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Original Research

Virgin plastic pellets may cause toxic effects to embryos of the sand dollar *Mellita quinquiesperforata*: a preliminary study

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Abstract

This study aimed to determine the toxicity of different virgin plastic pellets (Polypropylene with and without additive, Polyethylene and Polystyrene) on embryos of the sand dollar *Mellita quinquiesperforata*. Toxicity tests were carried out with each pellet's type. Adult individuals were collected at Itararé Beach (São Vicente, SP, Brazil) and gametes were obtained by osmotic induction. Elutriates (200 ml of each pellet type in 800 ml filtered seawater, agitated for 5 min and kept equilibrating for 10 days) were prepared. The tests were conducted in glass test tubes, with 4 replicates per treatment. The results were analysed by the generalized mixed model with Negative Binomial distribution ($p \leq 0.05$). Ratio Rate (RR) was calculated, to determine significant differences of the abnormal larval development between treatments and control (AIC = 548.9; X2 = 126; $p < 0.001$). All pellets' elutriates induced significant toxicity, and the presence of abnormal larvae was 3 to 4 times higher than in the control (RR = 3.13 - 4.06). Our results demonstrate that virgin pellets represent a potential threat for *Mellita quinquiesperforata* larvae.

Keywords: Additives, Ecotoxicology, Leachate, Microplastics, Plasticizers, Toxicity.

INTRODUCTION

Aquatic environments worldwide have been affected by a variety of anthropogenic pressures (Bebianno *et al.*, 2015). Poor management of solid waste generates a large amount of waste, called marine litter when it arrives in the ocean. Plastics represent about 90% of the total marine litter (Milijö, 2001; Rochman *et al.*, 2013) making them pollutants of priority concern. Microplastics (MPs, i.e., particles < 5 mm) are predominant among marine litter (Galloway *et al*., 2017), and may originate from primary and secondary sources. Primary source MPs are intentionally manufactured as small particles as raw material of other plastic-based materials or for direct use as abrasives in cleaning products, aesthetics. Secondary MPs result from the degradation of larger plastic pieces due to mechanical, photolytic and/or chemical processes and biological degradation in the environment (Andrady, 2011; Mathalon and Hill, 2014).

Plastic pellets constitute an important fraction primary sources of MPs. They consist of small granules, usually disc-shaped or cylindrical, composed of a variety of polymers, with polyethylene, polypropylene, and polystyrene being the most common (Ogata *et al.*, 2009). Plastic pellets are basically used as raw material for the manufacturing of plastic

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products, and during their production, handling, packaging and transportation, losses commonly occur and these materials end up in the environment (EPA, 1990; Mato *et al*., 2001). Plastic pellets can be released directly into the marine environment or can be carried by runoff, streams and river waters, reaching the estuaries and oceans (Ogata *et al.*, 2009; Karlsson *et al*., 2018).

Large quantities of plastic pellets have been found in coastal and other marine environments worldwide (Cole *et al.*, 2011; Ogata *et al.,* 2019), including Brazil (Ivar do Sul *et al*., 2009; Turra *et al.*, 2014; Izar *et al.*, 2019). Their presence in the environment causes not only physical impacts, but also can be associated with chemical contamination, because plastic pellets can sorb a range of chemicals from the environment, which can be carried and further released back to the ecosystem (Mato *et al*. 2001). The hydrophobic characteristic of plastic pellet polymers makes them prone to adsorb many substances present in the environment, including metals, polycyclic aromatic hydrocarbons (PAH), and polychlorinated biphenyls (PCBs) (Endo *et al.*, 2005; Ogata *et al*, 2009; Karapanagioti and Klontza, 2008; Teuten *et al.,* 2009; Fisner *et al.,* 2013). Additionally, virgin plastic pellets may contain a variety of chemical additives that are included during their production, and which depend on the final use of respective plastic and the type of the plastic polymer Groh *et al*., 2019). Wiesinger *et al*. (2021) reported that plastics may have more than 10,000 substances as additives, from which over 2,400 are of potential concern. Chemical additives are used to alter the physical characteristics of the polymer, such as the aesthetic (color and shape), physical (hardness, flexibility, and thermal resistance) properties, between others (EPA, 1990; Wiesinger *et al.,* 2021). These additives may be leached from the plastic pellets into the aquatic environment, being transferred to the water column or the aquatic biota (Akdogan *et al*., 2019).

Exposure of aquatic biota to contaminants from plastic pellets may occur through ingestion and/or absorption of dissolved substances, leading to bioaccumulation (Ogata *et al.,* 2009; Yamashita *et al.*, 2011). Besides, ingested microplastics may be transferred to predators, and during this process they can carry and transfer the contaminants sorbed on the pellets, as previously observed for plastic additives (Browne *et al.*, 2013; Chua *et al*., 2014). Because many of these additives are toxic, they may cause physiological effects when assimilated by the biota (Browne *et al*., 2013).

Although there are many studies addressing the physical and physiological impacts on marine organisms due to the ingestion of plastic pellets, the effects associated with substances leached from plastic pellets to the water column still need more attention. As demonstrated by Nobre *et al.* (2015), exposure to leachates of virgin and beached plastic pellets induced high toxicity to embryos of the sea urchin *Lytechinus variegatus*, with virgin plastic pellets exhibiting a higher chronic toxicity. Gandara e Silva *et al* (2016) demonstrated that the leachates from plastic pellets were toxic to embryos of the mussel *Perna perna*, while Izar *et al.* (2019) demonstrated that plastic pellets induced chronic toxicity to embryos of *L. variegatus* and the reproduction of the copepod *Nitokra* sp. at extremely high densities. Gewert *et al.* (2021) showed that leachates from virgin plastic polymers were toxic to the harpacticoid copepod *Nitocra spinipes*. These studies evidenced that the contamination and toxicity associated with virgin plastic pellets should be further investigated.

Because virgin plastic pellets may present a wide list of chemicals in their composition, and considering that some of such compounds may be leached from the pellets when they are introduced in the aquatic environment, this study aimed to assess the toxicity of four types of virgin plastic pellets (Polypropylene [PP] with and without additive, Polyethylene [PE] and Polystyrene [PS]) on the larval development of the sand-dollar *Mellita quinquiesperforata* (Echinoidea), in order to contribute to the construction of knowledge on this topic.

This species is highly abundant in the intertidal zone of the Brazilian coast (Diniz *et al*., 2021), inhabiting the surf zone of sandy beaches. This species' habitat makes *M. quinquiesperforata* prone to be directly or indirectly exposed with the MPs, since pellets can precipitate and accumulate in the sediment (Coppock *et al.* 2017; Imhof *et al.* 2018). Besides, *M. quinquiesperforata* is sensitive to contaminants justifying the choice for its use in ecotoxicological assays (Laitano *et al.*, 2008; Mello *et al.,* 2020).

MATERIALS AND METHODS

Basically, fertilized eggs of *M. quinquiesperforata* were exposed to aqueous solutions (elutriates) prepared from virgin plastic pellets and their embryolarval development was evaluated. The toxicity of different virgin pellets was assessed considering the potential leaching of additives, as suggested by Nobre et al. (2015). Polypropylene (PP) (officially with and without additives), polyethylene (PE) and polystyrene (PS) pellets with additives were obtained from the manufacturer (Braskem), stored in sealed amber glass jars and kept in the dark until the tests were performed.

The mean size of the plastic pellets was determined by dry sieving using a set of meshs (ϕ scale) to weight fractions, and then classified by Wentworth's scale (1992). To confirm the sizes of the pellets, 50 unities were measured using a pachymeter.

Plastic pellets elutriates were prepared following those procedures used by Nobre *et al.* (2015), which consists in an adaptation of the protocol proposed by Cesar *et. al* (2004) for sediments. Approximately 200 ml of pellets were supplied in 1 L beakers and supplemented with 800 ml of filtered seawater (0.45 µm polyester membrane). The solutions were stirred for 5 minutes and placed in static rest for at least 10 days, with the beakers wrapped in aluminium and kept inside a dark box at room temperature. Next, aliquots of elutriates were collected from the supernatant and placed in glass test tubes. For each

treatment, four replicates were prepared, consisting of test tubes with 10 ml of the respective elutriates: PP pellets with and without additives, PE and PS pellets and control (only clean and filtered seawater).

The toxicity tests followed the protocols of ABNT NBR 15350 (2012) and EPA (2002) for sea urchin eggs and embryos, with some adaptations proposed by Laitano et al. (2008) and Mello et al. (2020) for sand dollars. Approximately 100-120 adult individuals from *M. quinquiesperforata* were collected across the intertidal zones intertidal zones of Itararé Beach in São Paulo, Brazil. In laboratory, the organisms were placed inside tanks containing filtered seawater, and maintained under constant aeration, controlled temperature and photoperiod (12h:12h clear: dark). The conditions present during the acclimation are shown in the Supplementary Material (Table SM1).

The gametes of *M. quinquiesperforata* were obtained by osmotic induction. 0.5 ml of a solution of potassium chloride (1 M KCl) was injected into the aboral region of the organisms (Laitano et al., 2008), inducing the spawning. Gametes of at least 3 males and females were used (ABNT, 2012). The gametes were identified for sex according to their respective colours (whitish for sperm and reddish for ovules) and then examined for their integrity under microscope (i.e. for maturation stage and the presence of possible anomalies). The sperm were put in a beaker kept wrapped on ice, while the ovules were collected directly from the organisms in the Petri dishes and transferred to beakers with filtered seawater. For fertilization, 1-2 mL of sperm solution was added to the ovules solution, and agitated for 10 minutes to allow fertilization. Then, fertilized eggs were observed and quantified. This determination was made by the presence of the fertilization membrane around the eggs (Nascimento *et al.,* 2002). A minimum of 70% fertilization rate should be achieved in 3 sub-samples (Laitano *et al.*, 2015). Next, a volume containing 300 – 500 eggs was added to each test tube.

The test was performed under constant conditions (photoperiod 12h:12h light-dark, 25 ± 2ºC), for about 36-42 h, until the embryos have developed to the pluteus stage. Then, the contents of each replicate were fixed by adding 0.5 ml of 10% formaldehyde buffered solution. The first 100 embryos of each replicate were counted under an optical microscope and identified between normal and abnormal larvae (ABNT, 2012). The presence of stages prior to larval form, such as egg, morula, blastula and gastrula, as well any types of malformations, were considered abnormal larvae. Normal larvae are those with the length of the arms equal to or greater the length of the body (Nascimento *et al.,* 2002) (Figure 1). Three tests were performed with the four types of pellets. This was necessary to take into consideration the natural variability of sand-dollars' embryos.

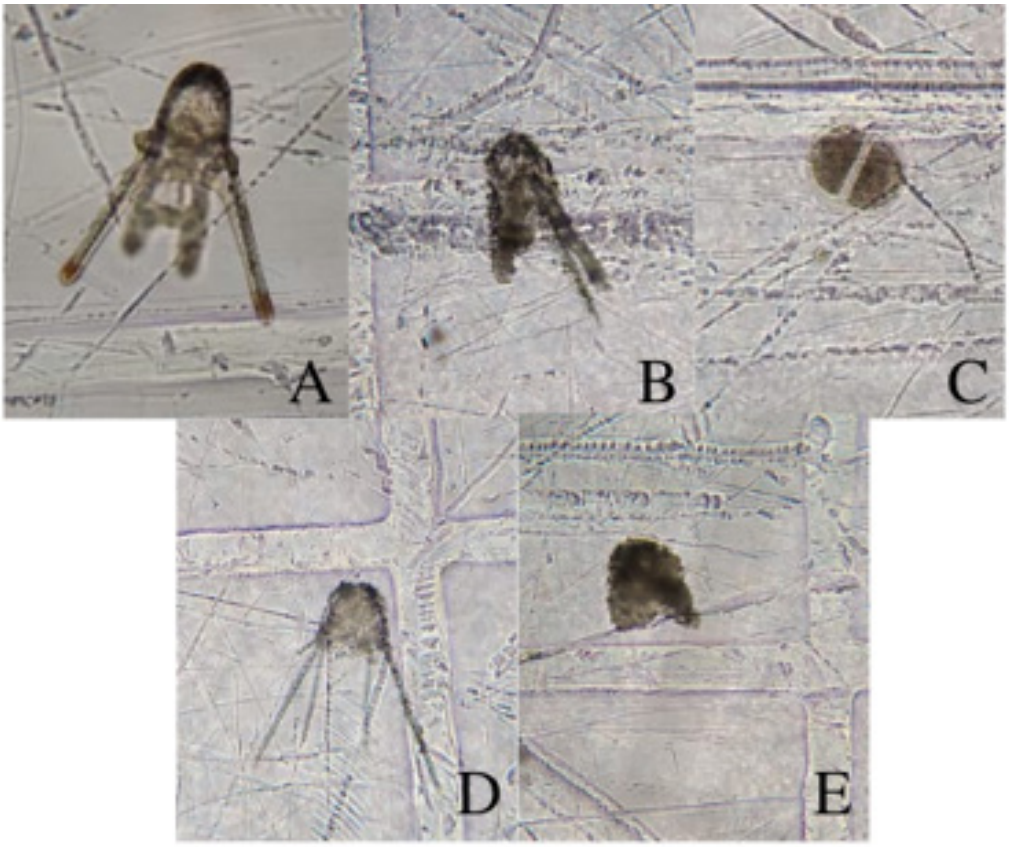

Figure 1. Larvae of Mellita quinquiesperforata in 10x increase; A: normal pluteus embryo after 36h; B: embryo exhibiting morphological anomaly, (malformed appendix); C: cell in the second cleavage after 36h; D: embryo with abnormal development of the appendages; E: embryo exhibiting delayed development. (Source: Letícia Albanit França)

To analyse the data, we built a generalized mixed model (GLMM) with Negative Binomial distribution (lower AIC, better fit to the model), in order to identify the differences of embryonic development between the treatments and the controls. The percentage of abnormal larvae was placed in the model as a dependent variable, with Tests (Factor 1, random, 3 levels) and Treatment (Factor 2, fixed, 5 levels) as independent variables. The significance level was 95% for all statistical analyses of this study ($p \leq 0.05$). Finally, to estimate the effect size, Ratio Rate (RR) was calculated for all treatments compared to the control. This analysis has a total of 60 replicates (N = 12 per treatment).

A second statistical analysis was performed per test to verify differences within each test separately, following the same previous model, however, without the random variable "Tests". In this analysis, the total number of replicates was 20 (N = 4 per treatment).

Results and Discussion

According to the Wentworth's scale (1992), the plastic pellets mean sizes were classified as coarse particles: PS, PP and PE were classified as fine gravel (100% PS and PP particles ranging between 4 and 5 mm; 100% PE particles higher than 5 mm), while PS "without" additive was mostly composed by particles at the range of fine gravel (79.1%) with contributions of very fine gravel (13.6%) and very coarse sand (7.3%).

The mean rate of abnormal larvae found in controls was 22.4% ± 7.19%, while in pellet treatments, the highest mean rate of abnormality was 90.9% ± 11.7% in the treatment of PP without additives. In the other treatments, the mean rate of abnormal larvae was less variable and only the PS showed a greater variation between the tests (Figure 2a); normal development rates ranged between 4.5% ± 5.4% and 80.0% ± 7.2%, with a mean of 70.2% ± 37.5%. All pellet treatments induced differences in the rate of abnormal larvae when compared to the control (AIC = 548.9; $X^2 = 126$; $p < 0.001$). The abnormal larvae rates were 3 to 4 times higher than in controls (RR = 3.13 - 4.06) (Figure 2b).

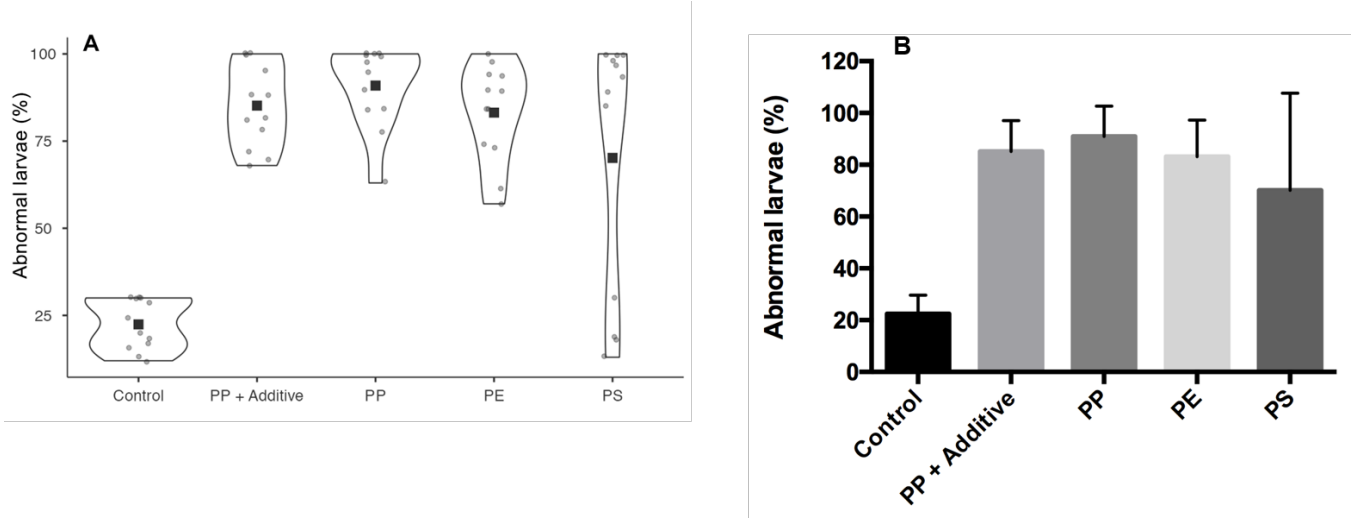

Figure 2. A: Percentage of abnormal larvae of *Mellita quinquiesperforata* exposed to different types of plastic pellets. B: Effect size on the development of *M. quinquiesperforata larvae*. PP = Polypropylene, PE = Polyethylene and PS = Polystyrene. Results are presented as means for each joint treatment. Error bars (B) are presented as 95% confidence intervals.

When the tests were analysed separately, the same pattern was exhibited by the controls. In pellets' treatments, results tended to be similar, excepting those from PS pellets in the test 1, in which such pellets were not toxic (Figure 3). The other pellets' treatments exhibited similar toxicity in the three experiments (Figure 3). The size of the effect in pellet treatments varied between the tests, with a Ratio Rate of 2.57 to 3.3 times more abnormal larvae in pellets treatment than in control in test 1, 5.49 to 6.23 times more in test 2 and 3.49 to 4.34 times more in test 3 (Figure 3). However, such variation was much more due to the variation of the percentage of abnormal embryos in the controls than in the pellets' treatments. Details of the results of each toxicity test are presented in the supplementary material.

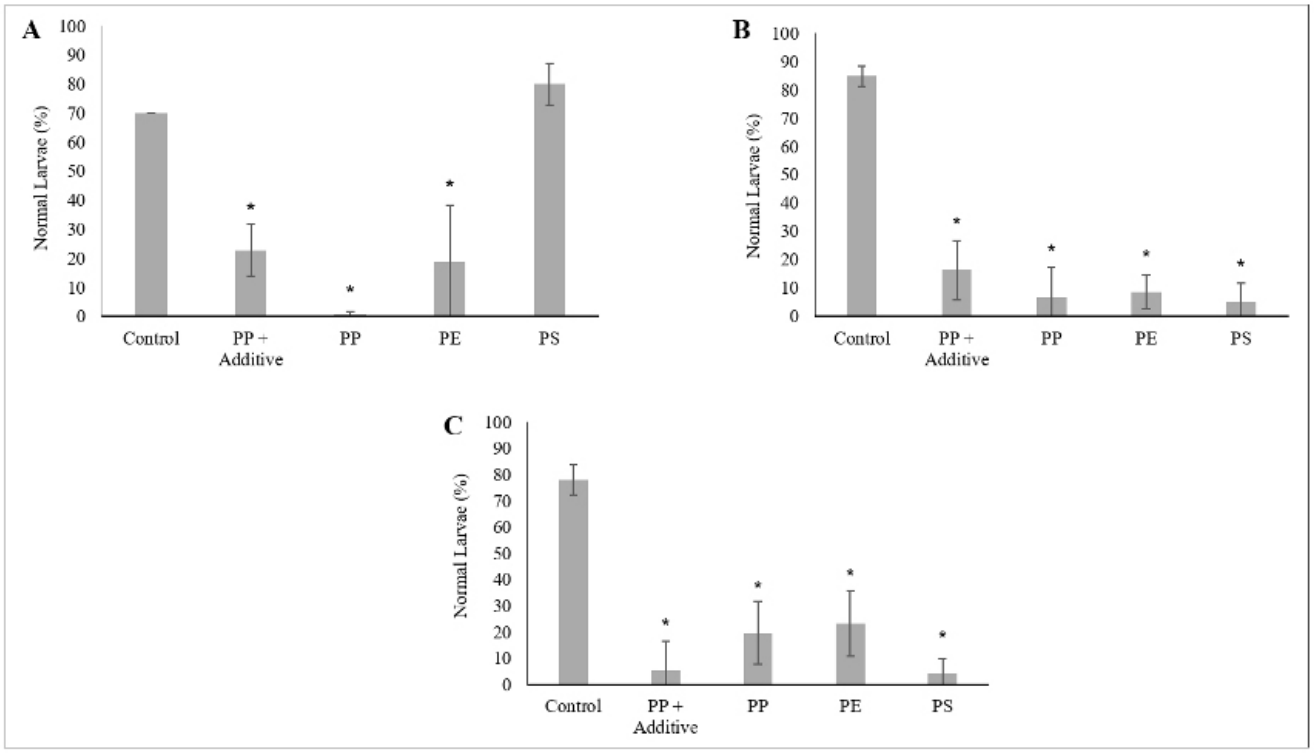

Figure 3. Effect size on the development of *Mellita quinquiesperforata* larvae in the different tests performed: A: Test 1, B: Test 2 and C: Test 3. PP = Polypropylene, PE = Polyethylene and PS = Polystyrene. White dots represent the means per treatment and error bars are 95% confidence interval.

The elutriates from all the plastic pellets tested caused adverse effects on the larval development of *M. quinquiesperforata*. The only exception was the toxicity of PS, which occurred in two out of three experiments, but the causes of this variation are unknown. When the overall results are analysed (Fig. 2), the polymers could be ranked according their toxicity as PP > PP+additive = PE > PS. This classification has some similarity with the study of Schiavo et al. (2018), in which PP was the most toxic, but PS was more toxic than PE.

The toxicity of elutriates produced from virgin plastic pellets suggest that these materials possibly contain chemicals that can be released to the water column and absorbed by the aquatic biota. Such chemicals can be added to the polymers during the manufacture process (Lithner *et al.,* 2011), and include stabilizers, plasticizers, flame retardants, pigments and fillers (Lithner *et al.*, 2011). As previously mentioned in this article, plastics may present over 2400 hazardous substances (Wiesinger *et al.,* 2021), which could explain the toxicity associated with plastic pellets leachates. However, our results differed from those reported by some authors. Rendell-Bhatti et al. (2021) observed absence of toxicity when studying virgin industrial pre-production plastic nurdles, in opposition to beached pellets and new white polyvinyl chloride (PVC) prime plasticised pellets, which were toxic. Beiras *et al.* (2018) also did not find toxicity associated to the ingestion of microplastics by the zooplankton while Koelmans *et al.* (2014) used a theoretical model to predict the leaching of nonylphenol and bisphenol A, concluding that the resulting leachates would not be toxic. On the other hand, Beiras et al. (2019) observed that leachates from virgin plastic polymers were toxic to the copepod *Acartia clausi* and embryos of the sea urchin *Paracentrotus lividus*, even in low concentrations. Thus, based on our results and the aforementioned literature, we can infer that the toxicity associated to plastic polymers seems to depend on the polymer (type and composition), the form of exposure and the species involved.

Some of the most dangerous health additives include polybrominated flame retardants (e.g., tetraBDE and pentaBDE), plasticizers such as phthalates, and lead-containing heat stabilizers (Lithener *et al.*, 2011). These additives are generally not bound to the polymer matrix, have low molecular weight and might be present in relatively large quantities. They can be more easily released from plastics, being emitted or leached from these materials (Lithener *et al.*, 2011). Literature shows that plastic products are reported to release hazardous substances, such as phthalates, brominated flame retardants, bisphenol A, formaldehyde, acetaldehyde, 4-nonylphenol and many volatile organic compounds (Brede *et al.*, 2003; Henneuse-Boxus and Pacary, 2003; Kim *et al.*, 2006; Mutsuga *et al*., 2006; Fernandes *et al.*, 2008; Tønning *et al*., 2010). Gewert *et al*. (2021) showed that leachates from virgin plastic polymers contained organic chemicals and were toxic to *Nitocra spinipes*, while Björnsdotter (2015) reported the release of organic chemicals from virgin plastics. Thus, the presence of such additives could explain the impairment of the embryonic development of *M. quinquiesperforata*.

Some of these chemicals have been associated with endocrine disrupting effects. Rochman *et al.* (2014) performed a 2-month dietary exposure with two treatments and control, one with virgin polyethylene plastic pellets and the other of marine plastics collected in the San Diego Bay, and showed that exposure to plastics was able to alter the functioning of the endocrine system of the fish *Oryzias latipes*, impairing the binding of endogenous estrogen to the estrogen receptor, reducing the fecundity of females. Endocrine disruption may also produce anomalies in the reproductive systems and changes of vitellogenesis (Bila and Dezotti, 2007), leading to reduction of reproduction rates and embryonic development. Some substances regularly associated with plastics and presenting evidences of being endocrine disruptors include bisphenol A, styrene, phthalates (BBP, DEHP, DBP, DEP and DCHP), and epicloridrin (Groshart and Okkerman, 2000; Okkerman and van der Putte, 2002, Cormier *et al.*, 2021). Rochman *et al*. (2014) observed that fish from treatment diet with virgin plastic pellets exhibited 2.4 times more total PAH and 2.2 times more total PBDE than the controls. Browne et al. (2013) observed that triclosan and PBDE-47 apparently caused more damage to organisms than other persistent pollutants, considering the ingestion of microplastics (PVC). Both additives reduced the feeding capacity of the tested organisms. Although we do not have information on the type and concentration of additives used in each of the pellets used in the study, we can assume that they possibly have some of these substances, and can release them to the point of harming the embryonic development of *M. quinquiesperforata*.

It is difficult to determine if virgin plastic pellets are free of chemicals, including those considered without additives. It is equally difficult to evaluate the nature and levels of additives, as thousands of compounds may be added to each type of polymer (Ascer, 2015; Verla *et al*., 2019; Wiesinger *et al.,* 2021). According to Brandon *et al*. (2016), polypropylene pellets tend to become more fragile under environmental conditions when compared to other polymers, increasing their ability to release or adsorb contaminants. Morerover PP is extremely sensitive to oxidation and requires large amounts of antioxidants and UV stabilizers, while PE requires low amounts of these chemicals (Zweifel, 2001). Moreover, the manufacturers classify the pellets "without additives" those containing antioxidants (Rummel *et al.*, 2019). This could be the case of our study, in which the effects observed in embryos exposed to PP without additives would be explained by the presence such and other substances which are not considered as additives by manufacturers, of their by-products. Rummel et al. (2019) observed that mono and dicarboxylic acids were formed as degradation products from plastic polymers, being associated with toxicity. The authors mentioned that UV-induced weathering increased the leachate toxicity (single cell CALUX assay) and the effects were likely caused by the liberation of unknown chemical mixtures. Further studies to identify the composition of pellets available on the market, as well as the potential toxicity of the associated substances, should be carried out in order to elucidate the toxic mechanisms related to virgin plastic pellets.

Nobre *et al.* (2015) observed that leachates from virgin PE pellets were more toxic to sea urchin embryos than the leachates of beached pellets. That study used the same concentrations of plastic pellets than ours (20%). On the other hand, Gandara e Silva *et al.* (2016) reported that the toxicity of the leachates from beached pellets to brown mussels was higher than that produced by virgin PP pellets. This evidences that the toxicity associated to virgin pellets may depend on several factors, such as type, concentration, organism tested, and nature and concentration of additives, among others.

Anyway, even virgin pellets can be lost and accumulate in the environment, especially on coastal regions, close to their main sources (Gregory, 1978; Izar *et al.,* 2019). The pellets' distribution logistics system which involves land and sea transport, and failures during this process may cause pellets' loss and environmental contamination (Turra *et al.,* 2008; Ogata *et al.,* 2009; Turner and Holmes, 2011). Izar *et al.* (2019) noticed high percentages of translucent and whitish plastic pellets on beaches from southeast Brazil, which suggests the input of virgin pellets to the environment. Based on their toxicities, the introduction of virgin plastic pellets may cause impacts to the aquatic biota due to physical effects and release of chemical substances.

The control of pollution by plastic pellets is under the responsibility of industries and maritime transport companies. However, the efforts to increase knowledge of the negative environmental impacts of plastics are limited, as well as to reduce losses and recycle discarded materials (Manzano, 2009). In Brazil there is not much information about management strategies directed to face this problem, reflecting the lack of public and/or private policies developed on the subject. Governments, economic sectors, academia and society should cooperate towards the comprehension of the problems caused by plastic pellets in the marine environment, the establishment and implementation of strategies to reduce impacts, as well as environmental education campaigns.

Elutriates from different types of virgin plastic affected the embryolarval development of the sand dollar *Mellita quinquiesperforata*, suggesting that these materials contain and can release chemicals to water column, representing thus potential threats for the marine species evaluated.

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Author contributions

LAF: Conceptualization, Methodology, Writing - Original Draft preparation; GMI: Methodology, Investigation, Writing - Reviewing and Editing; GTG: Conceptualization, Investigation; DMSA: Conceptualization, Supervision, Methodology, Writing - Reviewing and Editing

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Abbreviation

PP – Polypropylene
PE -Polyethylene
PS – Polystyrene

Table SM1. Physical-chemical parameters of the overlying water in the tanks where the adults of *M. quinquiesperforata* were acclimated. NM = not measured.

Test	DO ($mg.L^{-1}$)		pH		Salinity	
	Initial	Final	Initial	Final	Initial	Final
First	7.13	6.5	7.68	7.69	37	36
Second	7.92	7.22	6.92	7.25	33	35
Third	NM	NM	8.09	8.08	35	35

Table SM2. Normal development rates (%) of embryos of *M. quinquiesperforata* exposed to pellets in the first toxicity test.

	Normal development (%)					
Pellets	R1	R2	R3	R4	Mean	SD
PP with additive	0	0	1	2	0.75	0.9
PP without additive	19	28	32	12	22.75	8.10
PE	6	43	26	0	18.75	19.62
PS	82	87	81	70	80	7.2
Control	70	70	70	70	70	0

Table SM3. Physical chemical parameters of the first test

Pellets	DO ($mg.L^{-1}$)		pH			Salinity	
Initial	Final	Initial	Final	Initial	Final		
PP with additive	6.77	6.73	7.10	7.92		35	34
PP without additive	6.89	7.01	6.69	8.01		35	32
PE	7.09	6.61	7.49	8.05		35	33
PS	6.97	6.84	8.15	7.96		35	35
Control	7.13	6.5	7.68	7.69		37	36

Table SM4. Normal development rates (%) of embryos of *M. quinquiesperforata* exposed to pellets in the second toxicity test.

	Normal development (%)					
Pellets	R1	R2	R3	R4	Mean	SD
PP with additive	5	22	0	0	6.75	10.44
PP without additive	30	18	12	5	16.25	10.60
PE	2	16	6	10	8.5	5.98
PS	15	3	0	2	5	6.78
Control	87	80	88	84	84.75	3.60

Table SM5. Physical chemical parameters of the second test

Pellets	DO (mg.L^{-1})		pH		Salinity	
	Initial	Final	Initial	Final	Initial	Final
PP with additive	6.36	7.12	7.93	7.89	33	34
PP without additive	6.56	7.22	7.92	7.87	33	35
PE	7.09	6.61	7.49	8.05	35	33
PS	6.97	6.84	8.15	7.96	35	35
Control	7.92	7.22	6.92	7.25	33	35

Table SM6. Normal development rates (%) of embryos of *M. quinquiesperforata* exposed to pellets in the third toxicity test.

	Normal development (%)					
Pellets	R1	R2	R3	R4	Mean	SD
PP with additive	37	16	16	10	19.75	11.84
PP without additive	0	22	0	0	5.5	11
PE	39	11	16	27	23.25	12.45
PS	7	11	0	0	4.5	5.45
Control	83	71	82	76	78	5.60

Table SM7. Physical chemical parameters of the third test (nm = not measured).

Pellets	DO (mg.L^{-1})		pH		Salinity	
	Initial	Final	Initial	Final	Initial	Final
PP with additive	nm	nm	7.94	7.88	34	35
PP without additive	nm	nm	7.94	7.86	36	35
PE	nm	nm	7.92	7.83	35	35
PS	nm	nm	8.00	7.09	34	34
Control	nm	nm	8.09	8.08	35	35