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Microplastics Not Shown to Affect Coral Health in the Field Like in Laboratory Studies

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ABSTRACT

Microplastics, an emerging pollutant shorter than 5mm, pose a significant threat as their concentrations increase. With coral reef health in decline, particularly due to rising temperatures, concerns on the effect of microplastics on reef health have arisen. Previous studies investigating this relationship in laboratories have poorly imitated the complexity of field conditions. Therefore, this study explores the effect of microplastics on coral health in the field globally to better understand this pollutant's threat. By utilizing two databases and filtering data points to those within a 0.2 degree overlap (longitude and latitude), coral mortality, and secondarily, bleaching, were analyzed against the amount of microplastics. Data cleaning was performed to remove invalid or non-quantitative values such as "low" and "severe." Initial analysis revealed that all variables lacked a normal distribution, and various transformations failed to produce a normal curve. Hence, nonparametric statistical tests were used, such as Spearman's Rank Correlation. Mortality data exhibited a weak positive correlation to microplastics; however, at lower quantities of microplastics, there was a range in coral mortality, while at higher quantities, only high mortality values were present. The same analysis conducted with bleaching data showed no correlation. This suggests microplastics are a factor adversely affecting coral mortality but not bleaching in conjunction with other environmental concerns. Hence, these findings do not support that of laboratory studies, which find significant negative effects to coral. This study demonstrates the need for further field research to understand microplastics' role compared to other threats corals face.

Introduction

Plastic production has great potential for harm in the ocean, with estimates of over 5.25 trillion particles currently circulating (Eriksen et al., 2014). Microplastics are a type of plastic shorter than 5 mm, as measured by the longest dimension (Arthur, Baker, & Bamford 2009). They are a contaminant of emerging concern due to their abundance and ubiquity (Kroon et al., 2020). High concentrations increase the probability organisms contact and later ingest microplastics (Wright, R. C. Thompson, & T. S. Galloway 2013). Microplastics' small size gives them a comparatively large surface area to volume, allowing them to absorb significant amounts of toxic compounds (Brennecke et al., 2016). For example, they tend to concentrate persistent organic pollutants (POPs) such as polychlorinated byphenols (PCBs) and dichlorodiphenyltrichloroethane (DDT) due to their hydrophobicity (Cole et al., 2011). One type of POP, polycyclic aromatic hydrocarbons (PAHs), are of particular concern as they are mutagens and carcinogens (Kim et al., 2013). Microplastics may also be vectors for pathogens and microorganisms (Kirstein et al., 2016). These dangers are particularly worrisome due to the microplastics' small size, making them easily ingestible and more bioavailable compared to large plastics (Browne, T. Galloway, & R. Thompson 2007). They can biomagnify up food chains and pass through cell barriers as well (Brennecke et al., 2016).

Microplastics affect the feeding and energy dynamics of coral. Corals may ingest them through feeding, as they indiscriminately capture particles of plankton-like size, a category many microplastics fall into

(Corona et al., 2020). Ingestion of microplastics can not only cause the corals to waste energy digesting particles with no nutritional value, but also block metabolism of actual food sources. Likewise, corals that retain microplastics may simulate fullness, and corals exposed to microplastics have higher energy demands (Reichert et al., 2019). Corals exposed to microplastics are also shown to have compromised immune systems and decreased detoxication abilities (Tang et al., 2018). Other stress responses like decreased growth have been observed (Reichert et al., 2019). Microplastics can disrupt the coral's relationship with symbiotic algae and affect the algae's ability to photosynthesize (Reichert et al., 2018; Syakti et al., 2019).

Additionally, microplastics can adhere to the coral's surface, with concentrations higher than surrounding water (Corona et al., 2020). Existing environmental factors such as temperature stress and the accumulation of microplastics in sediment make corals particularly at risk (Hughes et al., 2018; Wang et al., 2016). Deadly effects like tissue necrosis (Reichert et al., 2018), significantly higher likelihood of disease (Lamb et al., 2018), disturbance of coral's relationship with symbiotic algae and possible bleaching (Okubo, Takahashi, & Nakano, 2018; Reichert et al., 2018) exist, among other concerns.

Recently, laboratory studies have shown the many negative effects of microplastics on coral (Reichert et al., 2018; Syakti et al., 2019; Tang et al., 2018). However, there is a lack of field research, and laboratory conditions do not accurately represent the field in terms of microplastic shapes, distributions, types, sizes, and amounts. Laboratory studies may have concentrations of microplastics more than thousands of times greater than the highest field concentrations (Phuong et al., 2016). Many negative health effects found from microplastics have only been correlated in labs, not in the field. This leaves many questions about the effects of microplastics in natural environments.

The existing field studies also present limitations. So far, field studies tend to focus more on abundance, as opposed to effect (Cheang, Ma, & Fok, 2018; Connors, 2017; Ding et al., 2019; Nie et al., 2019; Zhang et al., 2019). Research often includes only a small geographic area and lack a global scale (Connors, 2017). Thus, this study was undertaken to combat these two deficits in established research on microplastics and coral.

Materials and Methods

Data Acquisition via Online Databases

Coral data was obtained from the Coral Reef Watch, which contains numerous data products, including the "Coral Bleaching Database V1." This database was chosen for its global data collection, and because it has information on not only coral bleaching but also coral mortality, even to the percentage (Donner, Rickbeil, & Heron, 2017).

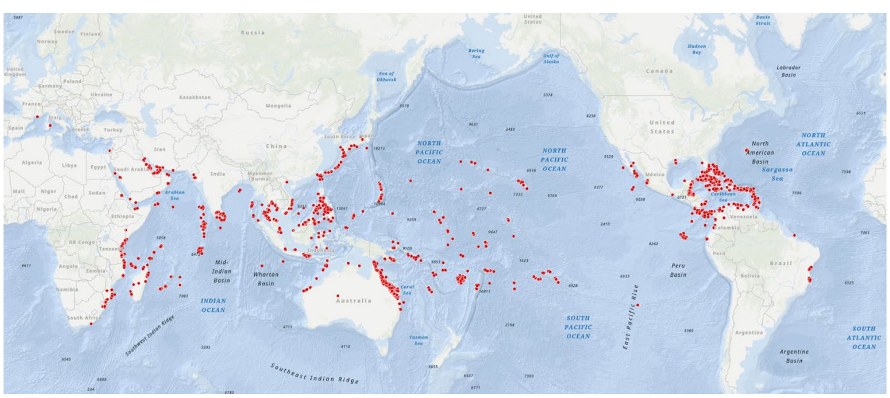

Figure 1. Map of coral reefs samples in the "Coral Bleaching Database V1."

From this database, three variables were used: Mortality Code, Severity Code, and Percent Mortality, where Severity Code refers to bleaching. Mortality Code consisted of five integers that represented from 0 to >50% coral mortality, with zero being 0% mortality, one 1-10% or "mild" mortality, two 11-50% or "moderate" mortality, three >50% or "severe" mortality, and four for "unknown." A similar ranking system was utilized for Severity Code, except with negative-one for "unknown," rather than four. When filtered for these variables, there were 594, 6685, and 3038 data points for Mortality Code, Severity Code, and Percent Mortality respectively.

For microplastics, NOAA's National Centers for Environmental Information (NCEI) has three publicly available databases: Adventure Scientists, SEA (Sea Education Association) Plastics Project, and GEOMAR (Research Center for Marine Geosciences). The Adventure Scientists has the units particles/m^3, SEA pieces/km^2, and GEOMAR particles/m^3 (Nyadjro et al. 2020; Law, S. Morét-Ferguson, et al. 2010; Law, S. E. Morét-Ferguson, et al. 2014; Tanhua, Gutekunst, and Biastoch 2020; Barrows, Cathey, and Petersen 2018). Additionally, the SEA Atlantic and Pacific data sets were combined into one.

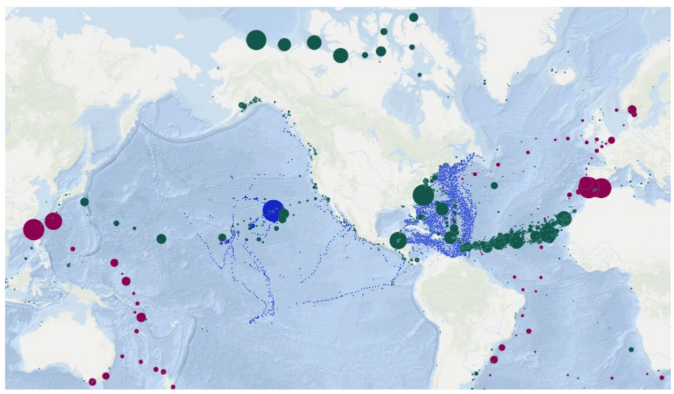

Figure 2. Comparison of the locations of the three microplastics data bases: Adventure Scientists (particles/m^3) in green, SEA (pieces/km^2) in blue, and GEOMAR (particles/m^3) in pink. The larger circles represent more microplastics, however, note the different scales.

Determining Overlap Between Databases

The different microplastic data sets had different units of measure and different sampling methodologies, so the three microplastics data sets could not be combined and analyses would have to be run separately. Thus, the critical consideration in whether a microplastics database could be used was overlap with the coral database, to maximize sample size and more precisely test the correlation. Overlap was determined by filtering out any data points from the microplastics datasets that lacked sufficient latitude and longitude overlap (0.2 degrees) with a coral data point. For each coral data point, if there are multiple microplastics values located within the overlap, then the average of the microplastics data is taken. A 0.2 degree overlap was chosen after various testing to find a satisfactory distance, about 20km.

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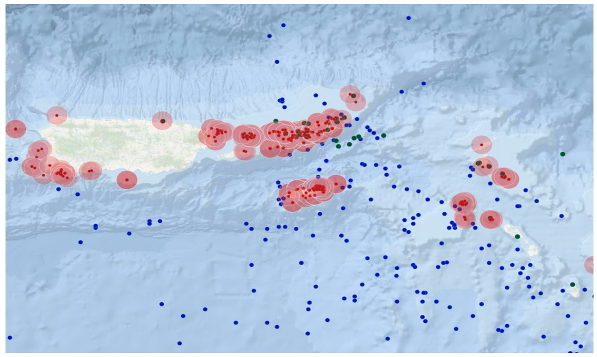

Figure 3. Map illustrating the overlap between where coral reef data was taken in the database (red dot), overlap radius (lighter red circle), and where microplastic data was (blue and green dots).

In general, overlap was limited because coral reefs only survive in water shallower than 70 meters, which is near the coast. Through this analysis, it was discovered that only the Adventure Scientists data set had sufficient overlap, with 1394 microplastics data points covering 1586 coral locations. There were more coral locations than microplastic data points because at some locations, multiple coral data points were within the overlap radius, so one microplastic data point corresponded to multiple samples in the reef. Despite GEOMAR and SEA having more data (358, 200 data points and 8667 data points respectively), they had less overlap with coral locations (3 and 1485 locations respectively. Note that while SEA had almost as many coral locations as Adventure Scientists, when filtered for data on mortality, too few data points remained). Ultimately, the lack of overlap was because GEOMAR took numerous samples around a single location, and SEA had many collection sites in the middle of the ocean.

Data Cleaning

After determining the data sets to be used, the data was then filtered to leave only usable data. Percent Mortality, unlike Mortality Code and Severity Code, contained less standard data, with some percentages only referring to a species or type of coral and others discussing a change in Percent Mortality over a specific time period. Data points that were not numeric, such as "low" or "severe" were removed to avoid subjective judgements. Filtering out data points that were blank, "N/A," or had a number corresponding to no data significantly reduced the usable data.

In the end, Mortality Code had 80 data points, Severity Code had 1444 data points, and Percent Mortality had 977 data points.

Data Analysis

R 4.1.0 was used for data analysis (R Core Team, 2016). To determine the appropriate tests to use, first the normality of data was investigated. Using the *ggpubr* package, it was found that all variables failed the Shapiro-Wilk normality test (Alboukaedel Kassambara, 2020). Normality plots using the *ggqqplot()* function demonstrated a far from normal distribution of the microplastics data and Percent Mortality data, which were closest to log-normal. The Mortality Code and Severity Code were closest to a uniform distribution, but due to the categorical nature of the values, the data lacked normality.

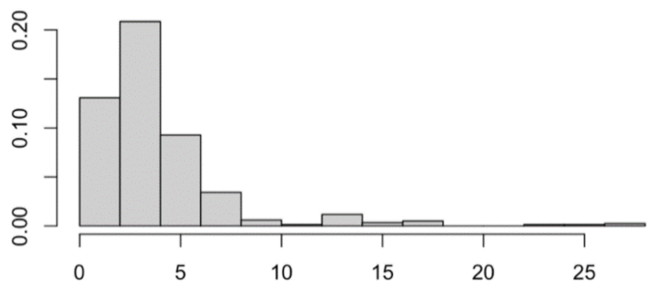

Figure 4. Histogram of the distribution of microplastics using a relative frequency density scale.

To combat this, the data was transformed to a more normalized distribution. Due to the high number of zero values (no microplastics or no mortality/bleaching), the Yeo-Johnson transformation was chosen. This was determined using the *bestNormalize* package (Peterson, 2021) to choose from hyperbolic arcsine, centering and scaling, exponential, log, square-root, and Yeo-Johnson transformations to estimate the normality of the different transformations to the data. For the microplastic data, the *yeojohnson()* function found the best lambda values to be *lambda* = -.0226 and *lambda* = -2.079 for Percent Mortality.

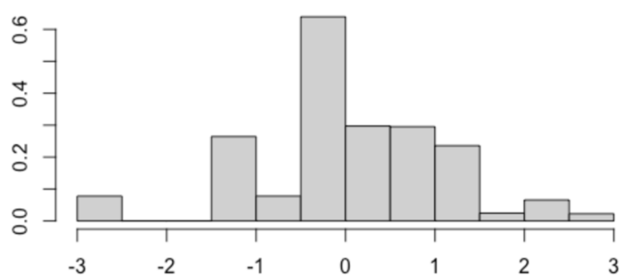

Figure 5. Histogram of microplastics data compared to a normal curve after the Yeo-Johnson transformation.

However, these transformations were unsuccessful with normality statistics (Pearson's P divided by degrees of freedom) an order of magnitude larger than that for a normal distribution. Code data could not be transformed by the Yeo-Johnson technique, and other transformations also proved ineffective. Hence, nonparametric tests were performed.

The nonparametric Spearman rank correlation coefficient for Spearman's rho was chosen to test the correlation between microplastics and the ordinal Mortality/Severity Code, using the *cor.test()* function. The nonparametric Pearson correlation coefficient for Pearson's product-moment correlation *r* was chosen to test the correlation between microplastics and the continuous Percent Mortality.

To further test whether the microplastics data across the different Mortality/Severity Codes are substantially different, Welch's ANOVA for homogeneity of variances was run. Microplastics data was separated into groups by Code and these groups were compared against each other to determine whether a substantial difference existed between them. If microplastics had no effect on Mortality/Severity Code, it would be expected that the distribution of microplastics within each Mortality/Severity Code is the same. A similar test was run with Percent Mortality with groups of 10%. Welch's ANOVA was selected because the data violated the assumption that the standard deviation across different Codes was the same. Lastly, to calculate which categories had significant differences, the post hoc Games-Howell test was run.

Results

Normality

It was found that the data for microplastics was not a normal distribution, being right skewed (Figure 6). The normality plot was most noticeably skewed by having a more than average number of data points without any microplastics. It also failed the Shapiro-Wilk normality test.

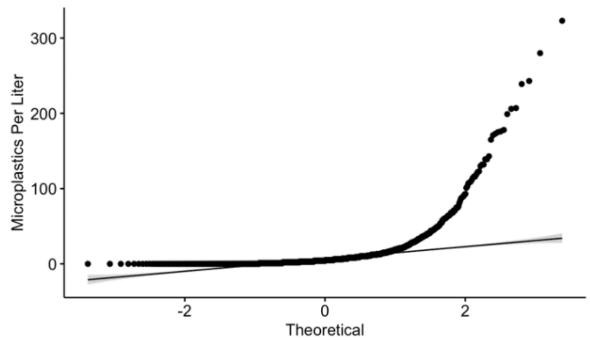

Figure 6. Distribution of microplastics data as compared to a normal distribution, the straight line. The data was extremely right skewed.

Mortality Code

The positive correlation between Mortality Code and Microplastics per Liter was weak (Spearman's rho = .28), but statistically significant, as defined by $p < .05$.

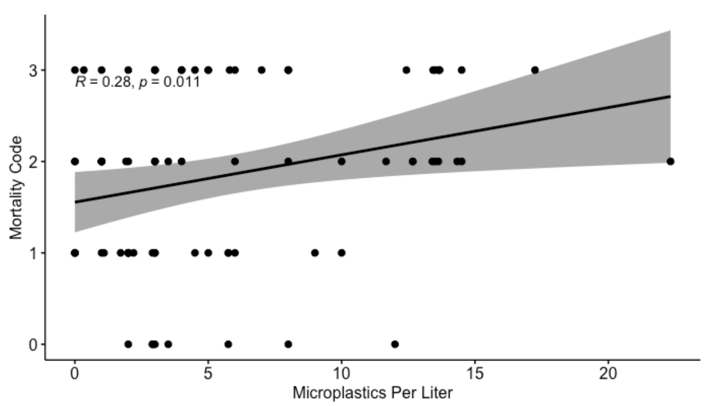

Figure 7. Spearman correlation of Microplastics Per Liter versus Mortality Code.

Despite the low correlation, there was an interesting trend. At lower quantities of microplastics, there was a range of Mortality Codes, but at higher quantities of microplastics, high Mortality Codes occupied a much larger share of the data. This observation was supported by Welch's ANOVA which found significant differences between microplastics data across Mortality Codes ($p < .05$). The post hoc Games-Howell test showed significant results for Mortality Code for codes of one and two (1-10% Mortality and 11-50% Mortality), and two and three (11-50% Mortality and >50% Mortality).

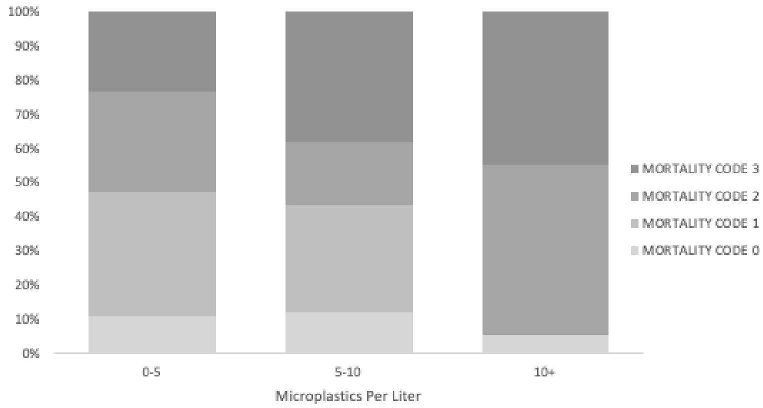

Figure 8. 100% stacked histogram graph of Mortality Code versus Microplastics Per Liter. Microplastics was grouped by 5s into three groups.

Percent Mortality

The Percent Mortality data was strongly skewed to the right. The Pearson correlation showed a weak positive correlation ($r = .24$, $p < .05$).

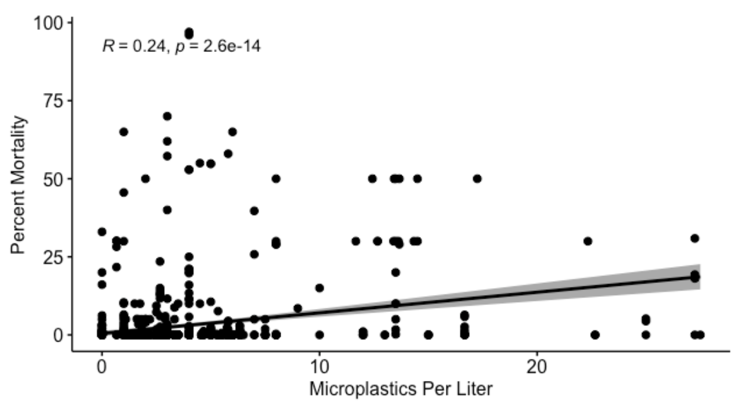

Figure 9. Pearson correlation of Percent Mortality versus Microplastics Per Liter.

When Percent Mortality was grouped into categories by 10%, it showed a similar trend as Mortality Code with a range in mortality at lower microplastic quantities while increased mortality values at higher amounts.

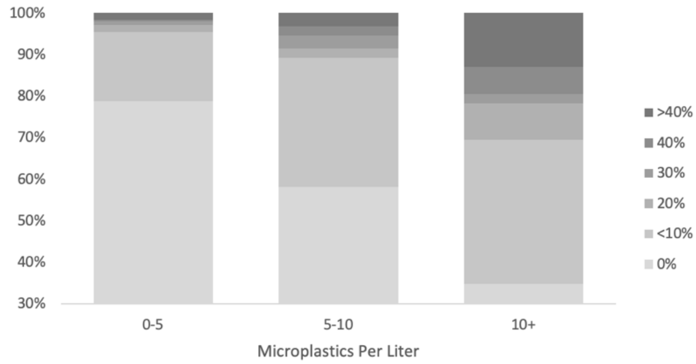

Figure 10. A 100% stacked histogram of Percent Mortality versus Microplastics. Note the scale.

Welch's ANOVA found significant differences between these categories of Percent Mortality ($p < .05$) and the post-hoc Games-Howell test found significant results between 0% and 10%, 0% and 30%, 0% and 40%, and 10% and 30%.

Severity Code

The Spearman rank correlation showed no correlation between Microplastics Per Liter and Severity Code (Spearman's rho = -.06, $p < .05$).

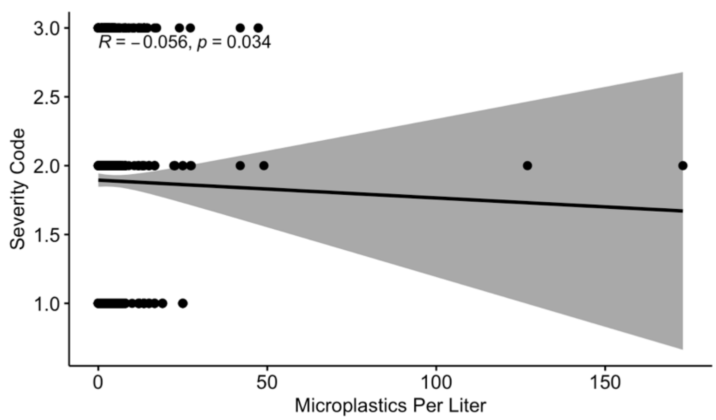

Figure 11. Spearman Rank Correlation of Microplastics Per Liter Versus Severity Code, showing no relationship between the variables.

Similarly, there was no correlation in the 100% stacked histogram, unlike Mortality Code. Welch's ANOVA performed for Severity Code found no significant difference ($p > .05$), and hence the post hoc Games-Howell reflected this as well.

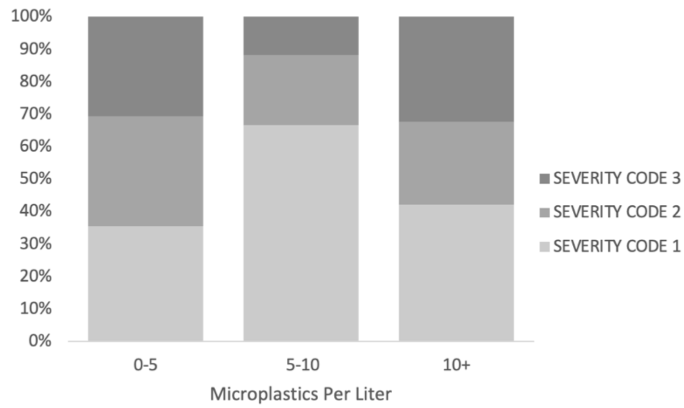

Figure 12. A 100% stacked histogram of Severity Code and Microplastics Per Liter does not show the same correlation as Mortality Code.

Discussion

The trend of a range in coral mortality at lower amounts of microplastics while at higher amounts, greater mortality occupied a larger share of the data, among other findings, suggests that microplastics are an additional environmental stressor. Recent temperature-stress and bleaching significantly reduces coral energy reserves which microplastics also contribute to (Grottoli, Rodrigues, & Palardy, 2006). In combination with other environmental issues like ocean acidification, other pollution, and climate change, microplastics can kill corals (Huang et al., 2020). For example, Okubu et al. found that thermally bleached coral does not expel ingested microplastics as efficiently as healthy ones (2020). However, any effect of microplastics on coral that goes beyond a contributing stressor in the field was not found in this study.

The results showed no correlation between bleaching and microplastics. This may have been affected by the fact that widespread coral bleaching due to warming temperatures may overshadow any affect from microplastics and hence this study draws no relation between the two.

The overall results of this study suggest that the effect of microplastics is much weaker in the field than in laboratory studies. Laboratory studies tend to find much stronger correlations between microplastics and coral health that were not found in this study. Given the low correlations and that microplastics in laboratory studies are of significantly higher quantities than in the field, it can be concluded that the state of ocean microplastics is not the same as in laboratory experiments.

Conclusion

This study finds that the effects of microplastics on coral health to be significantly less severe than previous laboratory studies have found. Comparing these results and the lower quantities of microplastics in ocean samples compared to laboratory studies, this research finds that laboratory studies do not mimic the current state of the ocean. However, with plastic production on the rise, future microplastic concentrations may one day reflect current laboratory research.

Considering that the state of the ocean is rapidly evolving due to acidification, warming, etc., field research is essential to understand the role of microplastics on coral. Studies of reefs containing abnormally high microplastics, for example near clothing factories or wastewater effluent discharges, in comparison to less polluted areas, will provide key information. Additionally, reefs with limited temperature increases and bleaching, or where other parameters (nutrients, etc.) are healthy, are important locations for understanding the effects on coral mortality. Further statistical research should incorporate environmental factors and account for different species of coral, as they vary in sensitivities to microplastics (Reichert et al., 2019).

Limitations

Since no physical field research was conducted, the results of this study are dependent on the quality and accuracy of the datasets which were not collected by the author. The "Coral Bleaching Database V1" is the product of many scientists' work, and despite efforts to make the data as uniform as possible, concerns are still held that certain percentages listed in the data reflected only a specific species when it was not recorded as such.

Additionally, even though the coral and microplastics have a 0.2 degree overlap, it is possible that the microplastics are simply a proxy for other types of pollution in that location and the microplastics are not the factor affecting coral health. There was no control to isolate the effects of microplastics from other environmental factors. Furthermore, unlike laboratory studies, it was impossible to study only one species of coral, but the magnitude and exact health effects of microplastics is species-specific, and different species vary in their ability to mitigate the toxicity of microplastics.

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