Abstract

Two experiments were executed to assess the feasibility of Polychlorinated Biphenyls (PCBs) transfer to fish tissues via MPs as a vector. PCBs that occur in the marine environment were tested for their adsorption to four different MP types. PCB congeners showed the highest adsorption levels to Polypropylene homo-polymer. The uptake of PCBs through MP ingestion was tested in an outdoor mesocosm using the herbivorous rabbitfish, Siganus rivulatus in the eastern Mediterranean Sea. Polypropylene homo-polymer particles (0.3-5.0 mm) pre saturated with 11 PCB congeners, in two concentrations (500 ng/g and 5000 ng/g), were mixed with dough and offered to the fish. PCBs were identified after two weeks in fish muscle tissues, but not in the liver. These results suggest that ingestion of contaminated MP by rabbitfish might harm them in the long run, and perhaps even those who consume them on a regular basis, e.g. rabbitfish predators and humans.

Key words: Microplastics, PCBs, Siganus rivulatus, Eastern Mediterranean Sea, contamination, Mesocosm.
1. Introduction

Marine plastic debris is widely distributed throughout the world oceans and encompasses large amounts in the water column and on the seafloor (Cózar et al., 2014; Eriksen et al., 2014; Jambeck et al., 2015). This phenomenon corresponds to the ever-increasing production of plastic, from 0.5 mt/y (million tons/year) in the mid-late 20th century to over 330 mt/y presently (Thompson et al., 2009; PlasticsEurope, 2017). The most abundant form of plastic debris is microplastics (MP, ranging from <0.3-5 mm) (Barnes et al., 2009) that are ingested by various organisms throughout the food web (Boerger et al., 2010; Cole et al., 2013; Foekema et al., 2013; Lusher et al., 2013; Allen et al., 2018; Guzzetti et al., 2018; López-López et al., 2018) and that may adhere to macroalgae and to benthic seagrasses (Gutow et al., 2016). Chemical analyses of the MP indicate that these particles adsorb metals and a variety of organic compounds including toxic persistent organic pollutants (POPs) (Mato et al., 2001; Pascall et al., 2005; Rios et al., 2007). Rochman et al. (2013a) found that in the sea, plastics reach equilibrium with POPs within 3-12 months, whereas under laboratory conditions equilibrium may be reached within 1-3 days (Teuten et al., 2007). Moreover, it appears that as particle size decreases, there is an increase in PCB adsorption rates (Zhan et al., 2016) onto the microplastics. This increase is thought to be related to the increase in hydrophobic (microplastics) surface area available to PCBs to absorb onto (Teuten et al., 2007) with the drop in particle size. According to several pioneering ecotoxicological studies (e.g. Alimba & Faggio, 2019) the increase in concentration of adsorbed PCBs appears to affect the biota that ingest them, but some of these studies were inconclusive (Teuten et al., 2007; Thompson et al., 2007; Browne et al., 2008). Recent ecotoxicological studies suggest that MP consumption may lead to POP poisoning in mussels (Jang et al., 2016; Rist et al., 2016, Faggio et al. 2018, Pittura et al., 2018), oysters (Green, 2016; Sussarellu et al., 2016), sea urchins (Della Torre et al., 2014) and fish (Rochman et al., 2013b; Rochman et al., 2014; Jin et al., 2018), resulting in reduced survival (Green, 2016; Rist et al., 2016), hepatic stress (Rochman et al., 2013b), endocrine disruption (Rochman et al., 2014), reproduction abnormalities (Della Torre et al., 2014; Sussarellu et al., 2016), immune system disabilities (Jang et al., 2016) and gut inflammation (Jin et al., 2018).

Investigations of the interaction between POPs and MPs have revealed a large range in adsorption rates, related most likely to the molecules in question, as well as the type and degradation state of the polymers tested, hydrophobic interactions and crystallinity of the
polymers, etc. (Mato et al., 2001; Pascall et al., 2005; Rios et al., 2007; Teuten et al., 2007; Ogata et al., 2009; Rochman et al., 2013a; Hüffer & Hofmann, 2016; Hartman et al., 2017; Rochman et al., 2017; Wang & Wang, 2018).

In this study, we wanted to test the hypothesis that PCBs adsorbed to MPs may leach from the MPs during passage though the gut of fish that consume MPs and enter fish tissues. The fish that we chose to study was the invasive herbivorous rabbitfish (Goren and Galil, 2001), *Siganus rivulatus*. In a previous study, we found particularly high abundances of MPs in the digestive tracts of rabbitfish caught in eastern Mediterranean coastal waters (Van der Hal et al., 2018); 90% of the rabbitfish examined had MPs in their digestive tracts and 15% of these had more than 100 MP particles per digestive tract, a relatively high number of particles compared to other studied fish species (Boerger et al., 2010; Güven et al., 2017; Peters et al., 2017; Ramon et al., 2018). In earlier work carried out along the Israeli Mediterranean coast, the mean abundance of floating MPs in coastal waters was 7.68 ± 2.38 particles/m³, with a maximal abundance of 324 particles/m³ (Van der Hal et al., 2017a), which is higher than the abundance recorded in other regions in the world (Cózar et al., 2014). The mean concentration of PCBs adsorbed to MPs collected in the Israeli coastal zone was 147 ng/g (maximal concentration was 423 ng/g; Van der Hal et al., 2017b), similar to the range of concentrations recorded elsewhere (Ogata et al., 2009).

In light of the high abundance of MPs and the presence of PCBs on them along the Israeli coast, and considering the relatively high MP ingestion rates by rabbitfish, we set out to examine: (i) whether PCBs will adsorb onto different plastic polymers; (ii) which of the plastic polymers tested had highest PCB adsorption rates; and (iii) whether PCBs adsorbed onto MPs are released following ingestion and transferred into rabbitfish body tissues. This study entailed feeding of locally caught siganids with PCB-enriched MPs and subsequently analyzing fish tissues for presence of the PCBs in order to understand whether the fish incorporate the PCBs into their tissues.

2. Methods

2.1. PCB adsorption onto MPs

A PCB-MP adsorption experiment was performed as follows: a mixture of 11 PCB congeners (Sigma Aldrich Mix 1, PCB congeners #18, 28, 44, 52, 101, 118, 138, 149, 153, 180 and 194) was dissolved in ethanol and added to four Erlenmeyer flasks (prebaked at 550°C) each containing 100 ml of saline water (39%, the mean salinity in the eastern Mediterranean). The final concentration of all PCBs was 5000 ng/g, with 1% ethanol in the saline water. After
stirring the samples for 1 hour, 7.5 g of 5.0 mm pristine plastic pellets of four different types were added (approximately 100 particles) to each of the flasks. The pellets used were: CRT 102 - low-density polyethylene (LDPE), CRT 103 - high-density polyethylene (HDPE); CRT 200 - polypropylene homo-polymer (PP homo-polymer) and CRT 201 - polypropylene co-polymer (PP co-polymer), representing four of the most common types of plastic found in the oceans (Rios et al., 2010). The flasks with pellets and PCBs and the control flasks (pellets without PCBs) were placed in a shaking bath (50 rpm) at 20°C for 24 hours. Following this, the plastic pellets were collected onto Whatman "1" glass fiber filters and transferred to 250 ml flasks. The PCB-treated plastic pellets and the untreated (control) plastic pellets were washed 3 X 30 ml with dichloromethane, followed by 3 X 30 ml hexane to extract the PCBs. The organic solvents were combined and concentrated to 1 ml using a rotary evaporator, followed by solvent drying with sodium sulfate. Detection and analysis of PCBs was carried out using an Agilent gas chromatograph (6890) connected to a mass spectrometer (5973) system (GC/MS) and quantified using the original external standard solution (Sigma Mix 1). The limit of detection of this method is 0.03 ppb and the limit of quantitation is 0.103 ppb.

2.2. PCB time-based adsorption to PP homo-polymer particles

To test the effect of the duration of exposure on the adsorption rate of PCBs to MP, we exposed one type of polymer (the one that showed the highest adsorption in the previous experiment, see below) to two concentrations of the PCB mixture: 500 and 5000 ng/g. Two shaded, round-bottle flasks were filled with 100 ml saline water (salinity 39%). PP homo-polymer particles (7.4 g each) were placed in the flasks and the PCB mixtures were added (as described above). A third flask containing saline water (39%) and PP homo-polymer particles only was used as a “no addition” control. The bottles were placed on a horizontal electric rotator and rotated at a speed of 12 rpm for 14 days. At specific time points, after 3 h, 24 h, 2 d, 9 d, and 14 d, 0.5 g particles were removed, extracted and analyzed for their PCB concentration.

2.3. Mesocosm experiment to test PCB uptake by siganids

An experiment to test the ingestion of PCB-laced MPs was carried out in a mesocosm facility at the National Institute of Oceanography, Israel Oceanographic and Limnological Research (IOLR) as follows. To this end, 103 siganids (average weight 14.65 g ± 4.67) were captured by hand-held nets while free-diving near the Haifa shore (32.49.34.94 N, 34.57.27.37 E; 2 m depth) on the night of August 1, 2017.
Capture of the rabbitfish was authorized by the Israeli National Parks Authority, which is responsible for the protection of all wildlife, and animal experimental procedures (below) were conducted in compliance with the Guidelines of the European Union Council (86/609/EU) for the use of laboratory animals. The collected rabbitfish were split into two groups and placed in two 1 m³ tanks for acclimation (August 1-August 12). Fifteen (15) individuals died during the first two days after capture, of which 7 were stored (-20°C) for subsequent analysis to test for non-induced PCBs.

Twelve 140 L mesocosm tanks supplied with ambient flowing seawater were stocked with 6–8 siganids/tank on August 12, 2017. The mesocosm tanks were situated in a pool supplied with flowing coastal seawater to maintain natural temperatures. Bricks were placed on the bottom of each tank to provide the fish with the sort of shelter that they naturally seek in the sea.

Pristine PP homo-polymer MP pellets (5.0 mm; CARAT GmbH) were ground using a food processor (Davo 250 grinder and chopper) and the ground plastic pieces were collected on a 125 µm sieve to obtain MP pellets within the 0.3-5.0 mm size range. MPs were embedded in dough made of white flour mixed with tap water; siganids are known to feed on this mix (Tacon & De Silva, 1997). The following four treatments (3 replicates each) were tested:

- Treatment A$_{1-3}$: Dough with MP, enriched with PCBs (500 ng/g)
- Treatment B$_{1-3}$: Dough with MP, enriched with PCBs (5000 ng/g)
- Treatment C$_{1-3}$: Dough with MP, without PCBs.
- Treatment D$_{1-3}$: Dough only.

Preliminary examination of the digestive tract of 88 siganids (mean fish weight - 15 g) revealed that these had, on average, 50 particles or =0.015 g particles/digestive tract. In order to mimic these conditions, the fish were offered the equivalent of 0.015 g MP/fish/day.

Every two days, fish were offered food (dough) at a rate of 20% of their weight in each of the treatments (Durai, 1998; Yousif et al., 2004). The feeding experiment lasted 12 days altogether. After 6 days, 20 fish were removed from each treatment (2-3 fish from each replicate) and after 12 days, the remaining fish (4-5 individuals from each replicate) were removed from the tanks. All fish were rapidly euthanized by immersion in icy water, and then decapitated (Blessing et al., 2010) and stored frozen (-20°C) until further processing.

2.4. Quantification of PCBs and MPs in the fish

All fish were measured (total length) and weighed (wet weight). Liver and muscle tissues were removed and pooled. Muscle tissue was dissected from the left side of the fish
between the lateral and dorsal lines. Pooled liver (0.53-2.47 g first week, 0.46-0.89 g second week) and muscle (2.93-5.53 g first week, 3.42-5.75 g second week) samples were ground (Revel wet & dry grinder chopper) with sodium sulfate until homogeneous, and then extracted by shaking the samples overnight in hexane (20°C). The solvent was filtered off through 0.47 mm GF/C membranes, processed and analyzed in a similar manner as described above for the adsorption tests. Following the GC/MS analysis (same protocol as described above), the digestive tract of each fish was removed and immersed in 10% potassium hydroxide (KOH) for two weeks (room temperature) in order to digest all tissue and facilitate enumeration of the MP particles (Foekema et al., 2013). It was not possible to collect and quantify MPs that had passed through the rabbitfish gut (feces) because the PP particles float and all the floating material in the fish tanks was continuously flushed out of the tanks in order to maintain water quality.

3. Results

3.1. PCB adsorption onto plastic polymers

All of the PCBs tested adsorbed onto at least 1 of the plastic polymers tested and most onto 2 or 3 polymer types (Fig 1). Maximal adsorption levels for the 11 PCB congeners tested (see Fig. 1) were found for the PP homo-polymer (4580 ng/g), followed by the PP co-polymer (2596 ng/g), LDPE (584 ng/g) and HDPE (40 ng/g). It is noteworthy that PCB congener 18 adsorbed only onto the PP homopolymer and that congener 28 adsorbed onto all 4 types tested.

![Figure 1](image_url)

Figure 1. Concentrations of the 11 PCB congeners (in ng/g) adsorbed on plastic polymer pellets after a 24-hour adsorption period. Each congener is presented individually showing the concentration on each type of polymer examined.
3.2. PCB time-based adsorption onto PP homo-polymer particles

The sorption of PCB congeners onto PP homo-polymer peaked after 1-2 days in both concentrations tested (500 ng/g and 5000 ng/g) (Fig. 2a and 2b). Saturation levels remained steady during the two weeks of the experiment, with a slight temporal decrease in the last 4 days. A higher level of adsorption was recorded for the heavy congeners (PCBs 194 and 180) relative to the lighter congeners, and was detected in the 500 ng/g solution where saturation was established after one day. In the 5000 ng/g solution, adsorption of heavy congeners continued to increase until 2 days of exposure, whereas other, lighter congeners reached saturation within 1 day. PCB 18 (the lightest molecule) was not detected in either of the two concentrations tested, yet small amounts were detected in the primary experiment as shown in Fig. 1.
3.3. Siganid uptake of MPs and PCBs

No traces of PCBs were found in the fish liver or muscles after the first week of treatment. By the end of the second week, traces of PCBs were found in fish muscle tissues in one of the three replicate tanks exposed to 5000 ng/g PCB. PCB congener 194 was detected at a concentration of 200 ng/g in this muscle tissue sample. Among the siganids that died before the start of the experiment, 2 out of the 7 fish examined had MP particles (2 and 3 MPs per fish) in their digestive tracts. MP particles were found in 8 out of the 25 fish collected after the first week that had been offered food with MP particles (Treatment A-C). They had between 1 and 2 MP particles/digestive tract, with an average of 1.57 particles per fish. After 12 days, 29 out of the 45 fish (64%) had between 1 and 10 MPs in their digestive tract. Exceptions to these findings were two fish (outliers) that had 27 and 48 particles per fish. The overall average abundance of particles was 5.06 MP per fish; if we exclude the 2 outliers, the average was 2.92 MPs per fish. There were no signs of the experimental MP particles in the dough-only control treatment, and there were no other particles in the digestive tracts of these fish.

4. Discussion

Our experiment focused on the most common plastic polymers found in the world’s oceans (Rios et al., 2010) and on PCB congeners that are found on some MP particles. We found that the highest and fastest adsorption of PCBs occurred on the PP homo-polymer, and that there was considerably less adsorption onto HDPE and LDPE. Our findings differ from other published data, which showed similar sorption rates of PCBs onto HDPE, LDPE and PP and an even higher sorption level onto MP particles for the polyethylene groups (Pascall et al., 2005; Teuten et al., 2007; Ogata et al., 2009; Rochman et al., 2013a). Although these studies suggest that there is greater POPs adsorption onto particles with larger surface area (Teuten et al., 2007), higher diffusivity characteristics (Pascall et al., 2005) and crystallinity (Mato et al., 2001), our measurements showed higher sorption on the lower surface area polymer (i.e., PP homo-polymer). Due to the high concentration of PCBs adsorbed, the PP homo-polymer was chosen for the dynamic adsorption and mesocosm experiments with the siganids.
Saturation of the PCB congeners onto PP homo-polymer occurred within 2 days but saturation levels did not vary substantially in the following 12 days, as also observed by Teuten et al. (2007). The slight decrease in saturation in both 500 ng/g and 5000 ng/g solutions, recorded during the experiment (Fig. 2) may have been caused by adherence of PCBs to the surfaces of the glass Erlenmeyer that was used, as also described in Teuten et al. (2007) and Phuong et al. (2016). Moreover, the heavier PCB congeners adsorbed more readily to the plastic particles as compared with the lighter congeners. These results contradict those reported by Pascall et al. (2005), who showed a decrease in the sorption of PCBs onto MPs with the increase in chlorine numbers (heavier congeners) in the congeners.

*Siganus rivulatus* is a schooling, generalist herbivore that feeds on macroalgae in the coastal zone (Bariche, 2006). This species is highly abundant along the Israeli coast, has commercial value (Goren & Galil, 2001, Rilov et al., 2017) and is a good model species to examine MP uptake due to its diet and plastic-enriched habitat. The reason that rabbitfish consume relatively large numbers of MPs is not clear, but it may be related to MP adherence to macroalgae (Gutow et al., 2016) or an attraction of the siganids to their taste or smell. Moreover, Savoca et al. (2017) found that fish preferentially consumed biofouled MP over clean (virgin) MP particles. In our study, MPs were found in the digestive tracts of the fishes taken from the experimental tanks and the average abundance in the gut increased from week 1 to week 2, possibly due to further acclimation of the fish to the mesocosm tanks (higher ingestion rates) or to blockage of the digestive tract.

When ingested by fish, POPs adsorbed to MP particles were reported to induce a greater toxicity level than when the contaminants were ingested separately (Rainieri et al., 2018). Moreover, polybrominated diphenyl ethers (PBDE’s); plastic additives which were sorbed to plastic microbeads were released from the beads when ingested by fish in controlled experiments (Wardrop et al., 2016). In contrast, other studies showed that the delivery of POPs to marine biota via MPs is negligible when compared to the uptake of these toxic compounds from water, sediments and organic materials (Bakir et al., 2016; Koelmans et al., 2016; Beckingham & Ghosh, 2017; Besseling et al., 2017). Similarly, Diepens & Koelmans (2018) suggested that PCBs adsorbed to MPs would bio-magnify less when compared to their ingestion via “normal diets”. Tivefälth et al. (2018) showed a similar pattern of increased gene expression and enzymatic changes when fish were exposed to POPs, both with and without MP.

Hartman et al. (2017), suggest that since many microplastics have POPs adsorbed to their surfaces, as the abundance of MPs in the water increases, the levels of exposure of
marine biota to MP, and to the adsorbed POPs will increase as well, i.e. there is a dose-response. It is also notable that there appears to be an increase in the adsorption rate of POPs in aged or weathered particles (Bandow et al., 2017). While these results display a trend in MP hydrophobic adsorption of PCBs, a detailed kinetic experiment is needed in order to calculate kinetic coefficients that will enable us to better understand the affinity of PCBs to different types of MP polymers.

A PCB concentration in fish higher than 50 ng/g is considered hazardous for human health; and higher than 2000 ng/g is considered toxic (Kassa & Bisesi, 2001). Tolerable concentrations in humans are set by the World Health Organization at 2 pg/kg body weight 'Toxic Equivalents' per day (EFSA, 2015). Although our findings are preliminary, 200 ng/g of PCB 194 found in the muscles of fish exposed to PCB-laced MPs for 2 weeks or less is a source of concern (see EFSA, 2015).

The results from this study demonstrate the effect that plastic pollution may have on commercially important herbivorous fish. Recent studies of both non-eviscerated canned fish and eviscerated fish consumed by humans have shown these to contain microplastics (Karami et al., 2017; Karami et al., 2018). This is also shown in another recent study that found MP ingestion occurring mostly in herbivorous fish (Peters et al., 2017). It is noteworthy that greater concern has been voiced in recent years with regard to exposure of humans to heavy metals and POPs that accumulate in carnivorous rather than herbivorous fish species, due to biomagnification (Harding et al., 2018). For this reason, herbivorous fish have been regarded as “safer”, but this dogma may not be valid, as we show here. Diepens & Koelmans (2018) propose that POPs adsorbed to MPs biomagnify less because carnivores consume these to a lesser degree than herbivores, for example. Whereas this may be true, we also need to consider that carnivores, such as groupers feed on rabbitfish (Aronov & Goren, 2008), and may therefore be exposed to high levels of POPs via their prey. In a food-web study, Batel et al. (2016) exposed brine shrimp to benzo[a]pyrene (BaP) spiked MPs, and these invertebrates were subsequently consumed by zebrafish. An examination of the zebrafish showed that there was a transfer of BaP to the fish tissues, demonstrating that POPs may indeed be biomagnified by this pathway. The anticipated increase in plastic production in the coming years (Hartman et al., 2017) and the likelihood that much of it will end up in the oceans (Jambeck et al., 2015) suggests that we should carry out more studies of this sort in the future in order to understand some of the human health risks in consuming seafood.

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