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Microplastics disrupt hermit crab shell selection

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Abstract

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

Introduction

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

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

Shell selection in common European hermit crabs (*Pagurus bernhardus*) is an essential survival behaviour, reliant on collecting accurate information about the new shell, assessing its quality, and deciding whether to change shells [24]. Hermit crabs inhabit empty gastropod shells to protect their soft abdomens from predators [25], with optimal shell weight determined by body weight [26]. The location and sensory perception of new shells represent aspects of cognition [21]. Hermit crabs then cognitively evaluate shell quality by investigating the interior and exterior with their chelipeds [24]. They decide to swap shells if the new one is assessed as an improvement over the current shell. Accurate assessments are highly adaptive, as lower quality shells reduce growth, fecundity, and survival [27]. Because hermit crabs gather information about the new shell, assess its quality compared to their current shell, and make a decision manifested in behaviour, shell selection offers a tractable model of cognitive assessments in marine environments.

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

Methods

Hermit crabs were collected from Ballywalter Beach, Northern Ireland, and maintained in Queen's University Belfast's animal behaviour laboratory at 11 °C with a 12/12 h light cycle. We randomly allocated subjects to either CTRL or PLAS treatments. For five days, we kept both groups in 0.028 m^3 glass tanks (45 cm × 25 cm × 25 cm). All tanks contained 10 l of aerated seawater and 80 g of bladder wrack seaweed (*Fucus vesiculosus*). The PLAS treatment also included 50 g of polyethylene spheres (Materialix Ltd., London, United Kingdom; size: 4 mm, 0.02 g; concentration: 25 particles/l, 5 g/l). Lower than most exposure studies, this concentration represented natural conditions more realistically [12]. Polyethylene is the most abundant microplastic found in marine organisms [28].

After five days, hermit crabs were removed from their current shell using a small bench-vice to crack the shell [29]. Each subject was then sexed and weighed [24]. We only selected non-gravid females for the study (n = 35 CTRL, 29 PLAS) to control for sex differences in behaviour [25]. Based on their body weight, each hermit crab was provided a suboptimal *Littorina obtusata* shell 50% of their preferred shell weight [26]. After two hours acclimating to the suboptimal shell, subjects were individually placed in a 15 cm-diameter crystallising dish 10 cm from an optimum-weight *L. obtusata* shell (i.e. 100% the preferred weight for the weight of the hermit crab). The dish contained aerated seawater to a depth of 7.5 cm. We recorded the latency to contact the optimal shell, time spent investigating the optimal shell, and latency to enter the optimal shell. If the hermit crab did not approach and enter a shell within 30 min, the session ended.

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

Results

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

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 (*n* = 35)	25 (71%)	21 (60%)
Plastic (*n* = 29)	10 (34%)	9 (31%)

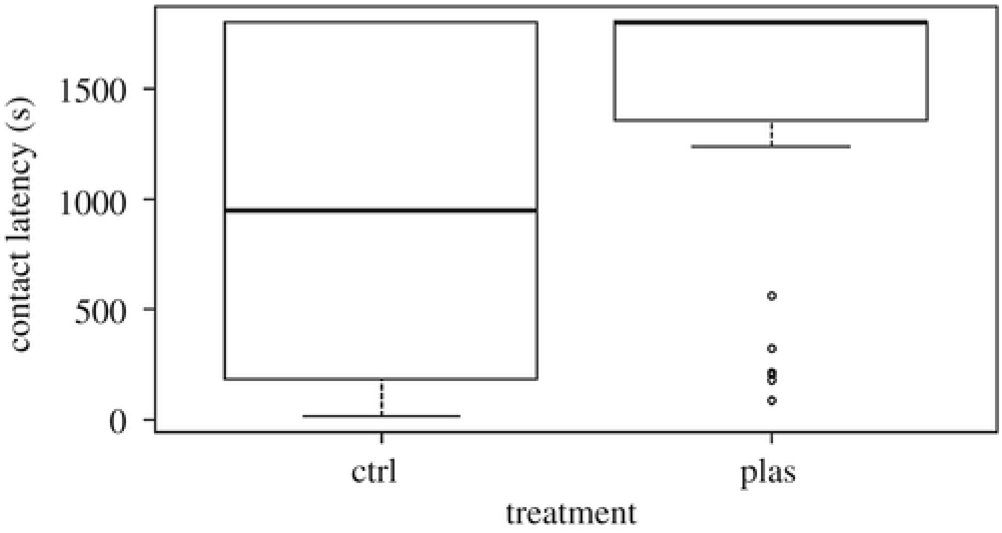

Figure 1. Latency (s; median, IQR) to contact the optimal shell for CTRL and PLAS treatments.

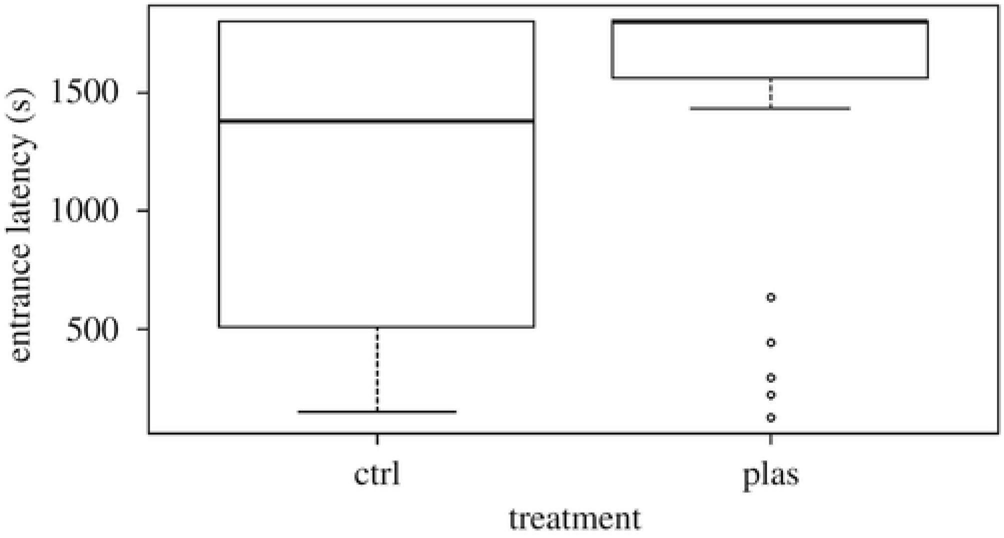

Figure 2. Latency (s; median, IQR) to enter the optimal shell for CTRL and PLAS treatments.

Discussion

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

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

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

Although this research was laboratory-based, our experimental design was more ecologically relevant than previous exposure studies. Microplastic exposure research typically uses unrepresentative concentrations and particle types [16]. Environmental microplastic concentrations range from 39-89 particles/l in effluent [37] to ~13 particles/l in the deep sea [38]. Whereas 100 particles/l is the highest concentration ever recorded in nature [14,39], 82% of exposure studies test > 100 particles/l [11]. Our 25 particles/l concentration was, thus, more realistic than most laboratory-based microplastic research. A recent meta-analysis reported more deleterious effects at higher concentrations [11], although others have found little evidence for concentration- or duration-dependent effects [12,13]. Microparticle shape also influences uptake and effects. Whilst fibres and fragments are more abundant in field observations [14,28], we used spheres, because they have more negative impacts on marine life [13]. However, microplastic pollution encompasses various shapes, sizes, and polymer types [40]. Future laboratory studies could replicate this heterogeneity.

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

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

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

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

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

Competing Interests. We declare we have no competing interests.

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References

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

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

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

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

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

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