

Research Article

Artificial water points for wildlife management facilitate the spread of red swamp crayfish (*Procambarus clarkii*)

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Abstract

The red swamp crayfish (*Procambarus clarkii*) has been widely introduced into aquatic systems all around the world, triggering multiple impacts. In Doñana Natural Area, Abalario and the Biological Reserve of Doñana, Spain (one of the most important biodiversity reserves in Europe), the red swamp crayfish was first introduced in 1974 and expanded its range quickly through marsh habitat, but distributed more slowly throughout scrubland ponds. Water persistence, salinity, and substrate can condition the presence of the species in the wild. Before the creation of the Natural Area and the Biological Reserve of Doñana, farmers dug artificial water points in some scrubland ponds to provide water throughout the year for livestock. Once declared a protected area, managers assumed control of the maintenance of the park and opened new water points as a wildlife management tool. The aim of the study presented here was to determine the environmental conditions that favor the spread of crayfish in the temporal ponds of Doñana and whether the occurrence of water points increases the spread of crayfish. For this purpose, we sampled crayfish using a sweep net in 60 randomly selected ponds in two different areas of Doñana. Crayfish occurrence was significantly related to the presence of seasonal streams connecting the ponds with refuge areas and to the presence of artificial water points within the pond. The probability of occurrence of red swamp crayfish in a pond without seasonal streams was more than 5 times lower than that in a pond with seasonal streams. The probability of crayfish occurrence in ponds without artificial water points was 2.5 times lower than that in ponds with artificial water points. We review the advantages and disadvantages of water points as a management tool.

Key words: pond, Special Protection Area, habitat management, engineering

Introduction

Alien species are considered to be one of the major drivers of the degradation of aquatic systems (Sala et al. 2000; Gherardi et al. 2009). Native to North America, the red swamp crayfish (*Procambarus clarkii* Girard, 1852) has been widely introduced into aquatic systems all around the world, triggering multiple impacts (Barbaresi and Gherardi 2000; Gherardi 2007; Savini et al. 2010). The species preys on native species (Gamradt et al. 1997; Cruz et al. 2006), decreases water quality (Rodríguez et al. 2003; Matsuzaki et al. 2009), reduces the content of organic matter in sediments (Angeler et al. 2001), and is thought to be responsible for transmitting diseases to related species (Diéguez-Uribeondo et al. 1997; Gil-Sánchez and Alba-Tercedor 2002). Also, it

has been shown to generate multiple cascade effects in some ecosystems (Geiger et al. 2005; Rodríguez et al. 2005; Tablado et al. 2010).

The Doñana Natural Area in SW Spain, which consists of a mixture of marshes and scrubland that also includes a National Park, is one of the most important biodiversity reserves in Europe. In the Doñana marshes, the red swamp crayfish was first introduced in 1974 (Habsburgo-Lorena 1979) and expanded quickly. At least since the early 1990s, the crayfish occupied temporal ponds within the sandy area, and its distribution showed remarkable inter-annual variation depending on the amount of rainfall recorded (Díaz-Paniagua et al. 2014). The most favourable conditions for the presence of the species in the wild are fresh water, clay soil for digging their galleries and permanent water (Montes et al. 1993). In drought years, its distribution in Doñana is restricted to sites

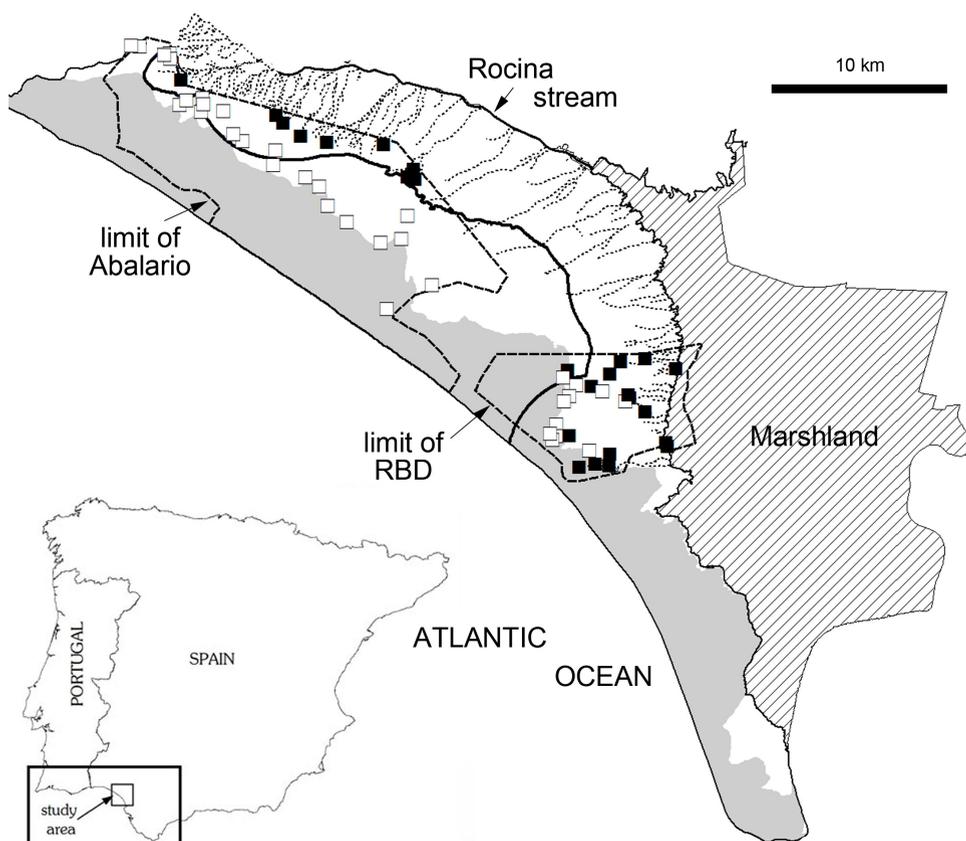


Figure 1. Study area of the Doñana Natural Area, Abalarío and the Biological Reserve of Doñana, Spain from March and April 1998. Black squares: ponds with red swamp crayfish; White squares: ponds without crayfish. The scrubland area is depicted in white; most of the ponds and temporal streams are located in this area. The gray area represents the most recent sandy deposits (Rodríguez 1998); ponds and streams are very scarce in this area. Rocina stream and Marshland are refuge areas. Abalarío and the Biological Reserve of Doñana (RBD) are the two study areas. Seasonal streams have been indicated with a dotted line.

where freshwater contributes to the marshland, either via surface runoff or from groundwater (Bravo-Utrera 2010). But in periods of high floods, the crayfish increase their distribution towards some of the abundant ($n > 600$; Bravo-Utrera 2010) temporary freshwater ponds with sandy substrate that are interspersed throughout the scrubland; these temporary freshwater ponds are of great importance for the conservation of Doñana's biodiversity (see Serrano et al. 2006 for a review). In these temporary ponds, the substrate and flooding regime (drying up in summer) prevent the long term survival of crayfish, which also limits species dispersal throughout Doñana (Díaz-Paniagua et al. 2014). In a previous study, Cruz and Rebelo (2007) described the ability of crayfish to colonize these temporary ponds in

Doñana at a distance of up to 1000 m from a refuge area. However, in wet years, we have observed freshwater ponds with crayfish over 5000 m away from these refuge areas, suggesting that distance is not the only factor involved in colonization. Another recent article suggests (but they did not empirically test) that artificial water points would promote the persistence of crayfish and contribute to its increased spread (Díaz-Paniagua et al. 2014). Thus, given that the impact of crayfish could be high even at very low density (Matsuzaki et al. 2009), analyzing the environmental conditions that favor the spread of crayfish in areas of the Doñana scrubland is a priority. The results of this study could help with the management of this important reserve and other protected areas of high biodiversity.

Methods

Study area

The study was conducted in two areas of stabilized dunes in the Doñana Natural Area, Abalario and the Biological Reserve of Doñana (RBD), in south-western Spain (Figure 1). Doñana is a flat region located along the right bank of the Guadalquivir River mouth. Before flowing into the Atlantic Ocean, the Guadalquivir River creates a marshland, which constitutes the eastern border of the study area. The whole study area is characterized by sandy soils of aeolian origin in a succession of ridges of different age running parallel to the coast (Rodríguez 1998; Zazo et al. 1999). The vegetation largely consists of scrubland sparsely populated with isolated large cork oaks (*Quercus suber*) and patches of pine plantations. The climate is Mediterranean sub-humid with marked seasons. Mean annual temperature is 17–19 °C. The mean minimum temperature of the coldest month is 4–10 °C, and the mean maximum temperature of the warmest month is 24–28 °C. Rain varies widely from year (range over the last 30 years: 170–1000 mm/m²; average about 550 mm/m²) falling almost exclusively in between autumn and winter. In years of high or average rainfall, and depending on the age of stabilized dunes, ponds and streams are formed. These streams are highly seasonal and appear almost exclusively in the oldest deposits (First Eolian System; Rodríguez 1998), located in the north of the Abalario, and in proximity to marshes in the RBD.

Previous studies have identified two areas of refuge, during dry periods, which are the centers of origin for the dispersal of crayfish: the marshland and Rocina stream. These refuge areas are the places where the crayfish survive even in the driest years, either in permanent water or in deep wet burrows (Figure 1; Montes et al. 1993).

Field sampling

We randomly sampled 33 ponds in Abalario and 27 in the RBD. In each pond, we sampled crayfish using a sweep net measuring 38×26 cm (mesh size = 2mm). Each time the net was inserted into the water, it was swept a length of about 1.5 m; this step was repeated three times on each occasion in order to create a stream of water to facilitate the capture of the specimens. This process was repeated on five consecutive occasions, sampling about 2 m² of water surface at each sampling point. Sampling points were

spaced at least 10 m apart and were distributed along two perpendicular transects oriented along the major axes of the pond. The number of sampling points was established according to the water surface, with a maximum of 10 sampling points per pond.

In order to determine the effectiveness of the sampling strategy, and to identify the probability of getting a false negative result, we estimated the probability of capturing at least 1 crayfish in a pond as: $p = (1 - (1 - F)^N)$, where F is the frequency of capture of crayfish and N is the number of sampling points.

The study was conducted during the last week of March and first week of April 1998. The period 1995–1998 was the wettest recorded in the region (2630 mm/m²), so it was particularly favorable for the expansion of the crayfish.

Habitat features

The presence of seasonal streams, distance to the nearest refuge area, and pond surface area were measured by GIS tools (ArcView 3.2). The hydrological regime of each of the ponds in both of the study areas was assigned according to Bravo and Montes (1993) in two groups: semi-permanent, defined as when flooding was probable for >8–9 months each year, and seasonal, defined as when flooding was probable for < 8–9 months. For ponds in Abalario, we assigned the northern ones (belonging to the Rivetehilos phreatic layer) as semi-permanent and the remaining ponds as seasonal. The presence of artificial water points was recorded in the field. These water points (locally known as "zacallones") are always excavations in the bottom of the pond usually made with an excavator bucket. They are around 5 m long, 2 m wide, and more than 1 meter deep. The number of sampling points in each pond was also included as a factor in the models (Table 1).

Generalized Linear Mixed Models (GLMMs) were used to describe the relationships between crayfish presence and pond features (presented in the Table 1). We used the procedure GLIMMIX of SAS 9.2. Crayfish presence/absence in the ponds was used the dependent factor. Area identification (RBD vs. Abalario) was introduced as a random factor to prevent pseudoreplication. We fitted a model with Binomial error and logit link, and the results of Type III analyses are presented. A forward stepwise procedure was used to add new variables into the model, using $p < 0.05$ and AIC values as criteria to evaluate whether the model should incorporate the new variable. The best set of

Table 1. Description and range of explanatory variables explored in the models to explore introduced crayfish (*Procambarus clarkii*) occurrence in the Doñana Natural Area, Abalario and the Biological Reserve of Doñana, Spain in March and April 1998. RBD= Biological Reserve of Doñana.

Code	Description	RBD n=27	Abalario n=33
<i>Location</i>			
<i>seasonal streams</i>	connection of the lagoon by a seasonal stream, with the nearest refuge area	with: n=12 without: n=15	with: n=13 without: n=20
<i>dist_ref</i>	distance (in km) to the nearest refuge area	3.7 ± 1.9	5.8 ± 2.1
<i>Pond features</i>			
<i>surface</i>	area of the pond (in hectares)	1.4 ± 2.1	2.0 ± 2.2
<i>flood</i>	hydrological regime	seasonal: n=14 semipermanent: n=13	seasonal: n=22 semipermanent: n=11
<i>water points</i>	man made water point	with: n=10 without: n=17	with: n=6 without: n=27
<i>Sampling</i>			
<i>intensity</i>	number of sampling points in each pond		1-10

candidate models for each response variable was tested by means of significance and AIC values. We also calculated their AIC weights (AICw; ranging from 0 to 1; highest being the best), which indicates the probability that the model was the best among the whole set of candidate models (Johnson and Omland 2004).

Results

The red swamp crayfish (*Procambarus clarkii*) was captured in 70 of 101 sampling points within ponds with crayfish, and therefore the probability of capture per sampling was $F=70/101=0.69$. Three sampling points within a single pond insured a probability of capture of 0.97. However, in three ponds without crayfish, we were only able to conduct three or less sampling points due to the limited area flooded. Even so, the probability of false negatives being incorporated into the data negligible. We detected the red swamp crayfish in 16 ponds in RBD (59.3%) and nine ponds in Abalario (27.3 %), 12 of which had a water point (75% of the ponds with water points).

Crayfish occurrence was positively related to the presence of seasonal streams connecting the ponds with refuge areas ($F_{1,57}=21.02$, $p<0.0001$) and to the presence of artificial water points within the pond ($F_{1,57}=5.48$, $p=0.023$). No additional variables were entered the model (Table 2). The probability of occurrence of red swamp crayfish in a pond without seasonal streams was 0.16 (0.06–0.38; Lsmeans, 95% confidence), which is

Table 2. Ranking of models for introduced crayfish (*Procambarus clarkii*) occurrence in the Doñana Natural Area, Abalario and the Biological Reserve of Doñana, Spain in March and April 1998. Only models with $\omega\text{AIC} > 0.001$ are shown. For brevity, we have used shortened variable names. Details can be found in Table 1.

Model no	Variables contained in the model	AIC	ΔAIC	ωAIC
1	seasonal stream + water point	46.87	0	0.876
2	seasonal stream	50.86	3.99	0.119
3	distance refuge + water point	57.66	10.79	0.004
4	distance refuge	61.18	14.31	0.001

more than five times lower than that in a pond with a seasonal stream (0.91, 0.72–0.98; Figure 2). The probability of crayfish occurrence in ponds without artificial water points was 0.31 (0.15–0.54) and 0.82 (0.47–0.96) in those with artificial water points. Therefore, the probability of crayfish occurrence is almost three times higher in ponds with artificial water points.

Discussion

Geomorphology facilitates the spread of introduced Procambarus clarkii

We found that introduced red swamp crayfish (*Procambarus clarkii*) were not able to naturally colonize some temporary ponds that are unconnected to refuge areas in the Doñana Natural Area,

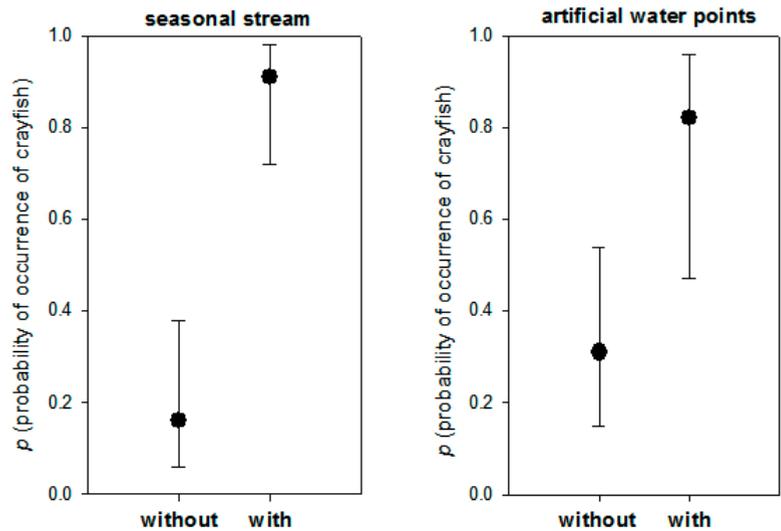


Figure 2. GLMM-derived probabilities of introduced crayfish (*Procambarus clarkii*) occurrence in the Doñana Natural Area, Abalarío and the Biological Reserve of Doñana, Spain in March and April 1998 as a function of artificial water points ($F_{1,57}=5.48$, $p=0.023$) and connection with refuge areas by a seasonal stream ($F_{1,57}=21.02$, $p<0.0001$).

Abalarío and the Biological Reserve of Doñana, Spain. Previous studies have described the ability of red swamp crayfish to move out of the water across land to other water bodies (Gherardi and Barbaresi 2000), which increases their ability to colonize and contributes to their high invasive potential (Gherardi 2007). In Doñana, Cruz and Rebelo (2007) indicated that crayfish colonize ponds located up to 1000 m away from refuge areas, but in our study, only three of the ponds with crayfish are located less than 1000 m from refuges ($3217\text{m} \pm 1701\text{m}$; mean \pm SD), suggesting that dispersal is mediated by ephemeral water courses (e.g. seasonal streams). In the course of our investigation we have repeatedly seen crayfish walking across the dam walls of rice cultures and crossing natural grasslands near the marshes, but not in the scrubland area away from water. In a study using pitfall traps in various terrestrial habitats throughout the region, we captured 2747 terrestrial vertebrates (including 28 species of amphibians, reptiles and mammals) and only one red swamp crayfish. This single crayfish was caught in a dry seasonal stream (J. Román, unpublished data). This observation highlights the importance of seasonal streams in the occupation of fresh water temporary ponds by the crayfish in Doñana. However, it is expected that all invading crayfish would disappear once the ponds dry up in summer, except in the few areas where water remains.

Water points facilitate the spread of introduced Procambarus clarkii

The presence of humans in Doñana for hundreds of years has resulted in the introduction of large numbers of livestock, mainly cows and horses. These herbivores are not naturally adapted to the temporal absence of water, and thus farmers dug artificial water points in some scrubland ponds to provide drinking water for cattle during dry periods. Artificial water points are also used as a source of drinking water by terrestrial wildlife. Following the declaration of the Doñana Natural Area, these water points were maintained and new ones were created by park rangers; in 2001 the number of water points in the National Park was 131 (Bañuls and Ramos 2002). These artificial water points allow for water persistence throughout the dry season, but also allow crayfish to thrive in areas where it would not normally be possible. Similar evidence has been found in Doñana for the North American corixid *Trichocorixa verticalis verticalis* (Fieber, 1851) for which the artificial water point might act as reservoirs of populations during the summer (Rodríguez-Pérez et al. 2009). However, the abundance of ponds in the region may facilitate the dispersion of individuals from one pond to another and serve as stepping stones to allow dispersal within the protected area. Although crayfish rarely reach ponds that are unconnected to refuge areas, once they do, the

presence of water allows them to survive droughts and facilitates dispersal to new locations. In arid environments of Australia, the spread of cane toads (*Bufo marinus*) was also mediated by artificial water points. In this case, limited access to artificial water points reduced the landscape connectivity between water points, which contained the spread of this invasive species (Florance et al. 2011).

Other negative effects associated with artificial water points have been reported and include changes in plant communities (Jeltsch et al. 1997; Todd 2006), soil nutrients (Tolsma et al. 1987; Rietkerk et al. 2000), and the distribution and movement of herbivores (Smit et al. 2007; Loarie et al. 2009; Fukuda et al. 2009), along with many other less obvious effects. For example, others have observed increases in predation rates due to the concentration of herbivores in the vicinity of water points (de Boer et al. 2010) or changes in the behavior of these herbivores associated with the risk of predation (Valeix et al. 2009). In Doñana, the spatial aggregation of cattle and wild ungulates at artificial water points makes both direct and indirect contacts more frequent and the transmission of bovine tuberculosis can be facilitated (Gortázar et al. 2008). This spatial aggregation of ungulates leads to severe alterations in pond vegetation as a result of trampling (Soriguer et al. 2001), as well as driving the local extinction of water vole (*Arvicola sapidus*) colonies (Román 2007).

Evidence documenting the benefits of the conservation of artificial water points for wildlife is merely circumstantial. For example, Iberian lynx (*Lynx pardinus*) usually drink water at these points and use the surrounding areas more frequently than expected by chance, but this occurs during both dry and rainy seasons (Palomares et al. 2001), and thus their use may be associated with other variables. Also, it has not been evaluated whether this use had a demographic effect on the species. In addition, previous studies have indicated that some scarce aquatic plants in the region, depending on permanent water, occur almost exclusively at these points in Doñana (Fernández-Zamudio et al. 2006; López-Albacete 2009).

In conclusion, this study presents clear evidence for the unexpected impact of a management tool in the Doñana Natural Area. It is possible that impacts observed here could be found in other natural reserves around the world, as the digging of water points is a widespread management tool across most dry areas, mainly in Africa and Australia. We believe this case provides an example

of the need for evidence-based conservation (Sutherland et al. 2004). Thus, further studies are needed to fully evaluate the pros and cons of these management tools, helping managers to make the right decisions concerning the maintenance of these artificial structures.

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