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# Evaluation of the exploitation potential of red swamp crayfish (*Procambarus clarkii*) in Portugal

Dissertação para obtenção do Grau de Mestre em Engenharia do Ambiente, perfil de Ordenamento do Território e Impactes Ambientais

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To my grandfather Manuel, who first introduced me to crayfish many years ago.

*Give a man a fish and you feed him for a day.*

*Teach a man to fish and you feed him for a lifetime.*

Chinese proverb

## Acknowlegements

To my advisor, nowadays a friend, João Gomes Ferreira. He initially told me that I have been five years being taught in the University, but he was there to unteach me. And thank goodness he did it.

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To my parents, who always provided me all the conditions and facilities to guarantee that I could win any challenge and build a stable life. Without their support I could never have become what I am today.

## Abstract

The red swamp crayfish (*Procambarus clarkii*) is a freshwater crustacean that was introduced in Portugal and became an invasive species. It brought several problems to ecosystems, causing serious environmental and economic damage, especially for rice farmers. It presents large populations and invaded all Portuguese territory. Currently there is no plan to control the species or any management plan that reduces the problems caused by it. However *P. clarkii* has a high commercial value in the various countries where it is consumed as an exquisite food. It is produced in aquaculture integrated with paddy fields in the United States, China and Spain, generating important amounts of annual sales and profit.

Several methods were used to identify the best crayfish production areas in Portugal, using a geographic information system, according to crayfish characteristics and ecology. It was created an individual growth model for the species and it was concluded that this model is well calibrated for Louisiana but presented a higher individual growth rate when in comparison with other studies for crayfish Portuguese populations. This individual model allowed to create a general population model to conclude about the biomass yields produced in paddy fields. The population model validation concluded that the final yields are according with the range of values produced in Louisiana and in southern Spain, but a bit higher than other Portuguese models.

The model suggests that Portugal can generate annually in aquaculture around 20000 tons of crayfish produced in nearly 23000 hectares of paddy fields. The average production of each paddy field can attain 870 kg $ha^{-1}$ $year^{-1}$ of crayfish. The best areas to produce *P. clarkii* are located in the paddy fields in the valleys of the rivers Mondego, Tagus, Sorraia and Sado, due to the availability of water and also to the proximity of the coast, which leads to milder temperatures. It is recommended that the species is used as a valuable economic resource, which culture can be made between December and April, when the paddy fields are fallow.

Crayfish aquaculture in Portugal can bring environmental and economic benefits. The population can be controlled and reduced through harvest. This can avoid the continuous spread of *P. clarkii* throughout the country because crayfish aquaculture method demands an annual restocking of the species in the paddy fields, which can be done using crayfish withdrawn from wild populations, improving the ecosystems quality by removing this harmful crustacean. Economically crayfish can generate important financial benefits to rice farmers. The product has a considerable market price in many countries, which can lead to an increase of the exportations and allow the creation of direct and indirect jobs, namely during the harvest, transformation and transportation of the product. Crayfish aquaculture industry can generate a total of 35 million € $year^{-1}$ in Portugal.

Keywords: invasive species, environmental problem, use of resources, geographic information system, population model.

## Resumo

O lagostim vermelho da Louisiana (*Procambarus clarkii*) é um crustáceo de água doce que foi introduzido em Portugal e se tornou uma espécie invasora. Trouxe vários problemas para os ecossistemas, causando graves danos ambientais e económicos, principalmente para os produtores de arroz. Apresenta grandes populações e invadiu todo o território nacional. Atualmente não existe qualquer plano de controlo da espécie ou forma de aproveitamento que reduza os prejuízos por esta causados. No entanto *P. clarkii* possui um elevado valor comercial em vários países que o consomem como alimento requintado e por isso é produzido em aquacultura integrada com arrozais nos Estados Unidos, na China e em Espanha, gerando anualmente importantes quantidades de vendas e lucros.

Foram utilizados vários métodos para identificar as melhores áreas de produção de lagostim em Portugal, recorrendo a um sistema de informação geográfica, de acordo com as características e ecologia do lagostim. Foi criado um modelo de crescimento individual para a espécie e concluiu-se que este modelo está bem calibrado para a Louisiana mas apresenta uma maior taxa de crescimento individual quando comparada com outros estudos sobre populações portuguesas de lagostins. Este modelo individual permitiu criar um modelo populacional geral para concluir acerca das quantidades de biomassa produzidas nos arrozais. Com a validação do modelo populacional concluiu-se que as quantidades finais estão de acordo com a gama de valores produzidos na Louisiana e no sul de Espanha, mas um pouco mais elevadas do que outros modelos portugueses.

O modelo sugere que Portugal pode gerar anualmente em aquacultura cerca de 20000 toneladas de lagostim, produzidas em pouco menos de 23000 hectares de arrozais. A produção média de cada arrozal pode atingir 870 kg $ha^{-1}$ $ano^{-1}$ de lagostim. As melhores áreas para produzir *P. clarkii* localizam-se nos arrozais dos vales dos rios Mondego, Tejo, Sorraia e Sado, devido à disponibilidade de água e também devido à proximidade da costa, o que leva a temperaturas mais amenas. Recomenda-se que a espécie seja aproveitada como um recurso económico valioso, cuja cultura pode ser feita entre Dezembro e Abril, quando os arrozais estão em pousio.

A aquacultura do lagostim vermelho da Louisiana em Portugal pode trazer benefícios ambientais e económicos. A população pode ser controlada e reduzida através da apanha para consumo humano. Isto evita a expansão contínua de *P. clarkii* por todo o País porque o método de aquacultura do lagostim necessita de uma introdução anual da espécie nos arrozais, que pode ser feita recorrendo a lagostins retirados de populações selvagens, melhorando a qualidade dos ecossistemas ao remover este crustáceo prejudicial. Economicamente o lagostim pode gerar importantes benefícios financeiros para os produtores de arroz. O produto tem um considerável valor comercial em vários países, o que pode levar ao aumento das exportações e permitir a criação de postos de trabalho diretos e indiretos, nomeadamente durante a apanha, transformação e transporte do produto. A indústria de produção de lagostim em aquacultura pode gerar no total 35 milhões de euros por ano em Portugal.

Palavras-chave: espécie invasora, problema ambiental, utilização de recursos, sistema de informação geográfica, modelo populacional.

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## Acronyms list

ANIA – Associação Nacional dos Industriais de Arroz

APA – Agência Portuguesa do Ambiente

ARH – Administração da Região Hidrográfica

CLC 2006 - CORINE Land Cover 2006

FAO – Food and Agriculture Organization

GIS – Geographic information system

GSI - Gonadosomatic Index

ICNF – Instituto de Conservação da Natureza e das Florestas

INE – Instituto Nacional de Estatística

IPMA – Instituto Português do Mar e Atmosfera

# 1. Introduction

## 1.1. Problem definition

The red swamp crayfish (*Procambarus clarkii*) is a freshwater crustacean, native to the southern United States which is currently present in almost every continent, being only absent from Oceania and the Poles (Anastácio, 1993; McClain & Romaire, 2007). It has been introduced in several countries, among them Portugal, due to its importance as food product (Fishar, 2006). *P. clarkii* is a highly harmful species to ecosystems it colonizes, due to its resistance, adaptation capacity and ability to reach new areas, competing directly with native species (Anastácio, 1993; Correia, 2002; Leitão, 2009).

The species was first introduced in 1973 in the area of Badajoz, in central Spain and in the Guadiana river basin. In 1974 a new introduction was made in the paddy fields of Seville, in southern Spain (Anastácio, 1993). The aim of this introduction was to produce a crayfish and rice double culture, as is done in the United States, resulting in extra money for the rice farmers and producing two cultures in one year in the same field. In 1979, crayfish populations in southern Spain increased without control, invading all the paddy fields in the region (Fishar, 2006). The population expanded to Portugal and became one of the largest worldwide. Thereafter the species began an effective expansion and colonized almost the entire Iberian Peninsula. In 2006 it had already reached the Salamanca region, in northern Spain (Anastácio, 1993; Fishar, 2006; Gil-Sanchez & Alba-Tercedor, 2002; Leitão, 2009).

After its introduction in Portugal, crayfish has expanded very quickly throughout the country, having become a dominant species and causing significant changes in many kinds of different ecosystems. As a consequence of the introduction of a new species and the great increase in its population, which occupies multiple habitats, there were serious problems caused directly by *P. clarkii* large populations (Cruz & Rebelo, 2006; Geiger *et al.*, 2005; Leitão, 2009). Economically, rice producers recorded significant losses and several problems in the paddy fields invaded by the species, where crayfish developed huge populations (Anastácio, 1993). Crayfish also brought many environmental problems and several impacts on native species, although there are favorable environmental conditions due to their presence, such as the change of the feeding regime of some important animals from the Portuguese fauna, now based on crayfish. On the whole, the impacts caused by the species are rather complex (Correia, 2002; Cruz, 2001; Cruz & Rebelo, 2006; Leitão, 2009). All around the world there are contention measures and many limitations to the imports and transport of live crayfish. In Europe the species occurs mainly in the lowest latitudes, with large populations living in the Iberian Peninsula, France and Italy (Fishar, 2006; Geiger *et al*., 2005; Gherardi, 2006).

Crayfish invaded paddy fields and all types of aquatic environments in Portugal. Some years after the introduction, the uncontrolled situation of the species led to the development of control measures. Initially, rice farmers resorted to chemicals with the aim of eliminating the crayfish from paddy fields but due to the resistance of the species, the situation has not changed, resulting in environmental damage due to the chemicals used. The chemicals used to eliminate the species killed other native species and polluted the ecosystems, while the crayfish survived (Anastácio *et al*., 1999b). In 1999, the red swamp crayfish was classified as an invasive species and any economic or other activities related to the species were forbidden, in an attempt to prevent its spread throughout the national territory. However, this prohibition in no way solved the problem and the species continued to expand and colonize new areas, being currently still considered a plague (Cruz & Rebelo, 2006; Decree 565/99; Leitão, 2009).

The abundance of crayfish in the Iberian Peninsula is mainly due to the mild temperatures of the air, the warmest area of Europe. This results in warmer water temperature and allows for better living conditions to the crayfish, which grows quickly and reproduces faster (FAO, 2013; McClain, 2010). Initially the species had no natural predators in this region, which contributed to the fast growth and expansion. Currently the situation has changed and many native animals started preying crayfish (Anastácio, 1993; Florêncio, 1993).

Nowadays crayfish remains a serious environmental problem in Portugal which lacks an effective solution, because so far, nothing specific has been done (Anastácio *et al*., 1999b). Although economic exploitation such as aquaculture is forbidden, the species is harvested and fished in rivers, paddy fields and lagoons and sent to Spain. There, crayfish is resold at higher prices or used to restock Spanish production fields (Gutierrez-Yurrita *et al*., 1998). Although the species is considered a plague in Portugal, Spain and other countries, Spanish authorities take control measures but also allow the harvesting and sale. Spaniards buy Portuguese production at low prices because it is not globally appreciated in this country and there is no legal crayfish industry and management, so the production has not a high commercial value (Anastácio *et al*., 1999b; Gutierrez-Yurrita *et al*., 1998; Ministerio de Agricultura Alimentación e Medio Ambiente, 2012**).**

Against this background, the introduction of an invasive species with disastrous environmental results and economic losses is a significant problem which leads directly to several other complex problems. Considering that the introduction of the species and its permanence in Portuguese territory appears to be irreversible, it is concluded that, given the impossibility of eradicating it, a solution that enables a correct management of the red swamp crayfish must be found, in order to minimize the damage caused by the species and obtain improvements over the current situation. At present, there is no plan for the use or management of the species in Portugal. This prevents effective control and fighting its territorial expansion and invasion of new areas and ecosystems, and also economic benefits.

## 1.2. Goals and scope

This dissertation aims to assess the potential value in aquaculture production integrated with paddy fields of the red swamp crayfish in Portugal, in order to understand whether it is suitable for economic exploitation and how it could be developed. Although this is the main aim of the work, the following specific objectives have been identified:

- Study the current distribution of the species in Portugal in order to conclude about the potential for control of the same as invasive species.
- Determine the capacity for production of the species at a national level, defining the best way to crop it and the most suitable production areas, using GIS.
- Develop a model to simulate crayfish population, growth and production in a paddy field.
- Propose a management strategy in order to harmonize the conflicts among production, economic value, and nature conservation, in order to understand also the economic market and do some kind of planning how to move forward.

## 1.3. Master Thesis layout

The work plan is divided into five steps. The first chapter is an introduction to the subject and aims to define the problem under study, presenting the objectives of the assignment and the scope in which it is inserted.

The second chapter provides a literature review on the topic. This chapter is divided into five main parts: the first part presents a framework to define the main themes in the current situation, showing its relevance, the second part presents general notes on the species, while the third part aims to define the current situation of it in Portugal. Its main objective is to better understand the ecology of the species, also reviewing aquaculture methods used and culture practices, in addition to defining the value and uses of the product in the current market. The fourth and fifth parts of this chapter present some references to Portuguese paddy fields and their most important aspects and a brief description on the study area and the climate.

The third chapter presents the methodology used to achieve the thesis goals. At this stage, it is intended to define a methodology to identify the best production areas of the species and the best way to proceed to its culture, using specific tools and methods. Initially the best production areas of the species at the national level will be identified, using GIS, and then it will be presented a population model for the exploitation of the species in these areas, depending on the modeling of various important factors selected to the survival and production of the species.

The fourth chapter presents the results and targets them to understand the best approach to the problem, in order to conclude whether the species is economically profitable to exploitation. This chapter also presents some critics to the results and comparisons with studies by other authors.

The fifth and last chapter presents the conclusions, taking into account the production process and the overall advantages and disadvantages of developing the economic exploitation of the species, making also some references to future developments, possible problems and recommendations.

# 2. State of the Art

## 2.1. Framework

The introduction of exotic species into new regions has occurred for thousands of years with the aim of benefiting man, though in different ways: usually due to food production, the attempt to control dangerous native species and pests or to meet the demand for exotic species by humans (Decree 565/99; Leitão, 2009). However the introduction of these species is now one of the biggest obstacles to the conservation of nature, bringing numerous impacts on ecosystems, significant changes in them and in the trophic relationships among species. It is currently one of the main causes of biodiversity loss worldwide. These species can also carry diseases and pests. The damage caused by invasive species costs many millions annually to each country to reduce its impacts, trying to eradicate the new generations and protect native species (Cruz & Rebelo, 2006; Decree 565/99; Leitão, 2009).

This problem is currently under study by the European Union, which plans to take measures to prevent the expansion and surpass of new borders by invasive species and also prevent the introduction of exotic plants and animals brought from other parts of the world. Invasive species are causing in Europe an annual loss estimated at least of 12 billion €, according to a 2010 study (Greensavers, 2013).

An invasive species is a non-native species that was introduced, through human transport, in a new geographical area, where it is not natural, having managed to stabilize and expand territorially. These species pose serious and diverse threats, damaging native flora and fauna, and affecting human health and economy. It is important to emphasize that the effects of these species on native species are significant and that has increased the number of species brought from their habitats to new geographical areas. Today it is very difficult to find areas that are not occupied by invasive species (Decree 565/99; Leitão, 2009).

Freshwater systems are extremely vulnerable to these issues and to be colonized by new invasive species, due to the numerous uses that man applies to them and also due to the easy dispersion in water (Leitão, 2009). Around the world numerous freshwater systems have dramatic ecological situations due to the introduction of exotic species, resulting in huge financial costs to attempt to recover them (Cruz & Rebelo, 2006; Fishar, 2006; Leitão, 2009). These systems were particularly damaged by the introduction of species of freshwater crustaceans, which caused numerous environmental problems in freshwater ecosystems worldwide because of their ecological characteristics (Cruz & Rebelo, 2006; Fishar, 2006; Leitão 2009). Although there have been different crayfish species introduced for the purpose of food production, red swamp crayfish is the species with the largest distribution worldwide, causing more problems due to its very specific ecological characteristics (Cruz & Rebelo, 2006; Fishar, 2006; Leitão 2009; Lodge *et al*., 2012).

The success of the red swamp crayfish as an ideal species for aquaculture is also the reason of the environmental problems caused by it: in both cases this is the result of strong biological characteristics, causing impacts throughout the food chain, becoming a dominant species in freshwater ecosystems, causing significant changes and affecting their conditions due to its rapid growth and massive reproduction rates (Ficetola *et al*., 2012). Attempts to eliminate the species using chemicals have proved fruitless because the species is extremely resistant after stabilized in a favorable habitat for its life cycle. Thus it is virtually impossible to eliminate it by any process after its stabilization and colonization of new areas. The expansion is usually extremely fast, with mild temperatures, being only slightly sensitive to other factors (Anastácio, 1993; Cruz & Rebelo, 2006; Leitão, 2009). The success of the species is based on the great adaptability to new areas and variable environmental and climatic parameters and the consequent development of resistance to variations in the environment and external factors. The species is extremely adaptable and physically strong at many different environments and has a very successful reproduction strategy (Cruz & Rebelo, 2006; Leitão, 2009). This guaranteed rapid dispersion throughout the Iberian Peninsula shortly after its introduction, developing large populations and bringing numerous problems for ecosystems (Anastácio *et al*., 1999b; Cruz & Rebelo, 2006; Fishar, 2006; Geiger *et al*., 2005; Leitão, 2009).

Figure 2.1 – *Procambarus clarkii* distribution in Europe

(Ministerio de Agricultura Alimentación e Medio Ambiente, 2012)

However, all these particular characteristics, very harmful to freshwater ecosystems, can be used to human benefit, making this species ideal for aquaculture. *P. clarkii* is the mostly produced worldwide freshwater crustacean (Fishar, 2006). The culture of the species is mainly made in the United States and China. These countries produce crayfish in two different ways: intensive monoculture in land ponds, where the species is produced all year round, and also in an integrated system, involving rice and crayfish double culture (FAO, 2013; Louisiana State University Agricultural Center, 2010). Due to the preference of the species for paddy fields, the aquaculture production of red swamp crayfish is done seamlessly with rice production, based on annual cycles. Rice and crayfish are a common culture in Louisiana and other United States southern states (FAO, 2013; Louisiana State University Agricultural Center, 2010). This method of production protects the paddy fields from damage done by crayfish burrowing activity, while using the natural range of crayfish biology, such as spawning and growth of the species, using the same field to produce two economic resources instead of one (Anastácio, 1993; FAO, 2013; Louisiana State University Agricultural Center, 2010).

Large amounts of crayfish currently exist in Portugal, which are caught and sold. Manual removal is the only way to combat the species, so Portuguese people can eat or improve the quality of the product to sell them to other countries where the species is appreciated as a food resource (Leitão, 2009).

Although economic activities with invasive species are forbidden in Portugal, an increase in harvesting of crayfish in the most productive paddy fields and ecosystems, and the consequent reduction of the populations, would help to reduce the large amounts of crayfish. This should be well received by the environmental management because one of the major goals of Portuguese environmental organizations is to combat invasive species in the country. One of the most important Portuguese non-governmental and environmental organization, Quercus, claims that nothing has been done by the authorities to eliminate these species, which costs millions of euro to the country (Greensavers, 2013).

Numerous profitable crops are species introduced by man that were not originally present in the territory of cultivation (Decree 565/99). Tomato, for example, is a species from Central and South America but is now cultivated throughout the world. It is one of the main agricultural products grown in Portugal (Expresso, 2013). The tomato is an introduced species, like almost all profitable crops, whose only difference is being no serious danger to ecosystems, while the red swamp crayfish is. Other introduced species became important resources to Portuguese agriculture and economic growth, such as potatoes and corn (Decree 565/99; Instituto Nacional de Estatística, 2012).

Crayfish can reach considerable market prices. It is an appreciated food in many countries and is a product with a high demand both in the European and the United States markets (Barbaresi & Gherardi, 2000; Fishar, 2006; Gutierrez-Yurrita *et al*., 1998; Louisiana State University Agricultural Center, 2010). The economic market of the species to export to other countries may be considerable. This can bring extra wealth to the producing countries.

Portuguese economy presents serious financial and production problems and it might be very important to exploit new resources with external markets, capable of generating wealth through national food production and allowing the creation of new jobs. In addition to the environmental benefits, Portugal can generate a new economic resource and valuable food, resulting in a reduction of food imports from abroad. It also could be exported a product with great market, helping to equalize the balance of trade of the country and reducing the excessive deficit. It is therefore essential to invest in the primary sector, able to directly create tradable goods. Regarding the crayfish, it is important to develop a truly national system of exploitation of the species, which may go beyond the current fishing and processing, without any planning or management. Serious exploitation can provide much larger amounts of crayfish and a better final product. The Portuguese government defined the aquaculture sector as a key priority for economic development. Portugal has a deficit trade balance in food and sea products. A goal of the Portuguese government is doubling the aquaculture production by 2015. Portugal currently produces only 3% of its consumption in aquaculture, contributing to the increase of sovereign debt and imports (Visão, 2013). One of the national priorities is the supply of the domestic market with national products, especially food and agricultural products. Currently, Portugal imports more food products of agricultural origin than exports (RTP, 2013).

With forecasts predicting that the world population will continue growing especially in developing countries, these countries will undoubtedly need to import food to meet the huge demands of a growing population (United Nations, 2004).Thus, in the short and medium term, crayfish can be very important both in domestic market, as replacement of shrimp imports, as in the international market, increasing exports to countries which have larger economies and populations. It will always be necessary to produce more and more food and animal protein to feed so many people. Portugal thus should avail a valuable food resource that will allow an increase in exports and the consequent enrichment of the country.

## 2.2. General notes on the species

### 2.2.1. Ecology

Red swamp crayfish is a highly adaptable species. It tolerates a wide range of environmental conditions, has high growth rates, and a very successful reproductive strategy, which helps to create large populations and significant changes in ecosystems (Anastácio *et al.*, 1999b; Geiger *et al.*, 2005). The main characteristics of the species and success factors that enable such a broad distribution and invasion of habitats, which can be used to promote a good aquaculture process, are:

• Broad range of diet - It is an omnivorous species, feeding on living or dead plants and meat (Anastácio, 1993; FAO, 2013). Crayfish are not active and fast predators because it cannot catch fish or insects that move rapidly. About 20% of the diet consists of worms and larvae, and the remaining 80% of plants (Martins *et al.*, 2009). The species may consume animal or plant detritus, vegetation and living animals. It presents a great variability in the diet, this being one of the key factors for its great success in adapting to new environments. Crayfish are scavengers, which mean they feed on debris, whether plant or animal (Ficetola *et al.*, 2012; Geiger *et al.*, 2005; McClain & Romaire, 2007). The animals preyed are molluscs, insects, worms, larvae and some fish (Anastácio, 1993; FAO, 2013). However the species consumes mainly plant foods and some authors report an increase in the consumption of plant material throughout life (Ficetola *et al.*, 2012; Geiger *et al.*, 2005; McClain & Romaire, 2007).

• Short life cycle - The life span of the species also varies with the climate: in tropical and warm temperate climates lives between 12 and 18 months, while in cold temperate climates can reach more than 36 months (Anastácio, 1993).  There are individuals known to have reached ages over six years in nature (anecdotal references). Generally the species presents high mortality rates, a maximum longevity of 4 years, and a low mean life-time of less than a year (Scalici & Gherardi, 2007).

• Large populations due to a very effective reproduction strategy - In Louisiana each female can reproduce up to three times a year and up to 500 juveniles each time. However the smaller females spawn only 200 or more eggs (FAO, 2013; McClain & Romaire, 2007). The species can produce between one and three generations per year, with the largest number of generations found in populations near the equator, due to high annual average temperature and the absence of seasons (FAO, 2013; McClain & Romaire, 2007). In 40 ° north latitude the number of generations is usually reduced to one, but can be more, dependent on the environment conditions. In Louisiana and Portugal the species mates during the summer and spawns in the autumn, but juveniles can be found all year. The number of annual generations is one in Portugal, while in Louisiana may be between one and three (Anastácio, 1993; Geiger *et al.*, 2005; McClain & Romaire, 2007). This is due to Portugal's latitude because, in general, the average annual temperature is higher at lower latitudes. Portugal is located approximately 10 º north of Louisiana, so it is expected that crayfish grow more slowly and reach sexual maturity later in Portugal, producing less generations per year. Some studies state that crayfish reproduction cycle may change and adapt after its introduction in different regions (Anastácio & Marques, 1995; Anastácio *et al.*, 1999b).

• High tolerance to varied climatic conditions – The species is able to tolerate a wide range of environmental conditions, including low oxygen levels (it is not a limiting factor for growth rate), polluted water and high temperatures (D'Abramo *et al.*, 2004).The life cycle of the red swamp crayfish is very well adapted to the annual sequence of winter flooding and summer dry period in the

United States southern swamps. During the summer it digs a burrow, waits there for the first rains and spawns safely in the protection of the burrow (FAO, 2013; McClain, 2010; McClain & Romaire, 2004). Around October or November, when the rain comes, they leave the burrow to seek for food. Crayfish spends periods of drought or cold safely protected within the burrow (FAO, 2013; McClain, 2010; McClain & Romaire, 2004). The species also adapted this way in Portugal and in the Mediterranean region (Florêncio, 1993; Ministerio de Agricultura Alimentación e Medio Ambiente, 2012). There is a direct relation between temperature and molting. Water temperature is a major determination of growth rate (Chen *et al*., 1995). The species is very tolerant to different ranges of water temperature, ranging from 10ºC to more than 30ºC (Anastácio, 1993; Geiger *et al*., 2005; McClain & Romaire, 2007).

• Extremely fast growth - Both males and females reach sexual maturity around 80 mm (total body length) (Anastácio 1993; Eversole & Mazlum, 2002). At the optimum temperature of 22 ° C embryonic development is extremely fast and can last between two and three weeks. In adequate conditions larvae are born after 21 days of incubation, growing 80 mm in 3 months (FAO, 2013; McClain, 2010). Temperature is an important growth factor for crayfish. Higher temperatures mean faster growth but also higher mortality too (Anastácio *et al*., 2009; Chen *et al*., 1995; Fidalgo *et al*. 2001; Martins *et al*., 2007; McClain, 2010).

This specific characteristic, which are the reason for the environmental problems caused by the species are also the key factor for its success in aquaculture production, being the most desirable and produced freshwater crustacean worldwide, mainly due to its extremely fast growth (Fishar, 2006).

Red swamp crayfish physical characteristics are summarized in Table 2.1, based on data provided by FAO and the works by Anastácio (1993), Dorr *et al*. (2006) and Florêncio (1993).

Table 2.1 – Physical characteristics of the red swamp crayfish

| Common total body length | Maximum total body length | Common body weight | Maximum body weight |
|---|---|---|---|
| 10.5 – 12 cm | 20 cm | 35 – 56 g | Over 60 g |

*P. clarkii* is found in every kind of freshwater ecosystems, although it prefers lentic habitats such as slow rivers and streams, flooded fields, marshes, ponds with vegetation and leaf protection and human habitats such as irrigation channels and reservoirs. It avoids rivers and streams with a strong flow (FAO, 2013; Leitão 2009).

The species is native to Louisiana swamps, where it usually mates and spawns between July and October. The highest recruitment peak occurs in the months of September, October and November. Other geographical regions exhibit different patterns of reproduction (Anastácio, 1993; FAO, 2013; McClain, 2010; McClain & Romaire, 2007).

Mature crayfish mate in open water and then the females seek the protection of the burrow while the eggs are attached to their abdomen. The main period of burrowing and spawning is during late spring and summer, although it can happens any time of the year (McClain, 2010; McClain & Romaire, 2004). The duration of embryonic development is a function of temperature, and below 10 ° C development is inhibited (Anastácio, 1993). Crayfish hatchlings remain attached to the female´s abdomen until they can feed their selves and become free (FAO, 2013; McClain, 2010; McClain & Romaire, 2004).

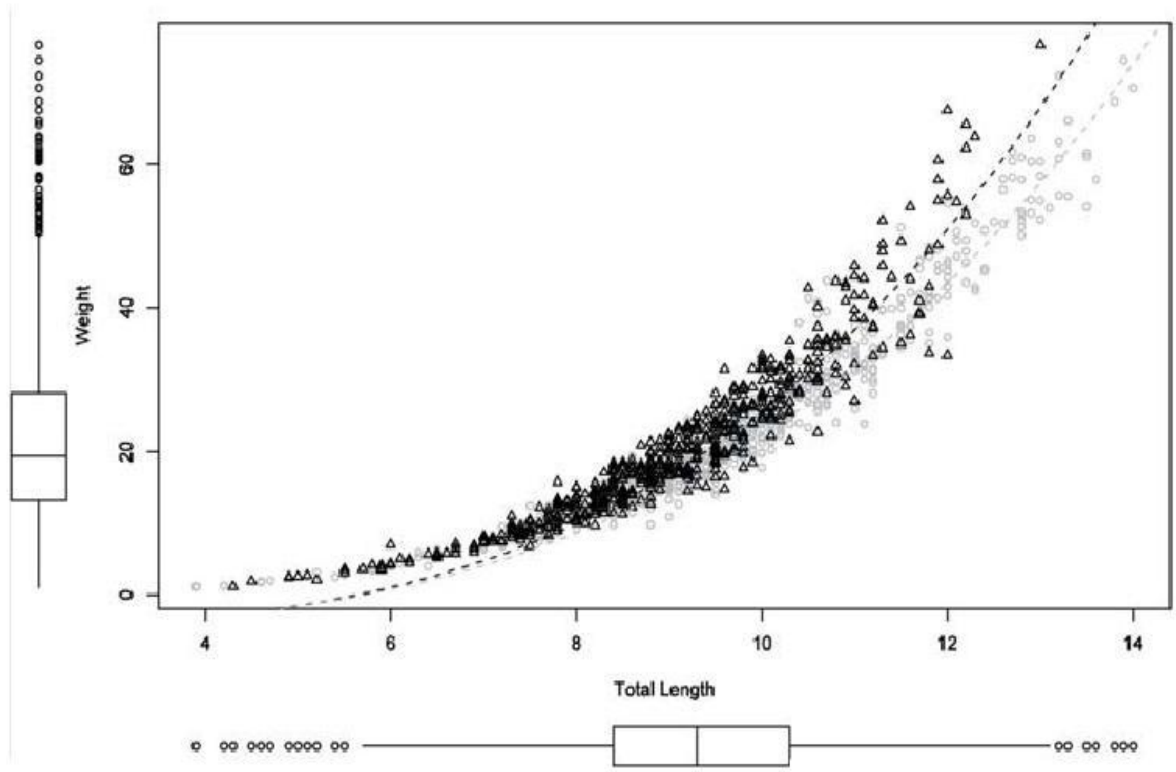


Figure 2.2 - Crayfish weight (g) and total body length (cm) relation

(Dorr *et al*., 2006)

Portuguese crayfish populations' recruitment may occur throughout most of the year but with seasonal peaks occurring between October to December, with a smaller period occurring in April to May and late summer (Anastácio, 1993; Anastácio *et al*., 2009). The species growth rates found in a study conducted in the Mondego valley were low, about 1.16 mm per week (Anastácio & Marques, 1995). This might be due to cold temperatures, but they are similar to the ones obtained by another authors for other areas of Portugal. When compared to another studies and authors from different part of the world it is clear that low growth rates can difficult the aquaculture of the species in Portugal (Anastácio, 1993; Anastácio *et al*., 2009; Fidalgo *et al*., 2001).

There seems to be a direct relationship between higher latitudes and higher female predomination, such as in Portugal. There is some contradiction in the literature: references to a greater number of males than females in Louisiana and in Kenya. Portuguese authors refer female predomination. (Anastácio, 1993; Anastácio *et al*., 2009; Anastácio *et al*., 1999b; Fidalgo *et al*., 2001).

In Portugal paddy fields and marshes are the areas preferred by the species. Crayfish have higher population densities, minor mortality rates and the life span of the species is higher in paddy fields and marshes, when in comparison to other habitats (Anastácio *et al*., 2009). Low densities in crayfish populations lead to larger individual sizes and faster growth (McClain, 2010; McClain & Romaire, 2009). Paddy fields are the most appropriate locations for crayfish populations due to the continuous flow of water, essential to the species life cycle, and food. Temperature and hydrology are fundamental to the structure of the population (Anastácio, 1993; Anastácio *et al*., 2009). Low population densities increase individual growth, so crayfish reach higher individual weights and grow faster, due to less competition (Anastácio *et al*., 2009; McClain, 2010; McClain & Romaire, 2009).

A study in a protected area in central Portugal, Paúl do Boquilobo, concluded that the relative abundance of crayfish individuals varies greatly throughout the year. The life cycle of crayfish is very dependent on the water level and temperature. There is an abundance of adults in the spring related to mating in the summer. There is a reduction of adults in summer related to burrowing activity, where the crayfish shelter to prevent dehydration in dry habitats and to spawn. In the winter there are low densities of the species, related with little activity and burrowing due to cold temperatures. Crayfish populations in this area shows a high density of juveniles in the winter, because when spawning occurs in early winter low temperatures delay development until the following spring and individuals grow very slowly (Florêncio, 1993).

### 2.2.2. Crayfish aquaculture and culture practices

Aquaculture is the cultivation and production of aquatic organisms for use and consumption by man (Kenyon College, Department of Biology). Aquaculture is increasing in importance due to the overexploitation of resources, which brings environmental changes and degradation of many ecosystems. According to FAO, in 2011 aquaculture equaled fisheries in terms of total production of fish and other aquatic species. The trend is for an increase in aquaculture and stabilization in fisheries (Ferreira *et al*., 2007; Ferreira *et al*., 2012). Aquaculture is currently one of the sectors of food production which had the highest growth in recent years, providing approximately 47% of world production of fish and other species from aquatic ecosystems (Silva *et al*., 2011).

Rice and crayfish integrated culture is done in the United States since the 18$^{th}$ century and in China since the last decades of the 20$^{th}$ century (McClain & Romaire, 2004). In the United States crayfish production usually attains very high yields: in 1985 the country produced 55000 tons, about 85% of the world market volume. In 2007, the United States produced 49500 tons of crayfish, which worth 85 million dollars (Fishar, 2006; McClain, 2010).

Crayfish and rice double culture is a common practice in the United States and China nowadays. In the United States, in 10 rice producers 7 also produce crayfish (Purina Mills, 2010). Although the greatest yields of crayfish are achieved when produced in large and specific earth ponds, rice and crayfish integrated production also produces large amounts of the crustacean (FAO, 2013; Louisiana State University Agricultural Center, 2010). This way of extensive aquaculture introduces the species in the paddy fields and respects an annual cycle for the growth of the plant and the crayfish. Both species perfectly complement themselves and use each other’s outputs (FAO, 2013; Louisiana State University Agricultural Center, 2010; Purina Mills, 2010).

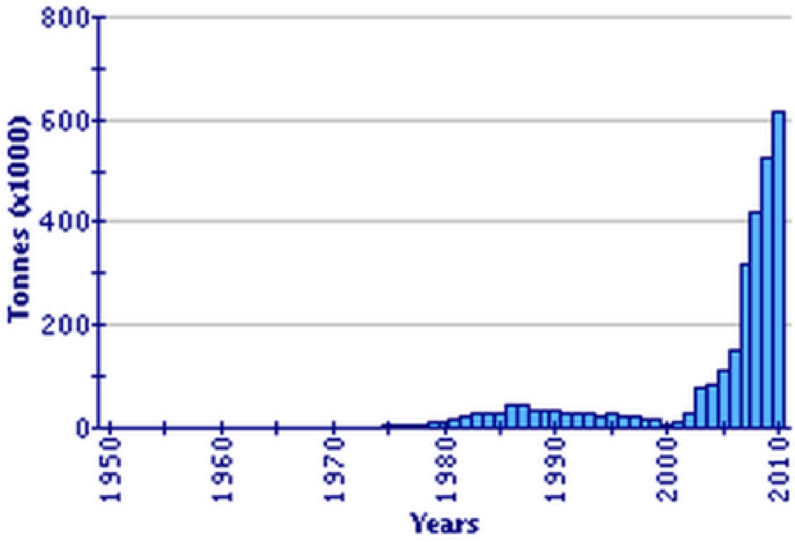


Figure 2.3 - Global aquaculture production of *Procambarus clarkii* in the world

(FAO, 2013)

Extensive aquaculture introduces crayfish at low densities, has no artificial inputs such as feed and has very little management by man (FAO, 2013). There are tables that define the periods of cultivation and harvesting of rice, as well as the introduction of crayfish, in addition to modeling temperature and water (Louisiana State University Agricultural Center, 2010). Currently the economic exploitation of the red swamp crayfish is done in the United States, China and in the marshes and paddy fields of the Guadalquivir river, in southern Spain. These three countries are the biggest worldwide producers (FAO, 2013; Gutierrez-Yurrita *et al*., 1998; McClain & Romaire, 2004).

In Louisiana, crayfish aquaculture practices are quite simple, using an annual calendar (Annex 1). Crayfish are restocked in paddy fields with natural recruitment from indigenous populations (FAO, 2013; Louisiana State University Agricultural Center, 2010). Recruitment can occur in any month during several months, always after flooding up the fields, but the bulk occurs usually from November to February. This happens because the spawning is not synchronized and occurs over several months and so there are multiple weight classes during the production season and the recruitment period is long (FAO, 2013; Louisiana State University Agricultural Center, 2010; McClain, 2010; McClain and Romaire, 2007).

Table 2.2 – Rice and crayfish integrated culture cycle

| Task/Month | Jan. | Feb. | Mar. | Apr. | May | Jun. | Jul. | Aug. | Sep. | Oct. | Nov. | Dec. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Field preparation for rice culture | | | | | | | | | | | | |
| Rice sowing | | | | | | | | | | | | |
| Rice growth | | | | | | | | | | | | |
| Restocking crayfish | | | | | | | | | | | | |
| Rice harvest | | | | | | | | | | | | |
| Flooding fields | | | | | | | | | | | | |
| Harvesting crayfish | | | | | | | | | | | | |

In April rice is sown and the fields are flooded. About 6 weeks later, in May or early June, medium crayfish are stocked into the fields. Crayfish are withdrawn from rivers and other water bodies. When the water level is low in the summer crayfish will burrow in irrigation channels to protect and reproduce themselves (FAO, 2013; Purina Mills, 2010). The rice harvest starts in August or September. In September and October the fields need to be artificially flooded, allowing the regrowth of foliage which serve as food resource for growing crayfish and protection. The fields must be flooded with a small layer of water around half meter tall (Auburn University & USDA-Natural Resources Conservation Service, 2004; Louisiana State University Agricultural Center, 2010; McClain & Romaire 2004). Crayfish leave their burrows and return to the fields where the remains of rice straw will serve as a natural food resource. The females leave the burrow with the newborn crayfish attached to them. During the harvest season, from November to March or April, crayfish will grow faster and bigger, due to better environmental conditions (McClain & Romaire, 2007; Purina Mills, 2010). This is a type of extensive aquaculture. Crayfish are removed from aquatic environments and are introduced in a field where it will grow and reproduce much faster, mainly due to the availability of food. This calendar can present some differences each year (FAO, 2013; McClain & Romaire, 2007; Purina Mills, 2010).

Although rice farmers in Portugal have recorded some serious damage to cultures, in the United States the production method allows the growth and harvest of crayfish, preventing any damages to the rice plants. In an email conversation with two American teachers who work with integrated production of crayfish and rice in Louisiana, professors Ray McClain and Robert Romaire, they stated that in Louisiana's paddy fields crayfish digs but do not cause serious problems. This personal communications led to the following conclusions about crayfish aquaculture in the United States:

- The losses in rice production can be avoided if big crayfish are totally absent from the paddy fields and separated from the rice seeds and young plants when the rice is sown. This can happen if before April the larger majority of crayfish was harvested for sell.
- When adult crayfish are stocked in the fields in May and June, usually 6 weeks to 8 weeks after the rice sow, the rice plants have grown 30 to 40 cm in height, and crayfish do very little or have no measurable damage to rice plants that large. There are other organisms in the flooded paddy fields that crayfish prefer to eat such as snails, aquatic insects, and other types of vegetation.
- Soon after crayfish are stocked in the flooded paddy fields, the mature crayfish seek levees where it can burrow for reproduction, usually one month after it have been stocked. Farmers don't prevent them from burrowing because it is in burrows where spawning occurs. Also the high density of rice plants relative to the low density of crayfish that are stocked (usually around 50 kg $ha^{-1}$) all collectively combine to have no adverse affect on the growing rice crop in Louisiana.
- There are no complaints from rice farmers in Louisiana of crayfish damaging rice production. Many rice farmers think that the presence of crayfish improves rice production by helping to control certain weeds that compete with rice and helping to potentially oxidize organic matter in the bottom sediments.

- Crayfish only dig in the levees and irrigation channels. Many rice farmers in Louisiana often re-build levees each year using specialized equipment and repair any damage crayfish may have done. The need for repair or re-build small levees each year is usually associated with erosion from wind and wave action and not from the burrowing activity of crayfish.

- If crayfish happen to be in the fields when rice is growing (in the water and not in burrows) and insecticides are applied to control insects, then most of those insecticides will kill crayfish that are in the water. If crayfish are in burrows when insecticides are applied then crayfish are not harmed.

- Few crayfish in Louisiana attain sizes over 40 g. The density of crayfish has to be very low for them to attain this size, because growth and size of maturity is density dependent. Larger crayfish are more valuable and desirable for live market sales.

- Crayfish usually damage the rice crop in only 2 ways: they eat the seeds or seedlings and their burrowing compromises levees, which can cause a field to be drained - upsetting weed control and nitrogen management measures. Since the fields are rarely flooded for any length of time when the seeds are sprouting or seedlings are becoming established, there is not much opportunity for crayfish to consume the seeds/seedlings. Once the fields receive the permanent flood, the rice plants are normally big enough and growing fast enough that any residual crayfish can do little harm. Most rice fields are fallow and often dry before planting and this prevents the buildup of crayfish populations in those fields.

Crayfish yields produced in the United States vary with the kind of culture and depends on the inputs and the management (FAO, 2013). Table 2.3 presents crayfish aquaculture characteristics based on data provided by FAO.

Table 2.3 - Crayfish aquaculture carachteristics

| | Crayfish monoculture | Rice and crayfish integrated culture |
|---|---|---|
| Common yields | 225 kg $ha^{-1}$ - 1300 kg $ha^{-1}$ | Lower than monoculture |
| Maximum yields | 2800 kg $ha^{-1}$ | Over 1000 kg $ha^{-1}$ |
| Disadvantages | • Need to construct large ponds.<br>• Costs of the land.<br>• Crayfish overcrowding, which leads to huge yields of small crayfish, difficult to sell. | • Need to restock every year<br>• Late-season harvest, which is done when prices are in decline because of the abundant supplies. |
| Vantages | • Stocking is only needed in new ponds, where crayfish are introduced in the spring between 45 – 89 kg $ha^{-1}$. | • Low population densities, which lead to higher individual sizes and heavier weights. |

Trap density can range from 20-60 $ha^{-1}$, depending on intensity of the operation. Harvesting begins as soon as the catch is justifiable - usually 2 to 4 months after flooding, depending on the meteorological conditions. Trapping frequency and duration also varies. Traps are emptied 2-7 days $week^{-1}$, often intermittently for 3 to 8 months. Frequency and duration of the harvest are influenced largely by cost of harvesting, marketing, price and catch. The daily catch rarely exceeds 1.0 kg $trap^{-1}$ on a sustainable basis (FAO, 2013; Purina Mills, 2010).

Most ponds larger than 2 or 3 hectares are harvested with the aid of a motorized, flat-bottom boat designed for shallow-water propulsion (FAO, 2013; Louisiana State University Agricultural Center, 2010; Purina Mills, 2010). Several boat types and propulsion designs are available, but the most widely used apparatus consists of an aluminium boat equipped with a hydraulically driven metal wheel that extends beyond the boat and either pushes or pulls it through the pond. The boat circulates through the irrigation channels in paddy fields. The traps are placed in the irrigation channels, where the boat can circulate (D'Abramo *et al*., 2004; FAO, 2013; Louisiana State University Agricultural Center, 2010; McClain & Romaire 2004; Purina Mills, 2010).

Commonly, the boat travels down the lanes of traps and a fisherman will empty and re-bait each trap from one side of the boat, often without stopping at the traps, placing harvested crayfish in a box in the boat (Louisiana State University Agricultural Center, 2010; Purina Mills, 2010). Two categories of bait are used to attract crayfish to the trap - natural baits of dead fish, and manufactured baits of proprietary formulations. A wire-mesh, 3-sided 'pyramid-shaped' trap is designed for use in shallow water and is effective and efficient to operate (D'Abramo *et al*. 2004; FAO, 2013; McClain & Romaire, 2004; Purina Mills, 2010).

To make the final product more appealing to the market some producers insert the crayfish in an indoor tank between 12 and 24 hours. During this period crayfish are not fed, allowing to clean the body, mud, debris and other impurities (Louisiana State University Agricultural Center, 2010; Purina Mills, 2010). More than 24 hours is not advised because mortality increases greatly. Anecdotal references state that crayfish can be inserted alive in tanks with weakly brackish water for some hours, which makes the flavor of the final product better (Louisiana State University Agricultural Center, 2010; Purina Mills, 2010; Romaire *et al*., 2005).

Using agricultural sub-products, such as rice straw and soybean, crayfish production can be increased between 1.5 and 2 times, which makes the paddy fields the best area to produce it (Martins et *al*., 2009). After the rice harvest the straw left in the fields serve as a food to crayfish populations. Rice is the best diet for crayfish growth, but only with low crayfish densities (FAO, 2013; Louisiana State University Agricultural Center, 2010). The greatest weight gain in crayfish individuals is achieved with soybeans, rice and potatoes, a good source of protein and carbon hydrates (McClain & Romaire, 2009). The best form of aquaculture production of the species is integrated in the paddy fields, because they are a natural food for crayfish, capable of a high body mass increase and preferred by the species (FAO, 2013; Louisiana State University Agricultural Center, 2010; Martins et *al*., 2009).

### 2.2.3. Crayfish as an economic resource and its market

Red swamp crayfish is a species with great market and commercial value, being widely consumed in the United States, Spain, China, Scandinavia, Russia and Ukraine, Nigeria and other African countries, Mexico, France, Australia, New Zealand, Caribbean islands, Canada and many other countries of northern and central Europe. Much money is annually spent in Europe to import crayfish (Barbaresi & Gherardi, 2000; Fishar, 2006; Gutierrez-Yurrita *et al*., 1998; Romaire *et al*., 2005). Crayfish larger than 20 g are desired in the United States market, but individuals greater than 30 g are preferred and have the highest value (McClain, 2010; personal communication). Martins *et al.*, (2009) concluded that each 100 g of raw wild crayfish has the proximate composition value presented in Table 2.4:

Table 2.4 – Nutritional value of the red swamp crayfish

| Calories | Total fat | Total protein | Fatty acid | Cholesterol | Sodium |
|---|---|---|---|---|---|
| 77 cal | 0.95 g | 15.9 g | 0.17 g | 114 mg | 58 mg |

The commercial exploitation of the species may be used not only for food production but also for other industries. In market terms, the species can be used for:

• Production of substances for dermal pharmaceutical interest and cosmetics from the animal's shell (chitin, chitosan and collagen).

• Extraction of pigments (astaxanthin) which can be used in cosmetics or as antioxidant.

• Production of animal protein in large quantities, which can be used for animal feed or human food. Crayfish are also used in industry, for the production of fish flour, among others (Martins *et al*., 2009).

The following box presents a simple summary about chitin, chitosan, astaxanthin and collagen:

- Chitin is a polysaccharide which can be found in the exoskeleton of crustaceans. Chemical treatment results in the production of chitosan, a polysaccharide widely used in the pharmaceutical and biomedical investigation areas due to possess unique characteristics in several respects. Astaxanthin is a red pigment that occurs in many living organisms. It is used in aquaculture of fish and shellfish and is added to animal feed to compensate the lack of a natural dietary source. Is essential for growth and survival. Astaxanthin is used as an antioxidant in the main developed countries for therapeutic and other uses (Martins *et al*., 2009).

- Collagen is the designation of a set of proteins which are tough and fibrous which are the main components of connective animal material. Collagen has a wide variety of applications, from food to medical. It is used in cosmetic and burns surgery and is widely used in the form of collagen casings for sausages, which are also used in the manufacture of musical string (anecdotal references).

## 2.3. Status of the species in Portugal

### 2.3.1. Introduction, expansion and current situation

The red swamp crayfish was first identified near the border between Portugal and Spain in Caia river, a tributary of the Guadiana river, in 1979, probably arriving from natural dispersion through the Guadiana river basin after a fast expansion in the southern Spain (Anastácio, 1993; Fishar, 2006; Leitão, 2009). From there it expanded quickly through all water lines and in 1986 had already been registered in almost all the southern Portugal, namely in the Guadiana, Tagus and Sado river basins. In 1987 it was first identified in the lower Mondego, causing the first serious damage to paddy fields in this area in 1990 (Anastácio, 1993; Fishar, 2006; Leitão, 2009).

Decree 565/99 confirms the presence of *P. clarkii* in Leça, Douro, Vouga, Mondego, Lis, Tagus, Sado, Mira and Guadiana river basins and also in ribeiras do Oeste and ribeiras do Algarve. In 1999, red swamp crayfish was already spread among almost all the Portuguese territory, being absent only in the extreme northern region of the country (ARH Centro, 2012; Leitão, 2009). However, in 2012, the species was present in Minho river basin, in the north border with Spain, so it may be assumed that crayfish has already invaded all Portuguese territory, being present in all river basins (Caminhense, 2012).

Portuguese hydrographic regions reports from the last years also indicate the presence of the species in all territory (ARH Centro, 2012). Portuguese populations are very variable. They can be huge and then present a reduction of 50% in one dry year (Leitão, 2009). This is mainly due to climatic factors, being extremely important the precipitation and amounts of water available in the ecosystems, which is essential to crayfish spawn and protection. The species has a strong preference for paddy fields and marshes, where it is always present at much higher densities than in other ecosystems (Anastácio, 1993; Anastácio *et al*., 2009; Florêncio, 1993).

### 2.3.2. Invasive species status

Portuguese law defines in Decree 565/99 that the introduction of non-indigenous species in nature can lead to situations of predation or competition with native species, transmission of pathogens or parasites and seriously affect biological diversity, economic activities or public health, with irreversible damage, difficult to quantify. Furthermore, when necessary, control or eradication of an introduced species that has become invasive is particularly complex and expensive. However, the introduction of some non-native species and their exploitation proved to be important factors for the development of the national economy, in particular to increase the variety and availability of food resources, as historical examples are potato, tomato and corn. Many non-native species were introduced in Portugal with benefits. However, many others can be very harmful, such as the perch (*Lepomis gibbosus*), water hyacinth (*Eichhornia crassipes*), highway ice plant (*Carpobrotus edulis*) and crayfish non-native species.

Instituto de Conservação da Natureza e das Florestas (ICNF) is the public entity which manages non indigenous species in Portuguese territory. The red swamp crayfish is considered by Portuguese law an invasive species, included in Annex I and Annex III (non-native species with known ecological risk) by Decree 565 from 21st of December 1999. These two annex defines the rules for the species included in them:

- Forbidden restocking
- It is prohibited to transfer, sale, purchase and the transportation, cultivation, breeding or detention in a confined space, economic exploitation and use as an ornamental plant or animal companion as a way of preventing introduction into the wild, or restocking.

Portuguese law also defines measures to control invasive species and states that the ones already introduced in nature are subject to a national plan with the aim of its eradication or control, promoted by the Ministry of Environment (Decree 565/99). Currently there is not any plan for management or eradication of crayfish, as defined by law. However there are important exceptions predicted by law. New introductions in the nature can be exceptionally allowed if verified each of the following situations:

a) There are clear advantages to humans;
b) There is no indigenous species suitable for the intended purpose;
c) Be preceded by the preparation of an environmental assessment, meticulously planned, with the conclusions being relevant to the authorization.

In other words, the dissemination or release of specimens of invasive species in nature is forbidden, with or without the purpose of establishing wild populations. The only exception, subject to authorization, is the spread or release in nature of invasive species specimens, for the establishment of wild populations, when there are clear advantages to humans, since there is no indigenous species suitable for the same purpose. A study of the environmental impact of the introduction must be elaborated before. It is covered by this exception the use of non-indigenous species for aquaculture and apiculture (Decree 565/99).

Currently fishing is allowed but the exploitation of the species in aquaculture production is not. It is also banned any kind of introduction in the nature. These points are also applicable to the economic exploitation of non-native species, including in aquaculture. If it is proved that crayfish aquaculture in Portugal would bring economic benefits and environmental improvements this could lead to a change: according to the law, it is currently possible to produce crayfish in aquaculture if it is established that there are clear advantages and great economic benefits, this being one of the most important goals of the work.

### 2.3.3. Comparison between Spanish and Portuguese situations

Much of the information about crayfish in Portugal came from anecdotal references, due to the law prohibition status. Some references such as local journals and blogs were used to meet the needs of information about the real current situation such as Jornal do Fundão (2010) and Abrangente (2003). Fishing activity is currently well documented in Portugal, mainly in Alqueva dam, Castelo de Bode dam, Mondego river and Sorraia river rice fields and river Tagus, mainly in central and southern Portugal. The final destination of every load is always Spain (Gutierrez-Yurrita *et al*., 1998; personal communication).

In Vila Velha de Rodão, a village in Tagus river and near the Spanish border, there is a factory which buys crayfish to local fisherman and sells them in the Spanish market. This factory receives the crayfish alive and in the industrial zone, which employs five people, crayfish is separated according to its size, it is weighted, treated and cleaned, packed and follows in wooden boxes for Spain to be resold in the Spanish market. The company was officially legalized in 2010 (Jornal do Fundão, 2010).

The factory manager affirms that crayfish fished in Tagus river is one of the best quality crayfish in Portugal. He claims that in the summer it is the best because it is clean, heavy and there is no hollow. The company buys crayfish to about twelve fishermen and in 2009 bought 300 tons of crayfish to the local fishermen only during the summer. They state that during the winter they buy crayfish to other fisherman, namely to the Figueira da Foz region (Mondego valley), in central Portugal but near the coast, because the local crayfish is very weak. They also say that if there were crayfish all the year they would buy it because the sell is assured in Spain, where it is a product with a very high demand (Jornal do Fundão, 2010).

In 2009 this company claims to have bought in Portugal 20000 tons of crayfish. As there is not currently any crayfish aquaculture this value is only referring to fisheries in aquatic environments without any planning or management. There are many fishermen who catch crayfish in this area all year, although the best months are from May to September (Jornal do Fundão, 2010). In the winter crayfish seek for shelter deeper in the river due to the strong flow and low temperatures. These fishermen sell the crayfish to the company for around 1.5 € $kg^{-1}$ and each one can catch around 15 kg $week^{-1}$ during the summer. There are also crayfish fishermen who make their only earning and life activity on fishing and selling the crustacean to the Spanish company (Jornal do Fundão, 2010). These fishermen can catch during the summer an average amount of 160 kg of crayfish $week^{-1}$. The importance of this activity is recognized by the direction of the Spanish company, which developed a good relation with the local fishermen because they know that there are whole families dependent on them and on the richness of the river to survive (Jornal do Fundão, 2010). Crayfish prices can be very variable, depending on the years, size and available amounts, but generally goes from 2 to 6 € $kg^{-1}$, although in Spain it can reach higher prices (Abrangente, 2003). Actually the price paid in Portugal for the crayfish is much lower than the one paid in Spain, where crayfish can be sold in the big cities much more expensive than the prices paid to Portuguese fishermen (Gutierrez-Yurrita *et al*., 1998).

There are also food festivals in Portugal that serve it as a delicacy, organized by the local community to promote local economy. These festivals have been a success in recent years, which should lead the way to the appreciation of this resource and extend to other areas of the country. Crayfish gradually have grown into a more appreciated specialty food. There is a crayfish festival in Ferreira do Zêzere, a village near Castelo de Bode dam. This festival has been growing every year since 2008 occurs always in April (RTP, 2010; Sapo, 2012).

In Spain, only the Guadalquivir valley has an important *P. clarkii* industry, able to support big markets. Data from 1986 refers yearly captures of 3000 tons of crayfish in Seville paddy fields, in southern Spain. It is also the only specific crayfish production zone in the Iberian Peninsula (Fishar, 2006; Gutierrez-Yurrita *et al*., 1998). In Portugal the species exploitation has been very weak, reduced to fisheries so, when in 1995 occurred a strong reduction in the production of Spanish crayfish, a businessman showed interest in installing a factory in the Mondego valley, to produce and sell frozen and canned crayfish to northern Europe, but the project did not go further. In 1997 Spaniards bought around 700 tons of crayfish from the Mondego valley, but it was calculated that the production from that region could reach 3000 tons (Gutierrez-Yurrita *et al*., 1998).

In November 2005 Spanish producers reported some problems in crayfish sells due to China's exportation to Louisiana, replacing crayfish produced in Spain. In this year, crayfish had a production higher than 3000 tons, only in Andalucía, with an industry of 8 companies, exporting internationally and also selling to the national market (Gutierrez-Yurrita *et al*., 1998; Seafood, 2005). It is important to note that due to the severe drought which affected southern Spain in the earl's 90's, the annual production of crayfish in the Guadalquivir marshes has been practically nil in those years. In these years all the production was assured by furtive fishing held by Spanish fishermen in Portugal. The animals were transported to Spain, processed and sold (Barbaresi & Gherardi, 2000; Gutierrez-Yurrita *et al*., 1998; Seafood, 2005).

Currently, some anecdotal references state that Spain produces more than 10000 tons of crayfish each year. Most of the production comes from the Guadalquivir river paddy fields and marshes, where crayfish and rice integrated production have reached incredible yields of 1100 kg $ha^{-1}$. The Guadalquivir river, in southern Spain, has about 35000 hectares of paddy fields, immediately southern of Seville (Myetymology, 2013). For example, in the small village of Isla Mayor, there are 28000 hectares of paddy fields which also produce crayfish. This village is totally dependent on rice culture, the only one which can be done in the area, and on crayfish industry, which depends on the rice culture because it is its only raw material. Crayfish and rice industries are the only wealth, economy and employment of this village (anecdotal references).

Spanish producers of red swamp crayfish sell the product to Louisiana, but also to European countries. Currently Spaniards sell around 301 tons yearly to European markets: 78% is exported to France, 13% to Sweden and the rest to Belgium and Netherlands (Gutierrez-Yurrita *et al*.,1998; Seafood, 2005).

### 2.3.4. Environmental and economic impacts

Crayfish introduction caused numerous impacts, presented in the following boxes.

Positive impacts

- Newborn crayfish are vulnerable to be eaten by many different species of fish in Portuguese freshwaters. Adult crayfish are mainly eaten by some important big birds, like herons and storks, and numerous mammals, most notably the otter *(Lutra lutra*), a protected species and very important in the Portuguese fauna. Studies conducted in Spain found that crayfish became the major source of food to the otter (Anastácio, 1993; Diário de Notícias, 2009; Florêncio, 1993; Geiger *et al*., 2005). Abundance of predacious wading birds and water birds increased after the establishment of red swamp crayfish in Italy and Spain (Lodge *et al*., 2012; Suite, 2010). Crayfish has also become the main food of the white stork, helping to make the species resident in certain areas of the Alentejo region, in southern Portugal, due to the abundance of the crustacean which is today the large basis of their diet throughout the national territory (anecdotal references).

- In Europe, the introductions benefited especially Spain, creating a successful crayfish industry and revitalizing the local economy in certain southern regions. It represents an important economic resource for the populations in poor areas of southern Spain, which work and earn money fishing and selling the crustacean (Barbaresi & Gherardi, 2000; Diário de Notícias, 2009; Fishar, 2006; Seafood, 2005).

Although there are some positive impacts, the species brought much more environmental and economic problems.

Negative impacts

- Its introduction in Portugal has seriously affected the native fauna and rice production, significantly changing the ecosystems due to its extremely rapid expansion and feeding habits (Anastácio, 1993; Cruz & Rebelo, 2006; Fishar, 2006;). The species caused important changes and trophic impact over other native populations in aquatic ecosystems affecting the natural status of the invaded habitats and changing the trophic chains (Ficetola *et al*., 2012; Geiger *et al*., 2005). Portuguese farmers tried to eliminate crayfish using chemicals which proved to be ineffective due to the resistance of the species, but resulting in severe impacts over other species and the environment (Anastácio *et al*., 1999b; Correia, 2002; Fishar, 2006). Impacts on community structure and food web interactions by red swamp crayfish are large and well documented (Lodge *et al*., 2012).

- Crayfish have negative effects over fish, invertebrates and amphibians due to its aggressive feeding habits. It also destroy and feeds on the aquatic vegetation (Ficetola *et al*., 2012; Gherardi, 2006; Ministerio de Agricultura Alimentación e Medio Ambiente, 2012). Commonly reduces the abundance of submersed and semiaquatic macrophytes by 50% to 100% via herbivore diet and stalk-cutting. In Europe, predation by red swamp crayfish reduces abundance of larval amphibians more than predation by native crayfishes. Overall, red swamp crayfish appear to replace many native species at lower and intermediate trophic positions, including food chains damaged by increased numbers of breeding storks and herons, shunting more energy directly to top vertebrate predators (Lodge *et al*., 2012).

- In the paddy fields, the problems are related to the digging and burrowing behavior of the species, damaging the irrigation channels and levees, changing the water circulation and affecting the plants roots. Rice plants may also be physically damaged and light penetration can be obstructed by suspension of sediments, which difficults rice growth in the young plants, very dependent on sunlight to a correct and fast growth to surface direction (Anastácio, 1993; Fishar, 2006). Rice culture suffered serious damages in the Mondego valley in 1990 (Anastácio, 1993). In Portuguese paddy fields crayfish burrows during the summer, mainly in the levees which serve as irrigation channels. They do not destroy the cultures directly neither eat it when the plants are tall enough, but causes some losses in water flow. However, adult crayfish may eat rice seeds or young seedlings when the rice is sown (Anastácio, 1993; Fishar, 2006; Ministerio de Agricultura Alimentación e Medio Ambiente, 2012).

- Some studies made in a Portuguese protected area, Reserva Natural do Paul do Boquilobo, concluded that crayfish were extremely abundant in the area and an important presence in the trophic chain and feeding regime of the park's animals. This led to important changes in a natural and unique ecosystem, and resulted in the loss of the natural values of the area. Crayfish also affected several species of amphibians in this protected area (Cruz, 2001; Florêncio, 1993).

- Red swamp crayfish also carries an important disease, the crayfish plague, which does not affect humans but can destroy completely native crayfish populations in Europe. This disease has totally changed worldwide ecosystems due to the weakness of native crayfish to face the plague. When the red swamp crayfish is introduced in a new ecosystem it brings the disease with it. Because native species does not have any resistance to this plague, they die and the red swamp crayfish substitutes them in the ecosystem, changing in entirely (Geiger *et al*., 2005; Gherardi, 2006; Gil-Sanchez & Alba-Tercedor, 2002).

- Contributed to the decline of the species of Portuguese native freshwater crayfish, lagostim-de-patas-brancas (*Austropotamobius pallipes*), due to the crayfish plague and direct competition. This species is protected by Decree and considered vulnerable by ICNF. Although it can be confirmed the apparent disappearance of the species, little populations may still exist in Douro river basin. However it is believed that the extinction of the species is truly irreversible, which is now on a status of pre-extinction (Geiger *et al*., 2005; Gil-Sanchez & Alba-Tercedor, 2002; ICNF; Ministerio de Agricultura Alimentación e Medio Ambiente, 2012).

## 2.4. Rice production and rice cycle in Portugal

Rice (*Oryza sativa*) is an important culture in Portugal with around 26800 hectares of paddy fields, mainly done in the most important river basins, with annual variations when some fields are directed to other cultures and several changes over the last years (Annex 2). Currently, rice culture is mainly done in three of the most important river valleys: Mondego, Tagus/Sorraia and Sado (Annex 3 and 4)( Almeida & Marques, 2013; ANIA, 2013; Público, 2010).

In an interview with a rice producer from the Tagus river basin (Coruche, near the Sorraia river), he stated that there are huge amounts of crayfish in the area, both in the paddy fields as in the levees and irrigation channels. Crayfish lives and spawn in the levees and irrigation channels all year and invade the fields when they are flooded. Crayfish burrowing and digging activity is mainly done in the levees and irrigation channels, resulting in problems related with irrigation and reducing rice production, because it affects the water circulation and the plants roots (personal communication).

This species does not eat or destroy the rice plant directly when they are tall (although they can destroy the seeds, the seedlings and young plants), but attracts big birds like herons, storks and seagulls. These birds seek for crayfish to feed in the rice fields which results in the destruction of rice plants due to the big and large paw of this kind of birds. While they advance through the paddy fields they step on the plants, causing serious damages and losses. Rice farmers tried to repel these birds but they couldn’t and is actually a serious problem (personal communication).

In this area rice is sown in April or May and is harvested in October. This period may present some annual variations related with climatic factors, mainly temperature and radiation. After the rice harvest the fields are burned or harvested with a machine which uses the agriculture remains to serve as food for the cattle. The fields can be prepared for the next year rice culture, standing in fallow for five or six months. Some producers may do another second culture, like corn or pea, but most of them don’t (personal communication).

There are some differences in the way of rice culture in Portugal: in Mondego valley, located in the central region of Portugal, there is lots of water all year and so the culture is done in a flooded field. In southern Portugal (Tagus and Sado river basins) there is much less water, so the culture is done in a dry field which is irrigated artificially, with higher production costs spent with the price of the water. The main difference is the rice sow, which can be done in a dry field and filled with water or in a flooded field. After the sow the fields are always flooded (personal communication).

No chemical products are currently used to eliminate crayfish from the fields, due to financial reasons and lack of important damages to the cultures to justify an investment. The only chemicals used are insecticides and herbicides allowed by European laws. Several trucks loaded with crayfish have been seen leaving paddy fields in Coruche frequently. This also occurs in the Mondego valley and that these trucks probably transport more than a ton of crayfish each time (personal communication).

The rice producer also stated that crayfish production might be a good idea for the region and it could bring more money and jobs to the area throughout the year. He said that a potential problem could be the mentality of rice producers who hate crayfish and would not be easily convinced to introduce crayfish in their paddy fields. They associate the species to a plague and a harmful animal. Restocking in May or June may not be accepted by farmers because they think the crayfish will destroy their crop and damage the paddy fields (personal communication).

Portuguese paddy fields are characterized by a continuously flooded spring and summer culture. The rice culture and growth is strongly influenced by water level, which is controlled during most of the rice growth period (Anastácio *et al.*, 2009). This is ideal for crayfish and rice integrated production in the same fields, due to the same need of water Rice culture in Portugal follows a simple annual cycle, similar to other countries. The following box presents a simple summary about rice production in Portugal, based on the anecdotal references from Borda do campo (2009) and personal communication from the rice producer.

- In the winter the fields remain fallow after the harvest period. As the rains start falling the fields gradually became flooded with huge amounts of water, almost turning lakes.

- In the spring begins a new cycle. In April the fields are prepared with machines which clear the irrigation channels to drain and irrigate the fields. After this, the farmers sow the rice, usually in late April or early May. There are several techniques used to sow the rice.

- Like other cultures, rice also needs some treatments for a healthy growth and a profitable production. In June, when the rice has already some height well above the water, it is fertilized. In late June the farmers proceeds to the weeding with chemicals, usually made by plane. For the chemicals to take effect, the fields should have little water.

- The plants grow until the summer, when the farmers reduce the water level.

- In September or October the rice is harvested and the rice straw is left in the fields or taken to feed the cattle. The fields are left fallow until the next year and the next cycle

In a wet seeded rice field, crayfish impact is stronger at the initial phases of rice growth and the impact on final rice grain production is smaller. The reduction in rice crop production due to crayfish presence might be linked to the traditional method of growing rice. In the Mondego valley, paddy fields are flooded before sowing, and the water level is kept as high as 10 cm or more. In Louisiana the fields are sown before flooding. If this method is used or if water levels are kept very low at the time of seedling emergence, most problems related to crayfish destruction of seed and young rice plants might be avoided (Anastácio *et al*., 2000).

## 2.5. Study area characterization – the climate

Portugal has a Mediterranean climate, Csa in the south and Csb in the north, according to Köppen climate classification (Annex 5) (IPMA, 2013). It is one of the mildest European countries. This guarantees annual mean temperatures around 17 ºC in most of the paddy fields, which are located mainly in the coastal zone and in the river valleys. The proximity to the coast also ensures high minimum temperatures in winter and milder maximum temperatures in the summer, unlike other European countries such as Spain, which have a more continental and harsh climate. The summers are generally mild in the north and very hot in the south. The fall and the winter are rainy and windy, with colder temperatures in the north. The spring and the summer are sunny and during the dry months of July and August the temperatures are very hot. In some areas, such as the Tagus valley the mean annual temperatures can reach 20ºC. The mean precipitation ranges between 3000 mm in the northern mountains to less the 600 mm in some Alentejo regions (Annex 6; IPMA, 2013).

Generally the mean temperature varies with the latitude and the more coastal or inland areas: higher latitudes have colder mean temperatures and the more inland regions have more extreme temperatures, with higher thermal amplitudes, lower minimums in the winter and higher maximums in the summer, when compared with coastal zones (Figure 2.4). The Annex 5 shows the different areas of the two main Portuguese climatic zones: both presents a temperate climate, but the Csa climate has warm and dry summers while the Csb climate has mild and dry summers. Further in this work, the study area will be focused on the paddy fields and the climate specifications in these areas.

This will be very important for the selection of the best paddy fields to produce crayfish. The main areas where paddy fields are located are inserted in the warmest zone, with an exception for the Mondego valley. The minimum mean temperature in these areas are around 10ºC, the maximum mean temperatures around 22ºC and the annual mean temperatures around 16ºC. In the more inland Portuguese areas, higher maximum temperatures, reached mainly during the summer, result to a faster growth on crayfish but also higher mortality rates. Also in the same regions, low minimum temperatures during winter, leads to a very slow or even null growth rate. This fact will be extremely important to the final crayfish best production areas selection.

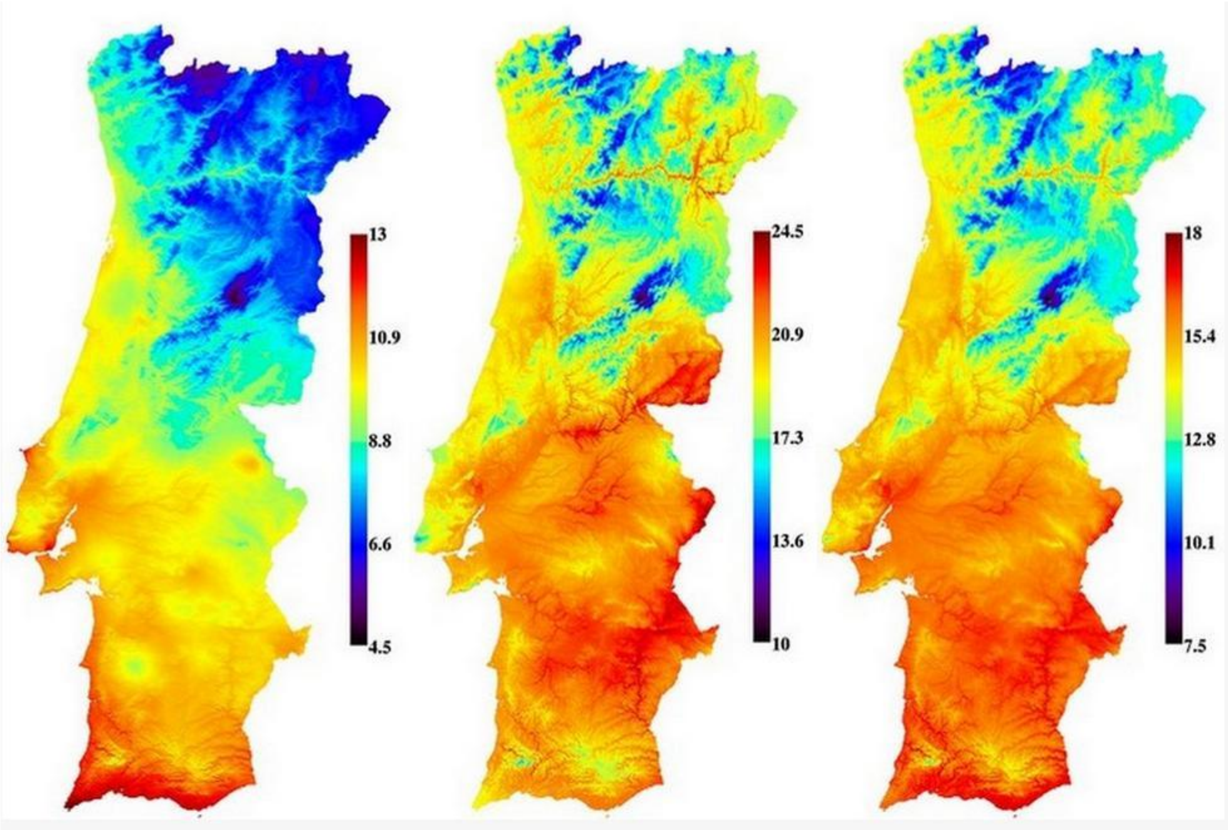


Figure 2.4 - From left to right: minimum mean temperature, maximum mean temperature and mean temperature in Portugal (ºC)

(Adapted from IPMA, 2013)

# 3. Methods

## 3.1. Tools and methods

The methodology used was divided in two main steps:

- Selection of the best locations for the species production integrated with paddy fields using GIS software.
- Creation of a population model to simulate the species production integrated with paddy fields using Insightmaker.

The work was developed between March 2013 and September 2013. The interview with the rice producer occurred in May 2013. The technical support and programs used were: Google Earth Pro 2013, ArcMap 10.1, Microsoft Excel 2007 and Insightmaker.

## 3.2. Methodological scheme

The following scheme presents a simple diagram to understand the methodology adopted in the thesis:

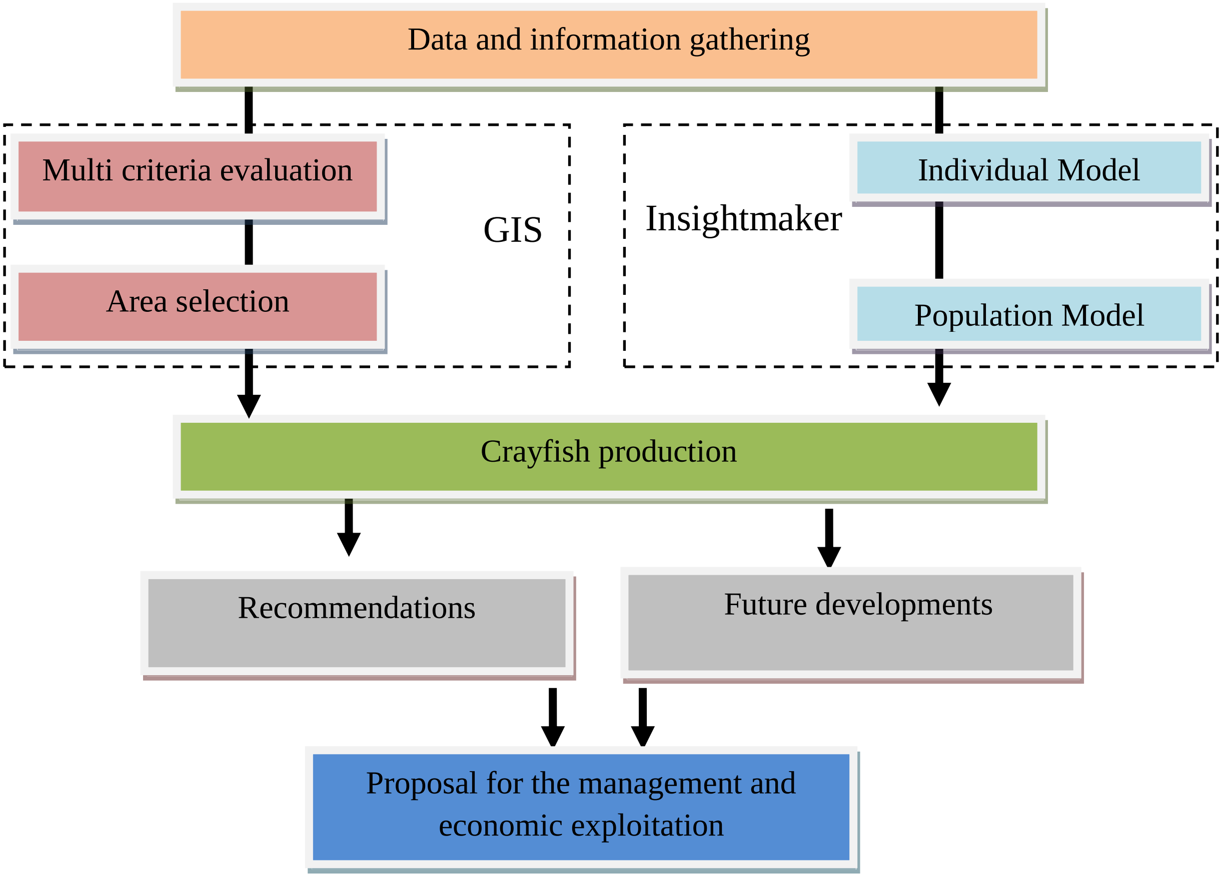


Figure 3.1 – Methodological scheme

## 3.3. Selection of the best locations using GIS

Initially it will be considered all the available area of paddy fields, applying the restrictions to them in the next phases. It were taken in account some restrictions which limited the total paddy fields area available for the species production. This work will only consider the method of extensive production, integrated with paddy fields, being the one that is closer to traditional harvesting and which has higher demands on the reintroduction of the species in culture system, therefore requiring crayfish removal from the natural ecosystems.

It was selected the total area of Portuguese paddy fields using CORINE Land Cover 2006 (CLC 2006), an European program which presents the data for an inventory of the uses of the land in Europe. The program was directly used to determine the entire area covered by paddy fields and make restrictions thereafter.

Although the main and initial study area is all Portuguese territory, some areas were selected using a multi criteria evaluation to conclude about the best areas to produce crayfish, due to its characteristics and ecology and how it is related to the climate. The species is very resistant and tolerates a wide range of environmental values for its growth. It will only be considered the most important factors influencing crayfish populations, such as temperature and water availability. Other important parameters, such as pH or dissolved oxygen concentration will be discarded due to the aim of producing the species integrated with paddy fields. The restrictions in the multi criteria evaluation used to select the best production areas were:

- Paddy fields areas greater than 100 hectares – crayfish live under different environmental conditions in Portugal from their native region, so it grows slower. If the area is too small, crayfish populations will not be able to regenerate or expand after the first harvest or the restocking and they might have difficulties in finding food. Also in small areas the population densities may be huge after reproduction, which leads to small individual sized crayfish, less valuable for the market, and higher mortality rates (McClain, 2010; McClain & Romaire, 2009).
- Mean temperatures, maximum temperatures and minimum temperatures – Generally, inland areas have large variations in temperature: very high peaks in the summer and low temperatures in winter with frozen minimums. This greatly reduces the growth of crayfish and increases the mortality of the animals.
- Precipitation and river basins with large area and flow - Reduced water levels also affect very negatively crayfish populations. As explained before crayfish populations and crayfish production are mostly dependent on the temperature and water availability. These two factors are extremely important when considering aquaculture of the species. Due to these problems of water availability, in this work the production areas will be restricted to the paddy fields located in the larger river basins.
- Protected areas – These areas are very sensitive to modifications in the fauna, which may lead to environmental problems and loss of the natural values of the area. The protected areas were considered as a restriction and all paddy fields located in them were excluded from the final production areas. The Natura 2000 ecological network was excluded from the multi criteria evaluation because of the many limitations and geographical areas occupied by these restrictions in Portugal.

Those restrictions aimed to eliminate the worst zones, such as small areas of paddy fields, areas exposed to frequent drought, where there might be problems with the supply of water, and regions with extreme temperatures, which can affect crayfish growth.

All small paddy fields in inland areas were eliminated, due to the strong temperature variation and peaks in the summer and winter, keeping the main areas of paddy fields in the valleys of the rivers, near the coastline. The final areas created in CLC 2006 were measured simply by the sum of all individual areas and then grouped into regions. In the last years these areas suffered some reductions and so it will be used the maps and data in the annex to achieve better results and present the current paddy fields area.

Because most of the paddy fields presented in the CLC 2006 maps currently do not exist anymore, it was decided to use other data and maps (Annex 3 and 4). Due to the need for water and temperature requirements only coastal areas embedded in large river basins with more than 100 hectares were considered. Chemical products in the fields and industries should have been studied but there was no specific information about them so these factors remained out of the evaluation.

The layers and data used to create the maps were:

- Paddy fields
- Protected areas
- Water lines/river basins
- Mean temperature
- Precipitation
- Urban areas

The maps were created in ArcMap. The original maps and databases were provided by CLC 2006 and by the Portuguese Environmental Agency (APA). The layers paddy fields, protected areas and urban areas were available in CORINE Land Cover, while APA provided the hydrography, temperature and precipitation maps.

## 3.4. Temperature model for Portugal

Based on the data provided by Instituto Português do Mar e da Atmosfera (IPMA) for the three main cities located near the most important paddy fields areas it was created a general model in Insightmaker, using a mathematical function. Only the values of average air temperature are going to be considered in this study, which directly influence the water temperature.

Figure 3.2 shows that the Portuguese city represented, Coimbra, presents very hot higher maximum temperatures, well over 35ºC. Generally the mean maximum and mean minimum temperatures are mild and quite acceptable to crayfish production. The highest temperatures occur in August.

Figure 3.2 is an example of the data inserted in the model to simulate the daily temperature variation. It was used to build a temperature model in the modeling platform Insightmaker, a free website, available at www.insightmaker.com, which allows building personal models, with many different applications. The other two cities temperature graphics are presented in Annex 7. These figures were the basis to the creation of the temperature model.

The daily temperature model variation during the year can be represented by a simple trigonometric function. It was chosen to use the temperature data for Setúbal and not for Évora (another important city located near the paddy fields areas) mainly due to the differences in the climate: Évora is located much more inland, while Setúbal is in the coast, and so the temperatures in Évora are much more continental and harsh. This does not happen in the paddy fields of that area, located in the Sado valley, near the coast (Annex 3).

The model simulates the temperature daily variation in a year and is presented in ºC. Figure 3.2 shows that the coldest month is January and the warmest month is August. The values used were always about the mean temperature in each month. It was delayed 30 days (Offset) so the minimum and maximum temperatures could be present in the right months. The coldest day of the year was considered to be the Julian day 30, the 30th of January, and the warmest day is the Julian day 213, which means the 2nd of August.

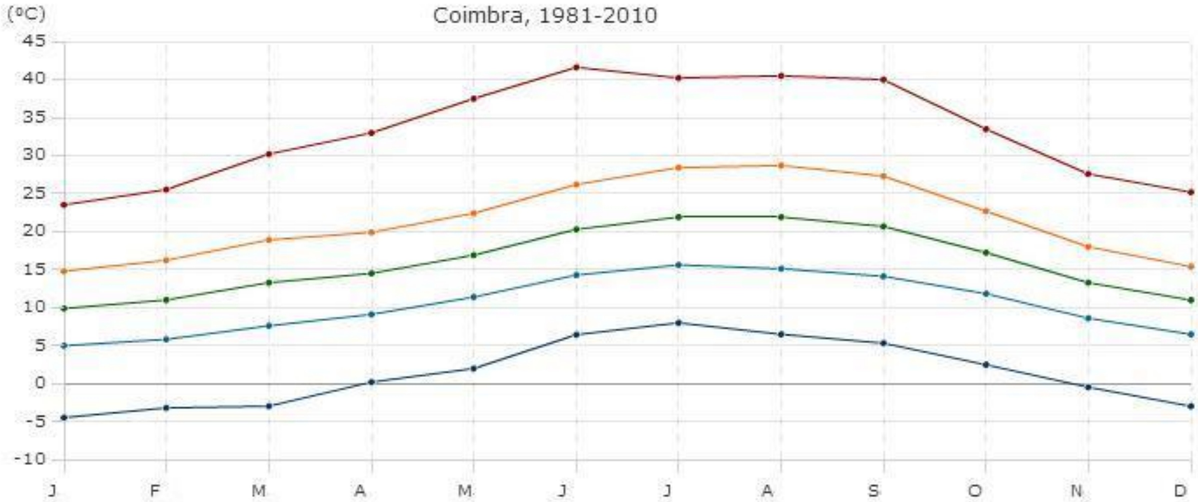


Figure 3.2 – From top to bottom: higher maximum temperature, mean maximum temperature, mean temperature, mean minimum temperature and lowest minimum temperature in Coimbra

(Adapted from IPMA, 2013)

The daily individual temperature model for each city temperature variation is based in the mean temperatures reached in the coldest month and in the warmest month. Those temperature extremes are used to create a variation which will be used by the trigonometric function to set a minimum and a maximum and a constant temperature increase and reduction through them. The coldest and warmest month data for each city are represented in the Table 3.1.

Table 3.1 - Coldest and warmest mean temperatures in the three selected cities

(IPMA, 2013)

| | Coimbra | Santarém | Setúbal |
|---|---|---|---|
| Coldest month mean temperature (ºC) | 9.9 | 10.4 | 10.1 |
| Warmest month mean temperature (ºC) | 21.9 | 23.8 | 23.2 |

The following equation presents the function used to simulate the daily temperature variation throughout the year:

$$\text{IfThenElse}(((\text{Days}-[\text{Offset}]) \bmod(365))\geq 0 \text{ and}((\text{Days}-[\text{Offset}]) \bmod(365)\leq 365, \text{SIN}(((\text{Days}-[\text{Offset}]) \bmod(365))\times\pi\div(365))\times 2\times[\text{Variation}]+[\text{Coldest month}], -\text{SIN}(((\text{Days}-[\text{Offset}]) \bmod(365))\times\pi\div(365))\times 2\times[\text{Variation}]+[\text{Coldest month}])$$

The model uses the trigonometric function SIN to create a variation. This variation is limited by the modulus, which turns to positive the negative function values. The period was delayed to span 365 days. This way, during the 365 days, the function has a variation dependent on the coldest and warmest month and maximum and minimum temperatures which will only be achieved one time in the year. The Offset delays in 30 days the coldest and warmest days. The original equation from Insightmaker is presented in Annex 8.

## 3.5. Crayfish population model

In order to simulate the evolution, growth, harvest and production of a crayfish population in Portuguese paddy fields, a simple population model was created in Insightmaker. This model is based on the following concepts:

- The paddy fields are used as a culture area, where the crayfish will grow faster;
- The initial restocking uses wild populations of the species, supporting the removal of crayfish from natural and valuable ecosystems and defining the potential control for the species;
- The size of crayfish population and the number of introduced crayfish might be modeled in each different production area due to the number of crayfish restocked and will be controlled as its use as an economic resource;
- The smallest amount of crayfish must be achieved in April, before rice is sown.

GIS was used for site selection but does not permit to conclude about production. So it was utilized an individual model, to simulate and conclude about individual growth, and a dynamic population model based on many factors that directly influence crayfish population in a paddy field. It is the following step after the selection of the best production areas with GIS, because the model allows simulating the production and separating the biomass by classes in order to be used by man to his own profit.

All the variables, stocks and equations present in the model represent crayfish population in one hectare paddy field, where there are five crayfish weight classes.

Table 3.2 – Crayfish weight classes present in the model

| | Weight class 1 | Weight class 2 | Weight class 3 | Weight class 4 | Weight class 5 |
|---|---|---|---|---|---|
| Weight range (g) | 0 - 10 | 10 - 20 | 20 - 30 | 30 - 40 | 40 -50 |
| Mean weight (g) | 5 | 15 | 25 | 35 | 45 |

These classes are directly influenced by growth rate, dependent on the temperature. Flow rate moves crayfish from one weight class to another when they are grown enough. Each class has a total biomass, which is the number of crayfish in the weight class multiplied by the mean weight of the class. The flow rate (weight class move from one to another) is given by the equation:

$$\text{Flow rate} = (\text{Weight class} \times \text{Growth rate}) / (\text{Mean weight 2} - \text{Mean weight 1})$$

Crayfish restocking will occur with low densities. This allows the crayfish to grow faster and reach higher individual weights, ideal for the market. Higher restocking densities lead to lower individual weight and slower growth (McClain & Romaire, 2009). The mean weight for the restocking class, weight class 2, is 15 g. So, if it will be restocked 45 kg of 15 g crayfish there will be 3000 crayfish in one hectare.

Anastácio (1993) found an average crayfish density of 5.4 g $m^{-2}$ in Mondego valley. It is considered in this model a crayfish introduction of 45 kg $ha^{-1}$. So if the field is restocked with 45 kg probably there will be no additional problems introducing crayfish in such low densities, because previous studies show that crayfish presents higher densities in some Portuguese areas.

The population model is directed for diary flows and stocks, which means that the model will run every quantity day by day. For example, crayfish harvested rates refers to crayfish taken from the paddy fields daily. The model cycle starts in the Julian day 150, the 1st of June. It is assumed that crayfish are restocked in the paddy fields in this date and that there were no crayfish in the area before. There are introduced a total of 3000 crayfish from weight class 2, 45 kg. All those crayfish are adult and able to reproduce themselves. So it is considered that crayfish will immediately reproduce in the following summer and that 60% of the population is constituted by females. These females will spawn between October 1st and December 1st. Each female will only spawn 200 eggs, due to their young age.

Because the model time units are set for days, it is considered that each female spawns during the two months, and not the total number of eggs at one time. It was also considered that females lose some of their body weight after spawning, although marginally. The newborn crayfish, or recruits, will flow directly to increase the number of animals in the weight class 1.

Crayfish will continue to grow and they will reach the next weight classes, while weight class 1 receives an enormous boom of recruits during the fall. In the winter the weight classes 4 and 5 can be harvested, removing from their stocks a certain number of bigger crayfish, leaving space so the smaller crayfish can have a continuous growth and being harvested after that.

It was considered that each weight class has a mortality rate of 0.008 $day^{-1}$, according to literature (Anastácio *et al*., 1999). The weight class mortality rate is equal for all weight classes, except for weight class 1. In this class it was considered a 5 time higher mortality rate, due to the higher vulnerability of little crayfish.

Crayfish mortality increases with higher temperatures. An explanation to justify the increase of mortality with high temperatures is that crayfish grow faster, reach the sexual maturity earlier and so became ready to spawn younger. The frequent spawning results in great populations and higher densities due to the big number of juveniles and leads to higher competition intra species, so the mortality is higher. Higher restocking densities also increases mortality rate. This explains the importance of working with crayfish low densities in this model, reducing the high mortality rates found in some Portuguese studies (Anastácio *et al*., 1999b; Anastácio *et al*., 2009).

Crayfish mortality also depends on the natural native predators present in the paddy fields fauna where crayfish might be produced. These predators can have vary variable populations or distributions throughout the country so they will not be considered in the model as a factor to increase or decrease mortality.

Table 3.3 - Table of parameters

| | |
|---|---|
| Reproduction | Crayfish reach sexual maturity around 80 mm, which means a mean weight of 10 g, according to the results of Dorr *et al*., 2006 (Figure 2.2). This means that all crayfish over that weight have reached sexual maturation. Considering that restocking is made with crayfish from weight class 2 and that this class has a mean weight of 15 g, it is concluded that every female is sexually mature and able to reproduce when introduced in the field. In Portugal it is referred one annual generation. So it will be considered that each female introduced in the paddy fields in June it is immediately able to spawn, due to its age, size and current season. Crayfish females often produce between 200 and 500 eggs but only bigger females produce more eggs. In this model, it will only be considered reproductive females in weight class 2, where they are smaller and so they will produce only 200 eggs. |
| Sex ratio | Sex ratio was an important parameter to calculate the number of spawning females in crayfish population. As stated before, in a common paddy field in Portuguese latitude, females outnumber males. It will be considered that the population proportion is 0.6 favorable to females (60% of crayfish population are females). |
| Mortality rate | It will be considered an equal mortality rate for the total of the crayfish population. Due to the low densities restocking and milder temperatures in the selected areas, the mortality rate considered is 0.008 $day^{-1}$, based in some literature references (Anastácio *et al*., 1999b; Anastácio *et al*., 2009).<br><br>Because newborn crayfish are much more vulnerable to be eaten by many other animals than adult crayfish, such as many kinds of fish, insects and amphibians, besides many mammals, it was considered that the mortality rate in weight class 1 is five times higher than the mortality rate for all other adult classes.<br><br>The present model adopted a low mortality rate of 0.008 $day^{-1}$ in all the classes, except for the weight class 1, which has a mortality rate of 0.04 $day^{-1}$. |
| Spawning | Crayfish spawning leads to a loss of biomass. Gonadosomatic Index (GSI) is the percentage of the egg weight to the total body weight of crayfish females. Red swamp crayfish females have a maximum GSI of 1.2% (Daniels *et al*., 1994). Crayfish spawning females belong to weight class 2, with a mean weight of 15 g.<br>During spawning females will lose 1.2% of its body weight. Although it is a marginal value, it was considered to keep the model simple and realistic, and because it can make difference in the biomass of great populations. |
| Growth rate | The growth rate used in the model was far superior to other Portuguese works because it was considered only dependent on temperature. It was not found any data about the species allometric growth. However, some studies refer that crayfish growth is somehow regular during its lifetime, and there are no strong variations in its weight and growth when related do body size (Figure 2.2 and Annex 9). In this model it is being assumed that adult crayfish have the same growth rate (in weight) as crayfish hatchlings. |

The minimum harvest size considered was 20 g in the weight class 3 (mean weight 25 g), according to market demands. The model considered two different harvest rates:

Table 3.4 – Harvest rate early season

| Period | Number of weight class 4 crayfish harvested daily ($ha^{-1}$) | Number of weight class 4 crayfish harvested daily ($ha^{-1}$) |
|---|---|---|
| Julian days 330 - 390 (1st of December – 31st January) | 50 | 50 |

Table 3.5 - Harvest rate late season

| Period | Number of weight class 4 crayfish harvested daily ($ha^{-1}$) | Number of weight class 4 crayfish harvested daily ($ha^{-1}$) | Number of weight class 4 crayfish harvested daily ($ha^{-1}$) |
|---|---|---|---|
| Julian days 390 – 450 (1st of February – 31st March) | 100 | 100 | 100 |

An individual biomass model was created to conclude about the growth of the species, using the general model, and presented in Figure 3.3. This model represents the individual growth and weight increase of one individual crayfish during its lifetime, based on the temperature variation.

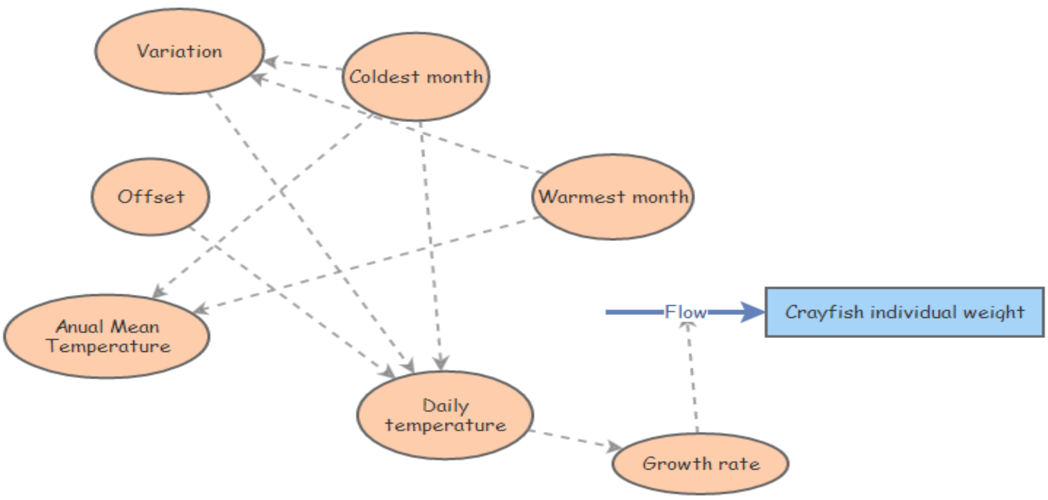


Figure 3.3– Individual growth model

The general scheme on the final population model is presented in Figure 3.4.

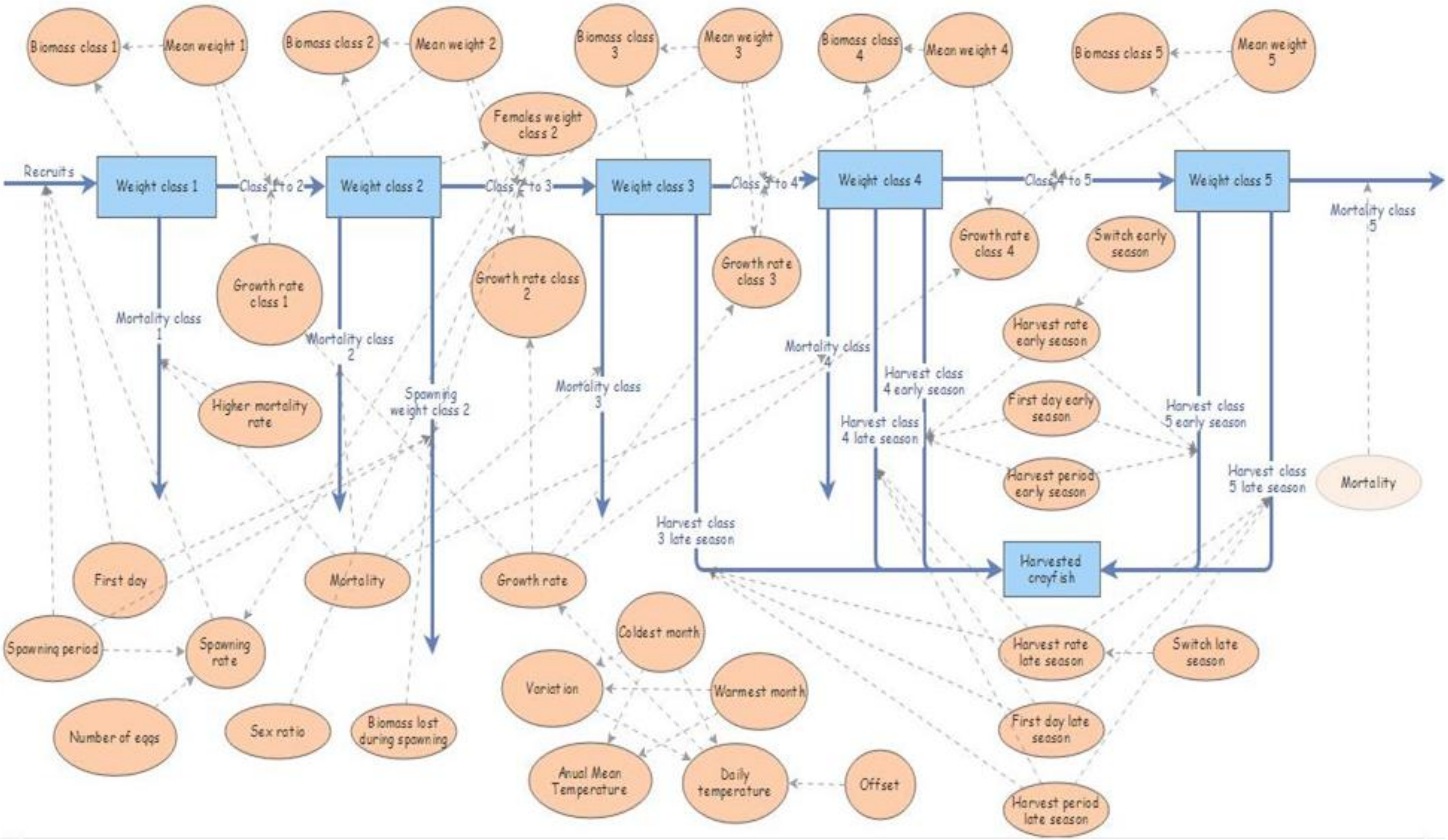


Figure 3.4 - Crayfish population model scheme

### 3.5.1. Temperature as a forcing function for growth

Considering that crayfish growth is strongly dependent on temperature, it was found a function to correlate crayfish growth and temperature. The data used was provided by McClain's work from 2010, "Seasonal Influences on Growth of *Procambarus clarkii* in Louisiana". This was the best article found that has the best information relating crayfish growth to seasonal changes in a non-laboratory environment. In this work the instantaneous growth of the species and the mean water temperature were measured at the same time, to provide data which could lead to a good correlation between the two variables. Table 3.6 and Table 3.7 present some of the results of the work which were used to seek for the relation and allowed the creation of a linear regression curve, using software Microsoft Excel 2007.

Because this work was made with crayfish hatchlings (0.5 g), the growth rate found, although dependent on the temperature, could not be perfectly matched for the model, because crayfish newborns have an extremely fast growth and adults may have not. It was not found any literature about differences in hatchlings and adults growth rates. Because it is intended to simulate a crayfish population, the linear regression growth line should be calibrated for the whole population, including adults.

Data from the same author was used only when more than 50% of the crayfish population was over 15 g, and so being considered an adult population, in order to understand if there were strong growth differences between crayfish hatchlings and adult crayfish for the same temperature values.

Table 3.6 - Crayfish instantaneous growth and temperatures for the general population

(Adapted from McClain, 2010)

| Temperature (ºC) | Growth (g $d^{-1}$) |
|---|---|
| 10.6 | 0.06 |
| 11.8 | 0.108 |
| 11.7 | 0.076 |
| 13.8 | 0.133 |
| 11.9 | 0.14 |
| 17.7 | 0.236 |
| 168 | 0.254 |
| 14.9 | 0.197 |
| 22.7 | 0.276 |
| 14.7 | 0.213 |
| 19.8 | 0.307 |
| 23.7 | 0.31 |
| 10.6 | 0.055 |
| 13.8 | 0.115 |
| 11.7 | 0.066 |
| 11.8 | 0.093 |
| 11.9 | 0.109 |
| 16.8 | 0.189 |
| 17.7 | 0.205 |
| 14.7 | 0.163 |
| 14.9 | 0.159 |
| 22.7 | 0.252 |
| 19.8 | 0.236 |
| 237 | 0.256 |

Table 3.7 - Crayfish instantaneous growth and temperatures for an adult population

(Adapted from McClain, 2010)

| Temperature (ºC) | Growth (g $d^{-1}$) |
|---|---|
| 16.8 | 0.254 |
| 14.9 | 0.197 |
| 22.7 | 0.276 |
| 14.7 | 0.213 |
| 19.8 | 0.307 |
| 23.7 | 0.31 |
| 22.7 | 0.252 |
| 19.8 | 0.236 |
| 23.7 | 0.256 |

# 4. Results

## 4.1. Selection of the best locations using GIS

The maps created show that there might be some problems in using CLC 2006 for this work. The total paddy fields area identified in this database is much bigger than the current area of existent paddy fields, according to the most recent works and maps provided (Annex 3 and 4). CLC 2006 does not identify some important paddy fields, such as the 200 hectares in Algarve, which exists at least since 2004. The problems with CLC 2006 might be due to an old identification of the areas, which later were probably converted into other land uses and the situation was not changed in the database.

Despite CLC 2006 identifies areas of paddy fields which currently do not exist, the best places for the culture of crayfish are the valleys of the great rivers, since they have more water and higher mean temperatures, due to the proximity to the coast. Therefore, the total area of paddy fields considered to crayfish production can be the total paddy fields areas identified in Annex 4 and 5, without the protected areas. They were an important help and represent the current situation.

In recent years the paddy fields located in inland areas were gradually reduced and eliminated. Those areas were very small when compared with the river valleys where the culture is currently is made. The inland paddy fields, represented in CLC 2006, were reduced and eliminated due to climatic problems that limited rice production, mainly due to drought and problems in the supply of water. Currently the production is focused in the most coastal areas and in the main river valleys (Annex 3 and 4).

The map provided by ANIA (Annex 3) considers the 200 hectares of paddy fields in Algarve, southern Portugal, although they are not represented in the maps. However due to the small area and the geomorphologic characteristics of the terrain, hydrology and rainfall in the area, these paddy fields were not considered a good option for crayfish aquaculture. The areas are too small, divided in three different and isolated fields, and the rice is sown in a dry field, with little water available.

Generally, it were excluded the more inland areas and defined but global regions for crayfish production. Initially all these maps will be presented without any of the restrictions made for the selection of the best areas. The two last maps data refers to period 1930-1961.

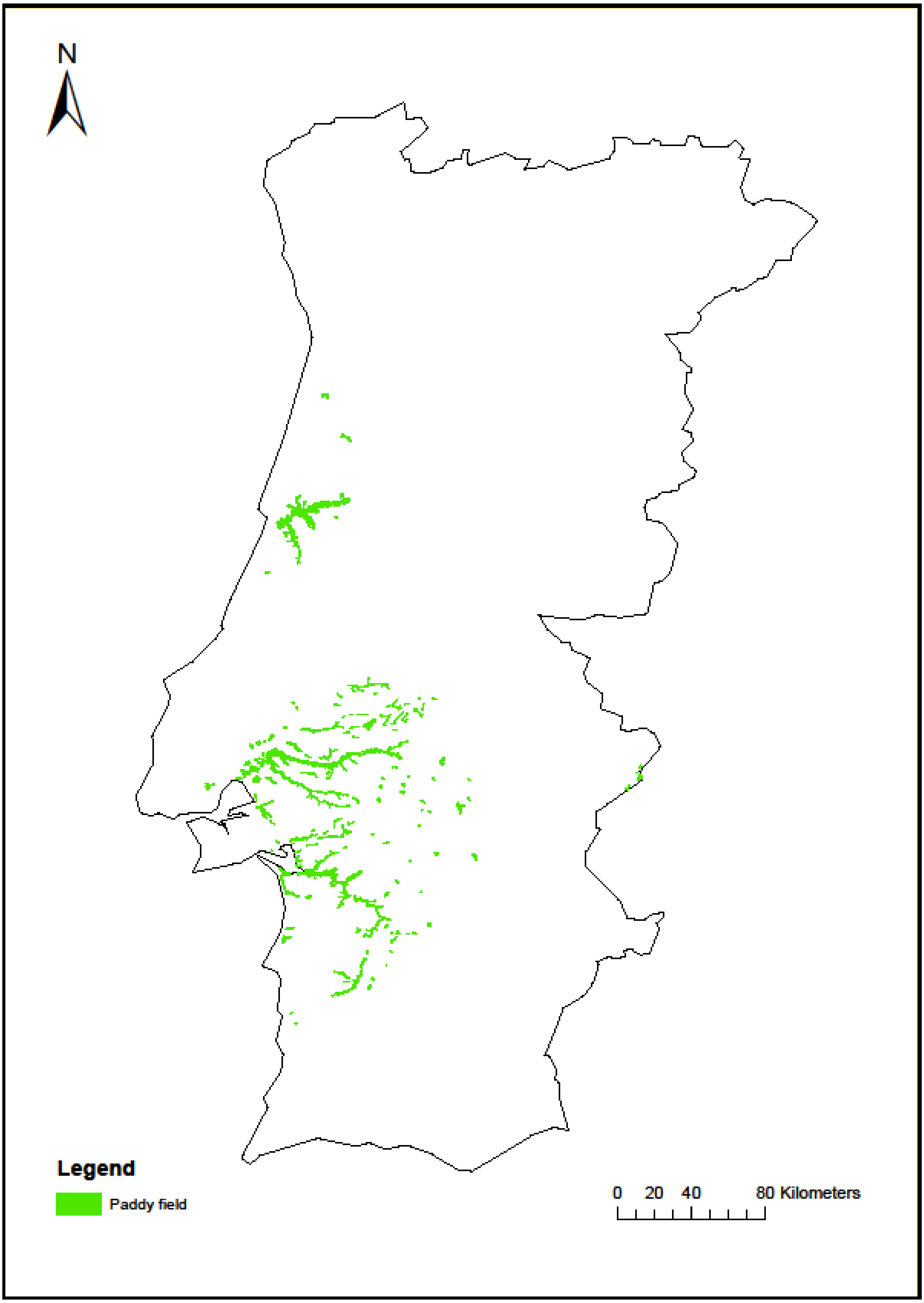


Figure 4.1 – Paddy fields identified in CLC 2006

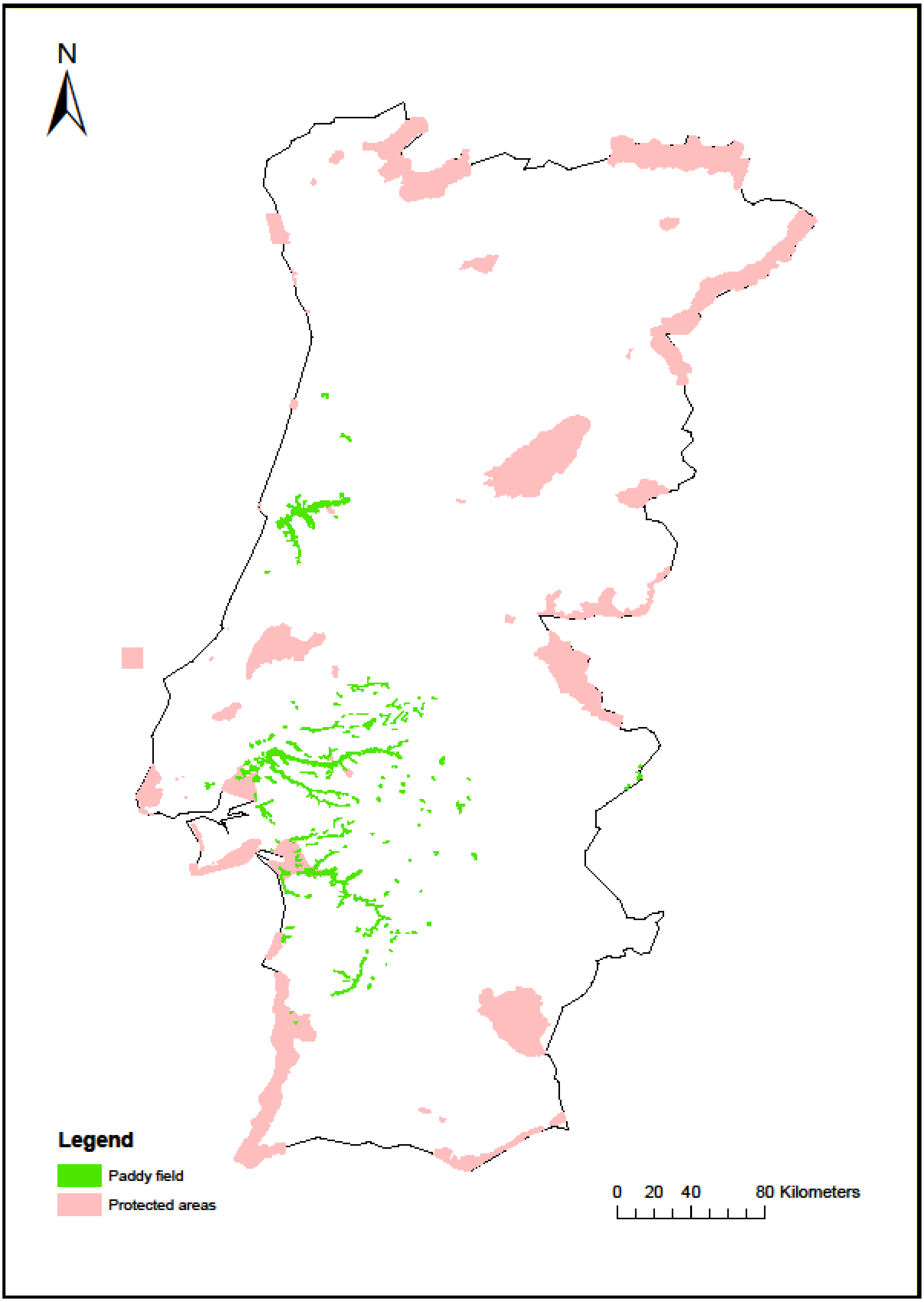


Figure 4.2 – Paddy fields and protected areas identified in CLC 2006

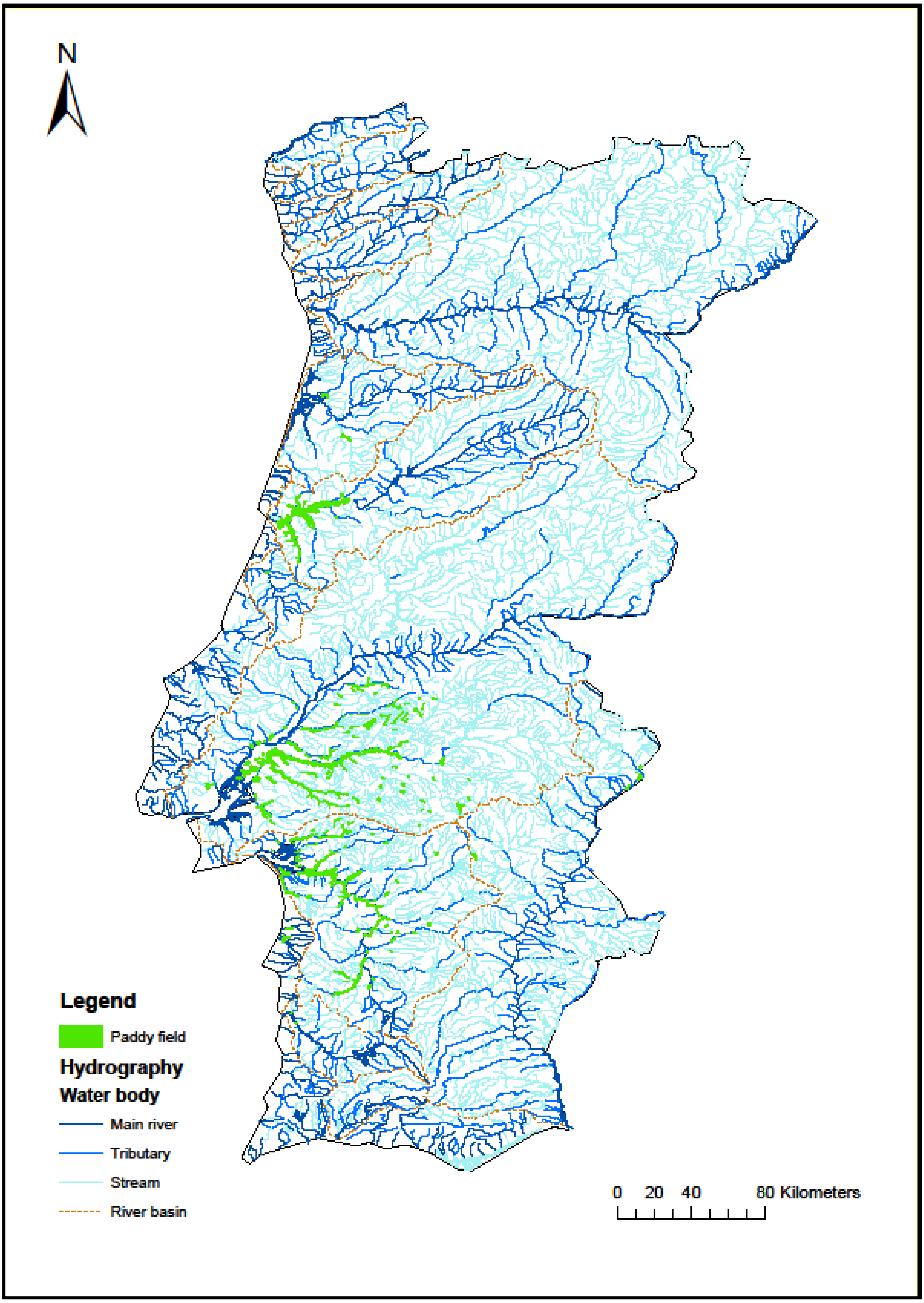


Figure 4.3 - Paddy fields identified in CLC 2006 and hydrography

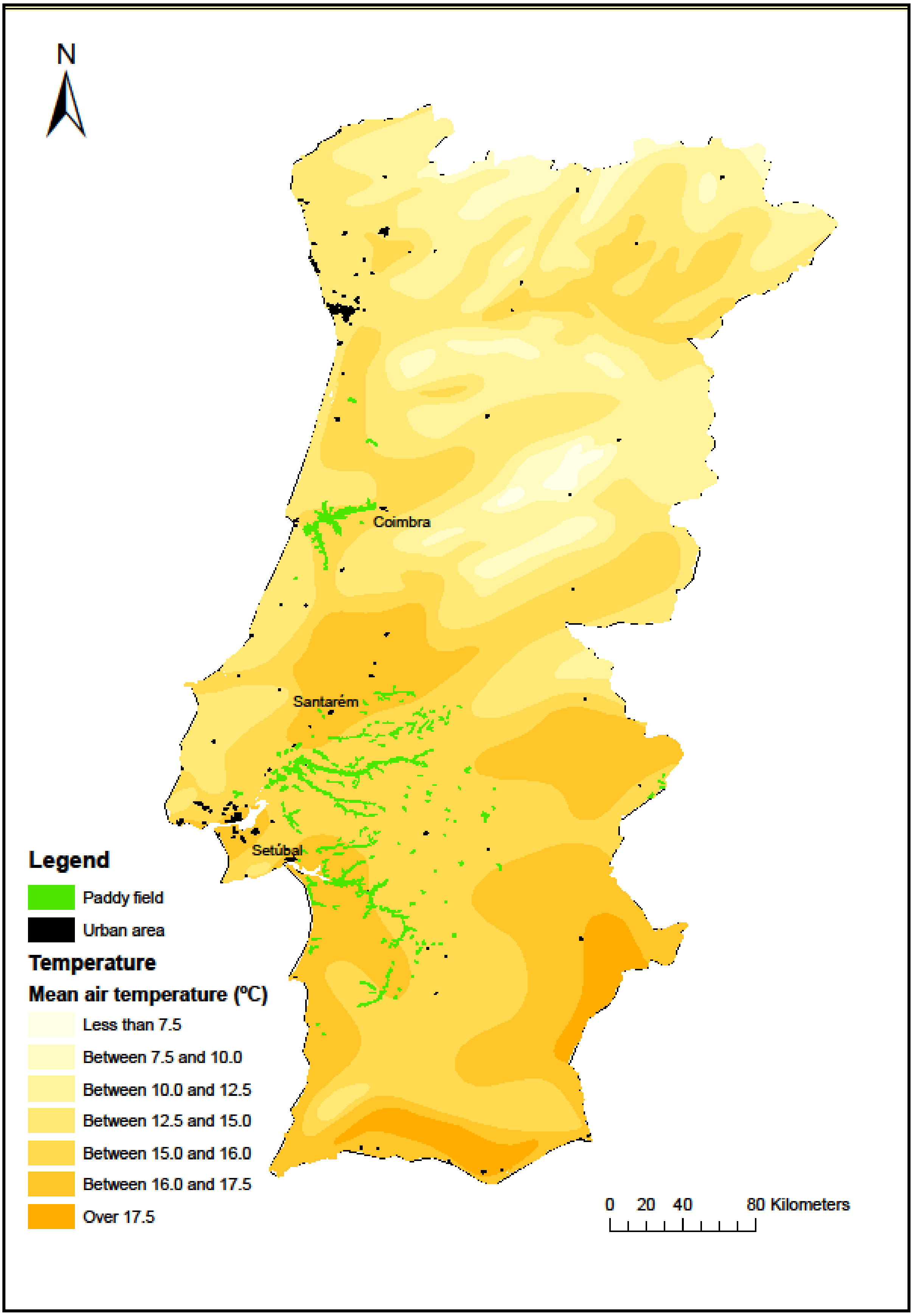


Figure 4.4 - Paddy fields identified in CLC 2006, mean air temperatures and major cities near the paddy fields

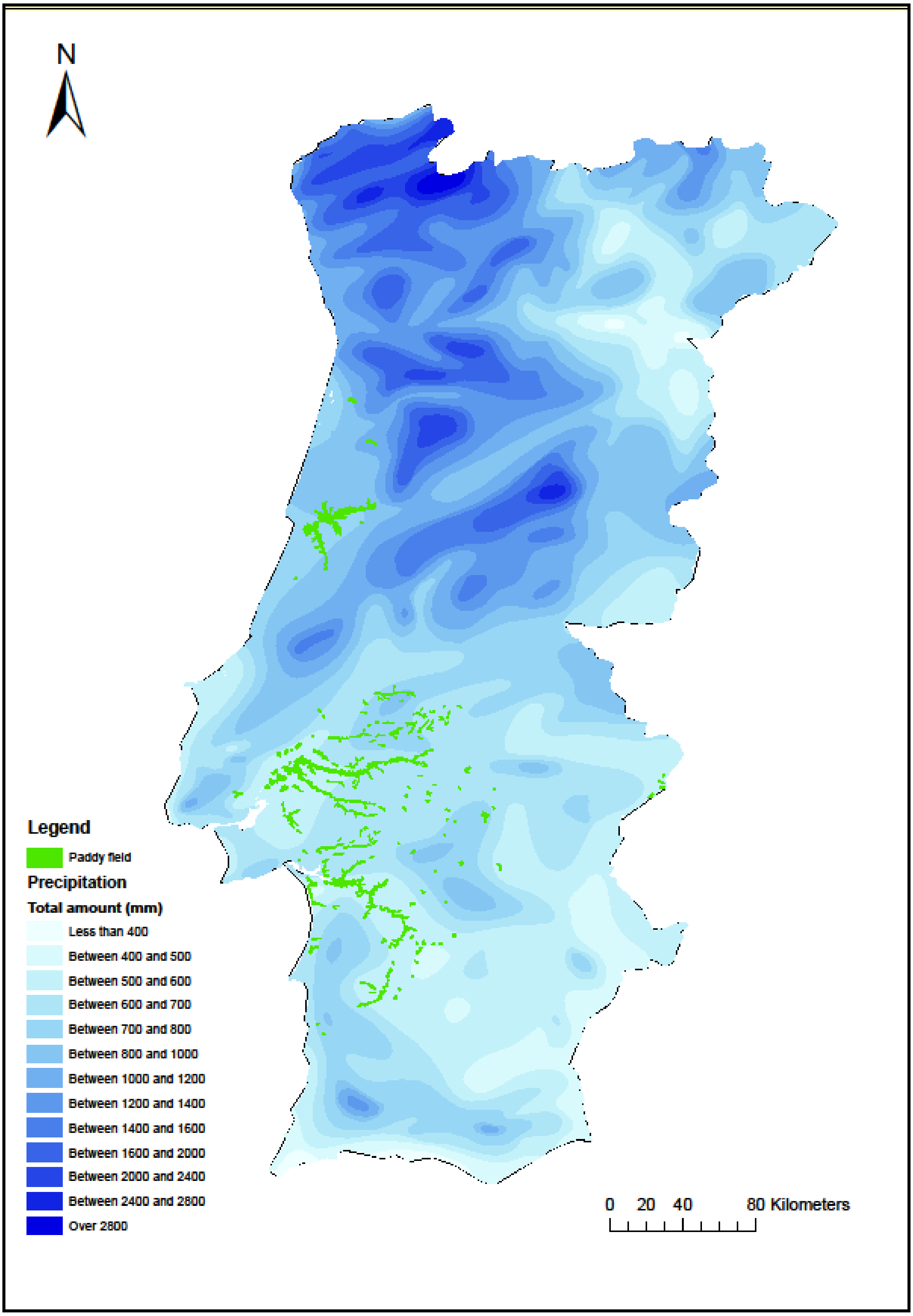


Figure 4.5 - Paddy fields identified in CLC 2006 and total precipitation

The temperature map eliminates areas further inland due to very high temperature peaks in summer and very low minimums in winter, when compared to most coastal areas, the ones that will be considered suitable for culture. Low temperatures inhibit growth and high temperatures increase mortality and lack of water. It is important to note that the selected areas are all located in the main river valleys, because these bigger rivers have bigger watersheds and so the water drained is far superior to the small basins and water courses, which can lead to problems in water supply and drought. Water is essential for crayfish life cycle and production. It were considered small areas of paddy fields when located near large areas and important rivers.

Figure 4.6 represents the final and the best zones for crayfish production, after applying the restrictions previously selected. The selected areas are according to the current largest rice production areas (Annex 3 and 4). It can be defined three main areas: Mondego, Tagus/Sorraia and Sado. These areas are the best due to the great paddy fields area, higher mean temperatures, related with the proximity to the coast, and higher amounts of water, due to the location in the biggest watersheds.

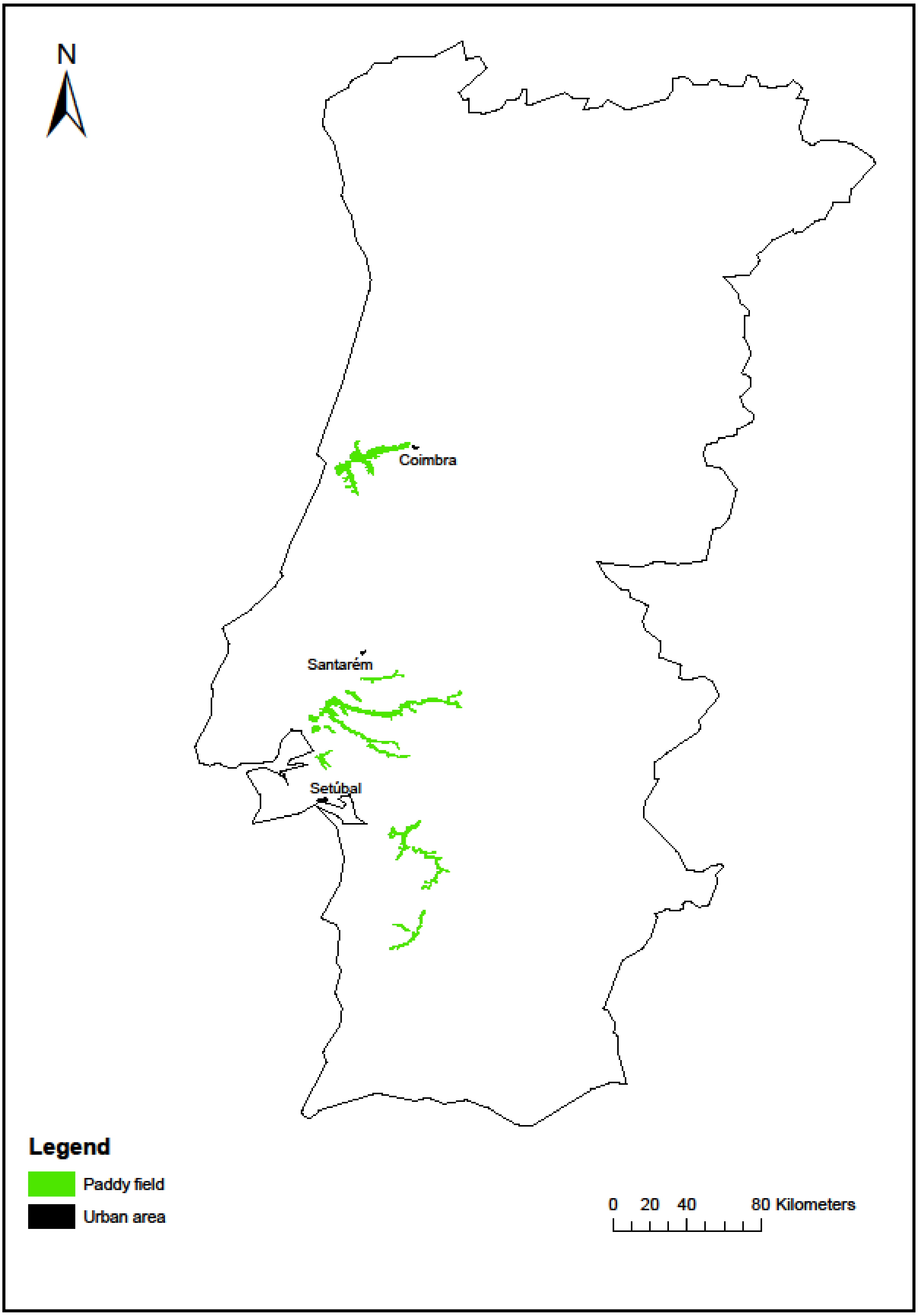


Figure 4.6 – Best paddy field areas for crayfish production

## 4.2. Best production areas

Three main study areas were defined for crayfish production, according to the methodology used and the climatic conditions. Although there are more paddy fields spread mainly in southern Portugal, it were only selected areas large enough to allow a serious study of the species potential, as they have greater production capacity. Because there are some differences in the paddy fields areas selected, the areas presented in bold in Table 4.1 were chosen as the final areas.

Table 4.1 - Final regions and areas selected for crayfish production

| Region | Final area in hectares (CLC 2006) | Final area in hectares (ANIA) | Final area in hectares (Almeida & Marques, 2013) |
|---|---|---|---|
| Mondego valley | 10840 | **6600** | 5448 |
| Tagus/Sorraia valley | 16914 | **9600** | 12652 |
| Sado valley | **6710** | 10400 | 8125 |

In the Mondego valley was chosen the area defined by ANIA because it is the mean value and very similar to the results by Almeida & Marques, so both could be accepted. The areas of the CLC 2006 are probably wrong and much of these must have been transformed in other land uses.

In the Tagus/Sorraia valley there are few restrictions with protected areas, but this lead to the selection of the 9600 hectares defined by ANIA. Almeida & Marques indentifies 12652 but much of this area is inserted in protected areas. Once again much of the areas identified in CLC 2006 must have been transformed in other land uses.

The great reduction in the Sado valley paddy fields area is mainly due to the protected estuarine area, Reserva Natural do Estuário do Sado, which led to the elimination of many hectares inserted in this area. So the chosen area is the one presented in the CLC 2006 final map, with the restrictions on protected areas.

The total final area selected for crayfish and rice integrated production in Portuguese paddy fields is 22910 hectares.

## 4.3. Growth dependence on temperature

Table 3.6 allowed the creation of a linear regression line, to conclude about crayfish growth relation to temperature. This relation will be essential to model calibration. The results are presented in Figure 4.7.

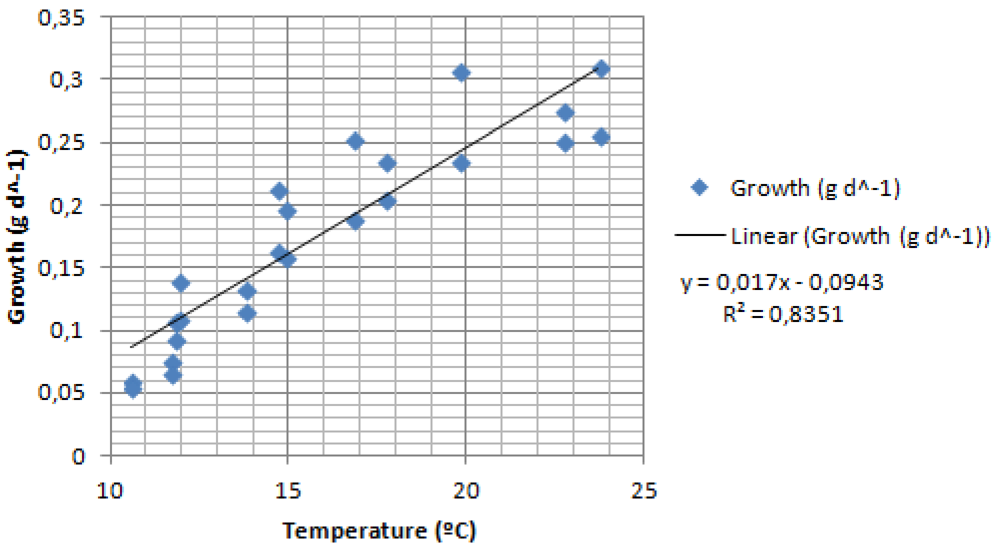


Figure 4.7 - Linear regression growth curve and equation

$R^2$ is the coefficient of determination. It indicates the proportion of the variation in the variable Y explained by the variable X. The linear regression line $R^2$ was 0.8351, which means that there is a strong percentage of the adjustment that explains the line. The r = 0.9138 is the correlation coefficient, a measure of the linear dependence between two variables. It concludes that there is a strong positive correlation between the two variables, growth and temperature. This means that the regression line equation can be inserted in the model and that crayfish growth is directly influenced by the daily temperature variation.

As previously done, the temperature and growth data only for adult population allowed the creation of a linear regression line, in order to understand the relation between the variables. The equation found in Figure 4.7 was used and inserted both in the individual model as in the population model in order to relate growth and temperature.

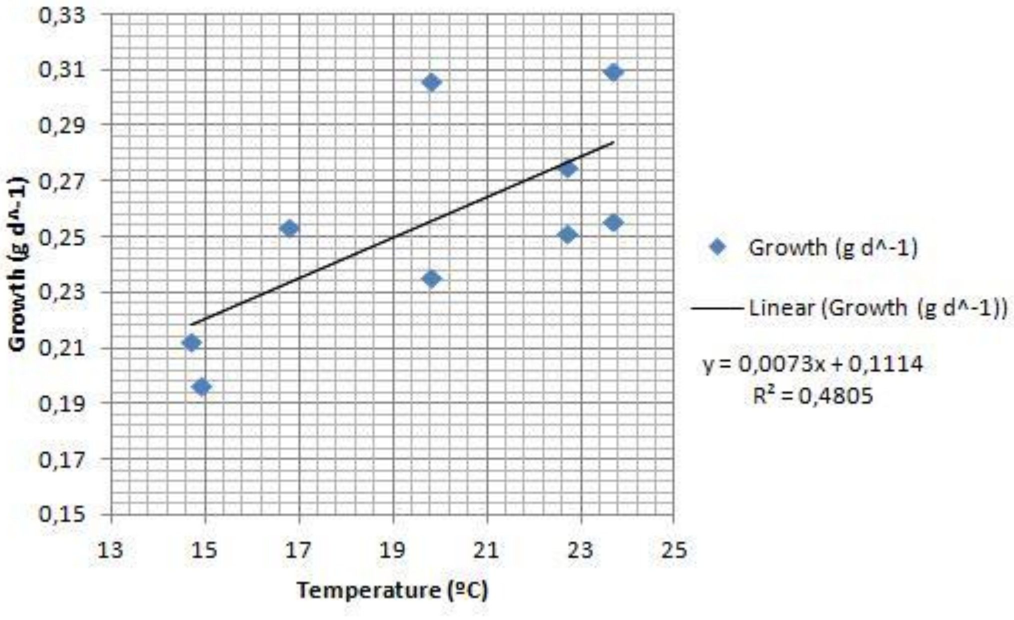


Figure 4.8 - Linear regression growth curve and equation for the adult population

Figure 4.8 shows that the $R^2$ is extremely weak so it cannot be used or accepted this line or adjustment. Even so, the linear regression line, if it was accepted, shows that adult crayfish growth rate is slightly higher than the total of the population with hatchlings and adults.

However it cannot be assumed that adult crayfish will have the same growth rates as hatchlings, because in the experience all adult crayfish lived in temperatures over 15 ºC, while the hatchlings lived in temperatures over 10 ºC. This data cannot allow to conclude about allometry because the growth rate and the mean weight of hatchlings and adult crayfish were never compared under the same temperatures.

In this work it will be considered that crayfish growth is independent from its age or weight and it is strongly dependent on the temperature. The temperature equation found provides the form of the growth relationship to temperature. The differences between temperatures will increase or decrease the growth rate. In the model it will be considered that all sized crayfish, from the hatchlings to the adults, will have the same growth rate, being only dependent on the temperature, the single forcing function for growth present in the model. Using the function previously created for the daily temperature variation and the equation found in Figure 4.7, the linear regression line was inserted in the crayfish and temperature models, in order to find a daily variation in the growth rate (g $d^{-1}$) during the year. It was used a single model for the daily temperature variation, explained in page 51.

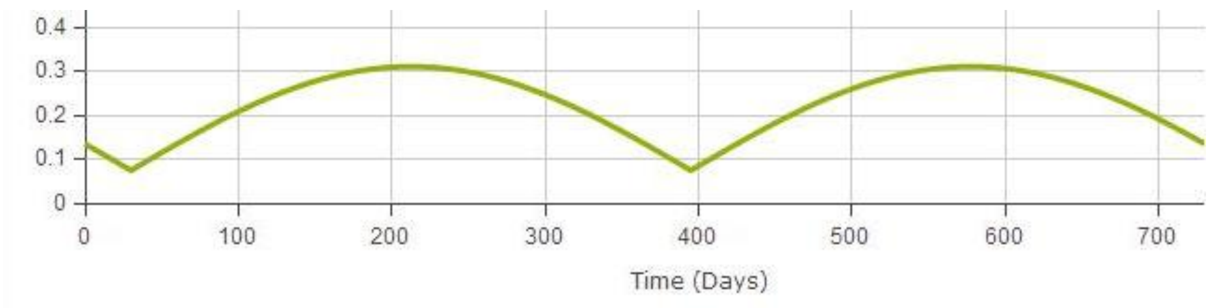


Figure 4.9 – Daily variation of growth rate (g $d^{-1}$) in Portugal

Figure 4.9 presents the final values found for crayfish growth dependent on the temperature daily variation (Figure 4.12), for the Portuguese climate. The instantaneous growth rate lies between 0.09 g $d^{-1}$ and 0.3 g $d^{-1}$.

In order to understand if the growth rate was dependent also on the weight of each crayfish it was made a multiple linear regression using the same data provided by the previous work. This proved to be wrong because it should be supposed that growth will be greater in the lightest and youngest crayfish. It turned to be the opposite. It is concluded that it is completely impossible to find the allometric growth with this data because the growth and the individual weight cannot be separated from the temperature and this influences both the results of daily growth and the weight. This only could have been made if the temperature was always constant. Because it is not, the correlation between growth and weight cannot be proved with this data.

### 4.3.1. Differences between real and modeled temperatures for Portugal

Figure 4.10 shows the results of the temperature model for the three selected Portuguese cities. It is important to present temperature variations according to the previous real data provided by IPMA, in order to allow that this daily temperature variation can be used in crayfish population model.

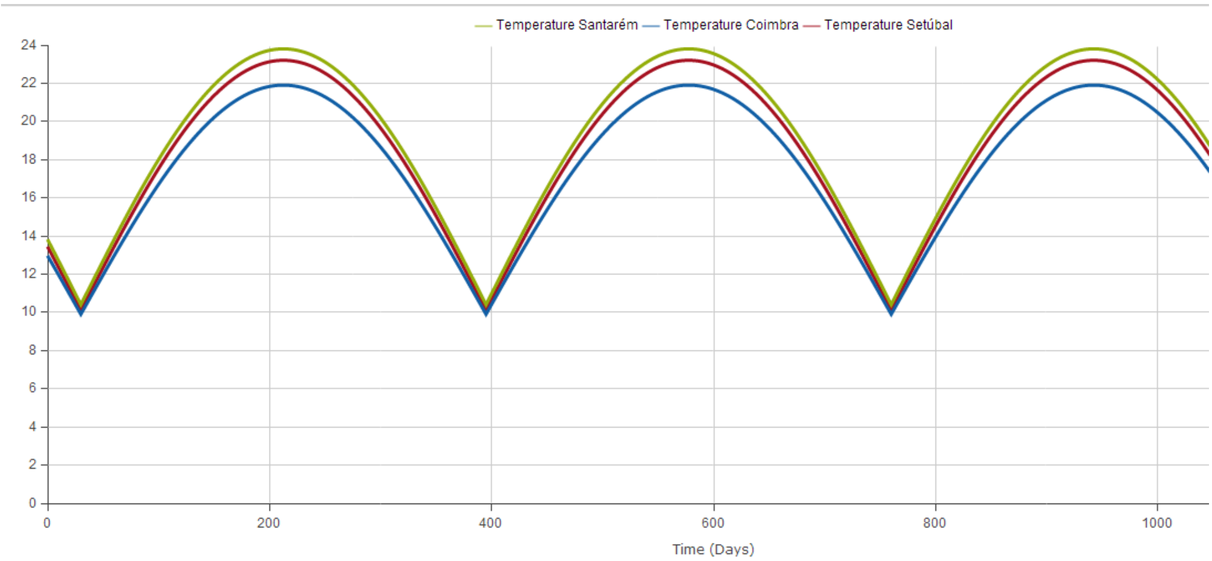


Figure 4.10 – Annual temperature variation in the three selected cities

Figure 4.10 and Figure 4.11 show that the model is a good fit for the daily variation temperature and for the mean annual temperature of the three selected cities, with minor differences, when compared to real temperatures in these cities.

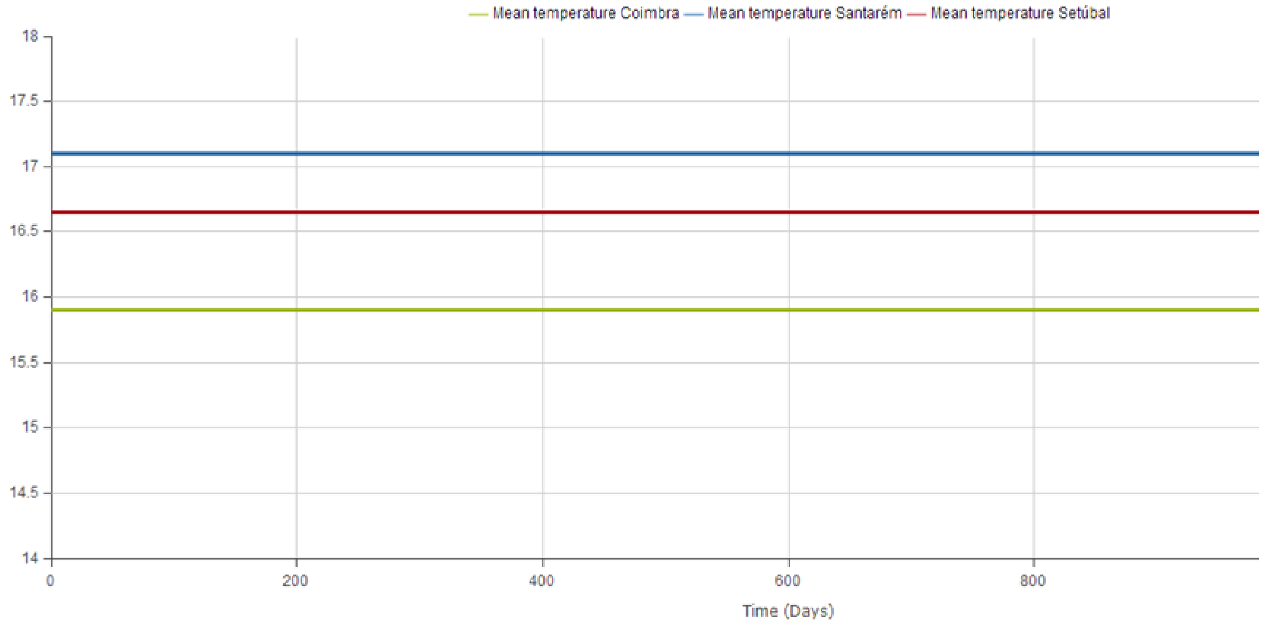


Figure 4.11 - Annual modeled mean temperature in the three selected cities

The differences among the cities temperature are only a few decimal degrees. The growth of crayfish was tested with three individual temperatures and its final number varied residually, with few more or less animals in each class, resulting in the same final yields. So it was decided to join the three lines in one single line. Regarding the fact that the annual mean temperatures are very close to each other due to the relative geographical proximity of the production areas, it was decided to insert in the crayfish population model one single model for the temperature. This single temperature model used the values from the warmest month (Santarém - 23.8 Cº) (Annex 7) and from the coldest month (Coimbra - 9.9 Cº).

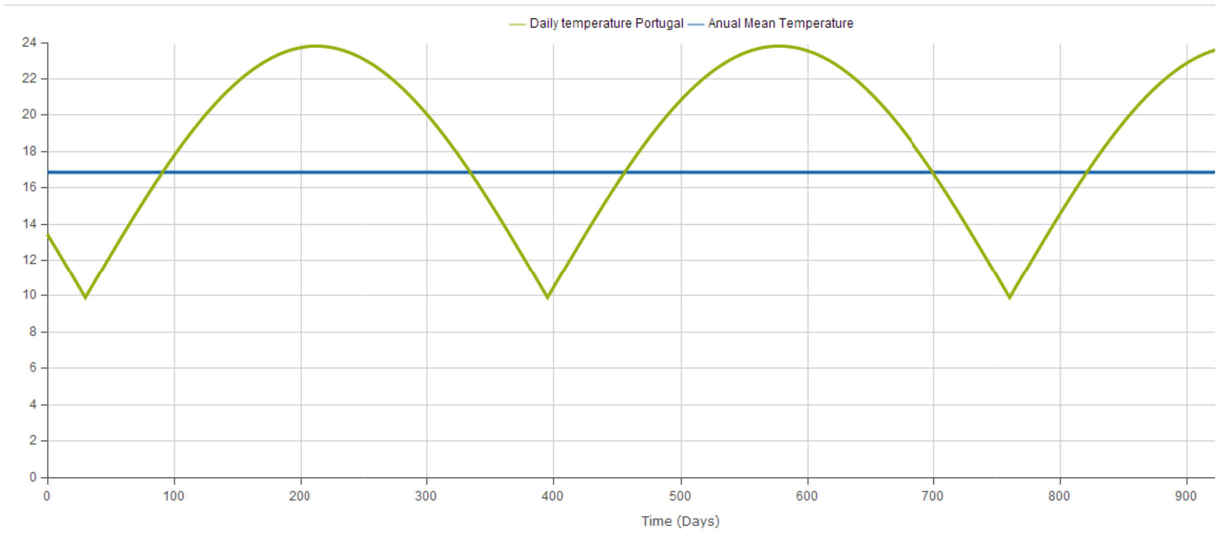


Figure 4.12 - Single temperature model for the three main cities near the selected paddy fields

Table 4.2 shows the temperature values used for growth forcing function on the single model of the species population. The new single mean temperature model presents a mean temperature of 16.9 ºC, which is considered a good approximation, and an approximately daily variation close to the original values.

Table 4.2 – Mean temperatures in the individual model and differences to original data

| City | Mean temperature individual model (ºC) | Difference to the mean temperature single model (ºC) | Mean temperature identified in the GIS vectorial map (ºC) |
|---|---|---|---|
| Coimbra | 15.9 | 1 | 15-16 |
| Santarém | 17.1 | 0.2 | 16-17-5 |
| Setúbal | 16.7 | 0.2 | 16-17.5 |

It can be concluded that the daily temperature variation single model is a good approximation and is well calibrated for the Portuguese study area and can be used further in this work. The comparison between Table 4.2, Figure 3.2, Table 3.1 and Figure 4.4 allows to conclude about the good temperature approximation between the measured data and the simulated data. All the results presented further in this work will be based on the daily temperature variation single model.

## 4.4. Individual model application to Louisiana

The model formulation was applied to Louisiana, in order to establish whether crayfish individual growth could be matched to measured data. This verification provides the basis for subsequent application of the validated model to the case study from Portugal that is the main object of the present work.

Portugal and Louisiana climates are different from each other, and so are the temperatures. The monthly daily average temperature in Louisiana ranges from 11.9 °C in January to 28.5 °C in August (National Weather Service, 2013). These months and temperatures were inserted in the model as the warmest and coldest month for Louisiana.

There are references to a very fast growth rate and weight increase in Louisiana, where crayfish can reach 8 cm in 3 months (FAO, 2013). At this size and age crayfish can attain 24 g (McClain, 2010). In McClain's work, crayfish hatchlings had a mean weight of 0.5 g, while in the best hypothesis crayfish reached a mean weight of 24 g in 90 days. Based in this literature references and considering that crayfish growth is regular throughout its life, it can be concluded that crayfish can attain 48 g in six months, on a regular growth basis.

Table 4.3 – Crayfish weight and age relation in Louisiana

| Weight (g) | Age (days) |
|---|---|
| 0.5 | hatchlings |
| 24 | 90 |
| 48 | 180 |

The individual model presented in Figure 3.3 allowed the comparison between the values of Table 4.3 and growth rates presented in the model using the temperatures from Louisiana, in order to understand if the model is well calibrated for the original area.

The results presented in Figure 4.13 show at Julian day 180 crayfish have a mean individual weight of 45 g. This result is in good agreement with measured data (Table 4.3). It can be concluded that the individual growth model is well calibrated for a crayfish population in Louisiana and that the equation used in the model, relating growth to temperature, is also correct. So this individual growth model can be used to build the general population model, because the individual growth pattern is correct.

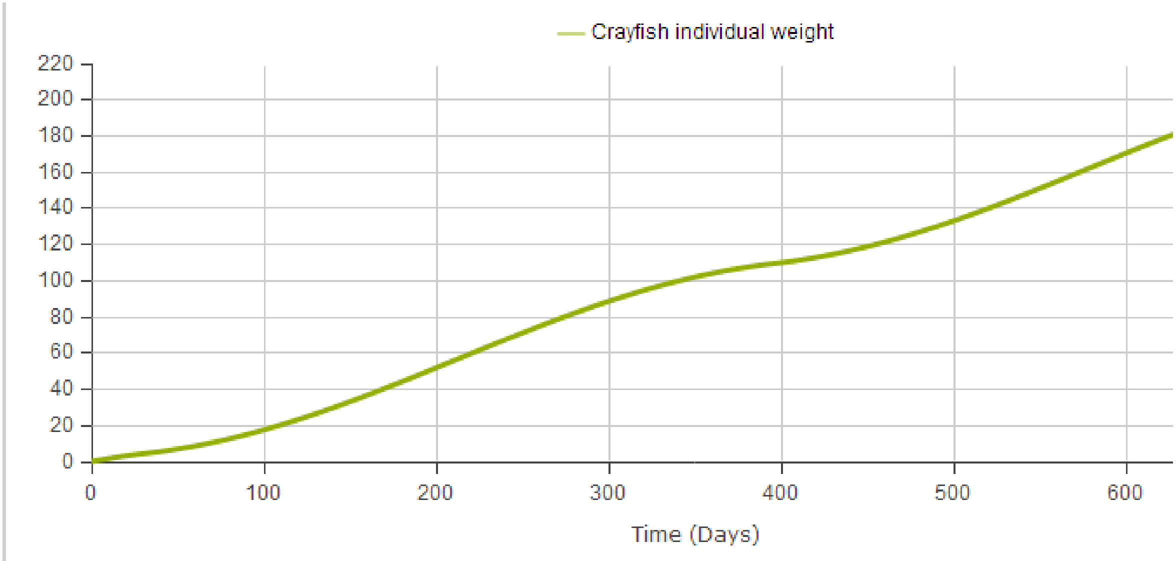


Figure 4.13 - Crayfish individual growth in Louisiana

## 4.5. Individual model application to Portugal

The individual biomass model was the applied to Portugal. The temperatures used were 23.8 ºC for the warmest month and 9.9 ºC for the coldest month. The objective was to examine whether the model provided an adequate description of individual growth in Portuguese areas.

Figure 4.14 shows that the crayfish individual weight increases over the simulation period. A comparison with another study from Portugal (Annex 9) shows that the growth rate used in this model is considerably higher, but the growth patterns are similar.


Figure 4.14 – Crayfish individual growth in Portugal

Crayfish body weight increases throughout the animal's life cycle. Annex 9 shows data from the Mondego valley (Anastácio, 1993) and supports this pattern. According to this model, Portuguese crayfish can reach 35 g in 6 months. This means a slower growth rate compared to Louisiana, as expected. However, the growth rate in this model is higher than the one found by Anastácio (1993).

This difference is directly related to the temperature. This model considers a trigonometrically variation in temperature, without peaks or strong variations that occur in reality. The temperatures inserted in the model are mean temperatures for each region over 30 years. In this model the temperatures can go up to 23.8 ºC. The work by Anastácio was made under low temperatures and 75% of the measurements reported temperatures under 20 ºC. This might explain the low growth rates field data found by this author.

## 4.6. Application of the population model to Portugal

The harvest rates and harvest periods considered in the model are strongly affected by crayfish individual and seasonal growth. The model run led to the following results:

In the first two months of harvest 50 crayfish of 35 g and 50 crayfish of 45 g each day can be caught, a total harvest of 240 kg $ha^{-1}$. In the second two months of harvest 100 crayfish of 25 g, 100 crayfish of 35 g and 100 crayfish of 45 g can be caught, a total amount of 630 kg $ha^{-1}$.

The model suggests that the total and final crayfish yields produced in Portuguese paddy fields can reach 870 kg $ha^{-1}$ $year^{-1}$. However in late March and early April there are still many adult crayfish in the fields and so the harvest rate can be strongly increased in this period to remove the maximum number of crayfish before sowing rice. The model started with default values, in order to compensate the high growth rates related to temperatures.

Figure 4.15 shows the peak of weight class 1 crayfish, related to spawning. This peak occurs around the Julian day 325, corresponding to the last week of November, and so it is correct according to the literature. The other weight classes remain relatively stable during the year. The increasing number of weight class 2 crayfish results from the growth of the weight class 1 crayfish, being obviously the most represented classes.

Figure 4.15 and Figure 4.16 represent the number of crayfish in each weight class and the biomass in each weight class, respectively. Because the peaks in the figures do not allow to have a detailed view on what happens in the first 150 days of the model, Table 4.4 presents the variation of each individual crayfish in the weight classes in the first 15 days after start running the model. All this figures and tables were taken during the run of the full model, so they include crayfish harvest.

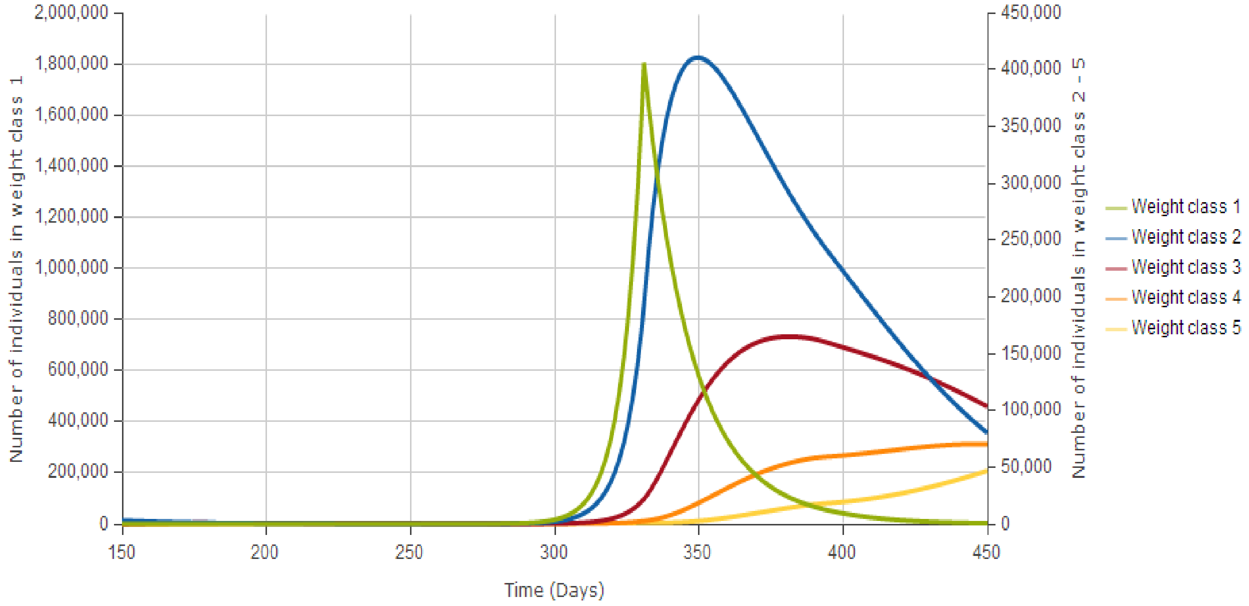


Figure 4.15 - Number of crayfish in each weight class

There are two main seasons of the year for the increase of the total biomass of the species. The first peak of biomass occurs in the fall, due to the spawning. Also this time of the year still presents mild temperatures, resulting in medium to high growth rates. The second peak of biomass occurs in the spring, due to the increase of the temperature, which lead to faster growth rates. In can be noted that the heavier weight classes are still increasing their weight in the spring.

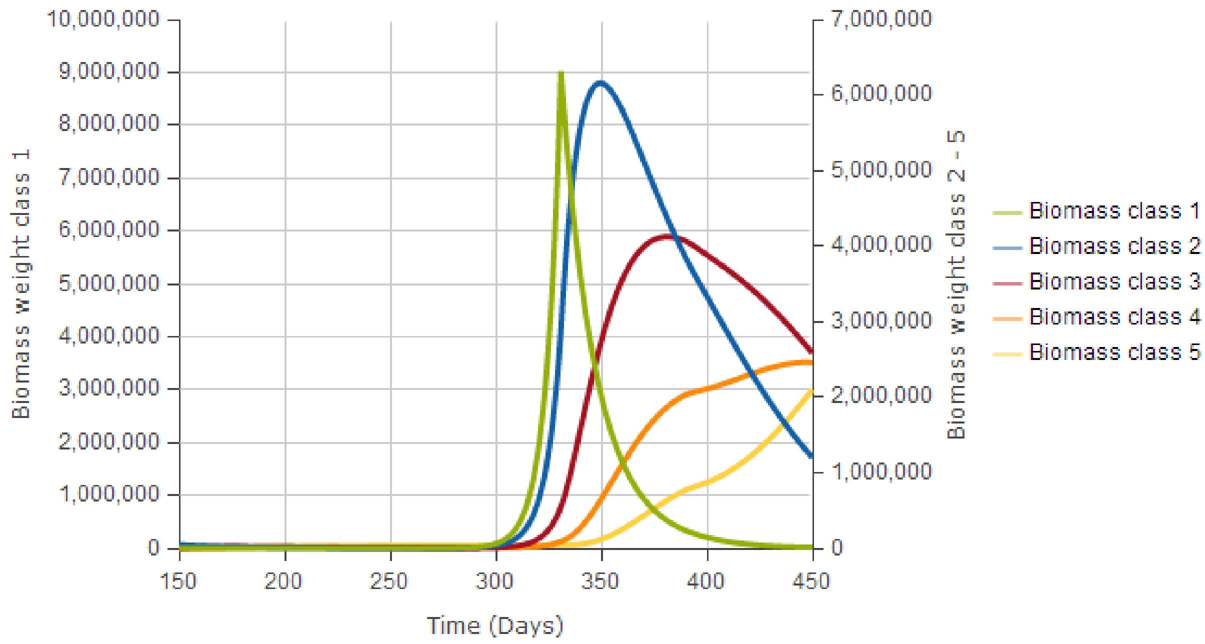


Figure 4.16 – Total biomass (g)

The values for the biomass in the weight classes are overestimated by the model. This discrepancy is mainly due to the total biomass value equation, weight classes and mean weight. For example, in the model, the biomass of the weight class 1 is given by multiplying the number of crayfish in the weight class 1 for the mean weight of the class, in this case 5 g. However, the newborn crayfish have a mean weight of 0.5 g. This would lead to a total biomass much lower than the one presented in Figure 4.16.

The harvested crayfish will be directly placed in a stock box without any other further movements, allowing the final count of total crayfish harvested. The harvested crayfish values are daily. For example, in spite of harvesting 100 individual crayfish during 7 days, it is more profitable harvesting 175 crayfish during 4 days, which results in the same 700 crayfish harvested. This allows the reduction of the costs with the harvesting and with labor. It can be made a mean week quantity and then divide to total amounts for some days to maximize the harvest productivity and reduce the production costs.

Table 4.4 shows that initially there are crayfish only in weight class 2. These crayfish will reach higher weights due to the fast growth in the spring and summer, and quickly move to superior weight classes. In the first days after the model starts there are no crayfish in the stock “Harvested crayfish” nor in “Weight class 1”, due to the lack of spawning and harvest in this period.

The results presented in Table 4.4 show that crayfish growth is overrated in the model. From the Julian day 150 to the Julian day 151, a total of 2076 g of crayfish biomass moved from weight class 2 to weight class 3. In 15 days the heaviest weight class reached 984 g of crayfish biomass. This can lead to excessive yields in the final simulated production. In reality crayfish will gradually increase its weight, while in the model there are only classes and so the crayfish immediately move to a heavier class and improve that class biomass, even if the individual weight is much lower than that class mean weight.

Table 4.4 - Crayfish stocks in the first days of the model run

| Time | Harvested crayfish | Biomass class 1 | Biomass class 2 | Biomass class 3 | Biomass class 4 | Biomass class 5 |
|---|---|---|---|---|---|---|
| 150 | 0 | 0 | 45,000 | 0 | 0 | 0 |
| 151 | 0 | 0 | 43,393.8333449... | 2,076.944425054 | 0 | 0 |
| 152 | 0 | 0 | 41,840.5055825... | 4,012.893181116 | 80.823187118 | 0 |
| 153 | 0 | 0 | 40,338.5153783... | 5,814.281966414 | 234.654132806 | 2.899034694 |
| 154 | 0 | 0 | 38,886.3925470... | 7,487.334847527 | 454.11394913 | 11.322914608 |
| 155 | 0 | 0 | 37,482.6979530... | 9,038.067874218 | 732.245322967 | 27.637255372 |
| 156 | 0 | 0 | 36,126.0233809... | 10,472.2928046... | 1,062.497641862 | 53.960279941 |
| 157 | 0 | 0 | 34,814.9913781... | 11,795.6209290... | 1,438.712238132 | 92.175376524 |
| 158 | 0 | 0 | 33,548.2550701... | 13,013.4669800... | 1,855.107776011 | 143.943396577 |
| 159 | 0 | 0 | 32,324.4979511... | 14,131.0531190... | 2,306.265804867 | 210.714678842 |
| 160 | 0 | 0 | 31,142.43365056 | 15,153.4129866... | 2,787.116499768 | 293.740786928 |
| 161 | 0 | 0 | 30,000.8056773... | 16,085.3958090... | 3,292.924608991 | 394.085949407 |
| 162 | 0 | 0 | 28,898.3871434... | 16,931.6705483... | 3,819.275626425 | 512.638192858 |
| 163 | 0 | 0 | 27,833.9804674... | 17,696.7300882... | 4,362.062205194 | 650.120159638 |
| 164 | 0 | 0 | 26,806.4170606... | 18,384.8954469... | 4,917.470827308 | 807.099603493 |
| 165 | 0 | 0 | 25,814.5569950... | 19,000.3200069... | 5,481.968742602 | 983.99955738 |

Table 4.5 represents the last days of crayfish harvest, untill the Julian day 450, the 1[st] of April. The final yields of crayfish caught are the 24100 individual crayfish present in the stock "Harvested crayfish" in the last day. It is important to note that there are still many crayfish that can be harvested in the higher weight classes and that they must be harvested without any specific rate in the last days before sowing rice.

Table 4.5 - Crayfish stocks in the last days of the model

| Time | Harvested crayfish | Weight class 1 | Weight class 2 | Weight class 3 | Weight class 4 | Weight class 5 |
|---|---|---|---|---|---|---|
| 435 | 19,600 | 6,342.113336... | 115,177.1847... | 122,332.7236... | 69,124.12547... | 35,493.39460... |
| 436 | 19,900 | 5,990.914585... | 112,582.3555... | 121,144.0406... | 69,289.25010... | 36,172.27692... |
| 437 | 20,200 | 5,658.018519... | 110,022.4070... | 119,941.6101... | 69,442.15024... | 36,861.51191... |
| 438 | 20,500 | 5,342.542305... | 107,497.7198... | 118,725.7767... | 69,582.35155... | 37,560.84525... |
| 439 | 20,800 | 5,043.64151282 | 105,008.6392... | 117,496.9133... | 69,709.39745... | 38,270.00880... |
| 440 | 21,100 | 4,760.508672... | 102,555.4758... | 116,255.4198... | 69,822.84987... | 38,988.72076... |
| 441 | 21,400 | 4,492.371882... | 100,138.5058... | 115,001.7216... | 69,922.28988... | 39,716.68573... |
| 442 | 21,700 | 4,238.493453... | 97,757.97153... | 113,736.2682... | 70,007.31842... | 40,453.59484... |
| 443 | 22,000 | 3,998.168593... | 95,414.08214... | 112,459.5319... | 70,077.55687... | 41,199.12597... |
| 444 | 22,300 | 3,770.724132... | 93,107.01421... | 111,172.0064... | 70,132.64766... | 41,952.94397... |
| 445 | 22,600 | 3,555.517287... | 90,836.91236... | 109,874.2052... | 70,172.25479... | 42,714.70081... |
| 446 | 22,900 | 3,351.934465... | 88,603.88988... | 108,566.6606... | 70,196.06432... | 43,484.03596... |
| 447 | 23,200 | 3,159.390105... | 86,408.02953... | 107,249.9215... | 70,203.78483... | 44,260.57664... |
| 448 | 23,500 | 2,977.325551... | 84,249.38421... | 105,924.5528... | 70,195.14781... | 45,043.93815... |
| 449 | 23,800 | 2,805.207972... | 82,127.97775... | 104,591.1333... | 70,169.90800... | 45,833.72424... |
| 450 | 24,100 | 2,642.529307... | 80,043.80568... | 103,250.2548... | 70,127.84370... | 46,629.52753... |

## 4.7. Application of the population model to Louisiana

The harvest rates were changed in the original population model created for Portugal in order to conclude about the harvest rates and yields in Louisiana. In this scenario, the changes in the model allowed the harvest of:

- Early season harvest rate: 100 crayfish $ha^{-1}$ $day^{-1}$ in weight class 4 and 5
- Late season harvest rate: 150 crayfish $ha^{-1}$ $day^{-1}$ in weight class 3, 4 and 5

This results in a total catch of 1425 kg $ha^{-1}$. Crayfish production integrated with paddy fields in the United States can be far superior to 1000 kg $ha^{-1}$, while in ponds can reach 2000 kg $ha^{-1}$. Both results of individual growth related to temperature and production yields are similar and in the range of values for Louisiana. This shows that the general model is well calibrated for this region.

## 4.8. Final results and discussion

The model suggests that crayfish activities in Portugal can generate annual yields of 19931 tons of crayfish produced in 22910 hectares of paddy fields, where up to 870 kg $ha^{-1}$ of crayfish can be produced. This crop would provide a considerable additional income for a farmer, dependent on the production costs and on the price of crayfish in the market. Three main production areas were selected, using a multi criteria evaluation: Mondego valley, Tagus/Sorraia valley and Sado valley.

A model aims to explain the reality in a simple way, making some adjustments. Models therefore present differences from the measured data. The differences are mainly in results, reflecting a simplification of processes and environmental data. In this case growth rates may be excessively high when compared with others measured in Portugal because the only data available to relate growth to temperature was from the United States. That work studied crayfish growth in a natural environment for the species, where crayfish are original, and so it is ideal for its growth. Data from Portugal result from fieldwork and may be more realistic. This means the total quantities of crayfish produced per hectare may be lower and the model may have to be adjusted and combined with field data.

Because the weight amplitude in each class is quite large (10 g), the model becomes faster and simple but less accurate. A more detailed and complex model would take much more time to run, but it would be more realistic. More weight classes would result in less amplitude in the animals' weight differences and the results would be more realistic. Few weight classes mean larger weight amplitude, which results in exaggerated final biomass values. The biomass pass between classes is too fast because the model assumes that all the crayfish in a weight class are considered to have that class mean weight and pass to the next class immediately, despising the intermediate weights, when in reality the animal weight increases gradually and slowly.

It was identified a problem in the model and results due to the fast pass of individuals between classes. The model was deliberately kept simple, but it is possible to retard the pass of individuals between classes using program options such as "Delay", which slows down the pass to the time step that is chosen. A more complex model can be developed in further works.

It was considered that only the females present in weight class 2 spawn because when the model starts there are no females in other classes. Nevertheless, the spawning period begins in October and by then there are females in all classes, so these should also spawn. However, as the pass between classes is too fast and does not correspond to reality, it was decided to keep that only the females present in weight class 2 in October will be able to spawn, in order to keep the model simple.

The individual growth model, the population model and final yields for Louisiana show good agreement with the literature. The application of the model to Portugal presents some problems. The individual model presents higher growth rates and high final yields of crayfish per hectare. This might be due to difficulties of the species outside its original distribution area, affecting its life cycle and resulting in different stress factors, reducing its growth. Nevertheless, the final yields are within the production range found in paddy fields in southern Spain.

The temperature data used in the model was always the mean temperature over the past 30 years. In reality the real temperatures have peaks and oscillations and so the growth should not present a continuous increase and decrease related to temperature, but higher and lower values, which will affect the final growth rate.

There might also be some differences in crayfish growth and mortality between the model and Portuguese paddy fields, since these are different from the natural environment of the species in Louisiana. There are also other factors that can change the growth rate of a crayfish population, such as allometry, availability of food and other stress factors that can influence and reduce individual growth. No data was available on these factors, either for the United States or Portugal, and this is clearly an area for further development of the model, on the basis of future experimental work.

### 4.8.1. Comparison with other authors

Anastácio (1993) estimated the production of the species as 272 kg $ha^{-1}$ $year^{-1}$ in a study for the Mondego valley. The same author created a general model (CRISP) which calculated the total crayfish production in that area to be in a range of 230 - 917 kg $ha^{-1}$ $year^{-1}$, but this higher amount could only be achieved if the mortality rate was reduced in 50% (Anastácio *et al*., 1999b). CRISP considered a maximum mortality rate of 0.6 crayfish $day^{-1}$ (only for the new crayfish recruits).

The main differences in the final yields result from the growth and the mortality rates assumed. The growth rate in this work is much higher than the one found by Anastácio because it was considered only dependent on temperature, while that author studied a population in a specific zone (Mondego valley, central Portugal). The author refers in his work that the low growth rates may be due to the low temperatures which occurred during the practical field work.

In southern Spain integrated production of rice and crayfish has reached maximum yields of 1100 kg $ha^{-1}$ (anecdotal references) but generally yields are smaller. In the United States crayfish production in paddy fields usually is not as high as in monoculture, but can exceed 1000 kg $ha^{-1}$. The model application for Portugal is in the range of the total yields produced in these countries.

### 4.8.2. Sensitivity analysis

Because the growth rate considered in the model is superior than the one found by other Portuguese authors, it was decided to do a sensitive analysis, in order to understand the changes in the final production if some parameters were modified.

The growth rate was reduced by 20% and the mortality rate was increased by 50%, to 0.012 crayfish $day^{-1}$. In this scenario, the changes in the model were:

- Early season harvest rate: 35 crayfish $ha^{-1}$ $day^{-1}$ in weight class 4 and 5.
- Late season harvest rate: 70 crayfish $ha^{-1}$ $day^{-1}$ in weight class 3, 4 and 5.

This results in a total crayfish yield of 607 kg $ha^{-1}$ $year^{-1}$. Even considering bad living conditions for crayfish, with changes in growth rate and mortality, the total yields are in agreement with the literature values.

The model indicates that if the growth rate of the species is reduced by 20% from the original model and the mortality rate increased by 50%, the final crayfish yields will present a reduction of 30%.

### 4.8.3. Potential control of the species

The eradication of the species in Portuguese territory is clearly an impossible task due to its widespread distribution throughout the country and all watersheds. So it should be proceeded an attempt to control the species in order to minimize the environmental and economic damage. There are three main methods to eliminate invasive species:

- Chemical control: the chemicals used to combat red swamp crayfish in Portugal have been unsuccessful, due to resistance of the species. These chemicals can also cause serious environmental damage and mortality of other species, besides the target species can develop resistance.
- Biological control: a major cause of the spread of invasive species is the lack of natural predators. This is one of the main reasons of the fast expansion of crayfish in Portugal. Although nowadays there are many native species who feed themselves on crayfish, this is not enough to combat the species great populations. It is important to introduce crayfish predators, such as native species or even the man.
- Physical control: Fishing can be considered a physical control method because it resorts to manual harvesting and extraction of invasive species from ecosystems.

The basic premise for this work and this kind of culture is that crayfish populations will be controlled by the need of restocking. If there are more hectares of paddy fields producing a second annual crop of crayfish, more crayfish will be withdrawn from ecosystems.

The control potential for the species in this model of management was calculated using the total amounts of crayfish restocked in paddy fields in May or June. If it will be restocked in each paddy field a total amount of 45 kg crayfish $ha^{-1}$, in the 22910 hectares of paddy fields available for the species production in Portugal there will be a need of 1031 tons $year^{-1}$ from natural aquatic ecosystems. All crayfish must come from wild populations, preferentially caught in natural ecosystems.

The culture of the species becomes the true potential of control: while there is culture there is also control, due to the annual need of restocking. The potential of control for the species is based on manual catches or fisheries, which removes crayfish from the natural and valuable ecosystems around the paddy fields. The fact that producers can fish them directly on the aquatic farms nearby or if they prefer to buy at low prices helps to generate a market flow and employment: crayfish are entered in the fields with nil or low costs and after a few months began to be harvested continuously, larger and heavier, to be sold at a profitable price.

### 4.8.4. Economic benefits and job opportunities

Crayfish can bring numerous profits in terms of job opportunities and revenue, due to export. The list below outlines some of the benefits of the creation of a crayfish production industry:

- Creation of direct jobs in harvesting, transformation and transportation of the product.
- Reduction of imports of similar kind of food (shrimp, animal protein).
- Supply of national food products to the domestic market.
- Increase of exports and a consequent increase in revenue for Portuguese economy.
- Control of this non-indigenous species and consequent environmental improvement.

The fishermen from Vila Velha de Rodão state they earn another salary during the summer by fishing and selling crayfish. If a real economic and well planed activity was legalized, this practice could occur in many other locations in Portugal, mainly in the rural areas, where income is lower and there are less job opportunities. During the spring and summer, from May to September, market needs can be met by fishing in certain areas such as Alqueva and Castelo de Bode dams and the Tagus river. This is ideal to complement crayfish production period in paddy fields from December to April.

Crayfish aquaculture legalization can lead to an increase of fisheries, due to the need of restocking in the spring. This will lead to a serious and effective national combat against an invasive species. Crayfish fishing would be encouraged throughout the country for sale to industries or rice producers who decide to co-cultivate crayfish.

Currently Portugal produces and sells to Spain at least 20000 tons year$^{-1}$, from fisheries alone. It was calculated in this work that the total aquaculture production in paddy fields could reach another 20000 tons year$^{-1}$. Portugal could double its product export.

Harvesting is the most labour intensive component of crayfish farming. Typically 60-80 percent of total direct expenses are associated with the harvest, due to the need of a boat and at least one person. Bait and the intense labour required for trapping are the greatest expenses (Bretonne *et al*., 1990; FAO, 2013).

The differences in the production costs will be based on the price paid by the crayfish restocking, the water flooding and the harvest costs. It will be important to conduct a market study to understand if crayfish is still profitable for the farmers after these costs, dependent on the prices paid in the international markets. There will be no cost for artificial feeding, since crayfish feed naturally on the remaining foliage and rice straw remaining in the fields (D'Abramo *et al*., 2004).

In terms of jobs crayfish industry could probably generate many direct jobs throughout the country. The factory in Vila Velha de Rodão currently has five full time employees, all working with crayfish transformation. If one more unit was created in each one of the three selected production areas, Mondego, Tagus/Sorraia and Sado, this could generate at least 15 direct jobs. Considering that each transformation unit may need two truck drivers to transport the product this would generate 6 more direct jobs.

The 23000 hectares of paddy fields to be harvested would additionally require staff, also providing jobs. Even with the help of a boat, probably 100 employees would be needed to harvest the 23000 hectares between December and April, considering each person would cover an area of 230 ha day$^{-1}$. This is equivalent to around 35 full time employees.

Those areas can be covered with the help of a boat, but if the producers choose to reduce the costs with the machines and fuel they will need much more people to cover the area. The crayfish industry could result in 56 direct jobs, probably ascending indirectly to much more. The benefits to the economy can be great.

The production costs estimative led to the following results:

- Restocking costs with small crayfish: considering that small crayfish are cheaper, an average price of 1 € $kg^{-1}$ is acceptable. Because the restocking is made with 45 kg this means a cost of 45 € $ha^{-1}$. In 230 ha farmers would spend 10350 € $year^{-1}$ with the restocking. However the farmers can fish crayfish in the water bodies by themselves, reducing the costs.

- Labor costs: considering that each 230 hectare paddy field needs one person for crayfish harvest with a minimum salary around 500 € $month^{-1}$, crayfish harvest for 5 months will result in a total labor cost of 2500 € $year^{-1}$ with labor in a 230 hectare paddy field.

- Fuel and bait: the costs with fuel may depend on the type of the boat and the engine. If the producers prefer they can use boats without fuel engines, but this will result in slower harvest and less area covered. Portuguese paddy fields may present differences from Louisiana paddy fields and the use of a boat may not be needed or cannot be adapted to Portuguese cultures. The bait used can be made of dead fish or other organic product and can be very variable in price. Because there is also homemade bait and the fuel prices and engine consumption are extremely variable, it will be considered a production cost of 50 € $ha^{-1}$ $year^{-1}$. The 230 hectare present a total cost of 11500 € $year^{-1}$.

- Water price can also be very variable, depending on the soil, areas, climate and precipitation. In a dry year the production costs in flooding the fields during the autumn can be expensive. It will be considered an average price of 0.005 € $m^{-3}$ (Vida Rural, 2008) due to the last years frequent tax and costs increase. If a producer needs to artificially flood the fields with a permanent 0.5 meter water layer, this means that each hectare needs to be permanent flooded with 5000 m3 of water. If a producer needs to artificially flood the fields during 2 months and each 2 weeks has to re-flood the fields due to evaporation, infiltration and other water losses, this means he will spent 100 € $ha^{-1}$ $year^{-1}$ with water. A 230 hectare paddy field will cost 23000 € $year^{-1}$.

Table 4.6 – Production costs and profit estimative

| Production costs of 230 hectare paddy field | Crayfish production in 1 hectare paddy field | Crayfish production in 230 hectare paddy field | Total revenue in 230 hectare paddy field (crayfish price 2 € $kg^{-1}$) | Profit in 230 hectare paddy field (with production costs) |
|---|---|---|---|---|
| 47350 € $year^{-1}$ | 870 kg $year^{-1}$ | 200100 kg $year^{-1}$ | 400200 € $year^{-1}$ | 352850 € $year^{-1}$ |

It can be concluded that the final profit, applying the production costs, can be 1534 € $ha^{-1}$.The total national 23000 paddy fields able to produce crayfish can generate 35 million € $year^{-1}$.

It is important to note that crayfish is considered a snack in Spain, a country with almost 50 million inhabitants and geographically ideal to strengthen the trade. Big crayfish can be priced up to 6 € $kg^{-1}$, so the total profit sales can be higher enough to justify the production costs.

Overall crayfish industry can bring job opportunities and extra money to many rural populations, generating new activities. Both the manufacturing sector and others such as transport and harvesting may also generate employment. Figure 4.17 presents a simple business diagram representing the economic process generated by crayfish activity legalization.

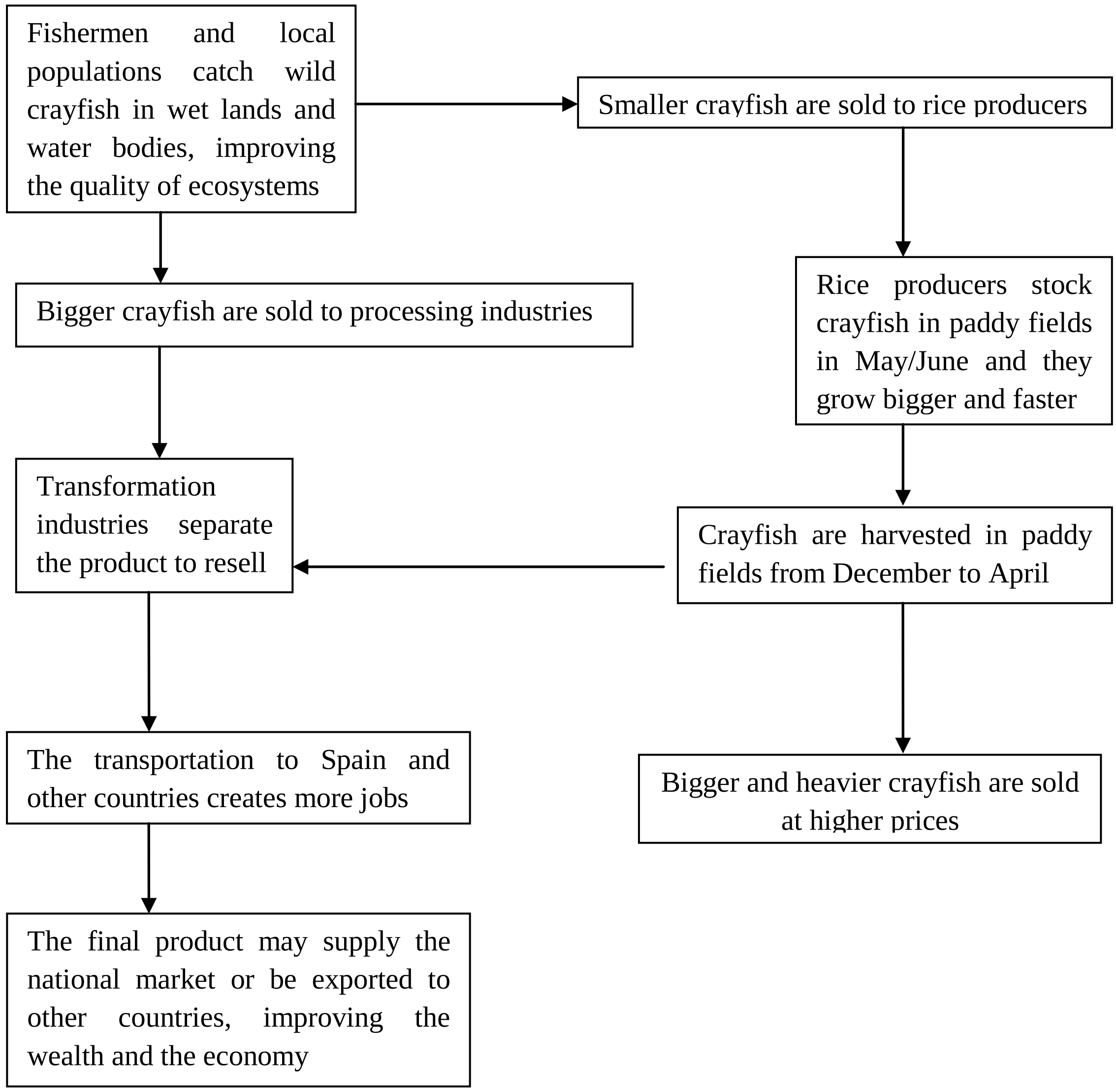


Figure 4.17 – Crayfish production diagram, jobs and benefits

# 5. Conclusions

## 5.1. Final considerations

Although crayfish introduction was aimed to produce food and bring wealth to rural and poor regions this never happened and the species brought more problems than benefits. However crayfish present a considerable economic and commercial value, being highly exploited in aquaculture and it is an important food resource in many regions of the globe. Considering this, and in the national current economic situation, the red swamp crayfish is a serious environmental problem that can be managed to provide economic benefits and also reduce the environmental damages of recent years. This management can go through the exploitation of the species as a food source, using aquaculture production as a mean of population control and reducing populations.

The basic premise to justify this work is the fact that it is totally impossible to eliminate the large populations of crayfish present throughout the country. These populations cannot be eliminated by any known means, so it must be used by man for his own environmental, economic and social benefit, taking advantage of a problem which can generate wealth. Crayfish consumption and exports as food can be the key to control the damages to the environment. In the current situation of uncontrolled population of the species, a management plan for crayfish which can make use of this feature as economical and environmental benefits is the only way to take advantage of this problem. The answer is not to ban, but to make the most of it and harmonize the different variables related to it.

The restocking with wild crayfish populations into the paddy fields can be simply seen as a transfer between two different areas: an area with natural values which is important to preserve and where crayfish will grow slower and have less value as a commercial product and another area without ecological richness but where crayfish will grow bigger and faster and became an important commercial product and valuable resource.

It is concluded that crayfish must be used as an economic and food resource. Crayfish restocking and harvest will help controlling populations. This is the only way of combating this serious environmental problem. Its consumption and industry will lead to an increase of the catches of the species, the only way of physical control. It is also an important way of biological control because the predator becomes the man. These premises should cheer the Portuguese environmental entities because this production method will continuously remove crayfish from the ecosystems, improving their natural values. Although the species is not globally appreciated in Portugal, there are lots of countries which buy large amounts of crayfish, because it is a very appreciated as a tasteful and luxury food.

The management proposal aims to produce rice and crayfish in the same field, resulting in two crops in one year and removing the crayfish from other water bodies and aquatic ecosystems, improving their environmental quality. Crayfish culture is a supplement to rice culture, taking advantage of the fallow fields to generate wealth in a second annual crop. The minimum number of crayfish in the paddy fields must be reached before sowing rice in April or May, to reduce the population individuals to the maximum and prevent digging and damage to paddy fields, without resorting to chemicals. Nothing is changed in rice cycle. Paddy fields in Portugal stand in fallow from November to April and have no economic exploitation during this period. It is a good period to use the agricultural remains let in the fields to serve as a natural food for the crayfish.

Although most of the population and farmers in Portugal hate crayfish and are unaware of his value and benefits, it was proved with this work that crayfish have an important market value in other countries and should be taken advantage of. Both fishing and aquaculture can co-exist without problems. Portugal has really good conditions to produce the species due to climate differences and variation in rainfall and temperatures when compared to southern Spain crayfish production areas, extremely hot in the summer and with much less rain because of the more continental climate, much drier, which in some years may result in a serious lack of precipitation. This leads to less water in the fields and strong reductions and variations in crayfish populations, as it occurred before.

Portugal can become an important producer of crayfish at a global level, supplying the European and international markets with a quality product. It could serve both the domestic and the international market, as the world's leading producers, have higher transportation costs due to distance, increasing the production costs. Louisiana frequently suffers tropical storms, hurricanes and serious flooding which can be very destructive and may affect crayfish production.

Crayfish reproduction and life cycle are very dependent on water availability. In abnormally dry hydrological years there might be problems with the culture of the species. Tagus/Sorraia and Sado production areas can present problems of water supply and high water prices paid by farmers. The Mondego valley area should not present problems with water supply. It will be the greater or lesser amount of rainfall and the flow drained by the watersheds that may reduce the production costs of farmers with the need for artificial flooding the fields. Probably crayfish production will be easier in the Mondego valley, central Portugal, due to the availability of water all year, although cold temperatures may lead to low growth rates.

Sowing rice differences from northern and southern Portugal may not affect crayfish because this method demands they are introduced in the paddy fields when the rice has around 6 weeks and the plants are tall enough not to be harmed by the species. By this time the paddy fields are always flooded. After the introduction, crayfish will burrow and seek for the levees and irrigation channels when the water level is reduced in the fields.

The model suggests that Portuguese paddy fields can also produce crayfish, as is done in southern Spain and in the United States. The comparison between Portuguese and Spanish paddy fields might be important to conclude about the calibration of the model for Portuguese and Mediterranean regions, both in the individual growth model as in the population model and final yields. It is important to note that the final yields suggested by the model for Portuguese areas are in the range of values of those produced in southern Spain.

All the goals of this work were attained. The current distribution of the species in Portugal was presented in the State of the Art chapter and the potential of control for the species was calculated in 1000 tons of crayfish removed from natural ecosystems each year. The production capacity at national level was calculated in 23000 hectares and the most suitable areas were identified. The model developed to simulate a crayfish population in a Portuguese paddy field suggests that can be produced up to 870 kg $ha^{-1}$. Crayfish industry can generate 35 million € $year^{-1}$, which means a high exploitation potential.

## 5.2. Recommendations

Because crayfish is present throughout the country it does not make sense to limit the trade. Fishing and industry should be encouraged and supported to enable the reduction of populations and allow exporting the product to other countries. This work suggests that a change in the legislation and the legalization of the practice of crayfish aquaculture in Portugal would bring great benefits on several levels, most notably the increase in exports and wealth, national food production, providing job opportunities in rural and poor areas and mitigation of environmental problems. The economic market generated would be responsible for helping to combat a plague and increase wealth. A real industry can be created and production can be assured all year by fisheries and aquaculture, supplying the markets demanding.

Due to the dangerous characteristics of the species, this works focused solely on the rice and crayfish integrated aquaculture with the aim of removing crayfish from ecosystems. It is important to inform Portuguese rice farmers about how things are done in the United States, their culture methods and how profitable crayfish can be.

In this current scenario, given the existence of a population model to simulate the species evolution in paddy fields, it only lacks a national strategy of exploitation for the species, which would benefit various sectors and activities. The environmental and governmental entities must reach an agreement in order to conduct a serious and official study on the species, requiring further and deeper developments. Potential problems that can difficult the creation of a strong crayfish industry exploitation in Portugal:

- Portuguese legislation – annual restocking, an essential step in crayfish production, is currently forbidden. Also the product may not be transported alive, which reduces the real market value of the species. Portuguese law clearly defines that an invasive species can be used if there are really benefits for humans.
- Chemical products used in paddy fields - Although these products don't affect humans, because they are not assimilated by rice plants, there may still be bioaccumulation by crayfish. The potential effect of the chemicals should be studied. The paddy fields are subject to national legislation that only allows the use of non-hazardous chemicals for humans and the environment. However, it is noted that the pesticides are very harmful to crayfish.
- Water price paid by the rice farmers - another potential problem for the implementation of this culture method in Portugal may be the water quantities and prices paid to irrigate the fields after the rice harvest. The price paid for water might be too expensive for the farmers, mainly in the areas located in southern Portugal. This can be a serious problem because crayfish production and sells may not compensate the costs in flooding the fields from October to December. Generally, from January to March the total amount of precipitation in Portugal is medium to high, which leads to the overflow of the main rivers and allows the fields to be naturally flooded and so the farmers may have little costs with water and crayfish production can be profitable. The total quantities of rain can directly affect the production costs. It is important to perform a financial analysis and a market study to understand if there is still a good profit with crayfish sells after the production costs

The model is well calibrated for Louisiana, both in the individual model as in the population model and crayfish produced yields. However it can be overrated to Portuguese populations due to the higher growth rates when compared with other Portuguese authors. The model needs to be calibrated to Portuguese populations with practical studies and field data in order to conclude about the real yields before start producing the species in aquaculture.

There are some differences in rice culture in Portugal and in the United States, mainly in the rice sown, which can be water seeded or drill-planted. However, if the crayfish justify profit and there is a rentable market, small adaptations might be needed to produce crayfish in paddy fields, such as the water level and the flooding in the fields in the autumn.

An important part of this management strategy is that crayfish must be introduced in the paddy fields at low densities (around 45 kg $ha^{-1}$). This assures less or none damage to the rice plants and allows crayfish to attain heavier weights and grow faster, also resulting in less mortality.

After the rice harvest, rice straw and other foliage must be left in the fields because it will guarantee food for crayfish. This allows an ideal culture without any costs with artificial food and good results in crayfish growth. When the fields are artificially flooded it results in a re-growth of the foliage providing the best possible conditions for crayfish growth and refugee.

The species has a higher market value if transported live (Diário de Notícias, 2013; Louisiana State University Agricultural Center, 2010; Romaire *et al.*, 2005). To prevent problems with leaks and colonization of new areas, transport lorries should be isolated, and the animals accommodated in bags, as is done in the United States (Louisiana State University Agricultural Center, 2010; Romaire *et al.*, 2005). The load will be safe and there won't be any problem with the propagation of the species, although every Portuguese watershed is currently colonized with *P. clarkii*.

## 5.3. Future developments

This research work shows that the red swamp crayfish can be used for the reason it was introduced, creating jobs and generating wealth, helping the poorest populations of rural areas. The potential to produce the species in Portugal is great. To be succeeded this study needs to get off the paper and become something achievable on the ground in order to make sense. The keyword is "to accomplish", because something must be done to combat an environmental plague that presents numerous opportunities for generating economic activities and that can help improving the Portuguese difficult financial situation. It is possible to reduce the problem with no additional cost to the environment.

The species production in aquaculture in Portugal will be strongly dependent on growth rate and mortality rate. In this work, the model used a growth rate dependent only on temperature. However, other Portuguese authors found low growth rates. This can difficult the species aquaculture process. Before the implementation of any strategy of crayfish use it is important to conclude if the growth rates used in this model can really happen in Portuguese paddy fields.

There is no data or references about crayfish yields produced in Portuguese paddy fields. It would be important to test this model in the paddy fields in order to conclude if it could really be produced the simulated yields. This could be an important achievement for Portuguese aquaculture and a beginning of a new industry with a great exporting market.

There are some important themes related to crayfish that might be important to study in further works, such as:

- Allometric growth and growth rates in Portuguese populations. No allometry data relating the weight increase with the size of the individual was found. In further works, if this model is used, the growth rates must be approximated to the studied populations in natural environmental conditions in Portugal.

- Influence of chemical products and rice fertilizers in crayfish.

- Economical analysis to conclude if the production is profitable after the production costs.

ProDeR (rural development program) is a strategic and financial support instrument which aims to help the rural development. This program was approved by the European Union and its goals are improving the competitiveness of agriculture and forestry, promote the sustainability of rural and natural resources and revitalize rural areas, economically and socially. This program may be an important help to gather financial support to the implementation of rice and crayfish integrated production in Portugal, since all its objectives are according to the proposed production of the species.

We should probably look to crayfish in another way, trying to solve a big problem with better imagination. The best way to remove a non-indigenous species is eating it. This was the solution that some northern Africa populations found for the plagues of locusts.

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# Annex 1 – Crayfish aquaculture cycle

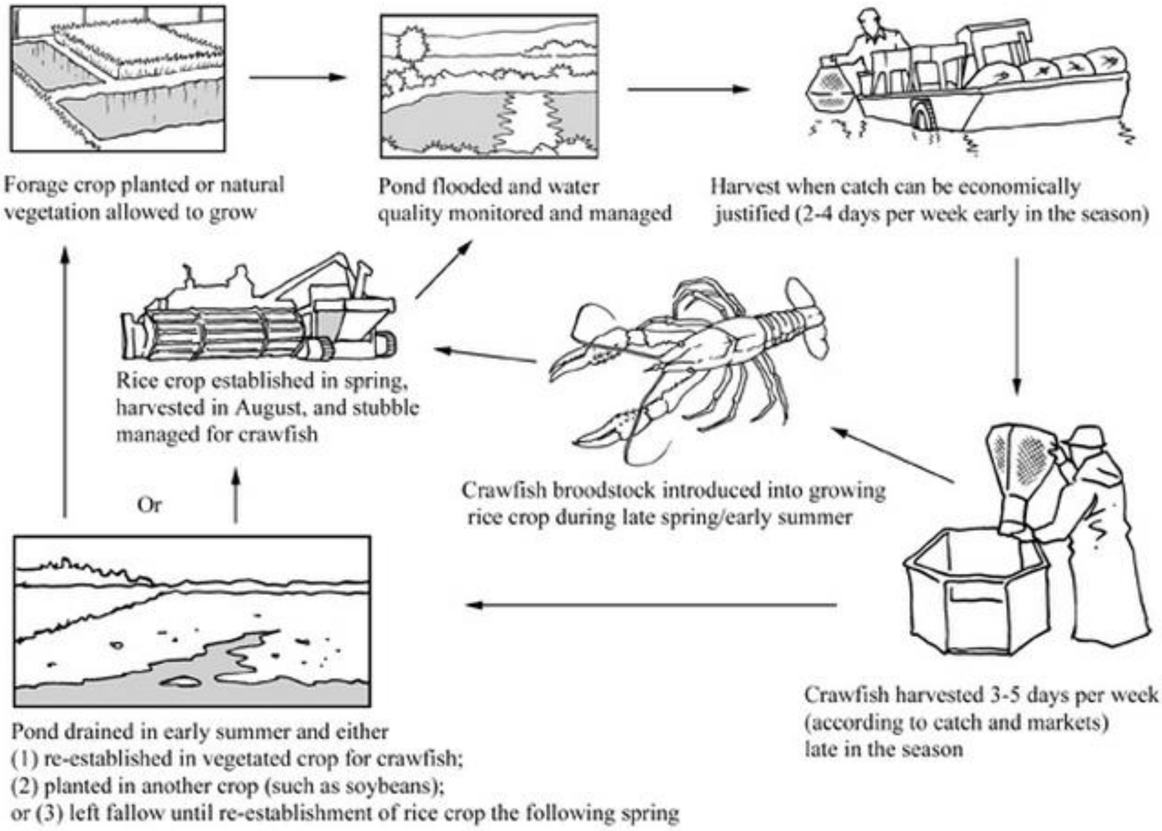



Figure A1.1 – Crayfish aquaculture cycle in the United States

(FAO, 2013)

## Annex 2 – Changes in Portuguese paddy fields area

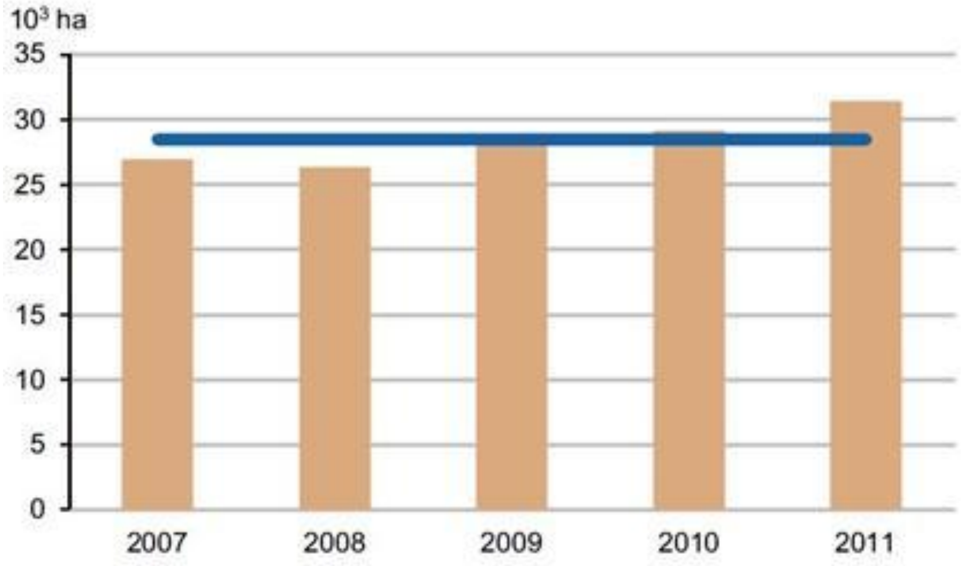



Figure A2.1 – Changes in Portuguese paddy fields area

(Adapted from INE, 2012)

# Annex 3 – Rice production areas in Portugal


Figure A3.1 – Rice production areas in Portugal

(Adapted from ANIA, 2013)

## Annex 4 – Portuguese paddy fields area in 2011

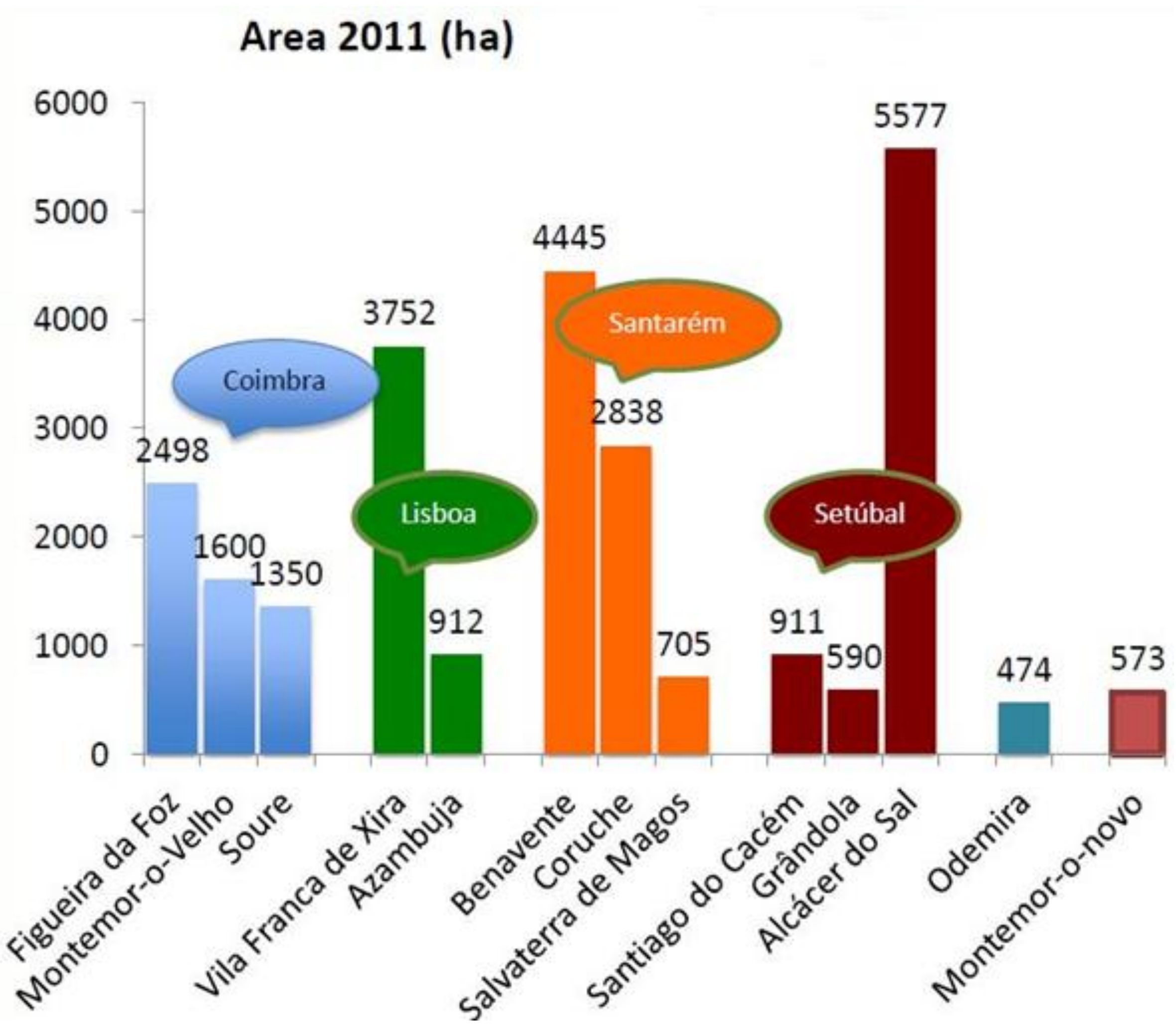



Figure A4.1 – Portuguese paddy fields area by municipality and region in 2011

(Adapted from Almeida & Marques, 2013)

# Annex 5 – Portuguese climate classification

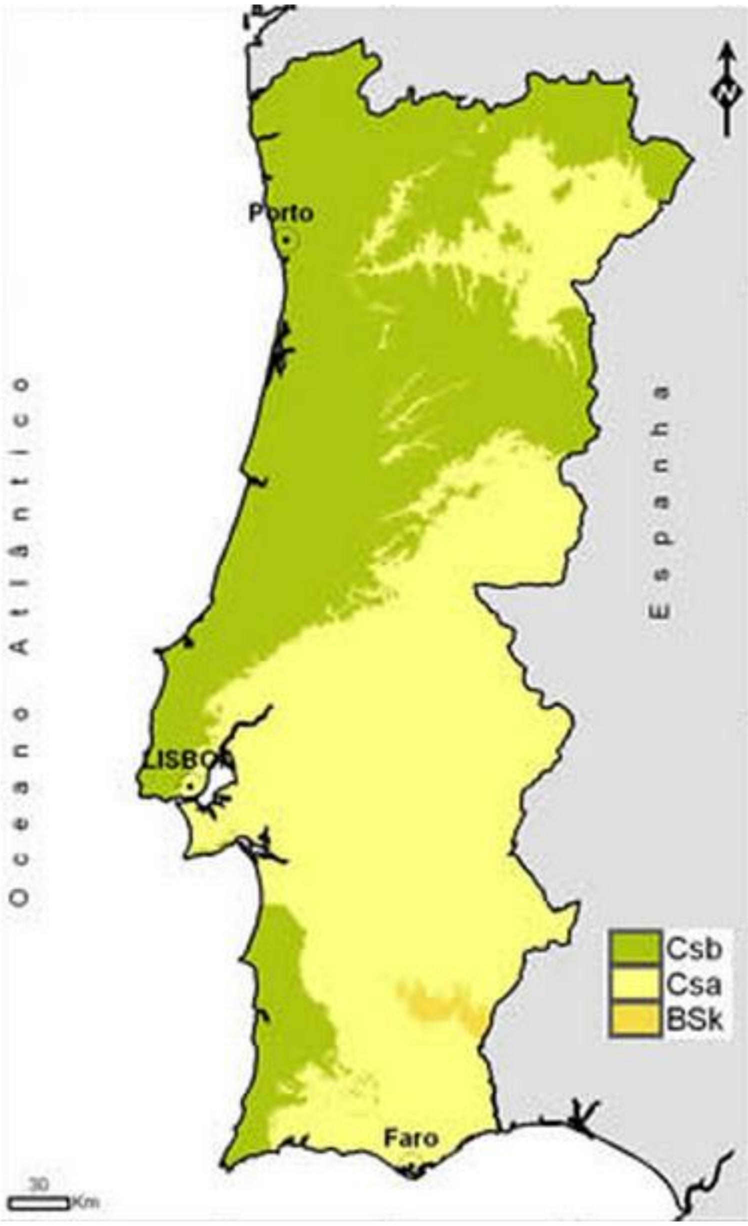



Figure A5.1 – Portuguese climate classification

(Adapted from IPMA, 2013)

# Annex 6 – Total annual precipitation in Portugal

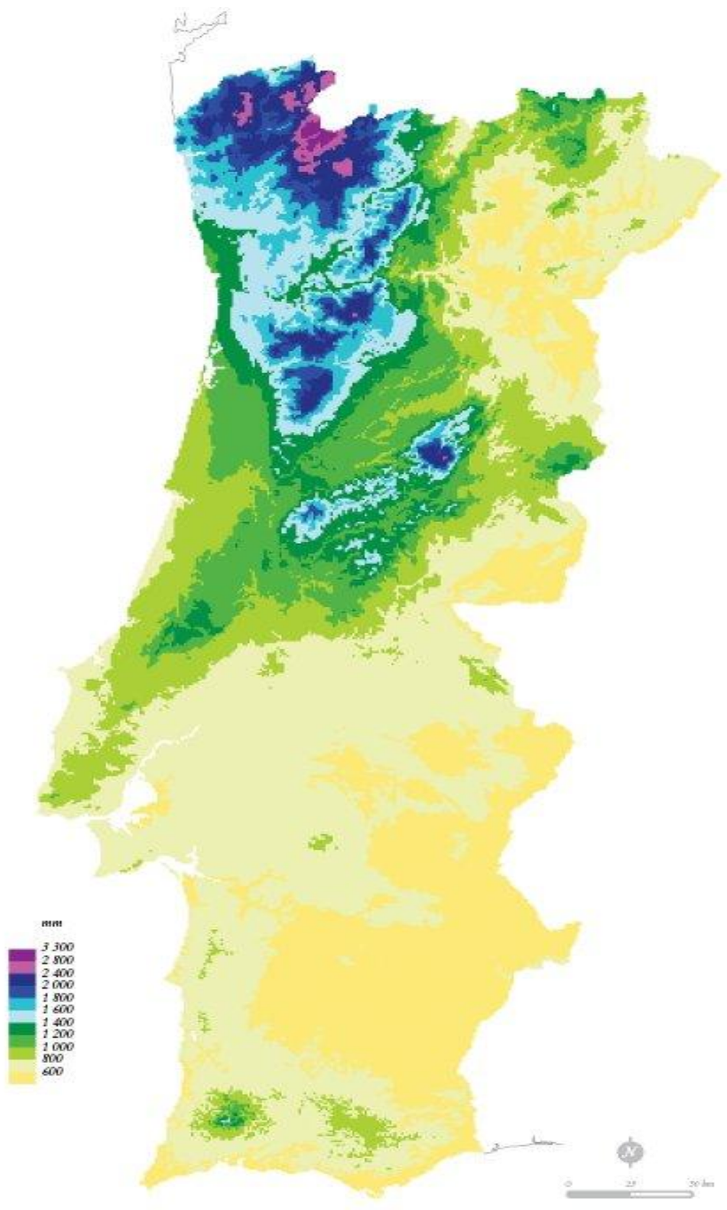
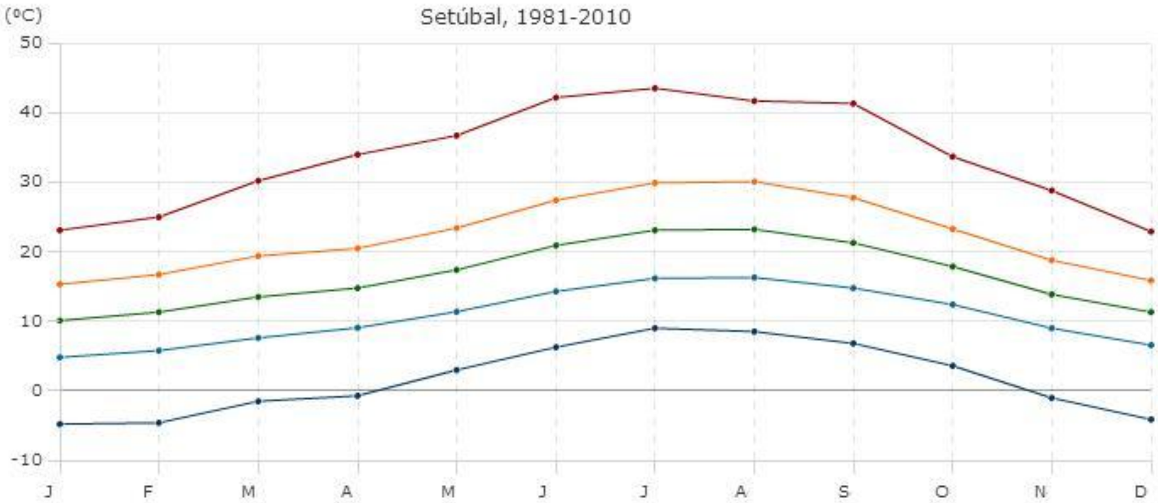



Figure A6.1 – Total annual precipitation (mm) 1960-1991

(IGEO, 2013)

# Annex 7 - Temperatures in the Setúbal and Santarém



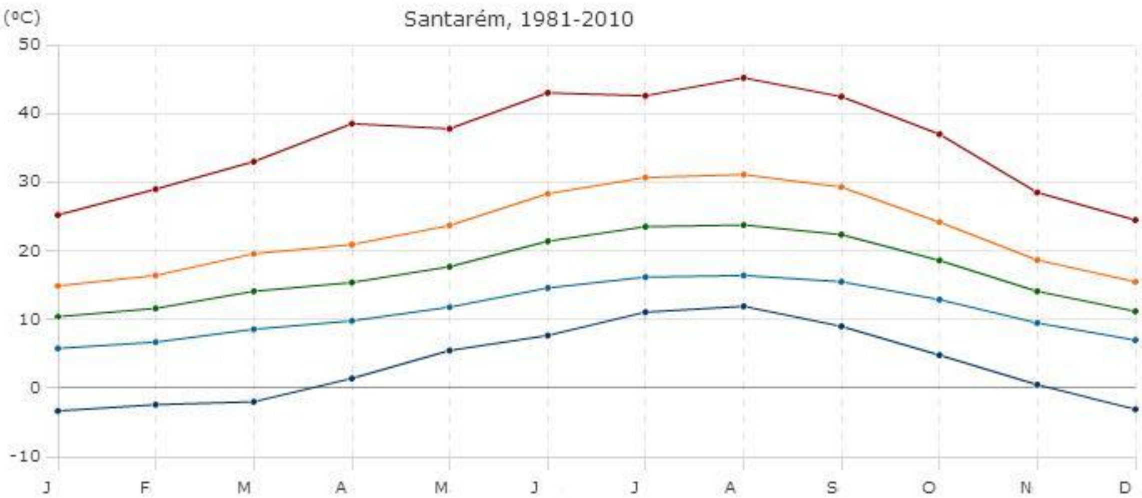




Figure A7.1 – From top to bottom: higher maximum temperature, mean maximum temperature, mean temperature, mean minimum temperature and lowest minimum temperature in Setúbal and Santarém

(Adapted from IPMA, 2013)

# Annex 8 – Daily temperature variation equation

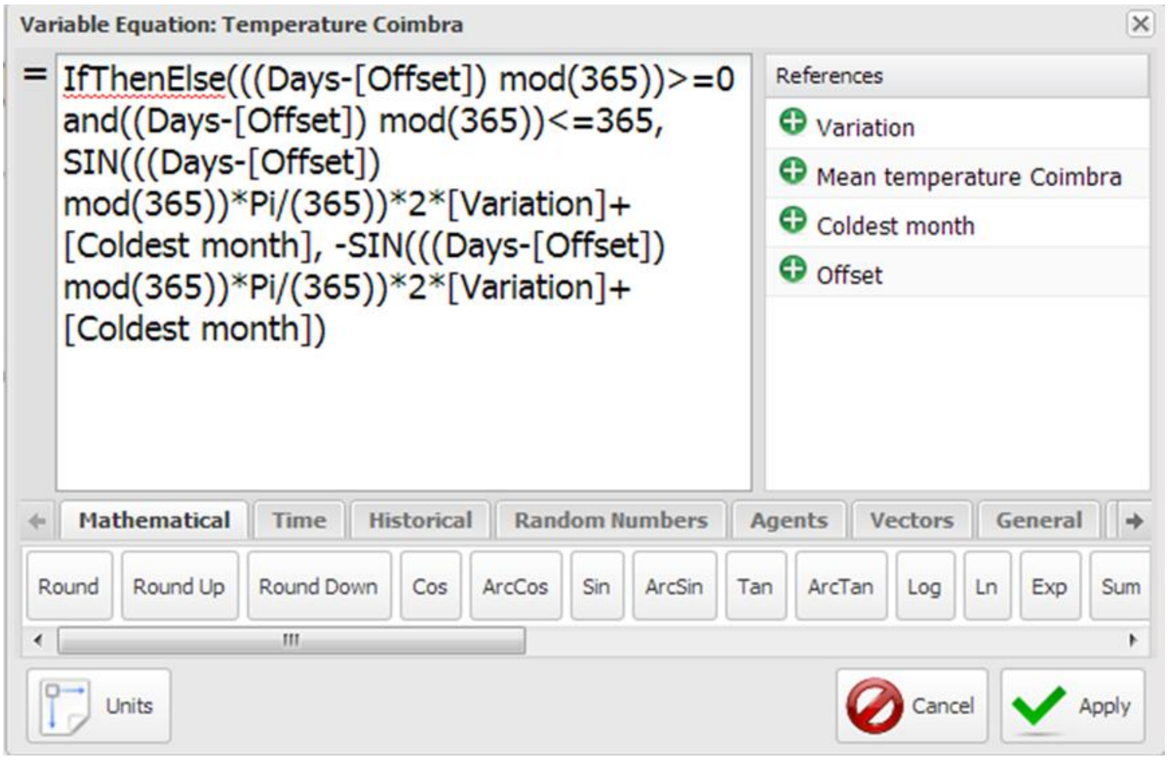



Figure A8.1 – Equation created in Insightmaker for daily temperature variation

# Annex 9 –Relation between crayfish weight and age

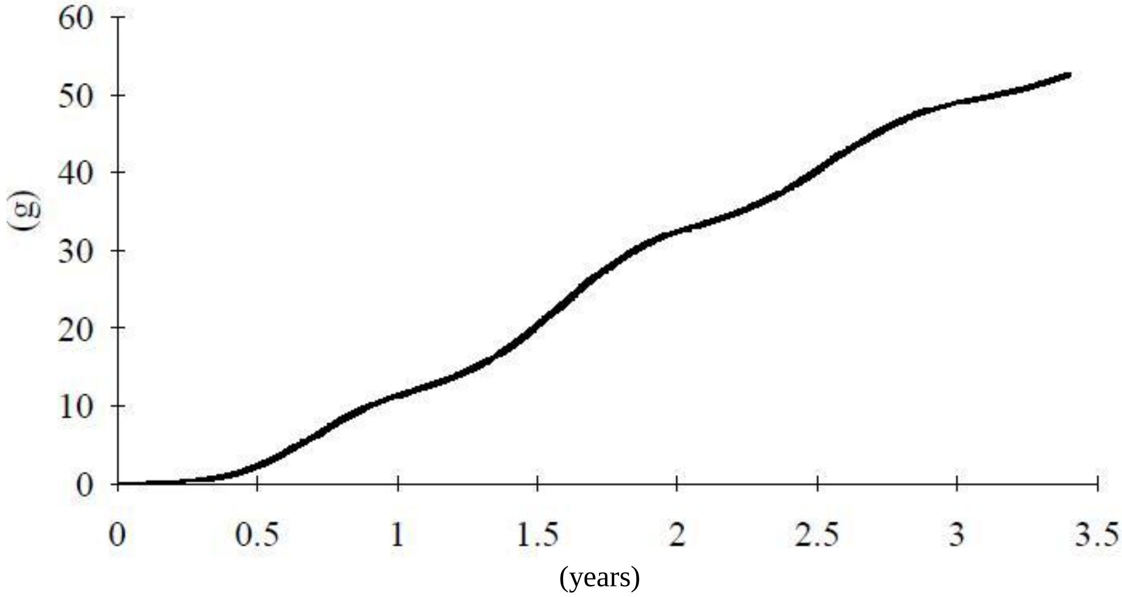



Figure A9.1 - Relation between crayfish weight (g) and age (years)

(Adapted from Anastácio, 1993)