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Assessment of microplastic toxicity to embryonic development of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea)

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ABSTRACT

Apart from the physiological impacts on marine organisms caused by ingesting microplastics, the toxicity caused by substances leaching from these particles into the environment requires investigation. To understand this potential risk, we evaluated the toxicity of virgin (raw) and beach-stranded plastic pellets to the development of embryos of *Lytechinus variegatus*, simulating transfers of chemical compounds to interstitial water and water column by assays of pellet–water interface and elutriate, respectively. Both assays showed that virgin pellets had toxic effects, increasing anomalous embryonic development by 58.1% and 66.5%, respectively. The toxicity of stranded pellets was lower than virgin pellets, and was observed only for pellet–water interface assay. These results show that (i) plastic pellets act as a vector of pollutants, especially for plastic additives found on virgin particles; and that (ii) the toxicity of leached chemicals from pellets depends on the exposure pathway and on the environmental compartment in which pellets accumulate.

1. Introduction

Plastic fragments comprise more than half of ocean litter (Derraik, 2002) and microplastics (plastic particles with a diameter of 5 mm or less; Cole et al., 2011) are now receiving attention from international agencies (GESAMP, 2010) and the scientific community (Wright et al., 2013; Ivar do Sul and Costa, 2014), because of the uncertainties regarding their environmental effects. Plastic pellets, or nibs, are a form of raw material for the manufacture of various plastic objects (EPA, 1990). They are small (⩽5 mm) and end up into marine environments by losses during the processes of production, transport and manufacturing (Thompson et al., 2005). Large quantities of plastic pellets are being found on coastal areas and other marine systems (Cole et al., 2011), and added to their potential ecological impacts and tendency to increase in production, plastic pellets have become an item of particular concern for marine environments (EPA, 1990).

The most common pellets are derived from polypropylene, polyethylene and polystyrene (EPA, 1992), and chemical compounds (such as emollients, colorants and antioxidants) are usually added to them in order to enhance their performance (EPA, 1990, 1992; Teuten et al., 2009). According to Ananthaswamy (2001), many of these additives are toxic and their effects on organisms can be severe. Browne et al. (2013) showed that additives can leach from ingested microplastics (PVC) into the bodies of worms, reducing their feeding activity and showing worse effects than other persistent anthropogenic pollutants. However, there is still controversy regarding the release of these additives, and therefore their biological impacts (Thompson et al., 2004; Teuten et al., 2009). Additives can be mixed with or chemically bound to polymers, which could substantially change their capacity of leaching and the potential toxicity of plastic pellets (EPA, 1992).

Plastic polymers, including nibs, can also act as vehicles for toxic hydrophobic compounds absorbed from the environment (Cole et al., 2011; Fisner et al., 2013a), potentially increasing the availability of these pollutants to the biota (GESAMP, 2010; Teuten et al., 2009; Browne et al., 2013). DDT, PCBs, PAHs, nonylphenols and other nonpolar substances can adsorb on plastic surfaces (Ward and Kach, 2009; Fisner et al., 2013a,b), becoming available for animals mainly through ingestion (Teuten et al., 2009; Browne et al., 2013; Chua et al., 2014). Most of these pollutants are toxic and bioaccumulative, and if leached from pellet and assimilated by an organism, can be introduced into

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the food chain, increasing their environmental persistence (Browne et al., 2013; Chua et al., 2014). Plastic pellets have the potential to accumulate these types of molecules at higher concentrations than sea water that increases according to the time of exposure, type of resin and its characteristics (Mato et al., 2001; McDermid and McMullen, 2004; Hirai et al., 2011).

Microplastics affect many species of marine organisms, from suspension- and filter-feeders to deposit feeders (Cole et al., 2011, 2013; Wright et al., 2013). However, their risks not only involve ingesting the plastic itself (a physical hazard) but also regard the contamination of organisms by chemical pollutants adsorbed on them (a potential chemical risk). Due to microplastics' ecological risks, toxicological effects have received growing attention (von Moos et al., 2012; Oliveira et al., 2012a,b), but these efforts usually involve organisms prone to ingest plastic particles, generating a lack of information about organisms that are unlikely to ingest them but have them in their surroundings.

Microplastics are being investigated solely as a vector of chemical compounds (additives of polymers and other pollutants sorbed from the environment) for marine biota, and the hazards would be directly related to the capacity of these compounds to be desorbed (leached) from them. Through this mechanism, a larger number of marine organisms could be affected by microplastic pollution, such as organisms from the meiofauna and microphytobenthos (that live in the interstitial water, where pellets sink and tend to accumulate) and their planktonic developmental stages (that live in the water column, where plastic pellets remain from their entry into the marine environment until their removal by deposition, ingestion or degradation). The use of planktonic larvae to evaluate the chemical toxicity of microplastics is important to help in the understanding of the impacts on critical biological processes such as larval development, which could change the structure of populations and communities.

The present study evaluated the toxicity of plastic pellets to the embryonic development of the sea urchin *Lytechinus variegatus,* using both virgin granules and pellets collected from sandy beaches. We hypothesized that virgin pellets would be more toxic due to leaching of additives than beach-stranded pellets, which would have already leached their additives, despite their potentially higher concentration of absorbed hydrophobic organic pollutants. We also hypothesized that the exposure pathway, i.e., simulating the release of pollutants to the interstitial water and water column, would influence the toxicity through the bioavailability of the chemical compounds to the biota.

2. Material and methods

To evaluate the external toxicity of plastic pellets, considering a non-ingestion pathway and therefore the effects through leaching of additives and absorbed pollutants into the water, embryos of *L. variegatus* were exposed to virgin and beach-collected granules. The virgin particles were used in order to analyze the potential toxicity of leaching additives (Halden, 2010; Lithner et al., 2011), while the beach-collected ones were chosen due to their potential toxicity arising from the desorption of sorbed hydrophobic pollutants from the environment (Ogata et al., 2009; Frias et al., 2010; Fisner et al., 2013a,b).

2.1. The plastic pellets

Virgin polyethylene granules were obtained from a petrochemical factory, and the beach-collected pellets were randomly sampled by sieving the surface sand in Santos Bay, on the southeastern coast of São Paulo state, Brazil (Fig. 1). The pellets were stored in sealed glass flasks and kept in the dark at room temperature until the assays (24–48 h).

The Santos Metropolitan Region is a densely urbanized and polluted area of high economic importance. In addition to the pollutants derived from the urban area (domestic sewage and burning of fossil fuels, for example), Santos is the largest port in Latin America (Santos Harbor) and is directly connected to one of the most important industrial complexes in Brazil, the Cubatão petrochemical pole (Fisner et al., 2013b). This link is through the Santos estuary and increases the input of pollutants into that marine area (Hortellani et al., 2008). Due to the transport of plastic pellets through Santos Harbor, which is responsible for 50,000 tons of granules/month (Fisner et al., 2013b), a large quantity of nibs has been reported previously in this bay (Turra et al., 2014). Pellets of different colors, ranging from translucent, similar to virgin ones, to darkened pellets with greater concentrations of hydrophobic pollutants (Endo et al., 2005; Fisner, 2012) have been found along its shores and in deep sediment layers (Manzano, 2009). Other studies have reported contamination of pellets by PAHs in this area (Fisner et al., 2013a,b).

2.2. Ecotoxicological assays

The assays (pellet–water interface and elutriate) followed the protocol described by the EPA (2002) and adapted by ABNT NBR 15350 (2012), and were employed in order to identify possible changes in the pellet toxicity from their source (water column) to their sink (beach interstitial water), respectively.

The pellet–water interface assay evaluated the toxicity of the sample subjected to upward movements of interstitial water and the consequent remobilization of contaminants from sediments (Ferraz et al., 2012). The elutriate assay assessed the toxicity of sediments after re-suspension episodes caused by natural events (e.g., waves) or human activities (e.g., dredging). For both assays, three trials were conducted with three treatments (negative control of uncontaminated seawater, virgin pellets and beach-collected pellets) and employed four replicates for each treatment. For each assay and replicate, 100 larvae were randomly selected and analyzed with a light microscope (400×) for anomalous development, resulting in a relative frequency (%) of normal (or reduction in normal) development caused by the compounds leached from the pellets.

For the pellet–water interface, the method of Cesar et al. (2004) was adapted. Thus, 2 ml of plastic pellets (virgin or beach-collected) was added in tubes and attached to a plankton net with a plastic ring, where 8 ml of seawater was added. The system remained static for 24 h, after which fertilized eggs were placed in the tubes. After a period of normal larval development (24 h), the larvae were fixed by addition of 4% formaldehyde for further analysis.

The elutriate treatment was adapted from ABNT NBR 15350 (2012); 200 ml of pellets was placed in 1 L beakers filled with 800 ml of filtered seawater. The solution was agitated in the jar-test equipment for 30 min and left to rest for 24 h (static condition). After this period, 4 aliquots of 10 ml each were collected from the supernatant and placed in assay tubes, where the fertilized eggs were also added. As performed for the pellet–water interface, after a period of normal larval development, the content of the assay tubes was fixed with 4% formaldehyde and then analyzed.

Temperature, dissolved oxygen, pH and salinity were monitored in all assays; their levels were within the standards required for normal development of *L. variegatus* embryos, according to ABNT NBR 15350 (2012).

Since the data satisfied the assumptions required for analyses of variance (ANOVA), a bi-factorial ANOVA was performed for each assay separately, comparing treatments (control without pellets,

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