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Original Article

Effects of Microplastics on Coral *Xenia elongata*: an Experimental Approach

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Abstract

Knowledge about the effects of plastic waste on the environment is relevant, an important raw material for the industry due to its low cost, high durability, and easy molding, but it has had drastic and irreversible consequences for ecosystems, including aquatic ones. It is known that in the oceans, this polymer is found in all shapes and sizes, and its action on marine biota has been widely described, among the findings being that corals ingest these microparticles when they confuse them with their prey, which induce the disruption of zooxanthellae leading to death, in addition to causing tissue damage and disease. Despite this, experimental research is still incipient, and this study fills the gap in the literature about what effects the microplastics (MPs) most commonly found in the oceans can cause in non-scleractinian corals. It was found that the concentration of microplastics used in this study caused physiological effects in the soft coral *Xenia elongata*, affecting respiratory functions, growth, and even leading to mortality.

Keywords: Non-scleractinian coral; Polypropylene; Polyethylene; Zooxanthellae.

INTRODUCTION

Due to its low cost, high resistance, and lightness, it is possible to find materials made from plastic polymer in various sectors, after all, it facilitated the automobile line, contributed to the durability of food in markets, revolutionized medicine, structured technological devices, among other applications (Derraik, 2002). However, although the widespread use of plastic materials began only half a century ago, its success generates social, economic, and environmental consequences (Debrot *et al.*, 2013). Once in the environment, plastic particles can reach a vast range of ecosystems, such as the seabed (Peeken *et al.*, 2018), mangroves (Maghsodian *et al.*, 2022), coral reefs (Imhof *et al.*, 2017), rivers (González-Pleiter *et al.*, 2020) and terrestrial ecosystems (Brahney *et al.*, 2020)

In the oceans, measuring and removing plastics becomes an almost impossible task, mainly due to their variety of sizes and shapes (nano- (<100 nm), micro- (0.0001–5 mm), meso- (5–25 mm) and macroparticles (> 25 mm). In general, these particles are being fragmented by photochemical and mechanical degradation (Ripken *et al.*, 2020), and as a consequence entering the food chain of marine ecosystems (Browne *et al.*, 2008). There are increasing studies showing that the ingestion of MPs in the marine environment can have a negative impact (von Moos *et al.*, 2012), however little is known about the effects on corals (Hall *et al.*, 2015).

Despite occupying a small territorial percentage of the oceans, coral reefs are home to the greatest biodiversity of marine ecosystems, which are very vulnerable to anthropogenic disturbances, such as domestic and industrial pollution, and climate change (Hughes *et al.*, 2018A). The main consequence has been the interruption of the symbiosis with dinoflagellate microalgae, causing bleaching and leading to their death (Hughes *et al.*, 2018B). More recently, plastic pollution has gained the attention of scientists, as it has been shown that this type of pollution is highly harmful to corals, from

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macroplastics, which are found to entangle animals resulting in their suffocation, to micro and nanoplastics, whose ingestion by corals (Hall *et al*., 2015) can increase the bleaching of these organisms (Chapron *et al*., 2018).

Studies on the effects of plastic MPs on marine ecology are more than a decade old (Kane *et al*., 2020; Thompson *et al.*, 2004) and because they are crucial ecosystems for marine biodiversity (Spalding and Brown, 2015), coral reefs have received attention from scientists regarding the effect of plastic pollution due to their vulnerability and the diverse ways in which the material is found in marine ecosystems (de Carvalho-Souza *et al*., 2018). Predictions indicate that by 2025 the amount of macroplastic adhered to reefs will increase by 40%, resulting in tissue damage and increasing the incidence of disease in corals (Lamb *et al.*, 2016).

Research in coral reef environments has been gradually increasing, but only seven years ago was the ingestion of polypropylene in a rocky, reef-building coral demonstrated in an experiment (Hall *et al.*, 2015). There is a diversity of coral species that respond differently to microplastics (Reichert *et al.,* 2018). In current studies of the effects of microplastics on corals, there are more experiments on stony corals, some zoanthus (Rocha *et al*., 2020), and a few on "soft" corals (Vencato *et al.*, 2021). The present study tests the hypothesis that microplastic particles present in ocean water can cause damage to soft corals, being the first to study the effects on the species *Xenia elongata* using virgin pellets. This work sought to test and identify the effects of microplastics on this species and fill the gap in the literature regarding the effects of the two plastics most found in the oceans, namely polyethylene (PE) and polypropylene (PP) (Thompson *et al*., 2004), and what they can cause in non-scleractinian corals.

MATERIAL AND METHODS

Animals

We conducted a series of three independent experiments using the coral species *Xenia elongata* belonging to the family Xeniidae commonly found on shallow reefs in the Indo-Pacific (Fabricius & De'ath *et al.*, 2008). The specimens used were obtained from the Ocyan Reef marine coral farm and transported to the experimental site at the Structural and Functional Ecology of Ecosystems Laboratory at Universidade Paulista (UNIP), Sorocaba campus, São Paulo, Brazil, under appropriate oxygen and temperature conditions. Immediately, they were acclimatized and housed in a holding aquarium, where they were kept for approximately 30 days for acclimatization. In total, sixteen colonies of the chosen species with similar sizes were used, each glued to a 03x03 cm cement base.

Aquariums Setup

The experiments were carried out using a hosting aquarium, three for tests and one as a control. The test aquariums did not have a filtration system, in order not to interfere with the dosage of plastic microparticles. The water used for cycling was taken from the aquarium where the corals are kept in the original breeding, so the salinity, phosphate, and nitrate levels were monitored and did not change. We perform partial water changes of 10% weekly and monitor the parameters before introducing them.

Each aquarium was illuminated with a 40W, 6400K white light bulb, and a timer programmed so that they remained on for approximately 12 hours, simulating the time that animals are exposed to sunlight in the oceans. The circulation and oxygenation of the control water was maintained through the booster system, and in the test aquariums we used boyu pumps with porous stone. The temperature was maintained at 25°C with a variation of ±1°C using a heater and thermostat.

Microplastic Preparation

Virgin PE and PP pellets were obtained from a manufacturer (Braskem/MAIS Polímeros do Brasil LTDA, Cajamar, São Paulo, Brazil). The pellets were mechanically fragmented and size-sorted using a 5-mm metal mesh sieve, with all particles used in the experiment being <5 mm in size. The administered microplastic concentration was set at ~200 particles L^{-1}. Since current data on microplastic quantities in coral reef environments remain inconsistent, this concentration was based on the experimental approach used by Reichert *et al*. (2019).

Experimental Design

Four specimens were distributed across three 10-liter aquariums, each representing a different experimental treatment: PP (~200 L^{-1} de polypropylene), PE (~200 L^{-1} de polyethylene), MIX (~100 L^{-1} de PP + ~100 L^{-1} de PE) and control group (no microplastics). They were exposed for 48 hours, and after this time, they were returned to the hosting aquarium, where they remained for 4 days, repeating this procedure for nine weeks. The corals were subjected to particles of PP and PE, considered the most abundant in marine ecosystems (Thompson *et al*., 2004). The description of the experimental design is presented in figure 1.

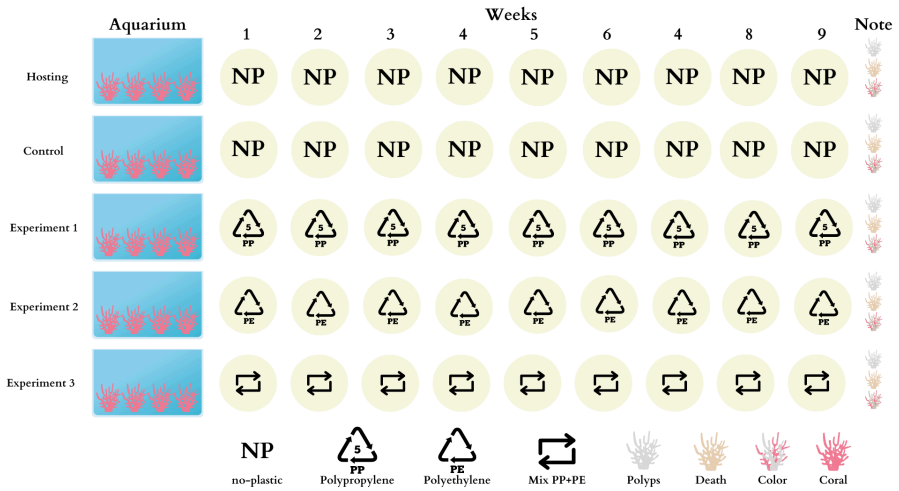

Figure 1. Experimental design and key parameters. Summary of three independent experimental tests of the effects of microplastics on coral health.

Biometric Measurements: Weight and Size

Size measurements were taken with a caliper, considering the tissue base to the polyps, whether or not the polyps were fully extended. They were measured after 48 hours of exposure, still inside the test aquarium under ideal temperature and circulation conditions so as not to cause possible stress. The weight was monitored with an analytical scale and was weighed 48 hours before returning to the aquarium.

Color and Health Analysis Through Photography

Through daily photographs and visual analyzes we compared the colors of the polyps, to quantify this information we considered the number 1 for animals in the healthy pattern (lighter pink) and 0 for those that presented the visual color characteristics considered as stressed (darker pink), there is evidence that *Xenia elongata* corals that grow in good lighting conditions are lighter compared to those that grow in less lighting, however there were no variations in lighting in the aquariums throughout the experiments, exposure time and type of lighting were the same for all test and control aquaria.

Pulsation Monitoring and Video Analysis

The photosynthetic rate of zooxanthellae was analyzed by observing the pulses through filming, as the decrease in pulse activity is a characteristic observed in Xeniidae corals and is related to the photosynthetic rate (Cohen *et al.*, 1977). Through the footage, we counted the act of pulsation made by the choir, for this we considered an average per minute, when completing the pincer movement, we counted 1 pulse.

Furthermore, the physiological condition of coral specimens in response to exposure to microplastics was monitored by filming and photographic records during the 48 hours of exposure. We later compared whether there was tissue development or disintegration, loss of pigments, necrosis, or death.

Statistical Analysis

Statistical analyses were performed in SAS 9.3 (Cary, NC). Percent symbiont loss data were transformed by arcsine-square root and analyzed using two-way ANOVA and Tukey post hoc tests to compare Corexit doses between the exposure times. Zooxanthellae density, normalized to protein content data, was analyzed using the same statistical tests. Pulse rate and relative oxygen saturation data were analyzed for a significant effect of dispersant concentration and exposure time with repeated measures ANOVAs. Pulse intensity data were analyzed for the treatments separately using two independent samples of Kruskal-Wallis tests. Alpha was set at 0.05.

RESULTS

During the experiment, the specimens kept in the control and PP aquariums showed no mortality throughout the nine weeks of testing. Although the PE group appeared to exhibit the same outcome by the ninth week, one individual died, leaving no tissue available for collection. The results observed in the MIX plastic exposure were entirely different; three individuals died in the third week, and in the following week, the only remaining *Xenia elongata* individual died, resulting in 100% mortality before the nine weeks of exposure. Figure 2 displays these data in the boxplot, with the Control and PP groups showing identical results, no data dispersion, and all specimens surviving until the end of the experiment. The PE group contained an outlier, indicating the loss of one specimen, resulting in 75% survival. The MIX group showed the most significant variability, revealing a progression of mortality where only one

individual survived beyond the first weeks of exposure. ANOVA indicated significant differences between groups ($F = 19.41$; $p < 0.0001$). A Tukey post-hoc test revealed significant differences ($p < 0.0001$) between all groups when compared to the MIX treatment (control vs. MIX, PP vs. MIX, and PE vs. MIX).

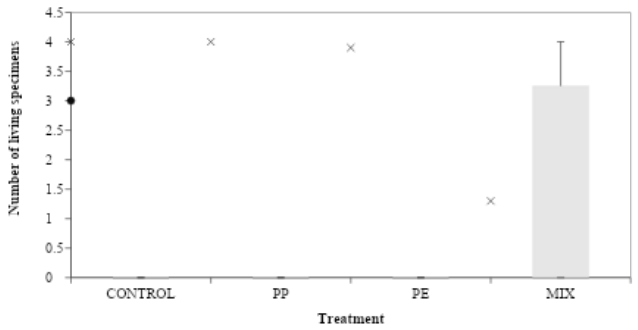

Figure 2. Living specimens during the weeks. PP - exposed to polypropylene; PE - exposed to polyethylene; MIX - exposed to the mixture of plastics.

All specimens exhibited a reduction in size throughout the tests, including those in the control group. The final mean values (±SD) for each group were: PP (5.90 ± 3.25), PE (3.2 ± 1.81), MIX (2.5 ± 2.03), and Control (6.1 ± 1.04). ANOVA revealed that corals exposed to the MIX treatment experienced the most severe length reduction, with an average decrease of 2.25 cm compared to the control. This reduction was significant from the second week of exposure onward. Corals exposed to PE also showed a negative impact, with an average reduction of 2cm starting in the fourth week. The PP group was the only one to show growth relative to the control during the initial weeks of exposure; however, they exhibited a significant reduction starting in the sixth week.

A separate ANOVA was conducted to evaluate the impact of plastic exposure on the weight of the tested corals. Corals exposed to MIX and PE did not show a significant weight reduction compared to the control group ($p = 0.3358$; $p = 0.21580$), while the effect of PP exposure was nearly negligible ($p = 0.94500$). However, exposure time had a significant impact on coral weight. From week 5 onward ($p = 0.04724$), coral weight began to decrease significantly compared to week 0. This effect was particularly evident in the interaction between treatments and weeks, especially in the MIX group, where the weight reduction was pronounced and significant over time.

Following the ANOVA, a Tukey post-hoc test revealed that, throughout the experiment, the MIX group consistently exhibited the greatest reduction in both weight and length compared to the other groups, with statistically significant differences ($p < 0.001$). From the second week onward, there was a clear separation between the MIX group and the other groups, with this difference becoming more pronounced over time. By the ninth week, the PE group also showed a significant reduction compared to the control ($p = 0.0122$) (Figure 3).

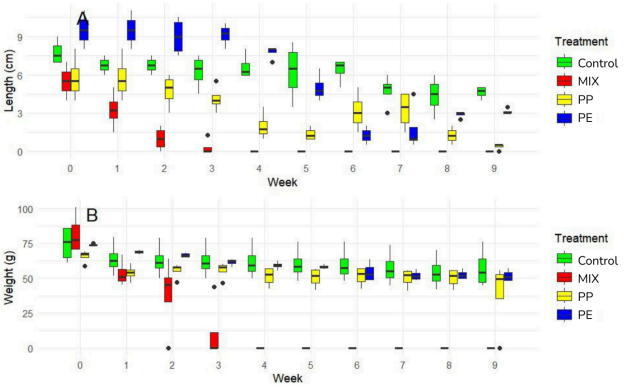

Figure 3. Box-plot weight and length during the weeks. PP - exposed to polypropylene; PE - exposed to polyethylene; MIX - exposed to the mixture of plastics.

Regarding weight loss, this was lower when exposed to PE and PP compared to the MIX. The control group showed a small loss in the first exposures, remaining linear. The experiment with the MIX showed a marked loss from the second exposure onwards. Despite the differences described throughout the experiment, there were no significant differences between the weights in the treatments over the exposure times ($F = 2.4491$, $p < 0.0819$). The final mean values (±SD) for each group were: PP (59.7 ± 7.62), PE (52.3 ± 6.37), MIX (46.2 ± 25.57), and Control (61.5 ± 5.24).

The photographic records showed that only one control individual showed color variation in the penultimate week of testing, but there was recovery. In the experiment with PP, the color variation began from the sixth week, and all individuals also showed recovery, while in the experiment with PE, the variation started in the fourth week, even though they recovered their healthy condition in the weeks following the last two weeks. Remained in a darker pink condition. In the experiment with the MIX in the first week, there was already a color variation, and no individual recovered (Figure 4).

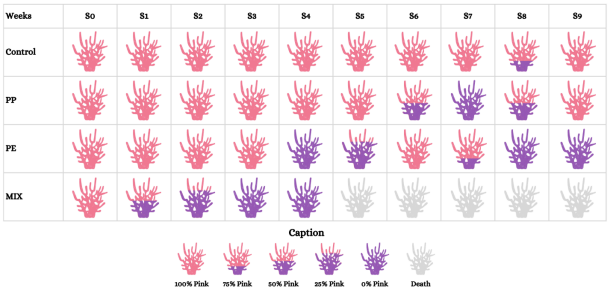

Figure 4. Color variation during the weeks. PP - exposed to polypropylene; PE - exposed to polyethylene; MIX - exposed to the mixture of plastics.

Through pulse counting we evaluated this movement on the first and second test day during the 9 weeks, the animals subjected to plastic exposure had a decrease in movement. It can be observed through Figure 5 that the mean pulse values per minute of the polyps varied between treatments. The control group remained with low variation between specimens, indicating stable physiological conditions throughout exposure. The PP and PE treatments had averages close to zero and little data variation, indicating strongly reduced pulsation in this group, but both with outliers, suggesting that some showed positive physiological activity at some moments. The group exposed to the mixture (MIX) had a higher average than PP and PE and greater data dispersion, which may be associated with early mortality of specimens in this exposure, yet the median is close to zero. When comparing the tests, an ANOVA revealed a significant difference among the groups ($F = 20.35$, $p < 0.001$). Tukey's post-hoc test showed that all groups exhibited significant differences when compared to the control ($p < 0.001$).

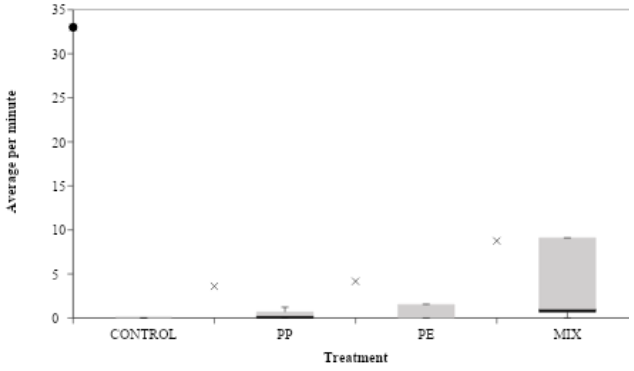

Figure 5. Average pulse rate per minute during the weeks. PP - exposed to polypropylene; PE - exposed to polyethylene; MIX - exposed to the mixture of plastics.

DISCUSSION

The experiment is groundbreaking in exposing the pulsating soft coral *Xenia elongata* to real quantities of MPs, both individually and in combination. This species was selected due to its high sensitivity to changes in water quality and its unique polyp pulsation behavior, which plays a role in aiding oxidative stress regulation. Furthermore, a literature review revealed that *Xenia elongata* has only been used in studies involving exposure to chemical dispersants (Studivan *et al*., 2015), highlighting the relevance and innovation of this research.

Other similar studies, such as that by Hierl and Westphal (2021) who exposed scleractinian corals to PET microparticles for 24h with weekly intervals over five months, also reported high survival rates and color changes, but did not observe bleaching. A pattern similar to that verified in the present study with the plastics tested separately (PP and PE). Convergent results were obtained by Reichert *et al*. (2019), who tested the same PE concentration used in this study on different scleractinian corals and observed changes in photosynthetic parameters after 12 weeks, with the first signs of health impairment appearing 6 weeks after exposure began. These findings reinforce the importance of studies with prolonged experimental periods, like that conducted in this research, and corroborate that individually tested plastics produce distinct effects from those observed in mixtures - a methodological gap still poorly explored in experimental coral studies, but essential for more realistic marine environment simulations.

Different experiments with corals have shown that MPs can affect energy, growth, cause pathogens, influence feeding and photosynthetic performance, and even cause bleaching and tissue necrosis (Lanctôt *et al*., 2020; Reichert *et al.*, 2018; Savinelli *et al.*, 2020), most tests are carried out with plastics individually, in which PP and PE are always associated, and it is reported that corals mistake them for prey (Hall *et al*., 2015; Savinelli *et al.*, 2020). It has also been shown in research that most ingested MPs can be expelled through the mucus that corals release (Rotjan *et al.*, 2019). In the study by Corinaldesi *et al*. (2021) where the coral *Corallium rubrum* was exposed to a mixture of MPs, with the highest concentrations being PE and PP, it was reported that the animals suffered tissue changes, necrosis, stress and total mortality after 14 days of exposure.

In this study, experimental and natural environments were both considered, as in other studies (Lamb *et al.,* 2018), it was shown that the MIX of plastics caused necrosis and death quickly, while individual plastics caused mild stress with the ability to reestablish healthy conditions, other experiments showed that a colony can survive an event light bleaching and eventually recapture zooxanthellae (Thornhill *et al.*, 2006) however some studies indicate that tissue necrosis impedes recovery and often leads to colony mortality (Rodolfo-Metalpa *et al*., 2005).

Other studies with soft corals showed that the species *Coelogorgia palmosa* ingested and adhered to plastic microparticles (Vencato *et al.*, 2021). Research carried out with corals with long polyps found that MPs can lead to damage in the energy metabolism of corals such as *Tubastrea aurea* (Liao *et al.*, 2021), which could be an explanation for the reduction in size and weight of *Xenia elongata* in experiments carried out by us.

Research with *Zoanthus sociatus* has shown that the adhesion of PVC microparticles can reduce the light that zooxanthellae absorb to carry out photosynthesis (Jiang *et al*., 2021) scleractinian corals have demonstrated in research a relationship between the surface covered by MPs and photosynthetic efficiency (Corona *et al.*, 2020) which may respond to color variation in the experiment, the coral under test may have had its surface influenced by plastic particles, decreasing the capacity of the

zooxanthellae to carry out photosynthesis.

There are studies that show that the photosynthetic and respiratory rate of the species *Xenia elongata* occurs through the pulsation of polyps (Cohen *et al.*, 1977). The stress observed through the reduction of polyps may also indicate that coral respiration has increased (Cook & Knap *et al.*, 1983), Lanctôt *et al.* (2020), when exposing the sclarectine coral *Stylophora pistillata* to PE microparticles, measured photosynthetic yields and showed that after 4 weeks of exposure, the coral also exhibited greater photosynthetic efficiency and decreased electron transport. Reichert *et al.* (2019) showed the same with six different species of corals with concentrations lower than that of Lanctôt for 24 weeks, which may answer the decrease in pulsation in this experiment as the tests progressed, especially on the first day of testing in the case of tests with PP and PE, studies with color.

CONCLUSION

This study showed that exposure to microparticles can affect coral activity, including respiratory functions, growth, and even species mortality. Because they are more resistant to variations, the species demonstrated an ability to reestablish their physiological conditions. As an animal with characteristic polyps, the pulsation indicates the rate and photosynthetic capacity, making the stress caused clearer. Few studies address reactions to MPs with non-scleractinian corals, making it necessary, since they are also important reef builders and because they absorb organic compounds they can serve as bioindicators. There is also a need for more experiments with real projections such as the MIX of plastics, with this information being scarce in the literature.

AUTHORS CONTRIBUTIONS

BCG: Conceptualization, Methodology, Data curation, Writing- Original draft preparation, Visualization, Investigation. **GMB:** Data curation, Visualization, Investigation.**WSS:** Supervision, Writing-Reviewing and Editing.

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