Marine Pollution Bulletin xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Trophic transference of microplastics under a low exposure scenario: Insights on the likelihood of particle cascading along marine food-webs

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ARTICLE INFO

Keywords:
Marine pollution
Microplastics
PVC
Food web
Trophic impacts
Biotransference

ABSTRACT

Microplastics are emergent pollutants in marine environments, whose risks along food-web still need to be understood. Within this knowledge gap, MPs transference and persistence along trophic levels are key processes. We assessed the potential occurrence of these processes considering a less extreme scenario of exposure than used previously, with microplastics present only in the hemolymph of prey (the mussel *Perna perna*) and absent in the gut cavity. Predators were the crab *Callinectes ornatus* and the puffer fish *Spheoeroides greeleyi*. Transference of microplastics occurred from prey to predators but without evidences of particle persistence in their tissues after 10 days of exposure. This suggests a reduced likelihood of trophic cascading of particles and, consequently, a reduced risk of direct impacts of microplastics on higher trophic levels. However, the contact with microplastics along food-webs is still concerning, modulated by the concentration of particles in prey and predators' depuration capacity and rate.

1. Introduction

Microplastics (MPs) are plastic particles minor than 5 mm (Arthur et al., 2009) industrially produced on the micro-sized scale or resulted from larger plastic's degradation (Andrady, 2011). The high volume of plastic consumed and their inadequate disposal made MPs a threat to different marine ecosystems, from coastal regions to open ocean and deep sea (Claessens et al., 2011; Van Cauwenberghe et al., 2013; Cózar et al., 2014). Their total quantity in oceans corresponds to 92.4% of the total plastic count (Eriksen et al., 2014), with an increasing tendency with time (Eriksen et al., 2014). Beyond abundance, MPs are of concern because of their small size and potential risks to wildlife. As plastics get smaller, their chances of being ingested and the range of exposed organisms increase (Cózar et al., 2014), potentially causing biological impacts from molecular and cellular levels to ecosystems (von Moos et al., 2012; Browne et al., 2013; Wright et al., 2013a, 2013b; Besseling et al., 2013; Rochman et al., 2014).

The ingestion of MPs was reported in a range of marine species, from pelagic and benthic habitats, with different feeding strategies and from different trophic levels (Graham and Thompson, 2009; Tourinho et al., 2010; Murray and Cowie, 2011; Lusher et al., 2013; Cole et al., 2013). However, most studies have associated it with a direct uptake from the environment (sediment or water), while the intake of MPs through trophic interactions (i.e., contaminated food) is still poorly understood. This knowledge gap made experimental works investigate MPs' biotransference (transfer of compounds from a food source to its consumer – Barwick and Maher, 2003). Using different polymers, along with benthic and pelagic species, studies have supported trophic transference based on observation of MPs in the gut cavity of predators after being fed with contaminated prey (Murray and Cowie, 2011; Watts et al., 2014; Setälä et al., 2014). These findings, though, suggest a temporary presence of MPs in consumers, with no evidence of persistence in their tissues, an important information to discuss trophic cascading of particles regarding its potential impacts along food-web. The further persistence of MPs after food intake may boost the risks to higher trophic levels (e.g. oxidative stress, inflammatory reactions, endocrine disruptions, fitness disturbances and transference of other toxic substances associated to the plastics – von Moos et al., 2012; Browne et al., 2013; Wright et al., 2013a, 2013b; Besseling et al., 2013, Rochman et al., 2014; Watts et al., 2014).

Two other studies, however, found MPs within tissues of predators (Farrell and Nelson, 2013; Batel et al., 2016), thereby increasing the effectiveness of MPs' biotransference and the risks associated to trophic cascading. Farrell and Nelson (2013) fed *C. maenas* with one single freshly contaminated mussel with high concentrations of polystyrene (PS) microspheres (0.5 μm) and observed the biotransference of MPs within predators' hepatopancreas, ovary and hemolymph. These records lasted for 21 days in the hemolymph with a peak after 24 h. Batel

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http://dx.doi.org/10.1016/j.marpolbul.2017.05.061
Received 7 December 2016; Received in revised form 25 May 2017; Accepted 26 May 2017

et al. (2016), in turn, identified 1-20 μm polyethylene particles (PE) taken up by epithelial cells of zebra fish's intestine after a chronic feeding regime with *Artemia nauplii* highly loaded with MPs. The observed.

Nevertheless, it is worth noting that the available data on MPs' trophic cascading consider a scenario where predators eat prey short after their MPs' intake, representing an extreme and not environmentally accurate exposure scenario. In fact, the presence of MPs in seawater seems to have a stochastic pattern, influenced by oceanographic biotic and abiotic forces, such as flood tide, winds, currents, frontal systems and the presence of biofilms (Eriksen et al., 2014; GESAMP, 2015; Gallagher et al., 2015). All these factors can temporally influence MP distribution in depth or along surface, raising the variability of MPs' abundance in the environment and, consequently, in the animals (Santana et al., 2016). Thus, apart from source regions (e.g. outputs of wastewater treatments and rivers or coastal areas with high population density without sewage treatment) and sink environments, such a variable exposure can promote short-term events of contact of MPs with organisms followed by longer periods without contact, which could increase their chances of depurate at least part of the ingested MPs prior to predation. This would reduce the probability of predators feeding on prey while they still have a digestive tract full of these particles, a scenario that can overestimate MPs impacts. Therefore, studies designed to understand the risks of this process are required under different scenarios of contamination, including less extreme conditions as those where MPs are only present in prey tissues but not in the gut.

Here we evaluated MPs' biotransference and persistence in a food-web using an experimental approach that considered a less extreme scenario than previous studies (e.g. Farrell and Nelson, 2013; Setälä et al., 2014; Batel et al., 2016; etc). Predators were fed with prey contaminated with a relatively low amount of plastic and then allowed to depurate for a subsequent period feeding upon uncontaminated prey in order to evaluate the persistence of particles. Brown mussels (*Perna perna*) were used as prey and a swimming crab (*Callinectes ornatus*) and a puffer fish (*Spheoeroides greeleyi*) as predator models. Mussels are found in abundance all over the world and are especially susceptible to ingest MPs (Browne et al., 2008; von Moos et al., 2012; Avio et al., 2015; Santana et al., 2016). They are also an important food source for organisms from higher trophic levels, including humans. Blue crabs and puffer fish were chosen as natural predators of bivalves, enabling a realistic simulation of a trophic chain. We hypothesized that MPs could be transferred to and persist in predators. We also assumed that these processes would occur in different ways between predator species due to their potentially distinct mechanisms of regulating the concentration of xenobiotics within their bodies. This study represents the first investigation of microplastics' trophic transfer and persistence for the two model types of predators (crab and fish).

2. Methods

2.1. Microplastic model

The Polyvinyl chloride (PVC) is one of the polymers most produced worldwide and is commonly encountered in marine environments (Andrady, 2011). Among the types of PVC produced, the experimental model was the Emulsion/Microsuspension (E/M), a spherical particle with a size range of 0.1 to 1.0 μm in diameter (Rodolfo et al., 2006). This PVC can be found in marine environments due to the loss during marine translocations, particularly when vessels are being loaded and unload with them (Pereira, 2014). The size approximates it to the nano scale, possibly increasing its similarity with other nanoparticles (of plastic or not) already observed to at least temporary persist in marine organisms (e.g. Browne et al., 2008; Pan et al., 2012; Hanna et al., 2012). The E/M PVC was a non-labelled MP, obtained directly from a polymer manufacturer, which hindered additional information related to the chemical composition due to market policies.

2.2. Biological models

Perna perna was chosen because its natural food ranges from nano- to micro-sizes (Maia et al., 2006), which is similar to the plastic model size range. In addition, this organism was already demonstrated to ingest such PVC particles (Santana et al., n.d.). In Brazil, this species can be found in abundance from the Espírito Santo to Rio Grande do Sul states (Fernandes et al., 2008) and is an important food source for marine organisms and humans. In 2006, 90 tons of mussels were cultured for human consumption in the State of São Paulo alone (IBAMA, 2008). For this study, about 200 mussels (4.98 ± 0.32 cm in shell length) were purchased from an aquaculture system (located at Lagoinha Beach, Ubatuba, northern coast of São Paulo, Brazil) and immediately taken to laboratory in a cooler lined with humid cotton towels.

Callinectes ornatus is a portunidae swimming crab, common in tropical marine and estuarine environments (Guerra-Castro et al., 2007). This species has an omnivorous diet, with predominance of crustaceans, molluscs (including bivalves) and fish (Mantelatto and Christofoletti, 2001). *Spheoeroides greeleyi* is a tetraodontidae puffer fish, abundant along the Brazilian coast (Figueiredo and Menezes, 2000), typically found in bays and estuaries (Schultz et al., 2002). They feed mostly on gastropods, bivalves and crustaceans (Targett, 1978) and are an important component of trophic chains in their natural habitats (Schultz et al., 2002). Here, 12 individuals of both species were collected at the Ubatuba research field station of the Oceanographic Institute of the University of São Paulo (Brazil), where assays were conducted. Sampling was done with a “trap” composed by nettings and fishing poles (the second being used to attract them with a prey attached to a hook). Sampled blue crabs (5.43 ± 0.77 cm in carapace width) and puffer fish (10.29 ± 0.69 cm in total length) were taken to the lab in glass aquaria to avoid prior contamination by MPs.

2.3. Contaminated mussels

Mussels were acclimatized for three days in a tank (1000 L) with open circulation system of natural seawater, where abiotic factors (temperature, pH and salinity) were monitored and food was the natural organic matter from water. After acclimation and prior to PVC exposure, hemolymph and soft tissues of three mussels were sampled to verify background contamination by MPs. Assessing it after mussels were acclimatized ensured the investigation of MPs presence in these organisms due both their growth in a farming system and their maintenance in the tank with an open circulation of natural seawater during acclimation. The method applied to process the tissues and identify the MPs is explained at the end of this section.

The remaining mussels were placed in 20 aquaria (12 L each; 10 mussels per aquarium) and exposed to 0.5 $g.L^{-1}$ of E/M PVC (approx. 4.4×10^{10} particles) for 3 h, following Browne et al. (2008). During exposure, mussels were feeding (e.g. opened valves and regular production of faeces and pseudofeces). Subsequently, they returned to the previous tank (1000 L) with clean seawater, and remained there for 12 days to ensure the digestive tract depuration and a high presence of MPs in the hemolymph, as observed for *M. edulis* (Browne et al., 2008). For depuration, mussels were kept under the same conditions as in acclimatization. By the end of 12th day, they were all collected, opened and had their soft tissues separated from their valves, weighed and then frozen to be further used as food (prey) for blue crabs and puffer fish.

Assuming that natural variations could act on mussels' uptake of MPs, other three *P. perna* were randomly selected to quantify the PVC present on their gut cavity, hemolymph and soft tissue after the 12 days of depuration. As for control group, mussels had the shell carefully and slightly opened (avoiding the rip of abductor muscle) and the intervalvular water drained to prevent samples' contamination with non-