and biotic alterations (Cruz & Rebelo, 2007). Freshwater crayfish are relatively long-lived and the largest invertebrates in temperate areas, considered as keystone species in aquatic communities (Horowirtz, 1990; Nystrom et al., 1996; Lodge et al., 2000). Their multi-level trophic interactions among several invasive and native species are far from being understood. Crayfish are omnivorous and may affect benthic communities through consumption and behavioural effects on other aquatic macroinvertebrates (Nystrom et al., 1999; Usio & Townsend, 2002).

The red swamp crayfish, *Procambarus clarkii* (Girard, 1852), is native from the Northeast Mexico and the Southeast U.S.A. (Huner, 1990) and it has been successfully introduced in all continents except Antarctica and Australia (Hobbs et al., 1989). This species has become the dominant freshwater crayfish in almost all areas where it was introduced (Henttonen & Huner, 1999), it is considered an ecologically plastic species, as well as successful invader (Gutiérrez-Yurrita et al., 1999). This is a generalist species, with active dispersal capabilities and highly adaptable to new environments (Gherardi & Barbaresi, 2000; Cruz & Rebelo, 2007). Because its dispersal ability may vary according to the habitat characteristics (Gherardi et al., 2000; Barbaresi et al., 2004), its effects on aquatic ecosystems are very difficult to predict (Parker et al. 1999; Kolar & Lodge, 2001; Moyle, 1999; Sakai et al., 2001; Heger & Trepl, 2003; Simon & Townsend, 2003). Along with habitat destruction, pollution and other negative impacts this species is an important threat to biodiversity because of its negative effects on other species such as amphibians and other native crayfish (Gherardi, 2006).
In Portugal, the introduction of *P. clarkii* occurred in 1979 (Ramos & Pereira, 1981), probably as a result of the natural expansion of spanish populations (Correia, 1993). It has increased and spread all over the country (Correia, 1993; Correia & Bandeira, 2004). In high densities *P. clarkii* has caused economic damages in agricultural areas, such as rice fields, because of its burrowing activity which increases water turbidity (Correia & Ferreira, 1995) and damages rice seedlings (Anastácio et al., 2005).

In the future, it is important to monitor the populations of *P. clarkii* to achieve new forms of control of their spread and dispersal. According to Gutiérrez-Yurrita & Montes (1998) the negative impact of *P. clarkii* highly depends on its reproductive strategy. Sommer (1984) referred that the reproductive cycle of *P. clarkii* seems to change geographically, mainly as a function of the hydrologic cycle and water temperature. The aims of the present study were to investigate the reproduction and growth of crayfish populations from two different marshes in order to understand how the reproductive success and growth change through time in areas with different environmental characteristics. The study of the reproduction of the populations of both marshes was based on the analyses of different biological parameters such as the maturation index, the evolution of the maturation state, the mean maturation size and recruitment patterns. The growth study of these populations was based on the analyses of growth parameters, such as growth rate, growth curve and growth of each cohort from each population.

**Material and Methodology**

**Crayfish sampling and laboratory analyses**

Crayfish were sampled once a month from May 2005 to May 2006, with a dip net (65 cm x 40 cm frame, 3 mm mesh size) and traps in two freshwater marshes. Both areas Paul de Cadaval (PCD) and Paul de Magos (PMG) are located in the Tejo river basin.
and belong to the Salvaterra de Magos County. PCD is a freshwater marsh of about 14 km² (39°N, 8°30’ W) and PMG a rice field area of 7 km² (38°58’N, 8°45’ W).

Sampling was replicated three times. In each replicate 3 traps with bait (canned sardine) were set out (with a distance of 5 m between them) at sunset covering a total area of 15 m². After 12 h crayfish were collected from the traps. At the same area of 15 m² crayfish were also sampled with the dip net using a catch per unit effort (CPUE) of 15 min. Samples obtained by both methods were separately kept in bags previously identified, and preserved in 70 % alcohol for further analysis.

PCD dried from July to October 2005 so, during these months, crayfish sampling and the measurement of environmental parameters did not take place.

In the laboratory, each specimen was analysed by measuring its carapace length (CL) to nearest the 0.01 mm and the whole animal was weighted to nearest the 0.001 g.

The sex was differentiated in individuals with a carapace length more than 1.3 cm. The sex of the individuals was distinguished by the presence of the gonopodia in males which results of the modification of the first pair of walking legs (Huner, 1978). The mature males (FI) were identified by the presence of enlarged claws and distinct hooks at the bases of the third and fourth pairs of walking legs. The juveniles were differentiated from sexual inactive males (FII) by the presence of a complete second pair of walking legs (Sukô, 1953; Hobbs, 1989). Females maturation was analysed according to gonad colour (white, yellow - immature females; orange and brown - mature females) (De La Bretonne & Avault, 1977).

**Analytical procedures**

**Reproduction**
The maturation index (MI) was estimated monthly for each sex and for each study area. According to Guerra & Niño (1995) the MI for each sex was obtained by the equation:

\[ MI = \frac{\sum (status\ K \times n'\ of\ K\ individuals)}{N} \]

The possible status K for males are: 1 - form II; 2 - form I; and for females are: 1 - white gonad; 2 - yellow gonad; 3 - orange gonad; 4 - brown gonad. The N are the total number of individuals of the population.

The mean size at sexual maturity for each sex was determined by the average length of mature individuals for each sex and for each area. At PCD, it was not possible to calculate the mean size at sexual maturity for females because there was only a mature female in May 2005 (with brown ovaries).

The proportion of mature males (F1) and immature males (F2) was also calculated (F1/F2).

Recruitment was assessed by determining the presence of individuals with less than 1.5 cm in each population (Huner & Barr, 1991). The analysis of the distribution of the recruits was based on the calculation of the percentage of offspring obtained for each month. Recruitment in each study area was also estimated by the FITSAT program for 12 months, based on a size-frequency distribution with 0.5 cm class interval (Gayanilo, 2005).

**Growth**

Growth parameters were estimated using the Gullant and Holt method through the FITSAT (FAO-ICLARM Stock Assessment Tools) computer program. The asymptotic length \( L_\infty \) was determined by the rule of Taylor (1988) using the “forced” Gulland and Holt method, in which the \( L_\infty \) was estimated by using the maximum length (Lmax) recorded by the Maximum Length Estimation’s method through the same program. The growth curve was determined by the application of the von Bertalanffy’s equation: \( L(t) \)
= \text{L}_\infty x [1 - \exp (- K x (t-t_0))] \text{ previously used for } P. \text{ clarkii} \text{ by e.g. Anastácio & Marques (1995) for the same species. L(t) is the crayfish carapace length (CL) at time t; L}_\infty \text{ is the CL of the oldest crayfish; K is the curvature parameter which records the rate at which the L}_\infty \text{ is obtained; and t}_0 \text{ is the initial condition parameter, when the CFL is equal to 0.}

All cohorts were identified through modal analysis by using the Bhattacharya’s method through the FITSAT computer program. The results of the Bhattacharya’s method routine were refined afterwards, by other routine of the FITSAT, the Normsep’s method. The growth increment of each cohort was determined for both marshes by the analysis of the mean CL estimated through the modal analysis after the application of the previous methods.

The growth curve of both populations was determined through the equation \( y = a + bx \), \( y \) was the length at instant \( t \) and \( x \) the growth rate. \( L(t) \) was calculated for each cohort using the equation \( L(t) = (L_1 + L_2) / 2 \). The growth rate for each cohort was calculated through the equation \( x = L(t) / a + b \), \( b \) was determined by \( k = -b \) and \( a \) was obtained using the equation \( L_\infty = -a / b \).

\textit{Statistical analysis}

The statistical analysis was performed using the software program Statistics version 7.0 and for that all data were grouped by season. The maturation indexes (MI) for both sexes were compared between marshes and seasons. Kolmogorov-Smirnov test were used to analyse the differences between the maturation indexes (MI) of both marshes (Sokal & Rohlf, 1981). To analyse the differences between seasons at each marsh the Kruskal-Wallis test was applied. When the null hypothesis was rejected, the
nonparametric multiple comparison test was applied (Siegel & Castellan, 1988) to better identify the differences between seasons.

**Results**

*Reproduction*

*Maturation index*

Immature animals represented the majority at PCD from December 2005 to February 2006 (Fig. 1). The mean maturation index of males (MIM) was 0.32 and ranged between 0.043, in November 2005, and 0.82, in May 2005. The mean maturation index of females (MIF) was 0.45 and ranged from 0.23, in January 2006, to 0.69, in February 2006.

![Graph showing MIF and MIM]

Figure 1. Variation of the maturation index for both sexes at PCD. MIM - Maturation index of males, MIF - Maturation index of females. From July 2005 to November 2005 no crayfish were captured at PCD.
Figure 2. Variation of the maturation index for both sexes PMG. MIM - Maturation index of males, MIF - Maturation index of females.

At PMG (Fig. 2) the variation of MIM was highly variable throughout the year. The mean MIM was 0.22 and varied between 0.02, in January 2006, and 0.62, in October 2005. The MIF was also variable, presenting a mean value of 0.55 and ranging between 0.37 in October/November 2005, and 0.7, in January 2006.

The maturation indexes of both sexes did not show significant differences between marshes (Kolmogorov-Smirnov, p = 0.01).

At PCD the MIM was not significantly different among seasons (Siegel & Castelan (z), df = 5, p = 0.0234). However, at PMG, MIM differences were significant (Siegel & Castelan (z), df = 5, p = 0.0234) among seasons. The MIM of spring 2005 significantly differed from the one in autumn 2005 and winter 2005 (Siegel & Castelan (z), df = 5, p = 0.0096). The MIM of winter 2006 significantly differed from the one in summer 2005 (df = 5, z = 0.046). At both marshes the MIF was not significantly different among seasons (PCD - Siegel & Castelan (z), df = 5, p = 0.316; PMG - Siegel & Castelan (z), df = 5, p = 0.331).
The evolution of the maturation Status

The temporal evolution of the maturation status of both sexes was analysed for each marsh. At PCD the mature males (FI) were abundant in spring 2005/spring 2006, which is in agreement with the high maturation index (MIM) calculated for the same season (Fig. 3). The mature females were abundant in spring 2005 and in this season a high maturation index (MIF) was also observed although the highest MIF was observed at the end of winter 2006 (February 2006) (Fig. 4). The immature individuals were more abundant in autumn/winter.

Figure 3. Variation of the maturation status of males at PCD. MM - mature males, IM - immature males, JUV - juveniles. From July 2005 to November 2005 no crayfish were captured at PCD.
Figure 4. Variation of the maturation status of females at PCD. IM - immature females, MF - mature females. From July 2005 to November 2005 no crayfish were captured at PCD.

At PMG, the mature males were abundant in autumn 2005 and in winter 2005, while in the same seasons very high MIM was observed (Fig. 5). Mature females were abundant in spring 2005 yet the MIF was high in winter 2006 (Fig. 6). Only in May 2005, August 2005 and September 2005 mature females were observed. Immature individuals of both sexes were abundant in spring.
Figure 5. Variation of the maturation status of males at PMG. MM - mature males, IM - immature males, JUV - juveniles.

Figure 6. Variation of the maturation status of females at PMG. IMF - immature females, MF - mature females.
Proportion of mature and immature males

The proportion between FI and FII males at PCD was 4.05 and at PMG, 2.48. At PCD, this proportion ranged from 0.19, in January 2005 to 6, in March 2006. From December 2005 to February 2006, no mature males observed. At PMG, the same proportion ranged from 0.08, in March 2006, to 8.25, in September 2005. From January 2005 to February 2005, mature males were not observed.

Mean size at sexual maturity

At PCD the mean size at sexual maturity of males (TM) was 4 cm. At PMG the mean size males at sexual maturity was 3.24 cm and for females, 2.90 cm.

Recruitment

Recently hatched crayfish (individuals with less than 1.5 cm of body length) were observed in the population at PCD mainly from November 2005 to March 2006 (Fig. 7). November 2005 was the month with the maximum recruitment percentage, 21.41 %. These results were in agreement with the relative recruitment values estimated through the FITSAT program. This analysis predicted to a maximum recruitment percentage of 37.69 % in October 2005 followed by November 2005, 23.14 %. The minimum recruitment percentage was estimated for May 2005, 0.21 %.
Figure 7. Recruitment analysis: estimation by the FITSAT program and observed results at PCD. From July 2005 to November 2005 no crayfish were captured at PCD.

Figure 8. Recruitment analysis: estimation by the FITSAT program and observed results at PMG.
Recently hatched crayfish were present in the PMG population along the year (Fig. 8), with the exception of September 2005 and October 2005. The maximum recruitment took place in May 2005, 11.69 % and the minimum in January 2006, 1.29 %. These results were not in agreement with the relative recruitment values predicted by the FITSAT. This analysis estimated a maximum recruitment percentage in October 2005, 22.41 %, followed by November 2005, 13.76 %. The minimum recruitment percentage was estimated for May 2005, 0.70 %.

Growth analysis
The observed extreme length determined for population at PCD was 6 cm and the predicted extreme length was 6.93 cm, which was used as “forced” \( L_\infty \) with a confidence interval of 95 % [5.58 - 8.27]. Therefore, the growth parameters of the PCD population were: \( L_\infty = 6.93 \); \( K = 1.315 \); \( t_0 = 0.0189 \); \( t = 0.11 \), \( L_{\text{max}} = 6.93 \); the Von Bertalanffy’s growth equation for the PCD population was:

\[
L(t) = 6.93 \left(1 - \exp(-1.315 (t - 0.0189))\right)
\]

The growth rate was 0.123 cm/year and 5.92 cm/week. The growth curve was \( L(t) = 9.1125 + 1.315 (0.123) \).

Four cohorts were detected in the PCD population (Fig. 9) but only in the fourth cohort was determined the increment growth which was represented in figure 10. From November 2005 to May 2006, the growth increment was 3.54 cm for the fourth cohort. This cohort had a size increment of 3.54 cm from November 2005 to May 2006. The growth rate was 0.214 for the first cohort. The growth rate was 0.068 for the second cohort, 0.184 for the third cohort and 0.123 for the fourth cohort.
Figure 9. Simulation of the von Bertalanffy’s growth curve for the PCD provided by the FITSAT program. Length - refers to the carapace length; C1 - Cohort 1 ; C2 - Cohort 2; C3 - Cohort 3; C4 - Cohort 4; C5 - Cohort 5.

Figure 10. Growth increment of the fourth cohort from the PCD population throughout the study period. From July 2005 to November 2005 no crayfish were captured at this study site. C4 - Cohort 4.

The observed extreme length measured for the PMG population was 6 cm (CL), while the predicted extreme length was 6.3 cm, which was used as “forced” $L_\infty$ with a confidence interval of 95% [5.75 – 6.87]. Therefore, the growth parameters from the
PMG population were: $L_\infty = 6.31$; $K = 0.845$; $t_0 = 0.017$; $t = 0.14$, $L_{\text{max}} = 6.31$, the Von Bertalanffy’s growth equation for PMG was:

$L(t) = 6.31 \left(1 - \exp\left(-0.845 \left(t - 0.017\right)\right)\right)$

The growth rate of PMG was 0.119 cm/year or 5.70 cm/week. The growth curve was

$L(t) = 5.332 + 0.845 \times (0.119)$

At PMG, the growth increment, represented in figure 12, was only observed for the third and the fourth cohort (Fig. 11). From June 2005 to November 2005, the growth increment was 1.58 for the third cohort and 2.04, for the fourth, from November 2005 to April 2006. The growth rate was 0.352 for the first cohort, 0.061 for the second, 0.057 for the third, 0.040 for the fourth, 0.107 for the fifth and 0.096 for the sixth.

![Figure 11. Simulation of the von Bertalanffy’s growth curve at PMG predicted by the FITSAT program. C1 - Cohort 1; C2 - Cohort 2; C3 - Cohort 3; C4 - Cohort 4; C5 - Cohort 5. Length - Carapace length.](image_url)
Figure 12. Growth analysis of each cohort from PMG population throughout the study period. C3 - Cohort 3; C4 - Cohort 4.

The asymptotic length ($L_\infty$) and the curvature parameter ($K$) revealed a faster growth of individuals from PCD than those from PMG. The growth rate was also faster at PCD than PMG.

**Discussion**

The study of the maturation state of individuals is very important to determine the reproductive cycle of a species. The analyses of the maturation index and the evolution of the maturation state of both sexes at Paul de Cadaval (PCD), indicated the spring 2005, after the flooding in the autumn 2005 (November) and the beginning of spring 2006, as reproductive periods. In Paul de Magos (PMG), the reproductive period occurred from the summer 2005 to the spring 2006. Correia (1995), referred the summer and autumn as the main reproductive period for the same populations, the spring as the preparation season and the winter as a rest season. Ligas (2008) collected high percentages of mature specimens in spring and summer in the Southern part of Tuscany
and these observations are confirmed by others studies in Italy and in the rest of Europe (Cano & Ocete, 1997; Gutiérrez-Yurrita & Montes, 1999; Chiesa et al., 2006). Gutiérrez-Yurrita et al. (1997) referred that in areas with a long flooding period, over than 6 months, there may be at least two reproductive periods (i.e. autumn and spring). In temporary freshwater marshes such as PCD, with a predictable variable hydrological regime over time (dry in summer and flooded in winter), the best strategy of females should be to associate ovarian maturation with periods of flooding and sufficiently high temperatures (Ilhéu & Bernardo, 1996; Gutiérrez-Yurrita et al., 1997). These coincide with the best availability periods in terms of food resources and refuge. It was also found that to find large females and for future offspring to have a greater probability of developing and surviving (Gutiérrez-Yurrita & Montes, 1998), it is necessary a hydroperiod longer than 4 months, temperature above 18°C and pH between 7 and 8 (Gutiérrez-Yurrita et al., 1997). At PCD, there was an extensive dry period and at PMG, there was permanent water, which may explain the higher abundance of mature individuals at PMG than at PCD.

The abundance of mature males is a good indicator of the reproduction state of a population (Huner & Romaine, 1979). The proportion between FI and FII males was higher at PCD than at PMG. At both marshes, there were FI males in winter and a great abundance in spring 2005/2006 and summer 2005 at PCD. The same happened in autumn 2005 and spring 2006 at PMG. In winter, there were few FI males in both sites for two reasons: some captured males were born in summer and had no time to mature until winter and other changed to FII males after mating in summer. Niquette & D’Abramo (1991) obtained similar results in Mississippi river, U.S.A.

According to present results at PCD, recruitment occurred from November 2005 to March 2006 but mainly in November 2005, which is in accordance with the results
estimated by the FITSAT program. In Central and Northern Portugal, Northern Italy and the United States the recruitment period also occurs in November (Huner & Barr, 1991; Anastácio & Marques, 1998; Gherardi et al, 1999; Fidalgo et al., 2001; Mueller, 2007). At PMG, recruitment occurred throughout most of the year (with the exception of September and October 2005). Correia (1995) observed the existence of mature and immature individuals, which may suggest the existence of a continuous reproductive period at this marsh. Other authors reported similar results in the Louisiana marshes (Penn, 1943) as well as in commercial ponds (Huner, 1978) and in Kenya (Oluoch, 1990). According to Anastácio & Marques (1995), this extension of the reproduction period is mainly, caused by more favourable climatic conditions.

The growth parameters, such as the asymptotic length ($L_{\infty}$), the curvature parameter ($K$) and the growth rate were higher at PCD than at PMG. Specifically, the asymptotic length computed was lower than the one obtained by Correia (1995) for the same marshes. Comparing the present work’s with those of Anastácio & Marques (1995) at the Mondego river, of Fidalgo et al. (2001) at the Aveiro region and of Chiesa et al. (2006) in Central Italy, it’s clear that they found a lower growth and a lower asymptotic length. However, in Italy Scalici & Gherardi (2007) obtained similar results for the asymptotic length. The curvature parameter obtained in the present study was higher than those obtained in the previously mentioned studies. Correia (1995) obtained higher results at PCD but like those of Fidalgo et al. (2001) and of Scalici & Gherardi (2007) were lower than ours. In addition, Dorr et al. (2006) obtained a growth rate of 1 which was similar to our results.

The extended recruitment period and the growth results observed in both populations of *P. clarkii* support the need of a proper management and control in this region. In
conclusion, this work confirmed once more the phenomenal reproductive capacity of the red swamp crayfish and partially explains its success as an invasive species.

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General Discussion

The knowledge of the population structure can determine its temporal evolution and allow for adequate models to be applied (Fenouil & Chaix, 1992). The populations from Paul de Cadaval (PCD) and Paul de Magos (PMG) presented several cohorts suggesting high productivity, because the production depends on the occurrence of multiple recruitment classes (De La Bretonne & Romaine, 1989; Correia, 1995). At PCD, the crayfish population was composed of large animals in spring and summer and juveniles by the end of autumn and winter. At PMG, the population presented newly hatched crayfish throughout the year while large individuals were more abundant in late spring, summer and early autumn. These results agree with the results of Beja (1996) and Correia (2001).

Density may control individual growth influencing the potential fecundity of population, which in turn determines recruitment, biomass and productivity (Momot & Gowing, 1977; Bostford, 1981). Crayfish populations are very sensitive to alterations on structure and density, the last one influencing their auto-regulation mechanisms (Momot, 1984). Correia (1995) obtained high densities at the same marshes considered in the present work and classified these as suitable habitats to the development of *Procambarus clarkii*. These habitats present a semi-temporary hydrologic regime and a high concentration of oxygen in water, in addition to the absence of predation and competition with other aquatic organisms. Furthermore, these studied biotopes exhibited conditions for a differentiated utilization of the habitat by the ovigerous females, males and juveniles, which are important aspects to survival and growth of the offspring (Blake & Hart, 1993).

The sex ratio for both marshes was favourable to females, which means that these populations were stable, according to Correia (1995). The abundance of females brings
stabilization of the population because they can re-establish the stock of juveniles even when high juvenile mortality occurs.

Productivity was lower for the PMG population than for the PCD’s, probably because the density of the first was higher and could have inhibited the increment in body size. However, both populations were considered productive because the results were high for the species.

The mortality rate and the mean lifetime of both populations were almost identical.

At both marshes, the highest values of maturation index and abundance of mature individuals were recorded mainly in the summer and autumn and the lowest values were recorded in winter. However, due to the presence of juveniles and mature individuals throughout the year the population from PMG appeared to have a continuous reproductive period. Correia (1995) described the same pattern for the same marshes. In her research, she described summer and autumn as the main reproductive seasons and spring as the preparation season for the reproduction. Winter was defined as the resting season.

The population continuity depends on the viability of the eggs and adequate recruitment (Momot & Gowing, 1977; Huner, 1978). The recruitment of the PCD crayfish population occurred mainly in spring and autumn and the recruitment of the PMG population occurred mostly all over the year. According to Correia (1995), a high recruitment indicates the reproductive potential and the success of a species when the environmental conditions are favourable to the development of the offspring. This may explain a more extended recruitment at PMG than at PCD.

The growth rates obtained by the Von Bertalanffy equation revealed a higher growth increment at PCD than at PMG. Correia (1995) described similar results for the same populations. Several authors referred the density, the water quality and the food
resources as limiting factors causing disparity in growth (Momot et al., 1978; Taylor, 1988; Correia, 1995). However, the environmental conditions at PMG did not seem to explain these results. On the other hand, the high densities may explain the lower values of growth rate comparing to those of the PCD population. In fact, if the density of juveniles is high and conditions are stressful, their growth may be deficient and they may reach maturity without being able to produce viable offspring (Fernandes et al., 1995; Gutiérrez-Yurrita & Montes, 1999).

The results of this work describe two stable populations of *P. clarkii* of the Tejo river basin. It seems that both populations present life history traits characteristic of this invasive species, such as a rapid growth, large number of offspring and a plastic life cycle. The knowledge of its biology reveals basic and essential information to understand how life history traits evolve and how biotic communities are assembled. Anyway, the present findings should already represent an interesting integration of the previous information on the distribution of this species in the Tejo river basin provided by Correia (1995).

This kind of study, based mainly in the analysis of dynamics population, reproduction and growth of invasive species such as *P. clarkii*, still remains useful and should be continued in the future. This work also revealed the urgent need for a proper management of this region’s populations, that may include its commercial use, as has been practiced in several countries, such as the United States (Huner, 1978; Hobbs et al., 1989) and Spain (Habsburgo-Lorena, 1986). Nevertheless, the fishing activity requires sanitary monitoring, due to the bioaccumulation capacities of this species (Alcorlo et al., 2006) and the demand by local fish markets. However, the crayfish fishing cannot eradicate naturalized populations unless coupled with other methods, either biological, chemical or physical (Kerby et al., 2005), which impacts on the
aquatic environment must be also carefully evaluated. Anyway, if crayfish are allowed to remain in freshwater ecosystems, the natural restoration of former habitats and the desired re-colonization by native species may become impossible (Kerby et al., 2005).

**General Conclusion**

This *Procambarus clarkii* study indicated that both populations were productive. The dynamics results confirmed indeed that the two marshes are suitable habitats to its rapid development. On the other hand, the analysis of the reproductive traits and growth rates found demonstrate the stability and spreading potential of *P. clarkii* in the Tejo river basin. Furthermore, this study of the biology of *P. clarkii* confirms the invasiveness of the species in the region.

To conclude, the evolution of these two populations since the last work in 1995 was very stable and outstanding which is a considerable problem to this region. Thus the urgent implementation of *Procambarus clarkii* populations monitory programmes in the affected areas, as it already happens in Italy (Ligas, 2008), aiming to control their evolution as well as to predict and avoid future invasions.

**References:**


