



HAL
open science

The tolerance of a keystone ecosystem engineer to extreme heat stress is hampered by microplastic leachates

Marine Uguen, Sylvie Gaudron, Katy Nicasastro, Gerardo Zardi, Nicolas Spilmont, Solène Henry, Laurent Seuront

► To cite this version:

Marine Uguen, Sylvie Gaudron, Katy Nicasastro, Gerardo Zardi, Nicolas Spilmont, et al.. The tolerance of a keystone ecosystem engineer to extreme heat stress is hampered by microplastic leachates. *Biology Letters*, 2024, 20 (3), pp.20230457. 10.1098/rsbl.2023.0457 . hal-04804435

HAL Id: hal-04804435

<https://hal.science/hal-04804435v1>

Submitted on 26 Nov 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

The tolerance of a keystone ecosystem engineer to extreme heat stress is hampered by microplastic leachates

Marine Uguen^{1,*}, Sylvie M Gaudron^{1,2}, Katy R Nicasro^{1,3,4}, Gerardo I Zardi^{3,5}, Nicolas Spilmont¹, Solène Henry¹, Laurent Seuront^{1,3,6}

(1) Univ. Lille, CNRS, Univ. Littoral Côte d'Opale, IRD, UMR 8187, LOG, Laboratoire d'Océanologie et de Géosciences, Station marine de Wimereux, F-59000 Lille, France.

(2) Sorbonne Université, UFR 927, F-75005 Paris, France.

(3) Department of Zoology and Entomology, Rhodes University, Grahamstown, 6140 South Africa

(4) CCMAR—Centro de Ciências do Mar, CIMAR Laboratório Associado, Universidade do Algarve, Campus de Gambelas, Faro, 8005-139 Portugal

(5) Laboratoire de Biologie des Organismes et Ecosystèmes Aquatiques (BOREA), Université de Caen-Normandie, MNHN, SU, UA, CNRS, IRD, Esplanade de la paix, 14032 Caen Cedex, France

(6) Department of Marine Resources and Energy, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo, 108-8477 Japan

* Corresponding author: marine.uguen@univ-lille.fr

ORCID IDs

MU: 0000-0001-5547-608X

SMG: 0000-0001-6189-3824

KRN: 0000-0002-7071-141X

GIZ: 0000-0001-8798-5794

NS: 0000-0002-2380-1113

SH: 0000-0002-6309-7159

LS:0000-0002-0051-5202

Abstract

Plastic pollution and ongoing climatic changes exert considerable pressure on coastal ecosystems. Unravelling the combined effects of these two threats is essential to management and conservation actions to reduce the overall environmental risks. We assessed the capacity of a coastal ecosystem engineer, the blue mussel *Mytilus edulis*, to cope with various levels of aerial heat stress (20, 25, 30 and 35°C) after an exposure to substances leached from beached and virgin low-density polyethylene pellets. Our results revealed a significant interaction between temperature and plastic leachates on mussel survival rates. Specifically, microplastic leachates had no effect on mussel survival at 20, 25 and 30°C. In turn, mussel survival rates significantly decreased at 35°C, and this decrease was even more significant following an exposure to leachates from beached pellets; these pellets had higher concentration of additives

40 compared to the virgin ones, potentially causing a bioenergetic imbalance. Our results stress
41 the importance of adopting integrated approaches combining the effects of multiple
42 environmental threats on key marine species to understand and mitigate their potential
43 synergistic effects on ecosystem dynamics and resilience in the face of the changing
44 environment.

45 **Keywords**

46 warm, global change, contaminant, emersion, keystone species

47

48 **1. Introduction**

49 Plastics and their associated chemicals have become a global concern from both scientific and
50 societal perspectives. The ever-increasing plastic production and poor waste management has
51 led to a permanent contamination of the biosphere by plastic items [1–3]. Coastal systems are
52 some of the most heavily plastic-polluted ecosystems [4,5]. Plastics may pose a serious threat
53 to organisms not only through physical damages [6,7], but also through the far less studied
54 consequences of their chemical content [8,9]. Plastic debris may noticeably act as vectors for
55 numerous chemical compounds that can be hazardous depending on the nature of the chemicals
56 and their concentrations [10,11], which can vary during plastic lifetime. Virgin plastics are
57 essentially composed of plastic polymers to which a range of additives are incorporated during
58 their manufacture in order to improve their performances [12], with *e.g.* phthalates making up
59 10 to 60% of the PVC weight [13]. Then, when released in the environment, a range of
60 environmental contaminants, potentially more toxic than additives (*e.g.* heavy metals,
61 pesticides), may be adsorbed on plastic particle surface and accumulate [14,15], at
62 concentrations up to 6 orders of magnitude higher than in the environment [16] and then be
63 desorbed [8]. These sorption/desorption phenomena noticeably increases as particle size
64 decreases [17]. As such, plastics in general and microplastics in particular (*i.e.* particles smaller

65 than 5 mm) may act both as a sink and a source of contaminants, exposing organisms to complex
66 and potentially toxic cocktails of chemical compounds [8,9,18].

67 As climate change progresses, the effects of extreme weather conditions, including the
68 increased frequency, intensity, and duration of heat stress, are expected to become more
69 pronounced [19–21]. In intertidal ecosystems, these events are particularly stressful, *e.g.* the
70 body temperature variation experienced by mussel specimens between emersion and immersion
71 can exceed 20°C (*e.g.* [22,23]), and can cause damages and mass mortality episodes, especially
72 in marine invertebrates such as mussels [22,24–26]. Noticeably, the temperature tolerance of
73 organisms is dependent on their health status, which can further be compromised by the
74 presence of contaminants in seawater [27–29]. Even though plastic pollution and heat stress *de*
75 *facto* frequently coincide, only a handful of studies have explored their combined repercussions.
76 These essentially include adverse effects on survival, physiological processes, and cellular
77 functions [30–33] though exceptions exist [34–36]. Despite the plethora of studies that assessed
78 the effects of microplastic leachates on various aspects of the biology and ecology of marine
79 organisms [8], to the best of our knowledge the combined impact of plastic leachates and heat
80 stress has not been addressed. Given the potentially high impact of these two stressors on
81 intertidal ecosystems, the objective of this study was to assess the impact of microplastic
82 leachates on the ability of a key intertidal ecosystem engineer [37], the blue mussel *Mytilus*
83 *edulis*, to survive to aerial heat stress. Leachate solutions were consistently prepared from low-
84 density polyethylene pellets that were either virgin (*i.e.* raw commercially available pellets) or
85 found stranded on the beach, and aerial heat stress was simulated at 4 different temperatures
86 (*i.e.* 20, 25, 30 or 35°C). We hypothesised that plastic leachate exposure alters the mussel ability
87 to withstand an aerial heat stress event and that leachates from beached pellets will have a more
88 deleterious impact than virgin ones because they have the potential to accumulate additional
89 toxicants when dispersed in the environment.

90

91 2. Material and Methods

92 a) *Study organisms*

93 A total of 720 specimens of the blue mussel *Mytilus edulis* (3 to 4 cm in shell length) were
94 collected in September 2022 from the intertidal rocky shore at Pointe aux Oies (Wimereux,
95 France; 50°47'08.3"N, 1°36'03.9"E) along the French coast of the eastern English Channel.
96 Prior to the experiments, mussels were acclimated in the laboratory for 24 h in 85 L tanks filled
97 with oxygen saturated natural seawater [38] representative of *in situ* conditions (T = 20°C;
98 S = 33).

99 b) *Microplastics and leachate solution preparation*

100 Microplastic leachate solutions (MPL) were prepared from either (i) commercially available
101 (Materialix Ltd., London, United Kingdom) low-density spheroidal polyethylene pellets (with
102 typical longest and shortest axes respectively 4.14 ± 0.21 mm and 1.89 ± 0.10 mm in length;
103 V-MPL treatment) or (ii) cylindrical beached pellets (typically 4.04 ± 0.56 mm in width and
104 3.03 ± 0.82 mm in height; B-MPL treatment) collected from the high-tide mark sediment
105 surface of the nearby beach (Ambleteuse, France; 50°80'61.9"N 1°60'31.34"E). Beached pellets
106 were consistently identified as low-density polyethylene using Fourier transform infrared
107 (FTIR) analysis with an Aldrich FTIR database search set at 97.5% correlation setting. GC-
108 HRMS analyses showed that virgin pellets were composed of 5 additives, including 4
109 plasticisers and 1 antioxidant. In contrast, beached pellets contained 7 additives, 4 plasticisers
110 and 3 antioxidants and in a quantity typically 2 to 4-fold higher than in virgin pellets. For more
111 details about the polymer and chemical content identification and the method used, see
112 Supplementary Material, S1.

113 Each type of pellets was mixed with natural aerated seawater for 24h [38–41] at 20°C at a

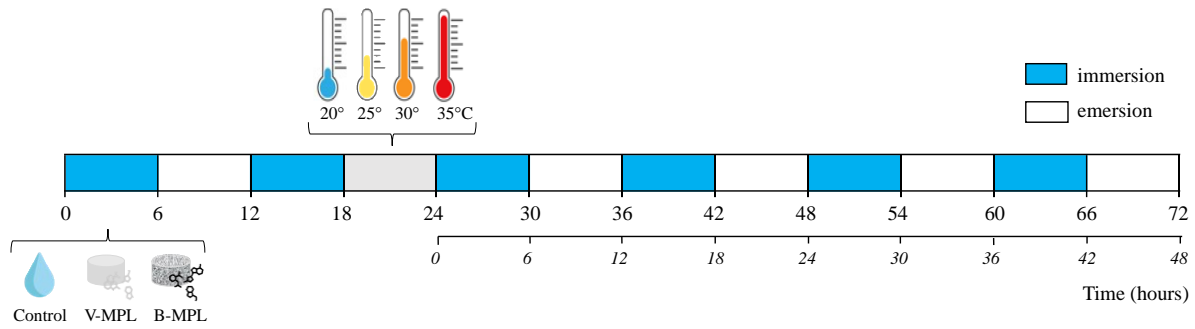
114 concentration of 10 g of pellets per litre (*ca.* 400 pellets per litre, or equivalently 20 mL of
115 pellets per litre; [39]). After 24 h, the experiment was performed using the solution alone (*i.e.*
116 without any pellets). A control solution (Control) was prepared by incubating aerated seawater
117 for 24 h at 20°C. Consistent oxygen saturation and seawater renewal were ensured for each
118 treatment during every immersion cycle.

119 *c) Experimental design*

120 The experiment was designed to mimic the temperature experienced by *M. edulis* during a
121 typical tidal cycle at the sampling site (*i.e.* 6 h immersion/emersion with a sea surface and air
122 temperature of 20°C; see SOMLIT 2022 data for Wimereux in September;
123 <https://www.somlit.fr/visualisation-des-donnees/>), with a one-off emersion heat stress event
124 representative of observed air temperature at the study site during the summer 2022 (25, 30 and
125 35°C; air temperature record = 39.6°C [42]). A previous study conducted at our study site, using
126 robomussels, revealed that mussel body temperatures occasionally exceeded 35°C, reaching a
127 maximum recorded temperature of 41.7°C, while the seawater temperature remained around
128 15°C, resulting in a delta temperature of over 20°C [22].

129 Specifically, the 72-hour experiment consisted of 6 successive immersion-emersion cycles
130 that were conducted (i) under naturally occurring seawater and air temperatures at our study
131 site in September (*i.e.* 20°C) as a control, and (ii) under a 6-hour aerial heat stress event of 25,
132 30 or 35°C after one immersion-emersion-immersion cycle at 20°C and followed by 4
133 immersion-emersion recovery events at 20°C (Fig. 1).

134



135

136 **Figure 1:** Schematic diagram of the immersion-emersion experiments. Mussels were immersed (blue rectangles)
 137 during 6 hours in Control, Virgin microplastic leachate (V-MPL) or Beached microplastic leachate (B-MPL)
 138 solutions and emerged (white rectangles) during 6 hours consistently at an aerial temperature of 20°C (control
 139 temperature) except during the second emersion (grey rectangle) where the mussels were exposed to a low (25°C),
 140 moderate (30°C) or high (35°C) aerial heat stress event. The numbers indicate the time in hours after the beginning
 141 of the microplastic leachate stress (normal font) or after the thermal stress (italic font).

142

143 For each experimental treatment (Temperature × Solution), 20 mussels were used in
 144 triplicates (*i.e.* $N = 60$ mussels for each of the 12 experimental trials). During the immersion
 145 phase, the mussels were incubated in a 1.5 L glass jar containing 1 L of Control, V-MPL, or B-
 146 MPL solutions. Immersions lasted for 6 hours at a temperature of 20 °C (Fig. 1). The emersion
 147 consisted in placing the mussels on natural seawater saturated paper towels in sealed 1.5 L glass
 148 jars to maintain around 95% relative humidity to avoid desiccation or evaporation [22]. For the
 149 aerial heat stress, the jars were placed in incubators (MIR-154, Panasonic, Japan; temperature
 150 resolution ± 0.3 °C) heated either at 20, 25, 30 or 35°C for 6 h (Fig. 1). Relative humidity and
 151 air temperature experienced by mussels inside the jars were monitored using Hygro Buttons 23
 152 (Proges-Plus, resolution 0.5°C and 1%) at 5 min interval (Supplementary Materials, S2).

153

154 *d) Endpoint and Statistical analyses*

155 After each immersion and emersion, the status of each mussel was visually checked. Opened
 156 mussels that did not respond to foot probing, *i.e.* no valve closure, were discarded and recorded

157 as dead [22,43–45].

158 To study the effects of the factors ‘Temperature’, ‘Solution’ and their interaction
159 ‘Temperature × Solution’, data were analysed using a 2-way ANOVA with Solution (Control,
160 V-MPL, B-MPL) and Temperature (20°C, 25°C, 30°C, 35°C) as fixed factors and percentage
161 of survived mussels at the end of the experiment as the dependent factor. Significant effects
162 were examined using Tukey-HSD post-hoc test. The data met the assumption required for the
163 ANOVAs; homogeneity of variances and dispersion of the residuals were checked using the
164 package ‘DHARMA’ (see Supplementary Materials, S3; [46]).

165 Lethal times at 50% of mortality (LT50; in hours) and their lower and upper fiducial
166 confidence limits (CL) for each solutions at 35°C — the only temperature where a significant
167 mortality was recorded — were estimated using a binomial generalised linear model with a
168 probit link function using the package ‘ecotox’ [47–50]. The data met the assumption required
169 for the generalised linear model; homogeneity of variances and dispersion of the residuals were
170 checked using the package ‘DHARMA’ (see Supplementary Materials, S4; [46]). To determine
171 the existence of differences between the LT50 at 35°C of each treatment, a ratio test was used
172 [49]. Exact *p* values are given in Supplementary Materials S5, S6 and S8. All statistical analyses
173 were performed using the R Core Team software (2022).

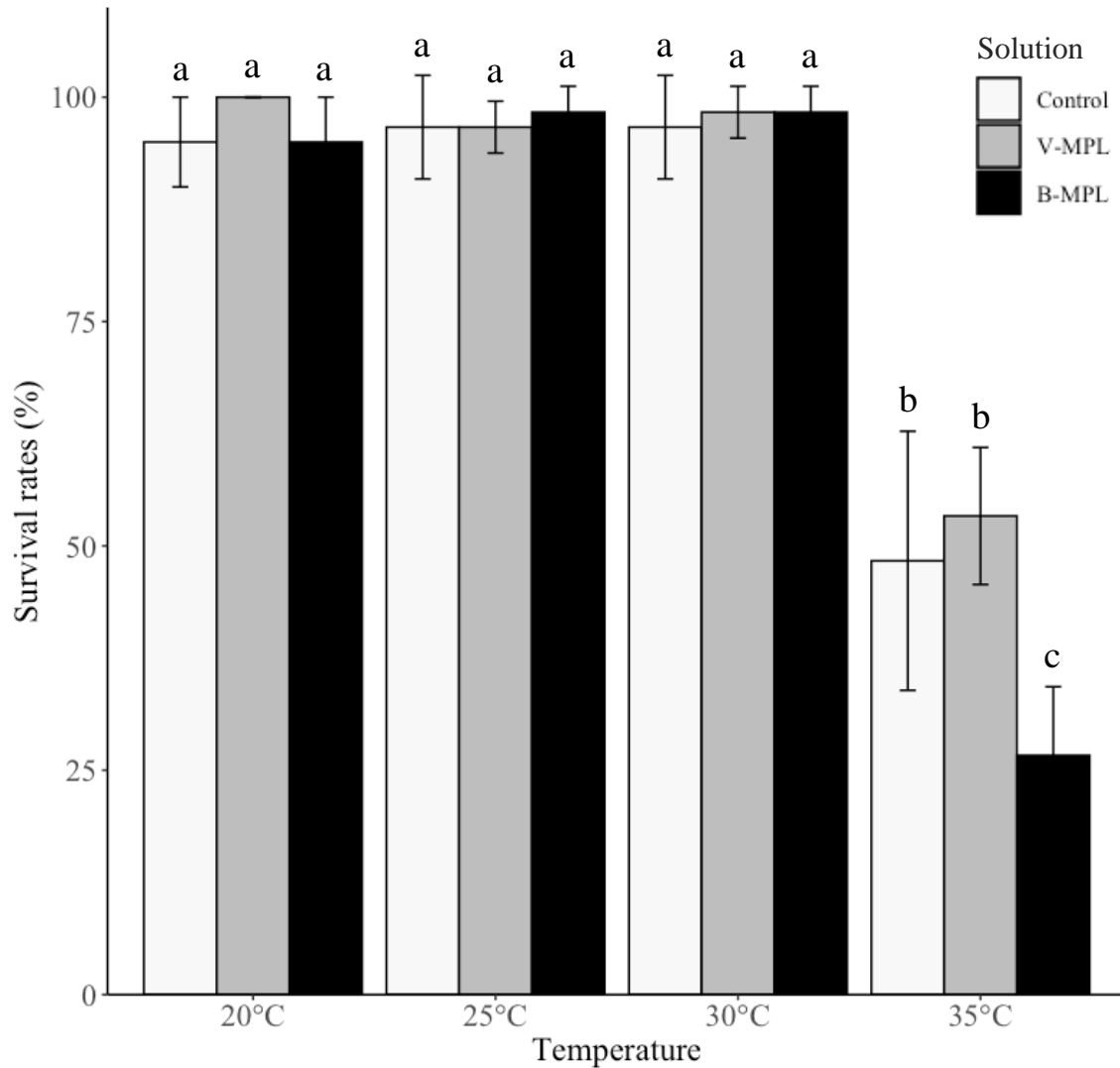
174

175 **3. Results**

176 *a) Survival rates*

177 Survival rates (mean ± SD) significantly decreased only after an aerial heat stress exposure at
178 35°C for all solutions, *i.e.* Control (48.3 ± 14.4%), V-MPL (53.3 ± 7.6 %) and B-MPL (26.7 ±
179 7.6%); Temperature × Solution, *p* < 0.01; Tukey test: *p* < 0.001; S5, S6; Fig. 2). Specifically,
180 at 35°C, significantly more mussels —5 on average— died when exposed to B-MPL compared
181 to those exposed to V-MPL and Control seawater (Temperature × Solution, *p* < 0.01; S6, Tukey

182 test: $p < 0.01$ and $p < 0.05$, respectively; Fig. 2). No significant differences in mussel mortality
 183 were found between V-MPL and Control seawater treatments at any of the aerial heat stress
 184 temperature tested (Temperature \times Solution, $p < 0.01$; S6; Tukey's test: $p > 0.05$; Fig. 2).
 185



186
 187 **Figure 2:** Survival rates (%; mean \pm Standard Deviation) of *Mytilus edulis* at the end of the 72 h experiment after
 188 an exposure to control seawater (light grey), virgin (grey) and beached (black) microplastic leachate solutions
 189 combined with an aerial heat stress event at different temperatures. Letters depict significant differences among
 190 Temperature \times Solution treatments ($p < 0.05$; Two-way ANOVA, Tukey post-hoc comparison). For details about
 191 the exact p -values, please refer to the Supplementary Materials S5, S6.

192
 193
 194 b) Lethal time after a 35°C aerial heat stress

195 When exposed to a 35°C aerial heat stress, the LT50 (CL) of mussels exposed to control
196 seawater, *i.e.* 69.5 h (64.4 – 78.0 h), was not significantly different from the LT50 recorded
197 following exposure to V-MPL, *i.e.* 71.1 h (67.3 – 77.0 h; ratio test: $p > 0.05$, S7, S8). In contrast,
198 mussels exposed to B-MPL were less resistant to a 35°C aerial heat stress, as LT50 was reached
199 in significantly less time, *i.e.* 59.4 h (57.2 – 62.0 h), than the other two treatments (ratio test: p
200 < 0.001 , S7, S8).

201 **4. Discussion**

202 We provide the first evidence of the combined effect of temperature and plastic leachates on
203 the survival rate of the keystone ecosystem engineer, *Mytilus edulis*. At temperatures ranging
204 from 20 to 30°C, the presence of leachates, either from virgin or beached microplastics, did not
205 affect the mortality rates of *M. edulis*. In sharp contrast, under conditions of high thermal stress
206 (35°C), and in agreement with previous studies [22,51], there was a significant decline in
207 survival rates for all treatments, especially when mussels were exposed to leachates from
208 beached microplastics. These results raise concern, given that thermal events with mussel body
209 temperature reaching more than 35°C during summer emersion at the study site occurred *ca.*
210 11 times over their 84-day time series [22], a scenario expected to intensify under future climate
211 projections [20]. Sub-lethal synergistic effect of temperature and contaminant, *e.g.* [52–56],
212 including plastics, *e.g.* [31,57–59], have previously been documented in mussels under
213 conditions of immersion. This present work, however, provides the first evidence of an
214 enhanced thermal mortality through an exposure to microplastic leachates.

215 The adverse synergistic effect observed here is likely due to a bioenergetic imbalance, *i.e.*
216 the energy costs exceed the available energy supply. Leachates originating from various plastics
217 such as car tire rubber, polypropylene, polyethylene terephthalate, polystyrene, polyvinyl
218 chloride, and noticeably even bioplastics are capable of harming mussel cells by interfering
219 with processes like lysosomal function, neurotransmission, oxidative stress and antioxidant

220 defences; see [60,61] for details on leachates composition. Under low and moderate thermal
221 stress conditions, the detoxification and repair cost may be low enough to supply basal
222 maintenance [29,62]. In turn, at high temperatures, the mussel heat shock response cost and cell
223 damages caused by aerial heat stress [63] are added to those of plastic leachates. In this context,
224 our results suggest that the processes used to counteract the cellular damage caused by these
225 combined stressors may become too costly and create a bioenergetic deficit, leading to high
226 mortality [29,62].

227 Noticeably, *M. edulis* survival rates following a 35°C aerial heat stress varied depending on
228 the pellet's history, with beached microplastic leachates (from pellets collected in the
229 environment and used at a locally realistic concentration [64]) being significantly more harmful
230 than virgin microplastic leachates (from commercially available pellets). The more severe
231 effect of B-MPL compared to V-MPL has previously been shown in *Perna perna* mussel
232 embryo [41] and also in a wide range of other species, *e.g.* in sea urchins (*Paracentrotus lividus*
233 [65,66]), jellyfish [65], gastropods [64] and even in dune plants [67], although exceptions exist
234 such as in zebrafish [65], copepods and another sea urchin (*Lytechinus variegatus* [68,69]).

235 The strongest effect observed for beached microplastic leachates compared to virgin ones
236 after the high aerial heat stress is likely related to the pellet chemical content. Indeed, virgin
237 pellets contained less additives, which were 2- to 4-fold less concentrated than in beached ones.
238 The additives characterised were mainly phthalate plasticisers (*i.e.* Dimethyl phthalate, DMP;
239 Diethyl phthalate, DEP; Di-n-butyl phthalate, DBP and Diisobutyl phthalate, DIBP) and
240 Bisphenol S antioxidant, which are known to have various detrimental effects on marine
241 invertebrates, for reviews see [70–72]. In addition, due to their permanence in the natural
242 environment, beached pellets are also likely to be loaded with a harmful cocktail of
243 environmental contaminants such as Persistent Organic Pollutants and heavy metals [73,74].
244 This is in sharp contrast with virgin pellets which are essentially composed of functional

245 additives (*e.g.* flame retardants, plasticisers, colorants [75]). Although mussel survival rates
246 were noticeably not affected at low temperatures when combined with either type of plastic
247 leachates, this does not rule out the existence of sublethal effects such as behaviour, growth and
248 reproduction which are likely to have longer term detrimental effects on the biology, ecology
249 and ultimately survival [62]. The resolution of these specific issues lies beyond the scope of the
250 present work, but warrants the need for further studies.

251

252 **5. Conclusion**

253 Thermal stress and plastic pollution are prominent challenges in the Anthropocene. In the
254 context of the anticipated rise in frequency, severity, and duration of extreme heat events [20],
255 coupled with the growing production of plastic and the subsequent accumulation of plastic
256 waste in the environment [76], our results raise concerns and highlight the critical need for
257 multi-stress studies to fully comprehend the impacts of these stressors on organisms. In turn,
258 increase in temperature has been shown to enhance the leaching of plastic associated chemical
259 compounds [77–79], and hence their potential toxicity, highlighting the need for further studies
260 to understand the fate of plastic-associated chemicals in warming ecosystems. Considering the
261 engineering role of the species used in this study, the combined impact of environmentally
262 realistic temperature [22], as well as the type and concentration of plastic pellet [64], could
263 pose a significant threat not only to the species itself but also to the overall ecosystem that relies
264 on these species for sustenance.

265

266

267 **Ethics**

268 Under French regulations, the present work did not require ethical approval from a human
269 subject or animal welfare committee.

270

271 **Data accessibility**

272 The data and scripts that support the findings of this study are available from the Dryad Digital

273 Repository: <https://doi.org/10.5061/dryad.gtht76hst>.

274 Supplementary material is available online.

275

276 **Declaration of competing interest**

277 The authors declare that they have no known competing financial interests or personal
278 relationships that could have appeared to influence the work reported in this paper.

279

280 **Author contributions**

281 **Marine Uguen:** Conceptualisation, Methodology, Investigation, Data curation, Formal

282 Analysis, Writing - Original Draft, Editing. **Sylvie M Gaudron:** Supervision, Resources,

283 Methodology, Investigation, Writing - Review & Editing. **Katy R Nicastro:** Supervision,

284 Methodology, Investigation, Writing - Review & Editing. **Gerardo I Zardi:** Methodology,

285 Investigation, Writing - Review & Editing. **Nicolas Spilmont:** Methodology, Investigation,

286 Writing - Review & Editing. **Solène Henry:** Investigation, Writing – Review & Editing.

287 **Laurent Seuront:** Supervision, Resources, Methodology, Investigation, Writing - Review &

288 Editing, Funding acquisition.

289

290 **Acknowledgements**

291 The authors are grateful to Sébastien Lefebvre for his assistance in statistics and to Kévin

292 Robache for his graphic design assistance. This study was supported by the EMIMA technical

293 platform of the University of Lille.

294

295 **Funding**

296 M. Uguen is funded by a PhD fellowship from the University of Lille and the Region-Hauts-
297 de-France. S. Henry is funded by a PhD fellowship from the University of Lille and the ANR
298 Project ECUME. This research was also financially supported by a grant (ANR-22-CE06-0029
299 ECUME) overseen by the French National Research Agency (ANR), both the European
300 Maritime and Fisheries Fund (FEAMP) and France Filière Pêche through the research project
301 SOLACE (“Resilience of the mussel *Mytilus edulis* to anthropogenic and climatic stressors”),
302 by the Fundação para a Ciência e a Tecnologia (FCT – MEC, Portugal, grant number:
303 UID/Multi/04326/2021 and EXPL/BIA-BMA/0682/2021) and by a Pierre Hubert Curien
304 PESSOA grant 2022-2023. This work has been partially financially supported by the European
305 Union (ERDF), the French Government, the Région Hauts-de-France and IFREMER, in the
306 framework of the project CPER IDEAL 2021–2027. Gerardo I. Zardi is a laureate of the
307 WINNING Normandy Program supported by the Normandy Region. This project has received
308 funding from the European Union’s Horizon 2020 research and innovation program under the
309 Marie Skłodowska-Curie grant agreement No 101034329.

310

311 **References**

- 312 1. Barnes DKA, Galgani F, Thompson RC, Barlaz M. 2009 Accumulation and
313 fragmentation of plastic debris in global environments. *Philos. Trans. R. Soc. B Biol. Sci.* **364**,
314 1985–1998. (doi:10.1098/rstb.2008.0205)
- 315 2. Geyer R, Jambeck JR, Law KL. 2017 Production, use, and fate of all plastics ever
316 made. *Sci. Adv.* **3**, e1700782. (doi:10.1126/sciadv.1700782)
- 317 3. Patil SM, Rane NR, Bankole PO, Krishnaiah P, Ahn Y, Park Y-K, Yadav KK, Amin
318 MA, Jeon B-H. 2022 An assessment of micro-and nanoplastics in the biosphere: A review of
319 detection, monitoring, and remediation technology. *Chem. Eng. J.* **430**, 132913.
- 320 4. Serra-Gonçalves C, Lavers JL, Bond AL. 2019 Global Review of Beach Debris
321 Monitoring and Future Recommendations. *Environ. Sci. Technol.* **53**, 12158–12167.
322 (doi:10.1021/acs.est.9b01424)
- 323 5. Wu P, Zhang H, Singh N, Tang Y, Cai Z. 2022 Intertidal zone effects on Occurrence,
324 fate and potential risks of microplastics with perspectives under COVID-19 pandemic. *Chem.*
325 *Eng. J.* **429**, 132351. (doi:10.1016/j.cej.2021.132351)
- 326 6. Derraik JGB. 2002 The pollution of the marine environment by plastic debris: a
327 review. *Mar. Pollut. Bull.* **44**, 842–852. (doi:10.1016/S0025-326X(02)00220-5)

- 328 7. Gall SC, Thompson RC. 2015 The impact of debris on marine life. *Mar. Pollut. Bull.*
329 **92**, 170–179. (doi:10.1016/j.marpolbul.2014.12.041)
- 330 8. Delaeter C, Spilmont N, Bouchet VMP, Seuront L. 2022 Plastic leachates: Bridging
331 the gap between a conspicuous pollution and its pernicious effects on marine life. *Sci. Total*
332 *Environ.* **826**, 154091. (doi:10.1016/j.scitotenv.2022.154091)
- 333 9. Seuront L, Zardi GI, Uguen M, Bouchet VMP, Delaeter C, Henry S, Spilmont N,
334 Nicastro KR. 2022 A whale of a plastic tale: A plea for interdisciplinary studies to tackle
335 micro- and nanoplastic pollution in the marine realm. *Sci. Total Environ.* **846**, 157187.
336 (doi:10.1016/j.scitotenv.2022.157187)
- 337 10. Koelmans AA, Bakir A, Burton GA, Janssen CR. 2016 Microplastic as a Vector for
338 Chemicals in the Aquatic Environment: Critical Review and Model-Supported
339 Reinterpretation of Empirical Studies. *Environ. Sci. Technol.* **50**, 3315–3326.
340 (doi:10.1021/acs.est.5b06069)
- 341 11. Diepens NJ, Koelmans AA. 2018 Accumulation of Plastic Debris and Associated
342 Contaminants in Aquatic Food Webs. *Environ. Sci. Technol.* **52**, 8510–8520.
343 (doi:10.1021/acs.est.8b02515)
- 344 12. Thompson RC, Moore CJ, vom Saal FS, Swan SH. 2009 Plastics, the environment and
345 human health: current consensus and future trends. *Philos. Trans. R. Soc. B Biol. Sci.* **364**,
346 2153–2166. (doi:10.1098/rstb.2009.0053)
- 347 13. Net S, Sempéré R, Delmont A, Paluselli A, Ouddane B. 2015 Occurrence, Fate,
348 Behavior and Ecotoxicological State of Phthalates in Different Environmental Matrices.
349 *Environ. Sci. Technol.* **49**, 4019–4035. (doi:10.1021/es505233b)
- 350 14. Rochman CM. 2015 *The complex mixture, fate and toxicity of chemicals associated*
351 *with plastic debris in the marine environment*. See <https://lillocat.univ-lille.fr>.
- 352 15. Thevenon F, Carroll C, Sousa J, editors. 2015 *Plastic debris in the ocean: the*
353 *characterization of marine plastics and their environmental impacts, situation analysis*
354 *report*. International Union for Conservation of Nature. (doi:10.2305/IUCN.CH.2014.03.en)
- 355 16. Mato Y, Isobe T, Takada H, Kanehiro H, Ohtake C, Kaminuma T. 2001 Plastic Resin
356 Pellets as a Transport Medium for Toxic Chemicals in the Marine Environment. *Environ. Sci.*
357 *Technol.* **35**, 318–324. (doi:10.1021/es0010498)
- 358 17. Mattsson K, Hansson L-A, Cedervall T. 2015 Nano-plastics in the aquatic
359 environment. *Environ. Sci. Process. Impacts* **17**, 1712–1721. (doi:10.1039/C5EM00227C)
- 360 18. Lynch JM, Knauer K, Shaw KR. 2022 Plastic Additives in the Ocean. In *Plastics and*
361 *the Ocean*, pp. 43–76. John Wiley & Sons, Ltd. (doi:10.1002/9781119768432.ch2)
- 362 19. Della-Marta PM, Haylock MR, Luterbacher J, Wanner H. 2007 Doubled length of
363 western European summer heat waves since 1880. *J. Geophys. Res. Atmospheres* **112**.
364 (doi:10.1029/2007JD008510)
- 365 20. IPCC. 2022 IPCC, 2022: Climate Change 2022: Impacts, Adaptation, and
366 Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the
367 Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S.
368 Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A.
369 Okem, B. Rama (eds.)]. Cambridge University Press. In Press.
- 370 21. Sun Q, Miao C, Hanel M, Borthwick AGL, Duan Q, Ji D, Li H. 2019 Global heat
371 stress on health, wildfires, and agricultural crops under different levels of climate warming.
372 *Environ. Int.* **128**, 125–136. (doi:10.1016/j.envint.2019.04.025)
- 373 22. Seuront L, Nicastro KR, Zardi GI, Goberville E. 2019 Decreased thermal tolerance
374 under recurrent heat stress conditions explains summer mass mortality of the blue mussel
375 *Mytilus edulis*. *Sci. Rep.* **9**, 17498. (doi:10.1038/s41598-019-53580-w)
- 376 23. Tsuchiya M. 1983 Mass mortality in a population of the mussel *Mytilus edulis* L.
377 Caused by high temperature on rocky shores. *J. Exp. Mar. Biol. Ecol.* **66**, 101–111.

- 378 (doi:10.1016/0022-0981(83)90032-1)
- 379 24. Galil BS, Mienis HK, Mendelson M, Gayer K, Goren M. 2022 Here today, gone
380 tomorrow-the Levantine population of the Brown mussel *Perna perna* obliterated by
381 unprecedented heatwave. *Aquat. Invasions* **17**.
- 382 25. Raymond WW *et al.* 2022 Assessment of the impacts of an unprecedented heatwave
383 on intertidal shellfish of the Salish Sea. *Ecology* **103**.
- 384 26. Zippay ML, Helmuth B. 2012 Effects of temperature change on mussel, *Mytilus*.
385 *Integr. Zool.* **7**, 312–327. (doi:10.1111/j.1749-4877.2012.00310.x)
- 386 27. Lavergne E, Pedron N, Calves I, Claireaux G, Mazurais D, Zambonino-Infante J, Le
387 Bayon N, Cahu C, Laroche J. 2015 Does the chronic chemical contamination of a European
388 flounder population decrease its thermal tolerance? *Mar. Pollut. Bull.* **95**, 658–664.
389 (doi:10.1016/j.marpolbul.2015.01.006)
- 390 28. Patra RW, Chapman JC, Lim RP, Gehrke PC. 2007 The effects of three organic
391 chemicals on the upper thermal tolerances of four freshwater fishes. *Environ. Toxicol. Chem.*
392 **26**, 1454–1459. (doi:10.1897/06-156R1.1)
- 393 29. Sokolova IM, Lannig G. 2008 Interactive effects of metal pollution and temperature
394 on metabolism in aquatic ectotherms: implications of global climate change. *Clim. Res.* **37**,
395 181–201. (doi:10.3354/cr00764)
- 396 30. Fonte E, Ferreira P, Guilhermino L. 2016 Temperature rise and microplastics interact
397 with the toxicity of the antibiotic cefalexin to juveniles of the common goby (*Pomatoschistus*
398 *microps*): Post-exposure predatory behaviour, acetylcholinesterase activity and lipid
399 peroxidation. *Aquat. Toxicol.* **180**, 173–185. (doi:10.1016/j.aquatox.2016.09.015)
- 400 31. Gomez A, Gonzales S, Francia-Quiroz J-C. 2021 Effect of temperature and
401 microplastic concentration on the filtration rate of the mussel *Semimytilus algosus* (Mytiloidea:
402 Mytilidae). *Rev. Biol. Trop.* **69**, 1242–1251. (doi:10.15517/rbt.v69i4.45426)
- 403 32. Kratina P, Watts TJ, Green DS, Kordas RL, O’Gorman EJ. 2019 Interactive effects of
404 warming and microplastics on metabolism but not feeding rates of a key freshwater
405 detritivore. *Environ. Pollut.* **255**, 113259. (doi:10.1016/j.envpol.2019.113259)
- 406 33. Wen B, Zhang N, Jin S-R, Chen Z-Z, Gao J-Z, Liu Y, Liu H-P, Xu Z. 2018
407 Microplastics have a more profound impact than elevated temperatures on the predatory
408 performance, digestion and energy metabolism of an Amazonian cichlid. *Aquat. Toxicol.* **195**,
409 67–76. (doi:10.1016/j.aquatox.2017.12.010)
- 410 34. Ferreira P, Fonte E, Soares ME, Carvalho F, Guilhermino L. 2016 Effects of multi-
411 stressors on juveniles of the marine fish *Pomatoschistus microps*: Gold nanoparticles,
412 microplastics and temperature. *Aquat. Toxicol.* **170**, 89–103.
413 (doi:10.1016/j.aquatox.2015.11.011)
- 414 35. Hoffschroer N, Grassl N, Steinmetz A, Sziegoleit L, Koch M, Zeis B. 2021
415 Microplastic burden in *Daphnia* is aggravated by elevated temperatures. *Zoology* **144**,
416 125881. (doi:10.1016/j.zool.2020.125881)
- 417 36. Plafcan MM, Stallings CD. 2022 Microplastics do not affect bleaching of *Acropora*
418 *cervicornis* at ambient or elevated temperatures. *PeerJ* **10**, e13578. (doi:10.7717/peerj.13578)
- 419 37. Buschbaum C, Dittmann S, Hong J-S, Hwang I-S, Strasser M, Thiel M, Valdivia N,
420 Yoon S-P, Reise K. 2009 Mytilid mussels: global habitat engineers in coastal sediments.
421 *Helgol. Mar. Res.* **63**, 47–58. (doi:10.1007/s10152-008-0139-2)
- 422 38. Uguen M, Nicastro KR, Zardi GI, Gaudron SM, Spilmont N, Akoueson F, Duflos G,
423 Seuront L. 2022 Microplastic leachates disrupt the chemotactic and chemokinetic behaviours
424 of an ecosystem engineer (*Mytilus edulis*). *Chemosphere* **306**, 135425.
425 (doi:10.1016/j.chemosphere.2022.135425)
- 426 39. Uguen M, Gaudron SM, Nicastro KR, Zardi GI, Spilmont N, Seuront L. 2023 Size-
427 dependent response of the mussel collective behaviour to plastic leachates and predator cues.

- 428 *Sci. Total Environ.* **888**, 164037. (doi:10.1016/j.scitotenv.2023.164037)
- 429 40. Seuront L, Nicastró KR, McQuaid CD, Zardi GI. 2021 Microplastic leachates induce
430 species-specific trait strengthening in intertidal mussels. *Ecol. Appl.* **31**, e02222.
431 (doi:10.1002/eap.2222)
- 432 41. Gandara e Silva PP, Nobre CR, Resaffe P, Pereira CDS, Gusmão F. 2016 Leachate
433 from microplastics impairs larval development in brown mussels. *Water Res.* **106**, 364–370.
434 (doi:10.1016/j.watres.2016.10.016)
- 435 42. Sorel M, Mittelberger S, Kreitz M. 2022 Un été 2022 extrêmement chaud. *La*
436 *Météorologie* **2022**, 99–106.
- 437 43. Martin MD, Mackie GL, Baker MA. 1993 Acute toxicity tests and pulsed-dose
438 delayed mortality at 12 and 22°C in the zebra mussel (*Dreissena polymorpha*). *Arch. Environ.*
439 *Contam. Toxicol.* **24**, 389–398. (doi:10.1007/BF01128739)
- 440 44. Waller DL, Rach JJ, Cope WG, Miller GA. 1995 Effects of Handling and Aerial
441 Exposure on the Survival of Unionid Mussels. *J. Freshw. Ecol.* **10**, 199–207.
442 (doi:10.1080/02705060.1995.9663439)
- 443 45. Wildridge PJ, Werner RG, Doherty FG, Neuhauser EF. 1998 Acute Toxicity of
444 Potassium to the Adult Zebra Mussel *Dreissena polymorpha*. *Arch. Environ. Contam. Toxicol.*
445 **34**, 265–270. (doi:10.1007/s002449900316)
- 446 46. Hartig F. 2022 DHARMa: residual diagnostics for hierarchical (multi-level / mixed)
447 regression models. R package version 0.4.6.
- 448 47. Finney DJ. 1971 *Probit Analysis*. Cambridge, England.
- 449 48. Robertson JL, Savin NE, Russell RM, Preisler HK. 2007 *Bioassays with arthropods*.
- 450 49. Wheeler MW, Park RM, Bailer AJ. 2006 Comparing median lethal concentration
451 values using confidence interval overlap or ratio tests. *Environ. Toxicol. Chem.* **25**, 1441–
452 1444. (doi:10.1897/05-320R.1)
- 453 50. Hlina BL. 2021 Package ‘ecotox’. Analysis of Ecotoxicology.
- 454 51. Jones SJ, Mieszkowska N, Wethey DS. 2009 Linking Thermal Tolerances and
455 Biogeography: *Mytilus edulis* (L.) at its Southern Limit on the East Coast of the United
456 States. *Biol. Bull.* **217**, 73–85. (doi:10.1086/BBLv217n1p73)
- 457 52. Andrade M, Soares AMVM, Solé M, Pereira E, Freitas R. 2022 Do climate change
458 related factors modify the response of *Mytilus galloprovincialis* to lanthanum? The case of
459 temperature rise. *Chemosphere* **307**, 135577. (doi:10.1016/j.chemosphere.2022.135577)
- 460 53. Bordalo D, Cuccaro A, Meucci V, De Marchi L, Soares AMVM, Pretti C, Freitas R.
461 2023 Will warmer summers increase the impact of UV filters on marine bivalves? *Sci. Total*
462 *Environ.* **872**, 162108. (doi:10.1016/j.scitotenv.2023.162108)
- 463 54. Leite C *et al.* 2020 Toxic impacts of rutile titanium dioxide in *Mytilus*
464 *galloprovincialis* exposed to warming conditions. *Chemosphere* **252**, 126563.
465 (doi:10.1016/j.chemosphere.2020.126563)
- 466 55. Lopes J, Coppola F, Soares AMVM, Meucci V, Pretti C, Polese G, Freitas R. 2022
467 How temperature rise will influence the toxic impacts of 17 α -ethinylestradiol in *Mytilus*
468 *galloprovincialis*? *Environ. Res.* **204**, 112279. (doi:10.1016/j.envres.2021.112279)
- 469 56. Moleiro P, Morais T, Leite C, Coppola F, Henriques B, Pinto J, Soares AMVM,
470 Pereira E, Freitas R. 2022 The effect of ocean warming on accumulation and cellular
471 responsiveness to cobalt in *Mytilus galloprovincialis*. *Mar. Pollut. Bull.* **182**, 113944.
472 (doi:10.1016/j.marpolbul.2022.113944)
- 473 57. Lenz M. 2016 GAME XIV: Auswirkungen von Wärmestress und Mikroplastik auf
474 marine Muscheln.
- 475 58. Pittura L *et al.* 2022 Cellular disturbance and thermal stress response in mussels
476 exposed to synthetic and natural microfibers. *Front. Mar. Sci.* **9**.
- 477 59. Weber A, Jeckel N, Wagner M. 2020 Combined effects of polystyrene microplastics

- 478 and thermal stress on the freshwater mussel *Dreissena polymorpha*. *Sci. Total Environ.* **718**,
479 137253. (doi:10.1016/j.scitotenv.2020.137253)
- 480 60. Capolupo M, Gunaalan K, Booth AM, Sørensen L, Valbonesi P, Fabbri E. 2021 The
481 sub-lethal impact of plastic and tire rubber leachates on the Mediterranean mussel *Mytilus*
482 *galloprovincialis*. *Environ. Pollut.* **283**, 117081. (doi:10.1016/j.envpol.2021.117081)
- 483 61. Capolupo M, Rafiq A, Coralli I, Alessandro T, Valbonesi P, Fabbri D, Fabbri E. 2023
484 Bioplastic leachates characterization and impacts on early larval stages and adult mussel
485 cellular, biochemical and physiological responses. *Environ. Pollut.* **319**, 120951.
486 (doi:10.1016/j.envpol.2022.120951)
- 487 62. Sokolova IM. 2013 Energy-Limited Tolerance to Stress as a Conceptual Framework to
488 Integrate the Effects of Multiple Stressors. *Integr. Comp. Biol.* **53**, 597–608.
489 (doi:10.1093/icb/ict028)
- 490 63. Nicastro KR, Pearson GA, Ramos X, Pearson V, McQuaid CD, Zardi GI. 2023
491 Transcriptome wide analyses reveal intraspecific diversity in thermal stress responses of a
492 dominant habitat-forming species. *Sci. Rep.* **13**, 5645. (doi:10.1038/s41598-023-32654-w)
- 493 64. Seuront L. 2018 Microplastic leachates impair behavioural vigilance and predator
494 avoidance in a temperate intertidal gastropod. *Biol. Lett.* **14**, 20180453.
495 (doi:10.1098/rsbl.2018.0453)
- 496 65. Cormier B *et al.* 2021 Chemicals sorbed to environmental microplastics are toxic to
497 early life stages of aquatic organisms. *Ecotoxicol. Environ. Saf.* **208**, 111665.
498 (doi:10.1016/j.ecoenv.2020.111665)
- 499 66. Rendell-Bhatti F, Paganos P, Pouch A, Mitchell C, D’Aniello S, Godley BJ, Pazdro K,
500 Arnone MI, Jimenez-Guri E. 2021 Developmental toxicity of plastic leachates on the sea
501 urchin *Paracentrotus lividus*. *Environ. Pollut.* **269**, 115744.
502 (doi:10.1016/j.envpol.2020.115744)
- 503 67. Menicagli V, Balestri E, Biale G, Corti A, La Nasa J, Modugno F, Castelvetro V,
504 Lardicci C. 2022 Leached degradation products from beached microplastics: A potential
505 threat to coastal dune plants. *Chemosphere* **303**, 135287.
506 (doi:10.1016/j.chemosphere.2022.135287)
- 507 68. Nobre CR, Santana MFM, Maluf A, Cortez FS, Cesar A, Pereira CDS, Turra A. 2015
508 Assessment of microplastic toxicity to embryonic development of the sea urchin *Lytechinus*
509 *variegatus* (Echinodermata: Echinoidea). *Mar. Pollut. Bull.* **92**, 99–104.
510 (doi:10.1016/j.marpolbul.2014.12.050)
- 511 69. Nobre CR, Moreno BBMB, Alves AV, Abessa DM de S, Cesar A, Choueri RB,
512 Choueri PKG, Pereira CDS. 2022 Can leachates of environmentally relevant concentrations
513 of microplastics in marine sediments affect the reproduction of an epibenthic copepod?
514 *Ecotoxicol. Environ. Contam.* **17**, 106–113. (doi:10.5132/eec.2022.01.10)
- 515 70. Burgos-Aceves MA, Abo-Al-Ela HG, Faggio C. 2021 Impact of phthalates and
516 bisphenols plasticizers on haemocyte immune function of aquatic invertebrates: A review on
517 physiological, biochemical, and genomic aspects. *J. Hazard. Mater.* **419**, 126426.
518 (doi:10.1016/j.jhazmat.2021.126426)
- 519 71. Liu J, Zhang L, Lu G, Jiang R, Yan Z, Li Y. 2021 Occurrence, toxicity and ecological
520 risk of Bisphenol A analogues in aquatic environment – A review. *Ecotoxicol. Environ. Saf.*
521 **208**, 111481. (doi:10.1016/j.ecoenv.2020.111481)
- 522 72. Oehlmann J *et al.* 2009 A critical analysis of the biological impacts of plasticizers on
523 wildlife. *Philos. Trans. R. Soc. B Biol. Sci.* **364**, 2047–2062. (doi:10.1098/rstb.2008.0242)
- 524 73. Khalid A, Zalouk-Vergnoux A, Benali S, Mincheva R, Raquez J-M, Bertrand S,
525 Poirier L. 2021 Are bio-based and biodegradable microplastics impacting for blue mussel
526 (*Mytilus edulis*). *Mar. Pollut. Bull.* **167**, 112295. (doi:10.1016/j.marpolbul.2021.112295)
- 527 74. Rodrigues JP, Duarte AC, Santos-Echeandía J, Rocha-Santos T. 2019 Significance of

528 interactions between microplastics and POPs in the marine environment: A critical overview.
529 *TrAC Trends Anal. Chem.* **111**, 252–260. (doi:10.1016/j.trac.2018.11.038)

530 75. Hermabessiere L, Dehaut A, Paul-Pont I, Lacroix C, Jezequel R, Soudant P, Duflos G.
531 2017 Occurrence and effects of plastic additives on marine environments and organisms: A
532 review. *Chemosphere* **182**, 781–793. (doi:10.1016/j.chemosphere.2017.05.096)

533 76. Geyer R. 2020 Chapter 2 - Production, use, and fate of synthetic polymers. In *Plastic*
534 *Waste and Recycling* (ed TM Letcher), pp. 13–32. Academic Press. (doi:10.1016/B978-0-12-
535 817880-5.00002-5)

536 77. Dhavamani J, Beck AJ, Gledhill M, El-Shahawi MS, Kadi MW, Ismail IMI,
537 Achterberg EP. 2022 The effects of salinity, temperature, and UV irradiation on leaching and
538 adsorption of phthalate esters from polyethylene in seawater. *Sci. Total Environ.* **838**, 155461.
539 (doi:10.1016/j.scitotenv.2022.155461)

540 78. Kida M, Ziembowicz S, Pochwat K, Koszelnik P. 2022 Experimental and
541 computational hazard prediction associated with reuse of recycled car tire material. *J. Hazard.*
542 *Mater.* **438**, 129489. (doi:10.1016/j.jhazmat.2022.129489)

543 79. Kida M, Koszelnik P. 2021 Investigation of the Presence and Possible Migration from
544 Microplastics of Phthalic Acid Esters and Polycyclic Aromatic Hydrocarbons. *J. Polym.*
545 *Environ.* **29**, 599–611. (doi:10.1007/s10924-020-01899-1)

546 80. Li C, Tang KHD. 2023 Effects of pH and Temperature on the Leaching of Di(2-
547 Ethylhexyl) Phthalate and Di-n-butyl Phthalate from Microplastics in Simulated Marine
548 Environment. *Biointerface Res. Appl. Chem.* **13**, 269. (doi:10.33263/BRIAC133.269)

549 81. Ye X, Wang P, Wu Y, Zhou Y, Sheng Y, Lao K. 2020 Microplastic acts as a vector
550 for contaminants: the release behavior of dibutyl phthalate from polyvinyl chloride pipe
551 fragments in water phase. *Environ. Sci. Pollut. Res.* **27**, 42082–42091. (doi:10.1007/s11356-
552 020-10136-0)

553 82. Zhang Y, Yuan Y, Tan W. 2022 Influences of humic acid on the release of
554 polybrominated diphenyl ethers from plastic waste in landfills under different environmental
555 conditions. *Ecotoxicol. Environ. Saf.* **230**, 113122. (doi:10.1016/j.ecoenv.2021.113122)

556 83. Godoy V, Martín-Lara MA, Calero M, Blázquez G. 2020 The relevance of interaction
557 of chemicals/pollutants and microplastic samples as route for transporting contaminants.
558 *Process Saf. Environ. Prot.* **138**, 312–323. (doi:10.1016/j.psep.2020.03.033)

559