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► **To cite this version:**

Marine Pyl, Angus Taylor, François Oberhänsli, Peter Swarzenski, Marc Besson, et al.. Evidence of microplastic-mediated transfer of PCB-153 to sea urchin tissues using radiotracers. *Marine Pollution Bulletin*, 2022, 185, pp.114322. 10.1016/j.marpolbul.2022.114322 . hal-03948652

**HAL Id: hal-03948652**

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Submitted on 20 Jan 2023

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## Evidence of microplastic-mediated transfer of PCB-153 to sea urchin tissues using radiotracers

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### ARTICLE INFO

#### Keywords:

Microplastics  
Polychlorinated biphenyls  
Bioaccumulation  
Bioavailability  
*Paracentrotus lividus*

### ABSTRACT

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

### 1. Introduction

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

Due to their small size, persistence and ubiquitous presence in aquatic ecosystems, MPs may present a significant threat to marine fauna. It is well documented that microplastics are available for ingestion to a broad range of neustonic (buoyant microplastics), pelagic (microplastics in suspension in the water column) and benthic species

(sinking microplastics), potentially affecting aquatic food webs at multiple trophic levels and ecological niches (Setälä et al., 2018). Accumulating within organisms, MPs may cause physical harm, such as feeding and digestion disruption, by generating blockages, false satiation and injuries in the digestive tract (Jabeen et al., 2018; Wright et al., 2013). In addition to the potential physical impacts of ingested microplastics, chemical toxicity could also arise from the leaching of plastic-associated contaminants (i.e. co-contaminants). Co-contaminants carried by MPs include chemicals incorporated during manufacture (e.g. additives such as flame retardants, plasticisers and stabilisers, Rochman, 2015) as well as chemicals sorbed from the environment (e.g. metals and organic pollutant such as polychlorinated biphenyls, Rodrigues et al., 2019; Rochman et al., 2013; Koelmans et al., 2016; Ogata et al., 2009). More than 250 organic compounds have been identified on plastic debris (Campanale et al., 2020). Based on both their ubiquity and abundance in the marine environment, as well as their ability to carry environmental contaminants and substantial proof of particle uptake by marine organisms, the hypothesis that MPs may play a role in aquatic ecotoxicology as vectors for toxic contaminants has arisen. Once ingested by organisms, as physico-chemical properties change, the myriad of

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contaminants associated with MPs can desorb from the plastic and potentially become bioavailable to organisms (Hartmann et al., 2017; Teuten et al., 2009; Engler, 2012). These co-contaminants may threaten marine organisms when MPs become inadvertently ingested and transferred up the food chain (Miller et al., 2020).

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

The Mediterranean sea urchin *Paracentrotus lividus* (Lamarck, 1816) was selected as a model organism for this study because it is a keystone species in Mediterranean coastal ecosystems where it plays a crucial ecological role (Boudouresque and Verlaque, 2020). As a consequence of its continuous benthic foraging activity, combined with its presence in highly anthropic areas, adult specimens of *P. lividus* are potential targets of MPs accumulation in marine benthic ecosystems (Manzo and Schiavo, 2022). Among the few studies that investigated the relationship between adult *P. lividus* and microplastics, ingestion of MPs has been demonstrated (Parolini et al., 2020; Murano et al., 2020; Murano et al., 2021; Porter et al., 2019; Hennicke et al., 2021) and small MPs (1–5 µm) have been recently proven to be able to cross their intestinal wall and reach their coelomic fluid (Pyl et al., 2022). Moreover, MPs have been shown to induce a slight modulation of oxidative status (Parolini et al., 2020), as well as to impact the circulating immune cells (Murano et al., 2020; Murano et al., 2021) of adults *P. lividus*. Nevertheless, no information is available to date on the potential “vector” effect of co-contaminants adsorbed onto MPs using adult specimens of *P. lividus* as a model, despite being recognised as an excellent model organism in marine ecotoxicology (Sugni et al., 2013) as well as species of great ecological and commercial importance (Pearse, 2006; Andrew et al., 2002).

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

## 2. Materials and methods

### 2.1. Sampling of the sea urchins and acclimatation

Specimens of adult sea urchins (*Paracentrotus lividus*, Lamarck) were collected by divers in the Bay of Villefranche-sur-Mer (NW Mediterranean coastline, France) from mid-October 2020 to the end of November 2020. This autumnal period corresponds to premature and beginning of gonadal maturation of the sea urchins from the Bay of Villefranche-sur-Mer (Fenaux, 1968). Prior to experimentation, specimens were acclimated to laboratory conditions for at least one week in constantly

aerated open circuit aquariums, with natural seawater (salinity = 38 ‰, UV-sterilized and 1 µm filtered) at 17 ± 0.5 °C, in a 12/12 h dark/light cycle. Sea urchins were fed *ad libitum* every two days with dry leaves of the algae *Saccharina latissima*, purchased from Biocean® during acclimatation.

### 2.2. Radiotracer and microplastics (MPs)

<sup>14</sup>C-labelled congener IUPAC-153 (2,2',4,4',5,5'-hexachlorobiphenyl) was selected as representative of PCBs for this study as it is one of the most bioaccumulative and the most abundant congener in marine biota (Safe, 1994; Stebbing et al., 1992). This β-emitting radiotracer, with a purity higher than 95 %, was purchased from American Radio-labelled Chemicals®, USA. The specific activity of this mother source was 12.6 mCi mmol<sup>-1</sup>.

The MPs considered in this study were high density polyethylene microplastics (HDPE MP), as polyethylene is the most common polymers found in the environment (Hidalgo-Ruz et al., 2012; PlasticsEurope, 2020) with the highest adsorption capacity (Rochman, 2015). These HDPE MP were microbeads purchased from Cospheric® as an isospheric homogeneous solid powder (size = 500–600 µm; 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>). These microplastics were specifically selected for this study in order (1) to be available to benthic organisms for ingestion as they are negatively buoyant and thus sink to the bottom of the aquarium, (2) to avoid the use of surfactant with its potential interference in the adsorption processes, and (3) for ease of visibility and detection. Preliminary tests showed that these microplastics are available to sea urchins for ingestion as they were readily detected in the gut of the organisms. Further details on the method and results of the verification of MPs uptake can be found in the Supplementary Information section.

### 2.3. Experimental procedure

Three different experiments were conducted to assess whether <sup>14</sup>C-PCB-153 adsorbed onto the surface of MPs is bioavailable for the sea urchin *P. lividus* during 15 days: (1) exposure of *P. lividus* to a low concentration of <sup>14</sup>C-PCB-153 sorbed onto a high number of virgin (i.e. non-aged) MPs (“lowPCB highMP” experiment), (2) exposure of *P. lividus* to a high concentration of <sup>14</sup>C-PCB-153 sorbed onto a relatively low number of virgin MPs (“highPCB lowMP” experiment) and (3) exposure to a low concentration of <sup>14</sup>C-PCB-153 sorbed onto a relatively low number of aged MP (“lowPCB lowMP” experiment). The concentrations of PCB-153 and amounts of MPs available per sea urchin within the aquariums of each experiment were selected to ensure the detection of any transfer of PCB-153 from MPs to sea urchin tissues while avoiding lethal effects.

The method applied for each experiment is composed of different phases, summarized as follows: (1) adsorption of <sup>14</sup>C-labelled PCB-153 onto MPs by applying the procedures described per Pyl et al. (2021); (2) 15 days exposure of *P. lividus* to <sup>14</sup>C-labelled PCB-153 adsorbed onto the surface of MPs; (3) dissection of the organisms and the extraction of the radiotracer among the different body compartments; (4) the use of β spectroscopy to determine the concentration of <sup>14</sup>C-PCB-153 on the particles and the distribution of PCBs within the different organs of the experimental model.

#### 2.3.1. Adsorption of <sup>14</sup>C-labelled PCB-153 onto MPs

The MPs used in the “lowPCB highMP” and in the “highPCB lowMP” experiment required the preparation of solutions and their incubation in order to enable the adsorption of <sup>14</sup>C-labelled PCB-153 onto the surface of MPs. For these two experiments, the adsorption of <sup>14</sup>C-labelled PCB-153 onto MPs were achieved (following the procedures described in Pyl et al. (2021) with adaptations detailed in the Supplementary Information) then the radio-labelled MPs were gently transferred from their vials to the bottom of the aquariums. The MPs used in the “lowPCB lowMP”

experiment were sampled at the end of the “highPCB lowMP” experiment from the bottom of the aquariums using glass volumetric bulb pipettes (10 mL) combined with a pipette filler. These MPs were collected in transparent containers, photographed, automatically counted using the software IMAGE J, then placed at the bottom of each aquarium. In order to assess the stability of the  $^{14}\text{C}$ -PCB-153 concentration per MP and the potential leaching of  $^{14}\text{C}$ -labelled PCB-153, samples of MPs were regularly collected and radioanalysed throughout the experiments. The average numbers and mass of MP available per aquariums at the beginning of the three experiments are displayed in Table 1. Details related to preparation of PCB-loaded MPs are reported in Supplementary Information.

### 2.3.2. Sea urchin exposure

All experiments were conducted in 20 L capacity glass aquariums constantly aerated via an open circuit (water renewal:  $20\text{ L h}^{-1}$ ) with natural seawater (UV-sterilized and  $1\ \mu\text{m}$  filtered, salinity = 38 ‰, pH = 8.1) at  $16.4 \pm 0.5\ ^\circ\text{C}$ , in a 12/12 h dark/light cycle. Sea urchins were fed every two days *ad libitum* with dry leaves of the algae *Saccharina latissima*, purchased from Biocean®, during all experiments. The duration of exposure of the sea urchin to MPs was 15 days. Within the experimental aquariums, sea urchins were placed at the bottom in order to allow direct contact between the radioactive MPs and the organisms. As presented in Fig. 1, for the “highPCB lowMP” and “lowPCB lowMP” experiments, one sea urchin was placed at the bottom of each exposure aquariums as enough aquariums were available to maximise the number of true replications. However, as only three exposure aquariums were available at the time of the “lowPCB highMP” experiment, three sea urchins were placed in each aquarium to increase the number of replicates of the exposure treatment (Fig. 1). Moreover, control sea urchins were placed in a separated compartment (termed “cage”, made of polyethylene with a mesh size of  $0.5\ \text{cm}^2$ ) within one aquarium of each

experiment (Fig. 1) to control possible cross-contamination through sea water via leaching of the  $^{14}\text{C}$ -PCB-153 from MPs. Since the amount of  $^{14}\text{C}$ -PCB-153 sorbed onto the aged MPs used for the “lowPCB lowMP” experiment was known to be stable (Fig. S1) and contamination via leaching deemed unlikely, one sea urchin placed in the “cage” was considered sufficient as a control. For the “highPCB lowMP” and “lowPCB lowMP” experiments, as the amount of  $^{14}\text{C}$ -PCB-153 sorbed onto virgin MPs was unknown to be stable, three sea urchins were placed in the “cage” within the control aquarium to account for potential contamination via leaching (Fig. 1).

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

For the “lowPCB highMP” experiment, twelve sea urchins *P. lividus* (ambital diameter  $56 \pm 2\ \text{mm}$ ) and four aquariums (three exposure and one control) filled with 10 L of seawater were used (Fig. 1). The entire water volume of each aquarium was renewed every 30 min during the 15 days of the experiment. At the beginning of the experiment, 5143 ( $\pm 30$ ) and 7550 ( $\pm 30$ ) MPs, each radiolabelled with 0.17 ng of  $^{14}\text{C}$ -PCB-153, were available at the bottom of each exposure aquarium and in the control aquarium, respectively (Table 1). The difference in the initial abundance of MPs between the control aquarium and the exposure aquariums is justified by the fact that the samples of MPs, in order to control the stability of the PCB adsorbed on the MPs, were carried out only in the control aquarium during the “lowPCB highMP” experiment. Based on both the initial concentration of  $^{14}\text{C}$ -PCB-153 per MP and on the number of MPs per aquarium (corrected for  $t_0$ ), a total amount of 871.56 ( $n = 1$ ) and 1279.51 ( $n = 1$ ) ng of  $^{14}\text{C}$ -PCB-153 was estimated to be available per exposure aquarium and in the control aquarium respectively, at the beginning of the experiment (Table 1). Once the MPs were deposited in the aquariums, the experiment was initiated by placing three sea urchins at the bottom of each exposure aquarium, as

**Table 1**  
Initial exposure conditions for each of the experiments.

	“lowPCB highMP” experiment	“highPCB lowMP” experiment	“lowPCB lowMP” experiment
Average number and mass of MPs available per aquarium ( $\pm$ SE)	Control <sup>a</sup> : 7550 ( $\pm 30$ ) MPs 0.793 ( $\pm 0.003$ ) g	Control <sup>a</sup> : 1170 ( $\pm 30$ ) MPs 0.123 ( $\pm 0.003$ ) g	Control <sup>b</sup> : 1226 ( $\pm 8$ ) MPs 0.129 ( $\pm 0.001$ ) g
	Exposure: 5143 ( $\pm 30$ ) MPs 0.540 ( $\pm 0.003$ ) g	Exposure: 1185 ( $\pm 30$ ) MPs 0.124 ( $\pm 0.003$ ) g	Exposure: 1216 ( $\pm 8$ , $n = 4$ ) MPs 0.128 ( $\pm 0.001$ , $n = 4$ ) g
Average amount of $^{14}\text{C}$ -PCB-153 sorbed onto MPs ( $\pm$ SE, $n$ ) [ng per MP]	Control <sup>a</sup> : 0.17 ( $n = 1$ )	Control <sup>b</sup> : 0.68 ( $\pm 0.04$ , $n = 3$ )	Control <sup>b</sup> : 0.15 ( $\pm 0.04$ , $n = 3$ )
	Exposure: –	Exposure: 0.49 ( $\pm 0.03$ , $n = 18$ )	Exposure: 0.15 ( $\pm 0.02$ , $n = 12$ )
Amount of $^{14}\text{C}$ -PCB-153 available per aquarium ( $\pm$ SE, $n$ ) [ng]	Control <sup>a</sup> : 1279.51 ( $n = 1$ )	Control <sup>b</sup> : 792.21 ( $\pm 46.01$ , $n = 3$ )	Control <sup>b</sup> : 188.24 ( $\pm 52.88$ , $n = 3$ )
	Exposure <sup>c</sup> : 871.56 ( $n = 1$ )	Exposure: 575.93 ( $\pm 32.86$ , $n = 18$ )	Exposure: 181.56 ( $\pm 22.15$ , $n = 12$ )
Average number and mass of MPs available per sea urchin in contact with MP ( $\pm$ SE)	1714 ( $\pm 30$ ) MPs 0.180 ( $\pm 0.003$ ) g	1185 ( $\pm 30$ ) MPs 0.124 ( $\pm 0.003$ ) g	1216 MPs ( $\pm 8$ , $n = 4$ ) 0.128 ( $\pm 0.0008$ , $n = 4$ ) g
Amount of $^{14}\text{C}$ -PCB-153 available per sea urchin in contact with MP ( $\pm$ SE, $n$ ) [ng]	290.52 ( $n = 1$ )	575.93 ( $\pm 32.86$ , $n = 18$ )	181.56 ( $\pm 22.15$ , $n = 12$ )

<sup>a</sup> The control aquariums of the “lowPCB highMP” and “highPCB lowMP” experiments contain 3 sea urchins placed in a “cage”.

<sup>b</sup> The control aquarium of the “lowPCB lowMP” experiment as stated here corresponds to the first aquarium, which contains one sea urchin placed in a “cage” and another at the bottom of the aquarium in contact with the MPs (Fig. 1).

<sup>c</sup> For the “lowPCB highMP” experiment, the amount of PCB available per exposure aquarium at the beginning of the experiment has been determined based on the amount of PCB sorbed onto MPs samples obtained from the control aquarium.



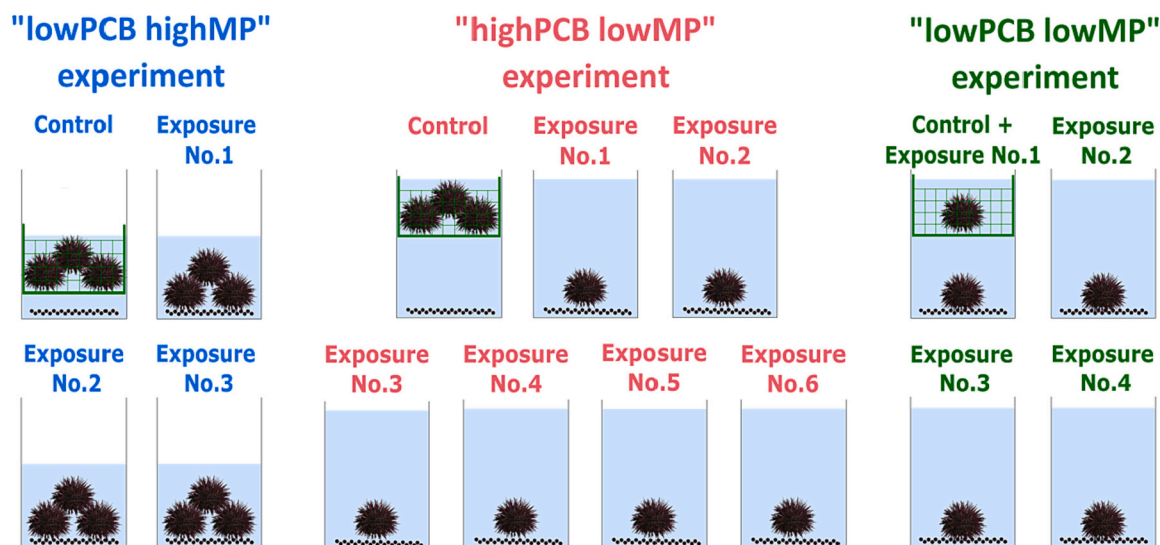


Fig. 1. Schematic representation of experimental setup of the three experiments.

well as three sea urchins within the cage of the control aquarium (Fig. 1). A total amount of 290.52 ng of  $^{14}\text{C}$ -PCB-153, loaded on an average number of 1714 ( $\pm 10$ ) MPs, were therefore available per sea urchin in direct contact with particles at the beginning of the experiment (Table 1). As the “lowPCB highMP” experiment was the first to assess the bioavailability of sorbed  $^{14}\text{C}$ -radiolabelled PCB onto MPs to marine organisms, both the minimal concentration of PCB-153 per MP and minimal number of radiolabelled MPs needed to ensure a detectable radiotracer signal following uptake and organ distribution by sea urchins was unknown. Therefore, high amounts of MPs available per sea urchin (average of 1714 ( $\pm 10$ ) MPs), each radiolabelled with an average activity of 0.17 Bq ( $\pm 0.02$ ,  $n = 6$ ), were selected to maximise the chance to detect the potential transfer of the radiotracer onto the tissues of sea urchins while avoiding lethal effects.

**2.3.2.2. “HighPCB lowMP” experiment: exposure of *P. lividus* to a high concentration of  $^{14}\text{C}$ -PCB-153 sorbed onto a relatively low number of virgin MPs.** For the “highPCB lowMP” experiment, nine sea urchins (ambital diameter  $62 \pm 2$  mm) and seven aquariums (six exposure and one control) filled with 20 L of seawater were used (Fig. 1). The entire volume of water in each aquarium was renewed every 60 min. At the beginning of the experiment, 1185 ( $\pm 30$ ) MPs, each radiolabelled with  $0.49 (\pm 0.03, n = 18)$  ng of  $^{14}\text{C}$ -PCB-153, were available at the bottom of each experimental aquarium. Similarly, 1170 ( $\pm 30$ ) MPs, each radiolabelled with  $0.68 (\pm 0.04, n = 3)$  ng of  $^{14}\text{C}$ -PCB-153, were placed at the bottom of the control aquarium (Table 1). Based on both the initial concentration of  $^{14}\text{C}$ -PCB-153 per MP and on the number of MPs per aquarium (corrected for  $t_0$ ), a total amount of  $575.93 (\pm 32.86, n = 18)$  and  $792.21 (\pm 46.01, n = 3)$  ng of  $^{14}\text{C}$ -PCB-153 was estimated to be available per exposure aquarium and in the control aquarium, respectively, at the beginning of the experiment (Table 1). One sea urchin was placed per exposure aquarium and three sea urchins were placed within the cage of the control aquarium (Fig. 1). A total amount of  $575.93 (\pm 32.86, n = 18)$  ng of  $^{14}\text{C}$ -PCB-153, loaded onto an average number of 1185 ( $\pm 30$ ) MPs, were therefore available per sea urchin in direct contact with particles at the beginning of the “highPCB lowMP” experiment (Table 1).

**2.3.2.3. “LowPCB lowMP” experiment: exposure to a low concentration of  $^{14}\text{C}$ -PCB-153 sorbed onto a relatively low number of aged MP.** At the end of the “highPCB lowMP” experiment, the MPs were collected and reused for the “lowPCB lowMP” experiment. This implies that, unlike the MPs used for the two other experiments which were virgin, the MPs used for

the “lowPCB lowMP” experiment were aged during 15 days. Solely MPs that were both (1) not contained in fecal pellets and (2) identical to virgin MPs (in terms of shape and size), were selected to be reused for the “lowPCB lowMP” experiment. This indicates that the aged MPs used in the “lowPCB lowMP” experiment did not pass through the digestive tract of sea urchins during the “highPCB lowMP” experiment. For the “lowPCB lowMP” experiment, five sea urchins *P. lividus* (ambital diameter  $58 \pm 2$  mm) and four aquariums filled with 20 L of seawater were used (Fig. 1). The entire water volume in each aquarium was renewed every 60 min. At the bottom of each aquarium, an average number of 1216 MPs ( $\pm 8, n = 4$ ), each radiolabelled with  $0.15 \text{ ng } (\pm 0.02, n = 12)$  of  $^{14}\text{C}$ -PCB-153, were available at the beginning of the experience (Table 1). Based on both the initial concentration of  $^{14}\text{C}$ -PCB-153 per MP and on the number of MPs per sea urchin (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 available per aquarium just prior to the beginning of the experiment (Table 1). One sea urchin was placed at the bottom of each aquarium and one control sea urchin was placed in the cage within the first aquarium (Fig. 1). A total amount of  $181.56 (\pm 22.15, n = 12)$  ng of  $^{14}\text{C}$ -PCB-153, loaded on an average number of 1216 ( $\pm 8, n = 4$ ) MPs, were therefore available per sea urchin in direct contact with particles at the beginning of the “lowPCB lowMP” experiment (Table 1).

#### 2.4. Dissection

After 15 days of exposure, all specimens were collected and rinsed abundantly with UV-sterilized  $1 \mu\text{m}$  filtered sea water in order to remove potential MPs in contact with the tegument and the peristomial membrane. The sea urchins were then measured, weighed, and sacrificed by cold exposure. The test of the sea urchin was then cut following the ambital diameter. Two mL of coelomic liquid were sampled and directly poured into a scintillation vial for radiocounting. Then, the entire digestive tract, from the oesophagus to the anus was collected, cut longitudinally and abundantly rinsed with sea water in order to remove all the digestive content and potentially ingested MPs. Once devoid of digestive content, the digestive tract walls were collected in scintillation vials and weighed. Then, the gonads and Aristotle’s lantern were sampled, rinsed with sea water, collected in scintillation vials and weighed. The sex of the specimen, determined based on the colour of the gonadal fluid, as well as the wet weight of the entire tegument including the spines were recorded. A small piece of tegument was then cut and weighed before and after the removal of its spines. Once weighed and the spines removed, the fragment of tegument was transferred into a

scintillation vial and cut into very small pieces for radiocounting. The dissection tools (scalpel, scissors, tweezers) were systematically decontaminated, both between the collection of different organs from the same urchin, and between the dissection of different urchins. To be decontaminated, tools were immersed successively into ethanol and osmotic water, then were left to dry before reuse.

## 2.5. Sample treatment and liquid scintillation counting

Radioactivity in samples was determined using a liquid scintillation counting method. While MPs and water samples were shortly analysed after the addition of 10 mL of Ultima Gold XR® (PerkinElmer®) scintillation liquid into the counting vials (PerkinElmer®), biological samples required preparation before counting. The extraction of  $^{14}\text{C}$ -PCB-153 from the four body compartments (digestive wall, gonads, Aristotle's lantern, and fragment of tegument) of the sea urchins was conducted by applying the methods described in Danis et al. (2005). A schematic diagram of the sample treatment is shown in Fig. 2. First, samples were ultrasonicated twice at room temperature for 10 min, each time with 2 mL of Acetonitrile® (Sigma-Aldrich®). The 4 mL of Acetonitrile® was mixed with 16 mL of filtered sea water contained within a

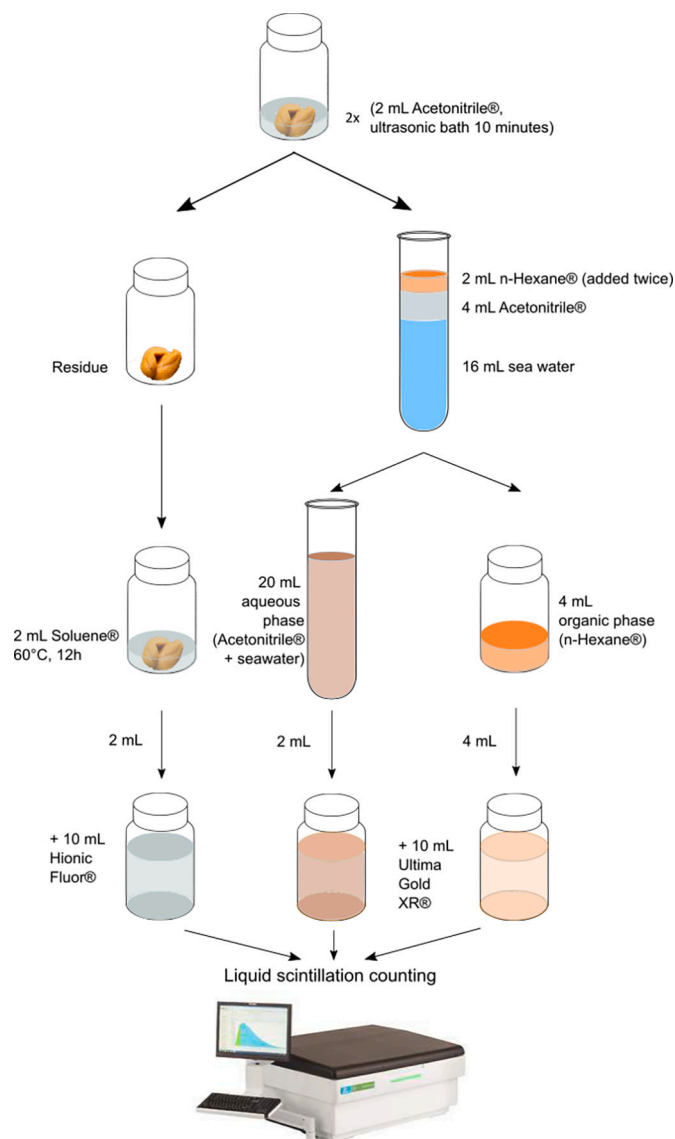


Fig. 2. Schematic representation of biological sample processing for the extraction of  $^{14}\text{C}$ -PCB-153, adapted from Danis et al. (2005).

glass tube (23 mL capacity, Fisher®). This treatment produced, for each sample, 20 mL of liquid phase containing the extracted  $^{14}\text{C}$ -PCB-153 and a residue.

The residue was digested with 2 mL of Soluene® (PerkinElmer®) at 60 °C for 12 h. Then, 10 mL of Hionic Fluor® (PerkinElmer®) scintillation liquid was added in each vial containing residue for counting.

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

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

## 2.6. Data analyses

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

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

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

**Table 2**  
Exposure conditions throughout the 15 days duration for each experiment.

	“lowPCB highMP” experiment	“highPCB lowMP” experiment	“lowPCB lowMP” experiment
Best fitting kinetic of <sup>14</sup> C-PCB-153 concentrations sorbed onto MP	Decreased linearly	Decreased exponentially	Stable
Average amount of <sup>14</sup> C-PCB-153 sorbed onto MPs (± SE, n) (ng per MP and ng per g of MP)	0.13 ng per MP (± 0.02, n = 6)	0.35 ng per MP (± 0.01, n = 120)	0.14 ng per MP (± 0.01, n = 36)
	1262.69 ng per g of MP (± 171.02, n = 6)	2515.96 ng per g of MP (± 156.17, n = 120)	1369.52 ng per g of MP (± 71.29, n = 36)
Average number and mass of MPs available per aquarium (± SE, n)	Control*: 4320 (± 1151, n = 6) MPs 0.454 (± 0.121, n = 6) g MP	Control*: 1065 (± 26, n = 8) MPs 0.112 (± 0.003, n = 8) g MP	Control**: 1211 (± 9, n = 3) MPs 0.127 (± 0.001, n = 3) g MP
	Exposure: 5143 (± 30) MPs 0.54 (± 0.003) g MP	Exposure: 1156 (± 4, n = 28) MPs 0.12 (± 0.001, n = 28) g MP	Exposure: 1201 (± 5, n = 12) MPs 0.13 (± 0.001, n = 12) g MP
Average amount of <sup>14</sup> C-PCB-153 available per aquarium throughout the experiment (± SE, n) (ng)	Control*: 572.75 (± 77.57, n = 6) <sup>a</sup>	Control*: 407.06 (± 39.86, n = 24) <sup>b</sup>	Control**: 172.36 (± 19.60, n = 9) <sup>c</sup>
	Exposure: 681.86 (± 92.35, n = 6) <sup>a</sup>	Exposure: 394.49 (± 16.36, n = 84) <sup>b</sup>	Exposure: 172.71 (± 9.12, n = 36) <sup>c</sup>
Average number and mass of MPs available per sea urchin in contact with MPs (± SE, n)	1714 (± 30) MPs 0.18 (± 0.003) g MP	1156 (± 4, n = 28) MPs 0.12 (± 0.001, n = 28) g MP	1201 (± 5, n = 12) MPs 0.13 (± 0.001, n = 12) g MP
Average amount of <sup>14</sup> C-PCB-153 available per sea urchin in contact with MPs throughout the experiment (± SE, n) (ng)	227.29 <sup>d</sup> (± 30.78, n = 6)	394.49 <sup>e</sup> (± 16.36, n = 84)	172.71 <sup>d</sup> (± 9.12, n = 36)

Average amount of <sup>14</sup>C-PCB-153 sharing the same superscript do not differ significantly between each other (1-way ANOVA, or Kruskal-Wallis test, or Dunn Test, p > 0.05).

\* The control aquariums of the “lowPCB highMP” and “highPCB lowMP” experiments contain 3 sea urchins placed in a “cage”.

\*\* The control aquarium of the “lowPCB lowMP” experiment as stated here corresponds to the first aquarium, which contains one sea urchin placed in a “cage” and another at the bottom of the aquarium in contact with the MPs.

Aristotle's lantern, body wall, and coelomic fluid).

The distribution of  $^{14}\text{C}$ -PCB-153 (%) among the body compartments was calculated, for each sea urchin in contact with the MPs, by reporting the amount of  $^{14}\text{C}$ -PCB-153 (ng) measured within the various body compartments (digestive wall, gonads, Aristotle's lantern, body wall, and coelomic fluid) by their sum, while taking into account the weighted contribution of each compartment.

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

Statistical analyses were performed using R statistical software (R Development Core Team), with a significance level of  $\alpha = 0.05$  for all analyses. Normality of the data and homoscedasticity were evaluated using Shapiro-Wilk's test and Levene's test, respectively. When the normality and homoscedasticity of the data could be assumed, parametric analyses were conducted to identify significant differences in the dataset. In particular, comparisons were conducted using a 1-way ANOVA analysis followed by a Tukey's pairwise test, while Pearson's tests were used to look for relationships. In contrast, when the data did not satisfy the assumption of normality and/or homoscedasticity, the

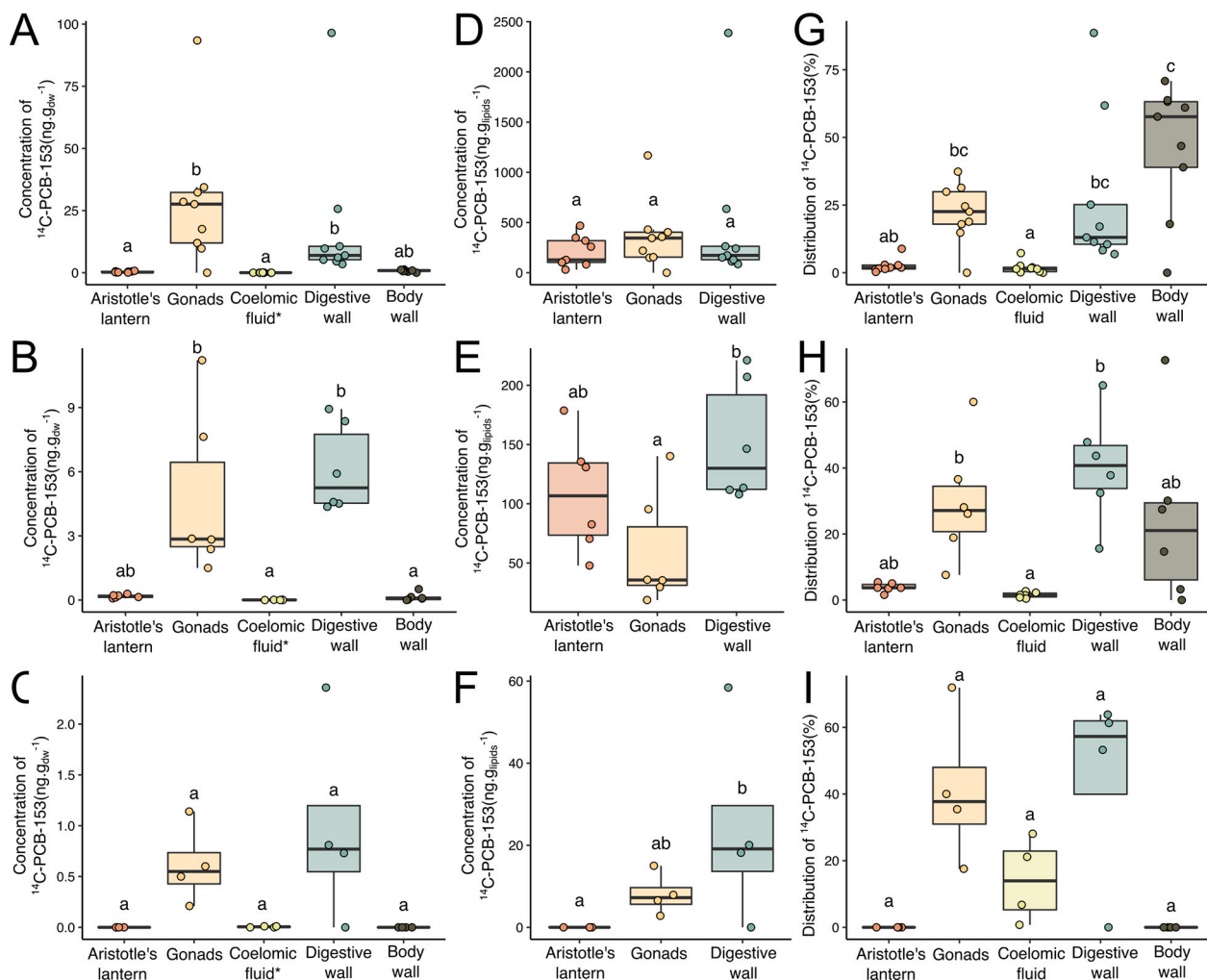
non-parametric Kruskal–Wallis tests for multiple comparisons, and the post-hoc Dunn tests with Bonferroni corrections for pairwise comparisons were conducted.

### 3. Results

#### 3.1. $^{14}\text{C}$ -PCB-153 sorbed onto MPs during the 15 days exposure

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

As leaching of  $^{14}\text{C}$ -PCB-153 from the MPs occurred in the “lowPCB highMP” and “highPCB lowMP” experiments, the average  $^{14}\text{C}$ -PCB-153 concentration per MP over the 15 days exposure has been taken into account for the rest of the calculations, and the total amounts of  $^{14}\text{C}$ -PCB-153 available per sea urchin through the three experiments have been recalculated accordingly (Table 2). In order to correctly assess the



**Fig. 3.**  $^{14}\text{C}$ -PCB-153 concentrations expressed in ng per gram of dry weight ( $\text{ng g}_{\text{dw}}^{-1}$ ) of each body compartment for (a) the “lowPCB highMP” experiment, (b) the “highPCB lowMP” experiment, and (c) the “lowPCB lowMP” experiment. Lipidic  $^{14}\text{C}$ -PCB-153 concentrations ( $\text{ng g}_{\text{lipids}}^{-1}$ ) in the different body compartments for (d) the “lowPCB highMP” experiment, (e) the “highPCB lowMP” experiment, and (f) the “lowPCB lowMP” experiment. Body distribution (%) of  $^{14}\text{C}$ -PCB-153 for (g) the “lowPCB highMP” experiment, (h) the “highPCB lowMP” experiment, and (i) the “lowPCB lowMP” experiment. Mean concentrations or distribution sharing the same superscript do not differ significantly between each other (Dunn test,  $p > 0.05$ ).



accumulation of  $^{14}\text{C}$ -PCB-153 within the sea urchin body due to direct contact with MPs, any contamination via seawater as a result of the leaching of the particles was quantified using control specimens and removed accordingly.

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

On average over the entire duration of the “highPCB lowMP” experiment, an amount of  $0.35 (\pm 0.01, n = 120)$  ng of  $^{14}\text{C}$ -PCB-153 was available per MP (Table 2). In addition, throughout this experiment, an average of 1156 MPs ( $\pm 4, n = 28$ ) and 1065 MPs ( $\pm 26, n = 8$ ) were available per experimental aquarium and in the control aquarium, respectively. Based on both the average  $^{14}\text{C}$ -PCB-153 concentration per MP and on the quantity of MPs available per aquarium over the 15 days exposure, 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 estimated to be available by experimental aquarium and in the control aquarium, respectively (Table 2). On average, during the 15 days of exposure, the total amount of  $^{14}\text{C}$ -PCB-153 available per aquarium was not significantly different between aquariums (Kruskal-Wallis  $X^2_2 = 3.854, p\text{-val} = 0.697$ ). Therefore, the six sea urchins in contact with the MPs during the “highPCB lowMP” experiment were all exposed to comparable  $^{14}\text{C}$ -PCB-153 levels. On average, over the duration of the experiment, a total amount of 394.49 ( $\pm 16.36, n = 84$ ) of  $^{14}\text{C}$ -PCB-153, loaded on an average number of 1156 ( $\pm 4, n = 28$ ) MPs, was available per sea urchin in contact with the MPs (Table 2). Finally, on average during the 15 days of exposures, the total amount of  $^{14}\text{C}$ -PCB-153 per sea urchin was higher during the “highPCB lowMP” experiment compared to the “lowPCB highMP” experiment (Dunn test,  $p\text{-val} = 0.001$ ).

On average over the duration of the “lowPCB lowMP” experiment, 1201 MPs ( $\pm 5, n = 12$ ) were available per sea urchin in direct contact with MPs. Since on average over the entire duration of the experiment, an amount of  $0.14 (\pm 0.01, n = 36)$  ng of  $^{14}\text{C}$ -PCB-153 was available per MP, this implies that a total amount of 172.71 ( $\pm 9.12, n = 36$ ) of  $^{14}\text{C}$ -PCB-153 was available per aquarium (Table 2). On average, during the 15 days of exposure, the total amount of  $^{14}\text{C}$ -PCB-153 available per aquarium was not significantly different between aquariums (Kruskal-Wallis  $X^2_2 = 2.782, p\text{-val} = 0.427$ ). Therefore, the four sea urchins in contact with the MPs during the “lowPCB lowMP” experiment were all exposed to comparable  $^{14}\text{C}$ -PCB-153 levels. Finally, on average during the 15 days of exposure, the total amount of  $^{14}\text{C}$ -PCB-153 per sea urchin during the “lowPCB lowMP” experiment was lower than that of the “highPCB lowMP” experiment (Dunn test,  $p\text{-val} = 3.3 \times 10^{-12}$ ) but not of the “lowPCB highMP” experiment (Dunn test,  $p\text{-val} = 0.113$ ) (Table 2).

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

No sea urchin mortality was detected, nor was there any evidence of a spontaneous spawning event during the course of the three

experiments. Based on daily observations, the sea urchins displayed optimal health conditions (e.g. climbing ability, straightened spines) throughout the exposure period of all experiments. It was noted that the sea urchins used in the “lowPCB highMP” experiment (three sea urchins per aquarium) were more mobile (i.e. increased displacement) during the whole experiment than the sea urchins used in the “highPCB lowMP” and the “lowPCB lowMP” experiments (one sea urchin per aquarium).

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

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

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

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

The body wall, digestive wall and gonads displayed the highest proportions of accumulated  $^{14}\text{C}$ -PCB-153 for all experiments, except for

the “lowPCB lowMP” experiment where  $^{14}\text{C}$ -PCB-153 was not found to concentrate within the body wall (Fig. 3). For all experiments, the body distribution of  $^{14}\text{C}$ -PCB-153 was not significantly different among the body wall, digestive wall, and gonads (p-values of Dunn test were all  $>0.05$ ; Tables S5a, S5b, S5c). For the “lowPCB highMP” experiment, the average proportion of  $^{14}\text{C}$ -PCB-153 accumulated by the body wall was not significantly higher than that of the digestive wall or gonads (Fig. 3G; Dunn test,  $p\text{-val}_{\text{TEG-TD}} = 1$ ,  $p\text{-val}_{\text{TEG-GON}} = 1$ ), but significantly higher than that of the Aristotle’s lantern and coelomic fluid (Fig. 3G; Dunn test,  $p\text{-val}_{\text{TEG-AL}} = 0.003$ ,  $p\text{-val}_{\text{TEG-LC}} = 0.001$ ). Moreover, in this experiment, the average proportions of  $^{14}\text{C}$ -PCB-153 accumulated by the digestive wall and by the gonads were significantly higher than that of coelomic liquid (Fig. 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 lantern (Fig. 3G; Dunn test,  $p\text{-val}_{\text{TD-AL}} = 0.105$ ,  $p\text{-val}_{\text{GON-AL}} = 0.129$ ). For the “highPCB lowMP” experiment, the average proportion of  $^{14}\text{C}$ -PCB-153 contained in the digestive wall and gonads were not significantly higher than that of the body wall and Aristotle’s lantern (Fig. 3H; Dunn test,  $p\text{-val}_{\text{TD-TEG}} = 1$ ,  $p\text{-val}_{\text{GON-TEG}} = 1$ ,  $p\text{-val}_{\text{TD-AL}} = 0.079$ ,  $p\text{-val}_{\text{GON-AL}} = 0.530$ ), but higher than that of the coelomic fluid (Fig. 3H; Dunn test,  $p\text{-val}_{\text{TD-LC}} = 0.001$ ,  $p\text{-val}_{\text{GON-LC}} = 0.016$ ). In this experiment, the distribution of  $^{14}\text{C}$ -PCB-153 was not significantly different between the body wall, Aristotle’s lantern and coelomic fluid (Fig. 3H; Dunn test,  $p\text{-val}_{\text{TEG-AL}} = 1$ ,  $p\text{-val}_{\text{TEG-LC}} = 0.236$ ,  $p\text{-val}_{\text{AL-LC}} = 1$ ). For the “lowPCB lowMP” experiment, the distribution of  $^{14}\text{C}$ -PCB-153 was different between the different body compartments (Kruskal-Wallis  $\chi^2_4 = 13.614$ ,  $p\text{-val} = 0.009$ ), but pairwise multiple comparison analyses are inconclusive (Fig. 3I; all p-values of Dunn test were all  $>0.05$ ; Table S5c).

#### 3.4. Assessment of the transfer efficiency of MP-loaded $^{14}\text{C}$ -PCB-153 to sea urchin tissues

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

#### 4. Discussion

Although globally banned since the 1980’s, polychlorinated biphenyls (PCBs) still persist in the marine environment (e.g. Beyer and Biziuk, 2009; Reddy et al., 2019) and are omnipresent on microplastics from all over the world (Ohgaki et al., 2021). In the present work, the most abundant PCB congener in marine biota (i.e. PCB-153) was shown to be transferred and accumulated by sea urchins from MPs in all experiments. These findings indicate that the  $^{14}\text{C}$ -PCB-153 adsorbed onto the MPs was bioavailable to sea urchins in direct contact with MPs and suggest that MPs located on the seafloor may act as vectors of PCB-153 contamination for sea urchins even during short-term exposure periods of only fifteen days. Moreover, the fact that a transfer of radiolabelled PCB-153 occurred in the three experiments suggest that the concentration gradient of PCB-153 was negative during each experiment (i.e., higher in MPs than in biological tissues), even though levels of native

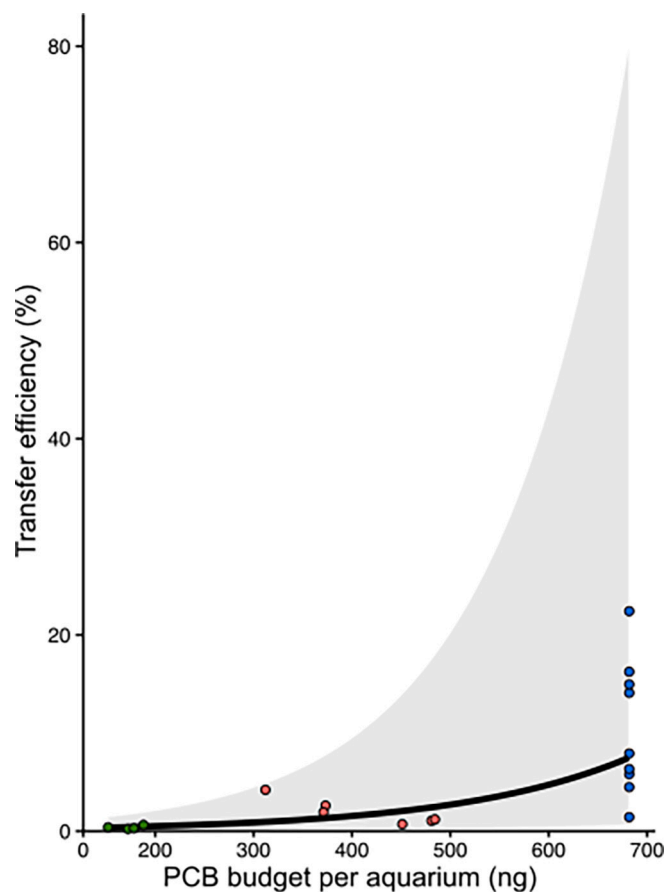


Fig. 4. Assessment of  $^{14}\text{C}$ -PCB-153 transfer efficiencies (%) from the MPs to sea urchins plotted against the  $^{14}\text{C}$ -PCB-153 amount available per aquarium throughout the three experiments. The blue dots correspond to the data of “lowPCB highMP” experiment, the red dots correspond to the data of “highPCB lowMP” experiment, and the green dots correspond to the data of the “lowPCB lowMP” experiment. Black line represents the fitted model with its 95 confidence interval (gray zone). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PCB-153 contained within urchins upon sampling were not analysed in this work.

While this work constitutes the first study on the potential “vector” effect of MPs using adult sea urchins, previous studies have also found a transfer of PCB sorbed onto MPs to biological tissues of seabird chicks (Teuten et al., 2009), lobsters (Devriese et al., 2017), goldfish (Grigorakis and Drouillard, 2018), lugworms (Besseling et al., 2013, 2017), while no transfer of PCB-153 from MPs was found into herring larvae (Norland et al., 2021). The studies that compared the MP-mediated uptake of PCBs to natural bioaccumulation pathways agreed that MP would be a minor contribution to total bioaccumulation comparatively to natural environmental uptake pathways (Devriese et al., 2017; Grigorakis and Drouillard, 2018; Besseling et al., 2013, 2017). Overall, there seems to be a growing consensus among various research groups that microplastics in the environment would have minor effects on bioaccumulation of associated chemicals in organisms (Koelmans et al., 2016, 2022; Besseling et al., 2013, 2017; Bakir et al., 2016; Beckingham and Ghosh, 2017; Devriese et al., 2017; Grigorakis and Drouillard, 2018). In contrast, it has been recently suggested that the current estimates of marine total plastic load could have been greatly underestimated (Lindeque et al., 2020), making the abundance of plastic debris and their relative importance compared to natural particles still unknown. Another challenge of clarifying the uncertainties regarding the importance of MPs as carriers of co-contaminants to marine biota is the lack of consistent approaches between experimental methodologies,

making direct comparison difficult if not impossible. Similar to the need of applying standard guidelines and procedures when studying the sorption-desorption kinetics of chemicals bound to MPs and natural particles (Pyl et al., 2021), there is an urgent need to standardise the metrics for the assessment of bioaccumulation when experimentally testing the importance of MPs as carriers of co-contaminants to marine biota (see Besseling et al., 2017 for comparison between different bioaccumulation metrics). The establishment of standardised methods and guidelines would give rise to improvement of the comparability between studies of different research groups, leading towards clarification regarding the uncertainty of the vector effects of MPs on bioaccumulation.

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

The sea urchins from the “lowPCB lowMP” experiment that were exposed to 15 days aged MPs were found to accumulate  $^{14}\text{C}$ -PCB-153 to the lowest degree when compared to the sea urchins from the “lowPCB highMP” and “highPCB lowMP” experiments that were exposed to virgin MPs. The low transfer of PCB from MPs to sea urchin during the “lowPCB lowMP” is most likely due to the fact that the average amount of  $^{14}\text{C}$ -PCB-153 available per aquarium throughout this experiment was lower than for the two other experiments. The variation of PCB accumulation in sea urchins from 15 days aged MPs (potentially coated with a biofilm) compared to virgin particles could be attributed to the difference in surface coating. Porter et al. (2019) showed that the grazing activity of *P. lividus* onto macroplastics was significantly higher when the plastic surface was biofouled rather than “clean” (i.e. not fouled). These authors also observed the highest grazing activity when sea urchins were exposed to both biofouled macroplastics and kelp simultaneously, which is in disagreement with our results of transfer. Our findings of transfer are also in contradiction with the statement that aged MPs would constitute a higher uptake pathway than virgin MPs, both given their higher absorbance capacity of co-contaminants (Johansen et al., 2018) and their higher likelihood to be ingested by organisms as they may not be recognised as inorganic matter (Fabra et al., 2021; Vroom et al., 2017; Porter et al., 2019). However, it is noteworthy that the MPs used in our “lowPCB lowMP” experiment were contaminated by PCB-153 before being left for 15 days to age in sea water, whereas the MPs used the previously cited work were contaminated by co-contaminants after the aging process. We speculate that aging the MPs prior to their contamination by co-contaminant, rather than aging the particles while the sorption of the co-contaminant already occurred, makes the sorbed co-contaminant more available to biological tissues upon ingestion by organisms. To test this hypothesis, further work comparing the bioavailability of chemicals loaded onto the two types of aged MPs is required. Nevertheless, our results suggest that PCB-loaded MPs, aged in a non-contaminated conditions are still a potential source of PCB-153 upon their ingestion by organisms.

Over the exposure period of the three experiments, digestive wall

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

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

In this work, the  $^{14}\text{C}$ -PCB-153 concentrations based on the total lipids contents did not allow to better discriminate between the different body compartments than the concentrations expressed in terms of dry weight. We hypothesize that a longer exposure time could trigger a predominant accumulation of  $^{14}\text{C}$ -PCB-153 within the body compartments containing the highest lipid contents (such as gonads), which could potentially lead to a better discrimination between the different body compartments. To test this hypothesis, further work investigating the influence of exposure time on the lipidic accumulation of PCB-153 is required. This is probably due to the fact that the total lipid contents were determined prior to experiments on individuals other than those exposed in this study, whereas the dry weights of each body compartment of exposed sea urchin were measured as part of the sample processing. Biological samples were not available in sufficient quantities to enable both the analysis of their total lipid content and their  $^{14}\text{C}$ -radioactivity measure in this work. In order to strengthen the findings of future studies, it is advised to analyse the total lipid content of every body compartment from every organism used experimentally when biological samples are available in sufficient quantities. It is also encouraged for future work to continue to express the PCB concentrations based on the total lipids content given the lipophilicity of the contaminants. In addition, the PCB concentrations expressed on a total lipid content basis are particularly valuable information given the seasonal transferability of lipids, and their associated contaminants, across the body parts observed for various organisms including for *Paracentrotus lividus* (Fernandez, 1998; Rocha et al., 2019; Anedda et al., 2021). In general for *Paracentrotus lividus*, the highest lipids content corresponds to a prevalence of recovery gametogenic stage (accumulation of reserves during the summer season), and the lowest lipids content in gonads corresponds to a high occurrence of mature gametogenic stages (reserves used for gamete development during the winter season) (Fernandez, 1998; Anedda et al., 2021). In order to investigate the seasonality of the MP-mediated transfer of PCB, further work mimicking the present study while using adult *P. lividus* in a different gonadic



maturation stage than ours is required.

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

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

## 5. Conclusion and perspectives

The present study reports for the first time a microplastic-mediated transfer of a key PCB congener into adult specimens of the sea urchin *Paracentrotus lividus*. These findings suggests that the <sup>14</sup>C-PCB-153 adsorbed onto the MPs was bioavailable to sea urchins across all experiments and that these MPs acted as vectors of <sup>14</sup>C-PCB-153 even during short-term exposure periods of only fifteen days. In order to clarify the uncertainty regarding the vector effects of MPs, further experimental studies investigating the extent at which MPs act as vectors for co-contaminants, compared to other naturally occurring particles, are much needed. It is advised for future studies to keep in mind that the relevance of MPs as vectors of co-contaminants depends on various factors such as the gradient of co-contaminant concentrations between MPs and the organisms (Koelmans et al., 2013). A truly comprehensive understanding of the importance of MPs as vectors for associated chemicals will, in turn, eventually give rise to a better characterisation of MPs as a threat to organisms, potentially leading towards more rigorous risk assessments of microplastic for human health and environment. The relevance of further study of MP pollution is evident when taking into account the predicted increase in MP abundance in the world's oceans in the coming years (Stollberg et al., 2021). In order to enhance our understanding regarding the extent at which MPs act as vectors for co-contaminants, further experimental studies investigating their role in the bioaccumulation of a wide range of contaminants, by different organisms, and at various pollution levels compared to other naturally occurring particles is much needed (Rodrigues et al., 2019). The use of radiolabelled chemicals to address this knowledge gap shows to be highly promising, as radiotracer techniques offer strong assets in terms of rapidity, accuracy and sensitivity (Besson et al., 2020; Johansen et al., 2018; Lanctôt et al., 2018). It is also advised for future work to assess the relevance of MPs for bioaccumulation of associated chemicals under environmentally realistic exposure conditions. In this work, as the concentrations of PCB-153 used were in the highest range of the PCBs concentration detected on MPs in the field, ranging from 0.2 to 7554 ng g<sup>-1</sup> (Campanale et al., 2020; Taniguchi et al., 2016), it is recommended

to improve the experimental set up of future studies by selecting environmentally realistic concentrations of PCBs or other co-contaminants. In addition, for studies aiming at further investigating the vector effect of sinking MPs to benthic grazers in particular, it is recommended to add sediments to the aquariums in order to enable appropriate comparison between the experimental and environmental levels of MPs (in terms of kg of dry sediment) and to mimic a more realistic situation.

## CRedit authorship contribution statement

**Marine Pyl:** Conceptualization, Investigation, Visualization, Data curation, Data analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Angus Taylor:** Conceptualization, Investigation, Visualization, Data curation, Methodology, Writing – review & editing. **François Oberhänsli:** Supervision, Writing – review & editing. **Marc Besson:** Data analysis, Visualization, Writing – review & editing. **Peter Swarzenski:** Writing – review & editing. **Bruno Danis:** Conceptualization, Supervision, Writing – review & editing. **Marc Metian:** Conceptualization, Methodology, Visualization, Resources, Supervision, Project administration, Funding acquisition, Writing – review & editing.

## Declaration of competing interest

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

## Data availability

Data will be made available on request.

## Acknowledgments

We would like to acknowledge financial support for the conduct of the study from the governments of the USA, Sweden and the UK under the IAEA's Peaceful Uses Initiative (PUI). We thank the divers of the Laboratoire d'Océanographie de Villefranche-sur-Mer for the collection of the sea urchins. The authors also would like to thank Imma Tolosa for her advice on the total lipid content analysis. The IAEA is grateful for the support provided to its Marine Environment Laboratories by the Government of the Principality of Monaco. This study was supported by the IAEA, the Marine Biology Laboratory of the Université Libre de Bruxelles and by the Fonds National de la Recherche Scientifique, Belgium (FNRS grant n°40004399).

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2022.114322>.

## References

- Adhikari, P.L., Bam, W., Campbell, P.L., Oberhänsli, F., Metian, M., Besson, M., Jacob, H., Swarzenski, P.W., 2022. Evaluating microplastic experimental design and exposure studies in aquatic organisms. In: *Microplastic in the Environment: Pattern and Process*. Springer, Cham, pp. 69–85.
- Andrew, N.L., Agatsuma, Y., Ballesteros, E., Bazhin, A.G., Creaser, E.P., Barnes, D.K.A., Botsford, L.W., Bradbury, A., Campbell, A., Dixon, J.D., Einarsson, S., 2002. Status and management of world sea urchin fisheries. *Oceanogr. Mar. Biol. Annu. Rev.* 40, 343–425.
- Anedda, R., Siliani, S., Melis, R., Loi, B., Baroli, M., 2021. Lipid metabolism of sea urchin *Paracentrotus lividus* in two contrasting natural habitats. *Sci. Rep.* 11, 14174. <https://doi.org/10.1038/s41598-021-93669-9>.
- Bakir, A., O'Connor, I.A., Rowland, S.J., Hendriks, A.J., Thompson, R.C., 2016. Relative importance of microplastics as a pathway for the transfer of hydrophobic organic chemicals to marine life. *Environ. Pollut.* 219, 56–65. <https://doi.org/10.1016/j.envpol.2016.09.046>.
- Beckingham, B., Ghosh, U., 2017. Differential bioavailability of polychlorinated biphenyls associated with environmental particles: microplastic in comparison to

- wood, coal and biochar. *Environ. Pollut.* 220, 150–158. <https://doi.org/10.1016/j.envpol.2016.09.033>.
- Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). *Environ. Sci. Technol.* 47, 593–600. <https://doi.org/10.1021/es302763x>.
- Besseling, E., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2017. The effect of microplastic on the uptake of chemicals by the lugworm *Arenicola marina* (L.) under environmentally relevant exposure conditions. *Environ. Sci. Technol.* 51, 8795–8804. <https://doi.org/10.1021/acs.est.7b02286>.
- Besson, M., Jacob, H., Oberhaensli, F., Taylor, A., Swarzenski, P.W., Metian, M., 2020. Preferential adsorption of Cd, Cs and Zn onto virgin polyethylene microplastic versus sediment particles. *Mar. Pollut. Bull.* 156, 111223 <https://doi.org/10.1016/j.marpolbul.2020.111223>.
- Beyer, A., Biziuk, M., 2009. Environmental fate and global distribution of polychlorinated biphenyls. In: Whitacre, D.M. (Ed.), *Reviews of Environmental Contamination and Toxicology*, 201. Springer, US, Boston, MA, pp. 137–158. [https://doi.org/10.1007/978-1-4419-0032-6\\_5](https://doi.org/10.1007/978-1-4419-0032-6_5).
- Boudouresque, C.F., Verlaque, M., 2020. Chapter 26 - Paracentrotus lividus. In: Lawrence, J.M. (Ed.), *Developments in Aquaculture and Fisheries Science, Sea Urchins: Biology and Ecology*. Elsevier, pp. 447–485. <https://doi.org/10.1016/B978-0-12-819570-3.00026-3>.
- Campanale, C., Dierkes, G., Massarelli, C., Bagnuolo, G., Uricchio, V.F., 2020. A relevant screening of organic contaminants present on freshwater and pre-production microplastics. *Toxics* 8, 100. <https://doi.org/10.3390/toxics8040100>.
- Claereboudt, M., Jangoux, M., 1985. Conditions de digestion et activité de quelques polysaccharidases dans le tube digestif de l'oursin *Paracentrotus lividus* (Echinodermata). *Biochem. Syst. Ecol.* 13, 51–54. [https://doi.org/10.1016/0305-1978\(85\)90013-4](https://doi.org/10.1016/0305-1978(85)90013-4).
- Cole, M., Lindeque, P., Halsband, C., Galloway, T.S., 2011. Microplastics as contaminants in the marine environment: a review. *Mar. Pollut. Bull.* 62, 2588–2597. <https://doi.org/10.1016/j.marpolbul.2011.09.025>.
- Collard, M., Laitat, K., Moulin, L., Catarino, A.I., Grosjean, P., Dubois, P., 2013. Buffer capacity of the coelomic fluid in echinoderms. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 166, 199–206. <https://doi.org/10.1016/j.cbpa.2013.06.002>.
- Coyle, R., Hardiman, G., Driscoll, K.O., 2020. Microplastics in the marine environment: a review of their sources, distribution processes, uptake and exchange in ecosystems. *Case Stud. Chem. Environ. Eng.* 2, 100010 <https://doi.org/10.1016/j.csee.2020.100010>.
- Danis, B., Cotret, O., Teyssié, J.L., Bustamante, P., Fowler, S.W., Warnau, M., 2005. Bioaccumulation of PCBs in the sea urchin *Paracentrotus lividus*: seawater and food exposures to a 14C-radiolabelled congener (PCB#153). *Environ. Pollut.* 135, 11–16. <https://doi.org/10.1016/j.envpol.2004.10.011>.
- de Sá, L.C., Oliveira, M., Ribeiro, F., Rocha, T.L., Futter, M.N., 2018. Studies of the effects of microplastics on aquatic organisms: what do we know and where should we focus our efforts in the future? *Sci. Total Environ.* 645, 1029–1039. <https://doi.org/10.1016/j.scitotenv.2018.07.207>.
- Devriese, L.I., De Witte, B., Vethaak, A.D., Hostens, K., Leslie, H.A., 2017. Bioaccumulation of PCBs from microplastics in Norway lobster (*Nephrops norvegicus*): an experimental study. *Chemosphere* 186, 10–16. <https://doi.org/10.1016/j.chemosphere.2017.07.121>.
- Engler, R.E., 2012. The complex interaction between marine debris and toxic chemicals in the ocean. *Environ. Sci. Technol.* 46, 12302–12315. <https://doi.org/10.1021/es3027105>.
- Fabra, M., Williams, L., Watts, J.E.M., Hale, M.S., Couceiro, F., Preston, J., 2021. The plastic trojan horse: biofilms increase microplastic uptake in marine filter feeders impacting microbial transfer and organism health. *Sci. Total Environ.* 797, 149217 <https://doi.org/10.1016/j.scitotenv.2021.149217>.
- Fenaux, L., 1968. Maturation des gonades et cycle saisonnier des larves chez *A. lixula*, *P. lividus* et *P. microtuberculatus* (Echinides) à Villefranche-sur-Mer. *Vie Milieu* 19 (A1), 1–52.
- Fernandez, C., 1998. Seasonal changes in the biochemical composition of the Edible Sea urchin *Paracentrotus lividus* echinodermata: echinoidea in a lagoonal environment. *Mar. Ecol.* 19, 1–11. <https://doi.org/10.1111/j.1439-0485.1998.tb00449.x>.
- Frias, J.P.G.L., Nash, R., 2019. Microplastics: finding a consensus on the definition. *Mar. Pollut. Bull.* 138, 145–147. <https://doi.org/10.1016/j.marpolbul.2018.11.022>.
- GESAMP, 2015. Sources, fate and effects of microplastics in the marine environment: part 2 of a global assessment. In: Kershaw, P.J., Rochman, C.M. (Eds.), (Eds.), *Reports and Studies - IMO/FAO/UNESCO-IOC/WMO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) Eng No. 93*.
- Grigorakis, S., Drouillard, K.G., 2018. Effect of microplastic amendment to food on diet assimilation efficiencies of PCBs by fish. *Environ. Sci. Technol.* 52, 10796–10802. <https://doi.org/10.1021/acs.est.8b02497>.
- Hartmann, N.B., Rist, S., Bodin, J., Jensen, L.H., Schmidt, S.N., Mayer, P., Meibom, A., Baun, A., 2017. Microplastics as vectors for environmental contaminants: exploring sorption, desorption, and transfer to biota. *Integr. Environ. Assess. Manag.* 13, 488–493. <https://doi.org/10.1002/ieam.1904>.
- Hartmann, N.B., Hüffer, T., Thompson, R.C., Hassellöv, M., Verschoor, A., Dagaard, A. E., Rist, S., Karlsson, T., Brennholt, N., Cole, M., Herrling, M.P., Hess, M.C., Ivleva, N. P., Lusher, A.L., Wagner, M., 2019. Are we speaking the same language? Recommendations for a definition and categorization framework for plastic debris. *Environ. Sci. Technol.* 53, 1039–1047. <https://doi.org/10.1021/acs.est.8b05297>.
- Hennicke, A., Macrina, L., Malcolm-Mckay, A., Miliou, A., 2021. Assessment of microplastic accumulation in wild *Paracentrotus lividus*, a commercially important sea urchin species, in the Eastern Aegean Sea, Greece. *Region. Stud. Mar. Sci.* 45, 101855. <https://doi.org/10.1016/j.rsma.2021.101855>.
- Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environ. Sci. Technol.* 46, 3060–3075. <https://doi.org/10.1021/es2031505>.
- Jabeen, K., Li, B., Chen, Q., Su, L., Wu, C., Hollert, H., Shi, H., 2018. Effects of virgin microplastics on goldfish (*Carassius auratus*). *Chemosphere* 213, 323–332. <https://doi.org/10.1016/j.chemosphere.2018.09.031>.
- Johansen, M.P., Prentice, E., Cresswell, T., Howell, N., 2018. Initial data on adsorption of Cs and Sr to the surfaces of microplastics with biofilm. *J. Environ. Radioact.* 190–191, 130–133. <https://doi.org/10.1016/j.jenvrad.2018.05.001>.
- Koelmans, A.A., Besseling, E., Wegner, A., Foekema, E.M., 2013. Plastic as a carrier of POPs to aquatic organisms: a model analysis. *Environ. Sci. Technol.* 47 (14), 7812–7820.
- Koelmans, A.A., Bakir, A., Burton, G.A., Janssen, C.R., 2016. Microplastic as a vector for chemicals in the aquatic environment: critical review and model-supported reinterpretation of empirical studies. *Environ. Sci. Technol.* 50, 3315–3326. <https://doi.org/10.1021/acs.est.5b06069>.
- Koelmans, A.A., Diepens, N.J., Mohamed Nor, N.H., 2022. Weight of evidence for the microplastic vector effect in the context of chemical risk assessment. In: Bank, M.S. (Ed.), *Microplastic in the Environment: Pattern and Process, Environmental Contamination Remediation and Management*. Springer International Publishing, Cham, pp. 155–197. [https://doi.org/10.1007/978-3-030-78627-4\\_6](https://doi.org/10.1007/978-3-030-78627-4_6).
- Lancôt, C.M., Al-Sid-Cheikh, M., Catarino, A.I., Cresswell, T., Danis, B., Karapanagioti, H.K., Mincer, T., Oberhansli, F., Swarzenski, P., Tolosa, I., Metian, M., 2018. Application of nuclear techniques to environmental plastics research. *J. Environ. Radioact.* 192, 368–375. <https://doi.org/10.1016/j.jenvrad.2018.07.019>.
- Lindeque, P.K., Cole, M., Coppock, R.L., Lewis, C.N., Miller, R.Z., Watts, A.J.R., Wilson-McNeal, A., Wright, S.L., Galloway, T.S., 2020. Are we underestimating microplastic abundance in the marine environment? A comparison of microplastic capture with nets of different mesh-size. *Environ. Pollut.* 265, 114721 <https://doi.org/10.1016/j.envpol.2020.114721>.
- Manzo, S., Schiavo, S., 2022. Physical and chemical threats posed by micro(nano)plastic to sea urchins. *Sci. Total Environ.* 808, 152105 <https://doi.org/10.1016/j.scitotenv.2021.152105>.
- Metian, M., Renaud, F., Oberhansli, F., Teyssié, J.-L., Temara, A., Warnau, M., 2019. Biokinetics of the anionic surfactant linear alkylbenzene sulfonate (LAS) in the marine fish *Sparus aurata*: investigation via seawater and food exposure pathways. *Aquat. Toxicol.* 216, 105316 <https://doi.org/10.1016/j.aquatox.2019.105316>.
- Meziti, A., Kormas, K.A., Pancucci-Papadopoulou, M.-A., Thessalou-Legaki, M., 2007. Bacterial phylotypes associated with the digestive tract of the sea urchin *Paracentrotus lividus* and the ascidian *Microcosmus* sp. *Russ. J. Mar. Biol.* 33, 84–91. <https://doi.org/10.1134/S1063074007020022>.
- Miller, M.E., Hamann, M., Kroon, F.J., 2020. Bioaccumulation and biomagnification of microplastics in marine organisms: a review and meta-analysis of current data. *PLOS ONE* 15, e0240792. <https://doi.org/10.1371/journal.pone.0240792>.
- Murano, C., Agnisola, C., Caramiello, D., Castellano, I., Casotti, R., Corsi, I., Palumbo, A., 2020. How sea urchins face microplastics: uptake, tissue distribution and immune system response. *Environ. Pollut.* 264, 114685 <https://doi.org/10.1016/j.envpol.2020.114685>.
- Murano, C., Donnarumma, V., Corsi, I., Casotti, R., Palumbo, A., 2021. Impact of microbial colonization of polystyrene microbeads on the toxicological responses in the sea urchin *Paracentrotus lividus*. *Environ. Sci. Technol.* <https://doi.org/10.1021/acs.est.1c00618>.
- Norland, S., Vorkamp, K., Bøgevik, A.S., Koelmans, A.A., Diepens, N.J., Burgerhout, E., Hansen, Ø.J., Puvanendran, V., Ronnestad, I., 2021. Assessing microplastic as a vector for chemical entry into fish larvae using a novel tube-feeding approach. *Chemosphere* 265, 129144. <https://doi.org/10.1016/j.chemosphere.2020.129144>.
- Ogata, Y., Takada, H., Mizukawa, K., Hirai, H., Iwasa, S., Endo, S., Mato, Y., Saha, M., Okuda, K., Nakashima, A., Murakami, M., Zurcher, N., Booyatumanondo, R., Zakaria, M.P., Dung, L.Q., Gordon, M., Miguez, C., Suzuki, S., Moore, C., Karapanagioti, H.K., Weerts, S., McClurg, T., Burres, E., Smith, W., Velkenburg, M. V., Lang, J.S., Lang, R.C., Laursen, D., Danner, B., Stewardson, N., Thompson, R.C., 2009. International pellet watch: global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. *Mar. Pollut. Bull.* 58, 1437–1446. <https://doi.org/10.1016/j.marpolbul.2009.06.014>.
- Ohgaki, T., Takada, H., Yoshida, R., Mizukawa, K., Yeo, B.G., Alidoust, M., Hirai, N., Yamashita, R., Tokumaru, T., Watanabe, I., Onwona-Agyeman, S., Gardiner, P., Eriksen, M., Kelly, J.F., Rodríguez-Sierra, C.J., Colasse, L., Baztan, J., Barretto, F.P., Izar, G.M., Abessa, D., Zakaria, M.P., Kwan, C.S., Saha, M., Ryan, P.G., Weerts, S., Ofosu-Anim, J., Sabi, E.B., Akita, L.G., Tait, H., Eriksson, C., Burton, H., 2021. International pellet watch: global monitoring of polybrominated diphenyl ethers (PBDEs) in plastic resin pellets. *Environ. Monit. Contam. Res.* 1, 75–90. <https://doi.org/10.5985/emcr.20210002>.
- Parolini, M., Ferrario, C., De Felice, B., Gazzotti, S., Bonasoro, F., Candia Carnevali, M.D., Ortenzi, M.A., Sugni, M., 2020. Interactive effects between sinking polyethylene terephthalate (PET) microplastics deriving from water bottles and a benthic grazer. *J. Hazard. Mater.* 398, 122848 <https://doi.org/10.1016/j.jhazmat.2020.122848>.
- Pearse, J.S., 2006. Ecological role of Purple Sea urchins. *Science* 314, 940–941. <https://doi.org/10.1126/science.1131888>.
- PlasticsEurope, 2020. Plastics - the facts 2020: an analysis of european plastics production, demand and waste data. <https://plasticseurope.org/knowledge-hub/plastics-the-facts-2020/> accessed 12.21.21.



- Porter, A., Smith, K.E., Lewis, C., 2019. The sea urchin *Paracentrotus lividus* as a bioeroder of plastic. *Sci. Total Environ.* 693, 133621 <https://doi.org/10.1016/j.scitotenv.2019.133621>.
- Pyl, M., Danis, B., Oberhänsli, F., Taylor, A., Swarzenski, P., Metian, M., 2021. An Effective Method to Assess the Sorption Dynamics of PCB Radiotracers Onto Plastic and Sediment Microparticles, 8. *MethodsX*. <https://doi.org/10.1016/j.mex.2021.101395>.
- Pyl, M., Taylor, A., Oberhänsli, F., Swarzenski, P., Hussamy, L., Besson, M., Danis, B., Metian, M., 2022. Size-dependent transfer of microplastics across the intestinal wall of the echinoid *Paracentrotus lividus*. *Aquat. Toxicol.* 106235 <https://doi.org/10.1016/j.aquatox.2022.106235>.
- Reddy, A.V.B., Moniruzzaman, M., Aminabhavi, T.M., 2019. Polychlorinated biphenyls (PCBs) in the environment: recent updates on sampling, pretreatment, cleanup technologies and their analysis. *Chem. Eng. J.* 358, 1186–1207. <https://doi.org/10.1016/j.cej.2018.09.205>.
- Rocha, F., Baião, L.F., Moutinho, S., Reis, B., Oliveira, A., Arenas, F., Maia, M.R.G., Fonseca, A.J.M., Pintado, M., Valente, L.M.P., 2019. The effect of sex, season and gametogenic cycle on gonad yield, biochemical composition and quality traits of *Paracentrotus lividus* along the North Atlantic coast of Portugal. *Sci. Rep.* 9, 2994. <https://doi.org/10.1038/s41598-019-39912-w>.
- Rochman, C.M., 2015. The complex mixture, fate and toxicity of chemicals associated with plastic debris in the marine environment. In: Bergmann, M., Gutow, L., Klages, M. (Eds.), *Marine Anthropogenic Litter*. Springer International Publishing, Cham, pp. 117–140. [https://doi.org/10.1007/978-3-319-16510-3\\_5](https://doi.org/10.1007/978-3-319-16510-3_5).
- Rochman, C.M., Hoh, E., Hentschel, B.T., Kaye, S., 2013. Long-term field measurement of sorption of organic contaminants to five types of plastic pellets: implications for plastic marine debris. *Environ. Sci. Technol.* 47, 1646–1654. <https://doi.org/10.1021/es303700s>.
- Rodrigues, J.P., Duarte, A.C., Santos-Echeandía, J., Rocha-Santos, T., 2019. Significance of interactions between microplastics and POPs in the marine environment: a critical overview. *TrAC Trends Anal. Chem.* 111, 252–260. <https://doi.org/10.1016/j.trac.2018.11.038>.
- Safe, S.H., 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit. Rev. Toxicol.* 24, 87–149. <https://doi.org/10.3109/10408449409049308>.
- Setälä, O., Lehtiniemi, M., Coppock, R., Cole, M., 2018. Chapter 11 - Microplastics in marine food webs. In: Zeng, E.Y. (Ed.), *Microplastic Contamination in Aquatic Environments*. Elsevier, pp. 339–363. <https://doi.org/10.1016/B978-0-12-813747-5.00011-4>.
- Stebbing, A.R.D., Carr, M., Dethlefsen, V., 1992. *Biological Effects of Contaminants in the North Sea: Results of the ICES/IOC Bremerhaven Workshop*. Inter-Research, 91.
- Stollberg, N., Kröger, S.D., Reininghaus, M., Forberger, J., Witt, G., Brenner, M., 2021. Uptake and absorption of fluoranthene from spiked microplastics into the digestive gland tissues of blue mussels, *Mytilus edulis* L. *Chemosphere* 279, 130480. <https://doi.org/10.1016/j.chemosphere.2021.130480>.
- Sugni, M., Barbaglio, A., Bonasoro, F., Gioria, M., Fasano, P., Pasini, M.E., 2013. In: The Role of Models in Science: A Multicomprehensive Experience with the Sea Urchin *Paracentrotus Lividus*. *Procedia - Social and Behavioral Sciences*, 3rd World Conference on Learning, Teaching and Educational Leadership, 93, pp. 1404–1408. <https://doi.org/10.1016/j.sbspro.2013.10.052>.
- Taniguchi, S., Colabuono, F.I., Dias, P.S., Oliveira, R., Fisner, M., Turra, A., Izar, G.M., Abessa, D.M.S., Saha, M., Hosoda, J., Yamashita, R., Takada, H., Lourenço, R.A., Magalhães, C.A., Bicego, M.C., Montone, R.C., 2016. Spatial variability in persistent organic pollutants and polycyclic aromatic hydrocarbons found in beach-stranded pellets along the coast of the state of São Paulo, southeastern Brazil. *Mar. Pollut. Bull.* 106, 87–94. <https://doi.org/10.1016/j.marpolbul.2016.03.024>.
- Teuten, E.L., Saquing, J.M., Knappe, D.R.U., Barlaz, M.A., Jonsson, S., Björn, A., Rowland, S.J., Thompson, R.C., Galloway, T.S., Yamashita, R., Ochi, D., Watanuki, Y., Moore, C., Viet, P.H., Tana, T.S., Prudente, M., Boonyatumanond, R., Zakaria, M.P., Akkavong, K., Ogata, Y., Hirai, H., Iwasa, S., Mizukawa, K., Hagino, Y., Imamura, A., Saha, M., Takada, H., 2009. Transport and release of chemicals from plastics to the environment and to wildlife. *Philos. Trans. R. Soc. B Biol. Sci.* 364, 2027–2045. <https://doi.org/10.1098/rstb.2008.0284>.
- Trenzado, C.E., Hidalgo, F., Villanueva, D., Furné, M., Díaz-Casado, M.E., Merino, R., Sanz, A., 2012. Study of the enzymatic digestive profile in three species of Mediterranean Sea urchins. *Aquaculture* 344–349, 174–180. <https://doi.org/10.1016/j.aquaculture.2012.03.027>.
- Vroom, R.J.E., Koelmans, A.A., Besseling, E., Halsband, C., 2017. Aging of microplastics promotes their ingestion by marine zooplankton. *Environ. Pollut.* 231, 987–996. <https://doi.org/10.1016/j.envpol.2017.08.088>.
- Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: a review. *Environ. Pollut.* 178, 483–492. <https://doi.org/10.1016/j.envpol.2013.02.031>.
- Zettler, E.R., Mincer, T.J., Amaral-Zettler, L.A., 2013. Life in the “plastisphere”: microbial communities on plastic marine debris. *Environ. Sci. Technol.* 47, 7137–7146. <https://doi.org/10.1021/es401288x>.