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# Unseen threats: negative effects of microplastic leachate on coral planulae settlement

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Coral reef ecosystems, especially those in Hawai'i, are increasingly threatened by marine plastic pollution, which may impair coral reproduction. Microplastics contain toxic, persistent, and endocrine-disrupting chemicals. While negative effects of microplastic ingestion have been observed on adult coral viability and health, very few studies have explored impacts on reproduction. Recent studies found that microplastic leachate negatively affects coral fertilization likely due to plastic additives incorporated into products during manufacturing. This study explored the effects of microplastic leachate on coral planula larvae settlement and survival. Planula larvae of the broadcast spawning species, *Montipora capitata*, and the brooding species, Harbor *Porites*, were exposed to microplastic leachate from four plastic types [nylon, polypropylene (PP), high-density polyethylene (HDPE), or low-density polyethylene (LDPE)] at three concentrations (50, 100, or 200 particles/L) for 7 days. Settlement and survival were affected by leachate concentration and polymer type strongly on Days 5 and 7 indicating delayed and/or potentially cumulative effects. The most pronounced negative effects on survival were observed with HDPE–100 and LDPE–200 treatments. One treatment unexpectedly promoted settlement (HDPE–200) likely due to attractive chemical cues released by the leachates. This is particularly concerning, as it suggests that planula larvae may be drawn to settle on degraded or suboptimal substrates affecting reef recruitment and replenishment. Species-specific responses were also found, with Harbor *Porites* exhibiting higher survival and variable settlement. Overall, microplastic leachate significantly impacted survival and settlement of both *Montipora capitata* and Harbor *Porites* planula larvae with complex temporal, species-specific, polymer-type, and concentration-dependent effects. Microplastic leachate presents an additional stressor to already threatened coral species making addressing both local and global stressors critical for the protection of coral reef ecosystems.

## KEYWORDS

microplastic leachate, *Montipora capitata*, Hawaii, Harbor *Porites*, plastic additives, coral planula settlement, marine plastic pollution, coral reproduction

# 1 Introduction

Coral reef ecosystems are among the most biodiverse ecosystems on our planet providing important ecological, economic, and cultural resources to millions of people (Cinner et al., 2016; Woodhead et al., 2019). These vital ecosystems are threatened by both global and local level stressors, including ocean warming and ocean acidification, pollution, habitat destruction, disease, eutrophication, and overfishing (Hughes et al., 2017; Good and Bahr, 2021). An additional threat of concern is marine plastic pollution, a prevalent contaminant on reefs, which can affect the health of multiple marine organisms in the ecosystem including corals (Lamb et al., 2018; Pantos, 2022; Pinheiro et al., 2023). Within the Hawaiian Archipelago, corals are especially threatened by plastic pollution because some of the highest rates of marine debris accumulation can be found here due to ocean circulation patterns (Brignac et al., 2019). A combination of the physical (size, shape, and color), chemical (polymer type, additives, and sorbed), and biological characteristics (biofilms, hitchhiking organisms, etc.) of plastics influences their effects on corals. Corals can not only be smothered or covered by larger macroplastics (Yoshikawa and Asoh, 2004; de Carvalho-Souza et al., 2018) but also experience adhesion (Martin et al., 2019; Corona et al., 2020; Kim et al., 2024), ingestion (Hall et al., 2015; Reichert et al., 2018; Rotjan et al., 2019), and incorporation of micro- and nanoplastics (Corinaldesi et al., 2021; Hierl et al., 2021; Jandang et al., 2024).

Previous studies focused on the effects of the ingestion of microplastics on coral health and resilience have shown a variety of effects due to the plastic concentration, type, size, and shape. Ingestion of microplastics has been found to have species-specific effects on health (growth and algal symbiosis), cellular/molecular responses (antioxidant and immune enzyme expression, detoxification, photosynthetic performance, and metabolite profiles), and stress responses (mucus production, bleaching, and tissue inflammation) (Chapron et al., 2018; Tang et al., 2018; Axworthy and Padilla-Gamiño, 2019; Mouchi et al., 2019; Reichert et al., 2019; Syakti et al., 2019; Lancôt et al., 2020; Savinelli et al., 2020; Tang et al., 2021). Most of the effects seen were due to physical characteristics (e.g., size, shape) raising questions about the effects of microplastic-associated chemicals.

Microplastics also pose a chemical threat to coral health due to both the toxic chemicals added during manufacturing (additives) and chemicals absorbed from the environment (sorbed) (Seidensticker et al., 2018; Caruso, 2019; Campanale et al., 2020). These chemicals include a variety of toxicants that are cancer causing, persistent, bioaccumulative, and endocrine disrupting (Gallo et al., 2018; Yu and Singh, 2023). Of the studies that explore the effects of microplastic-associated chemicals on coral health, bioaccumulation has been seen from hexabromocyclododecanes in polystyrene (Aminot et al., 2020). Microplastic-associated chemicals have also been observed inhibiting energy metabolism enzymes from chromium [Cr(III)] in polyethylene and apoptosis of the Symbiodiniaceae (Xiao et al., 2023). Additionally, the effects of Cr(III) were more obvious than the effects of polyethylene alone further suggesting that the physical threats of microplastic interactions are less severe than the effects of the chemical contaminants.

Although most of the current research has focused on microplastic effects on coral health, sublethal effects on coral reproduction could also be occurring. Corals have two main modes of sexual reproduction: brooding and spawning (Richmond, 1997). Brooding coral species internally produce eggs and sperm, which fertilize and develop into planula larvae within the coral. Alternatively, spawning coral species release egg and sperm into the water column where external fertilization and development occur. Planula larvae undergo settlement and metamorphosis where they change from a planktonic to benthic stage. The process of site selection and development is heavily mediated by chemical cues providing attractive substrates. Since corals are sessile after settlement, they likely encounter sinking particles or less dense, biofouled particles. Conversely, coral planula larvae move throughout the water column and likely encounter a variety of particles with different densities (i.e., sinking and floating plastics).

Only a few studies to date have explored the effects of microplastics on coral reproduction. Berry et al. (2019) found limited effects of weathered polypropylene on fertilization of the broadcast spawning coral, *Acropora tenuis*, but no significant effects on embryo development and larval settlement (Berry et al., 2019). There was a significant effect of particle size on abnormalities of the fertilized eggs exposed to large-weathered plastics resulting in significantly lower fertilization success. The mechanism for this decline in fertilization was assumed to be due to physical contact with the particles. More recently, Wilkins et al. (2024) found negative effects of microplastic leachate on fertilization and fatty acid quantity and composition of *Montipora capitata* eggs likely due to the endocrine-disruptive nature of many plastic additives (Wilkins et al., 2024). This study also tested the effects of microplastics alone and found more negative effects on fertilization due to the microplastic leachate likely containing associated chemicals. These two studies (Berry et al., 2019; Wilkins et al., 2024) focused on the effects of microplastic pollution on the gametes of broadcast spawning coral species and highlighted the need for further studies exploring chemical effects on coral reproduction. Based on information from these studies, chemical effects on planula larvae development have been unstudied leaving a gap in understanding the full potential effects on coral reproduction.

The purpose of this study was to test the effects of microplastic leachate from common polymer types (nylon, polypropylene, low-density polyethylene, and high-density polyethylene) at different concentrations (50, 100, and 200 particles/L) on coral settlement and development using planula larvae of the Hawaiian broadcast spawning coral *Montipora capitata* (Dana, 1846) and the Hawaiian brooding coral Harbor *Porites* (Spies, 2021). These polymer types were selected based on their environmental relevance, as they are commonly found in the Main Hawaiian Islands: polypropylene, high-density polyethylene, and low-density polyethylene are frequently found on windward beaches and sea surface samples, while nylon is most common on the seafloor (Brignac et al., 2019; Axworthy et al., 2024). The selected concentrations followed those of Berry et al. (2019) and represent a conservative estimate of

microplastic concentrations in surface waters by 2100 (Koelmans et al., 2016). Harbor *Porites* is a brooding coral species with no known lunar reproductive cycle, while *M. capitata* is a broadcast spawning coral species that reproduces around the new moon from May to August in Hawai'i. Harbor *Porites* is a recently described coral believed to be resilient due to its ability to withstand harsh environmental conditions and its widespread occurrence in low-water quality Honolulu Harbor, where it thrives with high levels of urban runoff, low circulation, sedimentation, sewage, and oil spills (AECOS, Inc 2014; Spies, 2021). *M. capitata* is a common coral species found throughout Hawai'i. We hypothesized that planula larvae of both *M. capitata* and Harbor *Porites* would exhibit reduced survival and settlement due to exposure to increasing concentrations of microplastic leachate likely containing microplastic-associated chemicals.

## 2 Materials and methods

Coral planula larvae from two species of Hawaiian corals with different sexual reproductive strategies were used: *Montipora capitata* and Harbor *Porites*. For *M. capitata*, positively buoyant gamete bundles (egg and sperm) were collected on the evenings around the June and July 2023 new moon from Lilipuna Pier in Kaneohe Bay, O'ahu (21.42973°N, 157.79220°W) by placing mesh traps over five adult colonies and collecting additional gamete bundles on the ocean surface. After bringing bundles back to Kewalo Marine Laboratory (Honolulu, HI), they were mixed and kept at ambient temperature in large coolers to fertilize and develop into planula larvae. After 7 days of development, they were used in experiments. For Harbor *Porites*, adult colonies were collected in June and September 2023 from Sand Island, transported to Kewalo Marine Laboratory, and maintained in outdoor water tables with filtered seawater (30 µm). Any produced planula larvae were collected and separated into 1-L beakers with filtered seawater (30 µm) until use. Planula larvae from both species were exposed to microplastic leachates for 7 days. Each solution consisted of a leachate that contained virgin, recently manufactured microspheres or resin balls (4.76 mm) of one of four polymer types [nylon, low-density polyethylene (LDPE), polypropylene (PP), or high-density polyethylene (HDPE)] at one of three concentrations (50, 100, and 200 particles/L). Microspheres (PP) were purchased from McMaster-Carr, USA, while the remaining resin balls were purchased from Plastic Ball Supply, USA. Each leachate was created by leaving these plastics in 2 L beakers with 2 L of filtered seawater (30 µm) for 1 month prior to the experiment. A leachate control was also created with just filtered seawater and no additional plastics (Control—Leachate). Due to differences in reproductive cycles of the two species, *M. capitata* experiments took place in June and July, while Harbor *Porites* experiments took place in June and September.

For both species, treatment solutions of 100-ml were added to 120-ml glass jars. A control with fresh filtered seawater was included in the experiment (Control—Fresh). A total of five swimming planula larvae were added to each jar along with a

pre-conditioned ceramic settlement plug conditioned by leaving it in an unfiltered seawater table to allow a biofilm to grow. All jars were placed into an outdoor, free-flowing water table to maintain ambient seawater temperature and light. The number of swimming, settled, and dead planula larvae were recorded every 2 days (Day 0, 1, 3, 5, and 7). Due to their size, sometimes planulae larvae could not be visualized and were initially labeled as “missing.” In case they were observed on later days, they were assigned as alive/swimming for the earlier measurements. If missing planula larvae were not subsequently found, they were recorded as dead. The experiments differed slightly between the species in terms of pre-conditioning of the settlement plugs and age of the planula. *M. capitata* planula larvae (7 days old) were used for the experiments, and the settlement plugs had been conditioned for 7 days. For Harbor *Porites*, planula larvae (0–6 days old) were used for the experiment, and settlement plugs had been conditioned for 11–12 days. Additionally, the number of replicates per species varied from month to month due to variability in production of planula larvae (refer to Supplementary Table S1).

### 2.1 Chemical analysis

The polymers used in this study to make the leachates were the same as those used by Wilkins et al. (2024) (Table 1). Based on the previous analysis using double-shot pyrolysis-gas chromatography/mass spectrometry, we know that several chemical additives were present in the plastics, but we cannot confirm that all these chemicals leached into the seawater.

### 2.2 Statistical analysis

Statistical analyses were performed to examine species-specific differences throughout the experiment to understand how different polymer types and concentrations affected survival and settlement for both species of coral planula larvae. The number of individuals per replicate was converted to a proportion for the analyses. Separate models were created for the survival and settlement analysis. For survival, the proportion of live individuals, which included both the swimming and settled planula larvae, was used as the dependent variable. For settlement, the proportion of settled

TABLE 1 Plastic additives found in the polymers through double-shot pyrolysis-gas chromatography/mass spectrometry.

Polymer type	Chemical(s) found
LDPE	No detectable chemicals
HDPE	Phenol, 2,4-bis (1,1-deimethyl)-,phosphite or “Alkanox 240”; aliphatic hydrocarbons (not a plastic additive)
PP	Phenol, 2,4-bis (1,1-deimethyl)-,phosphite or “Alkanox 240”
nylon	1,8-Diazacyclotetradecane-2,9-dione, aliphatic hydrocarbons

The results of this analysis were previously presented by Wilkins et al. (2024).

individuals only was used as the dependent variable. To reduce boundary issues with beta regressions, the response variables were adjusted to avoid values equal to 0 and 1 changing the range from 0–1 to 0.0005–0.9999.

Initially, two full models were created to determine the effects of month, species, day, and treatment on planula larvae survival and settlement. However, these models showed issues with the diagnostics (e.g., quantile deviations, patterns in the residuals, and issues with variance structure). Therefore, to better meet the model assumptions, individual Generalized Linear Mixed Models (GLMM) with beta distributions and a logit link function using the *glmmTMB* package in R were used. All models used survival or settlement as the response variable, with Treatment and Day interactions as fixed effects and Replicate as a random effect as needed. Model selection was determined by the Akaike Information Criterion. For survival, *M. capitata* and Harbor *Porites*—June used Treatment and Day as fixed effects with Replicate as a random effect. For Harbor *Porites*—September, a random slope model was used with the same predictors. For settlement, *M. capitata* used a similar model, which added Replicate as a random effect. For Harbor *Porites*, adding replicate in was unnecessary due to the small variability in replicates. *Post-hoc* comparisons were carried out as appropriate using Tukey's honestly significant difference (HSD) tests. All analyses were carried out in R studio (version 2024.12.0 + 467) and validated with Julius AI. Some *M. capitata* replicates could not be analyzed due to flooding of some of the jars on Day 6, so some counts on Day 7 are missing (see [Supplementary Table S1](#)). The data from the previous 5 days were still included in the analysis.

## 3 Results

### 3.1 Survival

July *M. capitata* experiments were removed from the analysis because the Control—Fresh treatment mean survival on Day 7 was very low ( $0.30 \pm 0.30$ ). All other experimental trials had higher planula larvae survival ( $>0.60$ , see [Table 2](#)). The Control—Leachate treatment mean survival percentages were consistently higher compared to the Control—Fresh treatments ([Table 2](#)).

Harbor *Porites* planula larvae showed consistently higher average survival for most treatments when compared to those of *M. capitata* ([Figure 1](#)). Additionally, results from each of the months seemed to have different trends and within days of the experiment (hereon referred to as Day).

#### 3.1.1 *M. capitata*

Planula larvae showed a strong significant decrease in survival over time that varied by treatment, not within. There was no significant difference between any treatment and the controls. Since flooding occurred on Day 6, several treatments had less replicates on Day 7 likely resulting in *post-hoc* comparisons showing significant differences only on Day 5. Planula larvae in the LDPE—100 treatment showed significantly higher survival than

**TABLE 2** The proportion of average survival on Day 7 of the experiments for the control treatments (Control—Fresh and Control—Leachate) for each month and species.

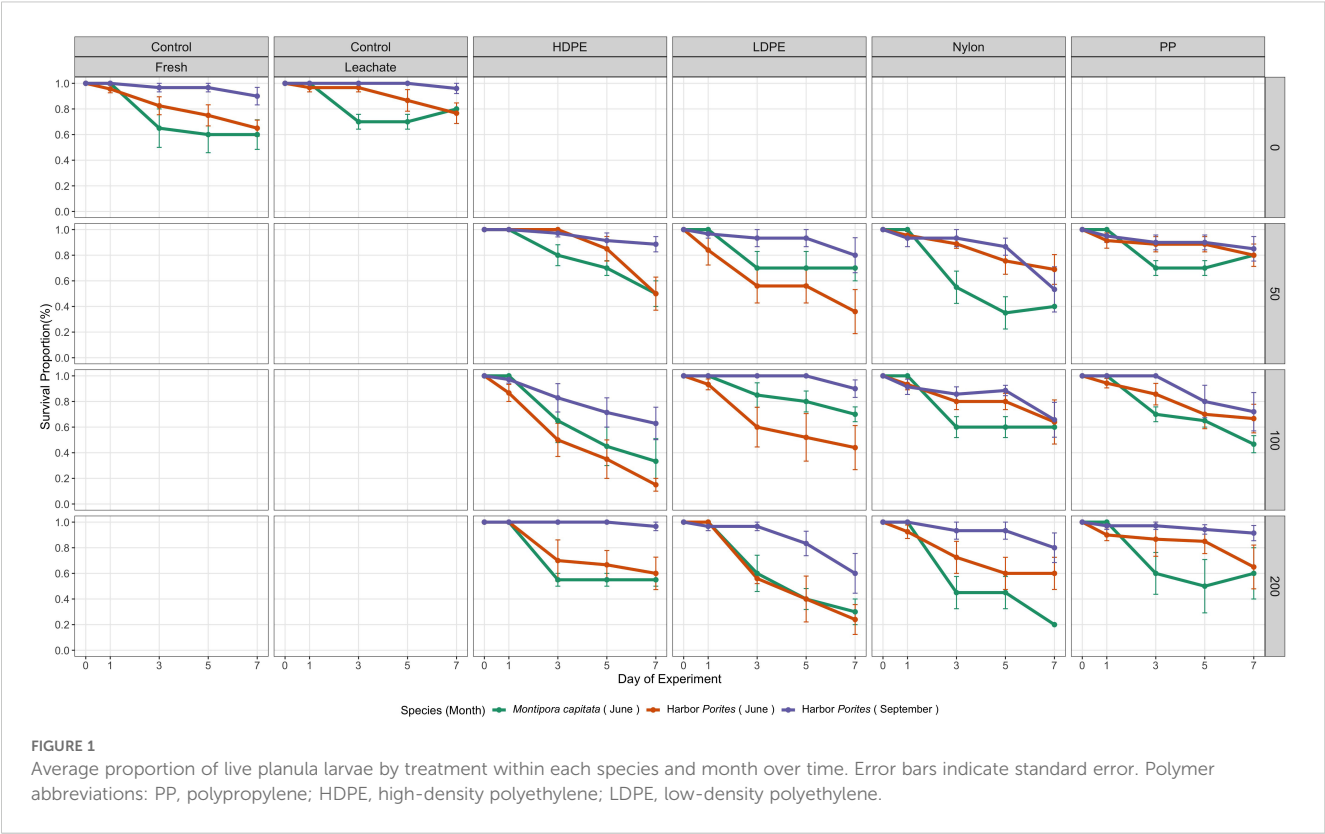
Control—Fresh				
Species	Month	Mean	SD	N
<i>Montipora capitata</i>	June	0.60	0.20	3
Harbor <i>Porites</i>	June	0.65	0.18	8
Harbor <i>Porites</i>	September	0.90	0.17	6
Control—Leachate				
Species	Month	Mean	SD	N
<i>Montipora capitata</i>	June	0.80	N/A	1
Harbor <i>Porites</i>	June	0.77	0.20	6
Harbor <i>Porites</i>	September	0.96	0.09	5

For *Montipora capitata* in June, the average only represents the one replicate that survived flooding that occurred on Day 6.

those in the HDPE—100 (p-value = 0.034) and LDPE—200 (p-value = 0.021) treatments on Day 5 ([Table 3](#)).

#### 3.1.2 Harbor *Porites*—June

Planula larvae survival showed a strong decrease over time (p-value < 0.001) and across the HDPE—100 (p-value = 0.017) and LDPE—200 (p-value = 0.006) treatments. Significant effects in survival were seen between treatments for Day 3, 5, and 7 ([Table 4](#)). For Day 3, planula larvae in the HDPE—100 treatment showed significantly lower survival than those in both control treatments (Control—Fresh p-value = 0.021; Control—Leachate p-value < 0.001). Planula larvae in the HDPE—100 treatment also showed lower survival than those exposed to HDPE at the lowest concentration (HDPE—50 p-value = 0.028) and a different polymer type at the same concentration (PP—100 p-value = 0.039). LDPE—100 and LDPE—200 treatment exposed planula larvae also showed lower survival compared to those in the Control—Leachate treatment (LDPE—100 p-value = 0.024; LDPE—200 p-value = 0.004). For Day 5, similar trends were seen within several treatments, with planula larvae having significantly higher survival than those in the HDPE—100 treatment, including both control treatments (Control—Fresh p-value = 0.015; Control—Leachate p-value < 0.001) and PP—100 treatment (p-value = 0.022). Planula larvae in the LDPE—200 treatment had significantly lower survival than those in both control treatments (Control—Fresh p-value = 0.012; Control—Leachate p-value < 0.001) and PP at the same concentration (PP—200 p-value = 0.003). Planula larvae in the LDPE—100 treatment also showed lower survival than those in the Control—Leachate treatment (p-value = 0.021). For Day 7, more significant differences were seen between treatments and HDPE and LDPE. Again, in the LDPE—200 treatment, planula larvae showed lower survival than those in both control treatments (Control—Fresh p-value = 0.034; Control—Leachate p-value = 0.001) and the PP treatment at the same concentration (PP—200 p-value = 0.007). Lastly, planula larvae in the HDPE—100 treatment showed lower survival compared to those in the Control—Leachate treatment (p-value = 0.002).



3.1.3 Harbor Porites—September

Like what was seen in June for Harbor *Porites*, significant declines in planula larvae survival were seen over time across the HDPE—100 (p-values = 0.005) and nylon—50 treatments (p = 0.024). Between treatments, significant effects were only seen for Day 5 and 7 (Table 5). Planula larvae exposed to several polymer types and concentrations had significantly lower survival than the controls. For both Day 5 and 7, HDPE—100 treatment exposed planula larvae had significantly lower survival than both control treatments. Nylon—50 treatment-exposed planula larvae also had significantly lower survival than those exposed to Control—Leachate on Day 5 only (p-value = 0.042). HDPE—100 treatment-exposed planula larvae had significantly lower survival than those in several other

treatments on both Day 5 and 7 including the other two HDPE concentrations and the LDPE—100 treatment.

3.2 Settlement

The June *M. capitata* experiment showed the highest mean settlement proportion in the Control—Fresh treatment (0.40 ± 0.20) (Table 6). The Control—Leachate treatment had the same average settlement proportion. For Harbor *Porites*, the June settlement proportions were higher for both controls. Interestingly, the June Control—Leachate treatment mean survival proportion (0.60) was higher than that of any other control or month for either species.

TABLE 3 Summary of significant model results and all post-hoc comparisons of *M. capitata* planula larvae survival across all days of the experiment.

Model results					
Predictor		Estimate	SE	Z ratio	P-value
Intercept		3.65	0.552	6.611	<0.001
Day		−0.76	0.174	−4.396	<0.001
Post-hoc comparisons					
Day	Contrasts	Estimate	SE	Z ratio	P-value
5	LDPE—100 and HDPE—100	2.06	0.592	3.475	0.034
5	LDPE—100 and LDPE—200	2.08	0.575	3.617	0.021

Additional non-significant comparisons between individual treatments and days of the experiment can be found in Supplementary Tables S2a, b.



TABLE 4 Summary of significant model results and *post-hoc* comparisons of Harbor *Porites* planula larvae survival across all days of the experiment in June.

Model results					
Predictor		Estimate	SE	Z ratio	P-value
Intercept		2.70	0.413	6.537	<0.001
Day		−0.42	0.126	−3.348	<0.001
HDPE—100 and Day		−0.50	0.209	−2.391	0.017
LDPE—200 and Day		−0.56	0.203	−2.756	0.006
Post-hoc comparisons					
Day	Contrasts	Estimate	SE	Z ratio	P-value
3	Control—Fresh and HDPE—100	1.10	0.304	3.618	0.021
3	Control—Leachate and HDPE—100	1.45	0.318	4.577	<0.001
3	Control—Leachate and LDPE—100	1.10	0.307	3.579	0.024
3	Control—Leachate and LDPE—200	1.23	0.302	4.081	0.004
3	HDPE—100 and HDPE—50	−1.21	0.342	−3.530	0.028
3	HDPE—100 and PP—100	−1.08	0.313	−3.436	0.039
5	Control—Fresh and HDPE—100	2.10	0.565	3.717	0.015
5	Control—Fresh and LDPE—200	2.00	0.530	3.773	0.012
5	Control—Leachate and HDPE—100	2.87	0.593	4.843	<0.001
5	Control—Leachate and LDPE—100	2.01	0.555	3.617	0.021
5	Control—Leachate and LDPE—200	2.77	0.557	4.981	<0.001
5	HDPE—100 and PP—100	−2.12	0.587	−3.602	0.022
5	LDPE—200 and PP—200	−2.57	0.625	−4.117	0.003
7	Control—Fresh and LDPE—200	3.12	0.900	3.470	0.034
7	Control—Leachate and HDPE—100	4.29	1.004	4.275	0.002
7	Control—Leachate and LDPE—200	4.31	0.955	4.512	0.001
7	LDPE—200 and PP—200	−4.08	1.046	−3.900	0.007

All additional non-significant comparisons can be found in [Supplementary Tables S3a, b](#).

Each species showed very different trends in the proportion of settled planula larvae with *M. capitata* showing generally higher settlement proportions (Figure 2). Additionally, each of the months and days within the experiment showed different trends.

### 3.2.1 *M. capitata*

Settlement proportions significantly increased over time ( $p$ -value = 0.001), and so did their variability (Table 7). Settlement significantly increased over time for planula larvae in both the HDPE—100 ( $p$ -value = 0.045) and nylon—50 ( $p$ -value = 0.052) treatments. Planula larvae in the Control—Fresh treatment showed the highest mean settlement proportion (0.395) on Day 7. Planula larvae in the HDPE—100 and nylon—50 treatments showed significant interactions with Day, but *post-hoc* comparisons did not find any significant treatment differences.

### 3.2.2 Harbor *Porites*—June

Settlement significantly increased over time ( $p$ -value = 0.001). There was no significant difference in planula larvae settlement between treatments and the Control—Fresh treatment (Table 8). The positive estimate and significant days suggest an increase in settlement as the experiment went on. Two treatments showed significantly lower planula larvae settlement rates on Day 5 compared to the Control—Leachate treatment: HDPE—100 ( $p$ -value = 0.043) and nylon—100 ( $p$ -value = 0.043) treatments.

### 3.2.3 Harbor *Porites*—September

Settlement significantly increased over time ( $p$ -value = 0.049) and within the HDPE—200 treatment ( $p$ -value = 0.004) (Table 9). Following *post-hoc* analysis, significant differences between treatments were seen for Day 3, 5, and 7. On Day 3, the planula

TABLE 5 Summary of significant model results and *post-hoc* comparisons of Harbor *Porites* planula larvae survival across days of the experiment in September.

Model results					
Predictor		Estimate	SE	Z ratio	P-value
Intercept		2.82	0.455	6.195	<0.001
HDPE—100 and Day		−0.52	0.186	−2.786	0.005
nylon—50 and Day		−0.56	0.247	−2.253	0.024
Post-hoc comparisons					
Day	Contrasts	Estimate	SE	Z ratio	P-value
5	Control—Fresh and HDPE—100	1.81	0.453	3.983	0.005
5	Control—Leachate and HDPE—100	1.92	0.469	4.094	0.003
5	Control—Leachate and nylon—50	2.03	0.596	3.411	0.042
5	HDPE—100 and HDPE—200	−1.96	0.444	−4.423	<0.001
5	HDPE—100 and HDPE—50	−1.65	0.433	−3.822	0.010
5	HDPE—100 and LDPE—100	−1.76	0.446	−3.947	0.006
7	Control—Fresh and HDPE—100	2.84	0.788	3.609	0.021
7	Control—Leachate and HDPE—100	3.02	0.820	3.676	0.017
7	HDPE—100 and HDPE—200	−3.08	0.777	−3.968	0.006
7	HDPE—100 and HDPE—50	−2.60	0.755	−3.429	0.039
7	HDPE—100 and LDPE—100	−2.74	0.784	−3.496	0.032

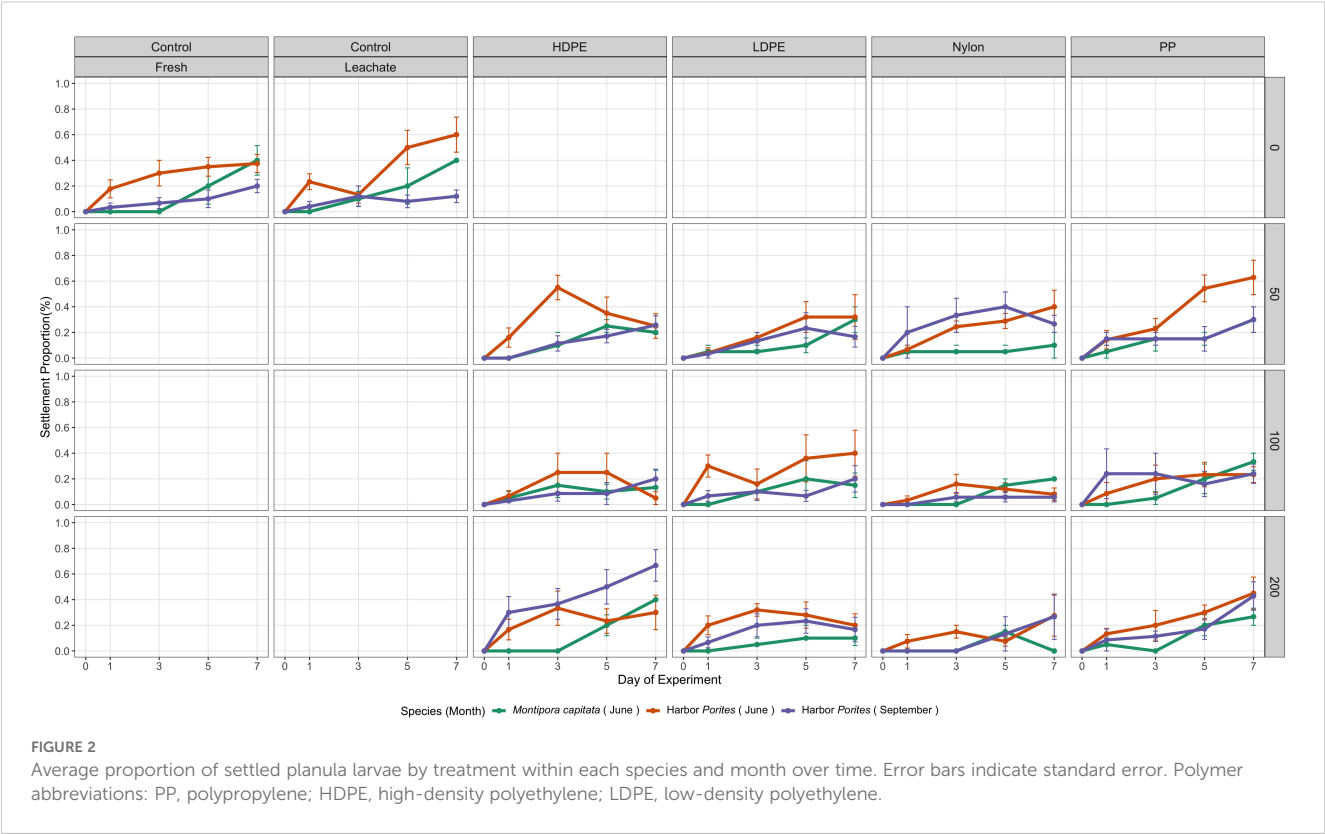
All additional non-significant comparisons can be found in [Supplementary Tables S4a, b](#).

larvae in the HDPE—200 treatment had significantly higher settlement than a lot of treatments including both control treatments (Control—Fresh p-value = 0.001, Control—Leachate p-value = 0.002) and the HDPE—100 treatment (p-value < 0.000). Compared to other polymers, planula larvae in the HDPE—200 treatment also had significantly higher settlement than other polymers at 200 particles/L concentration on Day 5 (LDPE—200 p-value = 0.012; nylon—200 p-value = 0.005). On Day 5, the

differences between treatments increased with planula larvae in the HDPE—200 treatment again showing the strongest differences compared to other treatments including both control treatments (Control—Fresh and Control—Leachate p-value < 0.0001) and the other HDPE concentrations (HDPE—50 p-value = 0.001; HDPE—100 p-value < 0.0001). Between polymers, planula larvae in the HDPE—200 treatment had higher settlement than all other polymer types at the same concentration: the LDPE—200

TABLE 6 Proportion of average settlement on Day 7 for the control treatments (Control-Fresh and Control-Leachate) for each month and species.

Control—Fresh				
Species	Month	Mean	SD	N
<i>M. capitata</i>	June	0.400	0.200	3
Harbor <i>Porites</i>	June	0.375	0.198	8
Harbor <i>Porites</i>	September	0.200	0.126	6
Control—Leachate				
Species	Month	Mean	SD	N
<i>M. capitata</i>	June	0.400	N/A	1
Harbor <i>Porites</i>	June	0.600	0.335	6
Harbor <i>Porites</i>	September	0.120	0.110	5



treatment (p-value < 0.0001), the PP—200 treatment (p-value = 0.043), and the nylon—200 treatment (p-value < 0.000) like what was seen for Day 3. The nylon—50 treatment-exposed planula larvae also showed significantly higher settlement than those in the nylon—100 treatment (p-value = 0.015). On Day 7, planula larvae in the HDPE—200 treatment again showed higher settlement than those in both control treatments (Control—Fresh p-value < 0.0001; Control—Leachate p-value < 0.0001), the other HDPE treatments (HDPE—50 p-value = 0.002; HDPE—200 p-value < 0.0001) and LDPE—200 (p-value < 0.0001).

4 Discussion

Microplastic leachate had negative effects on survival and settlement of *Montipora capitata* and *Harbor Porites* planula

larvae with species-specific and time-dependent effects seen. *Harbor Porites* planula larvae had higher survival and settlement compared to those of *M. capitata* in many circumstances. The effects seen vary by months and days of the experiment emphasizing the importance of time in survival and settlement analyses. Polymer type and concentration had significant effects with high-density polyethylene (HDPE) and low-density polyethylene (LDPE) treatments having the most significant effects across species and experiments. Overall, results from this study highlight that the relationship between microplastic leachate and coral planula larvae survival and settlement is complex with species-specific differences, temporal variability, and treatment effects.

Species-specific differences were seen in survival and settlement potentially due to biological differences between *M. capitata* and *Harbor Porites*. *M. capitata* is a broadcast spawning coral and is

TABLE 7 Summary of significant model results of *M. capitata* planula larvae settlement across all days of the experiment.

Model results				
Predictor	Estimate	SE	Z ratio	P-value
Intercept	−3.66	0.439	−8.338	<0.001
Day	0.355	0.108	3.292	0.001
HDPE—100 and Day	−0.279	0.14	−2.000	0.045
nylon—50 and Day	−0.289	0.149	−1.942	0.052

No significant *post-hoc* differences were found. All additional non-significant comparisons can be found in [Supplementary Tables S5a, b](#).



TABLE 8 Summary of significant model results and *post-hoc* comparisons of Harbor *Porites* planula settlement across all days of the experiment in June.

Model results					
Predictor		Estimate	SE	Z value	P-value
Intercept		−1.93	0.267	−7.246	<0.001
Day		0.23	0.068	3.371	0.001
Post-hoc comparisons					
Day	Contrasts	Estimate	SE	Z ratio	P-value
5	Control—Leachate and HDPE—100	1.37	0.402	3.400	0.043
5	Control—Leachate and nylon—100	1.30	0.382	3.398	0.043

All additional non-significant comparisons can be found in [Supplementary Tables S6a, b](#).

TABLE 9 Summary of significant model results and *post-hoc* comparisons of Harbor *Porites* planula settlement across all days of the experiment in September.

Model results					
Treatment		Estimate	SE	Z ratio	P-value
Intercept		−2.60	0.328	−7.937	<0.001
Day		0.156	0.800	1.961	0.049
HDPE—200 and Day		0.321	0.110	2.920	0.004
Post-hoc comparisons					
Day	Contrast	Estimate	SE	Z ratio	P-value
3	Control—Fresh and HDPE—200	−1.22	0.283	−4.33	0.001
3	Control—Leachate and HDPE—200	−1.27	0.296	−4.284	0.002
3	HDPE—100 and HDPE—200	−1.36	0.273	−4.982	0.000
3	HDPE—200 and HDPE—50	1.06	0.273	3.902	0.007
3	HDPE—200 and LDPE—200	1.07	0.283	3.765	0.012
3	HDPE—200 and nylon—200	1.37	0.343	3.987	0.005
5	Control—Fresh and HDPE—200	−1.87	0.347	−5.38	<0.001
5	Control—Leachate and HDPE—200	−2.03	0.361	−5.616	<0.001
5	HDPE—100 and HDPE—200	−2.13	0.334	−6.382	<0.001
5	HDPE—200 and HDPE—50	1.56	0.333	4.687	0.000
5	HDPE—200 and LDPE—200	1.81	0.343	5.284	<0.001
5	HDPE—200 and nylon—200	2.03	0.425	4.764	0.000
5	HDPE—200 and PP—200	1.11	0.327	3.402	0.043
5	nylon—100 and nylon—50	−1.58	0.425	−3.707	0.015
7	Control—Fresh and HDPE—200	−2.51	0.507	−4.944	0.000
7	Control—Leachate and HDPE—200	−2.79	0.524	−5.321	<0.001
7	HDPE—100 and HDPE—200	−2.90	0.486	−5.968	<0.001
7	HDPE—200 and HDPE—50	2.06	0.485	4.249	0.002
7	HDPE—200 and LDPE—200	2.56	0.492	5.194	<0.001

All additional non-significant comparisons can be found in [Supplementary Tables S7a, b](#).

generally considered more sensitive to environmental stressors. In contrast, Harbor *Porites* is a brooding coral that thrives and reproduces in a harbor habitat with wide salinity and water quality ranges that few other coral species have been seen in (Spies, 2021). There is very little current information about Harbor *Porites* in terms of genetic variability and its unique reproductive strategy as *Porites* corals are generally spawning corals. These differences in reproductive strategy, colony and larval physiology, and life history may have contributed to overall effects seen under certain leachate exposures.

Survival varied significantly by species, with Harbor *Porites* showing greater resilience than *M. capitata*. However, Harbor *Porites* planula larvae in the HDPE—100 treatment had consistently lower survival in both June and September with September showing the most negative effects compared to those of the other HDPE concentrations. Planula larvae in the LDPE—200 treatment showed lower survival than in several treatments in June for Harbor *Porites*. Between species, negative effects were seen in planula larvae survival of both species in the HDPE—100 and LDPE—200 treatments with more significant effects seen in Harbor *Porites*. Both the polymer type and concentration influenced survival with significant effects seen on the final days of the experiment (Day 5 and 7) suggesting a delayed or cumulative negative effect. A longer experiment could parse out the specific effects and any delayed effects seen. There may be a non-linear relationship or non-monotonic responses to concentration as commonly seen in studies on endocrine-disrupting chemicals due to the complex way in which hormones are mimicked within organisms (Vandenberg et al., 2012; Lagarde et al., 2015).

Settlement responses were not as consistent with Harbor *Porites* having generally higher settlement rates, while *M. capitata* had more variability in treatments and time. There were no matching effects seen between species or months. Unexpectedly, the most pronounced effects were seen in September for Harbor *Porites* with higher settlement seen in HDPE—200 treatment-exposed planula larvae over time and compared to the control treatments on Days 3, 5, and 7 suggesting that leachate may provide attractive chemical cues and/or induce a stress response. This is supported by the idea that microplastics give off chemical cues that make them attractive for ingestion by adult corals (i.e., phagostimulants) (Allen et al., 2017).

Planula larvae viability due to environmental conditions of the adult colonies also likely played a role in the different survival and settlement rates seen between months. During the summer of 2023, when these experiments took place, global sea surface temperatures recorded the warmest year since 1850 now referred to as a super-marine heatwave (Huang et al., 2024). The global mean sea surface temperatures reached record high levels in April 2023 and were broken again in July and August 2023 with high temperatures persisting throughout the remainder of 2023 (Huang et al., 2024). This high temperature corresponds with the time when the experiments took place, which could explain the month-specific responses seen, particularly for June. Planula larvae that are already impacted by other global or local stressors are more likely to be susceptible to microplastic pollution. This has been previously

suggested for adult colonies exposed to multiple stressors (Reichert et al., 2021; Bove et al., 2023). Microplastics present an additional stressor of concern, but likely do not have as significant of an impact on their own as suggested by the September survival data. This finding further supports the management strategy of focusing on mitigating local stressors to buy time to address the global stressors.

The findings of this study are not particularly surprising due to the negative effects of microplastic leachate seen on coral fertilization and fatty acids (Wilkins et al., 2024). Specifically, eggs from the HDPE—100 treatment showed significantly lower fertilization rates consistent with the survival and settlement trends seen in this study. However, this study did not look at fatty acids, so it is possible that sublethal effects may have been missed. Additionally, although microplastic leachate can negatively affect fertilization, survival, and settlement, these are only two points in the coral reproduction process. Exposure of either adults during the development of gametes or eggs and sperm into planula larvae may result in very different effects.

The settlement effects seen in this study are likely not representative of effects at other stages of reproduction. Since planula larvae were only tracked for 7 days, effects on metamorphosis and growth could not be fully assessed. If we simplify the life cycle of a coral into four key stages, adults, gametes, planula larvae, and juveniles, effects of microplastic-associated chemicals could have very different impacts at each life history stage. For example, ingestion of microplastics has a wide variety of negative effects on adult coral health and growth with implications for increased energy allocation to survival and, thus, likely reduced energy allocation to reproduction (Hankins et al., 2021). Exposure of adults to microplastic-associated chemicals through ingestion could impact the development of gametes and brooded planula larvae quality and production. Given the endocrine-disrupting and lipophilic nature of microplastics and their accumulation in the gastrovascular cavity where egg production occurs, these contaminants could accumulate in eggs and again affect quality and colony fecundity. Beyond gametogenesis, external fertilization during spawning has already been seen to be affected along with fatty acid quantity and composition, which are critical for early development (Wilkins et al., 2024). At the larval stage, planula larvae may settle in unfavorable and degraded conditions due to attractive chemical cues given off by chemicals or biofilms. In addition to effects in the water column, plastic can also be a misleading substrate that attracts settlement over natural substrates of corals (Bergami et al., 2021) and other species of marine larvae (Pinochet et al., 2020; Audrézet et al., 2022) due to its accumulation of attractive biofilms, which mimic natural settlement cues. While this may be seen as a beneficial aspect of plastic pollution, especially as a settlement promoter in degraded environments, plastics degrade over time, are easily transported, and may expose vulnerable settled larvae to new stressors. Finally, given that juveniles could also be ingesting nano and microplastics, the effects seen may affect growth and development like what has already been seen in adults.

While this study provides valuable insights, some limitations include the lack of direct, laboratory-based control testing of chemicals in the leachate and consideration of additional environmental variables. Of the chemicals found within the particles, there is no known toxicity information (Dopico-García et al., 2007). However, only chemicals within the specific plastics were tested, and none were tested within the leachate to confirm that they had leached and to determine their specific concentrations. Given our results, the concentrations used may also be misleading as an increased number of particles does not necessarily mean an increase in chemicals in the leachate. This is due to the complex leaching behavior of plastics, which depends on water parameters like pH, water temperature, and characteristics of the plastic, including age and texture (Koelmans et al., 2014; Li and Tang, 2023; Iftikhar et al., 2024). Additionally, the plastics used in this study were virgin plastics, but weathered plastics leach more additives (Luo et al., 2022) and can contain sorbed chemicals, which would likely have very different effects. Size of the particles might also be an important factor. Using smaller particles may have produced more dramatic effects due to more leaching of endocrine-disrupting compounds as seen in previous studies (Chen et al., 2019). Additionally, the microbial growth within the leachate was not considered as a potential influence on some of the positive or negative settlement effects seen. Taken altogether, future research should include a detailed chemical analysis of any microplastic leachates used, the use of environmental plastics, and the investigation of leachate from smaller particles for more realistic conditions.

The vulnerability of coral planula larvae to microplastic leachates seen here is consistent with effects seen for other marine animals. Microplastic leachate has negative effects on embryo development of the brown mussel, *Perna perna* (e Silva et al., 2016), the sea urchins, *Paracentrotus lividus* (Martínez-Gómez et al., 2017; Rendell-Bhatti et al., 2021) and *Strongylocentrotus purpuratus* (Paganos et al., 2023), the sea cucumber, *Apostichopus japonicus* (Wang et al., 2023), and the survival and settlement of barnacle nauplii, *Amphibalanus amphitrite* (Li et al., 2016). The complex effects of microplastic leachate demand more research and emphasize a knowledge gap on the most vulnerable stages of marine organism life cycles.

## 5 Conclusions

This is the first study to provide evidence of the effects of microplastic leachate on coral planula larvae survival and settlement. These species-specific, polymer-type, and time-dependent effects suggest potential delayed or cumulative effects. One leachate promoted settlement suggesting that attractive chemical cues from microplastics may be a concern. The findings from this study highlight the complex effects that marine plastic pollution could be having on the most vulnerable life stages of corals. Given the vulnerability of the early life stages of corals and the many other stressors that they currently face, a greater understanding of microplastic leachate effects on different life

history stages of corals is needed to understand potential effects on recruitment and replenishment of reefs. Future research must consider environmental plastics containing sorbed pollutants, different sizes of particles, and a better understanding of the leaching behavior of particles combined with a broader species analysis to truly understand the extent of marine microplastic pollution effects on corals.

## Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

## Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

## Author contributions

KW: Investigation, Data curation, Visualization, Methodology, Resources, Conceptualization, Funding acquisition, Project administration, Writing – original draft, Formal Analysis, Software, Writing – review & editing. RR: Validation, Writing – review & editing, Supervision.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2025.1596594/full#supplementary-material>

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