

1 **Maze learning and memory in a decapod crustacean**

2

3 **Ross Davies¹, Mary H Gagen¹, James C Bull¹ and Edward C Pope¹**

4

5 ¹College of Science, Swansea University, Singleton Park, Swansea SA2 8PP

6 Correspondence to: EC Pope (e.c.pope@swansea.ac.uk)

7 **Abstract**

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

19 **Keywords**

20 Crab, *Carcinus maenas*, spatial learning, maze

21 **Background**

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

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

48 **Methods**

49 **(a) Animals**

50 12 *Carcinus maenas* (mean carapace width, CW, $\pm 1SD = 54 \pm 16$ mm, range = 32–82mm; mean weight
51 $\pm 1SD = 28.7 \pm 13.0$ g, range = 6.2–43.3g) were collected from two locations in South Wales: Oxwich
52 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
53 individually in 30L tanks connected to a recirculating 40 000L seawater system. All crabs were
54 healthy with intact appendages and identified by the tank they were kept in (1-12). Animals
55 acclimated to this system for four weeks under an illumination cycle of 13:11 h light: dark and were
56 fed half a blue mussel, *Mytilus edulis*, twice a week before commencement of the study. No crabs
57 died or moulted during the study.

58 **(b) Maze design**

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

65 **(c) Conditioning study**

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

78 **(d) Trials without food**

79 Crabs from the conditioning study (hereafter “conditioned”) were tested again after six weeks (two
80 weeks after the last conditioning trial) in the absence of food. The trials were identical to the
81 conditioning study but with no mussel at the end-point. The maze was thoroughly cleaned with EtOH
82 in week 5 to remove any scent from the maze. To investigate whether another factor might attract the
83 crabs to the end-point, 12 new (naïve) *C. maenas* (mean CW \pm 1SD = 51 \pm 19mm, range = 34–89mm;
84 mean weight \pm 1SD = 26.1 \pm 14.6 g, range = 7.7–50.0g) were collected from Oxwich Bay and
85 maintained in individual tanks in the system for four weeks as before, then tested in the maze in the
86 absence of food. There was no significant difference in mean CW (unpaired *t*-test, $t_{df=22} = 0.522$, $p =$
87 0.607) or weight (unpaired *t*-test, $t_{df=22} = 0.474$, $p = 0.640$) between the naïve and conditioned crabs.

88 **(e) Data analysis**

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

101

102 **Results**

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

104 **(a) Conditioning study**

105 All crabs completed the maze within 25min when food was present. Crab weight did not significantly
106 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
107 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
108 showed a significant log-linear trend over time (slope = -0.634, SE = 0.079, $t_{df=11} = 7.98$, $p < 0.001$),
109 decreasing from 435 \pm 283s (mean \pm 1SD) in week 1 to 68 \pm 58s by week 4 (figure 1b). Crabs also took
110 fewer wrong turns in successive weeks; there was a significant, negative log-linear trend in the

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

114 Concordance correlation between individual crab performance and population average predictions
115 ranged between $\rho_c = 0.686$ – 0.977 (median = 0.923) for latency and $\rho_c = 0.623$ – 0.925 (median =
116 0.896) for the number of wrong turns, differences between slopes (latency: $cv_{slopes} = 24.6\%$; wrong
117 turns: $cv_{slopes} = 20.8\%$) dominated rather than intercepts (latency: $cv_{intercepts} = 5.75\%$; wrong turns:
118 $cv_{intercepts} = 2.28\%$). There was little rank correlation amongst individuals between concordance
119 correlation coefficients for latency and wrong turns (Kendall's $\tau = 0.091$, $p = 0.74$), nor between
120 individual response intercepts (Kendall's $\tau = -0.382$, $p = 0.09$) or individual slopes over time
121 (Kendall's $\tau = 0.030$, $p = 0.95$) for latency and wrong turns.

122 (b) Trials without food

123 All conditioned crabs moved to the end-point within 8min in the absence of food; mean ($\pm 1SD$)
124 latency for these animals was $276 \pm 95s$, which was significantly greater than in weeks 3 and 4 in the
125 presence of food (Tukey's multiple comparisons: week 3 vs. 6, mean difference = 181s, $p < 0.001$,
126 week 4 vs. 6, mean difference = 204s, $p < 0.001$) but not significantly different from crabs in weeks 1
127 or 2 (Tukey's multiple comparisons: week 1 vs. 6, mean difference = -108s, $p = 0.458$, week 2 vs. 6,
128 mean difference = 94.5s, $p = 0.193$). There was a significant difference in latency between naïve and
129 conditioned crabs (Mann-Whitney $U = 8$, $p < 0.0001$; figure 2) with only seven naïve crabs reaching
130 the end-point within the 60min trial and a mean ($\pm 1SD$) latency for all 12 naïve crabs of
131 $2,321 \pm 1,320s$.

132

133 Discussion

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

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

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

161 Decapod crustaceans display anxiety mediated by serotonin [19] so the maze conditions were as close
162 to those in the husbandry tanks as possible (i.e. same system water, no additional lighting) and the
163 experimental design included a substantial acclimation period to captivity. We believe these
164 accommodations contributed substantially to our results showing that although olfactory cues were
165 undoubtedly important in navigating the maze, the crabs clearly learned to move to the end-point of
166 the maze and improved their speed and efficiency during the four weeks. In addition, all conditioned
167 crabs showed some memory of the maze in the absence of food, with no significant difference in
168 latency between week 6 (food absent) and weeks 1 and 2 when food (and therefore an olfactory cue)
169 was present (figure 1b). The increase in latency and the number of wrong turns from week 4 to week
170 6 suggest, however, that some dishabituation occurred during the intervening two weeks. The
171 discovery that decapod crustaceans are able to learn mazes has important ecological implications but
172 will also allow the development of a model system to investigate the effects of waterborne
173 contaminants, or changes in water chemistry, on a sophisticated behaviour in ecologically and
174 economically important invertebrates.

175

176 **Acknowledgements**

177 Thanks to Keith Naylor and Hilary Williams for logistical support and Julian Kivell for building the
178 maze.

179 **Funding**

180 RD was supported by Swansea University College of Science and the Swansea University Science for
 181 Schools Scheme.

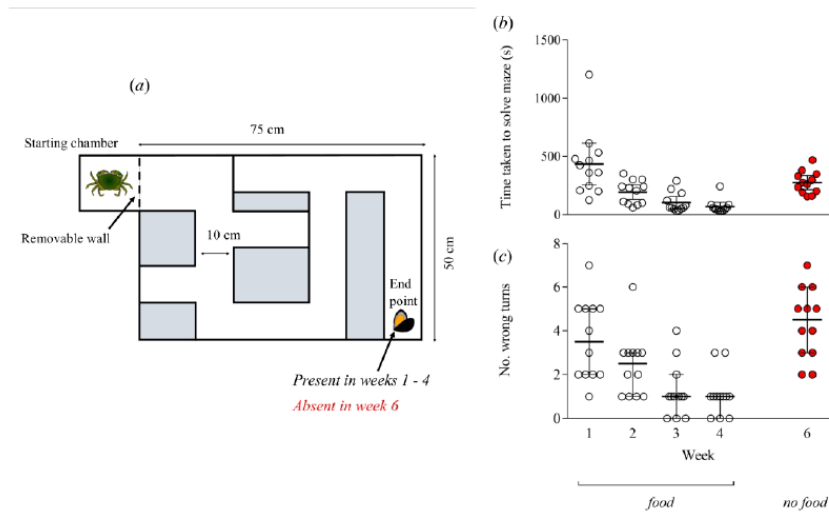


Figure 1. A) Scale schematic of the experimental maze showing an individual *Carcinus maenas* present in the starting chamber and a single, crushed *Mytilus edulis* (present in weeks 1-4, absent in week 6) at the end-point. B) Time taken to reach the end-point of the maze (latency; s) by *C. maenas*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

182

313x194mm (300 x 300 DPI)

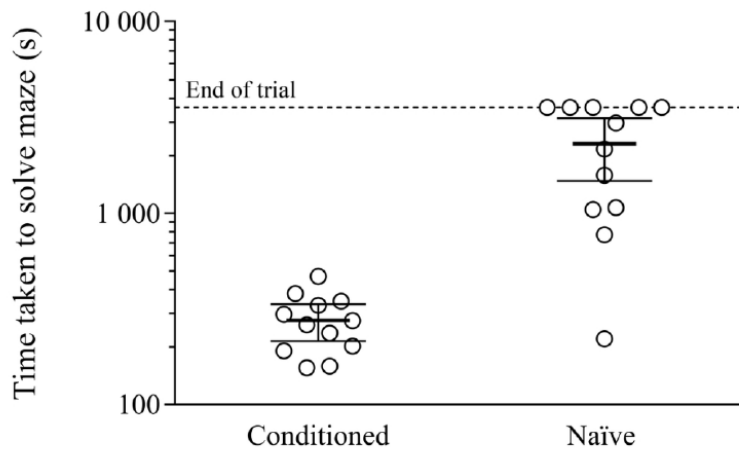


Figure 2. Time taken to reach the end-point of the maze (latency; s) for conditioned (n = 12) and naïve (n = 12) *C. maenas* individuals in week 6 (food absent). Lines shows means values \pm 95% CIs. The study was stopped after 1h (3 600 s) with animals that did not reach the end awarded this time.

112x66mm (300 x 300 DPI)

183

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*
189 individuals in weeks 1-6. Lines = mean \pm 95% confidence intervals (CIs), $n = 12$. C) The number of
190 wrong turns taken by individual *C. maenas* in weeks 1-6. Lines = median \pm 95% CIs, $n = 12$. *Carcinus*
191 *maenas* clipart courtesy of Tanya L. Rogers.

192

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

196 **References**

- 197 1. Perry CJ, Barron AB, Cheng K. 2013 Invertebrate learning and cognition: Relating phenomena
198 to neural substrate. *WIREs Cogn. Sci.* **4**, 561–582. (doi:10.1002/wcs.1248)
- 199 2. Boal JG, Dunham AW, Williams KT, Hanlon RT. 2000 Experimental evidence for spatial
200 learning in octopuses (*Octopus bimaculoides*). *J. Comp. Psychol.* **114**, 246–252.
201 (doi:10.1037/0735-7036.114.3.246)
- 202 3. Sharma S, Rakoczy S, Brown-Borg H. 2010 Assessment of spatial memory in mice. *Life Sci.*
203 **87**, 521–536. (doi:10.1016/j.lfs.2010.09.004)
- 204 4. Karson MA, Boal JG, Hanlon RT. 2003 Experimental evidence for spatial learning in
205 cuttlefish (*Sepia officinalis*). *J. Comp. Psychol.* **117**, 149–155.
206 (doi:10.1037/07357036.117.2.149)
- 207 5. Dale RH. 1988 Spatial memory in pigeons on a four-arm radial maze. *Can. J. Psychol.* **42**, 78–
208 83. (doi:10.1037/h0084177)
- 209 6. Collett M. 2009 Spatial memories in insects. *Curr. Biol.* **19**, 1103–1108.
210 (doi:10.1016/j.cub.2009.10.004)
- 211 7. Zhang SW, Bartsch K, Srinivasan M V. 1996 Maze learning by honeybees. *Neurobiol. Learn.*
212 *Mem.* **66**, 267–282. (doi:10.1006/nlme.1996.0069)
- 213 8. Chittka L, Niven J. 2009 Are bigger brains better? *Curr. Biol.* **19**, R995–R1008.
214 (doi:10.1016/j.cub.2009.08.023)
- 215 9. Strausfeld NJ. 1998 Crustacean-insect relationships: the use of brain characters to derive
216 phylogeny amongst segmented invertebrates. *Brain. Behav. Evol.* **52**, 186–206.
217 (doi:10.1159/000006563)
- 218 10. Wiersma CAG. 1957 On the number of nerve cells in a crustacean central nervous system.
219 *Acta Physiol. Pharmacol. Neer* **6**, 135–142.
- 220 11. Menzel R, Giurfa M. 2001 Cognitive architecture of a mini-brain: the honeybee. *TRENDS*
221 *Cogn. Sci.* **5**, 62–71. (doi:10.1001/jama.1969.03160200053027)
- 222 12. Vannini M, Cannicci S. 1995 Homing behaviour and possible cognitive maps in crustacean
223 decapods. *J. Exp. Mar. Bio. Ecol.* **193**, 67–91. (doi:10.1016/0022-0981(95)00111-5)
- 224 13. Zeil J. 1998 Homing in fiddler crabs (*Uca lactea annulipes* and *Uca vomeris*: *Ocypodidae*). *J.*
225 *Comp. Physiol. A* **183**, 367–377. (doi:10.1007/s003590050263)
- 226 14. Boles LC, Lohmann KJ. 2003 True navigation and magnetic map in spiny lobsters. *Nature*

- 227 **421**, 60–63. (doi:10.1038/nature01333.1.)
- 228 15. Zhang S, Mizutani A, Srinivasan M V. 2000 Maze navigation by honeybees: Learning path
229 regularity. *Learn. Mem.* **7**, 363–374. (doi:10.1101/lm.32900)
- 230 16. Mirwan HB, Kevan PG. 2015 Maze navigation and route memorization by worker bumblebees
231 (*Bombus impatiens* (Cresson) (Hymenoptera: Apidae). *J. Insect Behav.* **28**, 345–357.
232 (doi:10.1007/s10905-015-9507-3)
- 233 17. Tierney AJ, Lee J. 2011 Spatial learning in a T-maze by the crayfish *Orconectes rusticus*. *J.*
234 *Comp. Psychol.* **125**, 31–39. (doi:10.1037/a0020866)
- 235 18. Tierney AJ, Andrews K. 2013 Spatial behavior in male and female crayfish (*Orconectes*
236 *rusticus*): Learning strategies and memory duration. *Anim. Cogn.* **16**, 23–34.
237 (doi:10.1007/s10071-012-0547-1)
- 238 19. Fossat P, Bacque-Cazenave J, Du Deurwaerdere P, Delbecque J-P, Cattaert D. 2014 Anxiety-
239 like behavior in crayfish is controlled by serotonin. *Science* **344**, 1293–1298.
240 (doi:10.1126/science.1248811)
- 241 20. Shuranova Z, Burmistrov Y, Abramson CI. 2005 Habituation to a novel environment in the
242 crayfish *Procambrus cubensis*. *J. Crustac. Biol.* **25**, 488–494. (doi:10.1651/C-2556)
- 243 21. Yerkes R. 1902 Habit formation in the green crab, *Carcinus granulatus*. *Biol. Bull.* **3**, 241–
244 244.
- 245 22. van der Heyde A. 1920 Uber die Lernfahigkeit der Strankrabbe *Carcinus maenas*. *Biol. Zentr.*
246 **40**, 503–514.
- 247 23. Lin LI-K. 1989 A concordance correlation coefficient to evaluate reproducibility. *Biometrics*
248 **45**, 255–268. (doi:10.2307/2532051)
- 249 24. R Core Team. 2019 R: A language and environment for statistical computing. R Foundation
250 for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>
- 251 25. Tanner CJ, Jackson AL. 2012 Social structure emerges via the interaction between local
252 ecology and individual behaviour. *J. Anim. Ecol.* **81**, 260–267.
253 (doi:10.1111/j.13652656.2011.01879.x)
- 254 26. Fürtbauer I. 2015 Consistent individual differences in haemolymph density reflect risk
255 propensity in a marine invertebrate. *R. Soc. Open Sci.* **2** 140482 (doi:10.1098/rsos.140482)
- 256 27. Fürtbauer I, Fry A. 2018 Social conformity in solitary crabs, *Carcinus maenas*, is driven by
257 individual differences in behavioural plasticity. *Anim. Behav.* **135**, 131–137.

- 258 (doi:10.1016/j.anbehav.2017.11.010)
- 259 28. Huang S, Meng SX, Yang Y. 2009. Assessing the goodness of fit of forest models estimated
260 by nonlinear mixed-model methods. *Can. J. For. Res.* **39** 2418-2436. (doi:10.1139/X09-140)
- 261 29. Carrasco J, Jover L. 2003 The concordance correlation coefficient estimated through variance
262 components. *Biometrics* **59**, 849–858. (doi:10.1111/j.0006-341X.2003.00099.x)
- 263 30. Briffa M, Rundle SD, Fryer A. 2008 Comparing the strength of behavioural plasticity and
264 consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*.
265 *Proc. R. Soc. Lond. B* **275**, 1305–11. (doi:10.1098/rspb.2008.0025)
- 266 31. Cannicci S, Barelli C, Vannini M. 2000 Homing in the swimming crab *Thalamita crenata*: A
267 mechanism based on underwater landmark memory. *Anim. Behav.* **60**, 203–210.
268 (doi:10.1006/anbe.2000.1458)
- 269 32. Burrows MT, Kawai K, Hughes RN. 1999 Foraging by mobile predators on a rocky shore:
270 Underwater TV observations of movements of blennies *Lipophrys pholis* and crabs *Carcinus*
271 *maenas*. *Mar. Ecol. Prog. Ser.* **187**, 237–250. (doi:10.3354/meps187237)

272