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Factors affecting terrestrial movement in the amphibious mangrove rivulus (*Kryptolebias marmoratus*)

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Abstract

We hypothesised that the exploration tendency of the amphibious mangrove rivulus *Kryptolebias marmoratus* would be inhibited in the terrestrial environment because of constraints on terrestrial locomotion or orientation. Using a novel object test, we showed that the fish explored objects in the aquatic but not the terrestrial environment, supporting the existence of constraints on terrestrial exploration. In further tests of the effects of extrinsic factors on terrestrial movement between aquatic refuges, shallow water depth simulating desiccation risk and the presence of a conspecific simulating intraspecific competition increased emersion outside of refuges, while high water salinity had no effect. These extrinsic factors had little effect on terrestrial movement between different aquatic refuges, except possibly for the lowest water depth tested. A significant association observed between emersion activity and movement between aquatic refuges suggested that terrestrial movement in *K. marmoratus* might depend on the tendency of individuals to emerse.

KEYWORDS
competition, emersion behaviour, exploration, movement, personality, salinity

1 | INTRODUCTION

Animal movement is the change in spatial location of an animal across time (Nathan *et al*., 2008). Proximately, movement is governed by the interactions between intrinsic and extrinsic factors (Rasmussen & Belk, 2012). Intrinsic factors are unique individual traits, which include age, sex, personality, or motivational states. Certain aspects of personality, such as exploratory behaviour tendency, are valuable predictors of movement rates and direction (Réale *et al*., 2007). Notably, a positive correlation between exploratory behaviour and animal movement is observed in various vertebrates, including birds (Dingemanse *et al*., 2002), squirrels (Haughland & Larsen, 2004) and fishes (Rasmussen & Belk, 2012). Intrinsic exploratory tendencies help facilitate future movements as familiarity with the surrounding environment may help establish the most beneficial direction for travel. Extrinsic factors are environmental factors that influence movement, such as food availability, intraspecific aggression–competition and environmental deterioration (Bowler & Benton, 2005). Some factors, such as environmental

deterioration, can interact with intrinsic factors to encourage higher rates of movement (Hui *et al*., 2012), while others, such as predation risk, can negate the effects of personality and discourage movement (Cote *et al*., 2013). Extrinsic factors therefore play an important role in modifying animal movement.

Movement is often prompted by unfavourable habitats, where emigration from these habitats generally confers benefits to individuals that move. Movement is known to be beneficial in heterogeneous environments due to variation in habitat quality. Moving to a higher quality habitat can yield access to denser food resources and new mates (Hanski *et al*., 2002), as well as decrease intraspecific competition (Aars & Ims, 2000). Habitat quality also varies temporally so that strategic movement can be used to promote continued access or access to the best resources available at a given time (Boulinier & Lemel, 1996). In addition to benefits at the individual level, movement also offers advantages at the level of populations and communities by coupling spatially distant habitats (Johst & Brandl, 1997).

Movement can also be costly. Potential costs include energy expenditure, which varies depending on the mode of travel, the distance travelled and the relative difficulty of the route taken (Tucker, 1975). Individuals are subject to a higher risk of predation and mortality during movement between habitats due to potential lack of cover and increased exposure to predators (Waser *et al.*, 1994). Animals can also run the risk of immigration to a habitat of lower relative quality than their original habitat (Bonte *et al.*, 2012). Evidently, there are trade-offs to take into consideration when an individual is faced with the decision to move or stay.

Amphibious fishes have the option to move across the terrestrial environment and have access to microhabitats that are not typically available to fully aquatic fish (Taylor, 2000). This is especially notable in the mangrove rivulus, *Kryptolebias marmoratus* (Poey 1880), a fish species typically found in crab burrows or ephemeral pools in tropical mangrove forests of the western Atlantic Ocean (Taylor, 2000; Taylor *et al.*, 2008). *Kryptolebias marmoratus* have the impressive ability to colonise microhabitats in the terrestrial environment, including remnant insect galleries in rotting logs and leaf litter. These microhabitats are used during the dry season when the burrows or pools contain little to no standing water (Barr *et al.*, 2014; Hulsmans *et al.*, 2008). Low water levels during the dry season decrease access and increase the distance between aquatic habitats. Therefore, *K. marmoratus* must either travel across increasing distances to find suitable aquatic refuges or attempt to find alternative terrestrial microhabitats during dry periods. In addition to desiccation risk, decreasing water levels in aquatic refuges will increase the population density of fish and consequently increase intraspecific competition. Furthermore, increased water evaporation can increase water salinity (Hulsmans *et al.*, 2008). Any tendency to evade such deteriorating environmental conditions may be inhibited due to constraints on terrestrial movement. For example, the design of fishes for efficient aquatic locomotion is thought to constrain terrestrial locomotion performance (Gibb *et al.*, 2013). Furthermore, sensory constraints may also act to inhibit terrestrial movement as aerial exposure can affect vision, chemosensation, or lateral-line input (Sayer, 2005), which could potentially impair orientation of movement on land.

Our aim was to determine the key factors influencing the movement of *K. marmoratus* across the terrestrial landscape. We first tested the hypothesis that intrinsic exploration tendency would be inhibited by constraints on terrestrial locomotion or orientation. We then evaluated how terrestrial movement is influenced by extrinsic factors in a simulated terrestrial landscape. We expected that terrestrial exploration would be reduced relative to aquatic exploration in *K. marmoratus* and that extrinsic factors (desiccation risk, salinity and intraspecific competition) would increase terrestrial movements to different aquatic refuges distributed across the terrestrial landscape. Exploratory behaviour was measured in laboratory conditions simulating aquatic, semi-terrestrial and terrestrial conditions. Thereafter, we measured emersion events and movement between refuges in simulated terrestrial conditions for various treatments manipulating refuge water depth, salinity and intraspecific competition.

2 | MATERIALS AND METHODS

All procedures in this work were approved by the University of Guelph animal care committee (Animal Utilisation Protocol #2239) under the guidelines of the Canadian Council on Animal Care.
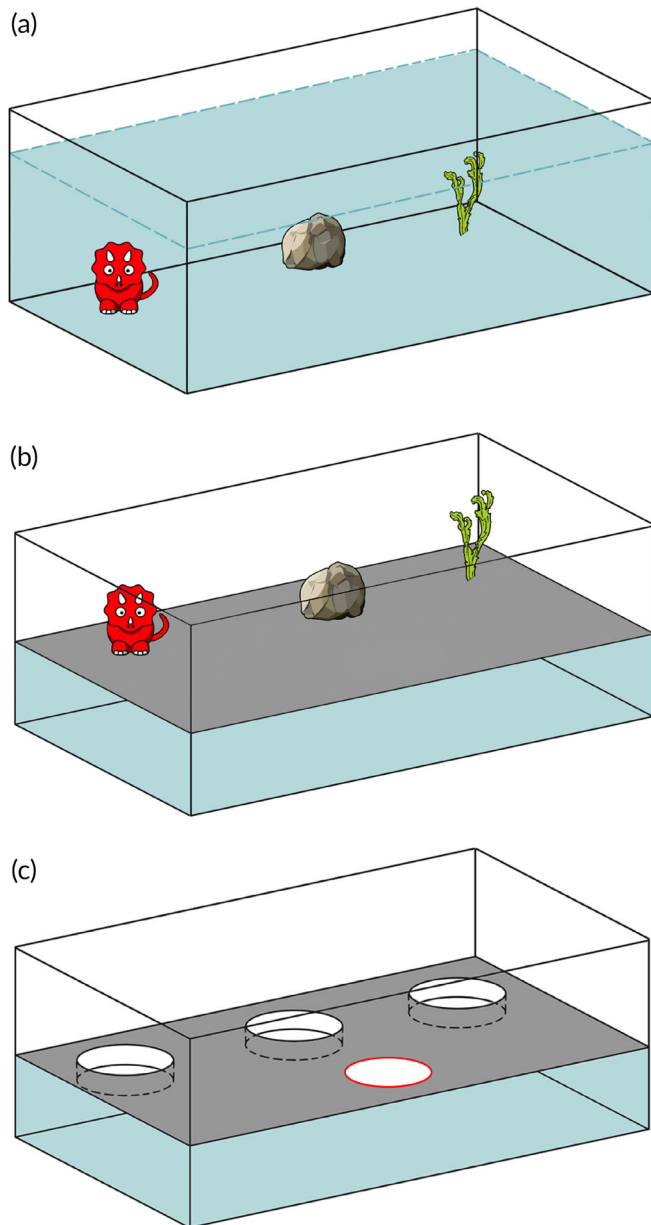
2.1 | Experimental animals

We used self-fertilising, hermaphroditic *K. marmoratus* originating from Honduras (strain HON9; Tatarenkov *et al.*, 2010) in these experiments. All fish were genetically identical at 32 microsatellite loci (Tatarenkov *et al.*, 2010), allowing us to focus on environmental factors. Fish were held separately in individual 100 ml plastic cups with 15 g l^{-1} artificial brackish water (Instant Ocean sea salt mixed with reverse osmosis water, United Pet Group Inc.; www.petfoodindustry.com) at the University of Guelph Hagen Aqualab (www.uoguelph.ca/ib/infrastructure_and_facilities/aqualab). Fish were maintained on a 12 h:12 h light: dark photoperiod at 25°C ambient temperature and they were fed twice weekly with live brine shrimp (*Artemia*).

2.2 | Object exploration

To test whether *K. marmoratus* display differential exploratory behaviour in aquatic *v.* terrestrial environments, we conducted a novel object test under three conditions: terrestrial, semi-terrestrial and aquatic (Figure 1a,b). All conditions were set up in 40 l glass tanks (length: 51 cm, width: 33 cm, depth: 28 cm). Terrestrial tanks contained a suspended platform made of PVC, with a moist filter pad placed on top to simulate the terrestrial substrate. Brackish water was added to the tank until the water level reached the suspended platform, such that water would be in contact with the filter pad *via* the perforations in the platform. This ensured that the substrate was kept moist throughout the experiments. Semi-terrestrial tanks were identical to terrestrial tanks in design; however, the water level was brought up *c.* 2 mm above the level of the filter pad substrate to stimulate 'squiggle' locomotion (*sensu* Pronko *et al.*, 2013), while still ensuring that more than half of the dorsal part of the fish was exposed to air. Aquatic tanks were filled with 20 l of 15 g l^{-1} brackish water and contained no platform.

Fish were randomly assigned to a terrestrial (*n* = 13), semi-terrestrial (*n* = 12), or aquatic (*n* = 11) condition. In this experiment, each fish was used only once for a test in one of the conditions stated above. Four fish were tested simultaneously in four separate tanks. Individual fish were placed in a tank and allowed a 24 h acclimation period to allow familiarisation with their new surroundings. Acclimation to a new environment is important, as the response to a novel object varies according to experience with the environment (*i.e.*, fish will often not explore if they have no experience with their current environment (Russell, 1967)). Following the acclimation period, three novel objects were placed in the tank. The objects used (green artificial plant, red plastic dinosaur toy, large white rock) were chosen to maximise colour and shape differentiation. Fish activity was recorded

FIGURE 1 Experimental tanks for studying the behavior of *Kryptolebias marmoratus* with: (a) novel objects within fully aquatic condition; (b) novel objects in semi-terrestrial and terrestrial conditions; (c) simulated crab burrows in terrestrial condition (○, the starting position in each assay, where focal fish were acclimated before beginning observations). A 2 cm radius surrounding objects in (a), (b) was used as the threshold distance to count *K. marmoratus* approaches

for 4 h following the introduction of the objects using a webcam (Logitech C270; ww.logitech.com) suspended over the tank. We analysed the video footage for exploratory behaviour, which was measured as the frequency of approaches made within an estimated 2 cm radius near an object using a superimposed image of a circle on objects in the video footage (object approaches h^{-1}) and the latency to explore all three objects (*i.e.*, the amount of time to approach each object at least once). More exploration was indicated by a higher frequency of object approaches and a lower latency to explore all three objects.

2.3 | Effect of extrinsic factors on movement in a terrestrial landscape

We performed three experiments to determine the effects of extrinsic factors on terrestrial emersion from aquatic refuges and movement. We additionally measured exploratory behaviours to see if conditions in aquatic refuges could stimulate terrestrial exploration. We defined emersion as fish fully emerging from the water, movement as the change in location from one aquatic refuge to another and exploration as the fish visiting each aquatic refuge present in the tank at least once. For this purpose, the terrestrial condition presented above was modified with the addition of artificial crab burrows to mimic micro-habitats found in the terrestrial landscape of mangrove forests. The burrows were depressions below the platform surface fitted with a plastic container of 6 cm diameter and a maximal depth of 8 cm (Figure 1c). Three burrows were lined along one side of the tank and one start burrow was placed in the middle on the opposite side of the tank. All burrows were filled with water such that the water level was flush with the substrate surface. At the beginning of experiments, fish were placed in the start burrow of each tank with a cover preventing emersion for a 10 min acclimation period. This shorter acclimation period in comparison with the experiment on object exploration behaviour was chosen after early trials showed no difference in activity following 24 h or 10 min acclimation. Following the acclimation period, the cover was removed and terrestrial activity was recorded for 8 h (water depth and salinity) or 4 h (competition). The recording period in the competition experiment was reduced to 4 h because preliminary observations showed that activity levels in the presence of a conspecific were high early but decreased greatly thereafter. The following variables were measured: the rate of emersion (emersion h^{-1}), latency to first emerse (min) and proportion of fish that successfully moved between burrows. Fish were considered to have successfully moved between burrows if they emersed from the start burrow and moved across the terrestrial substrate to a different burrow. We had originally planned to analyse the latency to explore all three burrows, but this exploratory behaviour was rare (2 out of 55 trials) under the testing conditions.

We tested the effect of water depth by varying burrow depth. In order to simulate different desiccation risks, a plastic Petri dish was inserted into the plastic containers to modify burrow depths (deep – 8 cm; intermediate – 3 cm; shallow – 1 cm). In this experiment, each fish (n = 8) was tested with the three burrow depths on consecutive days (one burrow depth day^{-1}) in a counterbalanced order. All burrows contained artificial brackish water at 15 g l^{-1} salinity and a temperature of 25 °C.

To test the effect of salinity, fish were held in tanks identical to those used in the water depth experiment, with the exception that burrow depth was kept constant at eight cm. Burrows contained either 15 g l^{-1} artificial brackish water (control, n = 4), or 60 g l^{-1} artificial seawater (high-salinity treatment, n = 8), the upper salinity limit

at which *K. marmoratus* are found in nature (Taylor, 2000). Controls and high-salinity treatments were run simultaneously using three tanks (one control and two high salinity treatments at a time) during 4 consecutive days. In this experiment, each fish was only tested once for a given treatment.

To test the effect of intraspecific competition, we used the same tank design as described above with a depth of 8 cm of 15 g l^{-1} salinity water in all burrows. In the control treatment (*n* = 8), one fish was placed in the start burrow, while two fish of the same HON9 strain were placed in the start burrow in the competition treatment (*n* = 11). Controls were run first followed by the competition treatment. A maximum of four individuals or four pairs of fish were run on a given testing day using four tanks. Only three tanks were used for the last day of testing due to a tank failure. In this experiment, each fish or pair of fish was only tested once in a given treatment. Emersion and movement rate in the competition treatment were determined as an average of the two fish due to difficulty differentiating between the individual fish in the video footage.

2.4 | Statistical analyses

Non-parametric ranked statistics were used as the data were not normally distributed. The Kruskal-Wallis test was used to compare exploratory behaviour variables in the object exploration experiment, where different fish were tested in three different conditions, while a Friedman test for non-independent data was used to compare emersion variables for fish tested across all three different conditions in the water depth experiment. Upon a significant Kruskal-Wallis or Friedman test (*P* < 0.05), a Dunn's multiple-comparison test was used to determine differences between conditions. A Mann–Whitney test was used when comparing emersion variables in experiments where different fish were used in only two conditions in the salinity and intraspecific competition experiments. The proportion of fish that explored objects or moved between burrows was analysed using Fisher's exact test, which is adequate for small sample sizes. A Bonferroni correction for multiple comparisons (α = 0.017) was applied when comparing proportions across three conditions. The effect of testing order in the water depth experiment was assessed using generalised estimating equations models with water depth and testing order as within-subject factors. The models used a normal distribution and an independent working correlation matrix structure. Finally, the relationship between the number of emersions and number of movements made by each fish was analysed using a Spearman correlation test on data pooled from experiments testing the effects of extrinsic factors on movement. For this purpose, only the first test of water depth was used to avoid using the same fish more than once. All fish from the salinity experiment were used (no differences between groups), but only controls in the competition experiment were used due to our inability to track individual fish behaviour in the competition treatment. For this analysis, emersion activity was defined as the number of emersion events and movement was defined as the number of movements between one burrow to a new burrow during the observation period, which allowed analysis of the relationship between

emersion and movement using a correlation approach. All tests were conducted in GraphPad Prism 5.04 (GraphPad Software; www.graphpad.com) and α was set at 0.05.

3 | RESULTS

3.1 | Object exploration

Kryptolebias marmoratus in the aquatic condition made more object approaches compared with the semi-terrestrial and terrestrial conditions (Figure 2a). This is supported by a significant Kruskal-Wallis test, $H(2) = 26.11$, $P < 0.001$ and Dunn's test showing significant differences between the aquatic and both semi-terrestrial and terrestrial conditions, but no significant difference between the semi-terrestrial and terrestrial conditions. In the same experiment, all 11 *K. marmoratus* in the aquatic condition approached each object at least once within a time of 35 ± 5.9 min (mean ± SE), while only one of 12 fish in the semi-terrestrial and none of the 13 fish in the terrestrial conditions explored all three objects during the experimental period (Figure 2b). Latency to investigate objects in the aquatic condition was significantly shorter than in the semi-terrestrial and terrestrial conditions, $H(2) = 31.76$, $P < 0.001$. There were significant differences in the proportion of fish exploring objects across the aquatic *v.* semi-terrestrial and aquatic *v.* terrestrial conditions, $P < 0.001$ in both cases.

3.2 | Effect of extrinsic factors on movement in a terrestrial landscape

3.2.1 | Water depth

Time to emerse from the burrows was significantly longer in the deep and intermediate water depths compared with the shallow depth, $\chi^2(2) = 13.0$, $P < 0.001$ (shallow *v.* deep, $P < 0.05$; shallow *v.* intermediate, $P < 0.05$), but there was no significant difference between the deep and intermediate water depths ($P > 0.05$; Figure 3a). Additionally, fish in the shallow depth emersed at a significantly higher rate than at deep or intermediate depths, $\chi^2(2) = 13.7$, $P < 0.001$ (shallow *v.* deep, $P < 0.05$; shallow *v.* intermediate, $P < 0.05$; Figure 3b). There was no significant difference between emersion rates at deep and intermediate depths ($P > 0.05$). All eight fish in shallow water depth moved between burrows successfully during the experimental period, while five out of eight and four out of eight fish moved between burrows at intermediate and deep-water depths, respectively. Fisher's exact test showed that these differences were not statistically significant: deep *v.* shallow, $P > 0.05$, intermediate *v.* shallow, $P > 0.05$, intermediate *v.* deep, $P > 0.05$. On the other hand, the number of movements by fish in the deep and intermediate depths were limited to once or twice, while the fish in the shallow-water depth made one to five movements during the experiment.

An additional Friedman's test followed by a Dunn's multiple-comparison test were used to analyse the effect of water depth on rate of movement. Fish in the shallow-water depth exhibited significantly higher rates of movement compared with fish in the intermediate depth, but not compared with fish in the deep water
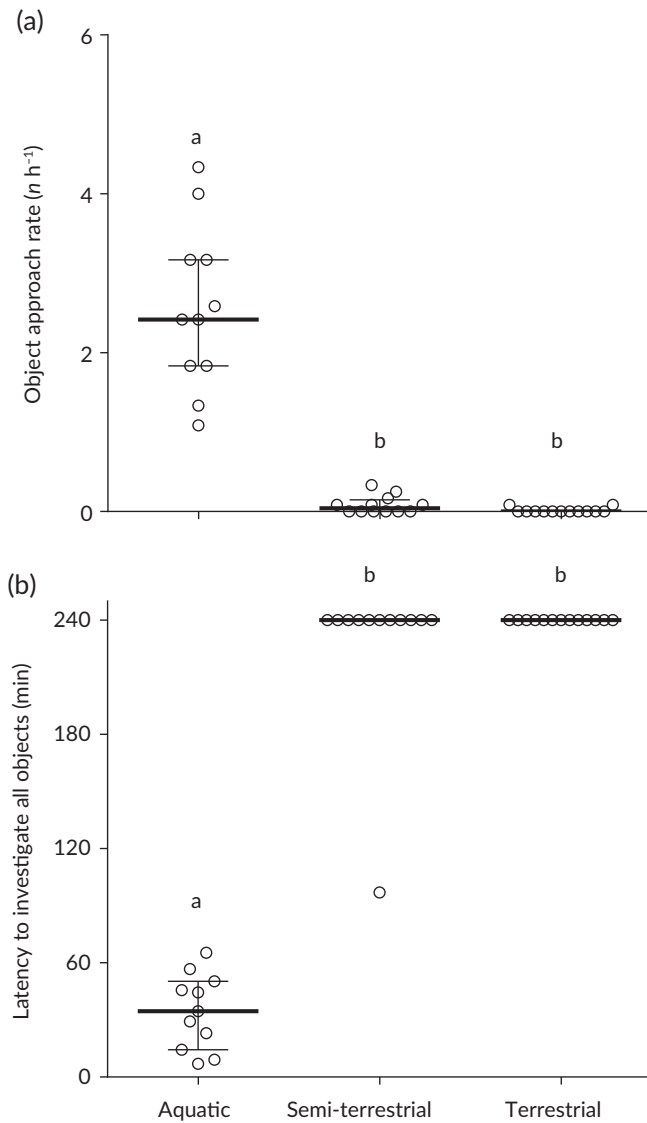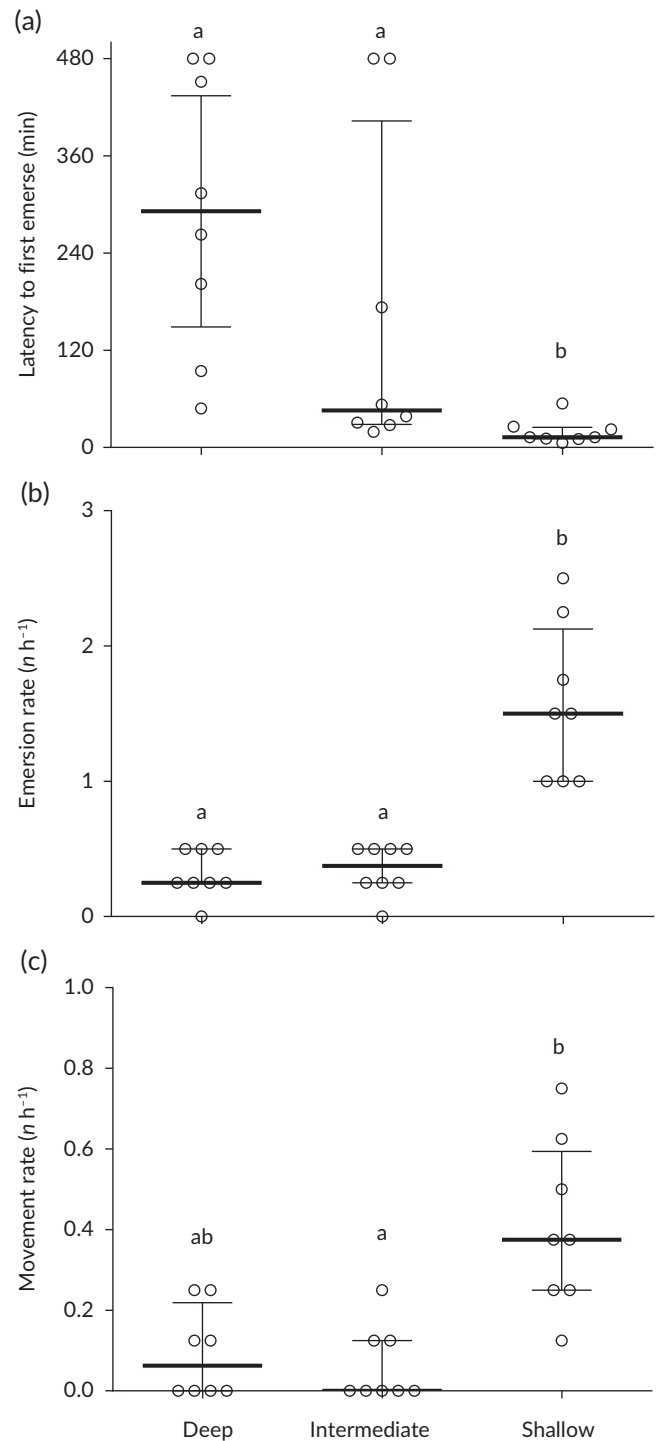
FIGURE 2 Median (± interquartile range; n = 11 for the aquatic condition, 12 for the semi-terrestrial condition, and 13 for the terrestrial condition shown in Figure 1) of (a) rate at which *Kryptolebias marmoratus* approach a novel object in the experimental tank and (b) the latency to investigate all three objects during a 4 h observation period. Only one fish in the semi-terrestrial or terrestrial conditions investigated all three objects during the observation period. Different lowercase letters indicate significant differences: Dunn's multiple comparison test, $P < 0.05$

depth, $\chi^2(2) = 9.9$, $P < 0.01$ (shallow $v.$ intermediate, $P < 0.05$; shallow $v.$ deep, $P > 0.05$; deep $v.$ intermediate, $P > 0.05$; Figure 3c). A separate analysis showed that testing order had no effect on time to emerse from burrows, $F(2) = 2.2$, $P > 0.05$) or rate of movement between burrows, $F(2) = 2.6$, $P > 0.05$, but emersion rate increased with subsequent testing, $F(2) = 10.1$, $P < 0.01$, pairwise comparisons: first $v.$ second, $P > 0.05$; first $v.$ third, $P < 0.01$, second $v.$ third, $P < 0.05$.
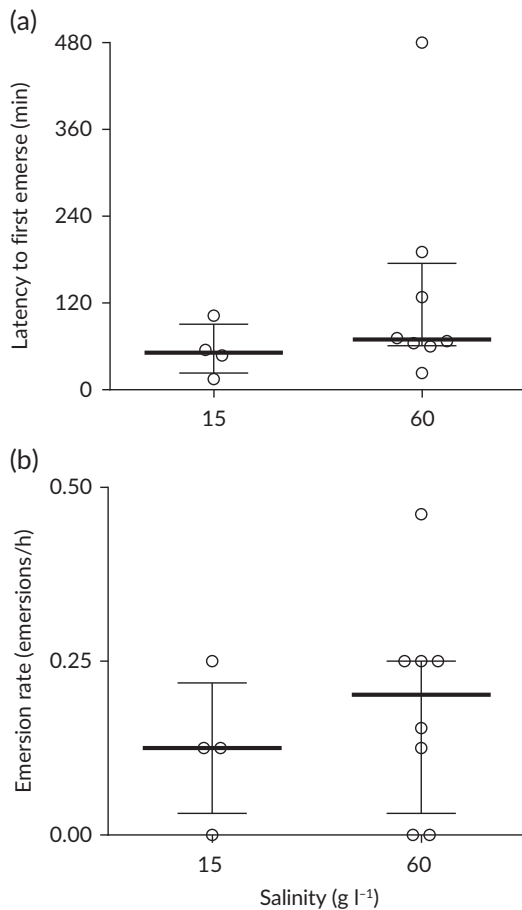
3.2.2 | Salinity

Time to emerse did not differ between salinities of 15 and 60 g l^{-1}, $U = 7$ ($n_{15} = 4$, $n_{60} = 8$), $P > 0.05$ (Figure 4a). Similarly, there was no

FIGURE 3 Median (± interquartile range; n = 8 at each depth) of (a) latency of *Kryptolebias marmoratus* to emerse from a simulated crab burrow during an 8 h observation period, (b) the emersion rate and (c) the rate of movement between simulated crab burrows. Different lowercase letters indicate significant differences: Dunn's multiple comparison test, $P < 0.05$

difference in emersion rates between fish that occupied burrows filled with water of different salinities (Figure 4b), $U = 11.5$ ($n_{15} = 4$, $n_{60} = 8$), $P > 0.05$. One of four fish in 15 g l^{-1} salinity moved between burrows successfully during the experimental period, while five out of
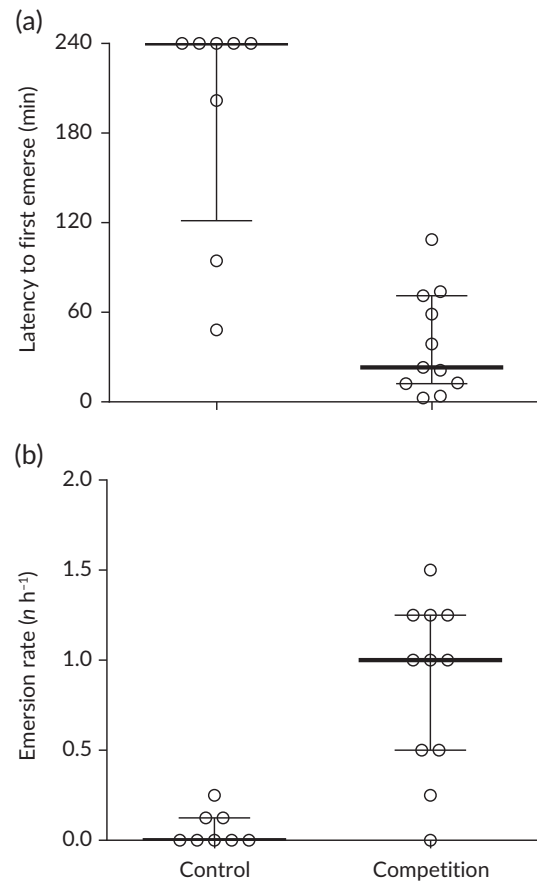
FIGURE 4 Median (±interquartile range; n = 4 at salinity 15 g l^{-1} and 8 at 60 g l^{-1}) for the (a) latency of *Kryptolebias marmoratus* to emerse from a simulated crab burrow during an 8 h observation period and (b) emersion rate. $P > 0.05$

FIGURE 5 Median (±interquartile range; n = 8 control and 11 for competition treatments) for the (a) latency of *Kryptolebias marmoratus* to emerse from a burrow during a 4 h observation period and (b) emersion rate. Emersion behavior was significantly increased by competition ($P < 0.05$)

eight fish moved between burrows in 60 g l^{-1} salinity. Fisher's exact test showed that this difference was not statistically significant, $P > 0.05$. With the exception of one fish that made two movements, each fish only made one movement or none at all, so no further analysis of movement was conducted for this experiment.

3.2.3 | Intraspecific competition

Kryptolebias marmoratus in the competition treatment emersed significantly faster than in the control treatment, $U = 5$ ($n_{control}$ = 8, $n_{competition}$ = 11), $P < 0.001$ (Figure 5a). In addition, fish in the competition treatment emersed at a significantly higher rate than in the control treatment, $U = 6$, ($n_{control}$ = 8, $n_{competition}$ = 11), $P < 0.01$ (Figure 5b). Four out of eight controls moved between burrows successfully during the experimental period, while five out of 11 fish moved in the competition treatment. Fisher's exact test showed that there was no difference in proportion of movement between treatments, $P > 0.05$. Each fish only made one movement or none at all, so no further analysis of movements was conducted for this experiment.
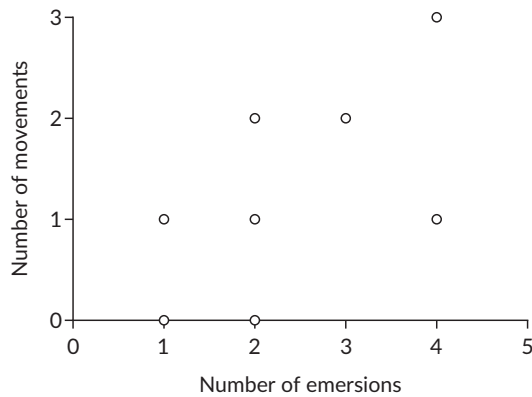
3.3 | Emersion and terrestrial movement

Because none of the extrinsic factors had much influence on terrestrial movement, we tested if there was a relationship between emersion behaviour and movement in *K. marmoratus* tested for the effects of extrinsic factors in this study. Fish used for novel object tests and fish in the competition treatment were excluded. Also note that only the first test in the water-depth experiment was used to assess this relationship. If a relationship between emersion behaviour and movement across fish were noted, it could imply a role for individual differences in determining population patterns in terrestrial movement in this species. The Spearman correlation test showed that the number of emersions made by *K. marmoratus* was positively associated with the number of movements between burrows when excluding the four fish that did not emerse during observations, $r_S(24) = 0.57$, $P < 0.01$ (Figure 6).

4 | DISCUSSION

Our findings are in line with the hypothesised inhibition of exploration behaviour in amphibious fish due to constraints posed by terrestrial

FIGURE 6 Data from the water depth, salinity and competition experiments with *Kryptolebias marmoratus* were used to assess the relationship between the number of emersions and terrestrial movements, but the second and third tests in the water-depth experiment and fish in the competition treatment were excluded. Four fish that did not emerse were also excluded from this analysis. A Spearman correlation test showed a positive relationship between emersion and terrestrial movements, $r_S(24) = 0.57$, $P < 0.01$. *n.b.* Many data points overlap

conditions, as exploratory behaviour in *K. marmoratus* was limited to the aquatic condition in the object exploration experiment. When assessing the role of extrinsic factors on terrestrial movement, we found that refuge water depth influenced emersion behaviour and terrestrial movement. However, water salinity and intraspecific competition did not influence terrestrial movement, which was contrary to our expectation. We also found an overall positive relationship between emersion and terrestrial movement independent of extrinsic factors, suggesting that individual differences in emersion behaviour could contribute to terrestrial colonisation in amphibious fish populations.

4.1 | Exploratory behaviour

As far as we are aware, our study was the first to investigate exploratory behaviour in different environment types (terrestrial *v.* aquatic) in an amphibious fish. *Kryptolebias marmoratus* displayed object exploration behaviour in an aquatic laboratory environment. This result confirms findings in non-amphibious fish that explored novel objects in similar situations (Brown *et al.*, 2007; Burns, 2008). Conversely, this exploratory tendency was not observed in the simulated terrestrial or semi-terrestrial environments, which suggests that exploratory behaviour is inhibited outside water. This decline in exploratory tendency may be due to higher energetic costs of locomotion in terrestrial compared to aquatic environments (Schmidt-Nielsen, 1972). Although *K. marmoratus* demonstrate a suite of terrestrial locomotory behaviours (Pronko *et al.*, 2013), terrestrially-acclimated individuals are exhausted less quickly than non-acclimated individuals (Brunt *et al.*, 2016). The goal of our semi-terrestrial condition was to alleviate some of these locomotory constraints on terrestrial movement of recently emersed fish. However, video analysis revealed that most of the fish locomotion in the semi-terrestrial condition consisted of tail flipping,

as opposed to lateral undulation swimming used in the aquatic environment. As fish in both conditions were still employing a relatively inefficient mode of locomotion (Gibb *et al.*, 2013), it could explain the lack of differences in exploratory behaviour between the semi-terrestrial and terrestrial conditions observed here.

In addition to locomotory constraints, potential sensory constraints may also have played a role in the diminished exploratory behaviour observed in terrestrial conditions. Being aerially exposed could potentially prevent *K. marmoratus* from assessing their surroundings through vision, chemosensation or lateral line input, thus reducing the likelihood of expressing exploratory behaviour on land. While Bressman *et al.* (2018) found that *K. marmoratus* demonstrated intentional movement towards broad visual cues in a terrestrial environment (*i.e.*, coloured areas or areas covered with aluminium foil in a laboratory setting), thus indicating an ability to orient in a terrestrial environment, they also noted that fish tended to move away from artificial plants and cited the possibility that individuals may have perceived these plants as an unfamiliar threat. Comparably, we observed that *K. marmoratus* did not move towards discrete, novel objects in a terrestrial environment. It is possible that *K. marmoratus* can use broad visual cues such as colour or shade to orient themselves on land, but aerial exposure may still impose sensory limitations on their ability to resolve more detailed visual cues. Other amphibious fish have evolved adaptations to mitigate such sensory constraints on land, such as the presence of a flattened cornea in the large-headed goby *Millerigobius macrocephalus* (Kolombatović 1891) (Graham, 1970; Graham & Rosenblatt, 1970) and pearl blenny *Entomacrodus nigricans* Gill 1859 (Graham *et al.*, 1985), or vertically variable lens thickness and double pupils in the largescale four-eyed fish *Anableps anableps* (L. 1758) (Swamynathan *et al.*, 2003). It is not known if *K. marmoratus* possess such adaptations.

4.2 | Extrinsic factors

Water depth had a strong effect on emersion behaviour and a subtle effect on terrestrial movement, suggesting that increased desiccation risk may encourage terrestrial movement in *K. marmoratus*. Future work with larger sample sizes may be needed to validate this last observation because terrestrial movements were observed at all water depths in the experiment. Furthermore, because there was no effect of testing order on the rate of movement between burrows in the water depth experiment, we reject the possibility that acclimation to the test conditions could release a propensity to explore the terrestrial landscape that would be limited by the short acclimation period we used. Nevertheless, if we accept that desiccation risk is a factor that promotes terrestrial movement, this activity might be explained by the patterns of water level fluctuation in fish habitats. Unlike intertidal sculpins that experience predictable daily tides that passively emerse individuals (Martin, 1991), mangrove forests experience seasonal changes in water level, with ephemeral pools containing little to no standing water for weeks or months in the dry season (Barr *et al.*, 2014; Hulsmans *et al.*, 2008). Thus, low water levels may signal the

need to find a moist terrestrial habitat in *K. marmoratus* to avoid being trapped in drying conditions.

In mangrove forests, as water levels drop during the dry season, salinity is increased as a result of rapid water evaporation (Hulsmans *et al.*, 2008; Robert *et al.*, 2009; Taylor, 2012). Thus, we expected that *K. marmoratus* would move to avoid osmotic stress due to high salinity, an idea supported by the findings of Brown *et al.* (1990) who showed that some species of intertidal blennies preferred water within a range of 30–40 g l^{-1} but avoided salinities >60 g l^{-1}. This salinity level is observed during the dry season in mangrove forests (Robert *et al.*, 2009). Surprisingly, *K. marmoratus* did not avoid 60 g l^{-1} water. This is possibly because they are euryhaline, tolerating up to 114 g l^{-1} seawater (King *et al.*, 1989). However, transfer to increased salinity immediately increased metabolic rate and swimming activity in *K. marmoratus* (Sutton *et al.*, 2018), suggesting that this species can tolerate high levels of salinity without emersion.

The presence of a conspecific in a burrow had a strong effect on emersion behaviour, but no effect on movement between burrows. Increased emersion rate may be an avoidance strategy used to escape short-term aggression displayed by conspecifics in the same burrow. This idea is supported by studies in *K. marmoratus* showing that intraspecific aggression is pervasive in water (Hsu & Wolf, 2001; Huehner *et al.*, 1985; Taylor, 1990), but ceases once fish are emersed (Taylor, 2012). Therefore, the observation of a selective increase in emersion without movement between burrows in the presence of a conspecific may simply serve to temporarily relieve fish from immediate intraspecific aggression, rather than prompt fish to move away to avoid further aggression in the future.

4.3 | Emersion *versus* terrestrial movement

When fish tested individually for the effects of extrinsic factors were analysed together, a positive relationship between emersion and terrestrial movement was found, which was independent of extrinsic factors. Such a relationship could indicate that individual differences in emersion behaviour could drive patterns of terrestrial movement and suggests that there could be behavioural phenotypes (*i.e.*, moving *v.* stationary individuals) in the isogenic lineage of *K. marmoratus* that was investigated. Behavioural phenotypes related to habitat movement have been observed in other vertebrates (Debeffe *et al.*, 2013; Dingemanse *et al.*, 2003; Rasmussen & Belk, 2012). Where movement phenotypes are found, movement is often promoted by density-dependent factors such as resource limitations and intraspecific competition (Bowler & Benton, 2005). In this context, once an appropriate number of individuals emigrate from the present habitat, these density-dependent factors are no longer detrimental, leaving stationary individuals with a habitat that is still sufficient for their needs. This would promote the evolution of distinct behavioural phenotypes: moving and stationary individuals, without the need for movement between habitats triggered by competitive encounters, as observed here. While individual differences in emersion behavior within isogenic lineages of *K. marmoratus* have previously been demonstrated (Turko *et al.*, 2011), more work is needed to demonstrate if behavioural phenotypes influence terrestrial movements in this species.

In conclusion, results of the present study support the idea that extrinsic factors that promote emersion behaviour do not directly promote terrestrial movement in *K. marmoratus*. However, it is possible that desiccation risk could influence terrestrial movement, as shallow water was associated with more movement between burrows in the water depth experiment. Nevertheless, terrestrial movement in this fish appears primarily influenced by intrinsic factors distinct from exploratory behaviour for two reasons: first, because exploration was suppressed in the terrestrial environment, possibly due to the high cost of locomotion on land or sensory constraints outside water and second, because there is an overall positive relationship between emersion and movement among individuals that does not depend on extrinsic factors. Future studies should examine how personality traits (*e.g.*, boldness, aggression) could affect emersion and terrestrial movement in *K. marmoratus* and if personality traits interact with extrinsic factors to modify movement. It would also be interesting to see if different genetic lineages of *K. marmoratus* differ in emersion and movement behaviours. While our findings may not be applicable to all amphibious fish species, such as those that use the terrestrial environment more regularly (*e.g.*, sculpins and mudskippers), they highlight important nuances that need to be considered if we are to understand how and why amphibious fish use terrestrial habitats.

CONTRIBUTIONS

I.Y.-L., ideas, data generation, data analysis and draft of manuscript; P.A.W., ideas, manuscript editing and funding; F.L., Ideas, data analysis, manuscript editing and funding.

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