

1 **Evidence of microplastic-mediated transfer of PCB-153 to sea urchin tissues using**  
2 **radiotracers**

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13 *Paracentrotus lividus*

14

15 **Abstract**

16

17 The present study reports the first experimental microplastic-mediated transfer of a key PCB  
18 congener into adult specimens of the sea urchin *Paracentrotus lividus*. Three experiments were  
19 conducted to assess whether <sup>14</sup>C-PCB-153 adsorbed onto negatively buoyant MPs (500-600 μm) is  
20 bioavailable to the sea urchin : (1) exposure to a low concentration of <sup>14</sup>C-PCB-153 sorbed onto a  
21 high number of virgin MPs (“lowPCB highMP” experiment), (2) exposure to a high concentration of  
22 <sup>14</sup>C-PCB-153 sorbed onto a relatively low number of virgin MPs (“highPCB lowMP” experiment), and  
23 (3) exposure to a low concentration of <sup>14</sup>C-PCB-153 sorbed onto a relatively low number of aged MP  
24 (“lowPCB lowMP” experiment). Results showed that the transfer of <sup>14</sup>C-PCB-153 from MPs to sea  
25 urchin tissues occurred in each of the three 15-day experiments, suggesting that MPs located on the  
26 seafloor may act as vectors of PCB-153 to sea urchins even during short-term exposure events.  
27

28 **Introduction**

29

30 The ubiquity and abundance of plastic litter in the marine environment has become a global  
31 environmental concern (Coyle *et al.*, 2020). Plastics in oceans are encountered in a range of different  
32 shapes and sizes (Cole *et al.*, 2011), influencing their behaviour and ultimate fate (de Sá *et al.*, 2018).  
33 Microplastics (MPs), defined as plastic items size ranging from 1 μm to 5 mm (Frias and Nash, 2019;  
34 Hartmann *et al.*, 2019), can be classified either as primary MPs, referring to manufactured particles  
35 produced as such (e.g. plastic pellets, exfoliating cosmetics or synthetic clothing fibres), or secondary  
36 MPs, which result from the breakdown of larger plastic debris (e.g. fragments; GESAMP, 2015). In  
37 marine environments, MP surfaces are relatively rapidly colonised by microorganisms termed as  
38 “plastisphere”, a process known as biofouling or biofilm formation (Zettler *et al.*, 2013).  
39

40 Due to their small size, persistence and ubiquitous presence in aquatic ecosystems, MPs may  
41 present a significant threat to marine fauna. It is well documented that microplastics are available  
42 for ingestion to a broad range of neustonic (buoyant microplastics), pelagic (microplastics in  
43 suspension in the water column) and benthic species (sinking microplastics), potentially affecting  
44 aquatic food webs at multiple trophic levels and ecological niches (Setälä *et al.*, 2018). Accumulating  
45 within organisms, MPs may cause physical harm, such as feeding and digestion disruption, by  
46 generating blockages, false satiation and injuries in the digestive tract (Jabeen *et al.*, 2018; Wright *et*  
47 *al.*, 2013). In addition to the potential physical impacts of ingested microplastics, chemical toxicity  
48 could also arise from the leaching of plastic-associated contaminants (i.e. co-contaminants). Co-  
49 contaminants carried by MPs include chemicals incorporated during manufacture (e.g. additives  
50 such as flame retardants, plasticisers and stabilisers, Rochman, 2015) as well as chemicals sorbed  
51 from the environment (e.g. metals and organic pollutant such as polychlorinated biphenyls,  
52 Rodrigues *et al.*, 2019; Rochman *et al.*, 2013; Koelmans *et al.*, 2016; Ogata *et al.*, 2009). More than  
53 250 organic compounds have been identified on plastic debris (Campanale *et al.*, 2020). Based on  
54 both their ubiquity and abundance in the marine environment, as well as their ability to carry  
55 environmental contaminants and substantial proof of particle uptake by marine organisms, the  
56 hypothesis that MPs may play a role in aquatic ecotoxicology as vectors for toxic contaminants has  
57 arisen. Once ingested by organisms, as physico-chemical properties change, the myriad of  
58 contaminants associated with MPs can desorb from the plastic and potentially become bioavailable  
59 to organisms (Hartmann *et al.*, 2017; Teuten *et al.*, 2009; Engler, 2012). These co-contaminants may  
60 threaten marine organisms when MPs become inadvertently ingested and transferred up the food  
61 chain (Miller *et al.*, 2020).

62  
63 However, there remains some uncertainties regarding the actual risks associated with exposure to  
64 MPs and associated co-contaminants. The relevance of MPs as vectors of has been challenged with  
65 the argument that plastics play a minor role as contaminant vectors compared to natural particles,  
66 such as suspended organic particles and natural prey, because of their relative abundance in the  
67 environment (Koelmans *et al.*, 2021; Koelmans *et al.*, 2016; Besson *et al.*, 2020). As a result, the  
68 extent to which microplastics act as vectors of adsorbed pollutants is currently a matter of debate  
69 and needs to be better understood in order to evaluate the potential response of marine  
70 ecosystems to MPs exposure (Rodrigues *et al.*, 2019). Improving our understanding of the  
71 bioavailability of MPs-associated co-contaminants upon their ingestion by organisms remains a  
72 prerequisite to accurately assess the importance of MPs as carriers of co-contaminants to marine  
73 biota. To address this knowledge gap, the use of radiotracer techniques has been recently  
74 recognised as a uniquely suited approach due to their rapidity, accuracy and sensitivity, allowing for  
75 the study of the bioavailability of MP-associated chemicals within environmentally realistic  
76 concentration ranges (Besson *et al.*, 2020; Johansen *et al.*, 2018; Lanctôt *et al.*, 2018; Adhikari *et al.*,  
77 2022).

78  
79 The Mediterranean sea urchin *Paracentrotus lividus* (Lamarck, 1816) was selected as a model  
80 organism for this study because it is a keystone species in Mediterranean coastal ecosystems where  
81 it plays a crucial ecological role (Boudouresque and Verlaque, 2020). As a consequence of its  
82 continuous benthic foraging activity, combined with its presence in highly anthropic areas, adult  
83 specimens of *P.lividus* are potential targets of MPs accumulation in marine benthic ecosystems  
84 (Manzo et Schiavo, 2022). Among the few studies that investigated the relationship between adult *P.*  
85 *lividus* and microplastics, ingestion of MPs has been demonstrated (Parolini *et al.*, 2020; Murano *et*  
86 *al.*, 2020; Murano *et al.*, 2021; Porter *et al.*, 2019; Hennicke *et al.*, 2021) and small MPs (1-5 µm)  
87 have been recently proven to be able to cross their intestinal wall and reach their coelomic fluid (Pyl  
88 *et al.*, 2022). Moreover, MPs have been shown to induce a slight modulation of oxidative status  
89 (Parolini *et al.*, 2020), as well as to impact the circulating immune cells (Murano *et al.*, 2020; Murano  
90 *et al.*, 2021) of adults *P. lividus*. Nevertheless, no information is available to date on the potential

91 "vector" effect of co-contaminants adsorbed onto MPs using adult specimens of *P.lividus* as a model,  
92 despite being recognised as an excellent model organism in marine ecotoxicology (Sugni *et al.*, 2013)  
93 as well as species of great ecological and commercial importance (Pearse, 2006; Andrew *et al.*,  
94 2002).

95  
96 The objective of this present work is to assess the potential of microplastics to act as vector for the  
97 transfer of PCB-153 to sea urchins *P.lividus* using radiotracers. More specifically, the bioavailability of  
98 <sup>14</sup>C-labelled PCB-153 sorbed onto negatively buoyant and relatively large polyethylene MPs, to sea  
99 urchin tissues was investigated under different experimental conditions. A transfer efficiency (%) of  
100 <sup>14</sup>C-PCB-153 was calculated to establish how much <sup>14</sup>C-PCB-153, initially sorbed onto the MPs, was  
101 transferred to sea urchins under the different experimental conditions.

## 102 **Materials and Methods**

### 104 **1. Sampling of the sea urchins and acclimatation**

105  
106 Specimens of adult sea urchins (*Paracentrotus lividus*, Lamarck) were collected by divers in the Bay  
107 of Villefranche-sur-mer (NW Mediterranean coastline, France) from mid-October 2020 to the end of  
108 November 2020. This autumnal period corresponds to premature and beginning of gonadal  
109 maturation of the sea urchins from the Bay of Villefranche-sur-mer (Fenaux, 1968). Prior to  
110 experimentation, specimens were acclimated to laboratory conditions for at least one week in  
111 constantly aerated open circuit aquaria, with natural seawater (salinity = 38‰, UV-sterilized and 1  
112  $\mu\text{m}$  filtered) at  $17 \pm 0.5$  °C, in a 12/12h dark/light cycle. Sea urchins were fed *ad libitum* every two  
113 days with dry leaves of the algae *Saccharina latissima*, purchased from Biocean® during  
114 acclimatation.

### 116 **2. Radiotracer and microplastics (MPs)**

117  
118  $^{14}\text{C}$ -labelled congener IUPAC-153 (2,2',4,4',5,5'-hexachlorobiphenyl) was selected as representative  
119 of PCBs for this study as it is one of the most bioaccumulative and the most abundant congener in  
120 marine biota (Safe, 1994; Stebbing *et al.*, 1992) and is considered a good indicator of PCB levels in  
121 contaminant monitoring studies (Danis *et al.*, 2005). This  $\beta$ -emitting radiotracer, with a purity higher  
122 than 95%, was purchased from American Radiolabelled Chemicals®, USA. The specific activity of this  
123 mother source was 12.6 mCi mmol<sup>-1</sup>.

124  
125 The MPs considered in this study were high density polyethylene microplastics (HDPE MP), as  
126 polyethylene is the most common polymers found in the environment (Hidalgo-Ruz *et al.*, 2012;  
127 PlasticsEurope, 2020) with the highest adsorption capacity (Rochman, 2015). These HDPE MP were  
128 microbeads purchased from Cospheric® as an isospheric homogeneous solid powder (size = 500-600  
129  $\mu\text{m}$ ; density = 1.2 g cm<sup>-3</sup>; containing black paramagnetic iron oxide; specific surface area = 9 m<sup>2</sup> kg<sup>-1</sup>).  
130 These microplastics were specifically selected for this study in order (1) to be available to benthic  
131 organisms for ingestion as they are negatively buoyant and thus sink to the bottom of the aquarium,  
132 (2) to avoid the use of surfactant with its potential interference in the adsorption processes, and (3)  
133 for ease of visibility and detection. Preliminary tests showed that these microplastics are available to  
134 sea urchins for ingestion as they were readily detected in the gut of the organisms. Further details  
135 on the method and results of the verification of MPs uptake can be found in the Supplementary  
136 Information section.

### 138 **3. Experimental procedure**

139  
140 Three different experiments were conducted to assess whether  $^{14}\text{C}$ -PCB-153 adsorbed onto the  
141 surface of MPs is bioavailable for the sea urchin *P. lividus* during 15 days : (1) exposure of *P. lividus* to  
142 a low concentration of  $^{14}\text{C}$ -PCB-153 sorbed onto a high number of virgin (i.e. non-aged) MPs  
143 ("lowPCB highMP" experiment), (2) exposure of *P. lividus* to a high concentration of  $^{14}\text{C}$ -PCB-153  
144 sorbed onto a relatively low number of virgin MPs ("highPCB lowMP" experiment) and (3) exposure  
145 to a low concentration of  $^{14}\text{C}$ -PCB-153 sorbed onto a relatively low number of aged MP ("lowPCB  
146 lowMP" experiment). The concentrations of PCB-153 and amounts of MPs available per sea urchin  
147 within the aquariums of each experiment were selected to ensure the detection of any transfer of  
148 PCB-153 from MPs to sea urchin tissues while avoiding lethal effects.

149  
150 The method applied for each experiment is composed of different phases, summarized as follows :  
151 (1) adsorption of  $^{14}\text{C}$ -labelled PCB-153 onto MPs by applying the procedures described per Pyl *et al.*  
152 (2021); (2) 15 days exposure of *P. lividus* to  $^{14}\text{C}$ -labelled PCB-153 adsorbed onto the surface of MPs;

153 (3) dissection of the organisms and the extraction of the radiotracer among the different body  
154 compartments; (4) the use of  $\beta$  spectroscopy to determine the concentration of  $^{14}\text{C}$ -PCB-153 on the  
155 particles and the distribution of PCBs within the different organs of the experimental model.

### 157 3.1. Adsorption of $^{14}\text{C}$ -labelled PCB-153 onto MPs

158  
159 The MPs used in the “lowPCB highMP” and in the “highPCB lowMP” experiment required the  
160 preparation of solutions and their incubation in order to enable the adsorption of  $^{14}\text{C}$ -labelled PCB-  
161 153 onto the surface of MPs. For these two experiments, the adsorption of  $^{14}\text{C}$ -labelled PCB-153  
162 onto MPs were achieved following the procedures described in Pyl et al. (2021) with adaptations  
163 detailed in the Supplementary Information. The radio-labelled MPs were then gently transferred  
164 from their vials to the bottom of the aquaria. The MPs used in the “lowPCB lowMP” experiment  
165 were sampled at the end of the “highPCB lowMP” experiment from the bottom of the aquariums  
166 using glass volumetric bulb pipettes (10 mL) combined with a pipette filler. These MPs were  
167 collected in transparent containers, photographed, automatically counted using the software IMAGE  
168 J, then placed at the bottom of each aquarium. In order to assess the stability of the  $^{14}\text{C}$ -PCB-153  
169 concentration per MP and the potential leaching of  $^{14}\text{C}$ -labelled PCB-153, samples of MPs were  
170 regularly collected and radioanalysed throughout the experiments. All sampling of MPs from the  
171 bottom of the aquaria were performed using glass volumetric bulb pipettes (10 mL) combined with a  
172 pipette filler. The average numbers and mass of MP available per aquaria at the beginning of the  
173 three experiments are displayed in Table 1. Details related to preparation of PCB-loaded MPs are  
174 reported in Supplementary Information.

### 176 3.2. Sea urchin exposure

177  
178 All experiments were conducted in 20L capacity glass aquaria constantly aerated via an open circuit  
179 (water renewal :  $20\text{L h}^{-1}$ ) with natural seawater (UV-sterilized and  $1\ \mu\text{m}$  filtered, salinity = 38‰, pH =  
180 8.1) at  $16.4 \pm 0.5\ ^\circ\text{C}$ , in a 12/12h dark/light cycle. Sea urchins were fed every two days *ad libitum*  
181 with dry leaves of the algae *Saccharina latissima*, purchased from Biocean®, during all experiments.  
182 The duration of exposure of the sea urchin to MPs was 15 days. Within the experimental aquaria,  
183 sea urchins were placed at the bottom in order to allow direct contact between the radioactive MPs  
184 and the organisms. As presented in Figure 1, for the “highPCB lowMP” and “lowPCB lowMP”  
185 experiments, one sea urchin was placed at the bottom of each exposure aquaria as enough  
186 aquariums were available to maximise the number of true replications. However, as only three  
187 exposure aquariums were available at the time of the “lowPCB highMP” experiment, three sea  
188 urchins were placed in each aquarium to increase the number of replicates of the exposure  
189 treatment (Figure 1). Moreover, control sea urchins were placed in a separated compartment  
190 (termed “cage”, made of polyethylene with a mesh size of  $0.5\ \text{cm}^2$ ) within one aquarium of each  
191 experiment (Figure 1) to control possible cross-contamination through sea water via leaching of the  
192  $^{14}\text{C}$ -PCB-153 from MPs. Since the amount of  $^{14}\text{C}$ -PCB-153 sorbed onto the aged MPs used for the  
193 “lowPCB lowMP” experiment was known to be stable (Figure S1) and contamination via leaching  
194 deemed unlikely, one sea urchin placed in the “cage” was considered sufficient as a control. For the  
195 “highPCB lowMP” and “lowPCB lowMP” experiments, as the amount of  $^{14}\text{C}$ -PCB-153 sorbed onto  
196 virgin MPs was unknown to be stable, three sea urchins were placed in the “cage” within the control  
197 aquarium to account for potential contamination via leaching (Figure 1).

#### 199 3.2.1. “LowPCB highMP” experiment : exposure of *P.lividus* to a low concentration of 200 $^{14}\text{C}$ -PCB-153 sorbed onto a high number of virgin MPs

201  
202 For the “lowPCB highMP” experiment, twelve sea urchins *P.lividus* (ambital diameter  $56 \pm 2\ \text{mm}$ ) and  
203 four aquariums (three exposure and one control) filled with 10 L of seawater were used (Figure 1).

204 The entire water volume of each aquarium was renewed every 30 minutes during the 15 days of the  
205 experiment. At the beginning of the experiment, 5143 ( $\pm 30$ ) and 7550 ( $\pm 30$ ) MPs, each  
206 radiolabelled with 0.17 ng of  $^{14}\text{C}$ -PCB-153, were available at the bottom of each exposure aquarium  
207 and in the control aquarium, respectively (Table 1). The difference in the initial abundance of MPs  
208 between the control aquarium and the exposure aquariums is justified by the fact that the samples  
209 of MPs, in order to control the stability of the PCB adsorbed on the MPs, were carried out only in the  
210 control aquarium during the “lowPCB highMP” experiment. Based on both the initial concentration  
211 of  $^{14}\text{C}$ -PCB-153 per MP and on the number of MPs per aquarium (corrected for  $t_0$ ), a total amount of  
212 871.56 ( $n=1$ ) and 1279.51 ( $n=1$ ) ng of  $^{14}\text{C}$ -PCB-153 was estimated to be available per exposure  
213 aquarium and in the control aquarium respectively, at the beginning of the experiment (Table 1).  
214 Once the MPs were deposited in the aquaria, the experiment was initiated by placing three sea  
215 urchins at the bottom of each exposure aquarium, as well as three sea urchins within the cage of the  
216 control aquarium (Figure 1). A total amount of 290.52 ng of  $^{14}\text{C}$ -PCB-153, loaded on an average  
217 number of 1714 ( $\pm 10$ ) MPs, were therefore available per sea urchin in direct contact with particles  
218 at the beginning of the experiment (Table 1). As the “lowPCB highMP” experiment was the first to  
219 assess the bioavailability of sorbed  $^{14}\text{C}$ -radiolabelled PCB onto MPs to marine organisms, both the  
220 minimal concentration of PCB-153 per MP and minimal number of radiolabelled MPs needed to  
221 ensure a detectable radiotracer signal following uptake and organ distribution by sea urchins was  
222 unknown. Therefore, high amounts of MPs available per sea urchin (average of 1714 ( $\pm 10$ ) MPs),  
223 each radiolabelled with an average activity of 0.17 Bq ( $\pm 0.02$ ,  $n=6$ ), were selected to maximise the  
224 chance to detect the potential transfer of the radiotracer onto the tissues of sea urchins while  
225 avoiding lethal effects.

226  
227 **3.2.2. “HighPCB lowMP” experiment : exposure of *P.lividus* to a high concentration of**  
228  **$^{14}\text{C}$ -PCB-153 sorbed onto a relatively low number of virgin MPs**

229  
230 For the “highPCB lowMP” experiment, nine sea urchins (ambital diameter  $62 \pm 2$  mm) and seven  
231 aquariums (six exposure and one control) filled with 20 L of seawater were used (Figure 1). The  
232 entire volume of water in each aquarium was renewed every 60 minutes. At the beginning of the  
233 experiment, 1185 ( $\pm 30$ ) MPs, each radiolabelled with 0.49 ( $\pm 0.03$ ,  $n=18$ ) ng of  $^{14}\text{C}$ -PCB-153, were  
234 available at the bottom of each experimental aquarium. Similarly, 1170 ( $\pm 30$ ) MPs, each  
235 radiolabelled with 0.68 ( $\pm 0.04$ ,  $n=3$ ) ng of  $^{14}\text{C}$ -PCB-153, were placed at the bottom of the control  
236 aquarium (Table 1). Based on both the initial concentration of  $^{14}\text{C}$ -PCB-153 per MP and on the  
237 number of MPs per aquarium (corrected for  $t_0$ ), a total amount of 575.93 ( $\pm 32.86$ ,  $n=18$ ) and 792.21  
238 ( $\pm 46.01$ ,  $n=3$ ) ng of  $^{14}\text{C}$ -PCB-153 was estimated to be available per exposure aquarium and in the  
239 control aquarium, respectively, at the beginning of the experiment (Table 1). One sea urchin was  
240 placed per exposure aquarium and three sea urchins were placed within the cage of the control  
241 aquarium (Figure 1). A total amount of 575.93 ( $\pm 32.86$ ,  $n=18$ ) ng of  $^{14}\text{C}$ -PCB-153, loaded onto an  
242 average number of 1185 ( $\pm 30$ ) MPs, were therefore available per sea urchin in direct contact with  
243 particles at the beginning of the “highPCB lowMP” experiment (Table 1).

244  
245 **3.2.3. “LowPCB lowMP” experiment : exposure to a low concentration of  $^{14}\text{C}$ -PCB-153**  
246 **sorbed onto a relatively low number of aged MP**

247  
248 At the end of the “highPCB lowMP” experiment, the MPs were collected and reused for the “lowPCB  
249 lowMP” experiment. This implies that, unlike the MPs used for the two other experiments which  
250 were virgin, the MPs used for the “lowPCB lowMP” experiment were aged during 15 days. Solely  
251 MPs that were both (1) not contained in fecal pellets and (2) identical to virgin MPs (in terms of  
252 shape and size), were selected to be reused for the “lowPCB lowMP” experiment. This indicates that  
253 the aged MPs used in the “lowPCB lowMP” experiment did not pass through the digestive tract of  
254 sea urchins during the “highPCB lowMP” experiment. For the “lowPCB lowMP” experiment, five sea

255 urchins *P. lividus* (ambital diameter  $58 \pm 2$  mm) and four aquariums filled with 20 L of seawater were  
256 used (Figure 1). The entire water volume in each aquarium was renewed every 60 minutes. At the  
257 bottom of each aquarium, an average number of 1216 MPs ( $\pm 8$ ,  $n=4$ ), each radiolabelled with 0.15  
258 ng ( $\pm 0.02$ ,  $n=12$ ) of  $^{14}\text{C}$ -PCB-153, were available at the beginning of the experience (Table 1). Based  
259 on both the initial concentration of  $^{14}\text{C}$ -PCB-153 per MP and on the number of MPs per sea urchin  
260 (corrected for  $t_0$ ), a total amount of 181.56 ( $\pm 22.15$ ,  $n=12$ ) ng of  $^{14}\text{C}$ -PCB-153 was estimated to be  
261 available per aquarium just prior to the beginning of the experiment (Table 1). One sea urchin was  
262 placed at the bottom of each aquarium and one control sea urchin was placed in the cage within the  
263 first aquarium (Figure 1). A total amount of 181.56 ( $\pm 22.15$ ,  $n=12$ ) ng of  $^{14}\text{C}$ -PCB-153, loaded on an  
264 average number of 1216 ( $\pm 8$ ,  $n=4$ ) MPs, were therefore available per sea urchin in direct contact  
265 with particles at the beginning of the “lowPCB lowMP” experiment (Table 1).  
266

#### 267 4. Dissection

268  
269 After 15 days of exposure, all specimens were collected and rinsed abundantly with UV-sterilized 1  
270  $\mu\text{m}$  filtered sea water in order to remove potential MPs in contact with the tegument and the  
271 peristomial membrane. The sea urchins were then measured, weighed, and sacrificed by cold  
272 exposure. The test of the sea urchin was then cut following the ambital diameter. Two mL of  
273 coelomic liquid were sampled and directly poured into a scintillation vial for radiocounting. Then,  
274 the entire digestive tract, from the oesophagus to the anus was collected, cut longitudinally and  
275 abundantly rinsed with sea water in order to remove all the digestive content and potentially  
276 ingested MPs. Once devoid of digestive content, the digestive tract walls were collected in  
277 scintillation vials and weighed. Then, the gonads and Aristotle's lantern were sampled, rinsed with  
278 sea water, collected in scintillation vials and weighed. The sex of the specimen, determined based on  
279 the colour of the gonadal fluid, as well as the wet weight of the entire tegument including the spines  
280 were recorded. A small piece of tegument was then cut and weighed before and after the removal of  
281 its spines. Once weighed and the spines removed, the fragment of tegument was transferred into a  
282 scintillation vial and cut into very small pieces for radiocounting. The dissection tools (scalpel,  
283 scissors, tweezers) were systematically decontaminated, both between the collection of different  
284 organs from the same urchin, and between the dissection of different urchins. To be  
285 decontaminated, tools were immersed successively into ethanol and osmotic water, then were left  
286 to dry before reuse.  
287

#### 288 5. Sample treatment and liquid scintillation counting

289  
290 Radioactivity in samples was determined using a liquid scintillation counting method. While MPs and  
291 water samples were shortly analysed after the addition of 10 mL of Ultima Gold XR<sup>®</sup> (PerkinElmer<sup>®</sup>)  
292 scintillation liquid into the counting vials (PerkinElmer<sup>®</sup>), biological samples required preparation  
293 before counting. The extraction of  $^{14}\text{C}$ -PCB-153 from the four body compartments (digestive wall,  
294 gonads, Aristotle's lantern, and fragment of tegument) of the sea urchins was conducted by applying  
295 the methods described in Danis *et al.* (2005). A schematic diagram of the sample treatment is shown  
296 in Figure 2. First, samples were ultrasonicated twice at room temperature for 10 minutes, each time  
297 with 2 mL of Acetonitrile<sup>®</sup> (Sigma-Aldrich<sup>®</sup>). The 4 mL of Acetonitrile<sup>®</sup> was mixed with 16 mL of  
298 filtered sea water contained within a glass tube (23 mL capacity, Fisher<sup>®</sup>). This treatment produced,  
299 for each sample, 20 mL of liquid phase containing the extracted  $^{14}\text{C}$ -PCB-153 and a residue.

300  
301 The residue was digested with 2 mL of Soluene<sup>®</sup> (PerkinElmer<sup>®</sup>) at 70°C for 24h. Then, 10 mL of  
302 Hionic Fluor<sup>®</sup> (PerkinElmer<sup>®</sup>) scintillation liquid was added in each vial containing residue for  
303 counting.  
304

305 From the liquid phase,  $^{14}\text{C}$ -PCB-153 was extracted twice, using 2 mL of n-Hexane<sup>®</sup> each time  
306 (Fisher<sup>®</sup>). To do so, 2 mL of n-Hexane<sup>®</sup> was added and mixed by vortex to the liquid phase of each  
307 tube. Prior to vortexing, the top of the tube was tightly wrapped with parafilm to prevent splashing  
308 of radioactive material. This treatment produced an organic phase (2 mL) and an aqueous phase (20  
309 mL). The organic phase (2 mL) was collected within a scintillation vial, and the extraction operation  
310 was repeated a second time. To do so, another 2 mL of n-Hexane<sup>®</sup> was added to the liquid phase of  
311 each tube, mixed by vortex, and extracted. The organic phase from the second extraction (2 mL) was  
312 combined with the first (2 mL) within the scintillation vial and 10 mL of Ultima Gold XR<sup>®</sup> scintillation  
313 liquid was added. Finally, 2 mL of the aqueous phase was collected in another scintillation vial and  
314 mixed with 10 mL of Ultima Gold XR<sup>®</sup> scintillation liquid.

315

316 Once the scintillation liquid was added to all radioactive samples, they were thoroughly mixed to  
317 homogenize the solution and kept in the dark for 24 h before radio counting. Maintaining samples in  
318 the dark for 24 hours before counting limited the phenomena of photoluminescence (potentially  
319 produced by the scintillation cocktail) as it decays in the dark.  $^{14}\text{C}$ -radioactivity was then measured in  
320 the samples using a Tri-Carb 4910 TR liquid scintillation counter (PerkinElmer<sup>®</sup>), compared to  
321 standards of known activities, and measurements were corrected for counting efficiency, physical  
322 radioactive decay and quenching effects (Metian *et al.*, 2019). Counting times were adapted to  
323 obtain counting rates with relative propagated errors less than 5% (maximal counting duration was  
324 90 min).

325

## 326 6. Data analyses

327

328 The total amount of  $^{14}\text{C}$ -PCB-153 (ng) accumulated per *P. lividus* body compartment was obtained by  
329 summation of the amounts of  $^{14}\text{C}$ -PCB-153 (ng) measured within the samples of organic phase, of  
330 the aqueous phase and of Soluene<sup>®</sup> of the body compartment (cfr section 3.4). The total amount of  
331  $^{14}\text{C}$ -PCB-153 (ng) accumulated by the whole body wall, as well as by the coelomic fluid, were  
332 estimated by extrapolating the amount of  $^{14}\text{C}$ -PCB-153 (ng) measured in the sub-samples to the  
333 weight of the total body wall, or to the entire volume of coelomic fluid, respectively. As the sampling  
334 of 0.5 mL of coelomic fluid represents about 2.5% of the total volume of *P. lividus* individuals of  
335 similar size (Collard *et al.*, 2013), the total volume of coelomic fluid per sea urchin was assumed to  
336 be 20 mL in this work.

337

338 The concentrations of  $^{14}\text{C}$ -PCB-153, reported as  $\text{ng g}_{\text{dw}}^{-1}$  or  $\text{ng g}_{\text{lipids}}^{-1}$ , were calculated for each sea  
339 urchin body compartment by dividing the total amount of  $^{14}\text{C}$ -PCB-153 (ng) accumulated into the  
340 body compartments by its dry weight ( $\text{g}_{\text{dw}}$ ) or its total lipid content ( $\text{g}_{\text{lipids}}$ ). The total lipid contents of  
341 the experimental body compartments were determined on the basis of their dry weight ( $\text{g}_{\text{dw}}$ ) and on  
342 the average total lipid content determined gravimetrically on lyophilized powder with  
343 hexane:dichloromethane (1:1) in prior experiments. Further details on the method and results of the  
344 total lipid content analyses can be found in the Supplementary Information.

345

346 As the objective of this study was to evaluate the potential of MPs to act as vectors of PCBs into sea  
347 urchins, as a result of direct physical contact with the particles or as a result of the ingestion of  
348 particles, the potential part of  $^{14}\text{C}$ -PCB-153 accumulation due to the contamination via seawater, as a  
349 result of the leaching of the particles, was removed. To do so, the average concentrations of  $^{14}\text{C}$ -PCB-  
350 153 ( $\text{ng g}_{\text{dw}}^{-1}$  or  $\text{ng g}_{\text{lipids}}^{-1}$ ) measured in the control sea urchins were subtracted from the  
351 concentrations measured for the sea urchins which had been in contact with the microplastics for all  
352 the body compartments (digestive wall, gonads, Aristotle's lantern, body wall, and coelomic fluid).

353

354 The distribution of  $^{14}\text{C}$ -PCB-153 (%) among the body compartments was calculated, for each sea  
355 urchin in contact with the MPs, by reporting the amount of  $^{14}\text{C}$ -PCB-153 (ng) measured within the

356 various body compartments (digestive wall, gonads, Aristotle's lantern, body wall, and coelomic  
357 fluid) by their sum, while taking into account the weighted contribution of each compartment.

358  
359 The transfer efficiency of  $^{14}\text{C}$ -PCB-153 (%) was calculated, for each sea urchin in contact with the  
360 MPs, by reporting the sum of the amount of  $^{14}\text{C}$ -PCB-153 (ng) measured within of the various body  
361 compartments (digestive wall, gonads, Aristotle's lantern, body wall, and coelomic fluid) by the total  
362 amount of  $^{14}\text{C}$ -PCB-153 available per sea urchin on average over the 15 days of exposure (ng).

363  
364 Statistical analyses were performed using R statistical software (R Development Core Team), with a  
365 significance level of  $\alpha=0.05$  for all analyses. Normality of the data and homoscedasticity were  
366 evaluated using Shapiro-Wilk's test and Levene's test, respectively. When the normality and  
367 homoscedasticity of the data could be assumed, parametric analyses were conducted to identify  
368 significant differences in the dataset. In particular, comparisons were conducted using a 1-way  
369 ANOVA analysis followed by a Tukey's pairwise test, while Pearson's tests were used to look for  
370 relationships. In contrast, when the data did not satisfy the assumption of normality and/or  
371 homoscedasticity, the non-parametric Kruskal–Wallis tests for multiple comparisons, and the post-  
372 hoc Dunn tests with Bonferroni corrections for pairwise comparisons were conducted.

373

## 374 Results

375

### 376 1. $^{14}\text{C}$ -PCB-153 sorbed onto MPs during the 15 days exposure

377

378 The concentration of  $^{14}\text{C}$ -PCB-153 sorbed onto MPs was monitored over the 15 days of exposure. In  
379 the “lowPCB highMP” and “highPCB lowMP” experiments, the  $^{14}\text{C}$ -PCB-153 concentrations per MP  
380 decreased linearly and exponentially as a function of time, respectively. Over the course of the  
381 “lowPCB lowMP” experiment, the amount of  $^{14}\text{C}$ -PCB-153 sorbed onto MPs remained constant  
382 throughout the 15 days exposure (Table 2). Parameters and statistics of kinetics, as well as further  
383 details on the calculations and statistical results can be found in the Supplementary Information  
384 (Figure S1; Table S3).

385

386 As leaching of  $^{14}\text{C}$ -PCB-153 from the MPs occurred in the “lowPCB highMP” and “highPCB lowMP”  
387 experiments, the average  $^{14}\text{C}$ -PCB-153 concentration per MP over the 15 days exposure has been  
388 taken into account for the rest of the calculations, and the total amounts of  $^{14}\text{C}$ -PCB-153 available  
389 per sea urchin through the three experiments have been recalculated accordingly (Table 2). In order  
390 to correctly assess the accumulation of  $^{14}\text{C}$ -PCB-153 within the sea urchin body due to direct contact  
391 with MPs, any contamination via seawater as a result of the leaching of the particles was quantified  
392 using control specimens and removed accordingly.

393

394 On average over the entire duration of the “lowPCB highMP” experiment, an amount of  $0.13 (\pm 0.02,$   
395  $n = 6)$  ng of  $^{14}\text{C}$ -PCB-153 was concentrated per MP (Table 2). An average number of 5143 MPs ( $\pm 30$ )  
396 and of 4320 MPs ( $\pm 1151, n = 6$ ) were available per experimental aquarium and in the control  
397 aquarium, respectively. Based on both the average  $^{14}\text{C}$ -PCB-153 concentration per MP and on the  
398 quantity of MPs available per aquarium over the 15 days exposure, a total amount of 681.86 ng ( $\pm$   
399  $92.35, n = 6$ ) and 572.75 ng ( $\pm 77.57, n = 6$ ) of  $^{14}\text{C}$ -PCB-153 was estimated to be available per  
400 experimental aquarium and in the control aquarium, respectively (Table 2). The total amount of  $^{14}\text{C}$ -  
401 PCB-153 available per aquarium during the “lowPCB highMP” experiment was not significantly  
402 different between aquariums (1-way Anova, p-val = 0.998). Therefore, the twelve sea urchins in the  
403 “lowPCB highMP” experiment were all exposed to comparable  $^{14}\text{C}$ -PCB-153 levels. On average, over  
404 the duration of the experiment, a total amount of 227.29 ng ( $\pm 30.78, n = 6$ ) of  $^{14}\text{C}$ -PCB-153, loaded  
405 on an average number of 1714 ( $\pm 30$ ) MPs, was available per sea urchin in contact with the MPs  
406 (Table 2).

407  
408 On average over the entire duration of the “highPCB lowMP” experiment, an amount of 0.35 ( $\pm$  0.01,  
409  $n = 120$ ) ng of  $^{14}\text{C}$ -PCB-153 was available per MP (Table 2). In addition, throughout this experiment,  
410 an average of 1156 MPs ( $\pm$  4,  $n = 28$ ) and 1065 MPs ( $\pm$  26,  $n = 8$ ) were available per experimental  
411 aquarium and in the control aquarium, respectively. Based on both the average  $^{14}\text{C}$ -PCB-153  
412 concentration per MP and on the quantity of MPs available per aquarium over the 15 days exposure,  
413 a total amount of 394.49 ng ( $\pm$  16.36,  $n = 84$ ) and 407.06 ng ( $\pm$  39.86,  $n = 24$ ) of  $^{14}\text{C}$ -PCB-153 was  
414 estimated to be available by experimental aquarium and in the control aquarium, respectively (Table  
415 2). On average, during the 15 days of exposure, the total amount of  $^{14}\text{C}$ -PCB-153 available per  
416 aquarium was not significantly different between aquariums (Kruskal-Wallis  $X^2_6 = 3.854$ ,  $p\text{-val} =$   
417  $0.697$ ). Therefore, the six sea urchins in contact with the MPs during the “highPCB lowMP”  
418 experiment were all exposed to comparable  $^{14}\text{C}$ -PCB-153 levels. On average, over the duration of the  
419 experiment, a total amount of 394.49 ( $\pm$  16.36,  $n = 84$ ) of  $^{14}\text{C}$ -PCB-153, loaded on an average number  
420 of 1156 ( $\pm$  4,  $n=28$ ) MPs, was available per sea urchin in contact with the MPs (Table 2). Finally, on  
421 average during the 15 days of exposures, the total amount of  $^{14}\text{C}$ -PCB-153 per sea urchin was higher  
422 during the “highPCB lowMP” experiment compared to the “lowPCB highMP” experiment (Dunn test,  
423  $p\text{-val} = 0.001$ ).

424  
425 On average over the duration of the “lowPCB lowMP” experiment, 1201 MPs ( $\pm$  5,  $n = 12$ ) were  
426 available per sea urchin in direct contact with MPs. Since on average over the entire duration of the  
427 experiment, an amount of 0.14 ( $\pm$  0.01,  $n = 36$ ) ng of  $^{14}\text{C}$ -PCB-153 was available per MP, this implies  
428 that a total amount of 172.71 ( $\pm$  9.12,  $n = 36$ ) of  $^{14}\text{C}$ -PCB-153 was available per aquarium (Table 2).  
429 On average, during the 15 days of exposure, the total amount of  $^{14}\text{C}$ -PCB-153 available per aquarium  
430 was not significantly different between aquariums (Kruskal-Wallis  $X^2_3 = 2.782$ ,  $p\text{-val} = 0.427$ ).  
431 Therefore, the four sea urchins in contact with the MPs during the “lowPCB lowMP” experiment  
432 were all exposed to comparable  $^{14}\text{C}$ -PCB-153 levels. Finally, on average during the 15 days of  
433 exposure, the total amount of  $^{14}\text{C}$ -PCB-153 per sea urchin during the “lowPCB lowMP” experiment  
434 was lower than that of the “highPCB lowMP” experiment (Dunn test,  $p\text{-val} = 3.3 \times 10^{-12}$ ) but not of the  
435 “lowPCB highMP” experiment (Dunn test,  $p\text{-val} = 0.113$ ) (Table 2).

## 436 437 **2. Concentration of $^{14}\text{C}$ -PCB-153 accumulated in sea urchin tissues (in $\text{ng g}_{\text{lipids}}^{-1}$ and in $\text{ng}$** 438 **$\text{g}_{\text{lipids}}^{-1}$ )**

439  
440 No sea urchin mortality was detected, nor was there any evidence of a spontaneous spawning event  
441 during the course of the three experiments. Based on daily observations, the sea urchins displayed  
442 optimal health conditions (e.g. climbing ability, straightened spines) throughout the exposure period  
443 of all experiments. It was noted that the sea urchins used in the “lowPCB highMP” experiment (three  
444 sea urchins per aquarium) were more mobile (i.e. increased displacement) during the whole  
445 experiment than the sea urchins used in the “highPCB lowMP” and the “lowPCB lowMP”  
446 experiments (one sea urchin per aquarium).

447  
448 The transfer of  $^{14}\text{C}$ -PCB-153 from MPs to sea urchin tissues occurred in each of the three  
449 experiments and in every body compartment (except in the body wall and Aristotle’s lantern of sea  
450 urchins from the “lowPCB lowMP” experiment, see Figure 3). Over the exposure period of the three  
451 experiments, the digestive wall and the gonads concentrated  $^{14}\text{C}$ -PCB-153 to the highest degree,  
452 reaching mean concentrations up to 18.76 and 28.39  $\text{ng g}^{-1}$  dry weight ( $\text{ng g}_{\text{dw}}^{-1}$ ), respectively (Figure  
453 3). For all experiments,  $^{14}\text{C}$ -PCB-153 was concentrated to a similar degree in the digestive wall and in  
454 the gonads ( $\text{ng g}_{\text{dw}}^{-1}$ ; Dunn test,  $p\text{-val}_{\text{lowPCB highMP}^{\text{exp}}} = 1$ ,  $p\text{-val}_{\text{highPCB lowMP}^{\text{exp}}} = 1$ ,  $p\text{-val}_{\text{lowPCB lowMP}^{\text{exp}}} =$   
455  $1$ ).

456

457 For the “lowPCB highMP” experiment, since there is no significant difference in terms of body  
458 concentration of  $^{14}\text{C}$ -PCB-153 ( $\text{ng g}_{\text{lipids}}^{-1}$  and  $\text{ng g}_{\text{dw}}^{-1}$ ) between aquariums (Kruskal-Wallis  $X^2_2 = 2.328$ ,  
459  $p\text{-val} = 0.312$ ), individuals from the same aquarium were considered as replicates in our calculations.  
460 During this experiment, both the digestive wall and gonads did concentrate significantly more  $^{14}\text{C}$ -  
461 PCB-153 than the Aristotle’s lantern and the coelomic fluid ( $\text{ng g}_{\text{dw}}^{-1}$ ; Figure 3A; Dunn test,  $p\text{-val}_{\text{TD-AL}} =$   
462  $0.042$ ,  $p\text{-val}_{\text{GON-AL}} = 0.018$ ,  $p\text{-val}_{\text{TD-LC}} = 8.1 \times 10^{-05}$ ,  $p\text{-val}_{\text{GON-LC}} = 2.4 \times 10^{-05}$ ). In addition, the average  $^{14}\text{C}$ -  
463 PCB-153 concentration of the body wall was not significantly different when compared to other  
464 body compartments ( $\text{ng g}_{\text{dw}}^{-1}$ ; Figure 3A; Dunn test,  $p\text{-val}_{\text{TEG-TD}} = 0.372$ ,  $p\text{-val}_{\text{TEG-GON}} = 0.195$ ,  $p\text{-val}_{\text{TEG-AL}}$   
465  $= 1$ ,  $p\text{-val}_{\text{TEG-LC}} = 0.173$ ). During the “highPCB lowMP” experiment,  $^{14}\text{C}$ -PCB-153 was concentrated to a  
466 similar degree in digestive wall, gonads and Aristotle’s lantern ( $\text{ng g}_{\text{dw}}^{-1}$ ; Figure 3B; Dunn test,  $p\text{-val}_{\text{TD-}}$   
467  $\text{GON} = 1$ ,  $p\text{-val}_{\text{TD-AL}} = 0.188$ ,  $p\text{-val}_{\text{GON-AL}} = 0.681$ ). Moreover, for this experiment, the  $^{14}\text{C}$ -PCB-153  
468 concentration of the digestive wall was significantly higher than body wall and coelomic fluid ( $\text{ng}$   
469  $\text{g}_{\text{dw}}^{-1}$ ; Figure 3B; Dunn test,  $p\text{-val}_{\text{TD-TEG}} = 0.019$ ;  $p\text{-val}_{\text{TD-LC}} = 0.0003$ ), whereas the gonads did not  
470 concentrate significantly more  $^{14}\text{C}$ -PCB-153 than the body wall but significantly more than the  
471 coelomic fluid ( $\text{ng g}_{\text{dw}}^{-1}$ ; Figure 3B; Dunn test,  $p\text{-val}_{\text{GON-TEG}} = 0.099$ ;  $p\text{-val}_{\text{GON-LC}} = 0.002$ ). For the  
472 “lowPCB lowMP” experiment,  $^{14}\text{C}$ -PCB-153 was concentrated to different levels in body  
473 compartments (Kruskal-Wallis  $X^2_4 = 13.004$ ,  $p\text{-val} = 0.011$ ), but pairwise multiple comparison  
474 analyses were inconclusive ( $\text{ng g}_{\text{dw}}^{-1}$ ; Figure 3C; Dunn test with  $p\text{-values}$  all  $> 0.05$ ; Table S4).

475

476 When the concentrations of  $^{14}\text{C}$ -PCB-153 were expressed in  $\text{ng g}^{-1}$  total lipids, the digestive wall  
477 concentrated  $^{14}\text{C}$ -PCB-153 to the highest degree over the exposure period of the three experiments,  
478 reaching mean concentrations up to  $464.53 \text{ ng g}^{-1}$  total lipids (Figure 3). However, no significant  
479 difference was found between the body compartments of the sea urchins from the “lowPCB  
480 highMP” experiment ( $\text{ng g}_{\text{lipids}}^{-1}$ ; Figure 3D; Kruskal-Wallis  $X^2_2 = 1.79$ ,  $p\text{-val} = 0.409$ ). For the “highPCB  
481 lowMP” experiment, the digestive wall was found to concentrate significantly more  $^{14}\text{C}$ -PCB-153  
482 than the gonads ( $\text{ng g}_{\text{lipids}}^{-1}$ ; Figure 3E; Dunn test,  $p\text{-val}_{\text{TD-GON}} = 0.014$ ). In addition, no significant  
483 difference was found between the  $^{14}\text{C}$ -PCB-153 concentrations of the digestive wall and Aristotle’s  
484 lantern ( $\text{ng g}_{\text{lipids}}^{-1}$ ; Figure 3E; Dunn test,  $p\text{-val}_{\text{TD-AL}} = 0.303$ ), nor between the Aristotle’s lantern and  
485 gonads ( $\text{ng g}_{\text{lipids}}^{-1}$ ; Figure 3E; Dunn test,  $p\text{-val}_{\text{AL-GON}} = 0.236$ ). For the “lowPCB lowMP” experiment,  
486 digestive wall did concentrate significantly more  $^{14}\text{C}$ -PCB-153 than the Aristotle’s lantern ( $\text{ng g}_{\text{lipids}}^{-1}$ ;  
487 Figure 3F; Dunn test,  $p\text{-val}_{\text{TD-AL}} = 0.044$ ), whereas the concentration of these two compartments was  
488 not significantly different than the gonads ( $\text{ng g}_{\text{lipids}}^{-1}$ ; Figure 3F; Dunn test,  $p\text{-val}_{\text{TD-GON}} = 1$ ,  $p\text{-val}_{\text{AL-GON}}$   
489  $= 0.202$ ).

490

491

492

493

### 3. Assessment of the body distribution of the accumulated $^{14}\text{C}$ -PCB-153

494

495 The body wall, digestive wall and gonads displayed the highest proportions of accumulated  $^{14}\text{C}$ -PCB-  
496 153 for all experiments, except for the “lowPCB lowMP” experiment where  $^{14}\text{C}$ -PCB-153 was not  
497 found to concentrate within the body wall (Figure 3). For all experiments, the body distribution of  
498  $^{14}\text{C}$ -PCB-153 was not significantly different among the body wall, digestive wall, and gonads ( $p\text{-values}$   
499 of Dunn test were all  $> 0.05$ ; Tables S5a, S5b, S5c). For the “lowPCB highMP” experiment, the  
500 average proportion of  $^{14}\text{C}$ -PCB-153 accumulated by the body wall was not significantly higher than  
501 that of the digestive wall or gonads (Figure 3G; Dunn test,  $p\text{-val}_{\text{TEG-TD}} = 1$ ,  $p\text{-val}_{\text{TEG-GON}} = 1$ ), but  
502 significantly higher than that of the Aristotle’s lantern and coelomic fluid (Figure 3G; Dunn test,  $p\text{-}$   
503  $\text{val}_{\text{TEG-AL}} = 0.003$ ,  $p\text{-val}_{\text{TEG-LC}} = 0.001$ ). Moreover, in this experiment, the average proportions of  $^{14}\text{C}$ -  
504 PCB-153 accumulated by the digestive wall and by the gonads were significantly higher than that of  
505 coelomic liquid (Figure 3G; Dunn test,  $p\text{-val}_{\text{TD-LC}} = 0.031$ ,  $p\text{-val}_{\text{GON-LC}} = 0.040$ ), but not that of Aristotle’s  
506 lantern (Figure 3G; Dunn test,  $p\text{-val}_{\text{TD-AL}} = 0.105$ ,  $p\text{-val}_{\text{GON-AL}} = 0.129$ ). For the “highPCB lowMP”  
507 experiment, the average proportion of  $^{14}\text{C}$ -PCB-153 contained in the digestive wall and gonads were

508 not significantly higher than that of the body wall and Aristotle's lantern (Figure 3H; Dunn test, p-  
509 val<sub>TD-TEG</sub> = 1, p-val<sub>GON-TEG</sub> = 1, p-val<sub>TD-AL</sub> = 0.079, p-val<sub>GON-AL</sub> = 0.530), but higher than that of the  
510 coelomic fluid (Figure 3H; Dunn test, p-val<sub>TD-LC</sub> = 0.001, p-val<sub>GON-LC</sub> = 0.016). In this experiment, the  
511 distribution of <sup>14</sup>C-PCB-153 was not significantly different between the body wall, Aristotle's lantern  
512 and coelomic fluid (Figure 3H; Dunn test, p-val<sub>TEG-AL</sub> = 1, p-val<sub>TEG-LC</sub> = 0.236, p-val<sub>AL-LC</sub> = 1). For the  
513 "lowPCB lowMP" experiment, the distribution of <sup>14</sup>C-PCB-153 was different between the different  
514 body compartments (Kruskal-Wallis  $X^2_4 = 13.614$ , p-val = 0.009), but pairwise multiple comparison  
515 analyses are inconclusive (Figure 3I; all p-values of Dunn test were all > 0.05; Table S5c).

516

#### 517 **4. Assessment of the transfer efficiency of MP-loaded <sup>14</sup>C-PCB-153 to sea urchin** 518 **tissues**

519

520 For the "lowPCB highMP" experiment, an average value of 10.41 % ( $\pm 2.28$ , n = 9) of <sup>14</sup>C-PCB-153 was  
521 transferred from the MPs to the body compartments of the sea urchins collected. Since there is no  
522 significant difference in terms of transfer efficiency between the aquariums of the first experiment  
523 (1-way ANOVA, p-val = 0.348), all individuals from the same aquarium were considered as replicates  
524 in our calculations. The sea urchins from "highPCB lowMP" and "lowPCB lowMP" experiments  
525 accumulated on average 1.95% ( $\pm 0.53$ , n = 6) and 0.39% ( $\pm 0.09$ , n = 4) of the <sup>14</sup>C-PCB-153 adsorbed  
526 on the MPs, respectively. Even though the three experiments were not directly comparable because  
527 of the differences in their experimental setup, the average transfer efficiency of <sup>14</sup>C-PCB-153 from  
528 MPs to body compartments of sea urchins in the "lowPCB highMP" experiment was notably higher  
529 than that of the "highPCB lowMP", which in turn were found to be higher than the "lowPCB lowMP"  
530 experiment. The transfer efficiency of <sup>14</sup>C-PCB-153 in urchins increased with the amount of <sup>14</sup>C-PCB-  
531 153 available in the aquarium ( $F_{1,17} = 36.802$ , p-val =  $1.258 \times 10^{-05}$ , Figure 4).

532

#### 533 **Discussion**

534

535 Although globally banned since the 1980's, polychlorinated biphenyls (PCBs) still persist in the  
536 marine environment (e.g. Beyer and Biziuk, 2009; Reddy *et al.*, 2019) and are omnipresent on  
537 microplastics from all over the world (Ohgaki *et al.*, 2021). In the present work, the most abundant  
538 PCB congener in marine biota (i.e. PCB-153) was shown to be transferred and accumulated by sea  
539 urchins from MPs in all experiments. These findings indicate that the <sup>14</sup>C-PCB-153 adsorbed onto the  
540 MPs was bioavailable to sea urchins in direct contact with MPs and suggest that MPs located on the  
541 seafloor may act as vectors of PCB-153 contamination for sea urchins even during short-term  
542 exposure periods of only fifteen days. Moreover, the fact that a transfer of radiolabelled PCB-153  
543 occurred in the three experiments suggest that the concentration gradient of PCB-153 was negative  
544 during each experiment (i.e., higher in MPs than in biological tissues), even though levels of native  
545 PCB-153 contained within urchins upon sampling were not analysed in this work.

546 While this work constitutes the first study on the potential "vector" effect of MPs using adult sea  
547 urchins, previous studies have also found a transfer of PCB sorbed onto MPs to biological tissues of  
548 seabird chicks (Teuten *et al.*, 2009), lobsters (Devriese *et al.*, 2017), goldfish (Grigorakis and  
549 Drouillard., 2018), lugworms (Besseling *et al.*, 2013; 2017), while no transfer of PCB-153 from MPs  
550 was found into herring larvae (Norland *et al.*, 2021). The studies that compared the MP-mediated  
551 uptake of PCBs to natural bioaccumulation pathways agreed that MP would be a minor contribution  
552 to total bioaccumulation comparatively to natural environmental uptake pathways (Devriese *et al.*,  
553 2017; Grigorakis and Drouillard., 2018; Besseling *et al.*, 2013; 2017). Overall, there seems to be a  
554 growing consensus among various research groups that microplastics in the environment would  
555 have minor effects on bioaccumulation of associated chemicals in organisms (Koelmans *et al.*, 2021;  
556 2016; Besseling *et al.*, 2013; 2017; Bakir *et al.*, 2016; Beckingham *et al.*, 2017; Devriese *et al.*, 2017;

557 Grigorakis and Drouillard., 2018). In contrast, it has been recently suggested that the current  
558 estimates of marine total plastic load could have been greatly underestimated (Lindeque *et al.*,  
559 2020), making the abundance of plastic debris and their relative importance compared to natural  
560 particles still unknown. Another challenge of clarifying the uncertainties regarding the importance of  
561 MPs as carriers of co-contaminants to marine biota is the lack of consistent approaches between  
562 experimental methodologies, making direct comparison difficult if not impossible. Similar to the  
563 need of applying standard guidelines and procedures when studying the sorption-desorption kinetics  
564 of chemicals bound to MPs and natural particles (Pyl *et al.*, 2021), there is an urgent need to  
565 standardise the metrics for the assessment of bioaccumulation when experimentally testing the  
566 importance of MPs as carriers of co-contaminants to marine biota (see Besseling *et al.*, 2017 for  
567 comparison between different bioaccumulation metrics). The establishment of standardised  
568 methods and guidelines would give rise to improvement of the comparability between studies of  
569 different research groups, leading towards clarification regarding the uncertainty of the vector  
570 effects of MPs on bioaccumulation.

571  
572 Among the three experiments carried out in this work, the sea urchins exposed to a low  
573 concentration of <sup>14</sup>C-PCB-153 sorbed onto a high number of virgin MPs (i.e. “lowPCB highMP”  
574 experiment) were found to accumulate <sup>14</sup>C-PCB-153 to the highest degree (Figure 3). This could be  
575 explained by the fact that the average amount of <sup>14</sup>C-PCB-153 available per aquarium throughout  
576 this experiment was higher than for the two other experiments. Three sea urchins were placed in  
577 each exposure aquarium in the “lowPCB highMP” experiment, which when compared to a single sea  
578 urchin in the two other experiments, results in reasonably similar <sup>14</sup>C-PCB-153 amount per urchin  
579 across all experiments. However, the individuals in the “lowPCB highMP” experiment had potential  
580 contact with a much greater number of MPs within the aquarium, which could explain the higher  
581 <sup>14</sup>C-PCB-153 accumulation in these individuals. Moreover, the high MP-mediated transfer of <sup>14</sup>C-PCB-  
582 153 that occurred in the “lowPCB highMP” experiment could also be related to the observed higher  
583 mobility of the individuals in the aquarium compared those in the other two experiments. We  
584 speculate that a high mobility could be due to inter-individual competition for food, that would lead  
585 to a high grazing activity of *P. lividus*.

586  
587 The sea urchins from the “lowPCB lowMP” experiment that were exposed to 15 days aged MPs were  
588 found to accumulate <sup>14</sup>C-PCB-153 to the lowest degree when compared to the sea urchins from the  
589 “lowPCB highMP” and “highPCB lowMP” experiments that were exposed to virgin MPs. The low  
590 transfer of PCB from MPs to sea urchin during the “lowPCB lowMP” is most likely due to the fact that  
591 the average amount of <sup>14</sup>C-PCB-153 available per aquarium throughout this experiment was lower  
592 than for the two other experiments. The variation of PCB accumulation in sea urchins from 15 days  
593 aged MPs (potentially coated with a biofilm) compared to virgin particles could be attributed to the  
594 difference in surface coating. Porter *et al.* (2019) showed that the grazing activity of *P. lividus* onto  
595 macroplastics was significantly higher when the plastic surface was biofouled rather than “clean”  
596 (i.e. not fouled). These authors also observed the highest grazing activity when sea urchins were  
597 exposed to both biofouled macroplastics and kelp simultaneously, which is in disagreement with our  
598 results of transfer. Our findings of transfer are also in contradiction with the statement that aged  
599 MPs would constitute a higher uptake pathway than virgin MPs, both given their higher absorbance  
600 capacity of co-contaminants (Johansen *et al.*, 2018) and their higher likelihood to be ingested by  
601 organisms as they may not be recognised as inorganic matter (Fabra *et al.*, 2021; Vroom *et al.*, 2017;  
602 Porter *et al.*, 2019). However, it is noteworthy that the MPs used in our “lowPCB lowMP”  
603 experiment were contaminated by PCB-153 before being left for 15 days to age in sea water,  
604 whereas the MPs used the previously cited work were contaminated by co-contaminants after the  
605 aging process. We speculate that aging the MPs prior to their contamination by co-contaminant,  
606 rather than aging the particles while the sorption of the co-contaminant already occurred, makes the  
607 sorbed co-contaminant more available to biological tissues upon ingestion by organisms. To test this

608 hypothesis, further work comparing the bioavailability of chemicals loaded onto the two types of  
609 aged MPs is required. Nevertheless, our results suggest that PCB-loaded MPs, aged in a non-  
610 contaminated conditions are still a potential source of PCB-153 upon their ingestion by organisms.  
611

612 Over the exposure period of the three experiments, digestive wall and the gonads concentrated  $^{14}\text{C}$ -  
613 PCB-153 ( $\text{ng g}_{\text{dw}}^{-1}$ ) to the highest degree. As PCB-153 is a highly hydrophobic and lipophilic  
614 compound, the higher  $^{14}\text{C}$ -PCB-153 concentrations ( $\text{ng g}_{\text{dw}}^{-1}$ ) in the sea urchin gonads and digestive  
615 walls compared to other body parts is probably related to the highest lipid content obtained for  
616 these two compartments. The lipidic concentrations of  $^{14}\text{C}$ -PCB-153 ( $\text{ng g}_{\text{lipids}}^{-1}$ ) indicates that the  
617 digestive wall incorporated  $^{14}\text{C}$ -PCB-153 to the highest degree over the exposure period of the three  
618 experiments, suggesting that the primary uptake pathway of  $^{14}\text{C}$ -PCB-153 could be attributed to the  
619 ingestion of MPs. This is in accordance with Danis et al. (2005) showing that the digestive wall of  
620 *P. lividus* displayed the highest concentrations of  $^{14}\text{C}$ -PCB-153 ( $\text{ng g}_{\text{lipids}}^{-1}$ ) when individuals were  
621 exposed via food. Overall, the fact that the digestive wall is the predominant compartment for body  
622 transfer of  $^{14}\text{C}$ -PCB-153 from MPs is likely due to the proximity of ingested MPs to the digestive walls  
623 for a long period of time. Furthermore, we speculate that the transit of PCB-loaded MPs through the  
624 slightly alkaline environment of the sea urchin gut (pH between 6.4 and 7.3; Claereboudt and  
625 Jangoux, 1985), coupled with digestive enzymes (Trenzado *et al.*, 2012) and microbiome activity  
626 (Meziti *et al.*, 2007) favoured the desorption of  $^{14}\text{C}$ -PCB-153 from MPs as well as its bioaccumulation  
627 into biological tissues.  
628

629 Although the  $^{14}\text{C}$ -PCB-153 concentrations ( $\text{ng g}_{\text{dw}}^{-1}$ ) and proportions (%) indicate that dermal  
630 sorption of  $^{14}\text{C}$ -PCB-153 from MPs was the secondary route of transfer during the “lowPCB highMP”  
631 and “lowPCB highMP” experiments, this was not the case for the “lowPCB highMP” experiment,  
632 where the body walls of urchins incorporated higher proportions of  $^{14}\text{C}$ -PCB-153 than the gonads  
633 and digestive wall. We speculate that these higher proportions of  $^{14}\text{C}$ -PCB-153 accumulated by the  
634 body wall during this experiment, compared to the two other experiments, might be due to more  
635 physical contact between the tegument and particles as a result of the high mobility of sea urchins  
636 observed during the “lowPCB highMP” experiment.  
637

638 In this work, the  $^{14}\text{C}$ -PCB-153 concentrations based on the total lipids contents did not allow to  
639 better discriminate between the different body compartments than the concentrations expressed in  
640 terms of dry weight. We hypothesize that a longer exposure time could trigger a predominant  
641 accumulation of  $^{14}\text{C}$ -PCB-153 within the body compartments containing the highest lipid contents  
642 (such as gonads), which could potentially lead to a better discrimination between the different body  
643 compartments. To test this hypothesis, further work investigating the influence of exposure time on  
644 the lipidic accumulation of PCB-153 is required. This is probably due to the fact that the total lipid  
645 contents were determined prior to experiments on individuals other than those exposed in this  
646 study, whereas the dry weights of each body compartment of exposed sea urchin were measured as  
647 part of the sample processing. Biological samples were not available in sufficient quantities to enable  
648 both the analysis of their total lipid content and their  $^{14}\text{C}$ -radioactivity measure in this work. In order  
649 to strengthen the findings of future studies, it is advised to analyse the total lipid content of every  
650 body compartment from every organism used experimentally when biological samples are available  
651 in sufficient quantities. It is also encouraged for future work to continue to express the PCB  
652 concentrations based on the total lipids content given the lipophilicity of the contaminants. In  
653 addition, the PCB concentrations expressed on a total lipid content basis are particularly valuable  
654 information given the seasonal transferability of lipids, and their associated contaminants, across the  
655 body parts observed for various organisms including for *Paracentrotus lividus* (Fernandez, 1997;  
656 Rocha *et al.*, 2019; Anedda *et al.*, 2021). In general for *Paracentrotus lividus*, the highest lipids  
657 content corresponds to a prevalence of recovery gametogenic stage (accumulation of reserves  
658 during the summer season), and the lowest lipids content in gonads corresponds to a high

659 occurrence of mature gametogenic stages (reserves used for gamete development during the winter  
660 season) (Fernandez, 1997; Anedda *et al.*, 2021). In order to investigate the seasonality of the MP-  
661 mediated transfer of PCB, further work mimicking the present study while using adult *P.lividus* in a  
662 different gonadic maturation stage than ours is required.

663  
664 Although the transfer efficiency of PCB appears to increase with the PCB amount available to urchins  
665 throughout the experiments (Figure 4), the high variability of transfer efficiencies observed  
666 at high PCB budgets suggests that further investigation is needed to clarify this trend. Transfer  
667 efficiencies cannot exceed 100% and PCB accumulation could be regulated by the urchins. For  
668 instance, Danis *et al.* (2005) showed a decrease of PCB-153 concentration in *P. lividus* tissues during  
669 the depuration phase of exposures, indicating the existence of detoxification mechanisms in these  
670 organisms.

671  
672 The MP-mediated uptake of <sup>14</sup>C-PCB-153 into sea urchin tissues, even in the case of the highest  
673 concentration on the HDPE particles, did not induce any lethal effects or any other external sign of  
674 unhealthy condition during the 15 day exposures. This is in line with Devriese *et al.* (2017) and  
675 Besseling *et al.* (2013; 2017), where longer exposures to PCB-loaded PE microplastics did not trigger  
676 noticeable effect on the survival of lobsters or lugworms, respectively. Although the sub-lethal  
677 effects of MP ingestion were not investigated in this work, sinking MPs have been recently shown to  
678 impair the *P.lividus*'s oxidative status at adult stage (Parolini *et al.*, 2020). However, the modulation  
679 of the sea urchin oxidative status obtained by Parolini *et al.* (2020) was triggered by a higher dose of  
680 MPs (daily exposure of 800 MPs during 7 days) than the MPs levels used in our study. In addition,  
681 the characteristics of MPs (irregular shaped PET-MPs of an average diameter  $316 \pm 12 \mu\text{m}$ ) used by  
682 Parolini *et al.* (2020) were different from ours, making the comparison between studies challenging.  
683 In general, the presence of MPs and associated contaminants within adult specimens of sea urchins  
684 and their associated biological effects remain poorly documented, indicating that this is an area  
685 requiring further research.

686

## 687 **Conclusion and perspectives**

688

689 The present study reports for the first time a microplastic-mediated transfer of a key PCB congener  
690 into adult specimens of the sea urchin *Paracentrotus lividus*. These findings suggests that the <sup>14</sup>C-  
691 PCB-153 adsorbed onto the MPs was bioavailable to sea urchins across all experiments and that  
692 these MPs acted as vectors of <sup>14</sup>C-PCB-153 even during short-term exposure periods of only fifteen  
693 days. In order to clarify the uncertainty regarding the vector effects of MPs, further experimental  
694 studies investigating the extent at which MPs act as vectors for co-contaminants, compared to other  
695 naturally occurring particles, are much needed. It is advised for future studies to keep in mind that  
696 the relevance of MPs as vectors of co-contaminants depends on various factors such as the gradient  
697 of co-contaminant concentrations between MPs and the organisms (Koelmans *et al.*, 2013). A truly  
698 comprehensive understanding of the importance of MPs as vectors for associated chemicals will, in  
699 turn, eventually give rise to a better characterisation of MPs as a threat to organisms, potentially  
700 leading towards more rigorous risk assessments of microplastic for human health and environment.  
701 The relevance of further study of MP pollution is evident when taking into account the predicted  
702 increase in MP abundance in the world's oceans in the coming years (Stollberg *et al.*, 2021). In order  
703 to enhance our understanding regarding the extent at which MPs act as vectors for co-contaminants,  
704 further experimental studies investigating their role in the bioaccumulation of a wide range of  
705 contaminants, by different organisms, and at various pollution levels compared to other naturally  
706 occurring particles is much needed (Rodrigues *et al.*, 2019). The use of radiolabelled chemicals to  
707 address this knowledge gap shows to be highly promising, as radiotracer techniques offer strong  
708 assets in terms of rapidity, accuracy and sensitivity (Besson *et al.*, 2020; Johansen *et al.*, 2018;

709 Lanctôt *et al.*, 2018). It is also advised for future work to assess the relevance of MPs for  
710 bioaccumulation of associated chemicals under environmentally realistic exposure conditions. In this  
711 work, as the concentrations of PCB-153 used were in the highest range of the PCBs concentration  
712 detected on MPs in the field, ranging from 0.2 to 7554 ng g<sup>-1</sup> (Campanale *et al.*, 2020, Taniguchi *et*  
713 *al.*, 2016), it is recommended to improve the experimental set up of future studies by selecting  
714 environmentally realistic concentrations of PCBs or other co-contaminants. In addition, for studies  
715 aiming at further investigating the vector effect of sinking MPs to benthic grazers in particular, it is  
716 recommended to add sediments to the aquariums in order to enable appropriate comparison  
717 between the experimental and environmental levels of MPs (in terms of kg of dry sediment) and to  
718 mimic a more realistic situation.

719

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721

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