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SHORT COMMUNICATION

Toxicity of glitter to marine organisms: a baseline study with embryos of the sand-dollar *Mellita quinquiesperforata*

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Abstract This study aimed to determine the toxicity of glitter particles of two colors (green and white) on the embryos of the sand dollar *Mellita quinquiesperforata*. Toxicity tests were performed using both green- and white-glitter particles. Adult sand dollar individuals were collected and gametes were obtained by osmotic induction. Each glitter was tested in five test solutions: 0.01, 0.1, 1, 10 and 100% (= mg L^{-1}). The results were analyzed by the Wilcoxon–Mann–Whitney test, allowing the determination of the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC). The LOECs calculated for the green glitter dispersions were 0.01 % ($P < 0.05$) in the first two tests, and 100% in the third test. The LOECs for the white-glitter dispersions ranged from 0.01 % to 0.1 %. Our results demonstrate that glitters of both colors are capable of affecting the embryonic development of *M. quinquiesperforata*.

Keywords Microplastics dispersion . Ecotoxicology . Marine pollution . Embryolarval test . Emerging pollutant . Glitter

Introduction

Plastic pollution is an increasing and priority issue of global concern (Xu et al. 2019). Plastic residues present wide variations in composition and size, and microplastics (MPs), that is, plastic particles ranging between 1 µm and 5 mm (Frias and Nash 2019), represent a predominant form of plastic litter in aquatic environments (Galloway et al. 2017). Microplastic pollution has become widespread in both terrestrial and aquatic environments (Yurtsever 2019a), reaching even remote regions such as islands, polar zones and deep oceans (Lusher et al. 2017). Moreover, plastics released into freshwater streams tend to be transported to the sea (Li et al. 2018), where they accumulate in large quantities.

Microplastics may have a primary or secondary origin. The first are MPs produced intentionally at the microscopic scale as precursors of other products or for direct use as abrasives in cleaning and aesthetic products. In turn, secondary MPs result from the degradation of macroplastics as a result of mechanical, photolytic, and chemical processes, which result in thousands or millions of MPs fragments from a larger piece of plastic (Alomar et al. 2016). Approximately 80% of MPs in oceans come from land-based sources, and rivers are the main routes for these residues to reach the oceans (Besseling et al. 2017a, b; Leslie et al. 2017; Rochman 2018). Most MPs float on the water surface (Oberbeckmann et al. 2014), but in the long term, many of them sink to the bottom and accumulate in sediments (Coppock et al. 2017; Imhof et al. 2018; Yurtsever 2019b).

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Microbeads consist of small plastic spheres ranging between 5 µm and 1 mm and are composed of various plastic polymers such as polyethylene (PE), polylactic acid (PLA), polypropylene (PP), polystyrene (PS), and polyethylene terephthalate (PET) (Rochman et al. 2015). Microbeads are manufactured and included in several personal care products, such as toothpastes, facial cleaners, soaps, and housing products, to maximize their cleaning or peeling properties (Rochman et al. 2015; Bhattacharya 2016; Lei et al. 2017); they are also designed to be washed and carried into the drains (Rochman et al. 2015). Because of their large-scale production and use, microbeads are potential contaminants in the marine environment; thus, there is increasing pressure to ban their use in cosmetic products (Rochman et al. 2015).

The glitter particle is formed by a set of plastic layers covered by thin metallic layers, similar to a sandwich, as described by Tagg and Ivar do Sul (2019). They include a variety of small, plain, and reflexive particles used in craftwork, textiles, and cosmetic products (Yurtsever 2019a). These particles have some similarities to microbeads; however, they have not received appropriate attention from the scientific community as a potential contaminant (Tagg and Ivar do Sul 2019).

Glitter is widely used in makeup, clothes and fancies, carnival floats, and other materials. Because of its small size, the glitter easily adheres to the human skin. They are removed by washing, are carried to domestic wastewaters, and thus can be released into natural aquatic environments (Tagg and Ivar do Sul 2019). In addition, glitter can fall from the skin and other surfaces, depositing on the streets, from where they can be removed by stormwater rainfall. Thus, urban drainage may represent an additional route for glitter entering the environment. Tagg and Ivar do Sul (2019) also suggested that glitter might be a good indicator of sewage in marine waters.

Glitter particles have already been found in samples from a wastewater plant in Norway (Lusher et al. 2017), and in sediments from UK rivers, in elevated quantities (Hurley et al. 2018). In addition, as increasing amounts of microplastics have been reported in lakes and rivers worldwide (Rios Mendoza and Balcer 2019), the quantities of glitter are likely to follow this trend. In a review discussing glitter as an environmental contaminant, Yurtsever (2019a) identified studies reporting the presence of glitter in environmental water samples, and addressed the potential environmental problems associated with its trade and use.

However, as noted by Tagg and Ivar do Sul (2019), glitter particles have historically been overlooked in investigations of microplastics as environmental contaminants. These authors also reported the lack of studies and information on the glitter sources, input rates, distribution across the environment, and potential effects on marine biota; they also addressed the relevance of glitter as an environmental pollutant, and the need for studies regarding these particles.

A major issue associated with pollution by MPs is the intoxication of the marine biota. Embryos and initial stages of the development of aquatic organisms are often more sensitive to such contaminants (Beiras et al. 2012; Martínez-Gómez et al. 2017). Kaposi et al. (2014) demonstrated that embryos of the sea urchin *Tripneustes gratilla* exposed to polyethylene microbeads were capable to ingest the particles, exhibiting sublethal effects such as reduction of the embryos sizes and development rates, when exposed to about 300 microbeads per milliliter. Green et al. (2021) compared the ecological impacts of the conventional PET glitter (not biodegradable) with glitters made of alternative materials (regenerated cellulose, natural and synthetic mica) on the biodiversity and functioning of freshwater ecosystems and found that all types of glitters tested could cause adverse effects on aquatic ecosystems.

Therefore, based on the likely widespread global glitter (Yurtsever 2019a), their association with sewage and urban drainage, and their potential toxicity (Tagg and Ivar do Sul 2019), these particles may represent contaminants of emerging concern, especially in aquatic environments. Still, because evaluations of glitter toxicity to marine organisms are lacking, ecotoxicological studies aimed at estimating its toxic potential to marine biota are urgently needed.

This investigation aimed to evaluate the toxicity of glitter particles of two colors (green and white) on the embryos of the sand-dollar *Mellita quinquiesperforata* and to observe potential differences in toxicity due to their colors.

Materials and methods

First, both glitters (from the same commercial brand) were characterized for their particle size distribution using the laser diffraction method ISO 13320 (ISO 2020). Particle sizes were examined using a laser granu-

lometer Mastersizer 2000 (Malvern Instruments Ltd, Worcestershire, UK) coupled with a wet unit (module Hydro 2000MU) in 10 repetitions. The particle size distribution was measured by the angular variation in the intensity of light as the laser interacted with the particles. Numerical values associated with the scattering pattern were recorded, allowing for glitter particle size characterization.

The white glitter was also analyzed for the type of polymer using pyrolysis coupled with Gas Chromatography and Mass Spectrometry (Py-GC/MS), following the protocols described by Gimiliani et al. (2020), Tsuge et al. (2011), and Zellner and Quarino (2009). This technique is powerful for MPs analysis, providing chromatographic separation and mass detection of all pyrolysis products (Matsui et al. 2020). About 0.2 mg of the sample was transferred to the Py-GC/MS sampler. The gases emitted by sample burning were transferred using helium as a carrier through an Ultra ALLOY-5 column. A Frontier Labs pyrolizer (model EGA/Py-3030D) was used as a micro-oven in the single-shot mode, which was connected to a Shimadzu GC/MS system (model QP5000). The resulting gases were separated, and their components were quantified. Concomitantly with these analyses, the particles were analyzed for the presence of poly vinyl chloride (PVC) in their composition. Before each analysis, a blank was analyzed following the same procedure and conditions to determine interference with the sample retention times, and the chromatograms were compared with those provided by Tsuge et al. (2011).

For the toxicity tests, glitter stock dispersions (SE100s) for each glitter type were prepared by adding 100 mg of particles into 1 L of filtered and autoclaved seawater (i.e., dilution water). The resulting mixtures (100 mg L^{-1}) were agitated and kept under agitation during the preparation of the test-dispersions. Prior to preparation of the test dispersions, the SE100s were sonicated for 8 min at 40 kHz. Then, the SE100s of each glitter were diluted in dilution water, producing five test dispersions: 0.01, 0.1, 1, 10, and 100%, plus a negative control (dilution water without glitter). Considering the concentration of the glitter stock dispersion (100 mg L^{-1}), the test concentrations were approximately 0.01, 0.1, 1 10, and 100 mg L^{-1} respectively. In this sense, organisms were expected to be exposed to the particles per se and the substances leached from the glitter, as it would occur in the natural environment. For each test dispersion, four replicates were prepared in glass tube tests, each receiving 10 ml of test dispersion.

The toxicity tests consisted of exposing eggs of the sand-dollar *M. quinquiesperforata* to glitter dispersions and observing their embryo development until pluteus larvae (ABNT 2012; Laitano et al. 2008). *M. quinquiesperforata* is abundant in sandy beaches along neotropical and subtropical regions (Laitano et al. 2008). These organisms are appropriate for testing the toxicity associated with glitter because they can be exposed to such particles (as well as other MPs) as both sand-dollar larvae and glitter particles remain for a period in the water column and then settle on the bottom sediments after reaching the marine environment (Coppock et al. 2017; Imhof et al. 2018). Moreover, embryos of *M. quinquiesperforata* are sensitive to contaminants (Laitano et al. 2008; Mello et al. 2020) and represent appropriate biological models to investigate the effects of glitter. The tests were performed following the NBR 15350 protocol (ABNT 2012), which describes toxicity tests with sea urchin embryos, with adaptations proposed by Laitano et al. (2008) and Mello et al. (2020) for *M.quinquiesperforata*. According to the test protocol, fertilized eggs were exposed to solutions containing the contaminants, and embryo development was evaluated after approximately 36 to 42 h. Three separate toxicity tests were performed for each type of glitter.

For each experiment, approximately 100 adult *M. quinquiesperforata* individuals were collected from the superior portion of the infra-littoral of sandy beaches on the coast of São Paulo. The animals were kept in tanks containing clean sediment and dilution water under constant conditions (photoperiod of 12h:12h clear-dark; temperature of 25 ± 2ºC, salinity ranging between 33 and 36, and constant gentle aeration). Gametes were obtained after osmotic induction (injection of 0.1-0.3 ml of 1M KCl solution into the organisms) (Laitano et al. 2008). Osmotic shock stimulates the release of gametes, which can be distinguished by their colors: sperm is whitish, while ovules are reddish to purple. Gametes from at least three males and three females were used in each experiment (ABNT 2012; Laitano et al. 2008). The gametes were checked for viability and then the fertilization was done, by adding a 0.5 ml of sperm solution to 24.5 ml of ovules solution. Fertilization success was observed by the presence of fertilization membranes. At least 90% of eggs should be fertilized to the test be valid (Laitano et al. 2008).

The tests were initiated by adding approximately 500 eggs to each test-chamber. The test system was kept under controlled conditions (photoperiod of 12h:12h clear-dark; 25 ± 2ºC) for approximately 36-42h, until the embryos developed to the pluteus stage. The physicochemical parameters of the overlying solu-

tions in the test chambers (i.e., salinity, dissolved oxygen, and pH) were measured only at the beginning of the tests, as the experiments were short-term and no great variations were expected. Then the contents of each replicate were fixed by adding 0.5 ml of tamponed formalin at 10%. The first 100 embryos of each replicate were counted under an optical microscope, to identify the normal larvae and those presenting any abnormalities (delayed development, morphological alterations) as recommended by ABNT (2012); for more details see Fig SM1.

The results of each experiment were organized and checked for normality and variance homogeneity using the Shapiro-Wilk test and Levene's test, respectively. Then they were analyzed by the Wilcoxon–Mann–Whitney test (controls x treatments), allowing the determination of the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC). Paleontological Statistical 4.03 – PAST software (Hammer et al. 2001) was used for data analysis.

Results and discussion

Particles sizes characterization showed that both glitters were composed of particles of various sizes (Table 1), and that the particle size distribution of the green glitter was more heterogeneous. According to the standard method NBR 6502 (ABNT 1995), green glitter was mainly composed of particles ranging between 0.002 and 0.006 mm and could be classified as similar to medium silt, according to the Wentworth's (1922) scale and the Shepard's (1954) classification (Table SM 1). The white glitter presented particle sizes between 0.06 and 2 mm, and could be classified as presenting texture similar to silty sand.

The polymeric composition of the white glitter obtained by Py-GC/MS is presented in Fig 1. The parti-

Table 1 Grain-size distribution and classification of green and white glitter particles

Grain size distribution statistics (Phi)	Glitter type	
	Green	White
Mean diameter	5.52	3.94
Standard deviation	0.94	0.84
Skewness	0.59	0.90
Kurtosis	3.53	4.19
Particle size range (Wenthworth)		
Medium sand (250 - 600 µm)	0.00	0.32
Fine sand (125 - 250 µm)	0.00	17.28
Very fine sand (63 - 125 µm)	4.24	43.93
Coarse silt (31 - 63 µm)	29.51	26.62
Medium silt (15.6 - 31 µm)	39.21	7.95
Fine silt (7.8 - 15.6 µm)	20.66	3.12
Very fine silt (3.9 - 7.8 µm)	4.94	0.73
Clay (<0.06 µm)	1.43	0.11

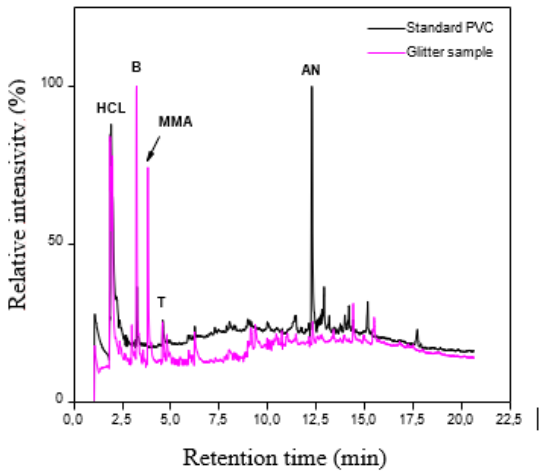

Fig. 1 Chromatograms of the glitter sample, showing the pyrolysis products identified. HCL: hydrogen chloride; B: benzene; MMA: methyl acrylate-vinyl chloride copolymer; T: toluene; AN: anthracene.

cles were composed of the methyl-acrylate vinyl chloride (MA-VC). The pyrolysis products showed peaks for the following compounds associated with PVC: hydrogen chloride (HCl), benzene (B), toluene (T) and anthracene (AN). Methyl acrylate (MM) was also identified, but this compound is not part of PVC; instead, it is an acrylic monomer used in polymers with distinct properties such as rigidity or flexibility, and hydrophobicity or hydrophilicity (McNeill et al. 1995). The literature shows that glitter particles may contain different types of polymers, such as polyester, polypropylene, polyvinyl chloride, and poly(methyl methacrylate), either single or combined (Vernoud et al. 2011; Gross et al. 2010). Besides, PVC can present various substances in its composition, such as HCl, benzene, toluene, styrene, and some poly aromatic hydrocarbons (PAHs) like indene, naphthalene, acenaphtene, fluorine, and anthracene (Tsuge et al. 2011), but not all compounds may be identified by Py-GC/MS.

The results of the toxicity tests with both types of glitter are shown in Fig. 2 and the Supplementary Material (Charts SM1 to SM6). The physicochemical parameters of the glitter suspensions tested were within accepTable ranges and are shown in the Supplementary Material (Tables SM2 to SM4). The results of the three experiments showed broad variation when compared to each other. The first tests (Fig. 2) did not show a clear dose-response; for the green glitter significant effects were detected only at 0.01 and 100 %; mean embryonic development was low at 0.1 and 1%, but not statistically different from the control. All concentrations tested for white glitter were toxic; however, the effects did not increase with the concentration. In the second test (Fig. 2), green glitter was toxic at all concentrations tested, and a clear dose-response was evidenced. For white glitter, significant effects occurred at 0.1 and 10 %. Finally, in the third test (Fig. 2), the green glitter was toxic only at the highest concentration tested (100 %), while the white glitter was toxic at most concentrations, with the exception of 0.1 %; however, the response tended to increase with increasing concentration. The LOECs calculated for the green glitter dispersions were 0.01 % ($P < 0.05$) in the first two tests (Fig. 2) and 100% in the third one (Fig. 2). In contrast, the LOECs for the white glitter

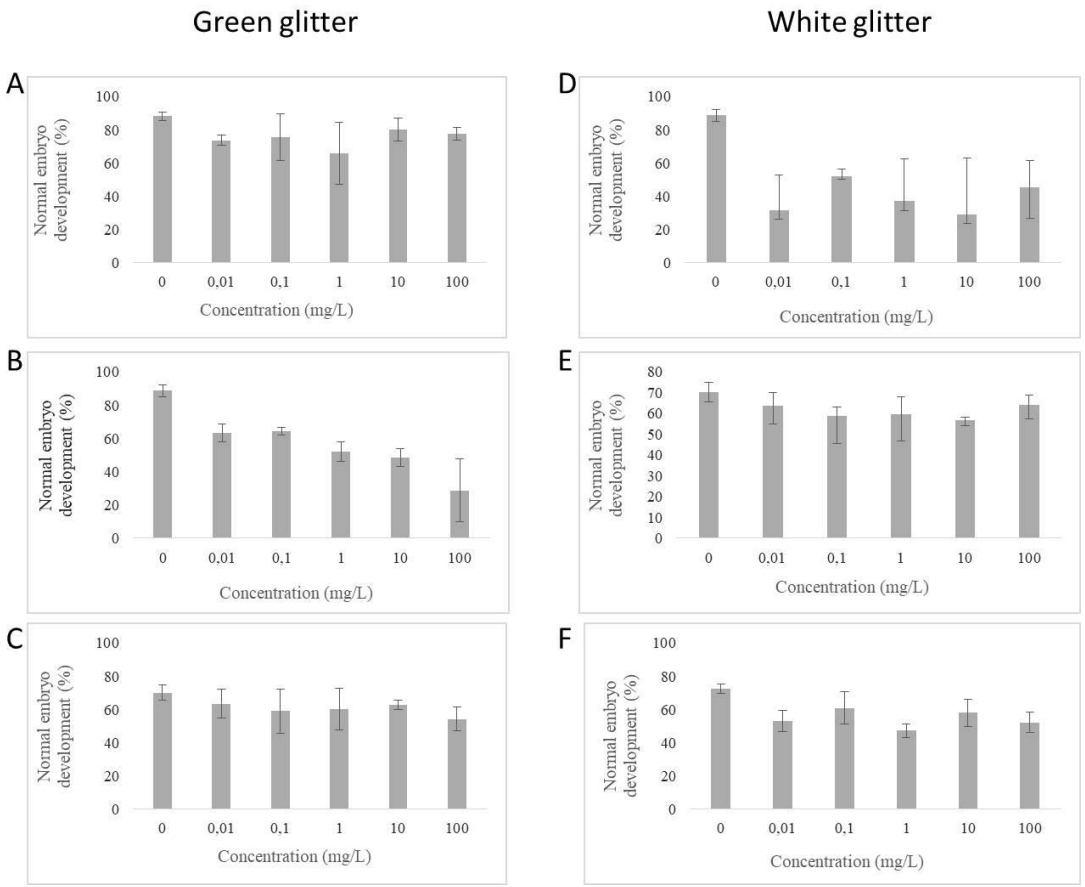

Fig. 2 Normal embryonic development of *Mellita quinquiesperforata* exposed to dispersions of green and white glitters. Asterisks indicate significant differences in relation to the control ($P < 0.05$). A, B, and C – green glitter, first, second, and third tests; D, E, and F – white glitter, first, second, and third tests. Error bars indicate standard deviations.

dispersions ranged between 0.01 % (tests 1 and 3) and 0.1 % (test 2), as shown in Fig. 2.

The results of the bioassays exhibited high variability, as observed by the LOECs, especially for green glitter. Some intra-replicate variability was also observed, as shown in the second test with white glitter. This variability may be caused by the difficulty of mixing the glitter homogeneously in the water, despite the procedures used to maximize the mixtures, and the consequent variation in the amount of leached substances in the water. In fact, the literature shows that microplastics often form aggregates in aquatic environments, although such phenomena is not well understood (Wang et al. 2021). Aggregation of MPs is a dominant physicochemical process that determines the behavior and overall fate of MPs in aquatic environments (Alimi et al. 2018), but the process in natural waters is still largely unknown (Singh et al. 2019). MPs aggregation may be influenced by multiple factors (Wang et al. 2021) and the resulting toxicity to aquatic biota can vary widely (Fan et al. 2019; Choi et al. 2019). Thus, the high toxicity variability in our results could be explained, at least in part, by the variable behavior of glitter particles in the aqueous medium.

According to the information displayed on the glitter packages, their compositions are different (Table 2), and only butylated hydroxytoluene (BHT), propylparaben, and talc were present in both types of glitter. The green glitter contained a higher number of compounds (12), while the white glitter contained only five compounds identified. These differences in composition and particle sizes may have influenced the dispersions' preparation and their toxicities; white glitter was easily mixed in the seawater, while green glitter formed a layer on the water surface. Anyway, both glitter dispersions caused toxic effects on the embryos of *M. quinquiesperforata*, and the dispersions from white glitter tended to be more toxic, possibly due to the fact that this glitter mixed more easily in the water.

Our results are similar to previous results obtained by our group (unpublished data), in which dispersions of glitter caused significant mortality and growth inhibition in brine shrimp *Artemia* sp. naupli at a concentration of 0.1%. In the present study, significant adverse effects on sand-dollars embryos occurred from 0.01%, in the tests 1 and 2.

Green et al. (2021) compared the toxicity of leachates (~435 mg kg^{-1}) obtained from conventional PET glitter and glitters of alternative biodegradable materials (modified regenerated cellulose; natural and synthetic mica) and observed similar effects, regardless of the type of glitter. The reported effects included lower levels of chlorophylls B and C in planktonic algae, and shorter root lengths of *Lemna minor* compared to the controls. Because the glitter sank to the bottom of the test chambers, the authors attributed the effects to the substances present in the leachates and not the physical effects.

The composition of glitters, given by both Py-GC/MS analysis and manufacturer information, includes substances that can be leached (Tables 1 and 2), yet the list of potential substances can be larger. The release of substances from plastics has been well documented in the literature (Brede et al. 2003; Hennesuse-Boxus and Pacary, 2003; Kim et al. 2006; Mutsuga et al. 2006; Fernandes et al. 2008; Tønning et al. 2010). Propylparaben (PPB) is used to extend the shelf life of personal care products. Once PPB leaches into the water, it has endocrine disruption properties and can affect the vitellogenesis and reproduction of exposed animals (Bila and Dezotti 2007), leading to a reduction in reproductive rates and embryo development. García-Espiñeira et al. (2018) observed that PPB affected the physiology of the nematode *Caenorhabditis elegans* in terms of growth, reproduction and gene expression. On its turn, the BHT is also used as additive to improve

Table 2 Composition of the glitters transcribed from the labels of each package. (CI 77019 = mica; CI 77891 = Titanium Dioxide)

Glitter type	Green Glitter	White Glitter
Formulation components	BHT	BHT
	Propylparaben	Propylparaben
	Talc	Talc
	Paraffinum Liquidum	CI 77019
	Methylparaben	CI 77891
	Caprylic/Capric Triglycerde	
	Hydrolyzed collagen	
	Magnesium Carbonate	
	Cyclomethicone	
	Serica Powder	
	Zinc Stearate	
	Dimethicone Crosspolymer	

plastics durability. It is considered safe for humans at the levels used, but there is some controversy regarding its presence and toxicity in the aquatic environment (Sarmah et al. 2020). Toxicity tests with embryos of zebrafish (*Danio rerio)* showed LC_{50} values after 96h of 4.39 mg L^{-1} and 200 µM, respectively (Sarmah et al. 2020; Yang et al. 2018), thereby demonstrating the teratogenic potential of BHT. Although the aforementioned investigations were conducted in a freshwater fish, we can assume that similar effects would be caused on marine organisms.

The green glitter package informs about the presence of methyl paraben (MeP), one of the most used parabens in cosmetic products (Marquez-Sillero et al. 2010). Carvalho et al. (2021) studied the MeP effects on larvae and adults of zebra fish and found median LC50 values of 105.1 mg L^{-1} for adults and 211.1 mg L^{-1} for larvae. The higher toxicity for adults was unexpected, as the initial developmental phases are normally more sensitive to pollutants. Li and Zhang (2017) discussed that the yolk seemed to be involved in the immune and antioxidant defenses in fish embryos, by the action of vitellogenin (Vtg) or Vtg-like proteins, explaining the results found.

In its turn, the methyl acrylate (MM), identified by the Py-GC/MS in the white glitter, was tested for its toxicity by Staples et al. (2000). The authors found lethal concentrations for rainbow trout *Oncorhynchus mykiss* (> 6.7 mg L^{-1} after 24h and 48h; 4.5 mg L^{-1} after 72h); and 3.4 mg L^{-1} after 96h); sheepshead minnow *Cyprinodon variegatus* (3.8 mg L^{-1} after 24h; 2.1 mg L^{-1} after 48h); 1.3 mg L^{-1} after 72h; and 1.1 mg L^{-1} after 96h); *Daphnia magna* (>6.4 mg L^{-1} after 24h; and 2.6 mg L^{-1} after 48h); and *Mysidopsis bahia* (>2.1 mg L^{-1} after 24h; >3.1 mg L^{-1} after 48h; 1.6 mg L^{-1} after 72h and 96h). Based on these results, we can assume that MM can be released from glitter particles and contribute to the toxic effects observed in sand-dollar embryos.

Other two compounds identified in the white glitter, benzene and toluene, had their toxicities described for the fathead minnow *Pimephales promelas* (Marchini et al. 1992), and benzene was more toxic to the larval stages (LC_{50} = 15.59 mg L^{-1} after 96h and 14.01 mg L^{-1} after 7 days). For toluene, the LC_{50} values were 17.03 and 9.38 mg L^{-1}, respectively, for 96h and 7d exposures). For the juvenile fish, LC_{50}s of benzene and toluene were 24.6 mg L^{-1} and 362 mg L^{-1}, respectively. These results indicated more severe effects during the first stages of development.

Finally, some differences in glitter toxicities may be due to the way they are mixed in seawater. The white glitter particles tended to mix more easily in the water, whereas the green glitter only mixed in the water after sonication; the green glitter particles also tended to be trapped on the water surface. Thus, white glitter may have leached chemical substances into water in a more effective way, explaining its higher toxicity.

To the best of our knowledge, this is the first study conducted with neotropical organisms focusing on the effects of glitter dispersion. As observed, both types of glitter suspensions were capable of causing adverse effects on the embryonic development of the sand dollar *M. quinquiesperforata*, and this may potentially affect other marine organisms. Further studies are required to assess the potential ecological risks associated with glitter particles, as well as the chemical substances associated with them.

Competing interests 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.

Author's contribution L.F. Albanit: Conceptualization, Methodology, Formal analysis, Investigation, Writing, I. Beverari: Methodology, Data analysis, manuscript editing; C. Cesar-Ribeiro: Methodology, data analysis, manuscript editing; G.T. Gimiliani: Conceptualization, Reviewing and Editing, D.M.S. Abessa: Conceptualization, Resources, Writing, Reviewing and Editing, Supervision.
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