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1 Microplastics disrupt hermit crab shell 2 selection

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10 **Key Words:** behaviour, cognition, crustacean, *Pagurus bernhardus*, plastic pollution, resource
11 assessment.

12 Abstract

13 Microplastics (plastics < 5 mm) are a potential threat to marine biodiversity. However, the
14 effects of microplastic pollution on animal behaviour and cognition are poorly understood.
15 We used shell selection in common European hermit crabs (*Pagurus bernhardus*) as a model
16 to test whether microplastic exposure impacts the essential survival behaviours of
17 contacting, investigating, and entering an optimal shell. We kept 64 female hermit crabs in
18 tanks containing either polyethylene spheres (n = 35) or no plastic (n = 29) for five days. We
19 then transferred subjects into suboptimal shells and placed them in an observation tank with
20 an optimal alternative shell. Plastic-exposed hermit crabs showed impaired shell selection:
21 they were less likely than controls to contact optimal shells or enter them. They also took
22 longer to contact and enter the optimal shell. Plastic exposure did not affect time spent
23 investigating the optimal shell. These results indicate that microplastics impair cognition
24 (information-gathering and processing), disrupting an essential survival behaviour in hermit
25 crabs.

26 **Introduction**

27 Microplastics (plastics < 5 mm in length [1]) are polluting oceans worldwide, causing
28 substantial scientific and societal concern [2-4]. Waste microplastics enter marine
29 environments either directly, as industry-made particles (primary microplastics [5]), or
30 indirectly, as plastics > 5 mm degrade (secondary microplastics [6]). In total, up to 10% of
31 global plastic production ends up in the ocean [2]. Microplastic exposure can reduce growth,
32 reproduction, and survival in diverse taxa, from corals to mammals [7-10]. However, the
33 ecological validity and scientific rigour of existing research is questionable, with recent
34 meta-analyses [11-13] and reviews [14-16] finding impacts equivocal and context-dependent.
35 As microplastic concentrations are highest along coastlines, littoral species face the greatest
36 potential risks [6].

37 To date, research into the effects of microplastic pollution on marine organisms has focused
38 on fitness and physiology [17]. A few studies have also investigated behavioural impacts on
39 marine organisms, indicating that microplastics disrupt feeding [18], locomotion [19], and
40 social behaviours [20]. Importantly, behaviour is underpinned by cognition: the mechanisms
41 animals use to acquire, process, store, and act on information from their environment [21].
42 This encompasses information-gathering, resource assessments, and decision-making.
43 Crooks *et al.* [22] identified ingested microplastics in the brains of velvet swimming crabs
44 (*Necora puber*) and suggested this could impact crucial survival behaviours. Microplastics
45 also transfer from blood to brain in Crucian carp (*Carassius carassius*), which may disrupt
46 feeding and swimming [23]. However, the effects of microplastic exposure on animal
47 cognition have not been explicitly tested.

48 Shell selection in common European hermit crabs (*Pagurus bernhardus*) is an essential
49 survival behaviour, reliant on collecting accurate information about the new shell, assessing
50 its quality, and deciding whether to change shells [24]. Hermit crabs inhabit empty
51 gastropod shells to protect their soft abdomens from predators [25], with optimal shell
52 weight determined by body weight [26]. The location and sensory perception of new shells
53 represent aspects of cognition [21]. Hermit crabs then cognitively evaluate shell quality by
54 investigating the interior and exterior with their chelipeds [24]. They decide to swap shells if

55 the new one is assessed as an improvement over the current shell. Accurate assessments are
56 highly adaptive, as lower quality shells reduce growth, fecundity, and survival [27]. Because
57 hermit crabs gather information about the new shell, assess its quality compared to their
58 current shell, and make a decision manifested in behaviour, shell selection offers a tractable
59 model of cognitive assessments in marine environments.

60 Here, we investigate whether microplastics affect hermit crab shell selection under
61 controlled conditions. After hermit crabs were kept in tanks either without microplastics
62 (CTRL) or with microplastics (PLAS), we transferred them into a suboptimal shell and
63 offered an optimal alternative. We hypothesised that, if plastic pollution impedes cognition,
64 the PLAS treatment would be less likely to find the optimal shell, accurately assess its
65 quality, and decide to change shells. Specifically, we predicted that CTRL hermit crabs
66 would be more likely and faster to contact, investigate, and enter the optimal shell than
67 PLAS hermit crabs.

68 **Methods**

69 Hermit crabs were collected from Ballywalter Beach, Northern Ireland, and maintained in
70 Queen's University Belfast's animal behaviour laboratory at 11 °C with a 12/12 h light cycle.
71 We randomly allocated subjects to either CTRL or PLAS treatments. For five days, we kept
72 both groups in 0.028 m³ glass tanks (45 cm × 25 cm × 25 cm). All tanks contained 10 l of
73 aerated seawater and 80 g of bladder wrack seaweed (*Fucus vesiculosus*). The PLAS treatment
74 also included 50 g of polyethylene spheres (Materialix Ltd., London, United Kingdom; size:
75 4 mm, 0.02 g; concentration: 25 particles/l, 5 g/l). Lower than most exposure studies, this
76 concentration represented natural conditions more realistically [12]. Polyethylene is the most
77 abundant microplastic found in marine organisms [28].

78 After five days, hermit crabs were removed from their current shell using a small bench-vice
79 to crack the shell [29]. Each subject was then sexed and weighed [24]. We only selected non-
80 gravid females for the study (n = 35 CTRL, 29 PLAS) to control for sex differences in
81 behaviour [25]. Based on their body weight, each hermit crab was provided a suboptimal
82 *Littorina obtusata* shell 50% of their preferred shell weight [26]. After two hours acclimating

83 to the suboptimal shell, subjects were individually placed in a 15 cm-diameter crystallising
84 dish 10 cm from an optimum-weight *L. obtusata* shell (i.e. 100% the preferred weight for the
85 weight of the hermit crab). The dish contained aerated seawater to a depth of 7.5 cm. We
86 recorded the latency to contact the optimal shell, time spent investigating the optimal shell,
87 and latency to enter the optimal shell. If the hermit crab did not approach and enter a shell
88 within 30 min, the session ended.

89 Statistical analyses were performed in R (R Core Team, Cran-r-project, Vienna, Austria,
90 version 3.4.4). Data were categorical (1/0) and continuous (latency). Kolmogorov-Smirnov
91 tests revealed our data were not normally distributed, so we used nonparametric tests
92 throughout. We analysed categorical data using Pearson's chi-squared tests and latency data
93 using Mann-Whitney *U* tests. If subjects did not contact or enter the optimal shell, we
94 assigned a ceiling latency of 30 min. We present data as medians \pm inter-quartile range and
95 consider $p < 0.05$ statistically significant.

96 **Results**

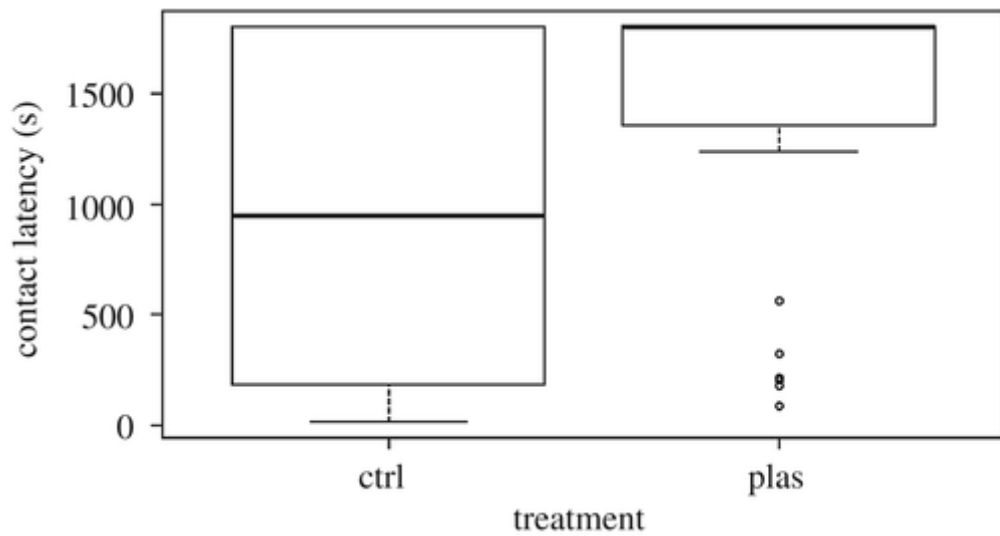
97 Compared to CTRL subjects, fewer hermit crabs in the PLAS treatment contacted the
98 optimal shell ($\chi^2_1 = 8.736$, $p < 0.005$; Table 1). The proportion entering the optimal shell was
99 also lower following microplastic exposure ($\chi^2_1 = 5.343$, $p = 0.021$; Table 1). Moreover, the
100 PLAS treatment had longer latencies to contact ($W = 290$, $p < 0.005$; CTRL median = 948 s,
101 IQR = 184-1800 s; PLAS median = 1800 s, IQR = 1356-1800 s; Figure 1) and enter the optimal
102 shell ($W = 349$, $p = 0.021$; CTRL median = 1379 s, IQR = 511-1800; PLAS median = 1800 s, IQR
103 = 1559-1800 s; Figure 2). Investigation time did not differ between treatments ($W = 142.5$, $p =$
104 0.406 ; CTRL median = 129.5 s, IQR = 74.75-195.5 s; PLAS median = 80.5 s, IQR = 70.75-183.5
105 s).

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107

Table 1. Number and percentage of hermit crabs that contacted and entered the optimal shell from CTRL and PLAS treatments.

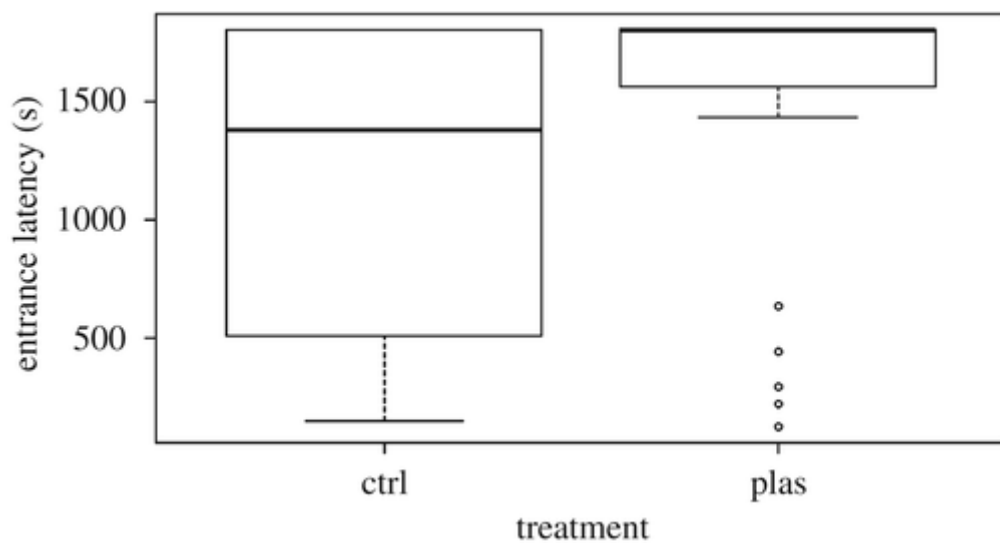
Treatment	Contact optimal shell (% contacting)	Enter optimal shell (% entering)
Control (<i>n</i> = 35)	25 (71%)	21 (60%)
Plastic (<i>n</i> = 29)	10 (34%)	9 (31%)

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Figure 1. Latency (s; median, IQR) to contact the optimal shell for CTRL and PLAS treatments.



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Figure 2. Latency (s; median, IQR) to enter the optimal shell for CTRL and PLAS treatments.

117 **Discussion**

118 We demonstrated that microplastic exposure impairs shell selection behaviour in hermit
119 crabs. Shell selection requires gathering and processing information about shell quality, so
120 our findings suggest microplastics inhibited aspects of cognition. To our knowledge, this is
121 the first study explicitly testing the cognitive effects of microplastic exposure, and the first
122 microplastic study on common European hermit crabs.

123 Despite microplastic exposure disrupting shell selection, the mechanism is unclear. Ingested
124 microplastics enter the brain in crabs [22] and carp [23], potentially impeding information-
125 gathering, resource assessments, decision-making, and behavioural responses. However,
126 both gut-brain studies used substantially smaller microparticles than the present study (0.5
127 μm [22] and 53 nm [23]). Smaller microparticles translocate more easily from the gut into
128 other tissues [30]. To establish whether microplastics passed through the gut membrane,
129 researchers could extract subjects' haemolymph after testing (e.g. [31]). More general
130 mechanisms may also be responsible for our results. Ingesting microplastics can induce false
131 satiation in crustaceans [32], reducing food intake, energy budgets, and growth [18,32-35].
132 Lower energy levels could, therefore, explain the PLAS treatment's tendency to avoid
133 changing shells. We hope that further studies address the effects of microplastic exposure on
134 specific cognitive processes.

135 Whilst contact and entrance latencies were shorter in the CTRL treatment than the PLAS
136 treatment, there was no difference in shell investigation duration. This may indicate that
137 microplastic exposure impaired the ability to assess shells from a distance (i.e. sensory
138 impairment). To some extent, hermit crabs can assess shell quality without contact. Elwood
139 and Stewart [36] observed more approach behaviour when shells were high-quality than
140 low-quality. Alternatively, the null results for shell investigation time may be due to sample
141 size, as only nine subjects in the PLAS treatment investigated the new shell.

142 Although this research was laboratory-based, our experimental design was more
143 ecologically relevant than previous exposure studies. Microplastic exposure research
144 typically uses unrepresentative concentrations and particle types [16]. Environmental

145 microplastic concentrations range from 39-89 particles/l in effluent [37] to ~13 particles/l in
146 the deep sea [38]. Whereas 100 particles/l is the highest concentration ever recorded in
147 nature [14,39], 82% of exposure studies test > 100 particles/l [11]. Our 25 particles/l
148 concentration was, thus, more realistic than most laboratory-based microplastic research. A
149 recent meta-analysis reported more deleterious effects at higher concentrations [11],
150 although others have found little evidence for concentration- or duration-dependent effects
151 [12,13]. Microparticle shape also influences uptake and effects. Whilst fibres and fragments
152 are more abundant in field observations [14,28], we used spheres, because they have more
153 negative impacts on marine life [13]. However, microplastic pollution encompasses various
154 shapes, sizes, and polymer types [40]. Future laboratory studies could replicate this
155 heterogeneity.

156 Our results contribute to previous research demonstrating the adverse effects of
157 microplastics [18,32-35]. Such findings have serious real-world applications: more than 10
158 countries have banned cosmetic microbeads since 2015, including the United States, United
159 Kingdom, France, Italy, New Zealand, and South Korea [3,4]. However, the overwhelming
160 majority of microplastic pollution is due to secondary microplastics. Lassen *et al.* [9]
161 attributed > 99% of Danish microplastic pollution to secondary sources and estimated that
162 cosmetic microbeads account for only 0.1%. At 60%, tyre dust was by far the biggest
163 contributor (see also [41-43]). Secondary microplastics represent an important prospective
164 avenue for research programs and legislative efforts [14,42].

165 In conclusion, hermit crabs exposed to polyethylene spheres were less likely to contact and
166 enter a better-quality shell than control animals, and took longer to do so. There was no
167 difference in time spent investigating the new shell. This proof-of-concept study indicates
168 that microplastic exposure impairs information-gathering, resource assessments, and
169 decision-making in hermit crabs. However, more research is needed to confirm the aspect of
170 cognition affected. Future studies could also establish the generality of our findings across
171 different species, cognitive processes, and microplastic exposures.

172 **Ethics.** Crustacean research is not regulated under UK law, but we followed the Association
173 for the Study of Animal Behaviour's Guidelines for the Use of Animals in Research. After the
174 experiment, all hermit crabs were returned to the shore unharmed.

175 **Data Accessibility.** Data are available in the electronic supplementary material.

176 **Authors' Contributions.** A.C., C.M. and G.A. designed the study; C.M. conducted the
177 experiments; A.C., E.J.B., E.M.C. and G.A. analysed and interpreted the data; A.C. prepared
178 the manuscript. All authors revised the manuscript, approved the final version, and agreed
179 to be held accountable for every aspect of the work.

180 **Competing Interests.** We declare we have no competing interests.

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183 **References**

- 184 1. Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W., ... &
185 Russell, A. E. (2004). Lost at sea: where is all the plastic?. *Science*, 304(5672), 838-838. DOI:
186 10.1126/science.1094559
- 187 2. Barnes, D. K., Galgani, F., Thompson, R. C., & Barlaz, M. (2009). Accumulation and
188 fragmentation of plastic debris in global environments. *Philosophical Transactions of the*
189 *Royal Society B: Biological Sciences*, 364(1526), 1985-1998. DOI: 10.1098/rstb.2008.0205
- 190 3. Lam, C. S., Ramanathan, S., Carbery, M., Gray, K., Vanka, K. S., Maurin, C., ... &
191 Palanisami, T. (2018). A Comprehensive Analysis of Plastics and Microplastic Legislation
192 Worldwide. *Water, Air, & Soil Pollution*, 229(11), 345. DOI: 10.1007/s11270-018-4002-z
- 193 4. Nelson, D., Sellers, K., Mackenzie, S., & Weinberg, N. (2019). Microbeads—a Case Study
194 in How Public Outrage Fueled the Emergence of New Regulations. *Current Pollution*
195 *Reports*, 5, 172-179. DOI: 10.1007/s40726-019-00114-7
- 196 5. Napper, I. E., Bakir, A., Rowland, S. J., & Thompson, R. C. (2015). Characterisation,
197 quantity and sorptive properties of microplastics extracted from cosmetics. *Marine*
198 *Pollution Bulletin*, 99(1-2), 178-185. DOI: 10.1016/j.marpolbul.2015.07.029

- 199 6. Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as
200 contaminants in the marine environment: a review. *Marine Pollution Bulletin*, 62(12),
201 2588-2597. DOI: 10.1016/j.marpolbul.2011.09.025
- 202 7. Anbumani, S., & Kakkar, P. (2018). Ecotoxicological effects of microplastics on biota: a
203 review. *Environmental Science and Pollution Research*, 25(15), 14373-14396. DOI:
204 10.1007/s11356-018-1999-x
- 205 8. Auta, H. S., Emenike, C. U., & Fauziah, S. H. (2017). Distribution and importance of
206 microplastics in the marine environment: a review of the sources, fate, effects, and
207 potential solutions. *Environment International*, 102, 165-176. DOI:
208 10.1016/j.envint.2017.02.013
- 209 9. Lassen, C., Hansen, S. F., Magnusson, K., Noren, F., Hartmann, N. I. B., Jensen, P. R.,
210 Nielsen, T. G., & Brinch, A. (2015). *Microplastics: Occurrence, effects and sources of releases to*
211 *the environment in Denmark. Environmental Project 1793*. Copenhagen, Denmark: Danish
212 Environmental Protection Agency.
- 213 10. Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of
214 microplastics on marine organisms: a review. *Environmental Pollution*, 178, 483-492. DOI:
215 10.1016/j.envpol.2013.02.031
- 216 11. Bucci, K., Tulio, M., & Rochman, C. M. (2019). What is known and unknown about the
217 effects of plastic pollution: A meta-analysis and systematic review. *Ecological Applications*,
218 0(0), e02044. DOI: 10.1002/eap.2044
- 219 12. Cunningham, E. M., & Sigwart, J. D. (2019). Environmentally accurate microplastic levels
220 and their absence from exposure studies on aquatic taxa. *Integrative and Comparative*
221 *Biology*, 59, 6, 1485-1496. DOI: 10.1093/icb/icz068
- 222 13. Foley, C. J., Feiner, Z. S., Malinich, T. D., & Höök, T. O. (2018). A meta-analysis of the
223 effects of exposure to microplastics on fish and aquatic invertebrates. *Science of the Total*
224 *Environment*, 631, 550-559. DOI: 10.1016/j.scitotenv.2018.03.046
- 225 14. Burns, E. E., & Boxall, A. B. (2018). Microplastics in the aquatic environment: Evidence
226 for or against adverse impacts and major knowledge gaps. *Environmental Toxicology and*
227 *Chemistry*, 37(11), 2776-2796. DOI: 10.1002/etc.4268

- 228 15. Connors, K. A., Dyer, S. D., & Belanger, S. E. (2017). Advancing the quality of
229 environmental microplastic research. *Environmental Toxicology and Chemistry*, 36(7), 1697-
230 1703. DOI: 10.1002/etc.3829
- 231 16. Phuong, N. N., Zalouk-Vergnoux, A., Poirier, L., Kamari, A., Châtel, A., Mouneyrac, C.,
232 & Lagarde, F. (2016). Is there any consistency between the microplastics found in the
233 field and those used in laboratory experiments?. *Environmental Pollution*, 211, 111-123.
234 DOI: 10.1016/j.envpol.2015.12.035
- 235 17. Franzellitti, S., Canesi, L., Auguste, M., Wathsala, R. H., & Fabbri, E. (2019). Microplastic
236 exposure and effects in aquatic organisms: a physiological perspective. *Environmental*
237 *Toxicology and Pharmacology*, 68, 37-51. DOI: 10.1016/j.etap.2019.03.009
- 238 18. Cole, M., Lindeque, P., Fileman, E., Halsband, C., & Galloway, T. S. (2015). The impact of
239 polystyrene microplastics on feeding, function and fecundity in the marine copepod
240 *Calanus helgolandicus*. *Environmental Science & Technology*, 49(2), 1130-1137. DOI:
241 10.1021/es504525u
- 242 19. Tosetto, L., Brown, C., & Williamson, J. E. (2016). Microplastics on beaches: ingestion and
243 behavioural consequences for beachhoppers. *Marine Biology*, 163(10), 199. DOI:
244 10.1007/s00227-016-2973-0
- 245 20. Mattsson, K., Ekvall, M. T., Hansson, L. A., Linse, S., Malmendal, A., & Cedervall, T.
246 (2014). Altered behavior, physiology, and metabolism in fish exposed to polystyrene
247 nanoparticles. *Environmental Science & Technology*, 49(1), 553-561. DOI: 10.1021/es5053655
- 248 21. Shettleworth, S. J. (1998). *Cognition, Evolution, and Behaviour (1st ed.)*. Oxford University
249 Press: New York, NY, USA.
- 250 22. Crooks, N., Parker, H., & Pernetta, A. P. (2019). Brain food? Trophic transfer and tissue
251 retention of microplastics by the velvet swimming crab (*Necora puber*). *Journal of*
252 *Experimental Marine Biology and Ecology*, 519, 151187. DOI: 10.1016/j.jembe.2019.151187
- 253 23. Mattsson, K., Johnson, E. V., Malmendal, A., Linse, S., Hansson, L. A., & Cedervall, T.
254 (2017). Brain damage and behavioural disorders in fish induced by plastic nanoparticles
255 delivered through the food chain. *Scientific Reports*, 7(1), 11452. DOI: 10.1038/s41598-017-
256 10813-0

- 257 24. Elwood, R. W. (2018). 10 Hermit Crabs–Information Gathering by the Hermit Crab,
258 Pagurus bernhardus. In *Field and Laboratory Methods in Animal Cognition* (eds. Bueno-
259 Guerra, N., & Amici, F.), pp. 222-243. Cambridge, UK: Cambridge University Press.
- 260 25. Elwood, R. W., & Neil, S. J. (1992). *Assessments and decisions: a study of information*
261 *gathering by hermit crabs*. Chapman and Hall: London, UK.
- 262 26. Elwood, R. W., McClean, A., & Webb, L. (1979). The development of shell preferences by
263 the hermit crab Pagurus bernhardus. *Animal Behaviour*, 27, 940-946.
- 264 27. Lancaster, I. (1990). Reproduction and life history strategy of the hermit crab Pagurus
265 bernhardus. *Journal of the Marine Biological Association of the United Kingdom*, 70(1), 129-
266 142. DOI: 10.1017/S0025315400034251
- 267 28. De Sá, L. C., Oliveira, M., Ribeiro, F., Rocha, T. L., & Fütter, M. N. (2018). Studies of the
268 effects of microplastics on aquatic organisms: what do we know and where should we
269 focus our efforts in the future?. *Science of the Total Environment*, 645, 1029-1039. DOI:
270 10.1016/j.scitotenv.2018.07.207
- 271 29. Walsh, E. P., Arnott, G., & Kunc, H. P. (2017). Noise affects resource assessment in an
272 invertebrate. *Biology Letters*, 13(4), 20170098. DOI: 10.1098/rsbl.2017.0098
- 273 30. Von Moos, N., Burkhardt-Holm, P., & Kohler, A. (2012). Uptake and effects of
274 microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an
275 experimental exposure. *Environmental Science & Technology*, 46(20), 11327-11335. DOI:
276 10.1021/es302332w
- 277 31. Farrell, P., & Nelson, K. (2013). Trophic level transfer of microplastic: *Mytilus edulis* (L.)
278 to *Carcinus maenas* (L.). *Environmental Pollution*, 177, 1-3. DOI:
279 10.1016/j.envpol.2013.01.046
- 280 32. Welden, N. A., & Cowie, P. R. (2016). Long-term microplastic retention causes reduced
281 body condition in the langoustine, *Nephrops norvegicus*. *Environmental Pollution*, 218,
282 895-900. DOI: 10.1016/j.envpol.2016.08.020
- 283 33. Au, S. Y., Bruce, T. F., Bridges, W. C., & Klaine, S. J. (2015). Responses of *Hyalella azteca*
284 to acute and chronic microplastic exposures. *Environmental Toxicology and Chemistry*,
285 34(11), 2564-2572. DOI: 10.1002/etc.3093

- 286 34. Blarer, P., & Burkhardt-Holm, P. (2016). Microplastics affect assimilation efficiency in the
287 freshwater amphipod *Gammarus fossarum*. *Environmental Science and Pollution Research*,
288 23(23), 23522-23532. DOI: 10.1007/s11356-016-7584-2
- 289 35. Watts, A. J., Urbina, M. A., Corr, S., Lewis, C., & Galloway, T. S. (2015). Ingestion of
290 plastic microfibers by the crab *Carcinus maenas* and its effect on food consumption and
291 energy balance. *Environmental Science & Technology*, 49(24), 14597-14604. DOI:
292 10.1021/acs.est.5b04026
- 293 36. Elwood, R. W., & Stewart, A. (1985). The timing of decisions during shell investigation
294 by the hermit crab, *Pagurus bernhardus*. *Animal Behaviour*, 33(2), 620-627. DOI:
295 10.1016/S0003-3472(85)80086-5
- 296 37. Verschoor, A.; De Poorter, L.; Dröge, R.; Kuenen, J.; De Valk, E. (2016). Emission of
297 Microplastics and Potential Mitigation Measures. Abrasive Cleaning Agents, Paints and
298 Tyre Wear; National Institute for Public Health and the Environment: Bilthoven, The
299 Netherlands.
- 300 38. Peng, X., Chen, M., Chen, S., Dasgupta, S., Xu, H., Ta, K., ... & Bai, S. (2018). Microplastics
301 contaminate the deepest part of the world's ocean. *Geochemical Perspectives Letters*, 9, 1-5.
302 DOI: 10.7185/geochemlet.1829
- 303 39. Leslie, H. A., Brandsma, S. H., Van Velzen, M. J. M., & Vethaak, A. D. (2017).
304 Microplastics en route: Field measurements in the Dutch river delta and Amsterdam
305 canals, wastewater treatment plants, North Sea sediments and biota. *Environment*
306 *international*, 101, 133-142. DOI: 10.1016/j.envint.2017.01.018
- 307 40. Rochman, C. M., Brookson, C., Bikker, J., Djuric, N., Earn, A., Bucci, K., ... & De Frond, H.
308 (2019). Rethinking microplastics as a diverse contaminant suite. *Environmental Toxicology*
309 *and Chemistry*, 38(4), 703-711. DOI: 10.1002/etc.4371
- 310 41. Eunomia (2016). *Plastics in the marine environment*. Bristol, UK. Available from:
311 www.eunomia.co.uk/reports-tools/plastics-in-the-marine-environment/ [accessed
312 November 29 2019].
- 313 42. Gouin, T., Avalos, J., Brunning, I., Brzuska, K., De Graaf, J., Kaumanns, J., ... & Thomas, J.
314 (2015). Use of micro-plastic beads in cosmetic products in Europe and their estimated
315 emissions to the North Sea environment. *SOFWJ*, 141(4), 40-46.

316 43. Sundt, P., Schulze, P., & Syversen, F. (2014). *Sources of microplastic pollution to the marine*
317 *environment. Report M-321,2015. Project 1032. Norwegian Environment Agency: Oslo,*
318 *Norway.*