

RESEARCH NOTE

Trail following differs between wild and captive-reared snails, *Lymnaea stagnalis*

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Trail following has been identified in a wide variety of gastropod species, in which individuals follow mucus trails laid down previously by themselves, conspecifics or heterospecifics. Trail following may have a variety of functions across species, including energy conservation through the reduction of mucus production (Davies & Blackwell, 2007) and energy gain through trail ingestion (Hutchinson *et al.*, 2007), nutrition by exploitation of food in mucus trails (Davies & Beckwith, 1999), homing behaviour (Cook & Cook, 1975; McFaruume, 1980), surfacing (Wells & Buckley, 1972) and locating trail-laying prey (Paine, 1963). However, a primary purpose of trail following is thought to be the location of conspecifics (Croll, 1983; Ng *et al.*, 2013).

In freshwater hermaphroditic gastropods, trail following has been demonstrated as a mate-location strategy in *Biomphalaria glabrata* (Townsend, 1974), but it is unknown whether this behaviour is widespread among freshwater gastropods. *Lymnaea stagnalis* (L.) is a preferentially outcrossing freshwater hermaphroditic gastropod (Puurtinen *et al.*, 2007) and is frequently used as a model species in neurobiology and behavioural ecology (Rundle *et al.*, 2004; Dalesman *et al.*, 2007; Benjamin & Kemenes, 2008). Research on reproduction in *L. stagnalis* has focused on mate choice rather than the mechanisms of mate location (De Boer *et al.*, 1997; Koene & Ter Maat, 2007). Here we investigated whether or not *L. stagnalis* exhibits trail-following behaviour and implements it as a mate-location strategy. Experiments were designed to compare a baseline of trail-following behaviour with that exhibited by the same individuals after increased sexual arousal resulting from a build-up of sperm during isolation (De Boer *et al.*, 1997). It was predicted that if trail following is used as a mate-location strategy in *L. stagnalis*, social isolation of individuals would increase trail-following behaviour. In fish, captive rearing is known to alter a number of behavioural traits, including conspecific interactions such as courtship (Kelley, Magurran & Macias-Garcia, 2005), aggression (Fleming *et al.*, 1996) and shoaling behaviour (Kydd & Brown, 2009). Therefore, we also assessed whether rearing in the laboratory had altered trail-following behaviour in *L. stagnalis* relative to wild counterparts originating from the same population.

Wild adult *L. stagnalis* were collected from the Somerset levels, Somerset, UK (South Drain: 51°10'50"N, 2°52'49"W) and acclimated to laboratory conditions for 1 week prior to any experimentation. Laboratory-reared snails originated from the same source, but were bred to *F*₂ generation in the laboratory.

In the laboratory snails were maintained in aquaria (30 × 19.5 × 20.5 cm deep) at 20 ± 1°C under a 14:10 light:dark cycle in aerated artificial pond water (APW) with 80 µg l⁻¹ Ca²⁺ (Dalesman & Lukowiak, 2010) and fed *ad libitum* on lettuce and trout pellets.

Snails were randomly selected from either the laboratory or wild stock, labelled using numbers printed on waterproof paper and attached to the shell using Loctite[®] 454 gel (Henkel Ltd., Hatfield, UK) and designated as marker snails (laying the mucus trail) or tracker snails (used to test if the trail was followed). All tracker snails were tested twice under both grouped and isolated conditions, randomizing the order in which they experienced each treatment (grouped *vs* isolated). Prior to behavioural trials, snails were maintained for 1 week in aerated APW in either grouped conditions (5 snails/l in 4-l aquaria) or isolated conditions (1 snail in 200 ml in 250-ml glass beakers). Snails were all of approximately the same size; there were no significant size differences between the treatment groups or between marker and tracker animals.

In total 20 laboratory-reared and 22 wild-caught snails were used as trackers. Behavioural trials were carried out in clear acrylic aquaria (30 × 19.5 × 20.5 cm deep, containing 400 ml APW) laid on A4 graph paper marked with 1-mm interval lines to enable the path of each snail to be recorded. The trail for each marker and tracker combination was recorded by the observer marking the position of the tip of the head continuously throughout the trials on separate graph paper, allowing accuracy of trail locations to within ± 1 mm. Trials were initiated by placing a marker snail in the centre of the aquarium, using a new individual on each occasion. On emergence (eyes and tentacles fully visible), the starting position of the head was recorded and animal movement mapped for 15 min. The marker snail was then removed and a tracker snail introduced into the centre of the aquarium at the marker snail's recorded start position (± 5 mm). Once the tracker snail emerged, its trail and direction were recorded in an alternative colour on the same graph paper as the marker snail for 15 min. Using this method, trails of marker and tracker snails were clearly recorded and any overlap could be established. The total distance (mm) and speed (mm s⁻¹) were calculated for each tracker.

The path of a tracker trail was defined as overlapping a marker trail if it was recorded within 5 mm of the marker snail's trail (mean foot width = 9.8 mm). Marker and tracker trail distance and overlap were calculated using the markings on the

graph paper as a distance reference; distance was recorded to an accuracy of ± 5 mm and overlap to an accuracy of ± 1 mm. Coincidence index (CI) was calculated as trail overlap distance divided by total marker-trail distance for each behavioural trial to indicate degree of trail following (Hutchinson *et al.*, 2007). Trackers that completely followed a marker trail had a CI value of 1 and 0 if they did not follow at all. Tracking decision was used to verify how tracker snails behaved on initially encountering the marker trail. Two aspects of the tracker's behaviour were recorded: (1) whether on the first instance the tracker snail encountered the marker trail it then followed it for a minimum of 2 cm (average foot length); this was scored as: 1 = followed for ≥ 2 cm; 0 = followed for < 2 cm; (2) whether the initial direction the tracker snail moved along the trail was the same as the direction that the marker snail had been moving; this was scored as: 1 = same direction; 0 = opposite direction.

CI and speed were analysed using rmANOVA in SPSS v. 20 (SPSS Inc., Chicago, IL, USA) with social treatment (socially isolated *vs* grouped) as the within-subject factor and rearing origin (laboratory *vs* wild) as the between-subject factor. Post-hoc Bonferroni and Student-Newman-Keuls (SNK) pairwise comparisons were carried out in SPSS when significant interactions were found. *G*-tests were performed to investigate whether tracker-snail rearing condition affected tracking decision and directionality within grouped and isolated treatment groups, and McNemar's test was used to assess whether tracking decision and directionality differed between grouped and isolated treatments in either wild-caught or laboratory-reared snails.

Overall, tracker snails moved significantly faster when maintained in grouped conditions than in socially isolated conditions (Fig. 1; rmANOVA: treatment, $F_{1,40} = 7.144$, $P = 0.011$). Wild-caught snails also moved significantly faster than laboratory-reared individuals (Fig. 1; rmANOVA: origin, $F_{1,40} = 6.858$, $P = 0.012$). However, the effect of isolation on crawling speed did not differ significantly between wild and laboratory-reared individuals.

Lymnaea stagnalis demonstrated trail-following behaviour and the overall mean CI of 0.21 was similar to that found in another freshwater gastropod, *Biomphalaria glabrata*, which demonstrated a CI of 0.19 following sexual arousal (Townsend, 1974), but considerably lower than levels demonstrated by marine gastropods, which vary from 0.45 to 0.75 (Edwards & Davies, 2002; Hutchinson *et al.*, 2007; Ng *et al.*, 2011). In laboratory-reared snails, isolation significantly reduced CI (Bonferroni: $P < 0.05$); however, CI did not alter between isolated and grouped conditions

in wild-caught snails (Bonferroni: $P > 0.05$; Fig. 2; rmANOVA: origin*treatment: $F_{1,40} = 4.934$, $P = 0.032$). Under grouped conditions, CI did not differ among laboratory and wild-caught snails (SNK: $P > 0.05$); whereas in isolated conditions wild-caught snails had a significantly higher CI than laboratory-reared snails (SNK: $P < 0.05$).

Whether snails followed the marker's trail or not, did not differ significantly between grouped and isolated conditions in wild-caught snails (grouped, 18/22; isolated, 19/22; McNemar's: $P > 0.99$), though there was a nonsignificant trend for laboratory-reared snails to reduce trail following when isolated (grouped, 13/20; isolated, 6/20; McNemar's: $P = 0.064$). In grouped conditions there was no significant difference in numbers that tracked on encountering the marker trail between laboratory-reared (13/20) and wild-caught (18/22) snails (*G*-test: $G = 1.54$, $P = 0.214$, d.f. = 1). However, in isolated conditions significantly fewer laboratory-reared (6/20) than wild-caught (19/22) snails tracked the marker-snail trail (*G*-test: $G = 14.731$, $P < 0.001$, d.f. = 1). Direction of tracking did not differ between groups, and trackers did not initially follow in the same direction as the marker significantly more than by chance (same direction, $n = 35$; opposite direction, $n = 22$).

These results show that *L. stagnalis* exhibited trail-following behaviour upon contact with a trail of a conspecific, agreeing with previous studies illustrating this behaviour in a range of gastropod species (Ng *et al.*, 2013). The display of trail-following behaviour by *L. stagnalis* suggests that this serves a function. The decrease in trail following by laboratory-reared populations following isolation, absence of change in wild snails from grouped to socially isolated conditions and no evidence that snails responded to direction of the marker, fails to support the prediction that it is a mate-location strategy as found in other freshwater gastropods (Townsend, 1974; Takeichi, Hirai & Yusa, 2007). The use of directional information in gastropod trails has been demonstrated in the freshwater gastropod *Physa fontinalis* (Wells & Buckley, 1972) and in many marine species (Cook & Cook, 1975; Ng *et al.*, 2013). While a greater proportion of tracker snails moved in the same direction as the marker snail on first encountering the trail, this was not significantly different from chance. This indicates that although *L. stagnalis* may be able to make use of directional information, it did not influence initial trail-following behaviour. This does not, however, exclude the possible use of trail following for locating conspecifics.

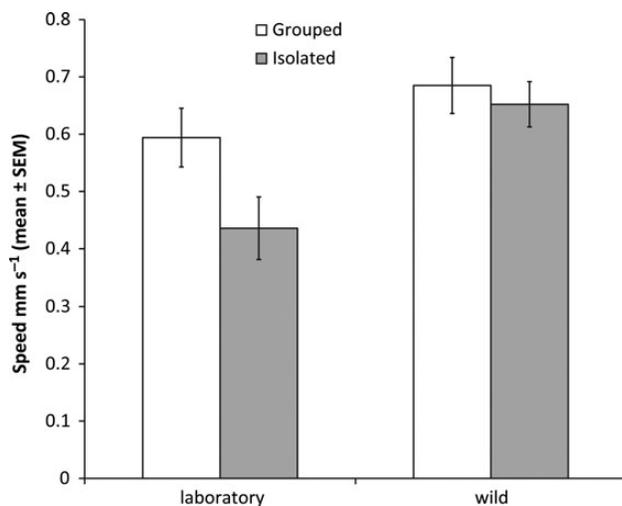


Figure 1. Mean ($\text{mm s}^{-1} \pm \text{SEM}$) tracker snail speed from socially isolated and grouped conditions of laboratory-reared and wild-derived *Lymnaea stagnalis*.

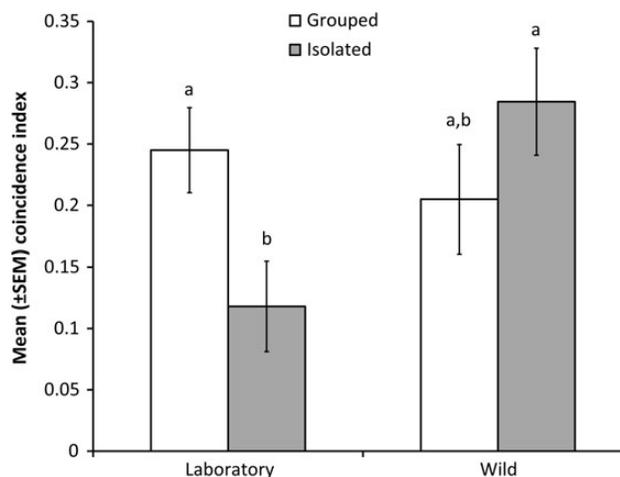


Figure 2. Mean coincidence indices (CI \pm SEM) for socially isolated and grouped conditions of laboratory-reared and wild-derived tracker snails (*Lymnaea stagnalis*). Significantly different values are indicated by different lettering.

Differences seen in trail-following behaviour between wild and laboratory-reared snails could be due to an environmental effect, where social contact during maturation and in adulthood may alter trail-following behaviour following isolation. In the wild, individuals are at a low density and so may spend significant periods without social contact and searching for conspecifics; however, under laboratory conditions population density is much higher and individuals will never go for long periods without contacting a conspecific. Mass rearing under high-density conditions in nonenriched environments has been found to extend time to copulation in tephritid flies (Diaz-Fleischer, Arredondo & Aluja, 2009). For laboratory populations, isolation for a period of a week may also act as a stress due to the regular contact individuals normally experience and effects of social isolation on memory formation indicates that this may occur (Dalesman & Lukowiak, 2011). A reduction in speed in isolated conditions in both wild and laboratory snails may be another indication of stress in these individuals; however, the average crawling speed (0.55 mm s^{-1}) was similar to speeds found in previous studies on *L. stagnalis* ($0.47\text{--}0.55 \text{ mm s}^{-1}$; Aono *et al.*, 2008; Dalesman & Lukowiak, 2010; Miyamae *et al.*, 2010).

Conspecific location strategies may not be directly related to mate location. Aggregation may decrease vulnerability to predators (Hager & Helfman, 1991; Spieler & Linsenmair, 1999; Hoare *et al.*, 2004) and is a defence strategy in marine gastropods (Ray & Stoner, 1994). In addition to variation in CI, we also found that wild snails moved significantly faster than laboratory-reared individuals. Rearing of tephritid flies under high-density conditions in nonenriched conditions has been found to reduce their activity levels (Weldon, Prenter & Taylor, 2010) and a similar effect could have operated in our study. If trail following is instigated as an antipredator response, the maintenance of fast crawling speeds and precise trail following in the absence of predation threat may have no benefit to fitness. *Lymnaea stagnalis* has been found to respond to experience of predation threat as juveniles (Dalesman *et al.*, 2006; Dalesman & Rundle, 2010) and so experience may also significantly alter antipredator-related behavioural traits in adults, including crawling rate.

In this study it was shown that *L. stagnalis* exhibits trail-following behaviour. However, while trail following is often thought to be a mate-location strategy for gastropods (Ng *et al.*, 2013), this was not supported by our data. Differences in trail following were found between laboratory and wild populations following isolation, indicating that captive rearing alters the effects of isolation on trail following in *L. stagnalis*. To assess whether this is due to an environmental effect, the next step will be to assess whether environmental enrichment and altering snail density affects trail following in captive-reared snails. This is the first study to demonstrate trail following in *L. stagnalis* and adds to a growing body of evidence that captive rearing may suppress behavioural traits exhibited by wild conspecifics.

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