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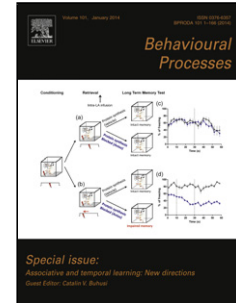
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Terrestrial dispersal of invasive signal crayfish during vulnerable life stages

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Highlights

- Juvenile crayfish reached a size threshold before moving on land
- Ovigerous crayfish were as likely to move overland as non-ovigerous individuals
- Terrestrial walking speed was similar between ovigerous and non-ovigerous crayfish

Abstract

1. Aquatic invertebrates commonly disperse between waterbodies by flight, though some decapods can emigrate from the water and walk overland.
2. The signal crayfish (*Pacifastacus leniusculus*, Dana) is a highly invasive species that can survive for days to weeks out of water, though it is unclear to what extent vulnerable life stages, such as ovigerous females or juvenile crayfish, terrestrially emigrate. Understanding this behaviour is important to consider during the management of crayfish stocks, since a single ovigerous female could potentially introduce hundreds of hatchlings to a new waterbody, which could themselves disperse overland.
3. Here, in a laboratory study, we examined the terrestrial emigration tendency of juvenile crayfish and compared the terrestrial emigration behaviour and overland walking speed of ovigerous and non-ovigerous females.
4. Size had a significant influence on juvenile crayfish terrestrial emigration tendency, where only those larger than 16.6 mm (carapace length) left the water. Ovigerous and non-ovigerous female signal crayfish showed no significant difference in terrestrial emigration tendency nor overland walking speed.
5. This is the first study to directly examine the terrestrial emigration behaviour of crayfish during these different life stages. These findings highlight the importance of considering animal behaviour during management strategies.

Keywords: dispersal; emigration; invasion; juvenile; ovigerous

1. Introduction

Invasive, non-native species are the second leading cause of biodiversity loss worldwide, with freshwater ecosystems particularly affected (Gherardi, 2010). Freshwaters are often invaded by fish, crustaceans or molluscs since these animals are most commonly accidentally or purposefully introduced for aquaculture, fishing or as ornamental species (Strayer, 2010). Invasive fish and molluscs can quickly disperse within invaded aquatic habitats (e.g. upstream or downstream from the site of introduction), though they are limited in terms of movement between separate waterbodies, relying on human mediated dispersal or hitch-hiking on other animals. On the other hand, decapod crustaceans can emigrate from the water and disperse overland, a behaviour which is relatively poorly understood.

Crayfish are especially successful invasive decapods due to their high fecundity, broad physiological tolerance and the relative ease with which they are translocated and disperse

(Gherardi, 2010; Westhoff & Rosenberger, 2016). Whilst crayfish can be carried to new waterbodies by anglers or animals (Anastácio et al., 2014), they also emigrate from water and walk overland (Holdich et al., 2014; Lodge et al., 2000). Introduced crayfish directly affect multiple levels of the food web through reducing invertebrate density, fish and macrophyte biomass, amphibian survival and significantly increasing decomposition rates (James et al., 2014; Lodge et al., 2000). In the UK, though several other crayfish species have successfully established, the signal crayfish (*Pacifastacus leniusculus*, Dana) is most common and widespread (Holdich et al., 2014), having caused dramatic losses of the native British white-clawed crayfish (*Austropotamobius pallipes*, Lereboullet) through direct competition and disease transmission (Holdich et al., 2009). As a result of these impacts and associated control measures, the estimated cost of signal crayfish in Great Britain is around £2.7 million per annum (Williams et al., 2010).

Developmental changes from juvenile to adult life-stages can alter dispersal strategies within species. The highly successful zebra mussel (*Dreissena polymorpha*, Pallas) disperses rapidly both during the planktonic larval stage, but also during juvenile and adult stages, attaching to motile surfaces using byssal threads (Johnson & Carlton, 1996). In crayfish, dispersal of small, juvenile individuals can occur by downstream drift through the water column at high flow, but they can also be carried to new waterbodies by clinging to the feathers of waterbirds (Anastácio et al., 2014). Whilst terrestrial dispersal has been observed in adult crayfish (Ramalho & Anastácio, 2015), whether small, juvenile crayfish also emigrate from the water is untested. Signal crayfish eggs in the UK hatch between late March and the end of July and hundreds of offspring may hatch synchronously from the same ovigerous female (Holdich et al., 2014), providing ample opportunity for overland dispersal events, should they occur in juvenile crayfish.

Within a population, some individuals are more likely to disperse than others. Emigration from a habitat can be driven by extrinsic factors including high population density (Enfjäll and Leimar 2005) and competition from aggressive conspecifics, which can drive out subordinate individuals (Hudina et al. 2014). Dispersal can also be dependent on the reproductive life-stage of the individual (Bowler & Benton, 2005). In birds, dispersal can be driven by reproductive failure; unsuccessful tree swallows (*Tachycineta bicolor*, Vieillot) are almost ten times more likely to disperse compared to successful individuals (Lagrange et al., 2017). In decapods, whilst terrestrial emigration facilitates the persistence of populations under environmental extremes and aids in the colonisation of new habitats (Cruz & Rebelo, 2007; Grey & Jackson, 2012), in most cases, several crayfish would need to successfully emigrate, walk overland (avoiding threats such as predation or desiccation) and arrive at another waterbody to establish a new population. Ovigerous (egg-carrying) females, however, could substantially increase the likelihood of further colonisation of nearby waterbodies if they are prone to such overland dispersal. Female signal crayfish can carry 200-400 eggs that are incubated for around six months before hatching (Holdich et al., 2014), and so there is a wide window of opportunity for ovigerous female crayfish to terrestrially emigrate and potentially introduce several hundred of her progeny to a new waterbody. It is currently unclear though whether ovigerous and non-ovigerous female crayfish differ in their tendency to emigrate and walk overland.

The present study aimed to 1) examine the terrestrial emigration tendency of juvenile signal crayfish, and 2) compare the terrestrial emigration tendency and walking speed of ovigerous and non-ovigerous female crayfish.

2. Methods

2.1 Crayfish origin and maintenance

Young, juvenile signal crayfish ($n = 17$) were caught in summer 2014 by hand-netting in the Bachawy River, Built Wells, South Wales. These crayfish ranged in carapace length from 8.5 - 26.5 mm and were estimated between 0 - 2 years old (hereafter referred to as juveniles; Guan, 1994). Adult, female signal crayfish, including ovigerous ($n = 10$) and non-ovigerous ($n = 7$) individuals were caught from a small recreational fishery pond within the same catchment using baited cylindrical crayfish traps ('Trappy Traps', Collins Nets Ltd., Dorset, UK) over a 1-week period during spring 2014. The carapace length of ovigerous crayfish was between 32.4 - 54.2 mm (average 44.1 mm) and non-ovigerous females 42.0 - 51.1 mm (average 47.4 mm). All ovigerous crayfish carried > 100 eggs per individual, though it was not possible to accurately count eggs without removing them. Previously, Hunt et al. (unpublished) showed no significant difference in terrestrial emigration tendency between male and female signal crayfish, and so the present study did not test male crayfish.

Crayfish were transported to the Cardiff University aquarium facility and maintained in 100 L holding tanks filled with 70 L dechlorinated water ($13^{\circ}\text{C}\pm 1$) for at least 2 weeks prior to the experiment. Holding tanks included gravel substrate (2 cm deep), plant pot and plastic tube refuges and the crayfish were fed daily with peas and defrosted *Tubifex* bloodworm. Weekly 50% water changes were performed to maintain water quality of holding tanks.

2.2 Ethical note

During the study all institutional and national guidelines (Animals [Scientific Procedures] Act 1986) for the care and use of animals were followed. All traps were checked on a daily basis under Natural Resources Wales trapping license CE068-N-315 and the crayfish were held under a Cefas licence (W C ILFA 002) at Cardiff University. As highly invasive species, all crayfish were humanely euthanized at the end of the experiment by freezing at -20°C to prevent their reintroduction to the wild (in accordance with the Wildlife and Countryside Act, 1981).

2.3 Experimental design

To investigate juvenile signal crayfish terrestrial movement, individual crayfish ($n = 17$, 8.5 - 26.6 mm carapace length) were placed in an observation arena containing pea gravel that gradually sloped ($\sim 35^{\circ}$) upwards towards the centre of the arena to create an equal amount of aquatic and terrestrial space (Fig. 1).

Adult female crayfish were observed in an experimental arena consisting of two separate aquaria (L 120 cm x W 28 cm x D 25 cm) connected by a terrestrial bridge (L 120 cm excluding slopes) with a gradual incline leading out of the water ($\sim 35^{\circ}$) allowing crayfish access to the terrestrial bridge (Fig. 2). The tanks were filled to a depth of 20 cm (leaving approx. 3 cm of the gravel slope out of water).

All crayfish were tested individually, measured (carapace length) at approx. 10:00 h, and then placed in the arena to acclimatise throughout the day. Then, for juvenile crayfish trials the lights switched off automatically at 20:00 h and juvenile crayfish behaviour was recorded until 08:00 h the next day (12 h observations per crayfish). At 08:00 h the following day, the juvenile crayfish was removed from the arena and the water replaced before the next trial began. For the adult female crayfish, the lights switched off at 22:00 h and behavioural observations occurred 08:00 h the next day (8 h observation per crayfish).

All behavioural observations occurred nocturnally, since overland dispersal occurs most often at night (Ramalho & Anastácio, 2015). Infrared LED security cameras (Swann PRO-735, Model: SWPRO-735CAM, Maplin UK) were used to record crayfish during observation periods using a digital video recorder (Swann DVR8-3450 8 Channel 960H Digital Video Recorder, Model: SWDVK-834504F, Maplin UK).

2.4 Statistical analyses

All focal crayfish were used once. For juvenile crayfish, the number of times an individual left the water and, for those crayfish that left at least once, the average time spent out of water per emergence were quantified per individual. Additionally, whether the crayfish spent any time in the terrestrial refuge was recorded. Due to the non-parametric nature of the data, Kendall's Tau correlation was used to examine the relationship between carapace length and the frequency of terrestrial emergences, as well as the time spent out of water per emergence.

For ovigerous and non-ovigerous female crayfish, the total number of times an individual left the water and time spent out of water was quantified. Additionally, the time taken for a crayfish to walk the full length of the bridge (120 cm) was used to estimate walking speed. Generalised Linear Models (GLM) with poisson distributions and 'log' link functions were used to determine whether carapace length or status (i.e. ovigerous or non-ovigerous) affected the frequency of terrestrial emergences and the time spent out of water per emergence. Data for the time spent in shelter by adult crayfish were first log-transformed, and subsequently analysed using a GLM with gaussian distribution and identity link function. A Wilcoxon test was used to compare the average walking speed of ovigerous and non-ovigerous crayfish that fully crossed the terrestrial bridge. All analyses were performed in R version 3.4.0 (R Core Team 2017).

3. Results

3.1 Juvenile crayfish

Overall, 53% of the juvenile signal crayfish left the water and there was a significant, positive relationship between carapace length and terrestrial emigration tendency (Fig. 3; $n = 17$, $z = 3.407$, $p < 0.001$). Crayfish that had a carapace length of 13.7 mm or less did not leave the water but were observed approaching the surface without emigrating on to land, whilst 90% of crayfish above this size left the water at least once (Fig. 3). The smallest crayfish to leave the water (16.6 mm carapace length) did so four times, spending on average 2 min 18 s out of water per terrestrial emergence. Of those crayfish that left the water, size did not have a significant influence on the time spent out of water per emergence ($n = 9$, $T = 36$, $p = 0.737$). Crayfish that left the water did so on average 13.1 (standard error ± 3.4) times over 12 h and spent an average of 2 min 20 s (± 10 s) on land per individual. Juvenile crayfish did not spend any time on the terrestrial refuge.

3.2 Female crayfish

Ovigerous signal crayfish emerged from the water 6.9 (± 2) times on average over 8 h and spent 2 min 15 s (± 20 s) out of water per terrestrial emergence whilst females that were not carrying eggs left the water on average 9.4 (± 3.3) times and spent 2 min 4 s (± 11 s) out of water per emergence. There was no significant difference in terrestrial emergence frequency ($F_{1,11} = 0.519$, $p = 0.482$), time spent out of water ($F_{1,14} = 0.120$, $p = 0.734$) or time spent in a refuge ($F_{1,15} = 0.001$, $p = 0.980$) between ovigerous and non-ovigerous female crayfish. Carapace length did not significantly affect the frequency of terrestrial emergences ($F_{1,14} = 0.266$, $p = 0.614$), time spent out of water per emergence ($F_{1,14} = 1.284$, $p = 0.275$) nor the time spent in a refuge ($F_{1,15} = 2.315$, $p = 0.149$). Of those that fully crossed the terrestrial bridge (120 cm), non-ovigerous females walked slightly faster (0.930 ± 0.083 cm s⁻¹) than ovigerous females (0.712 ± 0.043 cm s⁻¹) though this difference was not statistically significant (Wilcoxon test: $W = 8$, $p = 0.073$).

4. Discussion

The present study shows that size is a critical factor that determines the onset of terrestrial emigration in young, juvenile crayfish, where the smallest individual to leave the water was 16.6 mm in carapace length. Juvenile crayfish below this size threshold approached the surface

but did not leave the water. We also showed that ovigerous females terrestrially emigrate and explore terrestrial environments as frequently as non-ovigerous females, which could have important implications for the management and conservation of crayfish stocks.

Dispersal during larval or juvenile stages can be an important factor that contributes to the success of a species (Johnson & Carlton, 1996). The migration distance of young green turtles (*Chelonia mydas*, Linnaeus) away from natal hatching grounds, for example, significantly decreases with age (Okuyama et al., 2009). However, dispersal during such vulnerable life stages does present a significant risk, and moving overland is a highly risky behaviour for aquatic crustaceans due to the high probability of desiccation or predation (Claussen et al., 2000; Bubb et al., 2008). In the present study, size (and by proxy, age) determined the onset of terrestrial emigration behaviour in juvenile signal crayfish. This closely matches the results of a field study (Ramalho & Anastácio, 2015), where juvenile red swamp crayfish (< 16 mm carapace length) were present in the water but were not found on land. Aquatic crustacean movement on land is highly influenced by temperature and hydration state (Claussen et al., 2000; Allen et al., 2012). The terrestrial movement of male sand fiddler crab (*Uca pugilator*, Bosc) is significantly reduced after 7-10% loss of water (Allen et al., 2012), whilst crayfish walking speed is reduced by half for every 10% loss of water content due to evaporation at 25°C (Claussen et al., 2000). The risk of desiccation is especially problematic for smaller individuals due to their greater surface area for water loss via evaporation (Claussen et al., 2000; Allen et al., 2012). In their first year, juvenile signal crayfish moult up to 11 times (Holdich et al., 2014) and carapace thickness increases with age and maturity (Huner et al., 1976). The present study suggests that juvenile crayfish reach a threshold size before being able to terrestrially emigrate, most likely since reaching this size reduces the risk of desiccation.

The presence of eggs on the abdomen does not deter female signal crayfish from leaving the water and has no significant effect on their terrestrial walking speed. In red swamp crayfish, over a third of individuals dispersing overland were mature females, but only a tenth of these carried eggs or hatchlings (Ramalho & Anastácio, 2015). The present study highlights that ovigerous or hatchling-carrying individuals, despite perhaps being in relatively low abundance in the field compared to non-ovigerous females or males (Ramalho & Anastácio, 2015) may disperse overland. Crayfish that carry hundreds of eggs or hatchlings undoubtedly present the highest risk for colonising nearby waterbodies. A long egg incubation period, ranging from 166 to 280 days in the UK, presents a significant time during which ovigerous crayfish could colonise waterbodies via terrestrial dispersal. During winter, crayfish become less active and may not disperse far, although since hatching occurs as late as the end of July (Holdich et al., 2014), this still provides a long period of warmer temperatures when overland dispersal of ovigerous crayfish can occur.

In conclusion, this study has shown that juvenile, ovigerous and non-ovigerous crayfish emigrate from the water and that size is a critical factor that determines the onset of this behaviour. This highlights the importance of understanding specific dispersal strategies during different life stages of invaders (Strayer, 2010). Furthermore, the results have direct implications for the management of crayfish stocks, both in terms of conservation and invasive species control. Terrestrial movement of ovigerous crayfish should be considered during invasive crayfish population management strategies that include lake draw-down and biocide treatments, since crayfish disperse overland when water levels are lowered (Peay & Dunn, 2014; Ramalho & Anastácio, 2015), which could encourage ovigerous individuals to disperse to nearby waterbodies or remain out of water until treated lakes or ponds are refilled. On the other hand, smaller, juvenile crayfish that do not leave the water could be effectively targeted by biocides, especially since they are difficult to control by trapping alone (Stebbing et al. 2012). In terms of aquaculture, terrestrial movement by ovigerous crayfish from outdoor aquaculture ponds would not only reduce productivity, but also increase the risk of colonising

nearby freshwaters when such facilities exist within crayfish walking distances (up to 1 km in some cases; Souty-Grosset et al., 2016). Finally, future studies should assess the tendency of crayfish that are of conservation concern to emigrate from waterbodies, given the possibility that ovigerous (and therefore highly important) females could terrestrially emigrate from isolated sites in which they are often released.

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Conflict of interest: The authors declare no conflicts of interest.

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Figure 1 – Behavioural arena used to examine juvenile signal crayfish terrestrial movement. A PVC tube shelter was provided both in and out of the water. A gravel incline ($\sim 35^\circ$, dashed area) allowed crayfish to leave the water.

Figure 2 - Experimental arena (not to scale) used to investigate terrestrial movements of female signal crayfish. Two tanks including 2 cm of pea gravel were connected by a terrestrial bridge with a gradual gravel incline ($\sim 35^\circ$ incline, dashed area) leading out of the water to a gravel covered bridge.

Figure 3 – Number of terrestrial emigration events by juvenile signal crayfish over 12 h. All crayfish below 16.6 mm (carapace length) were observed approaching the surface (< 1 cm from surface) but did not leave the water. Dashed line shows terrestrial emigration size threshold, solid line shows the non-linear relationship between carapace length and terrestrial emigration.

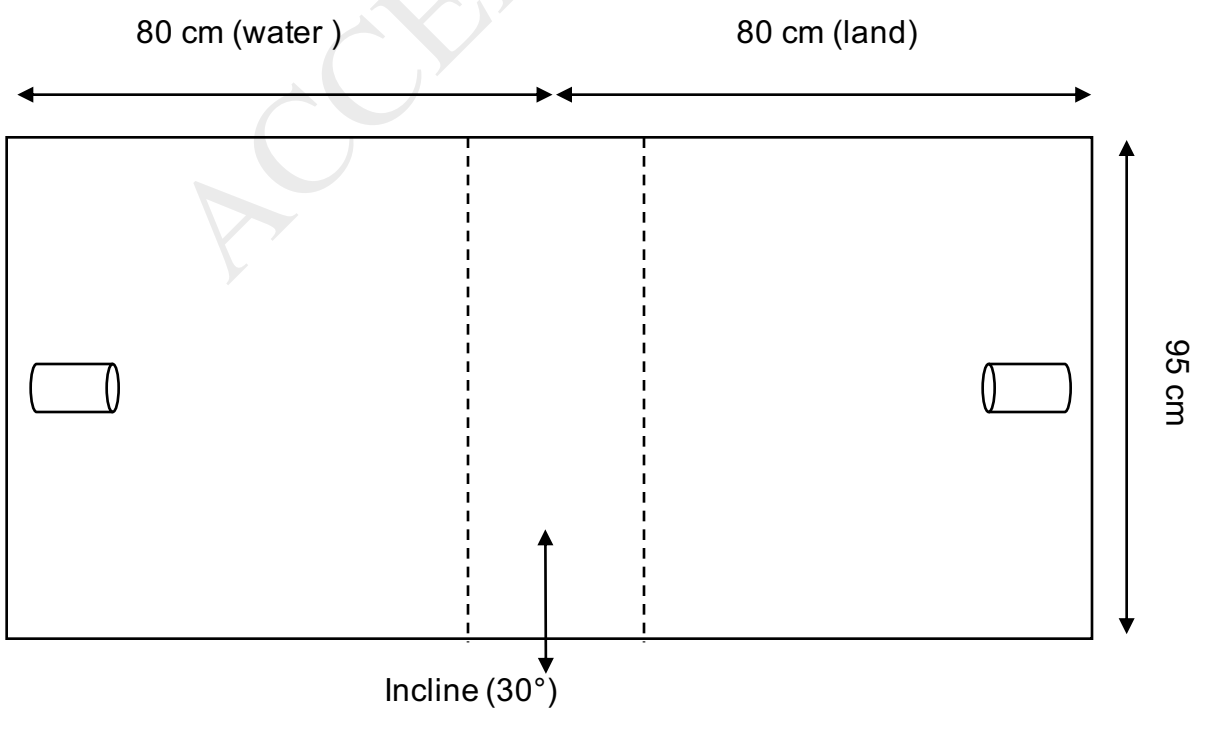
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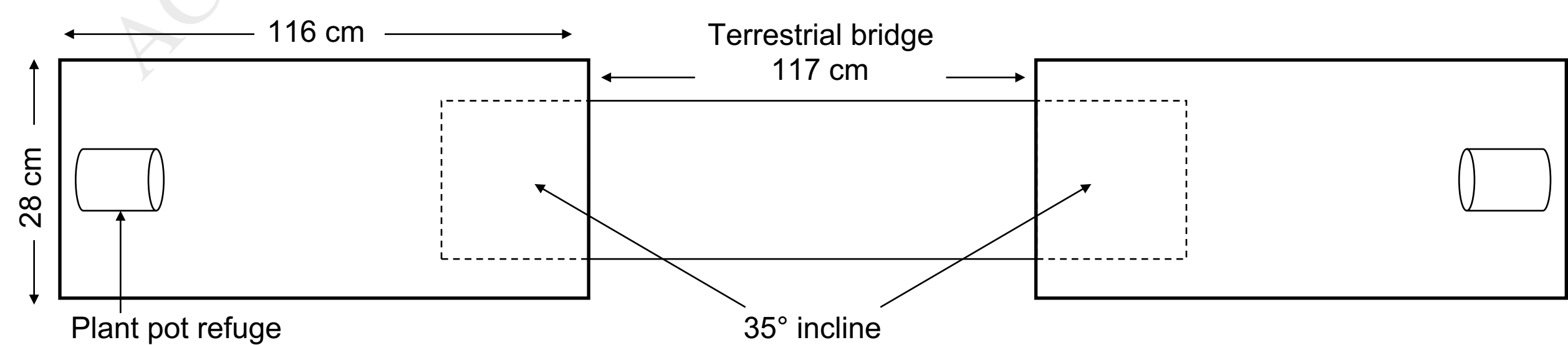
80 cm (water)

80 cm (land)

95 cm

Incline (30°)





Frequency of emergences

