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1 **Motion behavior and metabolic response to microplastic leachates in the benthic foraminifera**
2 ***Haynesina germanica***

3

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34

35 **Abstract**

36 Plastic is one of the major sources of pollution in modern oceans. When in seawater, toxic plasticizers
37 (the additives incorporated in plastic polymers during manufacturing processes) typically diffuse and
38 accumulate in sediments and in benthic and pelagic organisms' tissues. These plastic leachates affect
39 survival, behavior and metabolism of various marine metazoans, but little effort was placed in studying
40 their effect on protists. In this contribution we monitored the short-term effect of polypropylene (PP)
41 leachates at both environmentally realistic and chronic concentrations on *Haynesina germanica*
42 locomotion and metabolism. We found that PP leachates has no lethal nor effects on this species activity.
43 Taken together, these results suggest that benthic foraminifera may be more resistant than marine
44 metazoans to plasticizers pollutants.

45

46 **Keywords:** Benthic foraminifera, Plastic leachates, Polypropylene, Survival, Behavior, Respiration

47

48 **1 Introduction**

49 Plastics are acknowledged as one of the most ubiquitous and conspicuous sources of pollution of the
50 Anthropocene, especially in the marine environment (W. C. Li et al., 2016). Microplastics (MP) can
51 either be small plastic particles (smaller than 5mm) released in the environment or result from the
52 breakage and aging of macroplastics. They are now considered the most numerically abundant form of
53 solid waste on the planet (Eriksen et al., 2014) and a potential threat to marine ecosystems globally
54 (Galloway et al., 2017). Hence, they are widely observed from coastal waters to the deep-ocean floor
55 and from tropical to polar regions (Barnes, 2005; Chiba et al., 2018).

56 Microplastics are also responsible for a range of sub-lethal effects related to their pernicious role as a
57 vector of chemical pollutants. These pollutants leaching from MP to the marine environments originate
58 from the additives compounds (e.g. plasticizers, flame retardant, UV stabilizers, antioxidant, and
59 antistatic molecule) incorporated in plastics during the manufacturing process to modify the plastic
60 polymers physical properties and durability, but also from the chemical compounds already present in
61 the water (i.e. coming from another source of pollution) which are adsorbed at the MP's surface when
62 aging in the environments. Plastic additives such as phthalates, bisphenol A, nonyphenols and
63 brominated flame retardants can reach high concentrations in coastal waters (Hermabessiere et al., 2017;
64 Sánchez-Avila et al., 2012) and accumulate in marine organisms tissues (Vered et al., 2019). This work
65 specifically focuses on the toxicity of virgin MP leachates since they have recently been identified as
66 one of the most critical threat related to the presence of plastics in the ocean (Hahladakis et al., 2018;
67 Paluselli et al., 2019). The toxic effects of virgin microplastic leachates have been reported in various
68 marine faunal taxa, such as barnacles (H.-X. Li et al., 2016), crustacean larvae (Lithner et al., 2009),
69 gastropods (Seuront, 2018), bivalves (Ke et al., 2019) and sea urchins (Oliviero et al., 2019). Desorption
70 of these chemicals in the surrounding environment causes a range of harmful effects on embryo
71 development, reproduction, behavior or induce genetic aberrations (see Oehlmann et al. (2009) for a
72 review).

73 To date and to the best of our knowledge, there is still a critical lack of information available on effect
74 of microplastic on protists, despite a recent urge to fill this knowledge gap (Rillig and Bonkowski, 2018).
75 However, MP ingestion is likely to be common in protists (Setälä et al., 2014), including foraminifera
76 (Ciacci et al., 2019), and subsequently negatively impact their metabolic activity (Ciacci et al., 2019; Su
77 et al., 2020). Benthic foraminifera were targeted in this work due to their importance in the structure and
78 function of benthic ecosystems (Geslin et al., 2011; Gooday et al., 1992), their ability to respond to
79 various types of pollutant both under laboratory conditions (Denoyelle et al., 2012; Ernst et al., 2006;
80 Nigam et al., 2009) and *in situ* (see Alve, 1995 for a review). Like any benthic organisms, they are
81 directly exposed to the range of pollutants, including microplastics (Schwarz et al., 2019) which cannot
82 be degraded by bacteria (Nauendorf et al., 2016) and therefore accumulate in coastal sediments (Galgani
83 et al., 1996). In this context, the present study assessed the potential short-term effects of the leachates
84 from virgin polypropylene pellets considered at both environmentally realistic and chronic

85 concentrations on the stress level of the benthic foraminifera *Haynesina germanica*. Specifically,
86 movement behavior (Seuront, 2018) and respiration rate (Su et al., 2020) were considered as proxies of
87 the stress level of *H. germanica* following an exposure to polypropylene leachates. This foraminiferal
88 species and this plastic polymer were specifically chosen for their high abundances along the French
89 coast of the eastern English Channel (Armynot du Châtelet et al., 2018; Francescangeli et al., 2017;
90 Hermabessiere et al., 2019).

91 **2 Material and methods**

92 2.1 *Haynesina germanica* collection

93 Surface sediment (0-1cm) from Boulogne-sur-Mer harbor mudflat (eastern English Channel,
94 50°43'06.4"N 1°34'22.0"E) was sampled in June 2019 and stored in 100 ml polypropylene containers.
95 Sediment was kept at ambient temperature during transportation and placed within one hour in English
96 Channel seawater aquarium (12°C and 35 PSU) under a natural day-light cycle conditions until the
97 experiment took place. Sediment was sieved over a 125 µm stainless-steel mesh and colored-cytoplasm
98 *Haynesina germanica* were subsequently sorted. Only the active specimens (i.e. leaving a displacement
99 track on a thin layer of sediment) were considered as living and selected for the experiment. Living
100 individuals were transferred in artificial seawater (ASW) prepared with 35 grams of sea salt (RedSea
101 Fish Farm, Israel) per liter of Milli-Q water (Merck Millipore, Germany) and gently cleaned with a
102 brush to remove any surrounding particles.

103

104 2.2 Experimental conditions

105 Both behavioral experiments and metabolic measurements were conducted exposing *H. germanica* to
106 artificial seawater as control and to microplastic leachates seawater. Microplastic leachates seawater
107 was prepared from commercially available virgin polypropylene pellets (typically 3.3 to 4.7 mm in
108 diameter; Pemmiproductions, Germany) mixed with artificial seawater at a concentration of 20 ml and 200
109 ml of pellets per liter (hereafter respectively referred to as PP20 and PP200) and aerated for 24 h before
110 the beginning of the experiments following the protocol developed in Seuront (2018) to monitor the
111 effect of plastic leachates on a marine gastropod. Although not quantified in this experiment,
112 polypropylene leachates typically contain bisphenol A, octylphenol and nonylphenol (Hermabessiere et
113 al., 2017).

114

115 2.3 Behavioral experiment

116 For each experimental condition (i.e. control seawater and the two leachate treatments PP20 and PP200),
117 15 living *Haynesina germanica* (maximum diameter range: 300-440 µm) were spread randomly on the
118 bottom of 15-cm wide glass-Petri dishes filled with ASW, PP20 or PP200 (Figure 1). Petri dishes were
119 placed in a light and temperature-controlled incubator (MIR-154, Panasonic, Japan) set at 12°C. The
120 movements of *H. germanica* were recorded every 10 minutes using a digital camera (V1 with a 10-30

121 mm lens, Nikon, Japan) under homogenous dim light conditions (photosynthetically active radiation
122 $<100 \mu\text{mol photon m}^{-2} \text{ s}^{-1}$; SA-190 quantum sensor, LI-COR, USA) provided by a horizontal array of
123 LEDs (YN-160 III, Yongnuo, China). Each experiment lasted 10 hours.

124 Images were compiled in the open-source image analysis software Fiji (Schindelin et al., 2012) and (x, y)
125 coordinates were measured for each individual *H. germanica* using the *Manual Tracking* plugin (Figure
126 1). The distance travelled (D_t) by each individual between two images was calculated as: $D_t = \sqrt{[(x_t -$
127 $x_{t+1})^2 + (y_t - y_{t+1})^2]}$ where (x_t, y_t) and (x_{t+1}, y_{t+1}) are the coordinates between two successive images
128 taken at 10-minute intervals. The total distance travelled in 10 hours was calculated from the sum of all
129 D_t and subsequently converted to locomotion speed (mm h^{-1}). These behavioral parameters were
130 measured using *trajr* package (McLean and Skowron Volponi, 2018) in R v.3.5.3 (R Core Team, 2019).
131 Trajectories complexity was assessed using fractal analysis. The fractal dimensions of foraminifera
132 trajectories were estimated following the box dimension method (Seuront, 2015, 2010).

133

134 2.4 Respiration measurements

135 Five *H. germanica* specimens were randomly selected from the individuals used in behavioral
136 experiments and transferred from the Petri dish to a 1-mm wide glass microtube containing the three
137 tested seawater (ASW for control and PP20 and PP200 to test the effect of polypropylene leachates).
138 Steady-state oxygen consumption gradient (dC/dz , in pmol cm^{-4}) in the millimeter above the organisms
139 were measured using a 50- μm Clark-type oxygen microelectrode (Unisense, Denmark). Oxygen fluxes
140 (J , $\text{pmol cm}^{-1} \text{ s}^{-1}$) in the microtube were calculated using Fick's first law of free diffusion as $J = D \times dC/dz$
141 (Li and Gregory, 1974) with D being the free diffusion coefficient for oxygen ($D = 1.6 \cdot 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ at
142 12°C and 35PSU). Individual respiration rate (R , $\text{pmol ind}^{-1} \text{ day}^{-1}$) was then calculated as $R = J \times S/n$
143 (considering the microtube inner section $S = 7.9 \cdot 10^{-3} \text{ cm}^2$ and the number of individuals $n = 5$). Note
144 that our measurements were conducted on groups of 5 individuals both to take into account the low
145 individual respiration rate of benthic foraminifera and to overpass the sensor detection limit (Geslin et
146 al., 2011). Respiration rate measurements were replicated 6 times in control seawater and triplicated in
147 both P20 and PP200 leachate treatments. Since respiration is influenced by individual size, specimens
148 were measured to normalize the respiration rates by the foraminiferal biovolume ($8 \cdot 10^6 \mu\text{m}^3$ in average;
149 estimated following Geslin et al., 2011). All respiration measurements were carried out in the dark in a
150 12°C temperature-controlled water bath (Huber CC-K12, Germany).

151

152 2.5 Data analysis

153 Due to our small size samples, the effect of the 3 experimental conditions on movement speed, fractal
154 dimension and foraminiferal respiration rate was tested using Kruskal-Wallis test (Hollander and Wolfe,
155 1999) in R v.3.5.3 (R Core Team, 2019).

156

157 **3 Results**

158 Image analysis show that 100% of the individuals tested were moving throughout the experiments and
159 were still alive after being exposed to PP20 and PP200 for 10 hours. *Haynesina germanica* moved over
160 distances ranging from 7 to 32 mm, at locomotion speed ranging from 0.7 to 3.2 mm h⁻¹, 1.6 to 2.8 mm
161 h⁻¹ and 1.1 to 3.1 mm h⁻¹ for ASW, PP20 and PP200 respectively (Figure 2A). All the trajectories
162 considered in this work were significantly described in terms of fractal dimensions that ranged between
163 1.02 and 1.13 with average values of 1.07, 1.06 and 1.06 in ASW, PP20 and PP200 respectively (Figure
164 2B). Finally, respiration rate ranged from 41 to 114 10⁻⁶ pmol μm⁻³ day⁻¹ in the ASW control, from 66
165 to 164 10⁻⁶ pmol μm⁻³ day⁻¹ in PP20 and from 84 to 98 10⁻⁶ pmol μm⁻³ day⁻¹ in PP200 (Figure 2C).
166 Neither locomotion speed, fractal dimensions nor respiration rates exhibited any significant differences
167 between the three experimental conditions (Kruskall-Wallis-test: p>0.05; Table 1).

168

169 **4 Discussion**

170 The additives leaching from polypropylene (i.e. essentially antioxidant additives such as bisphenol A,
171 octylphenol and nonylphenol; Hermabessiere et al., 2017) have lethal effects on mollusks (Oehlmann et
172 al., 2000), barnacle larvae (H.-X. Li et al., 2016), amphibians (Hogan et al., 2006), annelids and
173 crustaceans (Staples et al., 2016). In contrast, the present work showed a lack of any lethal effect on
174 *Haynesina germanica* of PP leachates.

175 Similarly, no sublethal effect were perceptible through *H. germanica* locomotion and metabolism.
176 Specifically, locomotion speed was nearly 2-fold lower than those reported previously on the same
177 species (here ~2 mm h⁻¹ vs. ~4 mm h⁻¹ in Seuront and Bouchet, 2015) probably due to the lower
178 experimental temperature (12°C here vs. 22°C in Seuront and Bouchet, 2015) since decreasing
179 temperature is known to reduce foraminiferal activity (Bradshaw, 1961). Our results nevertheless clearly
180 indicated that PP leachates did not affect foraminiferal behavior (Figure 2A, B). This is consistent with
181 the observed lack of behavioral impairment in the intertidal gastropod *Littorina littorea*; as PP20
182 leachates impaired their chemosensory ability without impacting their neuromuscular abilities (Seuront,
183 2018). In turn, our results contrast with previous evidence that PP-plasticizers reduce fish larvae velocity
184 in the first days after hatching (Inagaki et al., 2016; Wang et al., 2013) and negatively impact adult-fish
185 locomotion and reproductive behavior after at least 2 months of exposure (Gray et al., 1999; Xia et al.,
186 2010). Note that the apparent discrepancy observed between the aforementioned studies and our
187 experiment might be due to differences in exposure duration as reported in Table 2.

188 Plasticizers have previously been reported to lead to an immediate increase followed by a decrease in
189 respiration rates with rising phenols concentration in mollusks (Levine and Cheney, 2000). They can
190 also induce energetical impairments in crustaceans anaerobic metabolism in less than 2 days (Nagato et
191 al., 2016). In contrast, we did not find any significant effects of PP leachates on foraminiferal respiration,
192 even under very high leachates concentrations, i.e. PP200 (Figure 2C). To the best of our knowledge,
193 the only other study that investigated the effect of PP leachates on a unicellular organism found a

194 decrease in dinoflagellate photosynthesis (M'Rabet et al., 2018) after 1 day of exposure, in accordance
195 with the reduced growth and oxygen production observed in the marine cyanobacteria *Prochlorococcus*
196 following a 24h-long exposure to leachates of common plastic items (i.e. HDPE shopping bags and PVC
197 matting; Tetu et al., 2019). Note that, conversely to M'Rabet et al. (2018) who specifically worked with
198 bisphenol A (Table 2), we did not have any control on the composition of the PP leachates. This is a
199 clear limitation of our study that will need to be improved in future works.

200 Overall, both the behavioral and metabolic activity data gathered in this preliminary study indicate that
201 the benthic foraminifera *Haynesina germanica* do not respond to MP unlike other unicellular and
202 metazoan organisms. Though this is highly speculative, this observation may suggest that their
203 resistance to leachates from virgin PP might induce a competitive advantage for benthic foraminifera.
204 Such a competitive advantage for foraminifera has previously been observed in relation to some
205 anthropo-natural phenomena such as organic-matter enrichment and anoxia (Langlet et al., 2013;
206 Stachowitsch, 2014). Note, however, that the observed lack of effect of MP on foraminiferal activity
207 may also be due to the relatively short-term exposure used in our experiments. More fundamentally, the
208 diversity of methods reported in the literature related to the type of polymer considered, the use of
209 unidentified leachates or a specific plasticizer, the pollutant concentrations, the duration of exposure as
210 well as the biology of the organisms considered (Table 2) dramatically prevent to reach a general
211 consensus when comparing the effect of MP on foraminifera with other organisms in our study. In this
212 context, future experiments aiming to assess the effects of MP leachates on benthic foraminifera should
213 benefit from (i) being more specific about the acute or chronic nature of their exposure, and (ii)
214 identifying and quantifying the plasticizers used or present in the leachates. Finally, further work is also
215 needed to assess the potential effects of leachates from (i) weathered PP in particular as they have shown
216 to have significantly stronger effects than virgin plastics (Bejgarn et al., 2015; Gandara e Silva et al.,
217 2016; Kedzierski et al., 2018; Nobre et al., 2015; Seuront, 2018), (ii) different plastic polymers (H.-X.
218 Li et al., 2016; Lithner et al., 2012, 2009; Tetu et al., 2019) and (iii) ingested plastic particles (Ciacci et
219 al., 2019).

220

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229 **References cited**

- 230 Alve, E., 1995. Benthic foraminiferal responses to estuarine pollution: a review. *J. Foraminifer. Res.* 25,
231 190–203.
- 232 Armynot du Châtelet, E., Francescangeli, F., Bouchet, V.M.P., Frontalini, F., 2018. Benthic foraminifera
233 in transitional environments in the English Channel and the southern North Sea: A proxy for
234 regional-scale environmental and paleo-environmental characterisations. *Mar. Environ. Res.*
235 137, 37–48. <https://doi.org/10.1016/j.marenvres.2018.02.021>
- 236 Barnes, D.K.A., 2005. Remote Islands Reveal Rapid Rise of Southern Hemisphere Sea Debris. *Sci.*
237 *World J.* 5, 915–921. <https://doi.org/10.1100/tsw.2005.120>
- 238 Bejgarn, S., MacLeod, M., Bogdal, C., Breitholtz, M., 2015. Toxicity of leachate from weathering
239 plastics: An exploratory screening study with *Nitocra spinipes*. *Chemosphere* 132, 114–119.
240 <https://doi.org/10.1016/j.chemosphere.2015.03.010>
- 241 Bradshaw, J.S., 1961. Laboratory experiment on the ecology of foraminifera. *Contrib. Cushman Found.*
242 *Foraminifer. Res.* 12, 87–106.
- 243 Chiba, S., Saito, H., Fletcher, R., Yogi, T., Kayo, M., Miyagi, S., Ogido, M., Fujikura, K., 2018. Human
244 footprint in the abyss: 30 year records of deep-sea plastic debris. *Mar. Policy* 96, 204–212.
245 <https://doi.org/10.1016/j.marpol.2018.03.022>
- 246 Ciacci, C., Grimmelpont, M.V., Corsi, I., Bergami, E., Curzi, D., Burini, D., Bouchet, V.M.P.,
247 Ambrogini, P., Gobbi, P., Ujiié, Y., Ishitani, Y., Coccioni, R., Bernhard, J.M., Frontalini, F.,
248 2019. Nanoparticle-Biological Interactions in a Marine Benthic Foraminifer. *Sci. Rep.* 9, 19441.
249 <https://doi.org/10.1038/s41598-019-56037-2>
- 250 Denoyelle, M., Geslin, E., Jorissen, F.J., Cazes, L., Galgani, F., 2012. Innovative use of foraminifera in
251 ecotoxicology: A marine chronic bioassay for testing potential toxicity of drilling muds. *Ecol.*
252 *Indic., Marine Benthic Indicators* 12, 17–25. <https://doi.org/10.1016/j.ecolind.2011.05.011>
- 253 Eriksen, M., Lebreton, L.C.M., Carson, H.S., Thiel, M., Moore, C.J., Borerro, J.C., Galgani, F., Ryan,
254 P.G., Reisser, J., 2014. Plastic Pollution in the World's Oceans: More than 5 Trillion Plastic
255 Pieces Weighing over 250,000 Tons Afloat at Sea. *PLOS ONE* 9, e111913.
256 <https://doi.org/10.1371/journal.pone.0111913>
- 257 Ernst, S.R., Morvan, J., Geslin, E., Le Bihan, A., Jorissen, F.J., 2006. Benthic foraminiferal response to
258 experimentally induced Erika oil pollution. *Mar. Micropaleontol.* 61, 76–93.
259 <https://doi.org/10.1016/j.marmicro.2006.05.005>
- 260 Francescangeli, F., Bouchet, V.M.P., Trentesaux, A., Armynot du Chatelet, E., 2017. Does elevation
261 matter? Living foraminiferal distribution in a hyper tidal salt marsh (Canche Estuary, Northern
262 France). *Estuar. Coast. Shelf Sci.* 194, 192–204. <https://doi.org/10.1016/j.ecss.2017.06.023>
- 263 Galgani, F., Souplet, A., Cadiou, Y., 1996. Accumulation of debris on the deep sea floor off the French
264 Mediterranean coast. *Mar. Ecol. Prog. Ser.* 142, 225–234. <https://doi.org/10.3354/meps142225>
- 265 Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine
266 ecosystem. *Nat. Ecol. Evol.* 1, 0116. <https://doi.org/10.1038/s41559-017-0116>
- 267 Gandara e Silva, P.P., Nobre, C.R., Resaffe, P., Pereira, C.D.S., Gusmão, F., 2016. Leachate from
268 microplastics impairs larval development in brown mussels. *Water Res.* 106, 364–370.
269 <https://doi.org/10.1016/j.watres.2016.10.016>
- 270 Geslin, E., Risgaard-Petersen, N., Lombard, F., Metzger, E., Langlet, D., Jorissen, F., 2011. Oxygen
271 respiration rates of benthic foraminifera as measured with oxygen microsensors. *J. Exp. Mar.*
272 *Biol. Ecol.* 396, 108–114. <https://doi.org/10.1016/j.jembe.2010.10.011>
- 273 Gooday, A.J., Levin, L.A., Linke, P., Heeger, T., 1992. The Role of Benthic Foraminifera in Deep-Sea
274 Food Webs and Carbon Cycling, in: Rowe, G.T., Pariente, V. (Eds.), *Deep-Sea Food Chains*
275 *and the Global Carbon Cycle*, NATO ASI Series. Springer Netherlands, pp. 63–91.
- 276 Gray, M.A., Teather, K.L., Metcalfe, C.D., 1999. Reproductive success and behavior of Japanese
277 medaka (*Oryzias latipes*) exposed to 4-tert-octylphenol. *Environ. Toxicol. Chem.* 18, 2587–
278 2594. <https://doi.org/10.1002/etc.5620181128>
- 279 Hahladakis, J.N., Velis, C.A., Weber, R., Iacovidou, E., Purnell, P., 2018. An overview of chemical
280 additives present in plastics: Migration, release, fate and environmental impact during their use,
281 disposal and recycling. *J. Hazard. Mater.* 344, 179–199.
282 <https://doi.org/10.1016/j.jhazmat.2017.10.014>

283 Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., Duflos, G., 2017.
284 Occurrence and effects of plastic additives on marine environments and organisms: A review.
285 Chemosphere 182, 781–793. <https://doi.org/10.1016/j.chemosphere.2017.05.096>

286 Hermabessiere, L., Paul-Pont, I., Cassone, A.-L., Himber, C., Receveur, J., Jezequel, R., El Rakwe, M.,
287 Rinnert, E., Rivièrè, G., Lambert, C., Huvet, A., Dehaut, A., Duflos, G., Soudant, P., 2019.
288 Microplastic contamination and pollutant levels in mussels and cockles collected along the
289 channel coasts. Environ. Pollut. 250, 807–819. <https://doi.org/10.1016/j.envpol.2019.04.051>

290 Hogan, N.S., Lean, D.R.S., Trudeau, V.L., 2006. Exposures to Estradiol, Ethinylestradiol and
291 Octylphenol Affect Survival and Growth of *Rana pipiens* and *Rana sylvatica* Tadpoles. J.
292 Toxicol. Environ. Health A 69, 1555–1569. <https://doi.org/10.1080/15287390500470759>

293 Hollander, M., Wolfe, D.A., 1999. Nonparametric Statistical Methods. Wiley.

294 Inagaki, T., Smith, N., Lee, E.K., Ramakrishnan, S., 2016. Low dose exposure to Bisphenol A alters
295 development of gonadotropin-releasing hormone 3 neurons and larval locomotor behavior in
296 Japanese Medaka. NeuroToxicology 52, 188–197. <https://doi.org/10.1016/j.neuro.2015.12.003>

297 Ke, A.-Y., Chen, J., Zhu, J., Wang, Y.-H., Hu, Y., Fan, Z.-L., Chen, M., Peng, P., Jiang, S.-W., Xu, X.-
298 R., Li, H.-X., 2019. Impacts of leachates from single-use polyethylene plastic bags on the early
299 development of clam *Meretrix meretrix* (Bivalvia: Veneridae). Mar. Pollut. Bull. 142, 54–57.
300 <https://doi.org/10.1016/j.marpolbul.2019.03.029>

301 Kedzierski, M., D’Almeida, M., Magueresse, A., Le Grand, A., Duval, H., César, G., Sire, O., Bruzard,
302 S., Le Tilly, V., 2018. Threat of plastic ageing in marine environment. Adsorption/desorption
303 of micropollutants. Mar. Pollut. Bull. 127, 684–694.
304 <https://doi.org/10.1016/j.marpolbul.2017.12.059>

305 Langlet, D., Geslin, E., Baal, C., Metzger, E., Lejzerowicz, F., Riedel, B., Zuschin, M., Pawlowski, J.,
306 Stachowitsch, M., Jorissen, F.J., 2013. Foraminiferal survival after long-term in situ
307 experimentally induced anoxia. Biogeosciences 10, 7463–7480. <https://doi.org/10.5194/bg-10-7463-2013>

309 Levine, C., Cheney, M.A., 2000. Metabolic responses to acute toxicity of alkylphenols and alkylphenol
310 polyethoxylates in *Elliptio complanata* measured by calorimetry. Environ. Toxicol. Chem.
311 19, 1906–1910. <https://doi.org/10.1002/etc.5620190727>

312 Li, H.-X., Getzinger, G.J., Ferguson, P.L., Orihuela, B., Zhu, M., Rittschof, D., 2016. Effects of Toxic
313 Leachate from Commercial Plastics on Larval Survival and Settlement of the Barnacle
314 *Amphibalanus amphitrite*. Environ. Sci. Technol. 50, 924–931.
315 <https://doi.org/10.1021/acs.est.5b02781>

316 Li, W.C., Tse, H.F., Fok, L., 2016. Plastic waste in the marine environment: A review of sources,
317 occurrence and effects. Sci. Total Environ. 566–567, 333–349.
318 <https://doi.org/10.1016/j.scitotenv.2016.05.084>

319 Li, Y.-H., Gregory, S., 1974. Diffusion of ions in sea water and in deep-sea sediments. Geochim.
320 Cosmochim. Acta 38, 703–714. [https://doi.org/10.1016/0016-7037\(74\)90145-8](https://doi.org/10.1016/0016-7037(74)90145-8)

321 Lithner, D., Damberg, J., Dave, G., Larsson, Å., 2009. Leachates from plastic consumer products –
322 Screening for toxicity with *Daphnia magna*. Chemosphere 74, 1195–1200.
323 <https://doi.org/10.1016/j.chemosphere.2008.11.022>

324 Lithner, D., Nordensvan, I., Dave, G., 2012. Comparative acute toxicity of leachates from plastic
325 products made of polypropylene, polyethylene, PVC, acrylonitrile–butadiene–styrene, and
326 epoxy to *Daphnia magna*. Environ. Sci. Pollut. Res. 19, 1763–1772.
327 <https://doi.org/10.1007/s11356-011-0663-5>

328 McLean, D.J., Skowron Volponi, M.A., 2018. trajr: An R package for characterisation of animal
329 trajectories. Ethology 124, 440–448. <https://doi.org/10.1111/eth.12739>

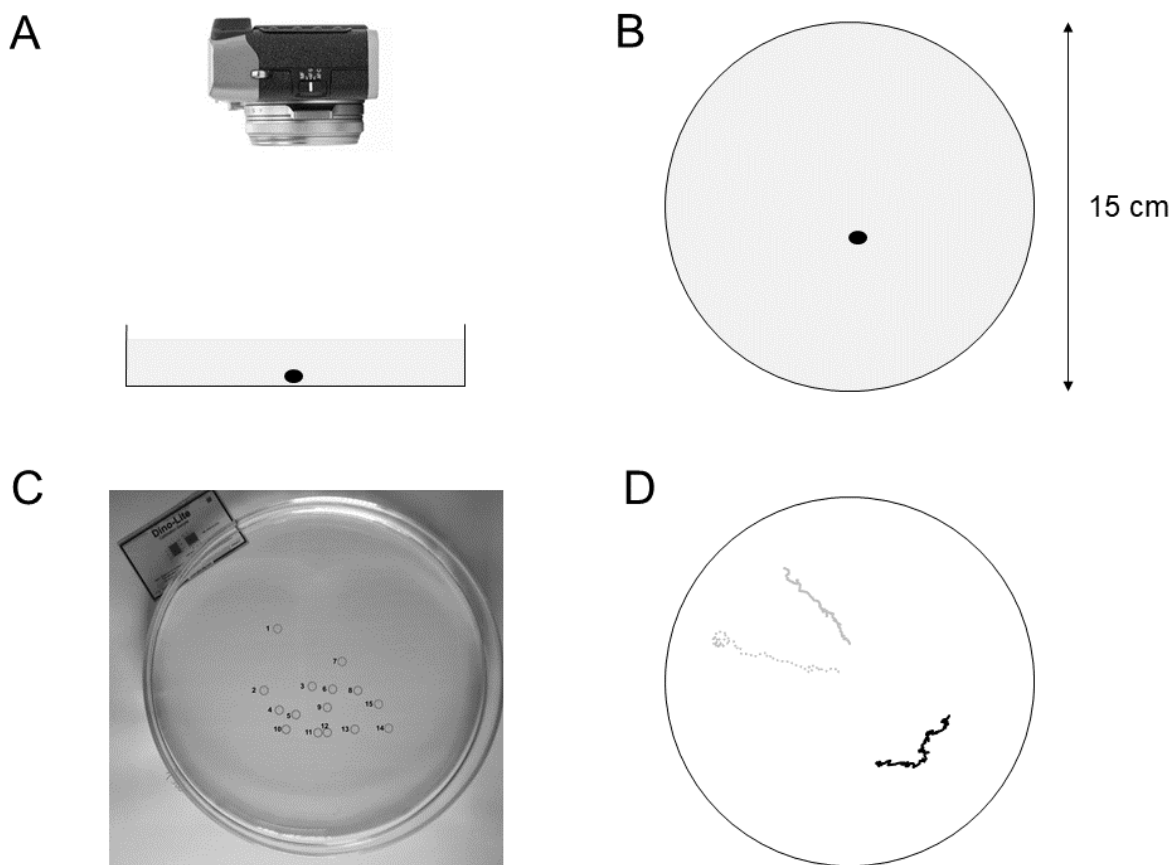
330 M’Rabet, C., Pringault, O., Zmerli-Triki, H., Ben Gharbia, H., Couet, D., Kéfi-Daly Yahia, O., 2018.
331 Impact of two plastic-derived chemicals, the Bisphenol A and the di-2-ethylhexyl phthalate,
332 exposure on the marine toxic dinoflagellate *Alexandrium pacificum*. Mar. Pollut. Bull. 126,
333 241–249. <https://doi.org/10.1016/j.marpolbul.2017.10.090>

334 Nagato, E.G., Simpson, A.J., Simpson, M.J., 2016. Metabolomics reveals energetic impairments in
335 *Daphnia magna* exposed to diazinon, malathion and bisphenol-A. Aquat. Toxicol. 170, 175–
336 186. <https://doi.org/10.1016/j.aquatox.2015.11.023>

337 Nauendorf, A., Krause, S., Bigalke, N.K., Gorb, E.V., Gorb, S.N., Haeckel, M., Wahl, M., Treude, T.,
338 2016. Microbial colonization and degradation of polyethylene and biodegradable plastic bags
339 in temperate fine-grained organic-rich marine sediments. *Mar. Pollut. Bull.* 11.
340 Nigam, R., Linshy, V.N., Kurtarkar, S.R., Saraswat, R., 2009. Effects of sudden stress due to heavy
341 metal mercury on benthic foraminifer *Rosalina leei*: Laboratory culture experiment. *Mar. Pollut.*
342 *Bull.*, Foraminifera and Marine Pollution 59, 362–368.
343 <https://doi.org/10.1016/j.marpolbul.2009.08.014>
344 Nobre, C.R., Santana, M.F.M., Maluf, A., Cortez, F.S., Cesar, A., Pereira, C.D.S., Turra, A., 2015.
345 Assessment of microplastic toxicity to embryonic development of the sea urchin *Lytechinus*
346 *variegatus* (Echinodermata: Echinoidea). *Mar. Pollut. Bull.* 92, 99–104.
347 <https://doi.org/10.1016/j.marpolbul.2014.12.050>
348 Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K.O., Wollenberger, L.,
349 Santos, E.M., Paull, G.C., Van Look, K.J.W., Tyler, C.R., 2009. A critical analysis of the
350 biological impacts of plasticizers on wildlife. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 2047–
351 2062. <https://doi.org/10.1098/rstb.2008.0242>
352 Oehlmann, J., Schulte-Oehlmann, U., Tillmann, M., Markert, B., 2000. Effects of Endocrine Disruptors
353 on Prosobranch Snails (Mollusca: Gastropoda) in the Laboratory. Part I: Bisphenol A and
354 Octylphenol as Xeno-Estrogens. *Ecotoxicology* 9, 383–397.
355 <https://doi.org/10.1023/A:1008972518019>
356 Oliviero, M., Tato, T., Schiavo, S., Fernández, V., Manzo, S., Beiras, R., 2019. Leachates of micronized
357 plastic toys provoke embryotoxic effects upon sea urchin *Paracentrotus lividus*. *Environ. Pollut.*
358 247, 706–715. <https://doi.org/10.1016/j.envpol.2019.01.098>
359 Paluselli, A., Fauvelle, V., Galgani, F., Sempéré, R., 2019. Phthalate Release from Plastic Fragments
360 and Degradation in Seawater. *Environ. Sci. Technol.* 53, 166–175.
361 <https://doi.org/10.1021/acs.est.8b05083>
362 R Core Team, 2019. R: A Language and Environment for Statistical Computing. R Foundation for
363 Statistical Computing, Vienna, Austria.
364 Rillig, M.C., Bonkowski, M., 2018. Microplastic and soil protists: A call for research. *Environ. Pollut.*
365 241, 1128–1131. <https://doi.org/10.1016/j.envpol.2018.04.147>
366 Sánchez-Avila, J., Tauler, R., Lacorte, S., 2012. Organic micropollutants in coastal waters from NW
367 Mediterranean Sea: Sources distribution and potential risk. *Environ. Int.* 46, 50–62.
368 <https://doi.org/10.1016/j.envint.2012.04.013>
369 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S.,
370 Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J.-Y., White, D.J., Hartenstein, V., Eliceiri, K.,
371 Tomancak, P., Cardona, A., 2012. Fiji: an open-source platform for biological-image analysis.
372 *Nat. Methods* 9, 676–682. <https://doi.org/10.1038/nmeth.2019>
373 Schwarz, A.E., Lighthart, T.N., Boukris, E., van Harmelen, T., 2019. Sources, transport, and
374 accumulation of different types of plastic litter in aquatic environments: A review study. *Mar.*
375 *Pollut. Bull.* 143, 92–100. <https://doi.org/10.1016/j.marpolbul.2019.04.029>
376 Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the
377 planktonic food web. *Environ. Pollut.* 185, 77–83. <https://doi.org/10.1016/j.envpol.2013.10.013>
378 Seuront, L., 2018. Microplastic leachates impair behavioural vigilance and predator avoidance in a
379 temperate intertidal gastropod. *Biol. Lett.* 14, 20180453. <https://doi.org/10.1098/rsbl.2018.0453>
380 Seuront, L., 2015. On uses, misuses and potential abuses of fractal analysis in zooplankton behavioral
381 studies: A review, a critique and a few recommendations. *Phys. A* 25.
382 Seuront, L., 2010. *Fractals and Multifractals in Ecology and Aquatic Science*, 1st ed. CRC Press, Boca
383 Raton.
384 Seuront, L., Bouchet, V.M.P., 2015. The devil lies in details: new insights into the behavioural ecology
385 of intertidal foraminifera. *J. Foraminifer. Res.* 45, 390–401.
386 <https://doi.org/10.2113/gsjfr.45.4.390>
387 Stachowitsch, M., 2014. Coastal hypoxia and anoxia: a multi-tiered holistic approach. *Biogeosciences*
388 11, 2281–2285.
389 Staples, C., Mihaich, E., Ortego, L., Caspers, N., Klečka, G., Woelz, J., Hentges, S., 2016.
390 Characterizing the effects of bisphenol A on sediment-dwelling benthic organisms. *Environ.*
391 *Toxicol. Chem.* 35, 652–659. <https://doi.org/10.1002/etc.3217>

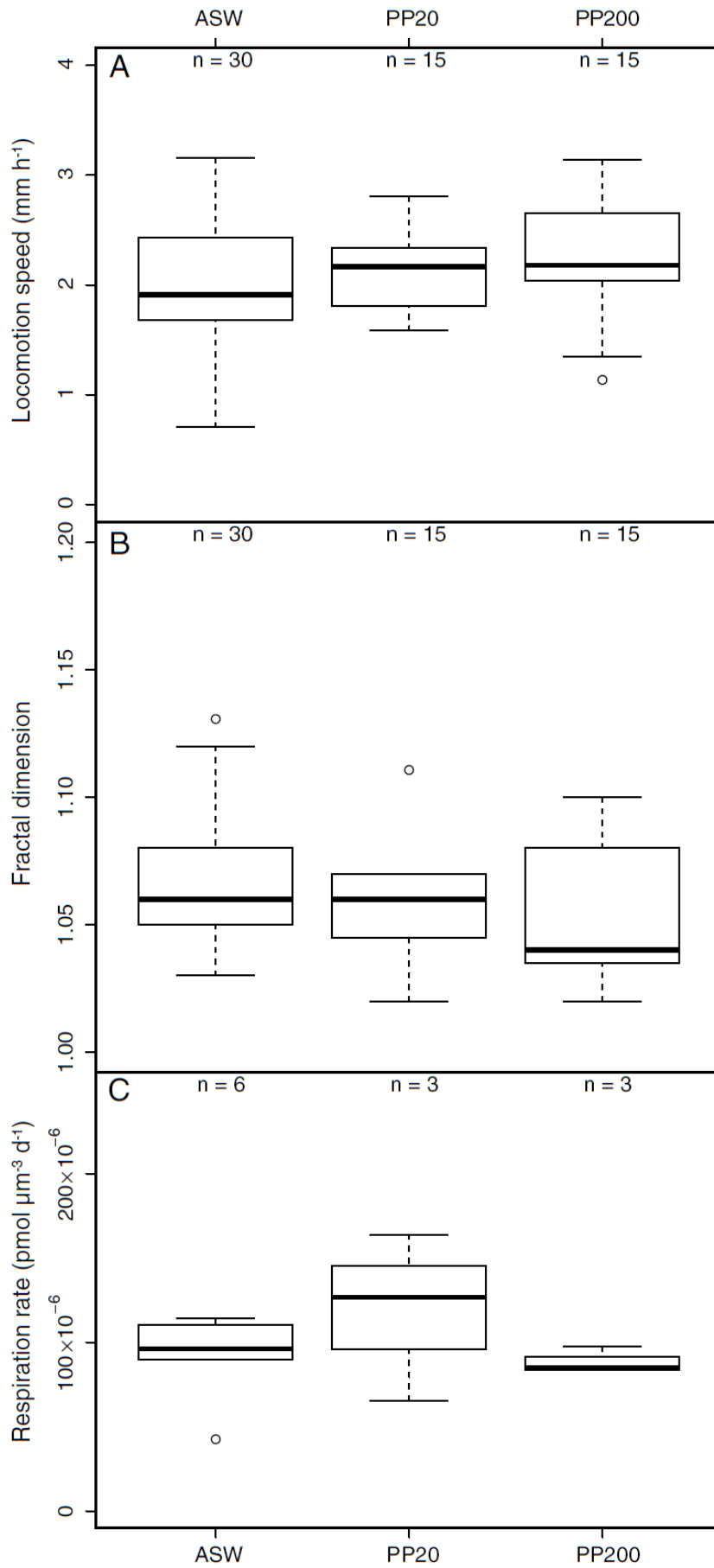
392 Su, Y., Zhang, K., Zhou, Z., Wang, J., Yang, X., Tang, J., Li, H., Lin, S., 2020. Microplastic exposure
 393 represses the growth of endosymbiotic dinoflagellate *Cladocopium goreai* in culture through
 394 affecting its apoptosis and metabolism. *Chemosphere* 244, 125485.
 395 <https://doi.org/10.1016/j.chemosphere.2019.125485>
 396 Tetu, S.G., Sarker, I., Schrameyer, V., Pickford, R., Elbourne, L.D.H., Moore, L.R., Paulsen, I.T., 2019.
 397 Plastic leachates impair growth and oxygen production in *Prochlorococcus*, the ocean's most
 398 abundant photosynthetic bacteria. *Commun. Biol.* 2, 184. [https://doi.org/10.1038/s42003-019-](https://doi.org/10.1038/s42003-019-0410-x)
 399 [0410-x](https://doi.org/10.1038/s42003-019-0410-x)
 400 Vered, G., Kaplan, A., Avisar, D., Shenkar, N., 2019. Using solitary ascidians to assess microplastic and
 401 phthalate plasticizers pollution among marine biota: A case study of the Eastern Mediterranean
 402 and Red Sea. *Mar. Pollut. Bull.* 138, 618–625. <https://doi.org/10.1016/j.marpolbul.2018.12.013>
 403 Wang, X., Dong, Q., Chen, Y., Jiang, H., Xiao, Q., Wang, Y., Li, W., Bai, C., Huang, C., Yang, D.,
 404 2013. Bisphenol A affects axonal growth, musculature and motor behavior in developing
 405 zebrafish. *Aquat. Toxicol.* 142–143, 104–113. <https://doi.org/10.1016/j.aquatox.2013.07.011>
 406 Xia, J., Niu, C., Pei, X., 2010. Effects of chronic exposure to nonylphenol on locomotor activity and
 407 social behavior in zebrafish (*Danio rerio*). *J. Environ. Sci.* 22, 1435–1440.
 408 [https://doi.org/10.1016/S1001-0742\(09\)60272-2](https://doi.org/10.1016/S1001-0742(09)60272-2)
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412 Figure 1 caption: A and B: schematic representation of the experimental setup with lateral view
 413 (A) and top view (B) of the position of the foraminifera (black ovoid shape) placed on the petri-
 414 dish. C: photograph of the initial position of the 15 individuals used in ASW control conditions.
 415 D: example of 3 extracted trajectories for ASW (full black line), PP20 (full grey line) and PP200
 416 (dotted grey line).



418 Figure 2: Locomotion speed (A), fractal dimension (B) and respiration rate (C) of *Haynesina germanica*
419 under the three experimental conditions (ASW: artificial seawater, i.e. control conditions; PP20 and
420 PP200: seawater prepared with 20 and 200 ml l⁻¹ polypropylene pellets, respectively). The box
421 represents the first, second and third quartiles and the whiskers extend to 1.5 times the interquartile
422 range. Values outside of this range are represented by open circles.

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426 Table 1 caption: results of the Kruskal-Wallis statistical analyses testing the effect of the
427 experimental conditions (ASW as a control, PP20 and PP200) on the three measured response
428 variables.

Response variable	Kruskal-Wallis X ²	degrees of freedom	p-value
Locomotion speed	1.6	2	0.44
Fractal dimension	3.9	2	0.14
Respiration rate	1.3	2	0.52

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430 Table 2 caption: organisms, response observed, type of pollutant, concentration, equivalent concentration in the present study and exposure duration
 431 tested in the literature cited in this article's discussion.

Reference	Organisms	Response observed	Pollutant type	Pollutant concentration	This study's equivalent	Exposure duration
Oehlmann et al. 2000	Mollusks	Mortality	Bisphenol A	1µg/L		5 months
		Mortality	Octylphenol	1µg/L		5 months
Li et al. 2016	Barnacle larvae	10% mortality	PP leachate	0.1 m ² /L	PP200 ~ 0.17m ² /L	1 day
Hogan et al. 2006	Amphibians	50% mortality	Octylphenol	1.4 µmol/L		2 weeks
Staples et al. 2016	Crustaceans	Mortality	Bisphenol A	78 mg/kg sedim dry weight		1 month
	Annelids	Mortality	Bisphenol A	60 mg/kg sedim dry weight		1 month
Seuront 2018	Gastropods	Behavior	PP leachate	20mL/L	PP20 = 20mL/L	3 hours
Inagaki et al. 2016	Fish larvae	Locomotion	Bisphenol A	200ng/mL		20 days
Wang et al. 2013	Fish larvae	Locomotion	Bisphenol A	15µmol/L		2 days
Gray et al. 1999	Adult fish	Reproductive behavior	Octylphenol	25µg/L		3 months
Xia et al. 2010	Adult fish	Locomotion	Nonylphenol	100µg/L		2 months
Levine and Cheney 2000	Mollusks	Respiration	Nonylphenol	10µmol/L		1 hour
Nagato et al. 2016	Crustaceans	Anaerobic metabolism	Bisphenol A	0.1mg/L		2 days
M'Rabet et al. 2018	Dinoflagellate	Respiration and photosynthesis	Bisphenol A	2µg/L		1 day
Tetu et al. 2019	Cyanobacteria	Photosynthesis	PVC leachate	1g/L	PP20 ~ 10g/L	3 hours

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