1 **Terrestrial emigration behaviour of two invasive crayfish species**

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16

17

Abstract

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21   To disperse between isolated waterbodies, freshwater organisms must often cross terrestrial
22   barriers and many freshwater animals that are incapable of flight must rely on transport via
23   flooding events, other animals or anthropogenic activity. Decapods such as crayfish, on the
24   other hand, can disperse to nearby waterbodies by walking on land, a behaviour that has
25   facilitated the spread of invasive species. Overland movement could play a key role in the
26   management of non-native crayfish, though to what extent terrestrial emigration occurs in
27   different species is poorly understood. Here, we directly compared the terrestrial emigration
28   tendency of two non-native crayfish species in Great Britain; red swamp (*Procambarus clarkii*)
29   and signal (*Pacifastacus leniusculus*) crayfish. We found that both species emigrated from the
30   water and that there was no significant difference in terms of their terrestrial emigration
31   tendency, suggesting that there is a risk both of these species will migrate overland and disperse
32   to new habitats. This study shows that terrestrial emigration is an important behavioural trait
33   to consider when preventing the escape of crayfish from aquaculture and further spread of
34   invasive species.

1. Introduction

Non-native species are common in freshwaters as humans have historically exploited these ecosystems for fishing, aquaculture and recreation, leading to the introduction of mammals, fish and invertebrates (Hulme et al. 2008; Strayer and Dudgeon 2010; Strayer 2010). Dispersal studies of aquatic species tend to focus on movement within a waterbody, particularly downstream migration (e.g. Bubb et al. 2002, 2004; Barson et al. 2009). The majority of introduced freshwater species must reach new habitats either by hitch-hiking on other animals or via human-mediated translocations (Shurin and Havel 2002; Anastácio et al. 2014) to become invasive. Dispersal is a three-stage process involving emigration, inter-patch movement and immigration. Factors such as population density and competition can drive emigration (Enfjäll and Leimar 2005; Hudina et al. 2014), though intrinsic differences in emigration tendency also exist amongst individuals, populations and species (Roland et al. 2000; Bowler and Benton 2005; Cote et al. 2010) and invasive species are generally considered to be better dispersers compared to native congeners due to higher levels of activity (Bubb et al. 2006) and boldness (Rehage and Sih 2004). Differences in the tendencies of non-native species to disperse, however, are less clear, though it is assumed that widespread species have a higher dispersal tendency.

Crayfish are commercially important freshwater crustaceans that are particularly pernicious invaders in some locations (Peay et al. 2010; Gherardi 2010). North American crayfish such as the red swamp (*Procambarus clarkii* Girard 1852) and signal crayfish (*Pacifastacus leniusculus* Dana 1852) are widely harvested species that have often escaped from aquaculture and established non-native populations (Holdich et al. 2009). The introduction of North American crayfish to Europe has led to the extirpation of native European species due to competitive displacement (Hill and Lodge 1999; Bubb et al. 2006; Hudina et al. 2011; Hanshew and Garcia 2012) and through the spread of the highly virulent crayfish plague, *Aphanomyces astaci* Schikora 1906 (see Holdich et al. 2014). In water, red swamp crayfish can disperse at a rate of up to 4 km in a single day (Gherardi & Barbaresi 2000) though movement rates of between 0.61-38 m day^{-1} are more commonly reported (Gherardi et al. 2000; Gherardi et al. 2002; Bubb et al. 2004; Aquiloni et al. 2005; Anastácio et al. 2015). Dispersal rates for signal crayfish in water are slightly lower, between 4.1 and 17.5 m day^{-1} (Bubb et al. 2004, 2006; Anastácio et al. 2015).

As well as their potential to rapidly disperse in water, the success of some invasive crayfish can, at least partially, be attributed to their ability to colonise nearby waterbodies, navigate barriers (weirs or falls) and escape from captivity by terrestrial emigration and overland dispersal (Kerby et al. 2005; Larson et al. 2009; Holdich et al. 2014; Puky 2014; Ramalho and Anastácio 2015). Some crayfish, particularly burrowing species, can survive for several months out of water in burrows (Huner and Linqvist 1995; Kouba et al. 2016) though all crayfish can tolerate some degree of terrestrial exposure. Of the nine non-native crayfish species in Great Britain, red swamp and signal crayfish are most frequently reported dispersing overland, at least in other parts of the world (Holdich et al. 2014; Ramalho and Anastácio 2015; Piersanti et al. 2018). Red swamp crayfish can move up to 1 km (Lutz and Wolters 1999; Souty-Grosset et al. 2016) at a speed of 90 m h^{-1} (Ramalho and Anastácio 2015). We are not aware of any direct study of signal crayfish overland movement, though Holdich et al. (2014) report that signal crayfish can 'survive for days to months' out of water.

Overall, there is a lack of information on crayfish terrestrial emigration, largely because this behaviour is only rarely directly observed, recorded or quantified in the field. A direct comparison of invasive red swamp and signal crayfish terrestrial emigration tendencies will provide important information for predicting their future potential rage expansion in Britain. Here, we tested the hypothesis that British populations of red swamp crayfish have a higher

84 terrestrial emigration tendency and are faster when walking on land compared to signal
85 crayfish.
86
87 **2. Materials and Methods**
88 Crayfish were trapped in spring 2016, from a small private pond (Powys, Wales; signal
89 crayfish) and from public recreational ponds (Hampstead Heath, London; red swamp crayfish).
90 Both species were caught using cylindrical crayfish traps ('Trappy Traps', Collins Nets Ltd.,
91 Dorset, UK) baited overnight with cat food and transported to the Cardiff University aquarium
92 facility, where they were maintained in a climate-controlled room set at $13\pm1°C$, 60% RH and
93 a 12 h light: 12 h dark cycle.
94
95 All crayfish were housed in single-species holding aquaria (density of 10 individuals per m^2)
96 filled with dechlorinated water, 2 cm gravel substrate and an excess of refugia (plant pots and
97 PVC tubes) with no terrestrial access. Holding aquarium water was biologically filtered and a
98 50% water replacement was performed weekly to maintain water quality. All crayfish were fed
99 *ad libitum* on a mix of frozen *Tubifex* bloodworm (Shirley Aquatics, Solihull, West Midlands,
100 U.K.) and frozen peas.
101   The crayfish were marked using a non-toxic yellow marker (Dykem, USA) on the
102 carapace to allow visual identification during video analysis. Furthermore, to allow individual
103 identification if crayfish lost the mark or moulted, all crayfish were individually tagged
104 following Bubb et al. (2002) using Passive Integrated Transponder (PIT) tags (7.5 mm PIT-
105 tags, ISO 11784 certified, Loligo Systems, Denmark).
106
107 *2.1 Experimental design*
108 To examine crayfish emigration tendency, an experimental arena was constructed consisting
109 of two aquaria (L100 cm x W48 cm x H53 cm) with moveable ramps (L43 cm x W29 cm; 30°
110 incline) that provided access to a terrestrial bridge (L240 cm x W20 cm x H20 cm) (Fig. 1).
111 Both the ramps and terrestrial bridge were coated with pea gravel to allow sufficient grip for
112 crayfish climbing out of the water and the area under each ramp provided a shared refuge. A
113 pea gravel bed (2 cm layer) was also provided in the aquaria. An infrared CCTV camera system
114 (Sentient Pro HDA DVR 8 Channel CCTV, Maplin) was suspended above the arena to monitor
115 crayfish behaviour in all experiments. All crayfish were sexed and measured (carapace length)
116 at the start of the experiment. Signal crayfish (n = 15; 5 males and 10 females) ranged from
117 38.6 – 59.3 mm (mean 47.9 mm) in carapace length, whilst red swamp crayfish (n = 17; 13
118 males and 4 females) ranged from 47.3 – 71.3 mm (mean 58.8 mm).
119   The terrestrial emigration propensity and walking speed of red swamp and signal
120 crayfish were quantified in the experimental arena (Fig. 1). At the start of each trial, individual
121 crayfish were placed in the water on one side of the arena at 09:00 h and allowed to acclimatise
122 until 20:00 h the same day. The lights were automatically turned off at 20:00 h and crayfish
123 behaviour was recorded until 08:00 h the next day (12 h recording).
124
125 *2.2 Ethical note*
126 This study was undertaken in accordance with ASAB/ABS guidelines for the use of animals in
127 teaching and research. All invasive crayfish were caught under a Natural Resources Wales
128 licence (Trapping licence number: NT/CW065-C-652/5706/01) and held under a Cefas licence
129 (W C ILFA 002) at Cardiff University. The crayfish were not exposed to harmful conditions
130 during the course of the experiment, however, both species of invasive crayfish were
131 euthanized humanely at -20°C in accordance with the Wildlife and Countryside Act, 1981.
132
133 *2.3 Statistical Analyses*

134 As crayfish are nocturnal, the 12 h observation period occurred overnight and for each
135 individual crayfish, the number of emergences from water and time spent out of water per
136 emergence were quantified from video recordings. A terrestrial emergence event was defined
137 as when more than half of the crayfish body was out of the water and on the bridge. Walking
138 speed (cm s^{-1}) was estimated over a set distance (i.e. the 240 cm bridge) and quantified only
139 for crayfish that fully crossed the bridge.
140 A negative binomial GLM (Generalised Linear Model) with a log link function (using
141 the MASS package; Venables and Ripley 2002) was used due to the zero-inflated nature of the
142 data to determine whether crayfish species or carapace length affected the total number of times
143 a crayfish left the water, including times they did not cross the bridge. Due to the availability
144 of crayfish, it was not possible to test a balanced number of male and female red swamp and
145 signal crayfish, but sex was included in the model as a nested term within species to account
146 for potential differences.
147 A gaussian GLM with 'identity' link function was used to examine whether crayfish
148 species or carapace length affected terrestrial walking speed (cm s^{-1}). Sex was not included in
149 this model since all red swamp crayfish that crossed the bridge (n = 4) were male. Model
150 selection and assumptions of normality were confirmed using residual diagnostic plots (Zuur
151 et al. 2010). All statistical analyses were performed in R 3.4.0 (R Core Team 2017).
152
153 **3. Results**
154 Overall, 35.3% of red swamp and 46.6% of signal crayfish left the water at least once over the
155 12 h nocturnal observation period. There was no significant difference in the tendency of either
156 species to emigrate from the water nor the time they spent out of water per emergence. Certain
157 individuals of both species tended to frequently leave the water; two red swamp crayfish left
158 the water 17 times each, whilst two signal crayfish left the water 20 and 14 times each. The
159 total number of emergences over the course of the experiment for both red swamp and signal
160 crayfish was 50 and 58, respectively. Carapace length had no significant effect on terrestrial
161 movement tendency.
162 Of the crayfish that emerged from the water during the 12 h observation period, red
163 swamp and signal crayfish spent on average 6 min 42 s (SD = 124 s) and 8 min 64 s (SD = 386
164 s) out of water, respectively. There was no significant difference in the walking speed of either
165 species on land, which was also unaffected by carapace length. Four male red swamp crayfish
166 were observed to fully cross the bridge (average speed 0.703 cm s^{-1}, SD = 0.07) and six signal
167 crayfish - two males and four females (0.601 cm s^{-1}, SD = 0.28)
168
169 **4. Discussion**
170 In the present study, terrestrial emigration occurred in both red swamp and signal crayfish from
171 invasive British populations, with no significant difference in their tendency to leave the water
172 or walking speed on land. These results suggest that although red swamp crayfish are generally
173 considered to have a higher tendency to walk overland due to their burrowing tendencies and
174 subsequent lowered risk of desiccation, signal crayfish are just as likely to walk overland, and
175 so overland dispersal could facilitate both species' spread. In terms of their walking speed, the
176 red swamp and signal crayfish tested here also crossed the bridge at similar speeds. Crayfish
177 do not move particularly quickly overland, especially compared to other decapods: ghost crabs
178 (*Ocypode* spp.), for example, can reach speeds of up to 2 m s^{-1} (Claussen et al. 2000).
179 Furthermore, when placed out of water, neither species of crayfish are able to direct their
180 movements towards nearby waterbodies (Marques et al. 2015) and their walking speed
181 decreases as they become dehydrated (Claussen et al. 2000). As such, crayfish are at a
182 significant risk of desiccation and need to cross terrestrial barriers as quickly as possible,
183 though neither species tested here appeared to have an advantage over the other.

Despite being the most widespread crayfish species globally, the red swamp crayfish has not yet spread far in Great Britain, potentially due to sub-optimal climate conditions (Ellis et al. 2012). Most established populations are currently found in ponds, canals and rivers around London, having first been discovered at Hampstead Heath in 1991 (Ellis et al. 2012; James et al. 2014). Signal crayfish, on the other hand, are by far the most abundant species in Britain, largely because they were introduced earlier (1970s) and on a larger scale for aquaculture (James et al. 2014; Holdich et al. 2014). The spread of signal crayfish has resulted in the widespread decline of native white-clawed crayfish (*Austropotamobius pallipes*). When tested in a similar experimental setting but with a shorter bridge length (Masefield, unpublished), a higher proportion (65%) of white-clawed crayfish left the water at least once compared to both invasive species tested in the present study (35.3% of red swamp and 46.6% of signal crayfish). Overall, however, white-clawed crayfish were found to leave the water less frequently than both invasive species. Further investigation of the tendency of native crayfish to leave the water is essential, given that conservation practices include the isolation of white-clawed crayfish in 'ark-sites', away from nearby waterbodies.

In terms of both red swamp and signal crayfish, the present study shows that terrestrial movement could be an equally important factor in the spread of both species, which are known to survive long periods out of water and in drought conditions (Cruz and Rebelo 2007; Holdich et al. 2014; Banha and Anastácio 2014). The red swamp crayfish, however, is generally considered to be more adept at overland dispersal, tolerating long periods out of water (Piersanti et al. 2018) and extending survival on land by constructing burrows and inhabiting small puddles whilst also feeding on terrestrial vegetation (Grey and Jackson 2012; Ramalho and Anastácio 2015; Kouba et al. 2016; Souty-Grosset et al. 2016). In their native North American range, burrowing has not been recorded in signal crayfish and some studies show that this species is incapable of constructing burrows (Kouba et al. 2016), suggesting it is less adapted to terrestrial habitats. However, in the Great Britain, invasive populations of signal crayfish frequently burrow in riverbanks and lakebeds (Holdich et al. 2014), which could explain their tendency to move overland in the present study, given the reduced risk of desiccation if they are able to construct burrows out of the water.

We have previously shown that ovigerous, non-ovigerous and juvenile signal crayfish from Britain also move out of water (Thomas et al. 2018) and in the present study we highlight that, at least in an experimental setting, signal crayfish are as likely to leave the water as red swamp crayfish, in the absence of competition or other stressors. As such, it is likely that signal crayfish leave the water and disperse overland more often than previously considered, which, coupled with its burrowing behaviour, could be a further factor contributing to its continuing invasion success in Great Britain (Holdich et al. 2014; Peay and Dunn 2014). Given that the distance travelled overland in the present study was limited, however, further research should estimate the potential distance both species could travel overland in a field environment, which would be a useful avenue for future research to inform management and control practices.

Overall, this study shows that both species of invasive crayfish tested here move overland to a similar degree. Previous studies have shown that red swamp and signal crayfish both emerge on to land in response to dewatering of habitats, which can occur naturally or before biocide management treatments (Peay and Dunn 2014; Ramalho and Anastácio 2015) and such overland movement is likely to reduce the efficacy of control methods. Individuals that are prone to leaving the water will also be more likely to escape from commercial and private aquaculture ponds and enter nearby watercourses, increasing the risks of introduction and further range expansion (Holdich et al. 2014; Marques et al. 2015). Furthermore, terrestrial emigration allows crayfish to navigate in-stream barriers such as weirs or waterfalls (Kerby et al. 2005). The current study highlights the importance of alternate dispersal mechanisms which,

233 despite not being widely reported, should be taken into consideration during management and
234 population control practices of invasive species.
235
236 **Declarations of interest**: None.
237
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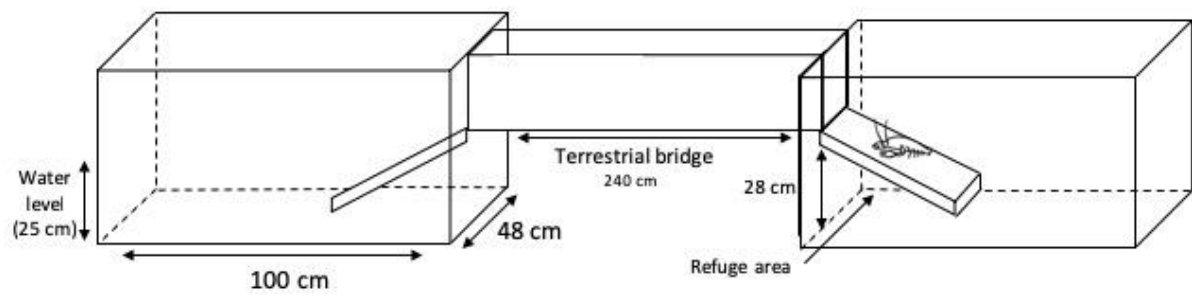444 210X.2009.

445
446 **Figure 1** – Experimental arena for assessing terrestrial emigration behaviour. Crayfish could
447 access the bridge (240 cm) using ramps, which also acted as a refuge. The water was filled to
448 approx. 3 cm below the level of the terrestrial bridge.