Escape rooms: behavioural response of two invasive crayfish species under water decline scenarios

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ABSTRACT

Climate change and invasive alien species pose a significant threat to biodiversity and the survival of native species. This study considered the impact of drought conditions on the escape behaviour through terrestrial dispersal ability of two invasive freshwater crayfish species, the red swamp crayfish (*Procambarus clarkii*) and signal crayfish (*Pacifastacus leniusculus*). Using an experimental design simulating drought conditions and Mediterranean summer temperatures, we tested the hypothesis that *P. clarkii* exhibits a greater tendency to terrestrial migration and a higher land-walking speed than the *P. leniusculus*. The results indicated that both species demonstrated strong escape behaviour, with *P. clarkii* showing escape success higher than *P. leniusculus*, and increasing with decreasing mean night temperature and crayfish weight. Although drought conditions did not trigger escape, invasive species showed increased resistance and the ability to move to more favourable environments, suggesting that drought is not an effective geographic barrier against the spread of these species. These results underline the importance of considering invasive species' ability to escape and climb as part of management and control strategies.

INTRODUCTION

Climate change and invasive alien species are the major drivers of biodiversity loss and species extinctions, after land use change (Turbelin *et al.*, 2017; Le Hen *et al.*, 2023). Invasive pathway, including arrival, survival and thrive in new environment, is affected by climate and landscape features by altering the geographical distribution of species and determining the seasonal conditions for survival and growth (Colwell *et al.*, 2008). Global warming will have a huge impact on ecosystems and native species, due to their thermal limits. The increasing of both average temperatures and frequency of extreme weather events, makes ecosystems vulnerable to invasions, providing many opportunities for dispersal and establishment of invasive species (Capinha *et al.*, 2013).

In the past 50 years, heat waves have become more frequent; precipitation has increased in northern Europe but has declined in the Mediterranean; the area affected by drought has increased since the 1970s in mid-latitudes and semi-arid low latitudes (Russo *et al.*, 2015). Under the projected scenarios, drought conditions are inducing an increase of freshwater species richness in the northern Europe and a decrease in the southern-west (Alcamo *et al.*, 2007). Freshwater ecosystems and their biodiversity are particularly vulnerable to climate change also due to limited abilities to disperse by many species (Dudgeon *et al.*, 2006; Deinet *et al.*, 2024). Indeed, rates of biodiversity loss in freshwater ecosystems in recent decades, far exceed those of terrestrial or marine species (Tickner *et al.*, 2020), even a global warming of 1.5°C poses a serious threat

to many freshwater species already threatening of extinction (Capon *et al.*, 2021).

The extent of species responses to climate warming is still greatly unknown; not all species will respond positively to warmer conditions (Román-Palacios and Wiens, 2020; Verberk *et al.*, 2021), and some species may be forced to migrate upstream in search of more suitable climatic conditions (Haubrock *et al.*, 2023). With decreasing rainfall, Mediterranean region will suffer increasingly severe droughts (Tramblay *et al.*, 2020), and many invasive species will benefit from these changes in many ecosystems.

Although the effects of climate warming on freshwater fauna have been well-studied (Wanders *et al.*, 2019; Johnson *et al.*, 2024), from oxygen- and capacity-limited thermal tolerance (Pörtner *et al.*, 2017), to community structure and ecosystem functioning (Friberg *et al.*, 2009), the effects of drought in Mediterranean regions are understudied (Bond *et al.*, 2008), especially those relative to invasive species that have higher resistance than native ones (Glon *et al.*, 2018).

In streams, the loss of water particularly affects habitat availability and stream connectivity (Bond *et al.*, 2008), and it also leads to increased water temperature due to reduced water flow and volume. Indirect effects also include water quality worsening, alteration of food resources, and changes in the strength and structure of interspecific interactions (Lake, 2003). Therefore, droughts and habitat fragmentation are both likely to be critical stressors in many freshwater ecosystems (Kingsford, 2006), conversely they might represent barriers to the spread of invasive species. Success factors for invasive species include the ability to survive in a variety of different habitat types and the ability to disperse in a short time (Zhang *et al.*, 2023).

Crayfish are among the largest freshwater macroinvertebrates as well as highly mobile crustaceans (Holdich, 2002). In addition to their potential to disperse rapidly in freshwater ecosystems, their success depends on their ability to colonise nearby waterbodies, pass weirs and falls, and escape from captivity thanks to their adaptability for terrestrial emigration and overland dispersal (Kerby *et al.*, 2005; Ramalho and Anastácio, 2015).

Escape behaviour as stereotyped escape response called the tail flip, peculiar of eucarids, has been intensively studied in crayfish and stomatopod, and both the behaviour and the underlying neural mechanisms are deeply examined and understood (Edwards *et al.*, 1999; Heitler *et al.*, 2000; Hunyadi *et al.*, 2020). The escape responses of crayfish in predator-prey interactions, particularly the tail-flip mechanism, is crucial for their survival (Dickey and McCarthy, 2007; Hunyadi *et al.*, 2020), allowing them to quickly prevent predation by dragonfly nymphs (Herberholz *et al.*, 2004). Whilst escape behaviour as tail flip requires the presence of water, many studies have investigated survival and resistance to dehydration, *e.g. Procambarus clarkii* (Girard 1852) survival to different relative humidity conditions (Piersanti *et al.*, 2018), native and non-native crayfish ability to survive desiccation by construction vertical burrows in the hyporheic zone (Kouba *et al.*, 2016), and exposure to air for long periods of time, thanks to their ability to continue absorbing oxygen even in the absence of dissolved oxygen (Banha and Anastácio, 2014).

To the best of our knowledge, no study to date has considered these two features simultaneously. Only few is known about the terrestrial dispersal of invasive crayfish (Thomas *et al.*, 2019), but no information on extreme environments such as unexpected droughts in perennial watercourses, which are increasingly common in the Mediterranean climate due to dry weather conditions. Existing studies on aquatic species, however, provide evidence that changes of environmental temperature can act as a stimulus on motor activity of crustacean (Lehti-Koivunen and Kivivuori, 1994).

The aim of this study was therefore to investigate the terrestrial dispersal ability of invasive crayfish species in drought conditions, when given an escape path. Invasive red swamp and signal crayfish males were exposed to water decreasing, simulating drought conditions, during Mediterranean summer temperatures, and providing them an escape ramp. We tested the hypothesis that the invasive crayfish, due to their dispersal behaviour, are trigged by drought conditions to escape.

METHODS

Study species

Signal crayfish *Pacifastacus leniusculus* (Dana, 1852) and red swamp crayfish *P. clarkii*, both with their native range in North America, have been widely established in freshwater European ecosystems. Both were selected as model species in this study for their invasiveness and behavioural plasticity features (Hanshew and Garcia, 2012; Ercoli *et al.*, 2021), but differ in their optimum temperature (Westhoff and Rosenberger, 2016). Indeed, signal crayfish, belonging to the Astacidae family, is endemic to northwestern USA and south-western Canada, and its optimum habitat in the Mediterranean region consists of the uppermost stretches by lower water temperatures (Vedia *et al.*, 2016). Red swamp crayfish, member of the Cambaridae family and endemic to the southeastern United States and Mexico, is instead considered as a "warm water" species (Veselý *et al.*, 2015; Haubrock *et al.*, 2019). Currently signal crayfish and red swamp crayfish are widespread all across Europe, with at least 29 and 15 respectively invaded countries, and they are classified as ones of the most ecologically impacting invasive crayfish (Kouba *et al.*, 2014) and therefore listed into the European Union Regulation on Invasive Alien Species 1143/2014 (European Union, 2014).

Crayfish collecting and holding conditions

Crayfish were collected from well-established populations in Northern Italy in June: *P. clarkii* from a channel in the Trebbia River basin (44.94934 °N, 9.5988 °E); and *P. leniusculus* from Valla stream (44.51971 °N, 8.3452 °E) (Fig. 1). Individuals from both species were caught using crayfish traps baited overnight and suddenly transported to the laboratory at the University of Pavia. Crayfish were sexed and only adult males, with both chelae and without mutilations or visible diseases, were tested in this study. We chose to test males to maximise their propensity to move over larger distance than females (Guan, 2000; Wutz and Geist, 2013). Then, males were measured with a digital calliper (accuracy ± 0.1 mm) to record the cephalothorax length (from the tip of the rostrum to the posterior median edge of the cephalothorax), and the length and width of both claws, to apply the Claw Index (CI) defined by "mean claw length x mean claw circumference" (van der Meeren and Uksnøy, 2000). In addition, wet weight (±0.1 g) was determined using handy spring balances and crayfish were individually marked on the cephalothorax using a waterproof pen. Red swamp crayfish (n=24 males)

ranged from 33.0 to 49.9 mm (mean 40.3±3.9 mm) in cephalothorax length, whilst signal crayfish (n=24 males) ranged from 39.2 to 58.1 mm (mean 47.0±4.6 mm). Wet weight of red swamp crayfish (n=24 males) ranged from 8.5 to 28.0 g (mean 16.3±4.4 g), whilst of signal crayfish (n=24 males) ranged from 17.0 to 57.0 g (mean 30.5±10.4 g).

Crayfish were housed in separate species small groups in plastic tanks (50x35x33 cm), with multi-hole bricks as shelters, each containing 35 L of constantly aerated dechlorinated tap water and maintained at room temperature (25-26.5°C) under a natural light/dark cycle. Each tank was covered with a plexiglas layer, secured with weighs to prevent crayfish escape. Crayfish were fed *ad libitum*, with food (carrots) always available and daily replaced. Acclimation lasted three weeks (Johović *et al*., 2020).

Experimental design

To test crayfish escape tendency in case of water decreasing to drought, an experimental set was constructed consisting of two aquaria (30x19x16 cm; A1 – start aquarium; A2 – final aquarium placed 8 cm higher than A1) with a ramp constituted by a 50 cm-long plastic drainpipe with a non-slip mat, placed in A1 and arriving at A2, with an inclination of 34° (Fig. 2). Twelve experimental sets were constructed to maximise and randomise the number of trials performed per night, following the nychthemeral rhythm peculiar to most freshwater decapods (Holdich, 2002; Sbragaglia and Breithaupt, 2022).

Before starting the experiment, each male was isolated in A1 and left to acclimatise for at least 1 h. Each tank contained 4 L of dechlorinated tap water, aerated *via* a 2 cm-long blue cylindrical aquarium air-stone placed, through a hole, at the bottom of the
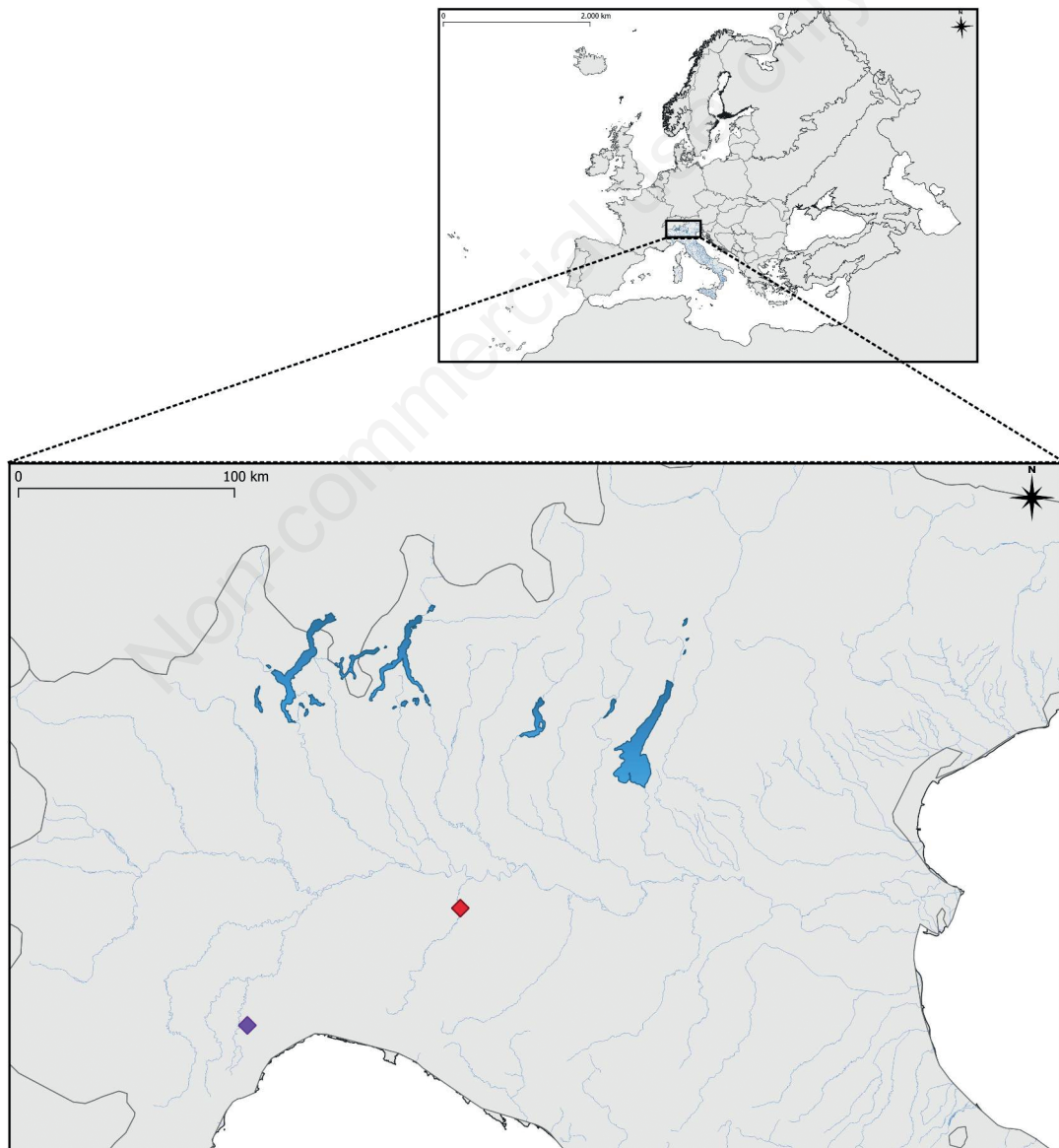

Fig. 1. Map of collecting crayfish sites belonging to Po River basin: *P. leniusculus* (purple diamond); *P. clarkii* (red diamond).

tank and connected to the aerator with a 5 mm-diameter silicone tube. After acclimation period, to simulate water decreasing (treatment) and to avoid further disturbing the crayfish in the aquarium, half of the aquaria A1 were put into dry conditions by removing the silicone tube from the aerator and so letting water drain into water collection tanks, positioned at a lower level. This procedure lasted 30 min. Then, a ramp was placed in every A1. The other half of the aquaria A1 continued to have aerated water throughout the experiment. Trials were recorded using a photo-trap camera (Maginon vision WK 3HD; RCP Handels GmbH & Co. KG, Norderstedt, Germany) sited 2.5 m above the centre of the experimental set, in order to control crayfish arrival at A2. The camera was programmed to take a photo and a 5-second video every 15 minutes, overnight, from 21:00 to 10:00, adding data, time and air temperature displayed on each photo. The air temperature was recorded using both the photo-trap camera thermometer and a Tinytag Aquatic 2 data logger (Gemini Data Loggers UK Ltd., Chichester, UK) at 30 min intervals. Air temperature was the temperature value at the time displayed on each photo showing the individual crayfish arrived at the A2.

In the morning, the crayfish were returned to housing tanks, while each aquarium (both A1 and A2) and the tubes were accurately washed to remove any trace eventually left by the crayfish and reassembled for the next test in the evening. At the end of each experimental night, all crayfish were found either in A1 or in A2, none remained inside the plastic drainpipe. Each crayfish was double tested, randomising the water decline treatment at the first or second trial, at a week interval. We performed a total of 96 trials, 48 per species. No crayfish died during the experiments. Thereafter, all crayfish were killed by hypothermia, freezing at -20°C in accordance with the European and Italian laws on animal use in scientific research (Tricarico and Zanetti, 2023).

Response variables and statistical analyses

All photos and videos were screened to check whether the escape event occurred and how long it took. An escape event was defined when the crayfish was found in the aquarium A2, where it could be reached by walking up the drainage ramp from the aquarium A1. The escape time (minutes) was only quantified for crayfish found in A2. Weight and claw index (CI) were standardized (mean=0 and sd=0) within species because their different

Fig. 2. Schematic experimental arena for assessing escape behaviour. Crayfish could escape from A1 to A2 through the drainpipe.

sizes (weight: Mann-Whitney U test = 38, p<0.001; CI: Mann-Whitney U test = 24, p<0.001). Since all but two (96%) *P. clarkii* males successfully escaped, we were unable to analyse the effect of the experimental treatment on the escape response. Consequently, in our initial analysis, we compare the escape response between species, regardless the experimental treatment. Subsequently, in the *P. leniusculus* subsample, we conducted a second analysis to assess the impact of the experimental treatment on the escape success.

We assessed the comparison of escape success responses (binary dependent variable) between species using a random intercept generalised linear mixed model (GLMM) with a logit link-function. The variables species (*P. clarkii* and *P. leniusculus*), weight, CI and night air temperature (mean air temperature measured for each experimental night) entered the model as fixed effects. Male identity (ID) entered the model as random effect on the intercept to account for repeated trials and all other individual traits, which remain constant (*e.g.* size, behavioural traits). The same model was used to evaluate the effect of the water decreasing (treatment) on the escape response by *P. leniusculus*: treatment, weight, CI and night air temperature (standardized to mean = 0 and sd=1) entered the model as fixed effects, while male identity (ID) was the random effect. GLMMs underwent a simplification procedure by removing non-significant effect (using LRχ^2 test) until obtaining the minimal significant model (Zuur *et al.*, 2009).

The escape times were assessed by using random intercept linear mixed models (LMM). In a first analysis, species, weight, CI and air temperature were the fixed effects, and ID the random effect. Then, we evaluated the effect of the water decreasing (treatment) on escape time using a second model, for each species, with treatment, weight, CI and air temperature as fixed effects, and ID as random effect. Visual inspection of residual plots (Q-Q plots) did not reveal any obvious deviations from homoscedasticity or normality for the response variables.

Analyses were performed using the packages *lme4* (Bates *et al.*, 2023) and *lmerTest* (Kuznetsova *et al.*, 2020), in R ver. 4.1.3 (R core Team 2022).

RESULTS

Overall, in 77 out of 96 trials (80%) crayfish escaped and were found in aquaria A2; the escaped crayfish were 46 out 48 (96%) for *P. clarkii*, while only 31 out 48 (65%) for *P. leniusculus* (Tab. 1). The GLMM for the escape success found highly significant effects for the species, weight and mean night temperature, whereas the effect of CI was negligible (Fig. 3, Tab. 2). The escape success was higher for *P. clarkii* than *P. leniusculus* ($\beta = 3.66 \pm 1.25$, z = 2.937, p=0.003; Fig. 3a), and increasing with both decreasing mean night temperature ($\beta = -0.68\pm0.35$, z = -1.902, p=0.057; Fig. 3b) and crayfish weight ($\beta = -0.86\pm0.38$, z = -2.246, p=0.025; Fig. 3c). Moreover, the GLMM for *P. leniusculus* escape success did not detect any significant effect (Tab. 2). Particularly, water decreasing did not affect signal crayfish escape success ($\beta = 1.26\pm0.79$, z = 1.600, p=0.11, Fig. 3d).

The escape time for *P. clarkii* was 81.2 ± 9.97 min (range 15-390), while mean *P. leniusculus* escape time was 197±17.9 min (range 45-405). The LMM found a highly significant effect
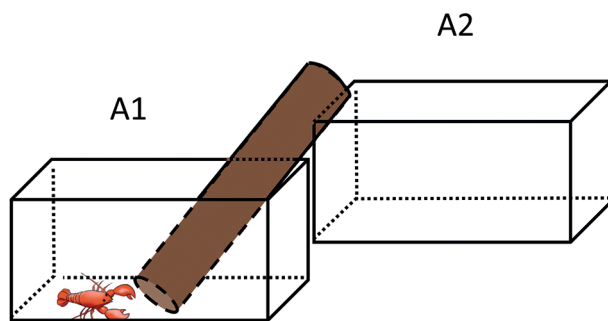
for the species (Tab. 3; Fig. 4a), confirming that *P. clarkii* was faster to escape than *P. leniusculus* ($\beta = 110\pm20.35$ min, $t_{34.34} = 5.44$, p<0.001; Fig. 4a). No significant effects were detected for

weight, CI and the air temperature when the crayfish arrived in A2 (Tab. 3). Moreover, the LMM for escape time of *P. leniusculus* did not detect any significant effect, except for the air temperature (Tab. 3): the escape time increased when air temperature decreased ($\beta = -86.71\pm25.13$, $t_{10.6} = 3.45$, p=0.006; Fig. 4b). The same analysis for *P. clarkii* did not detect any significant effect (Tab. 3). Particularly, water decreasing did not affect both *P. leniusculus* and *P. clarkii* escape time (Tab. 3, Fig. 4 c,d).
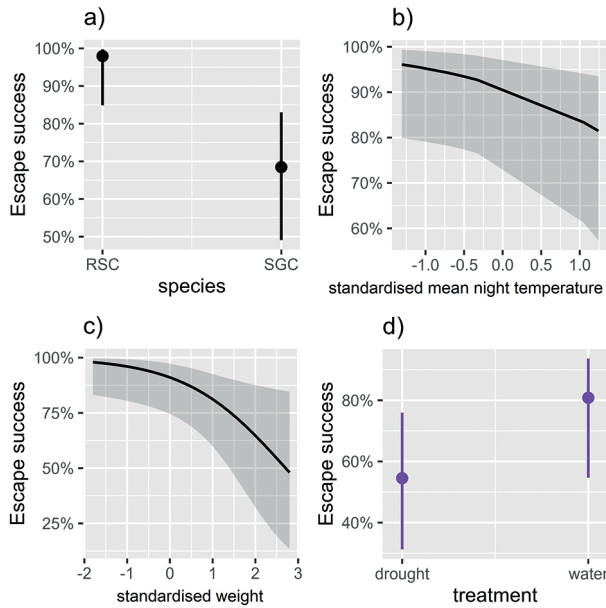

Fig. 3. GLMMs predicted probabilities of escape success: a) for the species; RSC, red swamp crayfish, *P. clarkii*; SGC, signal crayfish, *P. leniusculus*. b) For the standardised mean night temperature. c) For the standardised crayfish weight. d) For the treatment only for *P. leniusculus* escape success.

DISCUSSION

Regardless of the treatment (*i.e.*, good conditions with aerated water or drought conditions), our findings clearly revealed a strong escape behaviour and performance by these two invasive crayfish, when given the chance. *Procambarus clarkii* showed escape success higher than *P. leniusculus*, and it increased with both decreasing mean night temperature and crayfish weight. Indeed, many studies have analysed resistance to dehydration in *P. clarkii* (Banha and Anastácio, 2014; Piersanti *et al.*, 2018), without considering that in natural environment, when water flow is limited, crayfish move overland in response to hypoxia, seeking a more favourable environment, often moving over long distances (Gherardi *et al.*, 2002; Kouba *et al.*, 2016), overcoming obstacles (Kerby *et al.*, 2005), or climbing (Krieg and Zenker, 2020; Galib *et al.*, 2022).

To withstand desiccation in extreme environmental conditions, such as drought, some crayfish species were able to construct vertical burrows or to enter the first one vacant, as *P. clarkii* seems to behave in order to survive (Gherardi *et al.*, 2002; Kouba *et al.*, 2016), whilst *P. leniusculus* did not show any attempt at vertical burrowing under drought conditions (Kouba *et al.*, 2016), although it exhibited this burrowing behaviour in freshwater

Tab. 1. Results of the behaviour of crayfish males of *P. leniusculus* and *P. clarkii* exposed to the treatment.

| Species | Escape | Treatment | | Total |
		Drought	Control	
P. leniusculus	No	11	6	17
	Yes	13	18	31
P. clarkii	No	0	2	2
	Yes	24	22	46

Yes, crayfish escaped from aquarium A1 to aquarium A2; no, crayfish did not escape from A1; drought, water decreased simulating drought conditions; control, aerated water throughout the experiment.

Tab. 2. Results for the escape success response variables as estimated by GLMMs. Escape success was the full model, *P. leniusculus* was the model to test the treatment. Significant p-values are reported in bold.

Behavioural response	Fixed effect	χ^2	df	p
Escape success	Species	16.500	1	**<0.001**
	Mean night temp	4.340	1	**0.037**
	Weight	6.696	1	**0.010**
	CI	3.067	1	0.080
P. leniusculus	Treatment	3.026	1	0.082
	Mean night temp	2.398	1	0.121
	Weigh	3.121	1	0.077
	CI	1.071	1	0.301

Species, *P. leniusculus* and *P. clarkii*; mean night temp, mean air temperature measured for each experimental night from 9 p.m. until 10 a.m.; weight, crayfish weight; CI, crayfish claw index.

ecosystems in Great Britain (Holdich *et al.*, 2014). However, in our study the stronger escape success displayed by *P. clarkii* than by *P. leniusculus* would indicate a major propensity to explore environments in order to move to better conditions; moreover, lightweight crayfish were more successful in escaping. Previous studies indicated that smaller individuals of *Orconectes rusticus* (Girard, 1852) were slower and died faster out of water because of dehydration (Claussen *et al.*, 2000), whereas juvenile signal crayfish left the water, since having a carapace length of 16.6 mm (Thomas *et al.*, 2018). Crayfish involved in overland migrations can minimize the risks of desiccation by efficiently walking at

their maximum sustained speeds, and the smaller crayfish may partially compensate for this risk by increasing their speed relative to their body length (Claussen *et al.*, 2000).

Invasive crustacean species display increased resistance to adverse conditions when water levels decline in the dry season (Hänfling *et al.*, 2011). This is particularly relevant during periods of drought, which often occur along with high temperatures. Consequently, high water temperatures lead to lower oxygen levels and higher conductivity, severely stressing the aquatic fauna, especially fish (Bond *et al.*, 2008). In contrast, most crayfish are able to switch their oxygen source from water to air and thus able to climb banks and travel long distances out of the water (Holdich, 2002; Peay and Dunn, 2014).

In our experiment, all successfully escaped crayfish climbed 50 cm on a 34° inclined path. Although our experimental setup could not handle several slopes, the one used in this study was already a considerable slope for climbing. Indeed, *P. clarkii* recorded the lowest frequencies of upward movement at slopes of 20° and 30° on sandy soil and poor grassland substrates (Lemmers *et al.*, 2022). Instead, to test the ability of crayfish to return to water, when placed in a circular arena near the riverbank, *P. clarkii* did not move towards any particular direction and *P. leniusculus* moved towards a direction far from the water. These behaviours suggest that neither of the species could detect the presence of water in their environment and return to it (Marques *et al.*, 2015).

Concerning the times recorded by the crayfish to escape, these were significantly different between the two species, lower for *P. clarkii* and higher for *P. leniusculus*. The air temperature recorded on escape arrival showed to have an effect: in *P. leniusculus* the escape times increased when the air temperature decreased, in a range from 25.1°C to 26.5°C; conversely for *P. clarkii* none of the effects were significant. The claw index also never evidenced to be significant.

Our finding regarding *P. clarkii* escape behaviour was not surprising. *Procambarus clarkii* is adapted to habitats that have alternating dry and wet periods, and has the ability to exit the water and move overland (Holdich, 2002; Kerby *et al.*, 2005). Instead, in our study, *P. leniusculus* showed a high capacity to adapt to temperatures deviating from its optimum as a cool-water species. Within the limits of the species, the higher escape rate at higher
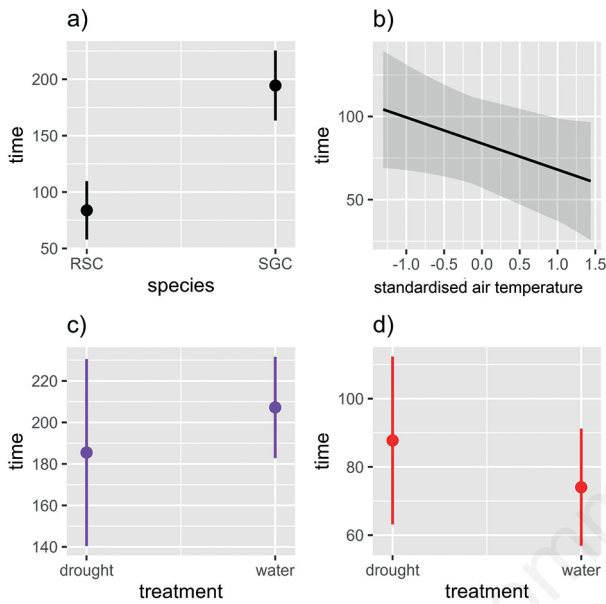

Fig. 4. LMMs predicted probabilities of escape time (minutes): a) for the species; RSC, red swamp crayfish, *P. clarkii*; SGC, Signal crayfish, *P. leniusculus*. b) For the standardised air temperature. c) For the treatment only for *P. leniusculus* escape time. c) For the treatment only for *P. clarkii* escape time.

Tab. 3. Results for the escape time response variables as estimated by LMMs. Escape time was the full model, *P. leniusculus* and *P. clarkii* were the models to test the treatment. Significant p-values are reported in bold.

Behavioural response	Fixed effect	F	df	p
Escape time	Species	29.58	1, 34.34	**<0.001**
	Air temp	3.204	1, 37.28	0.082
	Weight	0.001	1, 42.57	0.981
	CI	0.732	1, 43.22	0.397
P. leniusculus	Treatment	0.767	1, 10.80	0.400
	Air temp	11.91	1, 10.64	**0.006**
	Weigh	0.761	1, 16.13	0.396
	CI	0.581	1, 14.99	0.458
P. clarkii	Treatment	0.446	1, 41	0.508
	Air temp	0.030	1, 41	0.863
	Weigh	0.161	1, 41	0.691
	CI	1.484	1, 41	0.230

Species, *P. clarkii* and *P. leniusculus*; air temp, air temperature when the individual crayfish arrived at the final aquarium A2; weight, crayfish weight; CI, crayfish claw index.

air temperatures could indicate a behaviour that minimises the risk of desiccation. Crayfish species, such as *O. rusticus*, which predominantly inhabit intermittent streams, exhibit a particularly high reliance on terrestrial locomotion for their survival and ecological interactions. Their walking speeds, greatest at the intermediate temperatures (25-30°C) and decreasing at lower and higher temperatures, suggested that crayfish undertaken overland migrations can minimize the risk of desiccation by moving at their maximum sustainable speeds (Claussen *et al.*, 2000).

Moreover, our experiment was conducted also under dusk and night conditions, specifically to maximise the movement of both species, which have nocturnal habits, and therefore the success of escape. All the escaped crayfish, in fact, escaped within 405 min (6 h 45 min). Since the experiments started at 9 p.m., this means that after 3:45 a.m. no crayfish escaped. All crayfish that did not escape due to drought conditions belong to *P. leniusculus*. However, the 13-hour drought conditions did not lead to death or dehydration compromising their health and movement. Nocturnal underwater decapod activity is widely regarded as an adaptive behaviour (Holdich, 2002; Aquiloni *et al.*, 2005), whereas crayfish overland dispersion observed at night involved environmental variables (*e.g.*, high dew, fog and humidity) that enhance crayfish survival (Ramalho and Anastácio, 2015).

CONCLUSIONS

The condition of drought as a geographical barrier cannot be applied to all crayfish species, as the invasive ones have the potential not only to survive desiccation but also to move towards more favourable environments, colonizing nearby watercourses and increasing spread. Also from a behavioural ecology perspective, climbing ability should be consider in designing barriers to prevent the dispersal of non-native species. A positive relationship between climbing and dispersal rate recorded by Galib *et al.* (2022) could suggest that climbing behaviour plays a significant role in expanding the population's range through dispersal. Our findings contribute to taking into consideration the ability to escape, regardless of the environmental conditions, by the two globally recognized invasive crayfish species. When designing barriers to hinder the movement of crayfish species, it is crucial to consider not only the riverbeds but also the adjacent banks, which the crayfish can cross even in waterless conditions. Greater attention should be paid to *P. leniusculus*, which is currently present in Italy with few populations, but having a great potential for spreading further and invade new freshwater ecosystems.

ACKNOWLEDGMENTS

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