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Assessing microplastic as a vector for chemical entry into fish larvae using a novel tube-feeding approach

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HIGHLIGHTS

- Microplastic (MP) spheres loaded with PCB-153 were tube-fed to Atlantic herring.
- Fish larvae gut transit time is typically less than 24h.
- There was no transfer of PCB-153 from MP into the larval body.
- This is a novel approach to study absorption from contaminated MP into fish larvae.

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ABSTRACT

A tube-feeding model for administering microplastic (MP, Ø = 30 μm) spheres to fish larvae was employed to quantify the uptake of hydrophobic organic contaminants (HOCs) into the larval body through a single administration of MP. Polychlorinated biphenyl-153 (PCB-153) was used as a representative HOC that can be sorbed to MP in the sea. Atlantic herring (*Clupea harengus*) larvae (34–51 days post-hatching) were selected as the animal model. The herring larvae were tube-fed a single load of up to 200 polystyrene or polyethylene MP spheres spiked with ^{14}C-labelled PCB-153, and the control larvae were tube-fed an isotonic solution without MP. At the time of sampling (24 h post feeding), some larvae had evacuated all MP spheres from the gut, while others still had MP remaining in the gut. In larvae with a significant number of MP spheres still present in the gut, whole-body scintillation counting (including the MP in the gut lumen) showed elevated levels of the tracer compared to those in the control fish larvae. For larvae in which all or almost all MP had been evacuated by the time of sampling, the tracer levels of the whole body were not significantly different compared to those for the control fish larvae. These data indicate that there was no significant transfer of PCB-153 from contaminated MP into fish larvae within a gut-transit time of <24 h. This study suggests that the vector role of MP in HOC uptake and absorption may be minor compared to that of other HOC uptake pathways.

Author statement

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1. Introduction

Plastic debris accumulates in marine environments, ranging from coastal areas to deep sea regions, and poses a serious contamination issue (UNEP, 2016). In addition, plastic particles are known to absorb and perceived to act as vectors for hydrophobic organic contaminants (HOCs) in the environment (Mato et al., 2001; Ogata et al., 2009; Rochman et al., 2013a; Koelmans et al., 2016). HOCs, such as polychlorinated biphenyls (PCBs), have high hydrophobicity, high lipophilicity, low biotransformation, and low elimination rate. Hence, they bioaccumulate and biomagnify in the food chain upon ingestion (Järv et al., 2017; Nelms et al., 2018). PCB-153 is one of the most bioaccumulative and, thus, abundant PCB congeners in aquatic organisms (Safe, 1994).

The abovementioned physico-chemical properties of HOCs favour their sorption by organic materials, including synthetic polymers (Lohmann and Muir, 2010). The rate and extent of HOC sorption into MP depends on the molecular weight and concentration of the specific HOC in the water and plastic phases and the particle size and type of the plastic polymer, which eventually determine the polymer–water partition coefficient (Nau-Ritter et al., 1982; Pascall et al., 2005; Vonderheide et al., 2008; Teuten et al., 2009; Fries and Zarfl, 2012; Huffer and Hofmann, 2016; Zhan et al., 2016).

Microplastic (MP) particles may be contained in direct efflux from industries and households or originate from plastic debris that is slowly degraded by chemical and physical factors (including wind, waves, and UV light). It is well-documented that plastic debris, including MP particles, are ingested by marine organisms (Browne et al., 2008; Cole et al., 2015; Lusher et al., 2015, 2018) and even larvae of Atlantic herring (Hjelmeland et al., 1988). Upon ingestion, MP may not be broken down by enzymes and persist in the gut lumen or be expelled as indigested matter (Hjelmeland et al., 1988). It has been hypothesised that plastic debris may release sorbed HOCs into the digestive tract with a potential uptake into the tissues (Derraik, 2002; Teuten et al., 2009). It is important to understand the mechanisms underlying the release of MP-sorbed HOCs into and their potential absorption by an organism's digestive tract to increase our knowledge of the effects of plastic pollution, especially through the role of plastic as a vector for chemical contaminants.

The composition of the medium surrounding plastics in the digestive tract of an organism, including fatty acid profiles, enzymes, and bile levels, may lead to the release of sorbed HOCs, and potentially increase their bioaccumulation and toxic effects in the organism (Teuten et al., 2007; Gouin et al., 2011; Bakir et al., 2014; Tanaka et al., 2015, 2018). *In vitro* tests have studied desorption rates in artificial gut fluids (Bakir et al., 2014; Mohamed Nor and Koelmans, 2019). *In vivo* tests have been conducted to compare on HOC bioaccumulation in the presence and absence of MP (Rochman et al., 2013a; Besseling et al., 2017). Further, modelling studies have quantitatively evaluated the relative importance of exposure pathways of HOCs under natural conditions (Bakir et al., 2016; Koelmans et al., 2016). The kinetic data for this uptake process have been calculated for a range of chemicals, and an overall interpretation (modelling) framework has been developed (Mohamed Nor and Koelmans, 2019).

PCB-153 is one of the most abundant PCBs detected in organisms (Safe, 1994). Currently available data suggest that HOCs accumulated in marine organisms are derived to a very small extent from ingested MPs (Bakir et al., 2016; Koelmans et al., 2016). The main sources of HOCs in marine organisms are water, sediments, and food (Herzke et al., 2016; Koelmans et al., 2016; Besseling et al., 2017; Clukey et al., 2018). Diepens and Koelmans (2018) presented a theoretical food-web model and an example of an Arctic food web with PCBs and polycyclic aromatic hydrocarbons.

A challenge faced by current studies addressing the absorption of MP-associated HOCs by marine organisms is the lack of control over what is ingested and its quantity and time of ingestion, which are important factors for a proper assessment of the vectoral transfer of chemicals by MP. We have implemented an *in vivo* method for administering MP spheres spiked with HOCs directly into the digestive tract of fish larvae. Using this tube-feeding method, we can administer MP spheres of defined sizes and properties to fish larvae at a known time, instead of relying on voluntary ingestion, which depends on multiple factors, including the swimming ability of the organism and a specific sensory input, which lead to the successful capture and ingestion of MP. The method is based on a tube-feeding protocol originally outlined by Rust and co-workers (Rust et al., 1993) and further developed by Rønnestad and co-workers (Rønnestad et al., 2000b). It has been used to assess the digestion, absorption, and metabolism of proteins, free amino acids, and lipids in a range of species, including post-larval Senegalese sole *Solea senegalensis* (Rønnestad et al., 2000a, 2001a; Morais et al., 2007; Conceicao et al., 2010), Atlantic halibut *Hippoglossus hippoglossus* (Applebaum and Rønnestad, 2004) and Atlantic herring *Clupea harengus* (Koven et al., 2002; Rojas-García et al., 2016). It has been demonstrated that Atlantic herring larvae tolerate handling stress and stress imposed by the tube-feeding method and have low mortality (5–25%; Rønnestad et al., 2001b; Morais et al., 2005).

In this study, we extended the abovementioned tube-feeding method for the administration of MP spheres to study the *in vivo* transfer of HOCs from MP to herring larvae, using PCB-153 as the test HOC component. The results provide *in vivo* data for the release and absorption of PCB-153 from MP spheres into the larvae after a 24 h incubation period, when all or almost all the MPs had passed the gut of the larvae. Our findings provide insights into the chemical vector role of MP for fish larvae.

2. Materials and methods

All animal procedures and handling described in this study were carried out according to Norwegian National legislation via the Norwegian Animal Welfare Act (LOV-2015-06-09-16-65) and Regulations on the Use of Animals in Experiments (FOR-2017-04-05-451), as per the requirement in the EU (Directive 2010/63/EU) for animal experiments. The study was approved by the ethical review board at the University of Bergen and the National Animal Research Authority in Norway (FOTS ID: 15243).

2.1. Microplastic polymers

Polyethylene (PE) and polystyrene (PS) are two of the primary synthetic MP polymers present in the ocean (Erni-Cassola et al., 2019). Spherical MPs of both these polymers were used in the tube-feeding experiments. Low-density PE spheres (CPMS-0.96 27–32 μm) were obtained from Cospheric LLC, Santa Barbara, CA, USA. PS spheres (Calibre CS 30, Ø = 29.67–30.95 μm) were obtained from Microbeads AS, Skedsmokorset, Norway. The PE were shipped as a powder and were used as such, while the PS were delivered suspended in ion-exchanged water (with 0.03% sodium laureth sulfate). To clean the PS spheres, the suspension was filtered, and

the spheres were rinsed with MilliQ water (MilliporeSigma, Bedford, MA, USA) and vacuum filtered with a Durapore membrane filter of pore size 0.45 μm (MilliporeSigma, Bedford, MA., USA). The spheres on the filter were covered with aluminium foil and air dried for 3 d until they were completely dry (i.e., when the PS spheres no longer stuck to the filter) and were thereafter stored in glass vials.

2.2. Pollutant and spike stock solution of PCB-153

Prior to tube-feeding, the PE and PS spheres were spiked with native PCB-153 and ^{14}C-PCB-153. A stock solution was prepared with PCB-153 (2,2′,4,4′,5,5′-hexachlorobiphenyl; 97.7% purity; Chem Service Inc., West Chester, USA) in 2,2,4-trimethylpentane (Isooctane; Holger Hartmann AS, Langhus, Norway; was prepared at a concentration of 1 g L^{-1}). PCB-153 was diluted twice to reduce the amount of isooctane. First, 100 μL of the PCB-153 stock solution was diluted in 15 mL of acetone (Sigma–Aldrich, St. Louis, MO, USA; to obtain a solution with PCB concentration of 6.7 mg L^{-1}). Next, 100 μL of the first PCB diluted solution was diluted in 15 mL of acetone to obtain the spike solution with a PCB-153 concentration of 44 μg L^{-1} ^{14}C-labelled PCB-153 (2,2′,4,4′,5,5′-hexachlorobiphenyl [14C(U)]; 99% purity; specific activity of 0.466 GBq $mmol^{-1}$; dissolved in ethanol) was obtained from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA) and used as a tracer.

2.3. Sorption of contaminants into microplastics

Methanol was used as a co-solvent to load the MP spheres with PCBs (Smedes et al., 2009). The use of a co-solvent improves the sorption rate of PCBs into MP (Pignatello and Xing, 1996; Chawla et al., 2001; Seidensticker et al., 2017).

Clean PE (0.8 g) spheres, and PS (0.1 g) spheres retrieved from filtration, were weighed using a BP 615 scale (Sartorius, Göttingen, Germany). The measured amount of MP was transferred together into 100 mL glass bottles containing 15 mL of methanol, 100 μL of non-labelled PCB-153 spike solution (4.44 ng of PCB-153), and 20 μL of ^{14}C-labelled PCB-153 (74 kBq). The glass bottles were constantly shaken at 175 rpm. After 1 h, 20 mL of MilliQ water was added. This was repeated every hour for the next 4 h, until a 15/80 (*v/v*) methanol/water spike solution was obtained. The spike solution was shaken overnight in the dark at 175 rpm.

The next day, the MP were separated from the spike solution by filtration, washed with a minimum of 500 mL of MilliQ water, and processed as per the description above for the cleaning of PS (Section 2.1.). Since the spiked spheres were washed thoroughly with MilliQ water upon filtration and thereafter dried, any remnant of methanol was expected to have evaporated long before the spheres were used for tube-feeding and not have any influence on the outcome. Samples of filtrate from the spike solution (95 mL) were counted in a liquid scintillation analyser (Section 2.6). The remaining ^{14}C-PCB-153 in the spike solution did not adsorb to the plastic polymer, and the difference was considered as the amount of ^{14}C-PCB-153 that had sorbed to the MP (sorption efficiency).

2.4. Fish larvae

The digestion and transfer of MP-associated PCB-153 was studied in Atlantic herring, an ecologically and commercially relevant fish species (Overholtz and Friedland, 2002) and one of the preferred model species in previous tube-feeding experiments (Conceição et al., 2002; Koven et al., 2002; Morais et al., 2005; Rojas-García et al., 2016).

Norwegian spring spawning herring were caught outside Askøy, Norway (60°24′0″ N, 5°10′6″ E). Eggs (>10,000) and sperm were stripped from one female and one male and incubated at Bergen High Technology Centre, Bergen Norway. Upon hatching 14 d post fertilisation, the larvae were transferred to two 300 L rearing tanks and reared according to the standard protocol (Folkvord et al., 2009).

The larvae were reared in green water (algae-rich) containing *Rhodomonas* sp. algae (reared at Bergen High Technology Centre) and fed *ad libitum* with natural zooplankton, mainly copepod nauplii and copepodites collected from filtered seawater at Espegrend Marine Research Field Station (University of Bergen, Raunefjord, Norway), since 1 day post-hatching (DPH).

To measure the standard length (SL) of the larvae, they were sedated using MS-222 (Sigma–Aldrich, St. Louis, MO, USA) and imaged using a Nikon camera mounted on top of a Leica MZ95 stereomicroscope with graph paper in the background for scale. The SL was measured from the tip of the dorsal lip to the end of the notochord and the beginning of the tail fin using the *ImageJ* software (National Institutes of Health, USA).

2.5. Tube-feeding

The MP spheres were administered by tube-feeding, which allowed a liquid bolus to be deposited within the digestive system of the larvae. The original method involved the administration of a chemical/nutrient dissolved in isotonic solution (Rønnestad et al., 2001b). Additional adjustments and preparations for administering MP spheres in suspension are described in the Supplementary Information (SI Part A).

The time for tube-feeding of one larva was kept as short as possible to reduce handling stress for the larva. The herring larvae have several features that ease tube-feeding and shorten the handling time (Fig. 1A): transparency permits a clear observation of the insertion and localisation of the capillary tip; the gut is straight, and the midgut has an extended diameter compared to that of the foregut; and the filling of the gut can be observed and controlled (Rust et al., 1993; Rønnestad et al., 2001b; Conceição et al., 2007). The procedure was performed by a trained operator, and the total handling time per larva was typically less than 1 min.

Individual larvae were collected with a pipette and sedated with 0.1 g L^{-1} of MS-222 (Sigma–Aldrich, St. Louis, MO, USA) in a 20 mL glass vial, placed gently on a glass slide in a drop of clean seawater under a dissection microscope (Fig. S1D), and visually checked for quality (no deformities, no fin damage, and expected size) before administration of the bolus with MP spheres by tube-feeding. The slide with the larvae was kept cool using a cooling system (Fig. S1D iv) based on circulating water maintained at 8 °C in a Hetofrig CBN 18–30 water bath (Heto, Birkerød, Denmark). Cooled water was recirculated from the water bath to a transparent, flat-sided plastic bottle mounted on the dissection microscope.

The larvae were sampled and tested regularly after the onset of exogenous feeding to find the optimal window for tube-feeding, mainly based on larval robustness (survival), size, and transparency. Older larvae showed high stress resilience, particularly after 26 DPH, and tube-feeding trial with contaminated MP spheres was carried out using only good quality living Atlantic herring larvae between 34 and 51 DPH (n = 84).

The larvae that were collected for tube-feeding were sampled after the morning meal. Based on previous experience, fed larvae have a more easily distensible digestive tract that retains the administered volume better than that of fasted larvae (Rønnestad et al., 2001b). To ensure stability of the larvae on the slide during insertion of the capillary, the slide contained a series of parallelly glued glass capillaries (Fig. 1B) that formed chambers preventing the sideways movement of the larvae during handling and injection.

A glass capillary filled with the PCB-153-spiked MP suspension

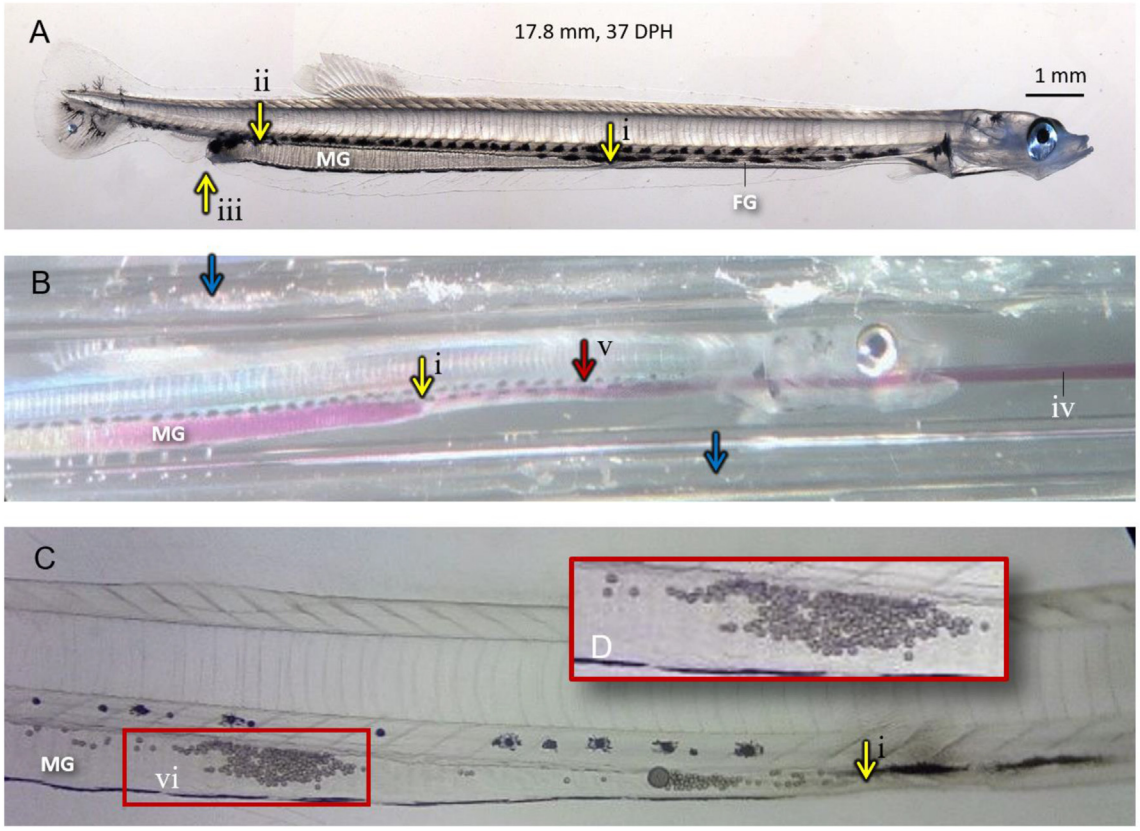

Fig. 1. A: Atlantic herring larva at 37 d post-hatching (photo from a separate batch of larvae). FG: foregut; MG: midgut. The digestive tract has three sphincters that are important for the tube-feeding procedure and are labelled with yellow arrows: valve between FG and MG (i); valve between MG and hindgut (ii); and the anal sphincter (iii). B: Training of the operator with the tube-feeding technique was done using a coloured solution (0.1% chlorophenol red (Sigma-Aldrich, St. Louis, MO, USA) dissolved in phosphate buffered water) to ensure that the capillary was properly inserted, and the MG was filled sufficiently. The capillary (iv) was gently inserted through the mouth and into the oesophagus, where the bolus was administered (Red arrow, v: the tip of the capillary). Blue arrows: a series of parallel glass capillaries glued to the slide. These formed a chamber that stabilised the larvae during handling and injection. C: MP spheres present in the midgut after tube-feeding. D: enlargement of area vi. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

was gently inserted through the mouth opening and halfway down the oesophagus (Fig. 1B), and ~460 nL of the suspension with an indefinite number of MP spheres (up to 200 spheres) was deposited in the gut. The injection volume was adjusted to the estimated maximal gut volume, which was calculated using the formula established for herring larvae: Gut volume (nL) = 0.079 $SL^{2.769}$ (Rojas-García et al., 2016). In addition, administration of the suspension into the gut was visually observed and controlled to ensure that the distension was acceptable, there was no reflux or evacuation, and a significant amount of MP, and not just aqueous solution, was administered (Fig. 1C and D).

After capillary withdrawal, the larvae were transferred to clean seawater for 2–3 min to allow them to recover from the anaesthetics, while the exact number of MP spheres in their digestive tract was counted. Next, the larvae were individually incubated for 24 h in vials containing 20 mL of clean seawater at 8 °C in a Hetofrig water bath. MilliQ water was used to rinse the slide between each larva. Over a course of 17 d, a total of 84 viable larvae were successfully tube-fed (~5 larvae day^{-1}), of which 22 larvae ("Control" group) were administered isotonic solution, while 62 larvae were administered suspended MP spheres.

2.6. Sampling and liquid scintillator detection

The larvae were sampled after 24 h of incubation, as earlier studies have shown that this is the gut-transit time of this species for ingested food (Mazurais et al., 2015; Bråte et al., 2016; Grigorakis et al., 2017). At sampling, the larvae were sacrificed with an overdose of MS-222 (1g L^{-1}), and the number of MP spheres still present in the digestive tract was counted under a stereo dissecting microscope.

Not all larvae had eliminated the MP after 24 h. The larvae were therefore categorised into four groups, depending on the number of MP spheres in the gut at the time of sampling: *"Control"*: tube-fed isotonic solution only; *"MP in gut"*: MP spheres $\geq$11; *"Almost empty"*: 1<MP $\leq$ 10; and *"Empty"*: MP = 0.

The larvae were transferred into individual liquid scintillation glass vials (20 mL, Sigma–Aldrich, St. Louis, MO, USA) with 1 mL of Soluene-350 (PerkinElmer Inc., Downers Grove, IL, USA) and stored for 24 h at room temperature (ca 20 °C), until they were fully dissolved. The vials were shaken vigorously by hand, 5 mL of scintillation cocktail (Ultima Gold XR, PerkinElmer Inc., Downers Grove, IL, USA) was added, and radioactivity in the samples was measured.

The larval incubation water with evacuated MP was shaken vigorously by hand for at least 10 s, 1 mL of the subsample was transferred into a separate scintillation glass vial (20 mL, Sigma–Aldrich, St. Louis, MO, USA), and 5 mL of Ultima Gold XR was added. The radioactivity of all samples was measured using a Tri-Carb 2900 TR Liquid Scintillation Analyser (PerkinElmer Inc., Downers Grove, IL, USA).

2.7. Statistics

Data were analysed for normality using the Shapiro-Wilk test for normal distribution, but the assumptions of normality were not met, and non-parametric tests were used. The statistical analysis for determining the significance level was therefore performed for paired, non-parametric data with the Wilcoxon test with Bonferroni correction ($\alpha = 0.05$) using the R software (R Core Team, Vienna, Austria, 2019). Outliers for the number of MP spheres passing through the gut were identified using linear regression and correlation coefficient ($R^2 > 0.90$) and were excluded from statistical analysis. We were unable to identify the underlying reason for the presence of outliers upon scrutiny of the data.

3. Results and discussion

3.1. Behaviour of plastic polymers during tube-feeding

The applied plastic polymers, PS and low-density PE, are two of the primary synthetic polymers found in the ocean, together with polypropylene (PP) and polyvinyl chloride (PVC) (Mato et al., 2001; Rios et al., 2007; Rochman et al., 2013b; do Sul and Costa, 2014). In line with the report by Baker and Mead (2000), we found that both PE and PS tended to float and aggregate on the surface of an isotonic solution, creating a heterogeneous MP layer on the surface. This tendency was much more pronounced for PE than for PS, most likely because the former has a specific density (0.96 kg L^{-1}) lower than that of water. For both MP, aggregation at the isotonic solution surface led to challenges in performing parts of the procedure of tube-feeding. The PE layer tended to fold around hypodermic steel needles and glass capillary tubes and moved upward, along the walls inside glass vials in which MP spheres were stored. This prevented the glass capillaries from being filled by suction. The PS MP spheres were easier to handle, formed weaker surface layers, and had a reduced tendency for clogging. The clogging behaviour was the main reason why variable amounts of MP spheres were administered during tube-feeding. The final method that worked best for both MP spheres is described in Section 2.5.

3.2. Spiking efficiency of PCB-153 to different plastic polymers

The sorption efficiency following this protocol was high. After spiking the MP spheres, the filtered spike solution contained only 3% and 4% of the original 74 kBq of ^{14}C-PCB-153 added for PS and PE, respectively. Thus, 97% and 96% of the added tracer would have bound to the PS and PE MP spheres, respectively, assuming negligible losses through glass adsorption. However, liquid scintillation counting (LSC) of the spiked MP spheres detected only 12% and 81% of the added ^{14}C-PCB-153 for PS and PE, respectively (for a more detailed description, see Supplementary Information - SI Part B). The remaining activity, which was not counted or recovered, was presumably that of the ^{14}C-PCB-153 absorbed into the MP matrix. LSC measures radioactivity (beta particles; electrons emitted by ^{14}C) by mixing the radioactive chemical with the liquid scintillator and counting the resultant emission of photons. Plastic is a well-known shield that efficiently absorbs beta particles. Thus, if a radioactive chemical is sorbed into a MP, the electrons from the tracer in the MP matrix will not react with the scintillator liquid at the surface of the particle. The split of countable and uncountable ^{14}C-PCB-153 also points towards the biphasic diffusion model, in which the sorption process of contaminants occurs as surface adhesion (adsorption) and diffusion into the inner plastic (absorption) (Pignatello and Xing, 1996; Teuten et al., 2009; Lee et al., 2014; Mohamed Nor and Koelmans, 2019). Desorption occurs in the reverse manner.

Any ^{14}C-PCB-153 that was absorbed into the larval tissue would have been detected efficiently by the LSC method. The different solvents in Soluene-350 (PerkinElmer) degraded and dissolved all tissues quickly. Thus, there was direct contact between ^{14}C-PCB-153 in the tissues and the liquid scintillator, resulting in light emission, which was detected by LSC.

3.3. Transfer of PCB-153 from microplastics

The number of MP spheres administered and counted in the digestive tract just after tube-feeding varied, ranging from 1 to 200 spheres, with 48 MP spheres counted per fish larva on average (Fig. 2).

With the current protocol, there was no practical approach to avoid the large variability in the number of administered MP spheres. Depending on its size, each larva was administered a set volume that contained the saline carrier as well as the MPs present in the saline carrier. The MP spheres tended to aggregate inside the syringe and glass capillary, and, if the number of spheres was too high, the narrow opening of the capillary was clogged. In addition, any aggregations that slowed or stopped the flow of MP spheres would still allow saline to pass between the MP spheres and enter the gut, until the set volume had been administered. Both these problems significantly lowered the number of MP spheres administered. Therefore, it was decided to change the strategy and not aim to deliver the same number of MP spheres to all the fish larvae, but to count the MP spheres present in the digestive tract just after tube-feeding.

After 24 h, a few fish larvae had empty guts and had evacuated all MP spheres (data presented by the blue line in Fig. 2). All the fish larvae had evacuated some MP spheres from the gut, but the number of evacuated MP spheres varied. The number of MP spheres

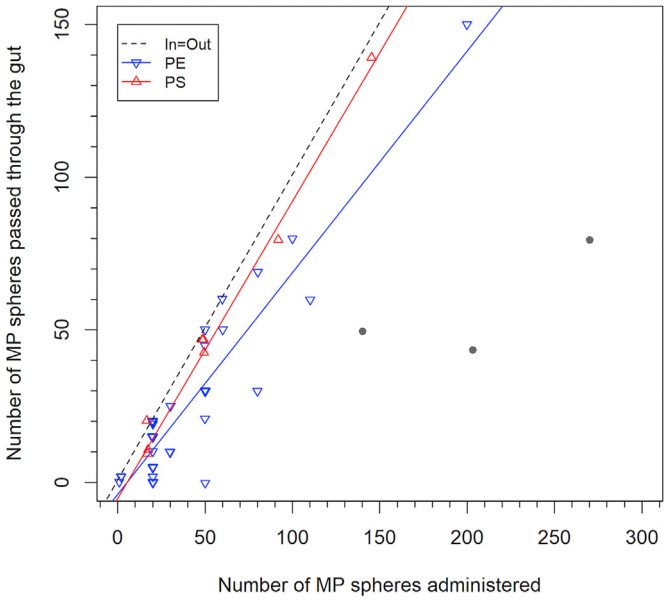

Fig. 2. Number of MP spheres that had passed through the digestive tract of Atlantic herring larvae 24 h after tube-feeding relative to the number of MP spheres administered at time 0 (n = 62). Any fish larva with data presented by the black dashed line (In=Out) had evacuated all the MP spheres administered. The larvae were fed either PE (n = 50, blue triangles) or PS (n = 9, red triangles) spheres. The blue and red lines form the linear regression model fitted to the data (PE: y = 0.73x - 3.81, R^2 = 0.84; PS: y = 0,97x - 5.05, R^2 = 0.99, PE and PS together: y = 0.88x − 8.50, R^2 = 0.91). Three datapoints were excluded in the analysis (grey circles). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

remaining in the guts of all the fish larvae was 13 on average (ranging from 0 to 50 spheres), which was significantly lower (Wilcoxon test, p-value = 0.0016) than the number of MP spheres administered (48). During the experiment, the evacuation time decreased with age of the larvae ($R^2 = 0.45$). However, the number of MP spheres administered remained stable ($R^2 = 0.02$) since time spent on handling the larva and limitations in the injection rate to prevent capillary clogging were standardized. However, we did not see an increase in the number of MP spheres passed through the gut with increasing size of the larvae ($R^2 = 0.1$).

As previously mentioned, we categorised the assessed fish larvae depending on the remaining number of MP spheres in the gut at sampling. The fish larvae in the category "*MP in gut*" (≥11 MP spheres remaining in the gut) had been administered 51 ± 36 MP spheres (mean ± SD) and had 24 ± 11 MP spheres in the gut at the end of the incubation time; thus, 26 ± 30 MP spheres had passed through their digestive tract during the 24 h incubation period. The fish larvae in the group "*Almost empty*" (1≤MP ≤ 10) had been tube-fed 57 ± 58 MP spheres, with only 6 ± 3 MP spheres remaining after incubation and 51 ± 56 MP spheres having passed through the digestive tract. The fish larvae in the last group, "*Empty*" (0 MP), had been fed fewer MP spheres, i.e., 23 ± 16 MP spheres, which had all passed through the gut during the 24 h incubation period.

For larvae that still had MP inside the gut at the end of incubation, we could not determine whether the tracer was in the MP or in the tissue of the fish (i.e., absorbed). However, the data showed that, in larvae in which all or almost all the administered MP had been completely evacuated (Groups "*Empty*" and "*Almost empty*"; Fig. 3), the tracer levels were similar to those in the larvae in the control group that had not been administered any MP, i.e., had no tracers (pairwise comparisons using Wilcoxon Rank Sum test, p-value > 0.79). Thus, we concluded that none of the ^{14}C-PCB-153 had been absorbed into the larval tissue during transit of MP through the gut. The fish larvae in the "*MP in gut*" group had significantly higher tracer counts than those in the control group and the larvae

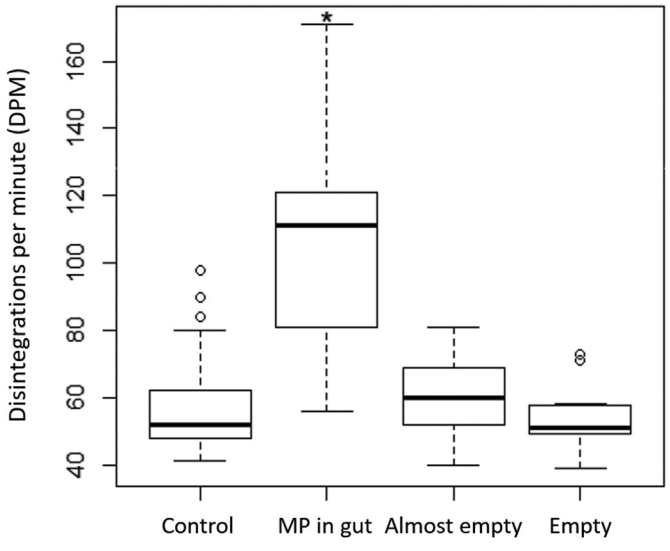

Fig. 3. Activity of ^{14}C-PCB-153 in fish larvae tube-fed with MP spheres compared to that in the larvae of the control group (n = 22). The tube-fed larvae were categorised based on how many MP spheres remained in the gut at sampling after 24 h of feeding: *Control* (0 MP, n = 22), *MP spheres in gut* (≥11 MP, n = 32); *Almost empty* (1≤MP ≤ 10 spheres, n = 19); and *Empty* (0 MP spheres, n = 11) groups. Statistically significant differences (pairwise comparisons using Wilcoxon Rank Sum test) between the groups are marked with an asterisk (*). The group "*MP in gut*" had p-values of 1.3×10^{-5}, 4.6×10^{-6}, and 4.1×10^{-7} compared to the groups "*Almost empty*", "*Empty*", and "*Control*," respectively.

in the "*Almost empty*" and "*Empty*" groups (pairwise comparisons using Wilcoxon Rank Sum test, p_{adj}-value <0.01) (Fig. 3). Thus, we could detect elevated levels of the tracer in the larvae only when the MP were inside the gut. Once the MP were evacuated, the tracer levels decreased to background levels. Taken together, these findings demonstrate that one-time ingestion of PCB-contaminated MP, with a gut-transit time of less than 24 h, will not significantly transfer the contaminant carried by the MP to fish larvae.

It should be noted that the numbers of MP spheres in the guts of the larvae in the present study, which reached up to 200, far exceed the numbers of MP particles observed in the guts of wild-caught larvae (Lusher et al., 2013, 2016; Neves et al., 2015; Nelms et al., 2018). However, since no transfer of PCB-153 to the larval tissue was observed even with such a high number of MP spheres, this factor does not seem to affect the results of the present study. The variability of the data did not allow any conclusion to be drawn regarding whether the properties of PE and PS affected the outcome of the study.

Tube-feeding refers to the direct administration of a bolus into the digestive tract of an animal, by a tube. There are shortfalls and limitations to this process, including the fact that the animal is anesthetised to reduce stress. However, tube-feeding offers the advantage of controlled administration of MP spheres, which will not be possible in set-ups that may be closer to the food uptake situation in the wild. Tube-feeding also allows the study of *in vivo* HOC uptake after MP ingestion. Moreover, digestion is mainly autonomously controlled by the digestive tract. Once an MP sphere is inside the gut, it initiates the secretion of enzymes, reflecting realistic gut fluid conditions. HOC uptake in the gut should thus not be significantly influenced by the method of MP administration. Herring larvae that ingested MP spheres from water had elevated trypsin secretion compared to that of non-feeding fish (Hjelmeland et al., 1988) but lower than that of larvae that ingested live food of the same size as that of MP spheres. Taken together, the data available for herring larvae show that the presence of MP in the gut activates digestion, supporting the physiological relevance of comparing the present data to those for larvae in the wild that ingest MP voluntarily.

The results of this study support those of other recent studies and calculations of vectoral transfer mechanisms for chemicals in nature, indicating that MP is not the main source of HOCs in biological tissues (Koelmans et al., 2013, 2016; Bakir et al., 2016; Besseling et al., 2017). Rather, natural pathways of bioaccumulation must be considered, such as water and, particularly, the contaminants already absorbed by the natural prey (Koelmans et al., 2016; Clukey et al., 2018). The accumulation of HOCs from ingested prey depends on several factors similar to accumulation from MP, such as diffusion over the microvillus surface area, cell permeability, and residence time in different gut segments (Lehman-McKeeman, 2010; Gouin et al., 2011). In addition, the mechanical and enzymatic digestion of the prey also plays a role in HOC accumulation. If HOCs cross the membranes of cells by diffusion or active transport, they enter the circulatory system and accumulate in fatty tissues. Some HOCs, such as PCBs, biomagnify in the food chain, reaching higher concentrations in predators than in prey (Järv et al., 2017; Nelms et al., 2018).

Whether plastics should be considered harmful themselves depends on the particle size and exposure time (Moore, 2006). We observed no visible breakdown or digestion of the MP in the herring gut after 24 h of feeding. This supports earlier findings that MP ingested by marine organisms are not broken down by digestive enzymes, but may rather accumulate and block the digestion and evacuation processes (Wright et al., 2013). Thus, MP may have direct physical effects once it is ingested into the digestive tract. Additionally, MP with sharp edges may lead to soreness of the

epithelium and cause indirect adverse effects. MP have been shown to lead to ulceration and potentially erroneous satiety signalling, decreased fecundity, decreased survival (Cole et al., 2015; Peda et al., 2016; Jovanovic, 2017; Rainieri et al., 2018; Yin et al., 2018), hyperplasia and inflammatory response in the intestine (Peda et al., 2016), induced microbiome dysbiosis, and altered mucosal cell composition (Jin et al., 2018). MP spheres that were used in the present study had a diameter of approximately 30 μm, and MP spheres around this size are quite abundant in the environment (Mintenig et al., 2020). These particles are too large to be absorbed into or penetrate the intestinal wall, as seen for nanoparticles and micro-sized particles with a diameter of approximately 10 μm (Brown et al., 2001; Hussain et al., 2005; Moore, 2006; Rothen-Rutishauser et al., 2007; Browne et al., 2008; Cedervall et al., 2012; Al-Bairuty et al., 2013; Al-Jubory and Handy, 2013). In nature, herring larvae of the size used in this study will actively select and ingest live prey significantly larger than 30 μm in size. In the study by Hjelmeland et al. (1988), 39-days-old herring larvae ingested live prey with a diameter of approximately 190 μm, and the MP used in that study was 94 μm in diameter. At the same time, fish larvae may also ingest smaller particles, either by active ingestion of a particle that is easy to capture, by accident, or with drinking water. It is not known if a larger MP than what is used in our study may have a longer residence time in the digestive tract and that may thus potentially increase any transfer of contaminants to the organism. However, a larger MP sphere may also have slower desorption and lower bioavailability.

4. Conclusions

A proper assessment of the vectoral transfer of chemicals by MP requires control of what is ingested, the quantity in which it is ingested, and the time at which it is ingested by an organism. In this study, we applied an *in vivo* tube-feeding method that permits the delivery of PE and PS spheres directly into the gut of Atlantic herring larvae to study the uptake of PCB-153, as a representative HOC, from contaminated MP spheres in a single administration into the digestive tract. The *in vivo* results indicate that one-time ingestion and complete gut transit of PCB-153-contaminated PE and PS spheres, with less than 24 h of retention time in the gut, do not significantly transfer PCB to fish larvae. This finding supports other studies in the field indicating that MP plays a minor role as a vector in contaminant uptake and accumulation by marine organisms compared to food chain exposure. Further studies are necessary to establish the effects of MP size and gut retention time, temperature, biofouling, and the presence of lipid-rich feed on the desorption of HOCs from MP. Tube-feeding is a novel experimental approach to study the vectoral transfer of chemicals by microplastics in the larval stages of fish under controlled conditions.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2020.129144.

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