

This article was downloaded by: [Arizona State University]

On: 31 March 2014, At: 12:48

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Ethology Ecology & Evolution

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/tee20>

### Burrowing activity of the invasive red swamp crayfish, *Procambarus clarkii*, in fishponds of La Brenne (France)

C. Souty-Grosset<sup>a</sup>, J. Reynolds<sup>b</sup>, F. Gherardi<sup>c</sup>, L. Aquiloni<sup>ce</sup>, A. Coignet<sup>d</sup>, F. Pinet<sup>d</sup> & M. Del Mar Mancha Cisneros<sup>ad</sup>

<sup>a</sup> Laboratoire Ecologie et Biologie des Interactions, Equipe Ecologie Evolution Symbiose, Université de Poitiers, UMR CNRS 7267, Poitiers, France

<sup>b</sup> Trinity College, University of Dublin, Dublin, Ireland

<sup>c</sup> Dipartimento di Biologia, Università degli Studi di Firenze, Firenze, Italy

<sup>d</sup> Parc Naturel Régional de la Brenne, Rosnay, France

<sup>e</sup> Itinera C.E.R.T.A. scarl, Via Isidoro del Lungo 58, 52025 Montevarchi (Arezzo), Italy

Published online: 18 Mar 2014.

To cite this article: C. Souty-Grosset, J. Reynolds, F. Gherardi, L. Aquiloni, A. Coignet, F. Pinet & M. Del Mar Mancha Cisneros (2014): Burrowing activity of the invasive red swamp crayfish, *Procambarus clarkii*, in fishponds of La Brenne (France), *Ethology Ecology & Evolution*, DOI: [10.1080/03949370.2014.892538](https://doi.org/10.1080/03949370.2014.892538)

To link to this article: <http://dx.doi.org/10.1080/03949370.2014.892538>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

## Burrowing activity of the invasive red swamp crayfish, *Procambarus clarkii*, in fishponds of La Brenne (France)

C. SOUTY-GROSSET<sup>1,6</sup>, J. REYNOLDS<sup>2</sup>, F. GHERARDI<sup>3</sup>, L. AQUILONI<sup>3,5</sup>, A. COIGNET<sup>4</sup>,  
F. PINET<sup>4</sup> and M. DEL MAR MANCHA CISNEROS<sup>1,4</sup>

<sup>1</sup> Laboratoire Ecologie et Biologie des Interactions, Equipe Ecologie Evolution Symbiose, Université de Poitiers, UMR CNRS 7267, Poitiers, France

<sup>2</sup> Trinity College, University of Dublin, Dublin, Ireland

<sup>3</sup> Dipartimento di Biologia, Università degli Studi di Firenze, Firenze, Italy

<sup>4</sup> Parc Naturel Régional de la Brenne, Rosnay, France

<sup>5</sup> Itinera C.E.R.T.A. scarl, Via Isidoro del Lungo 58, 52025 Montevarchi (Arezzo), Italy

Received 27 August 2013, accepted 13 January 2014

In 2007, the invasive crayfish *Procambarus clarkii* (Girard 1852) was identified for the first time in the "Parc Naturel Régional de la Brenne" (Indre department, Centre region, France). Ten infestation sites were found in the park, with 62 fishponds colonised. To better understand how the fishponds could be affected, and changes in invaded ponds, we followed crayfish burrowing activity in recently drained fishponds in two areas. Weekly investigations of burrowing activity by analysing their density, occupation and location with respect to the banks, and micro-habitat features were carried out for 2 months in spring. Burrow densities of 1.16 and 1.05 burrows m<sup>-2</sup> were found at Le Terrier Blanc and Coudreau 3 respectively. *Procambarus clarkii* sought humid soil in a dry pond and preferred burrowing on silt substrates. While no statistical differences were found between sites, some differences were observed among weeks independently of the sites. The mean mouth diameter of burrows in the two sites was 5.7 cm (SD = 2.17,  $n = 214$ ; min. value: 1.2 cm; max. value: 14 cm). Notwithstanding the situation in the last weeks of the study (when most chimneys were built), there was a certain turnover between open and plugged burrows, and in occupancy. Given the scale and duration of the study and the number of fishponds surveyed, this study successfully quantified the dynamics of burrowing in these ponds and conclusions provide a useful basis for future studies and give suggestions of how other colonised fishponds may be affected.

KEY WORDS: burrowing, wetlands, fishponds, *Procambarus clarkii*, red swamp crayfish, burrow size, bank damage.

---

<sup>6</sup> Corresponding author: Catherine Souty-Grosset, Université de Poitiers, UMR CNRS 7267, Laboratoire Ecologie et Biologie des Interactions, Equipe Ecologie Evolution Symbiose, 40 Avenue du Recteur Pineau, 86022 Poitiers Cedex, France (E-mail: [catherine.souty@univ-poitiers.fr](mailto:catherine.souty@univ-poitiers.fr)).

## INTRODUCTION

Ecologically important invaders in fresh waters include decapods that act as powerful omnivores, which probably have been underestimated as an ecological force (STRAYER 2010). At present, more than 20 species of freshwater crayfish have been introduced worldwide for human food, fish forage and bait (GHERARDI 2011). Most of these have become invasive, i.e. species that, after their establishment, exert negative impacts on biodiversity and ecosystem services. In Europe, the extinction of many native crayfish populations during the mid-nineteenth century had led to the introduction of alien species as a possible solution to replace native populations (LODGE et al. 2000; SOUTY-GROSSET et al. 2006; SAVINI et al. 2010). Nowadays most countries in Western Europe are affected by alien crayfish species (HOLDICH et al. 2009).

A notorious case study is the red swamp crayfish, *Procambarus clarkii* (Girard 1852), which is a paradigmatic example of the consequences of uncontrolled introductions of species (HOBBS et al. 1989), illustrating also the complexities of preventing, eradicating and controlling invasions (reviewed in GHERARDI 2007).

Native to south-central USA and northeastern Mexico, *P. clarkii* was first introduced into Spain in 1973 with the aim of improving the economy of a depressed area in Andalusia by developing crayfish commerce (HENTTONEN & HUNER 1999; HUNER 2002). The success of this initiative led to its illegal importation to France and Italy in the 1970s–1990s and other introductions to the UK, Germany, Cyprus, Switzerland, the Netherlands and Portugal (SOUTY-GROSSET et al. 2006; HOLDICH et al. 2009). Much concern was raised among farmers and fishermen, due on one hand to the extensive agricultural damage inflicted by its burrowing and destroying plants (REYNOLDS & SOUTY-GROSSET 2012), and on the other to interference with fishing (reviewed in REYNOLDS 2011; LODGE et al. 2012). From an ecological perspective, irreversible changes in native floras and faunas were induced by this species, food webs of Mediterranean wetlands were altered, and the structure and functioning of these ecosystems were dramatically modified (GUTIÉRREZ-YURRITA et al. 1998; GEIGER et al. 2005; GHERARDI & BARBARESI 2007), including the Doñana marshes in Spain (BRAVO et al. 1994). In lake Chozas (NW Spain), the red swamp crayfish induced a reduction in plant density from 97% to less than 10% surface cover 3 years after its introduction (RODRÍGUEZ et al. 2003). In such habitats, often deprived of water for some part of the year, the success of this species as an invader is associated with its elevated tolerance to drought and is facilitated by its large reproductive output, short development and flexible feeding habits (GHERARDI 2006). This species harbours the crayfish plague oomycete (listed among the 100 of the worst invasive alien species in Europe; LOWE et al. 2000; GHERARDI & PANOV 2009) and is also known to be responsible for the largest range of negative ecological impacts among the top 27 alien animals introduced into Europe for aquaculture and related activities (SAVINI et al. 2010). Consequently, its impacts range from outcompeting native species to altering food web composition and habitat structure. The role of crayfish herbivory on macrophyte destruction had a trophic cascade effect on the wetland ecosystem (RODRÍGUEZ et al. 2005). According to these authors, crayfish had a major role in submerged plant destruction and a potential effect on Amphibia and macroinvertebrate population decrease. Plant destruction (99% plant coverage reduction) was directly related to invertebrates (71% losses in macroinvertebrate genera), Amphibia (83% reductions in species) and waterfowl (52% reduction).

In 2007, *P. clarkii* was found for the first time in the territory of the “Parc Naturel Régional de la Brenne”, a wetland located in the southwestern area of the Indre

department, west of Chateauroux in the centre of France. Ten infestation sites have been identified in the park, including 62 fishponds colonised by *P. clarkii* (COIGNET et al. 2012). In this area, there is a need to better understand the current status of the ongoing invasion by *P. clarkii* with the aims of understanding the species that are being impacted by it and how the habitat could be affected, for example by burrowing.

This crayfish species is considered to be a tertiary burrower, living mostly in open water and retreating to burrows during dry and low water periods to avoid desiccation and find protective cover (HUNER & BARR 1991). Previous studies have quantified *P. clarkii*'s burrowing in different water bodies of Europe; burrows may be temporary or permanent, whether they persist or not from year to year (CORREIA & FERREIRA 1995; ILHEU et al. 2003; BARBARESI et al. 2004). In general, *P. clarkii*'s burrows are simple in their morphology, with one tunnel and a single entrance. Burrow tops can be covered with a mud plug or a chimney, and burrow bottoms are usually enlarged into a terminal chamber. Adult *P. clarkii* can survive for up to 1 year under simulated burrow conditions without any food due to their reduction in metabolism, as long as there is still some free water (HUNER & BARR 1991). Based on the burrow's relationship to the water table, WELCH & EVERSOLE (2006) indicated that *P. clarkii* retain water in the burrow, independent of the water table.

Our aim here is to develop methodology suited to conditions in La Brenne in order to better understand how the traditional fishponds may be affected, and changes in invaded ponds. We followed crayfish burrowing activity in recently drained ponds. The ultimate goal is to identify factors in this ecosystem inducing burrow digging, and habitat features that make a site susceptible to damage from crayfish burrowing.

## MATERIALS AND METHODS

### Study area

The region of La Brenne is one of the most important French humid continental zones. Its mosaic of landscapes, including grasslands, ponds, mires, forests, valleys and exceptional richness in fauna and flora, have led to its recognition as an International Ramsar wetland zone since 1991. There are around 3700 artificial fishponds (between 0.8 and 1.5 m in depth and from 1000 m<sup>2</sup> to 180 ha) organised in chains within watersheds. Some fishponds are traditionally used for fish-farming and fished every year between October and March, but can also be drained from autumn to spring. Generally, the furthest downstream ponds are first emptied so that they can be filled with the water coming from the next pond upstream. Fishponds are re-filled by rain and by water draining from the land around.

Crayfish burrowing activity was studied in two contrasting sites: Coudreau (two typical fishponds) and Le Terrier Blanc (one pond and its adjacent wet grassland). Both sites presented differences with respect to water availability. Their general characteristics are shown in Table 1. At the beginning of the study, the pond floors were covered with mud and almost no aquatic vegetation, probably due to the season. Crayfish burrows were not uniformly distributed along the banks of fishponds.

Coudreau Fishponds (X: 1°15'06.0"E; Y: 46°41'55.3"N): since 2009, 6357 crayfish have been trapped in Coudreau 1 and 22 641 in Coudreau 3, the latter having a zone where burrows were observed.

Le Terrier Blanc Fishpond (X: 1°10'25.0"E; Y: 46°34'30.2"N): *P. clarkii* was first detected in 2007 but trapping began in August 2010, yielding 7246 crayfish; this pond was mainly used for leisure purposes and had not been drained in 4 years. The water outlet is at the north end and there is a water inlet from a ditch in the eastern side, where burrows were also noticed. The southern area, where passing cattle had left many tracks and holes, was particularly suitable for burrowing.

Table 1.  
General characteristics of the studied ponds.

Site	Pond	Area (ha)	General description	Fish present
La Rucherie	Coudreau 1	0.93	Very steep banks flanking flood-gate, gentle flattening on opposite side. Small grasses and short vegetation on banks, no shrubs. Tall trees on south side and along eastern side. Water inlet from the northern portion through a ditch.	Pumpkin sunfish ( <i>Lepomis gibbosus</i> ) and common roach ( <i>Rutilus rutilus</i> ).
	Coudreau 3	1.36	Steep banks flanking flood-gate, gentle flattening on opposite side where also some shrubs grow. Rocks, detritus and some shrubs along the bank in northwestern side and much more shrub cover on eastern edge.	
Le Terrier Blanc	Le Terrier Blanc	1.69	Gradual slopes flanking flood-gate and all the way through eastern edge, but becoming steeper on western edge. Heavy shrub cover along most of the bank. Tall trees along western and northern sides.	Common carp ( <i>Cyprinus carpio</i> ), along with some common roach ( <i>Rutilus rutilus</i> ), tench ( <i>Tinca tinca</i> ), pike ( <i>Esox lucius</i> ), pikeperch ( <i>Sander lucioperca</i> ), common perch ( <i>Perca fluviatilis</i> ) and pumpkin sunfish ( <i>Lepomis gibbosus</i> ).
	Area to the south	–	Flat area in grassland zone with mostly grass vegetation in western side and zone of bare soil on eastern side. Tall trees along the western side, the eastern side extends into a larger grassland zone.	

This area retained standing water during most of the duration of the study, partly fed by water coming from a small ditch even after the draining of the fishpond.

#### *Methods for assessing burrowing impacts*

Two distinct areas were selected for survey in each pond: zones of 112 m<sup>2</sup> (16 × 7 m) and transects of 20 m<sup>2</sup> each (25 × 0.80 m) were defined along the banks.

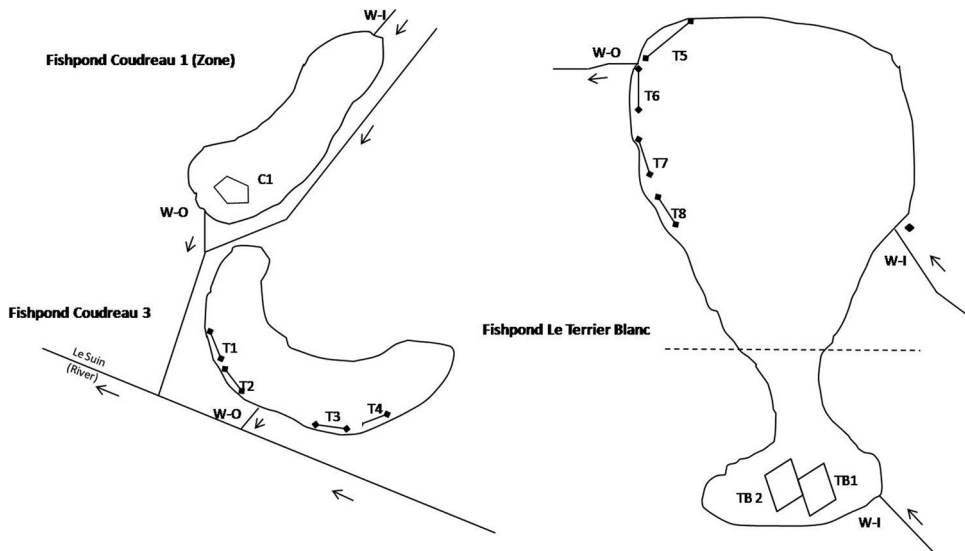


Fig. 1. — Two fishponds: Coudreau 1 with the studied Zone C1; Coudreau 3, showing the four transects T1, T2, T3 and T4. Fishpond Le Terrier Blanc: wet grassland area in the south where cattle walked through, showing zones TB1 and TB2; fishpond Le Terrier Blanc, showing the four transects T5, T6, T7 and T8. W-I and W-O indicate water inlets and outlets respectively.

Coudreau Fishponds (Fig. 1): In Coudreau 1, a zone with burrows (C1) was surveyed. In Coudreau 3, four transects (T1–T4) were selected based on the burrow density observed during the first survey.

Le Terrier Fishpond (Fig. 1): two neighbouring zones (TB1 and TB2) were surveyed in the wet grassland area with cattle tracks, and four transects (T5–T8) along the western side. The eastern side of TB1 had more standing water, but less vegetation, than TB2. During the first visit to the fishpond, most burrows were observed on the western side, and most of the eastern side had much fresh mud but not as many burrows.

On the first visit, burrows were marked with individual numbered pegs and their mouth diameter recorded. During each weekly inspection, they were checked for signs of crayfish occupation and signs of activity. When recording burrow occupation, crayfish were not extracted from the burrows to avoid disturbance and damage. Table 2 lists the terms used for burrow occupancy and burrowing activity.

At the end of the study, each burrow was manually inspected for occupation by digging the burrow out. Along the banks, each burrow was assigned to one of the following categories depending on its position: “Under the bank” when the burrow was situated in an area where the fishpond’s floor goes under the bank, “On the bank” when the burrow was on the bank, and “Open” when the burrow was out in the open yet inside the transect. Micro-habitats were also recorded for each burrow depending on whether the burrow was in an area of vegetation, clay or silt soils.

#### Data analysis

The 112-m<sup>2</sup> areas were compared for signs of burrow occupancy and burrowing activity in each week using G-tests for independent frequencies with Williams’s correction. McNemar’s test for change was used when comparing paired variables from the same site using the program R.

Table 2.

List of terms adopted for burrow occupancy and burrowing activity.

List of terms adopted for burrow occupancy and burrowing activity.		
Signs of occupation		
Occupied	crayfish occupant	seen from the exterior
	digging signs	burrow with fresh signs of digging
	mud plug	burrow with a mud plug
	compound signs	burrow showing either of the activities mentioned above
Non-occupied		burrow with no sign of occupation
Collapsed		burrow no longer visible or identifiable
Signs of activity		
	new	newly recorded burrow
	digging	burrow with fresh signs of digging
	mud plug	burrow with a mud plug
	compound signs	burrow showing either of the activities mentioned above
	inactive	burrow with no signs of activity at all

The hypothesis tested here was whether the number of occupied burrows or activity category changed significantly between the beginning and the end of the study. Independence of burrow location with respect to the two sites where transects were surveyed was tested using G-tests for independent frequencies with William's correction. The same test was performed on microhabitats. One-way repeated measures analyses of variance (ANOVAs) were used to analyse the relationships between independent and dependent variables. Finally, a Pearson correlation test ( $r$ ) was performed under SPSS software package (version 18, IBM, New York, USA).

## RESULTS

### *Signs of burrow occupancy and burrowing activity in transects*

There were no noticeable changes in any transects surveyed during the study. Therefore, signs of burrow occupancy and burrowing activity were analysed. Rain events decreased at both sites as the study progressed. The mud changed from a very moist texture with puddles to a very dry surface where one could easily walk. In weeks w3 and w5 feral pig footprints were found inside the zone in Coudreau 1 as well as many broken or displaced marker pegs and many collapsed burrows. Very little crayfish activity was recorded in the area afterwards. Burrow densities of the three zones and the eight transects are shown in Tables 3 and 4, respectively. A total of 93 burrows was counted along the transects of Le Terrier Blanc, and 84 along the transects of Coudreau 3. Pooling the number of burrows of all transects together, burrow densities of 1.16 and 1.05 burrows  $m^{-2}$  were found at Le Terrier Blanc and Coudreau 3 respectively.

In Le Terrier Blanc, an increase in occupied burrows during week 4 was recorded, mostly reflected by the increase of mud plugs and digging signs. The number of occupied, non-occupied and collapsed burrows showed no differences between TB 1 and TB 2



Table 3.

Burrow densities of the three zones i.e. number of burrows m<sup>-2</sup> over a zone of 112 m<sup>2</sup> recorded during a period of 7 weeks at Coudreau 1, and 5 weeks at Le Terrier Blanc.

Fishpond	Mean density (SE)	Minimum	Maximum
Coudreau 1	0.17 (0.05)	0.03	0.35
Le Terrier Blanc			
TB1	0.39 (0.01)	0.35	0.41
TB2	0.53 (0.02)	0.47	0.58

Table 4.

Burrow densities of transects shown as number of burrows m<sup>-2</sup> over a transect area of 20 m<sup>2</sup> and recorded during a period of 7 weeks at Coudreau 3 and 5 weeks at Le Terrier Blanc.

Fishpond		Mean density (SE)	Minimum	Maximum
Coudreau 3				
	T1	0.56	0.45	0.60
	T2	0.84	0.80	0.85
	T3	0.98	0.95	1.00
	T4	1.69	1.40	1.75
Le Terrier Blanc				
	T5	0.70		
	T6	0.70 (0.02)	0.65	0.75
	T7	2.25		
	T8	0.95		

whatever the week. Differences emerged only when comparing the three zones for the same week (week 1 for TB 1 and TB 2 at Le Terrier Blanc and week 2 for Coudreau 1; G-test,  $G = 12.981$ ,  $df = 4$ ,  $P < 0.05$ ). This was also the case when looking at all the different categories of burrowing activity (G-Test,  $G = 22.267$ ,  $df = 8$ ,  $P < 0.01$ ) as well as with the pooled data of active and inactive burrows (G-Test,  $G = 12.121$ ,  $df = 2$ ,  $P < 0.01$ ).

Tables 5 and 6 show the data expressed as percentages. When comparing the number of occupied and non-occupied burrows at the start and the end of the study,

Table 5.

Percentage of occupied, non-occupied and collapsed burrows during week 2.

%	TB1	TB2	Coudreau 1
Occupied	6.7	11.9	46.5
Non-occupied	93.3	88.1	48.8
Collapsed	0	0	4.7

Table 6.  
Percentage of inactive, new, digging, mud plugs, of burrowing activity during week 2.

%	TB1	TB2	Coudreau 1
Inactive	93.3	91.5	51.2
New	2.2	3.4	7.0
Digging	4.4	3.4	11.6
Mud plug	0	1.7	30.2

no significant difference was found at Le Terrier Blanc. However, significant differences resulted from the comparison of active and inactive burrows at zone TB 1 (McNemar,  $\chi^2 = 11.25$ ,  $df = 1$ ,  $P < 0.001$ ) and TB 2 at Le Terrier Blanc (McNemar,  $\chi^2 = 10.0833$ ,  $df = 1$ ,  $P < 0.01$ ), although this could be attributed to the new burrows being counted as a sign of burrowing activity and not of occupancy. A burrow may have been recent but it did not necessarily mean that it was occupied at that moment in time. Tests for change between the beginning and the end of the study were not performed at Coudreau 3 due to the intrusion of feral pigs in weeks 3 and 5.

#### *Burrows from the banks*

Burrow location within the bank showed differences between Le Terrier Blanc and Coudreau 3, particularly for the number of burrows in the open (G-Test,  $G = 6.970$ ,  $df = 2$ ,  $P < 0.05$ ; Fig. 2). Microhabitats also showed differences between the sites, particularly in the number of burrows on clay substrate and in vegetated zones (G-test,  $G = 14.156$ ,  $df = 2$ ,  $P < 0.001$ ; Fig. 3).

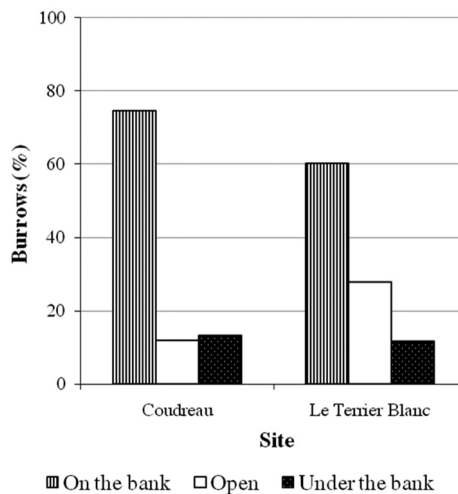


Fig. 2. — Percentage of burrows according to their location relative to the bank, per site.

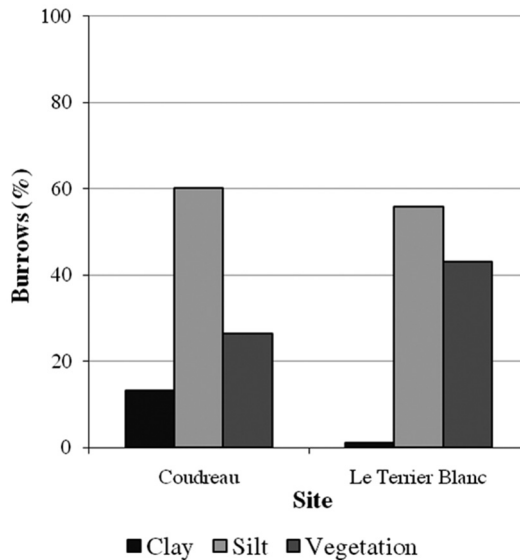


Fig. 3. — Percentage of burrows according to the microhabitat on the bank, per site.

#### *Comparison between open field and banks*

Since Coudreau was affected by feral pigs, a comparison between Coudreau and Le Terrier Blanc of mean numbers of burrows on the banks for the same six weeks was made with repeated measures ANOVA. The analysis showed no statistical difference between sites ( $F = 0.061$ ,  $df = 1,30$ ,  $P = 0.813$ ) but difference among weeks ( $F = 3.216$ ,  $df = 5,30$ ,  $P = 0.019$ ) independently of the sites (i.e. the two sites show the same difference with time) ( $F = 0.626$ ,  $df = 5,30$ ,  $P = 0.681$ ). The data from the transects have been pooled because the difference among them is minimal. The difference in density between the burrows made in the open (zone) and the burrows made on or near the banks (transects) was computed with data from La Terrier Blanc only. The comparison with repeated measures ANOVA showed no difference between burrows in the open and on banks ( $F = 1.477$ ,  $df = 1,4$ ,  $P = 0.291$ ), but the number of burrows increased over time ( $F = 5.008$ ,  $df = 5,20$ ,  $P = 0.004$ ) in both habitats ( $F = 1.380$ ,  $df = 5,20$ ,  $P = 0.274$ ). Since the two zones analysed in Le Terrier Blanc did not show any significant difference, the data from them were pooled. Analyses were done on the relative frequency (in %) of (1) occupied burrows (i.e. burrows that showed some signs of occupancy as above), (2) collapsed burrows, and (3) burrows with mud plugs (chimneys). In all cases, something happened at the fourth week. The Pearson correlation test showed no significant increase with time for the frequency of both occupancy ( $r = 0.658$ ,  $df = 4$ ,  $P = 0.155$ ) and the mud plugs ( $r = 0.5836$ ,  $df = 4$ ,  $P = 0.528$ ), but the difference was nearly significant for the frequency of collapsed burrows ( $r = 0.778$ ,  $df = 4$ ,  $P = 0.06$ ).

#### *Transitions between open and closed states of burrows*

The transition between two states of the burrow (mouth open and mouth closed with mud plugs) is shown in Table 7.

Table 7.

Transitions between two states of the burrow (mouth open and mouth closed with mud plugs).

	Open-closed	Closed-open	Closed-closed	Open-open
Obs	21	7	8	64
Exp	25	25	25	25
	$P > 0.05$	$P < 0.01$	$P < 0.01$	$P < 0.001$
		$G = 10.576$	$G = 9.056$	$G = 17.585$
		$df = 1$	$df = 1$	$df = 1$

The overall difference between the frequencies of transitions observed and expected was  $G = 76.31$ ,  $df = 3$ ,  $P < 0.001$ . More burrows than expected remained open but the frequency of burrows that switched from the open to the closed state was the same as expected. On the other hand, it was less obvious that the burrows that maintain the closed state were less frequent than expected. There were some instances of closed burrows that became open, although their observed frequency was less than expected.

#### *Diameters of burrow mouths*

No difference in mouth diameter was found in Coudreau (among nine transects: one-way ANOVA:  $F = 1.25$ ,  $df = 8,112$ ) and in Le Terrier Blanc (among four transects: one-way ANOVA:  $F = 4.75$ ,  $df = 3,89$ ). The data from all transects per site were therefore pooled. The two sites were compared for the size class distribution of the burrow mouth.

The mean mouth diameter in the two sites was 5.7 cm (SD = 2.17,  $n = 214$ ; min. value: 1.2 cm; max. value: 14 cm). No difference was found between the two frequency distributions ( $G = 3.499$ ,  $df = 7$ ,  $P > 0.1$ ). Both zones analysed in Le Terrier Blanc did not differ significantly for the mouth diameter (one-way ANOVA,  $F = 3.12$ ,  $df = 1,111$ ). The data from the two zones were therefore pooled and organised in a size class frequency distribution. The mean mouth diameter for Le Terrier Blanc was 6.11 cm (SD = 2.28 cm,  $n = 113$ ; min. value: 1.5 cm, max. value: 13 cm). Frequency distributions of mouth diameters were compared between transects and zones. No difference was found ( $G = 4.958$ ,  $df = 7$ ,  $P > 0.1$ ), although the burrows in the open that had larger mouths appeared to be more frequent.

## DISCUSSION

The present study was successful in quantifying aspects of the dynamics of burrowing, with implications for the impacts on fishponds in La Brenne. In Le Terrier Blanc, an increase in occupied burrows from the fourth week on was recorded, mostly reflected by the increase of mud plugs and digging signs. Significant differences were found when comparing active and inactive burrows. As already shown by HUNER & BARR (1991) *Procambarus clarkii* can survive low water conditions and dry periods by retreating into burrows, and low water oxygen concentrations by using atmospheric

oxygen. In La Brenne, we had an opportunity to analyse *P. clarkii*'s burrowing activity in dry fishponds involving two different situations with respect to water availability. It was suspected that *P. clarkii* would show higher burrowing activity in the case of Coudreau because they would experience the loss of water after draining and immediately retreat to burrows. The apparent increase in mud plugs and signs of digging in week 4 may be indicative of the loss of moisture in the soil and thus a change in behaviour of the crayfish to create mud plugs and prevent further evaporation. Since rain events ceased during the last three weeks of investigation, it was also expected that, as the fishponds continued to dry fully and the soil to lose moisture, burrowing activity would eventually cease because *P. clarkii* needs to have at least some moisture in the soil in order to survive. When burrows were manually inspected at the end, most of them retained moisture at their deepest point.

Hydrological features seem to be important factors in determining burrowing activity, as CORREIA & FERREIRA (1995) generally observed from May to October in Portugal. The first appearance of burrows was observed when the water levels began to recede; indeed, in sites prone to water fluctuations, burrowing depended partly on changed water levels whereas in sites where water was permanent, burrowing only related to the life cycle of *P. clarkii*. In our case, only one berried female was detected during the last inspection. Thus, the annual draining of some of the fishponds in La Brenne presents a unique opportunity to study the importance of hydrological features for the survival and proliferation of *P. clarkii*. Draining speed could be manipulated in order to observe how *P. clarkii*'s behaviour changes through the year.

Burrow location relative to the bank showed differences between Le Terrier Blanc and Coudreau 3, particularly for the number of burrows in the open field. Microhabitats also showed differences between both sites, particularly in the number of burrows on clay substrate and in vegetated zones. In the 20-m<sup>2</sup> transects along the banks, changes in the number of burrows remained constant through the study, and signs of occupancy and activity were never noticed. The differences with respect to the activity recorded in the 112-m<sup>2</sup> zones could be related to the structure of the banks. The bank of a fishpond is part of the terrestrial system surrounding the pond; thus, it is usually drier than the pond bottom. Also, when water recedes towards the flood-gate during the draining of a fishpond, concave areas in the middle of the pond are more likely to keep stagnant water than the slopes of the banks, especially if such slopes are steep. Thus humidity can be quickly lost at the level of the banks. The mud in the centre of Coudreau 3 remained very moist until week 3 or 4, whereas the banks dried rapidly after draining. It was the same for Le Terrier Blanc. It could be hypothesised that in a situation of rapid water loss, crayfish try to find the most humid substrate to hide in, which is why more activity was observed in the zones that retained water during the beginning of the study than in the transects where soil dried up faster. High densities of burrows were localised along the banks of each fishpond. While burrow density varied only slightly from transect to transect, not all parts of the bank showed burrows. This could indicate that some characteristics of the habitat are more suitable for burrowing than others. Location of the burrows with respect to the bank in the transect study reflects this. There were more burrows found out in the "open field" at Le Terrier Blanc than at Coudreau 3, which was characterised by small and medium sized rocks next to the bank; this type of environment is not suitable for crayfish digging. Although they can use rocks as shelters and hide underneath, crayfish seem to be more likely to dig on bare substrates. Further studies should follow how the structure of these banks changes through the years to see if a higher density of burrows actually damages the bank significantly more than just natural water erosion.

The analysis showed no statistical difference between sites but difference among weeks independently of the sites. There was no difference between burrows in the open field and in banks, but the number of burrows increased over time in both habitats. In the 4th week, there was no significant increase with time for the frequency of both burrow occupancy and the mud plugs, but the difference was nearly significant for the frequency of collapsed burrows. With respect to burrow microhabitats, CORREIA & FERREIRA (1995) and BARBARESI et al. (2004) found that burrow density increases with the amount of fine sediment in the soil. Due to time constraints, the present study included a simple inspection of substrate type with respect to silt and soil. We identified more burrows in clay substrates at Coudreau. It was also expected that trees along the banks probably made it more difficult to dig than in soil because the roots get in the way. Although the number of burrows in a vegetated microhabitat was not quantified for the 112-m<sup>2</sup> zones, Zone TB 2 had more grass cover and fewer burrows than Zone TB 1, which had more standing water and soft substrate as well as more burrows.

The overall difference between the frequencies of transitions (between mouth open and mouth closed with mud plugs) observed and expected was that more burrows than expected remained open but the frequency of burrows that switched from the open to the closed state was the same as expected. Usually, a chimney is built in unfavourable situations (no water, too cold, etc.). Most burrows remained open, possibly because at least some of them were not occupied. However, burrows that remained closed were less frequent than expected, indicating that some had been re-opened. There were some instances of closed burrows that became open, but their observed frequency was less than expected. Despite the adverse situation in the last weeks of the study (when most chimneys were built) there is, however, a certain turnover concerning observations of open or plugged.

Finally, no difference was found between the two frequency distributions of burrow mouth size at the two sites. Frequency distributions of mouth diameters were compared between transects and zones. Again, no difference was found, although the burrows in the open that had larger mouths appeared to be more frequent.

The present study shows that the ecological success of *P. clarkii* seems to depend on its ability to withstand harsh environmental conditions, especially during dry periods. In such situations, crayfish seem to burrow mainly for protection purposes against predators and from difficult environmental conditions such as water evaporation and hot temperatures (ILHEU et al. 2003). It seems likely that *P. clarkii* can successfully survive in dry fishponds of La Brenne so long as there is some water supply such as a small rivulet or rainwater. Their movement may be limited, however, due to low food resources and harsh environmental conditions. When such constraints remain for longer periods, *P. clarkii* seems to have lower chances of survival. Further long-term studies considering different draining speeds should be performed in order to reach more decisive conclusions with respect to what characteristics make a fishpond of La Brenne prone to be affected by *P. clarkii*'s burrowing activity.

To conclude, the present study was primarily successful in quantifying the dynamics of burrowing rather than the overall impact of *P. clarkii* on the fishponds of La Brenne, and it gives a view of how other colonised fishponds with similar characteristics may be affected. New protocols can now be developed based on the advantages and limitations found here. For future studies, the protocol followed by BARBARESI et al. (2004) in water-filled fishponds should be used in combination with the present study methodology during the summer season, to survey *P. clarkii*'s activity under various environmental conditions.

## ACKNOWLEDGEMENTS

Maria del Mar Mancha Cisneros was financially supported by the European Commission through the program Erasmus Mundus Master Course – “European Master in Applied Ecology” (EMMC-EMAE) (FPA 2008-0092/001 FRAME MUNDB123) – and by the Natural Park of Brenne.

Francesca Gherardi was grant-aided by the Regional Council of Poitou Charentes (France) within the “Programme Régional de Bourses de Chercheur Invité” at the University of Poitiers.

## REFERENCES

- BARBARESI S., TRICARICO E. & GHERARDI F. 2004. Factors inducing the intense burrowing activity of the red-swamp crayfish, *Procambarus clarkii*, an invasive species. *Naturwissenschaften* 91: 342–345. doi:10.1007/s00114-004-0533-9.
- BRAVO M.A., DUARTE C.M. & MONTES C. 1994. Environmental factors controlling the life history of *Procambarus clarkii* (Decapoda, Cambaridae) in temporary marsh of the Doñana National Park (SW Spain). *Verhandlungen der Internationale Vereinigung für Limnologie* 25: 2450–2453.
- COIGNET A., PINET F. & SOUTY-GROSSET C. 2012. Estimating population size of the red swamp crayfish (*Procambarus clarkii*) in fish-ponds (Brenne, Central France). *Knowledge and Management of Aquatic Ecosystems* 406: art. 02, 11 pp. doi:10.1051/kmae/2012019.
- CORREIA A.M. & FERREIRA O. 1995. Burrowing behavior of the introduced red swamp crayfish *Procambarus clarkii* (Decapoda: Cambaridae) in Portugal. *Journal of Crustacean Biology* 15 (2): 248–257. doi:10.2307/1548953.
- GEIGER W., ALCORLO P., BALTANÁS A. & MONTES C. 2005. Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands. *Biological Invasions* 7: 49–73. doi:10.1007/s10530-004-9635-8.
- GHERARDI F. 2006. Crayfish invading Europe: the case study of *Procambarus clarkii*. *Marine and Freshwater Behaviour and Physiology* 39: 175–191. doi:10.1080/10236240600869702.
- GHERARDI F. 2007. Understanding the impact of invasive crayfish, pp. 507–542. In: Gherardi F., Ed. *Biological invaders in inland waters: profiles, distribution, and threats. Invading nature. Vol. 2. The Netherlands: Springer Series in Invasion Ecology.*
- GHERARDI F. 2011. Crayfish, pp. 129–135. In: Simberloff D. & Rejmánek M., Eds. *Encyclopedia of biological invasions. Berkeley and Los Angeles: University of California Press.*
- GHERARDI F. & BARBARESI S. 2007. Feeding preferences of the invasive crayfish, *Procambarus clarkii*. *Bulletin Français de la Pêche et de la Pisciculture* 385: 7–20. doi:10.1051/kmae:2007014.
- GHERARDI F. & PANOVA V.E. 2009. Alien species fact sheets: *Procambarus clarkii* (Girard, 1852), red swamp crayfish/crawfish (Cambaridae, Crustacea), p. 316. In: Daisie P.P.E., Ed. *Handbook of alien species in Europe. Dordrecht, The Netherlands: Springer.*
- GUTIÉRREZ-YURRITA P.J., SANCHO G., BRAVO M.A., BALTANÁS A. & MONTES C. 1998. Diet of the Red Swamp Crayfish *Procambarus clarkii* in Natural Ecosystems of the Doñana National Park Temporary Fresh-Water Marsh (Spain). *Journal of Crustacean Biology* 18 (1): 120–127. doi:10.2307/1549526.
- HENTTONEN P. & HUNER J.V. 1999. The introduction of alien species of crayfish in Europe: a historical introduction, pp. 13–22. In: Gherardi F. & Holdich D.M., Eds. *Crayfish in Europe as alien species: how to make the best of a bad situation? Crustacean Issues* 11. *Rotterdam: H.A.A. Balkema.*
- HOBBS H.H., JASS J.P. & HUNER J.V. 1989. A review of global crayfish introductions with particular emphasis on two North American species (Decapoda, Cambaridae). *Crustaceana* 56: 299–316. doi:10.1163/156854089X00275.
- HOLDICH D.M., REYNOLDS J.D., SOUTY-GROSSET C. & SIBLEY P.J. 2009. A review of the ever increasing threat to European crayfish from non-indigenous crayfish species. *Knowledge and Management of Aquatic Ecosystems* 11 (394–395): 1–46.

- HUNER J.V. 2002. *Procambarus*, pp. 541–584. In: Holdich D.M., Ed. Biology of freshwater crayfish. Oxford: Blackwell Science.
- HUNER J.V. & BARR L.E. 1991. Red swamp Crawfish: Biology, culture, and exploitation. Baton Rouge, Louisiana: Louisiana State University Sea Grant College System, Louisiana State University, 128 pp.
- ILHÉU M., ACQUISTAPACE P., BENVENUTO C. & GHERARDI F. 2003. Shelter use of the Red-Swamp Crayfish (*Procambarus clarkii*) in dry-season stream pools. *Archiv für Hydrobiologie* 157 (4): 535–546. doi:10.1127/0003-9136/2003/0157-0535.
- LODGE D.M., DEINES A., GHERARDI F., YEO D.C.J., ARCELLA T., BALDRIDGE A.K., BARNES M.A., CHADDERTON W.L., FEDER J.L., GANTZ C.A., HOWARD G.W., JERDE C.L., PETERS B.W., PETERS J.A., SARGENT L.W., TURNER C.R., WITTMANN M.E. & ZENG Y. 2012. Global Introductions of Crayfishes: Evaluating the impact of species invasions on ecosystem services? *Annual Review of Ecology, Evolution, and Systematics* 43: 449–472. doi:10.1146/annurev-ecolsys-111511-103919.
- LODGE D.M., TAYLOR C.A., HOLDICH D.M. & SKURDAL J. 2000. Nonindigenous crayfishes threaten North American freshwater biodiversity: Lessons from Europe. *Fisheries* 25 (8): 7–20. doi:10.1577/1548-8446(2000)025<0007:NCTNAF>2.0.CO;2.
- LOWE S., BROWNE M., BOUDJELAS S. & DE POORTER M. 2000. 100 of the world's worst invasive alien species: A selection from the global invasive species database. *Hollands Printing Ltd, New Zealand: Invasive Species Specialist Group (ISSG) of the Species Survival Commission (SSC) of the World Conservation Union (IUCN)*, 12 pp.
- REYNOLDS J.D. 2011. A review of ecological interactions between crayfish and fish, indigenous and introduced. *Knowledge Management Aquatic Ecosystems* 401 (10): 21.
- REYNOLDS J. & SOUTY-GROSSET C. 2012. Management of freshwater biodiversity: crayfish as bioindicators. *Cambridge: Cambridge University Press*, 384 pp.
- RODRÍGUEZ C.F., BÉCARES E. & FERNÁNDEZ-ALÁEZ M. 2003. Shift from clear to turbid phase in Lake Chozas (NW Spain) due to the introduction of American red swamp crayfish (*Procambarus clarkii*). *Hydrobiologia* 506-509: 421–426. doi:10.1023/B:HYDR.0000008626.07042.87.
- RODRÍGUEZ C.F., BÉCARES E., FERNÁNDEZ-ALÁEZ M. & FERNÁNDEZ-ALÁEZ C. 2005. Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biological Invasions* 7: 75–85. doi:10.1007/s10530-004-9636-7.
- SAVINI D., OCCHIPINTI-AMBROGI A., MARCHINI A., TRICARICO E., GHERARDI F., OLENIN S. & GOLLASCH S. 2010. The top 27 animal alien species introduced into Europe for aquaculture and related activities. *Journal of Applied Ichthyology* 26 (2): 1–7. doi:10.1111/j.1439-0426.2010.01503.x.
- SOUTY-GROSSET C., HOLDICH D.M., NOËL P.Y., REYNOLDS J.D. & HAFFNER P. 2006. Atlas of crayfish in Europe. Paris: Muséum National d'Histoire Naturelle, 187 pp. (Patrimoines naturels, 64).
- STRAYER D.L. 2010. Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* 55 (Suppl. 1): 152–174. doi:10.1111/j.1365-2427.2009.02380.x.
- WELCH S. & EVERSOLE A.G. 2006. Comparison of two burrowing crayfish trapping methods. *Southeastern Naturalist* 5: 27–30. doi:10.1656/1528-7092(2006)5[27:COTBCT]2.0.CO;2.