

## Resistance to dehydration and positive hygrotaxis in the invasive red swamp crayfish *Procambarus clarkii*

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**Abstract** – The red swamp crayfish *Procambarus clarkii* is the most cosmopolitan invasive freshwater crayfish species in the world. High tolerance to a wide range of environmental conditions, among which its great ability to survive drought conditions, and the economic importance are the main factors of its spreading success.

The present study tests for the first time the survival of this species to different relative humidity (RH) conditions (plastic boxes with humidity controlling salt solutions at 30%, 50%, 70%, 100% RH) and its ability to orient in humidity gradients (dual-choice chambers 50–78% RH, 60–75% RH). *P. clarkii* shows a great ability to survive out of water, positively related to the RH conditions, surviving more than 2 days at very low humidity (30% RH) and more than 1 month at 100% RH. In addition the crayfish showed a positive hygrotaxis spending most of their time in the area of the choice chambers with a higher RH.

The results of the present investigation are particularly relevant considering that one of the major challenges to predict and control biological invasion lies in understanding which biological and ecological features might favor the colonization of alien invasive species in new areas.

**Keywords:** Behavior / Crustacea / freshwater alien species / desiccation / hygrosensors

**Résumé – Résistance à la déshydratation et hygrotaxie positive chez l'écrevisse de Louisiane envahissante *Procambarus clarkii*.** L'écrevisse de Louisiane *Procambarus clarkii* est l'espèce d'écrevisse d'eau douce la plus cosmopolite au monde. Sa grande tolérance à un large éventail de conditions environnementales, parmi lesquelles sa grande capacité à survivre aux conditions de sécheresse, et son importance économique sont les principaux facteurs de son succès de propagation.

La présente étude teste pour la première fois la survie de cette espèce à différentes conditions d'humidité relative (RH) (boîtes en plastique avec des solutions salines contrôlant l'humidité à 30%, 50%, 70%, 100% RH) et sa capacité à s'orienter dans des gradients d'humidité (chambres à double choix 50–78% RH, 60–75% RH). *P. clarkii* montre une grande capacité à survivre hors de l'eau, positivement liée aux conditions d'humidité relative, survivant plus de deux jours à très faible humidité (30% d'humidité relative) et plus d'un mois à 100% d'humidité relative. En outre, les écrevisses ont montré une hygrotaxie positive en passant la plupart de leur temps dans la zone des chambres de choix avec une HR plus élevée.

Les résultats de la présente étude sont particulièrement pertinents étant donné que l'un des principaux défis à relever pour prédire et contrôler l'invasion biologique réside dans la compréhension des caractéristiques biologiques et écologiques susceptibles de favoriser la colonisation d'espèces exotiques envahissantes dans de nouvelles zones.

**Mots-clés :** Comportement / Crustacé / espèce exotique d'eau douce / dessiccation / hygro-récepteurs

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## 1 Introduction

Alien species introduction is threatening biodiversity in various ways: genetic variation reduction and erosion of gene pools through the extinction of endemic species, and modification of habitat and ecosystem functioning (see review in Vilà *et al.*, 2009). The North American crayfish *Procambarus clarkii* (Girard, 1852) (Decapoda, Cambaridae), also known as red swamp crayfish or Louisiana crayfish (see review in Loureiro *et al.*, 2015), is native to northeastern Mexico and the southern USA and has been introduced all over the world except Antarctica and Oceania. This cosmopolitan freshwater crayfish (Hobbs, 1988; Gutiérrez-Yurrita *et al.*, 1999; Lindqvist and Huner, 1999; Gherardi, 2006; Chucholl, 2011), is the most widely introduced freshwater crayfish species in the world. The main purpose of the introduction in Europe was initially its expected commercial exploitation in professional fisheries and freshwater aquaculture (Souty-Grosset *et al.*, 2006). In invaded areas *P. clarkii* is responsible for pronounced environmental modifications causing severe damages in agricultural ponds and natural systems, like bank collapse (Correia and Ferreira, 1995) and negative ecological and economic consequences, such as the decline of native counterparts (Lodge *et al.*, 2000; see review in Souty-Grosset *et al.*, 2016). Furthermore, *P. clarkii* is a potential carrier of the crayfish plague (*Aphanomyces astaci*), lethal for the indigenous crayfish species and an importer of pathogenic micro-fungal taxa such as *Phoma glomerata* (Coelomycetes), potentially harmful to human health, flora and fauna (Dörr *et al.*, 2011; 2012a and b). The possibility of spreading diseases by this species is an additional negative element linked to its presence on the territory.

Its great ability to colonize a wide range of environments is due to its peculiar behavioral and biological characteristics among which its aggressiveness inducing competitive displacement of native species (Dick *et al.*, 1995; Gamradt *et al.*, 1997; Usio *et al.*, 2001). Moreover, its parental care improves reproductive fitness increasing offspring survival (Marchetti *et al.*, 2004). Locomotion and dispersal ability increase rates of spread and spatial patterns of invasion (Johnson and Carlton, 1996; Lewis, 1998), while burrowing behavior helps coping with environmental stress factors (Gherardi, 2001). Definitely, *P. clarkii* is an efficient digger using burrows as refuge to protect from predators, to nest and protect offspring, and to deal with various environmental stresses like high temperatures and dehydration (Huner and Barr, 1991; Barbaresi and Gherardi, 2006; Carreira *et al.*, 2017). The great ability of *P. clarkii* to withstand dehydration by dwelling in the hyporheic zone through vertical burrowing has been highlighted in a laboratory investigation simulating a period of 1-week drought at a relative humidity (RH) of 99% (Kouba *et al.*, 2016). A previous laboratory investigation on the dehydration survival capacities of the same species, performed only at 30% RH, reported that the red swamp crayfish began to die after 6 h of desiccation (with a  $LT_{50}$  of 11.9 h and a  $LT_{90}$  of 17.6 h) (Banha and Anastácio, 2014).

This is the first detailed study performed under controlled conditions showing the resistance to dehydration and the positive hygrotaxis behaviour in the invasive red swamp crayfish. The investigation was achieved testing survival of

adult males to different RH values obtained with humidity controlling salt solutions (30%, 50%, 70%, 100% RH), and their ability to orient inside humidity gradients (50–78% RH, 60–75% RH).

## 2 Material and methods

### 2.1 Experimental animals

*P. clarkii* was collected in Lake Trasimeno (Umbria, Central Italy) from November 2016 to July 2017. The lake is an ideal habitat for this species and has been colonized successfully since 1999 (Dörr *et al.*, 2001; Dörr and Scalici, 2013). After a rapid expansion, nowadays its hale population is well established and economically important for the local fish market and even for recreational activities (Dörr *et al.*, 2006). To avoid any bias in the experiment, we selected intermolt and intact (integral walking legs, chelae, antennae and antennules) adult males (sexually active showing distinct grasping hooks on the ischia of the 3rd and 4th pairs of pereopods). Specimens wet weight ranged between 17.2 and 49.4 g with a carapace length between 39.60 and 55.75 mm. Crayfish were acclimatized for at least 2 weeks in glass aquaria (70 cm × 30 cm × 40 cm) in laboratory. Twenty crayfish were collocated in each aquarium supplied with oxygenated tap water (previously exposed to air to remove chlorine), and fed with commercial aquaculture pellets *ad libitum*. Metal net was added to provide shelters. No special authorisation was required for collecting specimens in field and for laboratory activities.

### 2.2 Resistance to dehydration

Crayfish survival out of water was analysed in various conditions of RH, obtained by using aqueous solutions of NaOH at different concentration values (Madge, 1961). The experiments were carried out in square polypropylene plastic containers (18 cm × 18 cm × 11 cm) separated into a lower and an upper compartment by a median horizontal diaphragm of a plastic gauze (2 mm mesh). Humidity controlling substances were placed in the lower compartment of each container. A single crayfish was removed from water and placed on the gauze in the upper compartment of each cage, free to walk in the square arena without direct contact with the solution. Total wet weight and carapace length of each crayfish were recorded. Each container was covered with a tight-fitting lid.

Crayfish survival was tested at 30%, 50%, 70% and 100% RH. Fifteen specimens for each RH value were tested. Health condition of crayfish inside each box, as dead or alive (life criteria: locomotion and/or active motion of appendages when touched with a sterile glass stick), was checked once a day in the afternoon. The containers were opened at each observation time to remove dead specimens and to ensure oxygen replacement. Survival data were censored after 30 days of follow up. Before and at the end of the experiment, temperature and RH inside one container for each condition were monitored for 24 h using a thermo-hygrometer (HOBO® H08-004-02 Onset Computer Corporation RH range 0–95% accuracy ±5%, temperature range −20 °C/+70 °C). Fifteen crayfish were kept individually in polypropylene containers

(18 cm × 18 cm × 11 cm) filled with aged tap water as control. Control water was oxygenated daily. During the acclimation period of at least 2 weeks *P. clarkii* was fed *ad libitum*. In consideration that this species is known to be able to survive for relatively long dry periods without food, and that the presence of diet could create bias in our experimental conditions crayfish were not fed during the trial.

Bioassays were performed in a thermostatically controlled experimental room at 25 °C and 50–60% RH.

### 2.3 Ability to orient in a humidity gradient

The crayfish response in different humidity gradients was tested in dual-choice chambers similar to those described by Arbogast and Carthon (1971). Each chamber consisted of two parts made using boxes of clear polypropylene plastic (23 cm × 30 cm × 6 cm). The lower part was a box divided by a median wall into two compartments. The upper part was a walking arena consisting of an identical box with a floor of plastic gauze (1-mm mesh), and covered with a glass plate. Solutions with different concentrations of NaOH (Madge, 1961) filled the two compartments of the lower box to provide contrasting humidity on the two sides of the walking arena placed over it. The fine plastic mesh on the arena floor allowed diffusion of water vapour preventing contact of the crayfish with the caustic solutions. Temperature and RH inside each side of the arena were monitored using a thermo-hygrometer.

Two bioassays were performed in two different gradients. In the first set of chambers the solutions provided a diffusion gradient from 50 to 78% RH on the two sides of the walking arena; in the second set of chambers the gradient was from 60 to 75 % RH. To allow for sufficient stabilization, 12 h before the experiments, the two compartments of the lower box of each chamber were filled with the solutions and the arena was mounted and closed with the glass plate. In each replicate, one crayfish was removed from water and maintained half an hour in an empty polypropylene box to dry out. Later, it was placed in the centre of the walking arena and covered with the glass plate. After 5 min of acclimation, the movements of the specimen (walking and staying in the two sides of the arena or on the separation line) were directly observed from a distance of 3 m and recorded for 1.5 h. At the end of each bioassay the net of the walking arena was removed and cleaned with water and odour-free detergent; the whole walking arena was washed with the same detergent every 2–3 bioassays.

The possible presence of bias was evaluated by running controls consisting of chambers with the two compartments in the lower box filled with distilled water, to obtain a walking arena with around 100% RH everywhere. For the bioassays we used intact crayfish and ablated crayfish, whose antennae and antennulae were gently removed by dissection scissors 1 day before the bioassays. Ablated crayfish were tested only for the higher humidity gradient in consideration that our aim was simply to understand if they were able or unable to perceive humidity in relation to the possible presence of hygroreceptors on antennae and antennulae.

We removed antennae and antennulae because both are important sensory organs in Crustacea (antennae mainly for mechanoreception and antennulae mainly for chemoreception, Koch *et al.*, 2006; Derby and Sorensen, 2008) and no data are

available on the presence or location of hygroreceptors in crayfish. Intact crayfish were tested in both humidity gradients.

Sixteen replicates were performed for each gradient and 18 replicates for the controls with intact crayfish. Twelve replicates were performed both for the gradient from 50 to 78% RH and for the controls with ablated crayfish. One crayfish was tested in each replicate. All tests were conducted between 09.00 and 15.00 h (CEST). The bioassay room was maintained at 25 °C and 50% RH.

### 2.4 Statistical analysis

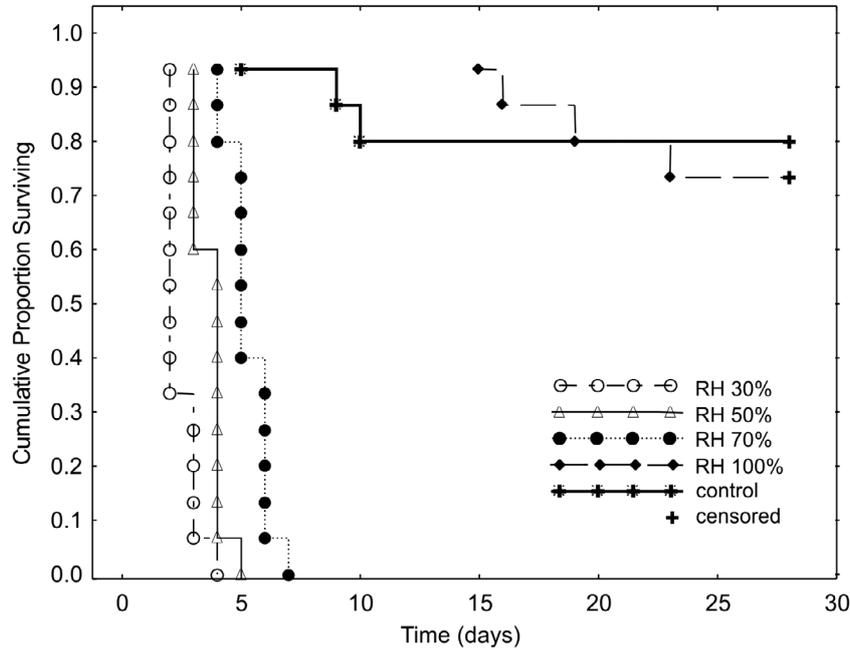
The survival analysis for multiple samples, using an extension of Peto and Peto's generalized Wilcoxon test, was applied to compare crayfish survival at the different experimental conditions. Survival functions for the different groups were plotted using the cumulative proportion of surviving (Kaplan–Meier curves) (Statistica 6.0, Statsoft Inc., 2001). The crayfish mean longevity at the various conditions of humidity and their weight was analysed by one-way ANOVA (Statistica 6.0, Statsoft Inc., 2001). Each crayfish was considered as a replicate.

The percentage of time (residence time) spent in each of the two sides of the arena was compared using the *t*-test for dependent samples (Statistica 6.0, Statsoft Inc., 2001). Data were reported and analysed as percentage of time spent on the total time (90 min) or on the first minute of the experiment. The percentage of time (residence time) spent by crayfish on the separation line (no choice) was not considered in the analysis and was not shown in figures because very low. *t*-Test for independent samples was used to compare the residence time and the activity (defined as the number of transitions between the two sides of the walking arena) of intact and ablated crayfish in the control situation both for the total duration and for the first minute of the experiment. One-way ANOVA was used to compare the residence time and the activity in the different RH gradients both for the total duration and for the first minute of the experiment. All internal comparisons were submitted to unequal N HSD Tukey test (Statistica 6.0, Statsoft Inc., 2001). Before the analysis, Box–Cox transformation was used to reduce data heteroscedasticity (Sokal and Rohlf, 1998).

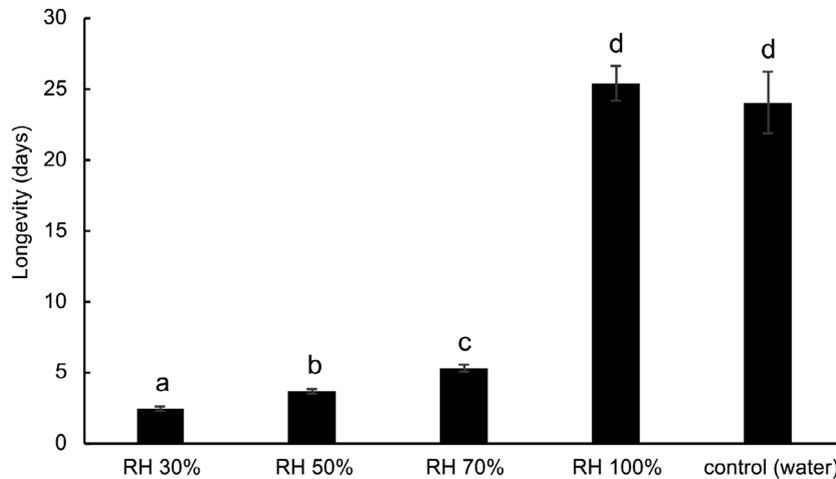
## 3 Results

### 3.1 Resistance to dehydration

Crayfish survival at the different humidity and in the control water was significantly different ( $\chi^2=66.55$ ,  $df=4$ ,  $P < 0.001$ ). The longevity curves (proportion surviving plotted against time) appeared similar in shape at 30%, 50% and 70% RH, with a great slope and the death of all crayfish nearly simultaneous at each RH level. Differently, curves at 100% RH and the control showed a flat shape and the surviving of the most part of specimens for all the experimental period (30 days); (Fig. 1). In particular, at 100% RH, 73% of the tested crayfish survived more than 30 days, similarly to the control (water), where 80% of crayfish survived more than 30 days. At 30%, 50% and 70% RH, 100% of tested individuals died between 2 and 7 days (Fig. 1). The mean longevity recorded in the different tested conditions was statistically different



**Fig. 1.** Cumulative proportion of *P. clarkii* surviving at different humidity (30–50–70–100% RH) and for control (not fed) at 25 °C. Note that at 100% relative humidity (RH) most of the crayfish (73%) survived longer than the experimental time (30 days) and no crayfish died before 48 h for any condition. Data were censored for 30 days.



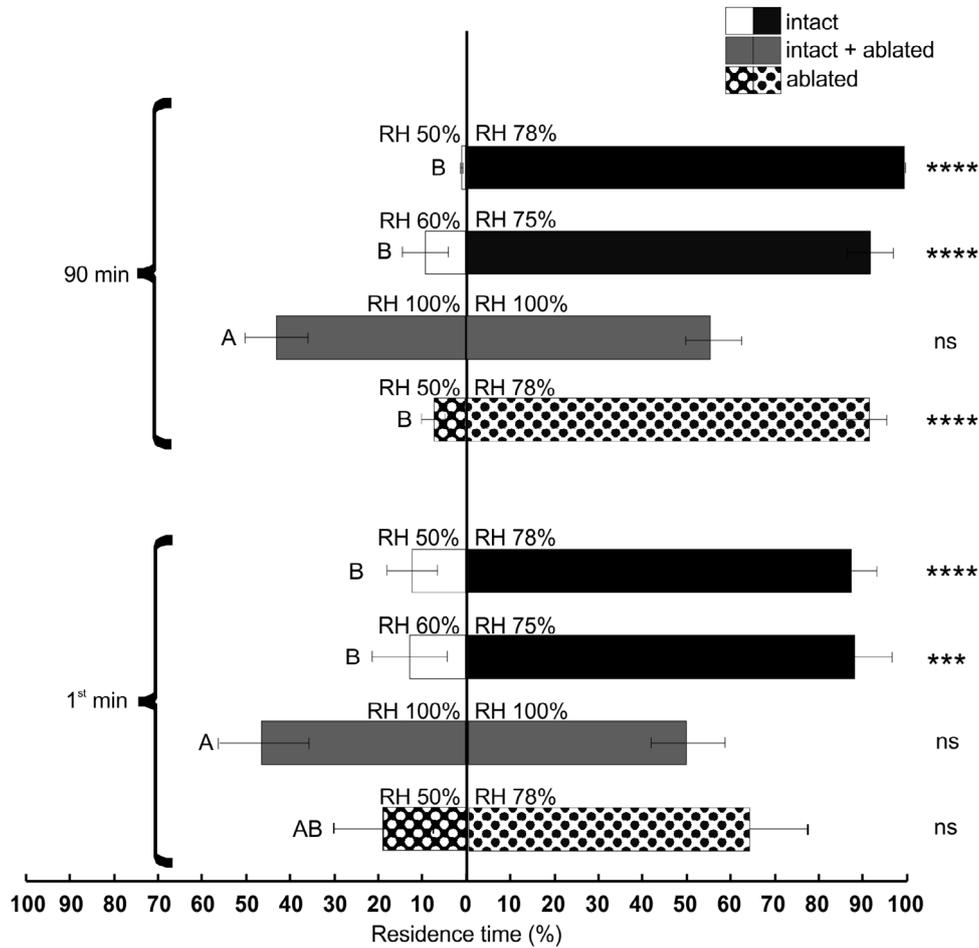
**Fig. 2.** Mean time of crayfish longevity at different humidity (30–50–70–100% RH) and for control (not fed) at 25 °C. Bars indicate mean  $\pm$  SE. Data with different letters are significantly different at  $P < 0.05$  (one-way ANOVA, unequal N HSD Tukey test).

( $F = 176.68$ ;  $df = 4, 70$ ;  $P < 0.001$ ) (Fig. 2). Indeed, the longevity of crayfish tested at 100% RH and in the control was not different and significantly higher than that recorded at lower RH, where it increased significantly together with the RH values (Fig. 2). Weight ( $29.46 \pm 0.89$ , mean  $\pm$  SE) of crayfish tested in various conditions of humidity was not statistically different ( $F = 0.44$ ;  $df = 4, 70$ ;  $P = 0.777$ ).

### 3.2 Ability to orient in a humidity gradient

Considering the total period of the bioassays in the dual-choice chambers, intact crayfish spent most of their time in the side of the arena with a higher RH, both in 50–78% RH

( $t = 166.51$ ;  $df = 15$ ;  $P < 0.001$ ) and in 60–75% RH gradient ( $t = 7.47$ ;  $df = 15$ ;  $P < 0.001$ ). Also for ablated crayfish, the residence time was significantly higher inside of the arena with higher RH in the tested gradient (50–78% RH) ( $t = 8.07$ ;  $df = 11$ ;  $P < 0.001$ ) (Fig. 3). Intact crayfish spent more time in the moister area of the two gradients, also considering only the first minute of the bioassay (50–78% RH gradient:  $t = 6.44$ ;  $df = 15$ ;  $P < 0.001$ . 60–75% RH gradient:  $t = -4.39$ ;  $df = 15$ ;  $P = 0.0005$ ), while ablated crayfish did not show any preference during the first minute of the test ( $t = 1.98$ ;  $df = 11$ ;  $P = 0.073$ ) (Fig. 3). For control both intact and ablated specimens showed no significant difference between time spent in each of the two sides of the arena considering the



**Fig. 3.** Time percentage (mean  $\pm$  SE) spent by intact and ablated crayfish in each of the two sides of the walking arena in 50–78% RH chambers ( $n = 16$  for the intact crayfish and  $n = 12$  for the ablated ones), 60–75% RH chambers ( $n = 16$ , only intact crayfish), and in the control ( $n = 18$  for intact crayfish and  $n = 12$  for ablated ones). For controls, no significant differences were recorded between behaviour of ablated and intact crayfish so they were pooled together. Data from the whole experimental time, 90 min, and from the first minute of the test are analysed independently. \*\*\*\* $P < 0.0001$ , \*\*\* $0.0001 < P < 0.001$ ; ns, not significant ( $t$ -tests for dependent samples). Data with different letters are significantly different at  $P < 0.05$  (one-way ANOVA, unequal N HSD Tukey test).

whole experimental time (intact:  $t = -0.74$ ;  $df = 17$ ;  $P = 0.468$ , ablated:  $t = -0.59$ ;  $df = 11$ ;  $P = 0.568$ ) and the first minute (intact:  $t = -0.01$ ;  $df = 17$ ;  $P = 0.990$ , ablated:  $t = -0.29$ ;  $df = 11$ ;  $P = 0.777$ ). In consideration that in controls no significant differences in the response of ablated crayfish and intact ones were registered, both for the whole experimental time ( $t = -0.53$ ;  $df = 29$ ;  $P = 0.599$ ) and for the first minute ( $t = -0.53$ ;  $df = 29$ ;  $P = 0.601$ ), we pooled them together in the comparison among the residence times in the arenas with different RH values (Fig. 3).

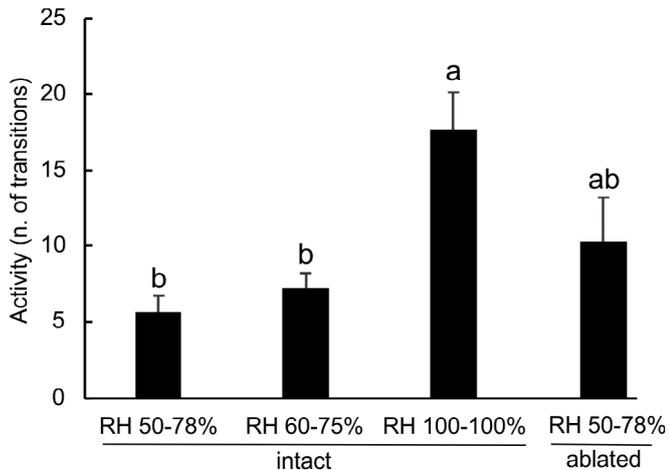
When comparing the residence time in the side of the arena with lower RH of intact and ablated crayfish in the tested gradients, considering the whole experimental time, it was significantly lower compared to the control in all the tested gradients, both for intact and ablated crayfish, without any difference among the different conditions ( $F = 23.25$ ;  $df = 3, 70$ ;  $P < 0.001$ ) (Fig. 3). In the first minute, the residence time in the side of the arena with lower RH in the tested gradients was lower compared to the control for intact crayfish but intermediate for ablated ones ( $F = 5.05$ ;  $df = 3, 70$ ;

$P = 0.003$ ) (Fig. 3). Percentage of time spent by crayfish on the separation line (no choice) was almost negligible (maximum 3%), except in the case of the first minute of experiment with ablated crayfish ( $16.7\% \pm 11.2\%$ ).

Crayfish activity was significantly different in the various tests ( $F = 7.69$ ;  $df = 3, 70$ ;  $P < 0.001$ ) (Fig. 4). In consideration that in the controls there was no significant difference in the activity of ablated crayfish and intact ones ( $t = 1.99$ ;  $df = 28$ ;  $P = 0.065$ ), we pooled them together. Intact crayfish activity tested in the two RH gradients was lower than activity in the control. Ablated crayfish activity was intermediate between the activity of intact specimens in the two RH gradients and that of the control. Moreover, there was no difference between the activity of the intact crayfish in the two RH gradients (Fig. 4).

#### 4 Discussion and conclusions

Our data show a great ability of the crayfish *P. clarkii* to survive out of water, strongly related to the RH conditions. Indeed, even at a very low level of humidity such as 30% RH



**Fig. 4.** Crayfish activity, defined as the number of transitions between the two sides of the walking arena, of ablated and intact crayfish in the tested types of dual-choice chambers (50–78% RH and 60–75% RH gradients) and in the control (100–100% RH). For controls, no significant differences were recorded between behaviour of ablated and intact crayfish so they were pooled together. Data with different letters are significantly different at  $P < 0.05$  (one-way ANOVA, unequal N HSD Tukey test).

(which is not frequent in the areas surrounding inland waters), none of our specimens died before 48 hours and more than 70% of the crayfish maintained at 100% RH survived more than 30 days, without any difference with the crayfish kept in water (controls). These data are in agreement with those reported by Kouba *et al.* (2016) in a laboratory investigation simulating a period of 1-week drought at a 99% RH, but are highly different from those reported in another laboratory investigation where at 30% RH *P. clarkii* began to die after 6 h and did not survive 1 day of desiccation (Banha and Anastácio, 2014). The differences concerning our results, when compared to the Banha and Anastácio studies, are likely due to different experimental conditions.

Other investigations on the ability to survive drought conditions in aquatic arthropods, which can face dehydration, such as dragonfly larvae living in small astatic pools have been performed in the past using the same method (Piersanti *et al.*, 2007; Reborá *et al.*, 2007). Among crustaceans a similar investigation has been carried out only on three species of Talitridae, typically living in the intertidal zone, showing a survival time ranging between one and 48 h, and strongly dependent on the RH values (Williamson, 1951). The resistance to dehydration shown by *P. clarkii* in our investigation is similar to that reported for some terrestrial crabs (about 3–4 days at an RH of 78–90% RH values) (Burggren and McMahon, 1981) even if, as far as we know, no detailed investigation testing the survival under different controlled RH conditions has been performed in Decapoda. In relation with the high resistance shown by *P. clarkii*, it is important to remember that many crayfish species developed strategies to become the dominant decapods not only in freshwater but also in the surrounding terrestrial habitats (Reynolds *et al.*, 2013). *P. clarkii* is considered a “tertiary burrower” (Hobbs, 1981), spending most of its life in open waters and only retreating in burrows to avoid predators and

dehydration or when breeding (Gherardi, 2001). On the other hand, a terrestrial behavior was reported by Huner (2002), Chucholl (2011) and Abdel-Kader (2016) who observed *P. clarkii* migrating overland to explore new habitats in response to poor water quality (McCarthy *et al.*, 2014) or for dispersal among waterways (Morgan, 1991; Furse *et al.*, 2004; Herrmann *et al.*, 2018).

The colonization of terrestrial habitats by some decapods has required considerable morphological, physiological and behavioral modification, mainly concerning ion regulation and gas exchange. These processes have been investigated extensively in terrestrial crabs (Burggren and McMahon, 1981). Crayfish can breathe out of water since their gills are protected by the carapace and do not collapse in air for some time (Reynolds *et al.*, 2013). Most crayfish can switch from water to air as an oxygen source (McMahon, 2002) and may leave water bodies when their oxygen levels have become depleted. The shape of our longevity curves, with a great slope and the death of all the crayfish nearly simultaneous at 30%, 50% and 70% RH and with a flat shape at 100% RH, could be in agreement with a death due to gill dehydration, but further investigations are necessary to test this hypothesis.

Our bioassays in the dual-choice chambers showed that crayfish spent most of their time in the side of the arena with a higher RH. A recent study on environmental cues driving overland dispersal by *P. clarkii* reports that movement direction and dispersal are influenced by gradients of temperature and elevation while, unexpectedly, they are not related with RH values (Marques *et al.*, 2015). These variances, if compared to our results, can be due to lower humidity values (32–55%) as hypothesized by the same authors or may depend on differences in the experimental setup. In fact our crayfish were tested in small (30 cm × 23 cm) dual choice chambers where RH was constant and controlled by saline solutions, while Marques *et al.* (2015) used 200 cm metal gutters humidity stimuli produced by nebulizers collocated only at their ends and not monitored in the centre. The positive hygrotaxis shown by *P. clarkii* in air is surely relevant to orientate its burrowing behavior. In a behavioural study investigating the ability of this species to learn the position of a wet burrow hidden to its sight and move towards it. Barbaresi and Gherardi (2006) hypothesized the use of a combination of tactile and visual information jointly with the potential use of humidity cues, but the authors highlight that the study was not designed to investigate the sensory channels used by *P. clarkii* to orient. The ability to orient in humidity gradients at a microclimatic scale is a crucial aspect in arthropod habitat selection in order to regulate body water loss, especially in terrestrial species such as insects and arachnids, where thermo-hygroreceptors have been extensively investigated, and are typically constituted of two antagonistic hygroreceptor neurons (moist and dry) and one cold neuron forming a triad in a single antennal sensillum (Tichy and Loftus, 1996; Steinbrecht, 1998; Reborá *et al.*, 2007; Piersanti *et al.*, 2011). Among Crustacea, putative hygroreceptors have been reported only in the terrestrial isopod *Porcellio scaber* showing numerous cuticular tricorn sensilla on the surface of the whole body with a morphology resembling insect hygroreceptors (Ziegler and Altner, 1995). In any case, these authors suggest that structural criteria for thermo-hygrosensitivity used in insects cannot simply be applied to crustaceans.

Recent investigations on humidity sensation in *Coenorhabditis elegans* (Russell *et al.*, 2014) and humans (Filingeri *et al.*, 2014) confirmed that in some animals humidity may be perceived as the synthesis of mechanical and thermal signals without a specific sensory organ, thus revealing a remarkable similarity in the hygrosensation transduction mechanism used by hygroreceptors provided (*e.g.* insects) and hygroreceptors lacking (*e.g.* roundworms and humans) species, all able to detect humidity (Filingeri, 2015). Our data on crayfish with ablated antennae show that they are still able to orient and select for a high RH value, but this hygrotaxis needs more than one minute to be expressed, while one minute is enough for the positive hygrotaxis of intact specimens. In addition, these crayfish showed a level of activity in the bioassays similar to that of control. We can exclude that the stress due to the amputation affects these results, because no difference in activity and hygrotaxis occurred between the control bioassays of ablated and intact crayfish. These results suggest that *P. clarkii* could have specific hygroreceptors on the antennae and somewhere else on the body surface, but we cannot exclude a different pathway for hygrosensitivity in these arthropods as suggested for other animals (Filingeri, 2015). Further morphological and physiological investigations could clarify this interesting aspect in Crustacea.

In conclusion, the present study represents the first detailed description of the ability of the red swamp crayfish to survive at different degrees of dehydration, and to select moist environments following humidity cues, which allow this species to survive periods of drought by constructing burrows, and also disperse migrating overland. Positive hygrotaxis and the aptitude to withstand dehydration are relevant to confirm the notable ecological plasticity of the red swamp crayfish, and this knowledge can be a useful tool serving to control and to contain this resilient invasive alien crayfish.

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