Maze learning and memory in a decapod crustacean

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Abstract

Spatial learning is an ecologically important trait well studied in vertebrates and a few invertebrates yet poorly understood in crustaceans. We investigated the ability of European shore crabs, *Carcinus maenas*, to learn a complex maze over four consecutive weeks using food as a motivator. Crabs showed steady improvement during this conditioning period in both the time taken to find the food and in the number of wrong turns taken. Crabs also clearly remembered the maze as when returned two weeks later but without any food, they all returned to the end of the maze in under eight minutes. Crabs that had not been conditioned to the maze (naïve animals) took far longer to reach the end and many (42%) did not venture to the end of the maze at all during the one-hour study period. This study provides an initial description of spatial learning in a benthic decapod; a better appreciation of this adaptive trait in these animals will develop our understanding of resource exploitation by benthic crustaceans and their ecological roles.

Keywords

Crab, *Carcinus maenas*, spatial learning, maze

Background

Some forms of learning, for instance habituation and sensitisation, are evident throughout the animal kingdom [1]. More complex forms of learning, such as spatial learning, have so far been demonstrated in only vertebrates and a select number of invertebrate species [2–7]. Insects, for example, display an extensive repertoire of learned behaviours and some impressive cognitive abilities [6,8] but aquatic arthropods, such as crustaceans, are poorly studied despite their key roles in marine and freshwater ecosystems. The substantial differences between crustacean and insectan brains [9], especially the much lower neuronal counts in crustaceans (for example, *ca*. 90 000 neurons in a crayfish brain [10], *cf.* with *ca*. 1 million in a honey bee brain [11]), might predict a diminished level of behavioural complexity in Crustacea but the relationship between brain size (measured by either volume or the number of neurons) and behavioural complexity is far from consistent [8]. Decapod crustaceans, for example, show a variety of sophisticated navigational behaviours, including homing [12], path integration [13] and true navigation [14].

Decapod crustaceans often live in complex, three-dimensional, benthic habitats. Learning the location of, and routes to, resources should therefore be an adaptive trait that we can investigate using mazes. Mazes provide a quantifiable measure of an animal's performance and whilst investigations into spatial learning in insects have used some quite complex maze configurations [7,15,16], crustacean studies have used much simpler arrangements (cross-, Y- or T-shaped mazes [17–20]) and the ability of crustaceans to solve more complex mazes has not been explored since some very limited studies in the early 20th Century [21,22]. We therefore used a more complex, multiple-turn maze, resembling those used in classic mouse studies (reviewed in [3]), to investigate spatial learning in the European shore crab, *Carcinus maenas*; an important generalist predator and scavenger in intertidal and shallow sea ecosystems. Our experimental design differed from many spatial learning studies in that animals were tested weekly, rather than several times a day, to investigate the formation of memory over longer timescales. A better appreciation of spatial learning in decapods will develop our understanding of resource exploitation by benthic crustaceans and their ecological roles, as well as leading to potential comparative studies with other animals, especially their insectan allies.

Methods

(a) Animals

12 *Carcinus maenas* (mean carapace width, CW, ±1SD = 54±16mm, range = 32–82mm; mean weight ±1SD = 28.7±13.0g, range = 6.2–43.3g) were collected from two locations in South Wales: Oxwich Bay (51°32'48.04"N, 4° 8'38.41"W) and Swansea Docks (51°36'59.26" N, 3°55'6.38" W) and kept individually in 30L tanks connected to a recirculating 40 000L seawater system. All crabs were healthy with intact appendages and identified by the tank they were kept in (1-12). Animals acclimated to this system for four weeks under an illumination cycle of 13:11 h light: dark and were fed half a blue mussel, *Mytilus edulis*, twice a week before commencement of the study. No crabs died or moulted during the study.

(b) Maze design

A maze with external dimensions 75cm x 50cm x 12.5cm high was constructed from 8mm opaque black Perspex (see figure 1a). A starting chamber (15cm x 15cm x 12.5cm high) was positioned adjacent to the entrance and separated from the main maze with a removable piece of black 8mm Perspex. The maze had a single correct path to the end-point, requiring five changes of direction, and included three dead ends. All passages were 10cm wide and a direct route from the starting box to the end-point required the crabs to traverse *ca*. 2m.

(c) Conditioning study

Crabs were tested weekly on the same day for four weeks; all crabs were fasted for a minimum of three days (d) before they were tested, with some fasted for 5d. The maze was placed in a large raceway tank (1.5m x 1m) in the same room as the holding tanks and both the maze and raceway were filled with still system water to a depth of 10cm. Individual crabs were placed in the starting chamber and a single crushed mussel was placed at the maze end-point. After a 60s acclimation period, the wall between the starting chamber and maze was removed. Movements of the crab were recorded using a Praktica DVC5.1 high definition video camera mounted on a tripod without additional lighting. The trial stopped when the crab located the food and started to feed, or after 60min had elapsed. Nobody was present in the laboratory during the trial, with the maze checked after 30min and then every 15min until the end of the trial. The maze and raceway were emptied, cleaned and refilled between each trial. The video was used to calculate latency (defined as the time elapsed) and the number of wrong turns taken whilst trying to reach the end of the maze.

(d) Trials without food

Crabs from the conditioning study (hereafter "conditioned") were tested again after six weeks (two weeks after the last conditioning trial) in the absence of food. The trials were identical to the conditioning study but with no mussel at the end-point. The maze was thoroughly cleaned with EtOH in week 5 to remove any scent from the maze. To investigate whether another factor might attract the crabs to the end-point, 12 new (naïve) *C. maenas* (mean CW±1SD = 51±19mm, range = 34–89mm; mean weight ±1SD = 26.1±14.6 g, range = 7.7–50.0g) were collected from Oxwich Bay and maintained in individual tanks in the system for four weeks as before, then tested in the maze in the absence of food. There was no significant difference in mean CW (unpaired *t*-test, $t_{df=22} = 0.522$, $p = 0.607$) or weight (unpaired t-test, $t_{df=22} = 0.474$, $p = 0.640$) between the naïve and conditioned crabs.

(e) Data analysis

Latency and number of wrong turns were analysed using separate generalised linear mixed-effects models. Latency was natural logarithm-transformed and modelled as a Gaussian process. The number of wrong turns was modelled as a Poisson process. Week was initially treated as a categorical variable and crab weight as a continuous variable; both as main effects and interacting. Data were grouped by individual crab, fitted as random intercepts. The significance of fixed effects was tested using likelihood ratios tests. Pairwise comparisons between weeks were assessed using *post hoc* Tukey tests. Subsequently, week 6 was dropped from the model and week was refitted as a linear response, interacting with weight. Here, week was modelled with random intercepts and slopes, by crab. The degree to which individuals deviated from population average model predictions was quantified using concordance correlation coefficients (ρ_c) [23]. The latency of conditioned and naïve crabs in the absence of food was compared using a Mann-Whitney U test. Statistical analyses were performed using R version 3.6.0 [24] and GraphPad Prism 7.

Results

Data available on Dryad (doi.org/10.5061/dryad.h2cp37f doi:xx).

(a) Conditioning study

All crabs completed the maze within 25min when food was present. Crab weight did not significantly affect latency (weight x week: $\chi^2_{df=1} = 0.004$, p = 0.95, weight: $\chi^2_{df=1} = 0.046$, p = 0.83) or the number of wrong turns (weight x week: $\chi^2_{df=1} = 1.62$, p = 0.20, weight: $\chi^2_{df=1} = 0.009$, p = 0.92). Latency showed a significant log-linear trend over time (slope = -0.634, SE = 0.079, $t_{df=11} = 7.98$, $p < 0.001$), decreasing from 435±283s (mean ± 1SD) in week 1 to 68±58s by week 4 (figure 1b). Crabs also took fewer wrong turns in successive weeks; there was a significant, negative log-linear trend in the

number of wrong turns over time (slope = -0.455, SE = 0.107, z = 4.24, $p < 0.001$), with the median number of wrong turns decreasing from 3.5 (interquartile range, IQR 2-5) in week 1 to 1 (IQR 0.25-1) in week 4 (figure 1c).

Concordance correlation betweeen individual crab performance and population average predictions ranged between $\rho_c = 0.686–0.977$ (median = 0.923) for latency and $\rho_c = 0.623–0.925$ (median = 0.896) for the number of wrong turns, differences between slopes (latency: $cv_{slopes} = 24.6\%$; wrong turns: $cv_{slopes} = 20.8\%$) dominated rather than intercepts (latency: $cv_{intercepts} = 5.75\%$; wrong turns: $cv_{intercepts} = 2.28\%$). There was little rank correlation amongst individuals between concordance correlation coefficients for latency and wrong turns (Kendall's $\tau = 0.091$, $p = 0.74$), nor between individual response intercepts (Kendall's $\tau = -0.382$, $p = 0.09$) or individual slopes over time (Kendall's $\tau = 0.030$, $p = 0.95$) for latency and wrong turns.

(b) Trials without food

All conditioned crabs moved to the end-point within 8min in the absence of food; mean (±1SD) latency for these animals was 276±95s, which was significantly greater than in weeks 3 and 4 in the presence of food (Tukey's multiple comparisons: week 3 *vs.* 6, mean difference = 181s, $p < 0.001$, week 4 *vs.* 6, mean difference = 204s, $p < 0.001$) but not significantly different from crabs in weeks 1 or 2 (Tukey's multiple comparisons: week 1 vs. 6, mean difference = -108s, $p = 0.458$, week 2 vs. 6, mean difference = 94.5s, $p = 0.193$). There was a significant difference in latency between naïve and conditioned crabs (Mann-Whitney $U = 8$, $p < 0.0001$; figure 2) with only seven naïve crabs reaching the end-point within the 60min trial and a mean (±1SD) latency for all 12 naïve crabs of 2,321±1,320s.

Discussion

Crabs showed a strong capacity for spatial learning over the timescale of this work. This learning ability was consistent across all animals, with individuals highly correlated against population average predictions. Consistency in behaviour, including exploratory behaviour, has been demonstrated in *C. maenas* before [25–27] but not in learning, and studies investigating invertebrate learning often record high levels of behavioural variability [2,18], which could be attributed to either behavioural plasticity or consistent individual differences (sometimes referred to as personality). We used concordance correlation coefficients to quantify individual differences [23,28] then compared rank concordance amongst individuals for consistent (intercepts) and plastic (slopes) changes over time [29,30]. There was a very weak correlation between individual differences in latency and wrong turns and this was dominated by idiosyncracies in plasticity rather than consistent differences between individuals – an individual that habituates to its environment strongly is not necessarily a faster learner. Caution is

needed in ascribing behavioural mechanisms to observed responses but these findings suggest maze learning in crabs is not simply accounted for by boldness or habituation to their environment.

Navigation in invertebrates is known to rely on several principles: compass directions, landmarks, path integration and magnetic maps [6,12,14,31]. The crabs did not complete the maze without error until week 3, suggesting either adoption of a search strategy or memory of approximate distance travelled and sequential turn direction. *C. maenas* shows strong thigmotactic behaviour in natural and tank conditions [32] which could manifest in our study as wall-hugging. Consistently following a wall on either the right or left would result in one or two wrong turns respectively, however, and we therefore propose the crabs displayed a degree of spatial learning. We looked solely at egocentric learning as visual and tactile cues were minimised, as were olfactory cues, other than from the food, so a response strategy based on sequential learning (in this case, right turn, ignore two openings, left turn, left turn, right turn, right turn) is possible. The potential for allocentric (the use of landmarks) learning cannot be entirely discarded, however, as crabs may have used the position of the camera, or other overhead features. Future work using other experimental designs, including placing food in more than one location, and maze configurations, such as consecutive T-mazes, might further elaborate spatial learning in these animals.

Decapod crustaceans display anxiety mediated by serotonin [19] so the maze conditions were as close to those in the husbandry tanks as possible (i.e. same system water, no additional lighting) and the experimental design included a substantial acclimation period to captivity. We believe these accommodations contributed substantially to our results showing that although olfactory cues were undoubtedly important in navigating the maze, the crabs clearly learned to move to the end-point of the maze and improved their speed and efficiency during the four weeks. In addition, all conditioned crabs showed some memory of the maze in the absence of food, with no significant difference in latency between week 6 (food absent) and weeks 1 and 2 when food (and therefore an olfactory cue) was present (figure 1b). The increase in latency and the number of wrong turns from week 4 to week 6 suggest, however, that some dishabituation occurred during the intervening two weeks. The discovery that decapod crustaceans are able to learn mazes has important ecological implications but will also allow the development of a model system to investigate the effects of waterborne contaminants, or changes in water chemistry, on a sophisticated behaviour in ecologically and economically important invertebrates.

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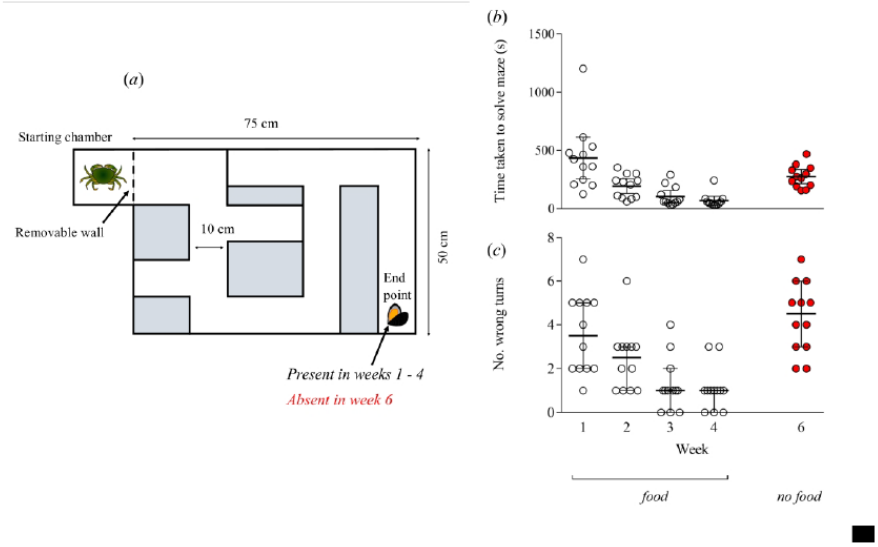

Figure 1. A) Scale schematic of the experimental maze showing an individual Carcinus maenas present in the starting chamber and a single, crushed Mytilus edulis (present in weeks 1-4, absent in week 6) at the end-point. B) Time taken to reach the end-point of the maze (latency; s) by C. maenas individuals in weeks 1-6. Lines = mean±95% confidence intervals (CIs), n = 12. C) The number of wrong turns taken by individual C. maenas in weeks 1-6. Lines = median±95% CIs, n = 12. Carcinus maenas clipart courtesy of Tanya L. Rogers.

313x194mm (300 x 300 DPI)

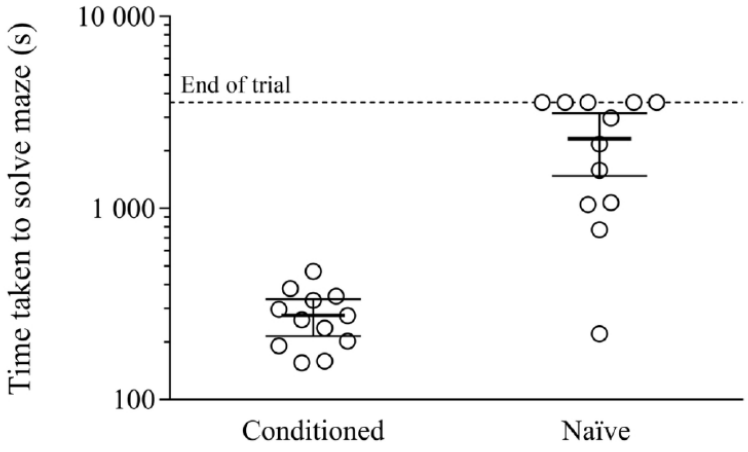

Figure 2. Time taken to reach the end-point of the maze (latency; s) for conditioned (n = 12) and naive (n = 12) C. maenas individuals in week 6 (food absent). Lines shows means values ±95%CIs. The study was stopped after 1h (3 600 s) with animals that did not reach the end awarded this time.

112x66mm (300 x 300 DPI)

184

185 **Legends**

186 **Figure 1**. A) Scale schematic of the experimental maze showing an individual *Carcinus maenas*
187 present in the starting chamber and a single, crushed *Mytilus edulis* (present in weeks 1-4, absent in
188 week 6) at the end-point. B) Time taken to reach the end-point of the maze (latency; s) by *C. maenas*
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