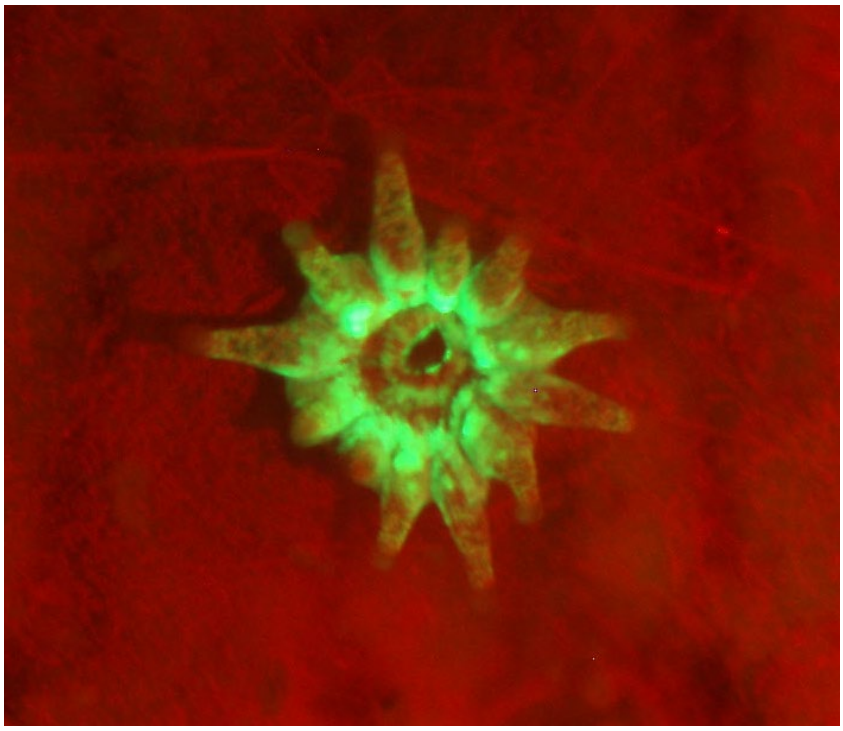
Chemical Contaminant Inhibition of Settlement and Survivorship in Corals: Phase 1



Chemical Contaminant Inhibition of Settlement and Survivorship in Corals: Phase 1

Final Report

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Management Summary (300 words or less)

Coral recruitment on Florida's Coral Reef (FCR) remains critically low, limiting the long-term success of restoration efforts. This project evaluated whether chemical contaminants contribute to recruitment failure by assessing their impacts on coral larval settlement and post-settlement survivorship in two key reef-building species, *Montastraea cavernosa* and *Diploria labyrinthiformis*. The study examined environmentally relevant concentrations of metals, hydrocarbons, and organic contaminants through both dissolved exposure pathways and contaminated settlement substrates. Results demonstrate that multiple contaminants can reduce larval settlement and increase mortality, often at concentrations at or below current regulatory thresholds. Contaminants such as fluoranthene, phenanthrene, pyrene, octocrylene, diuron and tributyltin showed consistent negative effects on settlement success. Substrate-associated contamination produced particularly strong impacts, with several contaminants inhibiting settlement or causing mortality at low concentrations, indicating that contamination of reef sediments and hardbottom surfaces can be a significant barrier to coral recruitment. Post-settlement survival was also influenced by exposure type. Coral recruits exposed to dissolved contaminants that survived the initial month generally remained viable and continued to grow. In contrast, those exposed to contaminated substrates exhibited near-zero survival within one month, suggesting persistent or cumulative effects associated with substrate-bound contaminants.

Importantly, observed adverse effects frequently occurred at concentrations below existing EPA water quality criteria and Florida sediment threshold effect levels (TELs/PELs), indicating that current regulatory benchmarks may not be sufficiently protective of early coral life stages. These findings highlight the need to incorporate coral-specific toxicity thresholds into water quality and sediment management frameworks. Management applications include prioritizing restoration sites with low contaminant loads, evaluating sediment and substrate quality, and revising regulatory thresholds based on coral early life-stage sensitivity.

Executive Summary (max 1 page)

Florida's Coral Reef (FCR) has experienced substantial decline due to multiple environmental stressors, including disease, climate change, and degraded water quality. While restoration efforts increasingly rely on coral outplanting, long-term reef recovery depends on successful natural recruitment through larval settlement and survival. However, recruitment rates remain low, and the role of chemical contaminants in inhibiting early life stages of corals is not well understood. This Phase 1 study addresses this critical knowledge gap by evaluating the effects of common inorganic and organic chemical contaminants on coral larval settlement and post-settlement survivorship. The project focused on two reef-building coral species, *M. cavernosa* and *D. labyrinthiformis* and assessed their responses to environmentally relevant concentrations of metals (copper, zinc, arsenic), hydrocarbons (including pyrene, phenanthrene, and fluoranthene), and organic contaminants (tributyltin, diuron, and octocrylene). Larval settlement experiments were conducted using both dissolved contaminants and contaminated settlement substrates to simulate different exposure pathways, and a methodology was developed to quantitatively assess impacts on settlement success, larval mortality, and longer-term juvenile survival and growth.

Multiple contaminants reduced larval settlement and increased mortality in both species, often at concentrations at or below currently established regulatory thresholds. Hydrocarbons, organics, and some metals produced consistent, dose-dependent reductions in settlement success and increases in larval mortality. Tributyltin was particularly toxic, significantly impairing both settlement and survival at very low concentrations. Effects were observed for both dissolved exposures and for contaminated substrates, highlighting the importance of sediment-associated contamination as a barrier to recruitment. Juvenile survivorship was generally low across treatments, with differences between exposure pathways. Recruits exposed to dissolved contaminants that survived beyond one month typically remained viable and continued to grow, while recruits exposed to contaminated substrates exhibited near-zero survival within the first month, suggesting persistent effects associated with substrate-bound contaminants. These findings indicate that even when settlement occurs, contaminated substrates may prevent successful recruitment by limiting early post-settlement survival. Importantly, observed biological effects frequently occurred at concentrations below existing EPA water quality criteria and Florida sediment threshold effect levels (TELs/PELs), suggesting that current regulatory benchmarks may not be sufficiently protective for early coral life stages.

This study provides region-specific toxicity data for Atlantic coral species and contributes to the development of standardized testing methodologies for coral early life stages. The findings have direct management implications, including the need to reassess water quality and sediment guidelines, incorporate coral-specific thresholds into regulatory frameworks, and prioritize site selection for restoration based on contamination profiles.

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1. BACKROUND/INTRODUCTION

Stony coral cover on Florida's Coral Reef (FCR) has been severely reduced due to local and global stressors and disease, resulting in deteriorated reefs where self-recovery is difficult or impossible. This has led to the development of robust coral restoration efforts and while these interventions are promising, success is predicated on reef-building corals eventually self-recruiting to ensure reef resiliency. Present management activity is focused on rebuilding population sizes through restorative outplanting of asexual coral fragments and ex-situ produced juveniles, with the hope that mature, sexually reproducing colonies will be able to self-recruit and increase coral cover. However, recruitment remains low on FCR and it is unclear whether this is a result of remnant coral populations which are

sufficiently separated to limit sexual reproduction, or reproductive failure due to environmental stressors through inhibition of fertilization, settlement, and survival.

Early life stages of scleractinian corals (i.e., planula larvae, early post-settlement recruits) are generally regarded as more sensitive to environmental stressors including chemical contaminants, compared to adults. Spawning and larval development of most coral species on FCR occurs through the summer and fall, which coincides with substantial rain-event driven terrestrial discharges, associated with risk of exposure to both dissolved chemical contaminants in the water column and sediment/substrate associated chemical contaminants. Impacts of environmental stressors on the early life stages of corals can lead to death or sublethal effects that can lead to severe structural impacts on populations and communities. The inhibition of larval settlement and/or metamorphosis, which are crucial developmental steps and ultimately determine survival, have been examined for only a very limited range of chemical contaminants. However, these studies have primarily been conducted with Indo-Pacific corals, thus this project will provide new, standardized data specific to Atlantic/Caribbean corals (summarized in Ouédraogo et al. 2023). This project adds substantially to the current state of knowledge by determining acute and subacute threshold of effect concentrations of multiple inorganic and organic chemical contaminant classes for early life stages of two species of coral on FCR. Furthermore, this data contributes to the establishment of standardized toxicity test methods for early life stages of coral, a current need, which is essential for determining the environmental risk of chemical contaminants in coral species (Burns and Davies 2021).

A recent meta-analysis of environmental contaminants on FCR found that several inorganic chemical contaminants (metals and non-metals) and organics (hydrocarbons, pesticides, and industrial chemicals) have been detected at concentrations which exceed both EPA water quality standards and State of Florida threshold and/or probable effects levels in waters and sediments across the southeast coast of Florida (Skelton, 2024). Very few studies have examined the effects of exposure to chemical contaminants for Atlantic corals, including those identified as a potential concern for coral larval settlement and survivorship, therefore potential impacts remain largely unknown and may have significant management implications for restoration.

1.1. Project Goals

The central goal of this project was to evaluate how inorganic and organic chemical contaminants may affect the settlement success and juvenile survivorship of priority coral species on FCR, and the first step in understanding this is through well-controlled settlement and survival assays, which provide scientifically defensible acute and subacute threshold estimates based on population-relevant metrics.

The objectives for this project were to 1) establish standard testing procedures to reliably identify acute and sublethal effects to coral larvae, and 2) determine the acute (mortality) and subacute/chronic (settlement and survivorship) toxicity thresholds of metals (copper, arsenic, and zinc), hydrocarbons (chrysene, fluoranthene, phenanthrene, and pyrene), and common organic pollutants (tributyltin, diuron, and octocrylene) to larvae of two priority coral species for which larvae are available via reproduction in the ex-situ nursery at NSU: *Montastraea cavernosa* and *Diploria labyrinthiformis*. The chemical contaminants chosen reflect three separate groupings of contaminant classes, with three individual contaminants in each class. These include metals, hydrocarbons and other ubiquitous organic contaminants representing a variety of potential historic and current pollution sources.

1.2. Priorities addressed

This project addresses FY 2025-2026 CPR Research Priorities: ER1. Better understand coral sexual reproduction and recruitment (or lack thereof) in *in situ* coral populations, with a particular interest in the factor(s) causing reproductive and/or settlement failure at different life stages. Specifically, inhibitors of reproduction and settlement (e.g., toxicants in the water column, turf algae, sediment toxicity). WQ1. Conduct research and evidence mapping to prioritize pollutants for study and management along Florida's Coral Reef. Investigations should assess both chronic and acute pollutant effects on coral physiological and reproductive processes, identifying relevant lethal and sublethal toxicity thresholds. Studies should prioritize sensitive early life stages of corals, with other life stages or reef species considered with justification. From the Resilience Action Plan for Florida's Coral Reef (2021-2026), this project also addresses: 1) Goal 1: Enable Resilience-based Management of Florida's Coral Reef, Objective 1, Abate threats to corals reefs and reduce water quality impacts, establish coral-specific water quality standards; Objective 2, Enhance reef ecosystem condition with disease interventions and restoration, restoration planning; 2) Goal 2: Support Public Policy that Creates the Enabling Conditions for Reef Recovery, Objective 2: Educate Florida's leaders on coral reef-related issues, Water quality regulations.

1.3. Reef Management Application

The focus of this project is on the impacts to coral larvae; this has the highest consequences to future restoration planning in that while outplant survival is achievable, without unassisted larval settlement, restoration efforts are unlikely to be successful long-term. Decades of sampling have detected significant amounts and accumulation of a variety of chemical contaminants in seawater and sediments in Florida marine environments, but few studies have considered the effects of exposure in the early life stages of corals in Florida (Ouédraogo et al. 2023, Skelton 2024). Exposure pathways may occur through multiple

routes, including chemical contaminants in the water column (either in dissolved or particulate forms), or chemical contaminants associated with reef sediments/substrates. Consequently, potential impacts remain largely unknown and may have significant management implications. Direct management applications of this project include, support for restoration-related management actions, such as the identification of likely successful outplant sites. These assessments will be based on tested (known in current monitoring programs), and/or projected levels of sediment/substrate contamination. These datasets will also allow us to identify the selection and distribution of species for outplants based on species which are more resistant to the specific chemical contaminant exposures at those locations. Specific management applications for water quality center on providing updated, applicable data for the revision of EPA water quality standards and State of Florida sediment thresholds of effect which will improve restoration outcomes. The establishment of these response thresholds support the creation of biological benchmarks, which for natural resource managers is important in addressing both chronic and acute water quality issues on FCR. Further, the project's contributions to development of a formal standard toxicity test methodology for examining contaminant impacts on coral larval settlement will advance our ability to provide definitive contamination thresholds that can be identified as a barrier to coral recovery and restoration.

2. METHODS

The purpose of this project was to evaluate how inorganic and organic chemical contaminants may affect the settlement success and juvenile survivorship of priority coral species on Florida's Coral Reef (FCR), and the first step in understanding this is through well-controlled settlement and survival assays, which provide scientifically defensible acute and subacute threshold estimates based on population-relevant metrics.

2.1. Task 1: Quality Assurance Plan and Reporting

A Quality Assurance plan was developed with input from Dr. Carys Mitchelmore (UMCES) and with the assistance of Ellen Skelton, MSc.; the plan describes the project purpose, background and anticipated results in addition to the experimental methodology, quality control mechanisms and data handling procedures. The initially submitted draft QA plan was evaluated by the DEP DEAR, revised and approved. The subcontract with UMCES was developed and submitted.

2.2. Task 2: Assess contaminant effects on settlement success of two coral species

Coral larvae (raised to the swimming stage/competency to settle) were exposed to nominal dissolved concentrations or contaminated settlement substrate [three exposure concentrations (low, medium and high) and a seawater control], with three replicates per

concentration of each exposure type of the target analyte in settlement chambers which contained settlement tiles (detailed in Table 1).

Table 1. *Concentration details for contaminant exposures.*

Contaminant	Dissolved – low conc.	Dissolved-mid conc.	Dissolved – high conc.	Contaminated settlement tile – low conc.	Contaminated settlement tile – mid conc.	Contaminated settlement tile – high conc
Metals:						
Arsenic	1 ug/L	10 ug/L	100 ug/L	2 ug/g	20 ug/g	200 ug/g
Copper	1 ug/L	10 ug/L	100 ug/L	5 ug/g	50 ug/g	500 ug/g
Zinc	5 ug/L	50 ug/L	500 ug/L	5 ug/g	50 ug/g	500 ug/g
Hydrocarbons:						
Chrysene	0.1 ug/L	1 ug/L	10 ug/L	0.1 ug/g	1 ug/g	10 ug/g
Fluoroanthene	0.1 ug/L	1 ug/L	10 ug/L	0.1 ug/g	1 ug/g	10 ug/g
Phenanthrene	0.1 ug/L	1 ug/L	10 ug/L	0.1 ug/g	1 ug/g	10 ug/g
Pyrene	0.1 ug/L	1 ug/L	10 ug/L	0.1 ug/g	1 ug/g	10 ug/g
Organics:						
Diuron	1 ug/L	10 ug/L	100 ug/L	5 ug/g	50 ug/g	500 ug/g
Octocrylene	1 ug/L	10 ug/L	100 ug/L	5 ug/g	50 ug/g	500 ug/g
Tributyltin	0.1 ug/L	1 ug/L	10 ug/L	5 ug/g	50 ug/g	500 ug/g

For the dissolved exposures, exposure media stock solutions were prepared in 500 mL volumetric flasks by adding the calculated amounts of each chemical contaminant for each test concentration to filtered artificial seawater from the coral spawning systems. Each test chamber was prepared by adding 100 ml of prepared exposure media and one settlement tile (3.2 x 3.2 cm ceramic tiles, Oceans Wonders®) to the appropriate chambers. For the contaminated substrate exposures, stock solutions were prepared at the specified concentrations for each individual chemical contaminant, in volumetric flasks with either reagent grade water (for water soluble contaminants), MeOH (for water insoluble contaminants), or EtOH (for tributyltin). To prepare the contaminated settlement tiles (3.2 x 3.2 cm ceramic tiles, Oceans Wonders®), 1 mL of the chemical contaminant stock solutions were applied to the surface of each tile and allowed to dry completely before use (USEPA OCSPP 850.1000 2016). Each test chamber was then prepared by adding 100 mL of filtered artificial seawater from the coral spawning system and one contaminated settlement tile to the appropriate chambers.

Three replicate settlement test chambers were used for each dissolved or contaminated substate exposure concentration, with an additional three replicate control/solvent control chambers per contaminant, for a total of 120 test chambers used for the dissolved concentration exposures and 120 test chambers used for the contaminated settlement substrate chambers across the 10 laboratory trials per coral species. A standardized amount (100 uL, Ionata pers. comm.) of finely crushed crustose-coralline algae (CCA) slurry was applied to the upper surface of each tile to induce settlement. Fifteen competent larvae

were then added to each 120 mL individual settlement chamber (120 mL glass jars with lid, Figure 1). Competency refers to a larva's ability to respond to settlement cues and undergo metamorphosis; larval competency will be evaluated by visual observations of swimming behavior (time to competency after fertilization is approximately 3 days) (Walker and Figueiredo 2019). Settlement chambers were randomly placed into 35 L water baths with small pump and a small 50W heater to maintain temperature at 28ºC throughout the exposures (Figure 1).

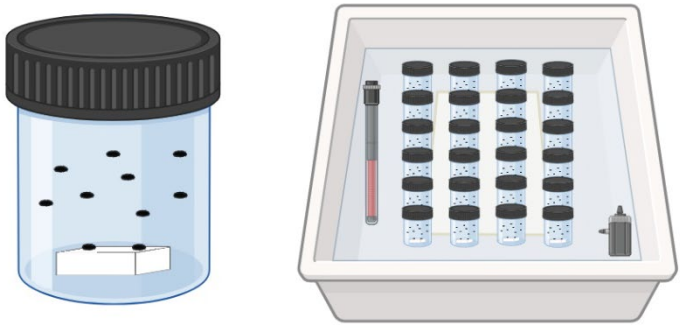

Figure 1. *Exposure chambers and water bath for larval settlement trials.*

Tiles were checked for settlement and metamorphosis daily until no or few observable swimming larvae were found. NIGHTSEA™ Xite Portable Fluorescence Flashlight System in Royal Blue (RB) and matching barrier filter glasses were used to assist counting of larvae.

Threshold nominal concentrations for larval mortality (LC50) and settlement success (EC50) was determined from counts of settled larvae and nominal exposure concentrations with log-logistic 4-parameter dose-response models using GraphPad Prism 10. Kruskal–Wallis analysis of variance (ANOVA) on ranks (α=0.05) with untransformed data was used to compare larval settlement % and mortality between treatments, and a Mann-Whitney U-test was used to determine significant differences between treatments and the controls (NOEC or LOEC) when significant test results were found

2.3. Task 3: Assess contaminant effects on survival of coral juveniles

After settlement was complete, tiles with settled larvae were transferred to grow-out tanks and to evaluate post-settlement survival and growth of surviving juveniles. Tiles were photographed with an Olympus LC20 digital camera attached to an Olympus SZ61 dissecting microscope equipped with a NIGHTSEA™ Stereo Fluorescence Adapter Standard Lamp Base, and a Royal Blue Barrier Filter in a darkened laboratory to detect natural fluorescence emitted by coral recruits. The position of settled corals on each tile was mapped (the specific location of each settled coral on the tile was recorded), and for each settled coral, survivorship and growth (increase in surface area over time, in mm^2)

was tracked over time (3 months for MCAV, and 1 month for DLAB, as a result of spawning time).

3. RESULTS

3.1. Task 2: Contaminant effects on settlement success

For *M. cavernosa*, settlement of the controls in previous studies has indicated typical values of 20% or higher (Walker and Figueiredo 2019, Figueiredo and Gilliam 2024). Control settlement of *M. cavernosa* in these trials ranged from 25.33-44.44%, indicating typical settlement success rate for these trials with this species. Dose-related reductions in settlement were observed for all dissolved and contaminated substrate exposures. Dose-response modeling did not generate consistently significant model fits, therefore Kruskal–Wallis analysis of variance (ANOVA) on ranks (α=0.05) with untransformed data was used to compare larval settlement % between treatments, and a Mann-Whitney U-test was used to determine significant differences between treatments and the controls (NOEC or LOEC) when significant test results were found. For dissolved exposures, significant reductions in settlement were observed for fluoranthene, phenanthrene, pyrene, diuron, octocrylene, and tributyltin. For contaminated settlement tiles, significant reductions in settlement were observed for zinc, pyrene, and octocrylene. Non-significant dose-related reductions in settlement were also observed for other dissolved and contaminated settlement tile exposures (Table 2, and Figures 2-3); many of these were likely to have been significant given a larger sample size.

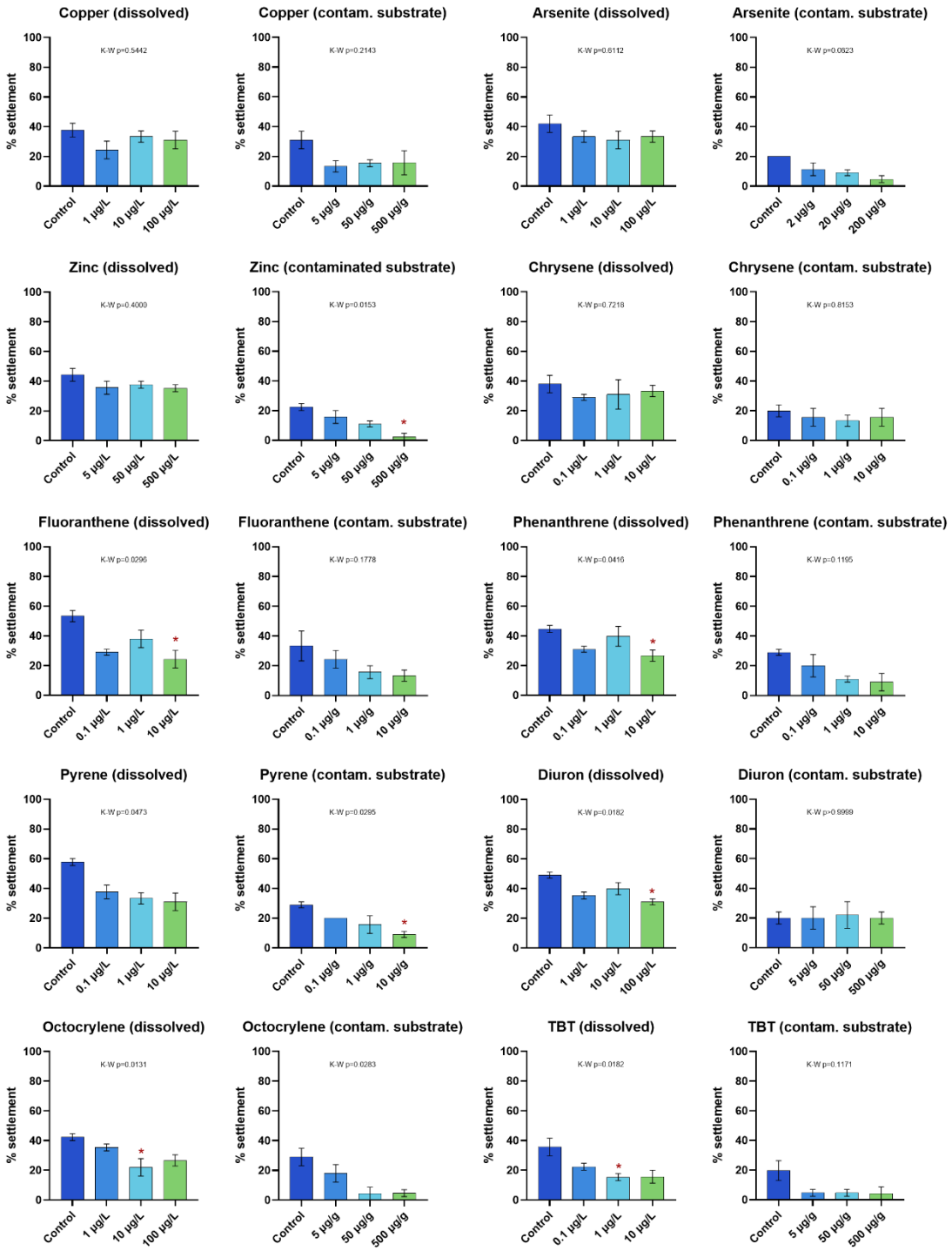

Figure 2. *4 day/96-hour percent settlement success for larvae of Montastraea cavernosa exposed to dissolved contaminants or contaminated substrate. *=significantly different from control.*

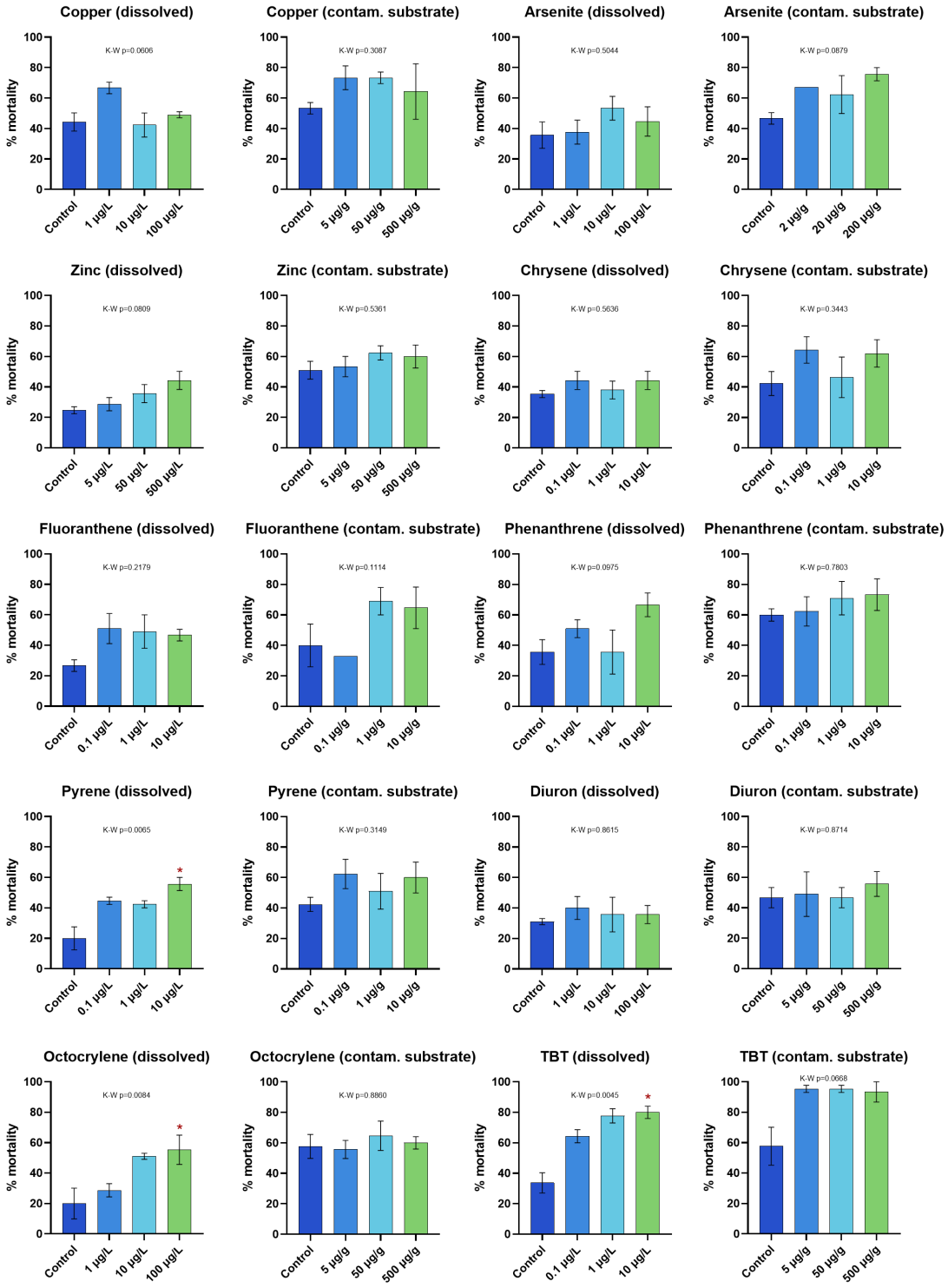

Figure 3. *4 day/96-hour percent mortality for larvae of Montastraea cavernosa exposed to dissolved contaminants or contaminated substrate. *=significantly different from control.*

As a number of still-swimming larvae were observed in the test chambers after 4 days, 5 days, and 6 days after the exposure commenced, the test with *D. labyrinthiformis* was extended to 7 days. For this species, settlement of the controls in previous studies has indicated typical values of 5-50% (Garcia Lara, 2023). Control settlement of *D. labyrinthiformis* (May 2026 spawning) in these trials ranged from 20-60%, within the range of typical settlement success for these trials with this species, although a larger proportion of larvae settled on the glass jar (21-47%) instead of the settlement tile (5-8%) for this species. Settlement for the same cohort in the Marine Larval Ecology Laboratory was <10 %.

For *D. labyrinthiformis*, dose-related reductions in settlement were observed for all dissolved and contaminated substrate exposures. Dose-response modeling did not generate consistently significant model fits, therefore Kruskal–Wallis analysis of variance (ANOVA) on ranks (α=0.05) with untransformed data was used to compare larval settlement % between treatments, and a Mann-Whitney U-test was used to determine significant differences between treatments and the controls (NOEC or LOEC) when significant test results were found. For dissolved exposures, significant reductions in settlement were found only for tributyltin. For contaminated settlement tiles, significant reductions in settlement were observed for copper, chrysene, and tributyltin. Non-significant dose-related reductions in settlement were also observed for other dissolved and contaminated settlement tile exposures (Table 3, and Figures 4-5); as with the results for *M. cavernosa*, many of these were likely to have been significant given a larger sample size.

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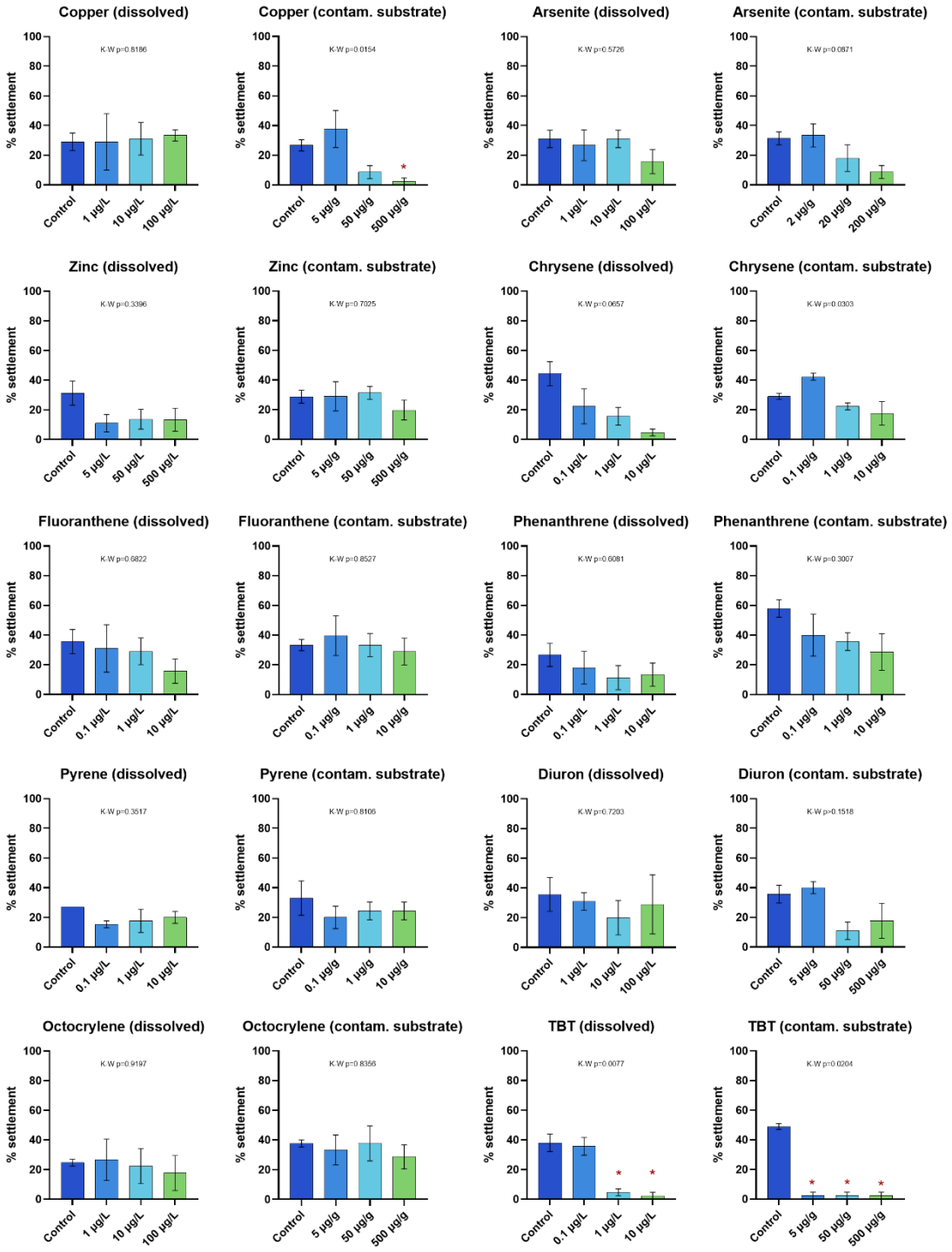

Figure 4. *7-day percent settlement success for larvae of Diploria labyrinthiformis exposed to dissolved contaminants or contaminated substrate. *=significantly different from control.*

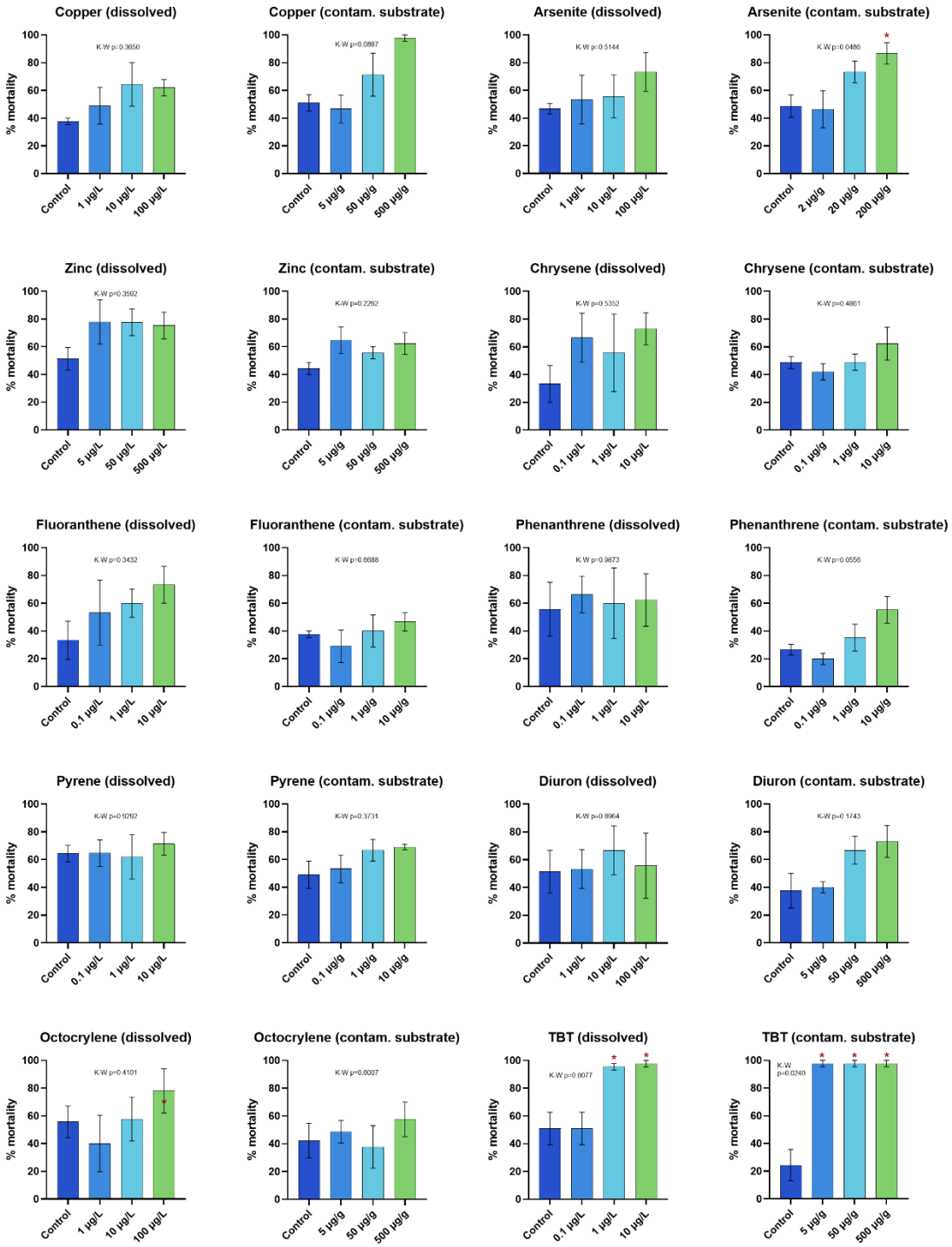

Figure 5. *7-day percent mortality for larvae of Diploria labyrinthiformis exposed to dissolved contaminants or contaminated substrate. *=significantly different from control.*

3.1.1. Summary Tables

Table 2. *Summary of 4 day/96 hour larval settlement test results for Montastraea cavernosa, with NOEC (No observed effect concentration) and LOEC (Lowest observed effect concentration). N/A = not applicable, CNC=could not calculate*

Contaminant	Exposure type	Assessment metric	Kruskal–Wallis ANOVA	NOEC	LOEC	4-day EC50 or LC50 (settlement or mortality)
Metals:						
Arsenite	dissolved	settlement	p=0.6112	N/A	N/A	CNC
Arsenite	dissolved	mortality	p=0.5044	N/A	N/A	CNC
Arsenite	substrate	settlement	p=0.0623	N/A	N/A	5.47 (0.53±45.43)
Arsenite	substrate	mortality	p=0.0879	N/A	N/A	>200
Copper	dissolved	settlement	p=0.5442	N/A	N/A	CNC
Copper	dissolved	mortality	p=0.0606	N/A	N/A	CNC
Copper	substrate	settlement	p=0.2143	N/A	N/A	CNC
Copper	substrate	mortality	p=0.3087	N/A	N/A	CNC
Zinc	dissolved	settlement	p=0.4000	N/A	N/A	CNC
Zinc	dissolved	mortality	p=0.0809	N/A	N/A	>500
Zinc	substrate	settlement	p=0.0153	50 µg/g	500 µg/g	13.40 (2.86-54.65)
Zinc	substrate	mortality	p=0.5361	N/A	N/A	CNC
Hydrocarbons:						
Chrysene	dissolved	settlement	p=0.7218	N/A	N/A	CNC
Chrysene	dissolved	mortality	p=0.5636	N/A	N/A	CNC
Chrysene	substrate	settlement	p=0.8153	N/A	N/A	CNC
Chrysene	substrate	mortality	p=0.3443	N/A	N/A	CNC
Fluoroanthene	dissolved	settlement	p=0.0296	1 µg/L	10 µg/L	1.49 (CNC)
Fluoroanthene	dissolved	mortality	p=0.2179	N/A	N/A	CNC
Fluoroanthene	substrate	settlement	p=0.1778	N/A	N/A	0.034 (0.0002-1.76)
Fluoroanthene	substrate	mortality	p=0.1114	N/A	N/A	8.72 (0.65-CNC)
Phenanthrene	dissolved	settlement	p=0.0416	1 µg/L	10 µg/L	>10
Phenanthrene	dissolved	mortality	p=0.0975	N/A	N/A	73.67 (2.57-CNC)
Phenanthrene	substrate	settlement	p=0.1185	N/A	N/A	0.25 (0.02-2.65)
Phenanthrene	substrate	mortality	p=0.7803	N/A	N/A	CNC
Pyrene	dissolved	settlement	p=0.0473	CNC	CNC	16.43 (0.63-497.8)
Pyrene	dissolved	mortality	p=0.0065	1 µg/L	10 µg/L	44.94 (1.68-CNC)
Pyrene	substrate	settlement	p=0.0295	1 µg/g	10 µg/g	0.46 (0.08-2.57)
Pyrene	substrate	mortality	p=0.3149	N/A	N/A	CNC
Organics:						
Diuron	dissolved	settlement	p=0.0182	10 µg/L	100 µg/L	>100
Diuron	dissolved	mortality	p=0.8615	N/A	N/A	CNC
Diuron	substrate	settlement	p=0.9999	N/A	N/A	CNC
Diuron	substrate	mortality	p=0.8714	N/A	N/A	CNC
Octocrylene	dissolved	settlement	p=0.0131	1 µg/L	10 µg/L	128.3 (7.34-2988)
Octocrylene	dissolved	mortality	p=0.0084	10 µg/L	100 µg/L	23.69 (CNC)
Octocrylene	substrate	settlement	p=0.0283	CNC	CNC	3.25 (0.35-15.36)

Octocrylene	substrate	mortality	p=0.8860	N/A	N/A	CNC
Tributyltin	dissolved	settlement	p=0.0182	0.1 µg/L	1 µg/L	CNC
Tributyltin	dissolved	mortality	p=0.0045	1 µg/L	10 µg/L	2.42 (0.15-CNC)
Tributyltin	substrate	settlement	p=0.1171	N/A	N/A	CNC
Tributyltin	substrate	mortality	p=0.0668	N/A	N/A	CNC

Table 3. *Summary of 7-day larval settlement test results for Diploria labyrinthiformis, with NOEC (No observed effect concentration) and LOEC (Lowest observed effect concentration). N/A = not applicable, CNC=could not calculate*

Contaminant	Exposure type	Assessment metric	Kruskal–Wallis ANOVA	NOEC	LOEC	7-day EC50 or LC50 (settlement or mortality)
Metals:						
Arsenite	dissolved	settlement	p=0.5726	N/A	N/A	58.17 (CNC)
Arsenite	dissolved	mortality	p=0.5144	N/A	N/A	> 100 ug/L
Arsenite	substrate	settlement	p=0.0871	N/A	N/A	19.43 (0.95-388.6)
Arsenite	substrate	mortality	p=0.0486	20 µg/g	200 µg/g	103.8 (21.96-152416)
Copper	dissolved	settlement	p=0.8186	N/A	N/A	0.0011 (CNC)
Copper	dissolved	mortality	p=0.3650	N/A	N/A	> 100 ug/L
Copper	substrate	settlement	p=0.0154	50 µg/g	500 µg/g	42.13 (CNC-114.3)
Copper	substrate	mortality	p=0.0887	N/A	N/A	261.1 (86.96-2804)
Zinc	dissolved	settlement	p=0.3396	N/A	N/A	CNC
Zinc	dissolved	mortality	p=0.3592	N/A	N/A	CNC
Zinc	substrate	settlement	p=0.7025	N/A	N/A	> 500 ug/g
Zinc	substrate	mortality	p=0.2262	N/A	N/A	CNC
Hydrocarbons:						
Chrysene	dissolved	settlement	p=0.0657	N/A	N/A	0.027 (CNC-0.37)
Chrysene	dissolved	mortality	p=0.5352	N/A	N/A	6.91 (CNC)
Chrysene	substrate	settlement	p=0.0303	CNC	CNC	9.31 (1.565-CNC)
Chrysene	substrate	mortality	p=0.4861	N/A	N/A	> 10 ug/g
Fluoroanthene	dissolved	settlement	p=0.6822	N/A	N/A	11.19 (CNC)
Fluoroanthene	dissolved	mortality	p=0.3432	N/A	N/A	0.30 (CNC)
Fluoroanthene	substrate	settlement	p=0.8527	N/A	N/A	> 10 ug/g
Fluoroanthene	substrate	mortality	p=0.6688	N/A	N/A	> 10 ug/g
Phenanthrene	dissolved	settlement	p=0.6081	N/A	N/A	0.005 (CNC-77.04)
Phenanthrene	dissolved	mortality	p=0.9873	N/A	N/A	CNC
Phenanthrene	substrate	settlement	p=0.3007	N/A	N/A	1.40 (0.10-20.58)
Phenanthrene	substrate	mortality	p=0.0556	N/A	N/A	29.74 (9.67-7635)
Pyrene	dissolved	settlement	p=0.3517	N/A	N/A	> 10 ug/L
Pyrene	dissolved	mortality	p=0.9292	N/A	N/A	CNC
Pyrene	substrate	settlement	p=0.8106	N/A	N/A	0.30 (0.002-16.49)
Pyrene	substrate	mortality	p=0.3731	N/A	N/A	> 10 ug/g
Organics:						
Diuron	dissolved	settlement	p=0.7203	N/A	N/A	> 100 ug/L
Diuron	dissolved	mortality	p=0.8964	N/A	N/A	> 100 ug/L

Diuron	substrate	settlement	p=0.1518	N/A	N/A	35.88(5.953-CNC)
Diuron	substrate	mortality	p=0.1743	N/A	N/A	80.11 (CNC)
Octocrylene	dissolved	settlement	p=0.9197	N/A	N/A	> 100 ug/L
Octocrylene	dissolved	mortality	p=0.4101	N/A	N/A	196.7 (13.88-CNC)
Octocrylene	substrate	settlement	p=0.8356	N/A	N/A	> 500 ug/g
Octocrylene	substrate	mortality	p=0.6007	N/A	N/A	> 500 ug/g
Tributyltin	dissolved	settlement	p=0.0077	0.1 µg/L	1 µg/L	0.22 (CNC-0.53)
Tributyltin	dissolved	mortality	p=0.0077	0.1 µg/L	1 µg/L	0.57 (0.03-3.88)
Tributyltin	substrate	settlement	p=0.0204	N/A	5 µg/g	< 0.1
Tributyltin	substrate	mortality	p=0.0240	N/A	5 µg/g	< 0.01

3.2. Task 3: Contaminant effects on survival of coral juveniles

Montastraea cavernosa coral recruits on tiles were microscopically assessed in October 2025, November 2025, and January 2026 using an Olympus SZX7 stereomicroscope and NIGHTSEA™ Stereo Fluorescence Adapter Standard Lamp Base, equipped with a Royal Blue Barrier Filter in a darkened laboratory to detect natural fluorescence emitted by coral recruits. Corals were photographed with an Olympus DP21 digital camera attached to an Olympus SZ61 dissecting microscope, and the position of settled corals on each tile was mapped (where the specific location of each settled coral on the tile is recorded). For each settled coral, survivorship, initial surface area (mm^2), and increase in surface area (mm^2) was assessed over time.

In general, low (20-50%) post-settlement survivorship has been observed across all treatments. However, post-exposure survivorship for the dissolved exposures was typically stable after 1 month, i.e. if recruits survived for 1 month after settlement tiles were transferred to the grow-out system, then those recruits tended to continue to survive and grow. This was not observed for post-settlement survivorship for the contaminated substrate exposures, where coral recruits, regardless of exposure level, typically did not survive to 1 month (Table 4, Figure 6).

Diploria labyrinthiformis coral recruits on tiles were microscopically assessed in June 2026 using the same approach as for M. cavernosa. Although low overall % settlement was observed across treatments, relatively high post settlement survivorship has been observed after 1 month (Table 5), for both dissolved exposures and contaminated settlement exposures, i.e if a coral successfully settled on the settlement tile, is was still present after 1 month.

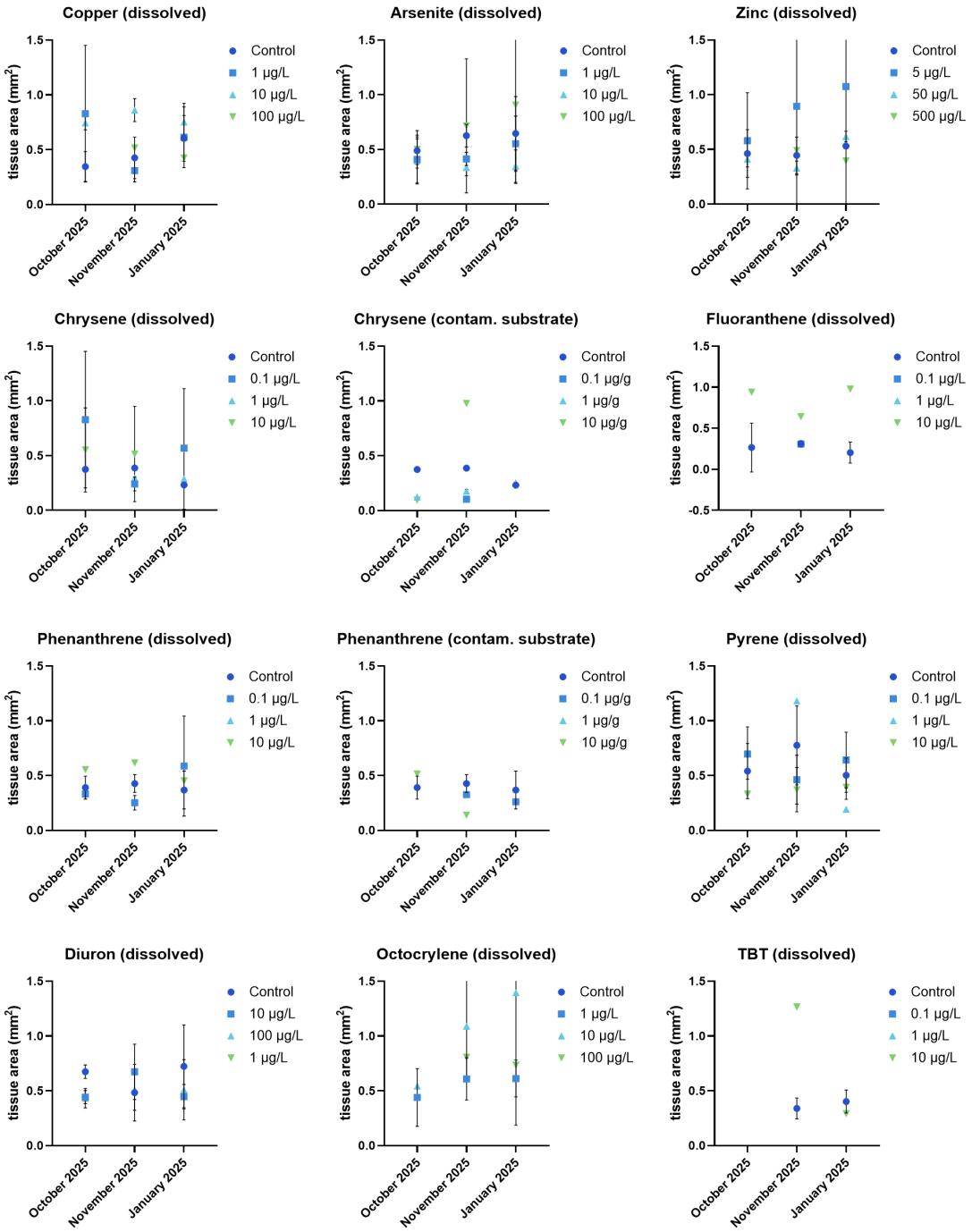

Figure 6. *Post-settlement growth (tissue area) of Montastraea cavernosa settlers exposed to dissolved contaminants or contaminated substrate over time.*

Table 4. *Post-settlement percent survivorship for Montastraea cavernosa dissolved and contaminated substrate exposures.*

Contaminant		Survivorship 1 month	Survivorship 2 months	Survivorship 3.5 months	Survivorship 5 months	Survivorship 6 months
Metals:						
	Controls	27.97	27.97	27.27	27.27	27.27
Arsenite	Dissolved low	25	25	25	8.33	8.33
	Dissolved mid	27.27	27.27	27.27	27.27	27.27
	Dissolved high	45.45	45.45	45.45	36.36	36.36
	Substrate low	0	0	0	0	0
	Substrate mid	0	0	0	0	0
	Substrate high	0	0	0	0	0
Copper	Dissolved low	62.5	62.5	62.5	62.5	62.5
	Dissolved mid	42.85	42.85	42.85	42.85	42.85
	Dissolved high	14.28	14.28	14.28	14.28	14.28
	Substrate low	0	0	0	0	0
	Substrate mid	0	0	0	0	0
	Substrate high	0	0	0	0	0
Zinc	Dissolved low	25	25	25	18.75	18.75
	Dissolved mid	15.38	15.38	15.38	15.38	15.38
	Dissolved high	7.14	7.147	7.14	7.14	7.14
	Substrate low	0	0	0	0	0
	Substrate mid	0	0	0	0	0
	Substrate high	66.66	0	0	0	0
Hydrocarbons:						
	Controls	17.46	16.86	13.85	13.85	13.85
Chrysene	Dissolved low	28.57	28.57	14.28	14.28	14.28
	Dissolved mid	10	10	10	10	10
	Dissolved high	30	30	20	20	20
	Substrate low	14.28	14.28	0	0	0
	Substrate mid	40	40	20	20	20
	Substrate high	50	25	0	0	0
Fluoroanthene	Dissolved low	6.66	6.66	0	0	0
	Dissolved mid	0	0	0	0	0
	Dissolved high	16.66	16.66	16.66	16.66	16.66
	Substrate low	0	0	0	0	0
	Substrate mid	0	0	0	0	0
	Substrate high	20	0	0	0	0
Phenanthrene	Dissolved low	27.27	18.18	18.18	9.09	9.09
	Dissolved mid	0	0	0	0	0
	Dissolved high	33.33	33.33	33.33	0	0
	Substrate low	14.28	14.28	14.28	0	0
	Substrate mid	0	0	0	0	0
	Substrate high	40	20	0	0	0
Pyrene	Dissolved low	33.33	33.33	33.33	11.11	11.11
	Dissolved mid	28.57	14.28	14.28	0	0

	Dissolved high	100	100	50	25	25
	Substrate low	0	0	0	0	0
	Substrate mid	0	0	0	0	0
	Substrate high	0	0	0	0	0
Organics:						
	Controls	23.76	23.76	22.77	22.77	22.77
Diuron	Dissolved low	0	0	0	0	0
	Dissolved mid	30	30	30	30	30
	Dissolved high	75	75	75	75	75
	Substrate low	0	0	0	0	0
	Substrate mid	0	0	0	0	0
	Substrate high	0	0	0	0	0
Octocrylene	Dissolved low	54.54	54.54	54.54	54.54	54.54
	Dissolved mid	33.33	33.33	33.33	33.33	33.33
	Dissolved high	50	50	50	50	50
	Substrate low	0	0	0	0	0
	Substrate mid	0	0	0	0	0
	Substrate high	0	0	0	0	0
Tributyltin	Dissolved low	0	0	0	0	0
	Dissolved mid	0	0	0	0	0
	Dissolved high	0	0	0	0	0
	Substrate low	0	0	0	0	0
	Substrate mid	0	0	0	0	0
	Substrate high	0	0	0	0	0

Table 5*. Post-settlement percent survivorship for Diploria labyrinthiformis dissolved and contaminated substrate exposures.*

Contaminant		Survivorship 1 month
Metals:		
	Controls	85.70
Arsenite	Dissolved low	66.67
	Dissolved mid	57.14
	Dissolved high	100
	Substrate low	71.42
	Substrate mid	100
	Substrate high	66.67
Copper	Dissolved low	100
	Dissolved mid	100
	Dissolved high	50
	Substrate low	100
	Substrate mid	100
	Substrate high	100
Zinc	Dissolved low	100

	Dissolved mid	50
	Dissolved high	100
	Substrate low	100
	Substrate mid	100
	Substrate high	75
Hydrocarbons:		
	Controls	80.63
Chrysene	Dissolved low	75
	Dissolved mid	66.67
	Dissolved high	100
	Substrate low	80
	Substrate mid	100
	Substrate high	100
Fluoroanthene	Dissolved low	100
	Dissolved mid	100
	Dissolved high	100
	Substrate low	100
	Substrate mid	60
	Substrate high	50
Phenanthrene	Dissolved low	100
	Dissolved mid	100
	Dissolved high	100
	Substrate low	100
	Substrate mid	50
	Substrate high	100
Pyrene	Dissolved low	60
	Dissolved mid	85.71
	Dissolved high	100
	Substrate low	100
	Substrate mid	100
	Substrate high	100
Organics:		
	Controls	55.47
Diuron	Dissolved low	75
	Dissolved mid	100
	Dissolved high	80
	Substrate low	83.33
	Substrate mid	100
	Substrate high	25
Octocrylene	Dissolved low	100
	Dissolved mid	75
	Dissolved high	100
	Substrate low	88.89
	Substrate mid	100
	Substrate high	100
Tributyltin	Dissolved low	100
	Dissolved mid	100

	Dissolved high	0
	Substrate low	0
	Substrate mid	0
	Substrate high	100

4. DISCUSSION

4.1. Contaminant effects on coral settlement

The Florida Department of Environmental Protection (FDEP) have listed general threshold effect levels (TELs) and probable effects levels (PELs) for 10 metals, 13 polycyclic aromatic hydrocarbons, seven pesticides, total PCBS, and one phthalate (MacDonald, 1994). These thresholds estimate the concentrations of a contaminant where effects may be seen (TELs) and are likely to be seen (PELs). Similarly, EPA chronic-acute ranges and maximum allowable concentrations in marine water exists for metals and non-metals in the state of Florida. These values for the contaminants tested here are provided in Table 6.

***Table 6**. Available State of Florida threshold effect levels (TELs) and probable effects levels (PEL) for sediment, and the available EPA maximum allowable concentrations in marine water and EPA marine Chronic-acute Aquatic Life Criteria ranges or Aquatic Life Benchmarks.*

Contaminant	TEL (ug/g) [1]	PEL (ug/g) [1]	Effect of contaminated substrate exposure	EPA chronic-acute (ug/L) [2]	EPA maximum (ug/L) [3, 4]	Effect of dissolved exposure
Arsenic	7.24	41.6	≥ 2 ug/g (LOEC: 200 ug/g)	36-69	36	≥ 10 ug/L
Copper	18.7	108	≥ 5 ug/g (LOEC: 500 ug/g)	3.1-4.8	3.7	≥ 1 ug/L
Zinc	124	271	≥ 5 ug/g (LOEC: 500 ug/g)	81-90	86	≥ 5 ug/L
Chrysene	0.108	0.846	≥ 0.1 ug/g	N/A	N/A	≥ 0.1 ug/L
Fluoranthene	0.113	1.494	≥ 0.1 ug/g	N/A	370	≥ 0.1 ug/L (LOEC: 10 ug/L)
Phenanthrene	0.086	0.544	≥ 0.1 ug/g	N/A	N/A	≥ 0.1 ug/L (LOEC: 10 ug/L)
Pyrene	0.153	1.398	≥ 0.1 ug/g (LOEC: 10 ug/g)	N/A	11000	≥ 0.1 ug/L (LOEC: 10 ug/L)
Diuron			≥ 50 ug/g	600 (acute)	N/A	≥ 1 ug/L (LOEC: 100 ug/L)

Octocrylene			≥ 1 ug/g	N/A	N/A	≥ 1 ug/L (LOEC: 10 ug/L)
Tributyltin			≥ 0.1 ug/g (LOEC: 5 ug/g)	0.0074-0.42	N/A	≥ 0.1 ug/L (LOEC: 1 ug/L)

[1] - https://floridadep.gov/dear/watershed-monitoring-section/content/sediment-guidelines
[2] - https://www.epa.gov/wqc/national-recommended-water-quality-criteria-aquatic-life-criteria-table
[3] - https://www.epa.gov/pesticide-science-and-assessing-pesticide-risks/aquatic-life-benchmarks-and-ecological-risk
[4] - https://www.epa.gov/wqs-tech/water-quality-standards-regulations-florida

4.1.1. Metals

The effects of copper on corals are well studied and it is often used as a reference toxicant in other ecotoxicological studies (Summer et al., 2019). It is extremely toxic to corals with adverse effects being observed at concentrations as low as 1 µg/L (Fonseca et al., 2017; Nalley et al., 2021) and mortality observed at concentrations as low as 10 µg/L for larva (Hedouin et al., 2016; Nalley et al., 2021; Reichelt-Brushett & Harrison, 2004). Dissolved copper is consistently shown to reduce or inhibit coral larval settlement in a concentration-dependent manner, even at relatively low µg L^{-1} levels. In *Acropora tenuis,* settlement was not affected at ≤20 µg L^{-1}, but was significantly reduced at 42–81 µg/L, with a 48-h EC_{50} of ~35 µg L^{-1}. At higher concentrations (~200 µg/L), complete mortality occurred, eliminating settlement entirely (Reichelt-Brushett & Harrison, 2004). Although settlement inhibition often occurs at sublethal concentrations, copper exposure also leads to dose-dependent mortality, which further depresses recruitment. In this study, exposure to dissolved copper resulted in slightly reduced settlement at ≥ 1 ug/L for MCAV, but no apparent reduction in DLAB. Increased mortality was observed at ≥ 1 ug/L for DLAB, but no apparent increase in mortality was observed for MCAV. Both species demonstrated reduced settlement and increased mortality in response to settlement substrate contaminated with copper, at concentrations ≥ 5 ug/g for MCAV and ≥ 50 ug/g for DLAB, indicating that current TEL and PEL values for marine sediments may not be protective of coral settlement.

There are currently no published toxicology data on the effects of arsenic on larval settlement. In this study, exposure to dissolved arsenite reduced slightly reduced settlement at > 1 ug/L for MCAV, and reduced settlement at 100 ug/L for DLAB. Increased mortality for both species was observed at ≥ 10 ug/L, which is lower than the EPA chronic/acute and EPA maximum allowable concentrations in seawater (Table 5), suggesting that EPA thresholds may need to be reduced to be protective of coral larvae. Contaminated substrate reduced settlement and increased mortality in MCAV at concentrations ≥ 2 ug/g, and in DLAB at concentrations ≥ 20 ug/g, indicating that current TEL and PEL values of 7.24 ug/g and 41.6 ug/g, respectively, may not be protective of coral settlement.

There are currently no published toxicology data on the effects of zinc on larval settlement, however gamete fertilization success in *Goniastrea aspera* was not affected by nominal concentrations of zinc up to 500 µg/L (Reichelt-Brushett & Harrison, 1999), while *Acropora tenuis* gametes showed decreased fertilization rates at nominal concentrations of 10 µg/L with no fertilization occurring at nominal concentrations of 5000 µg/L (Reichelt-Brushett & Harrison, 2005). In this study, exposure to dissolved zinc reduced settlement and increased mortality in both species in concentrations ≥ 5 ug/L, thus the EPA chronic/acute and maximum allowable concentrations of 81-90 ug/L and 86 ug/L, respectively, may need revision. Contaminated substrate reduced settlement in MCAV at concentrations ≥ 5 ug/g (this effect was significant at 500 ug/g), and at 500 ug/g in DLAB; increased mortality was observed in both species at concentration ≥ 5 ug/g, indicating that current TEL and PEL values of 124 ug/g and 271 ug/g, respectively, may not be protective of settlement for MCAV at least and can result in larval mortality in both species.

For all three metals, MCAV was overall more sensitive to settlement substrates contaminated with metals, i.e. effects on settlement and mortality occurred at lower concentrations compared to DLAB. indicating the possibility of species-specific differences in sensitivity to metal contamination. Further, reductions in settlement and increased mortality of larvae for one or both species indicates that current management threshold values for the tested metals may not be protective of coral larval stages.

4.1.2. Hydrocarbons

The effect of hydrocarbons on corals is increasing well studied, but few studies examine the effects of single hydrocarbons on larval settlement. Hydrocarbons, primarily oil and spill response products, have been found to cause inhibition of metamorphosis, fertilization, and reduced settlement in early life stages of corals (Epstein et al. 2000, Negri & Heyward 2000, Lane & Harrison 2002, Goodbody-Gringley et al. 2013, Negri et al. 2018, Nordborg et al. 2018, Overmans et al. 2018). No published toxicity studies are available for chrysene, fluoranthene, or pyrene on coral larval settlement, however phenanthrene was found to slightly reduce survival of *A. tenuis* larvae at 872 ug/L, with an EC50 for metamorphosis of 91 ug/L (Overmans et al. 2018).

In this study, exposure to dissolved chrysene had no apparent effect on settlement in MCAV, but slightly increased mortality ≥ 0.01 ug/L. Both reduced settlement and increased mortality were observed for DLAB at ≥ 0.1 ug/L. For the contaminated substrate exposure, reduced settlement occurred at concentrations ≥ 1 ug/g for both species, and increased mortality at concentrations ≥ 0.1 ug/g for MCAV, and ≥ 10 ug/g for DLAB. Effects of contaminated substrate were thus observed at concentrations within the lower range of TEL and PEL values of 0.108 ug/g and 0.846 ug/g, respectively, for marine sediment. No EPA dissolved threshold values are available for comparison.

Exposure to dissolved fluoranthene reduced settlement and increased mortality at ≥ 0.1 ug/L for both species, with effects seen at concentrations well below the EPA maximum concentration of 370 ug/L; the reduction in settlement was significant for MCAV at 10 mg/L compared to the controls Settlement tiles contaminated with fluoranthene reduced settlement at concentrations ≥ 0.1 ug/g for MCAV, and ≥ 10 ug/g for DLAB, and increased mortality at concentrations ≥ 1 ug/g for MCAV, and ≥ 10 ug/g for DLAB. As observed for chrysene, effects of contaminated substrate were found at concentrations within the lower range of TEL and PEL values of 0.113 ug/g and 1.494 ug/g.

Dissolved phenanthrene reduced settlement and increased mortality at ≥ 0.1 ug/L for both species; contaminated settlement substrate reduced settlement at concentrations ≥ 0.1 ug/g and increased mortality at concentrations ≥ 1 ug/g for both species. No EPA threshold for dissolved exposures exists for comparison, but effects of contaminated substrate were found at concentrations between the TEL and PEL values of 0.086 ug/g and 0.544 ug/g. Phenanthrene has the lowest state threshold sediment concentrations of the tested hydrocarbons, and the consistent high sensitivity of coral larvae to low concentrations of phenanthrene in these screening exposures indicates that further testing is warranted to assess the possible need to lower threshold values further.

Dissolved pyrene reduced settlement and increased mortality at ≥ 0.1 ug/L for MCAV. For DLAB, settlement was also reduced at ≥ 0.1 ug/L, and mortality was slightly increased at ≥ 10 ug/L. The effects of contaminated settlement substrate were consistent for both species, with reduced settlement at concentrations ≥ 0.1 ug/g and increased mortality at concentrations ≥ 0.1 ug/g. The effects of dissolved exposure occurred well below the EPA maximum threshold level of 11,000 ug/L, effects of contaminated substrate on both species were found at concentrations below the TEL and PEL values of 0.153 ug/g and 1.398 ug/g.

For all tested hydrocarbons, effects of contaminated substrate on settlement and/or mortality were found at concentrations within the range of or below current state of Florida TEL and PEL concentrations, indicating that these thresholds may not be protective of coral larval settlement and survival. The same was true for dissolved hydrocarbons, where effects were seen well below the EPA maximum allowable concentrations for fluoranthene and pyrene.

4.1.3. Organics

The effects of diuron are well understood, as it is often used as a reference toxicant as it can affect photosynthetic efficiency in corals at measured concentrations as low as 1 µg/L, with measured EC50 values of ranging from 2.03-24.2 µg/L (Jones 2004, Jones and Kerswell 2003, Negri et al. 2011, Negri and Heyward 2001). The majority of these studies

focused on adult corals, as these herbicides target photosynthesis, which is not yet a concern in larval or gametic stages (Jones 2004). However, recruits as young as two weeks were just as sensitive to diuron as adult colonies (Negri et al. 2005). In this study, exposure to dissolved diuron reduced settlement and slightly increased mortality at ≥ 1 ug/L for both species. Settlement tiles contaminated with diuron reduced settlement and increased mortality at concentrations ≥ 50 ug/g for DLAB; no apparent reduction in settlement was observed for MCAV, with only a slight increase in mortality compared to the control at the highest tested concentration of ≥ 500 ug/g for MCAV. There are no sediment concentration thresholds for diuron, however effects of dissolved diuron were observed at concentrations less than the EPA acute threshold of 600 ug/L.

Tributyltin (TBT) has been shown to be extremely toxic to early life stages of corals. Juvenile growth and symbiont density of *A. tenuis* decreased significantly at nominal concentrations of 0.4 µg/L and 1 µg/L, respectively (Watanabe et al., 2007; Watanabe et al., 2006). Larval metamorphosis of *A. millepora* had an IC50 value of 2.0 µg/L (nominal), which was significantly lower than the IC50 value of copper in the same experiment (110 µg/L) (Negri and Heyward 2001). In this study, exposure to dissolved TBT reduced settlement and increased mortailty at ≥ 0.1 ug/L for MCAV, and significantly reduced settlement and mortality at ≥ 1 ug/L for DLAB. TBT contaminated substrate tiles reduced settlement and increased mortality at concentrations ≥ 5 ug/g for both species, this effect was significant for DLAB. Substantial effects were seen from dissolved TBT at concentration within the EPA acute-chronic range of 0.0074 – 0.42 ug/L; there are no marine sediment threshold concentrations established for Florida, however the results of this study indicate that the establishment of TEL and PEL values for sediment could provide a valuable indicator of environmental quality and suitability for coral reef restoration.

Organic UV filters have recently emerged as a contaminant of concern in the aquatic environment. Toxicity studies have been conducted in 7 coral species using either adult fragments, early-life stages (i.e., larvae/planula), or both, for twelve organic UV filters (octocrylene, avobenzone, uvinal T150, mexoryl XL, mexoryl SX, octinoxate, oxybenzone, ethylhexyl salicylate, and benzophenone -2, -1, -4, and -8) (Mitchelmore et al. 2021, Skelton, 2024). Currently, no published studies exist for the effects of octocrylene on coral larvae settlement or survival. Here, dissolved octocrylene reduced settlement at ≥ 10 ug/L for both species, and increased mortality at ≥ 1 ug/L for MCAV, and at ≥ 100 ug/L for DLAB. Exposure to contaminated substrate reduced settlement at concentrations ≥ 5 ug/g for MCAV, with no apparent increase in mortality; reduced settlement and a slight increase in mortality compared to the controls was observed at ≥ 500 ug/g for DLAB.

4.2. Contaminant effects on coral juvenile survival and growth

For MCAV, low (20-50%) post-settlement survivorship was observed across all treatments. Post-settlement mortality rates in this species are typically high, but tend to decrease over time; the typical mortality rates of newly settled recruits on the reef are estimated to be above 95% (Walker and Figueiredo 2019). A difference was observed between dissolved and contaminated substrate exposures, where post-exposure survivorship for the dissolved exposures was relatively stable after 1 month, i.e. if recruits survived for 1 month after settlement tiles were transferred to the grow-out system, then those recruits tended to continue to survive and grow. This contrasted with post-settlement survivorship for the contaminated substrate exposures, where coral recruits, regardless of exposure level, typically did not survive to 1 month (Table 4). Thus, even when settlement occurs, contaminated substrates may prevent successful recruitment by limiting early post-settlement survival. Reduced survivorship on the contaminated settlement tiles may also be due to one or more factors, including alkalinity changes or lack of biofilm associated with the use of dry tiles in the preparation of contaminated substrate. Assessment of post-settlement growth has indicated no clear dose- or treatment-related effects for surviving MCAV juveniles. The settlement experiment with DLAB concluded on May 20, 2026, survivorship will be assessed on June 22, 2026.

4.3. Test method development

The development of coral larval settlement test methods for chemical contaminants has progressed to increasingly standardized, quantitative bioassays that use settlement inhibition as a sensitive endpoint. Few studies have evaluated post-settlement survival and growth after chemical contaminant exposure, as most assessment studies utilize only well-plates with CCA chips for settlement induction, which prevents the transfer to grow-out systems and tracking of larval survival and growth after settlement, and the accurate assessment of metamorphosis and attachment (Miller et al. 2022). Although initial settlement rates were within typical ranges for both species, low survivorship post-transfer to grow-out system were found, particularly on the contaminated substrate tiles. In order to generate the contaminated substate, dry tiles must be used in the preparation. It was noted that dry tiles reduced alkalinity in the settlement jars, which may have limited tile attachment and early calcification during metamorphosis, thus larvae were dislodged when the tiles were moved from the settlement jar to the grow-out system. We are at present evaluating different tile materials (ceramic vs. aragonite), and tile preparation techniques to alleviate this reduction in alkalinity without causing concomitant changes in pH.

In this study, settlement tiles were used in order to permit assessment of post settlement survival and growth. Ceramic tiles were used, as is typically used for settlement across multiple laboratories. The metal analysis described above found high concentrations of vanadium in these tiles, the effect of which larval settlement is unknown. For this reason,

we recommend the use of aragonite tiles in future static, closed system settlement tests eliminate the potential effects of this contamination.

The overall test approach generated consistent dose-related responses, both for dissolved and contaminated substrate exposures. Although analytical verification of exposures was not a part of this project, preliminary analysis of concentrations of metals used in the MCAV experiment showed that all dissolved stocks measured were 92-108% of nominal concentrations. As the preparation of contaminated substrate tiles was an entirely new approach, two extractions were prepared 1) an extraction in a warm 10% solution of hydrochloric and nitric acids to see what was readily leachable from the tiles, and 2) grinding of the tile into powder and microwave assisted acid digestion. The total metal concentrations (sum of the leachable and ground tile extractions) showed good recovery compared to nominal concentrations (i.e. 88-95% for copper, 103-108% for zinc and 68-133% for arsenic). For all metals, more was in the leachate than residual in the tile (i.e., copper 4-5x, zinc 1-3x and arsenic 3-4x more in the leachate than the tile residual). This could be investigated further using other leachate conditions that are more environmentally relevant.

For both species, settlement occurred not only on the tile, but also on the settlement jar glass as well. Inclusion of all settlers in endpoint calculations is a common practice, however in this study, which specifically examined substrate contamination as a variable, results indicated that some contaminants induced avoidance of the contaminated settlement tile as evidenced by the difference between overall settlement and settlement on the tiles only, particularly for MCAV.

5. MANAGEMENT RECOMMENDATIONS

Because successful coral recruitment requires both survival and settlement, even moderate concentrations of metals, hydrocarbons, and other organics can create a recruitment bottleneck, limiting population replenishment even without mortality. Chronic low-level exposures are especially concerning because these overlap with environmentally relevant concentrations near urbanized coasts. The results of this screening study indicate several potential management applications:

- The sensitivity of the two tested species to contaminated substrate indicates that sediment and or hardbottom contamination may be a significant barrier to larval recruitment on the reef framework.
- For metals and hydrocarbons, reduced settlement and mortality after exposure to dissolved contaminants and contaminated substrate for one or both species was observed at concentrations within the range of or below threshold effect

levels (TELs) and probable effects levels (PELs) for marine sediments, and for EPA chronic/acute and EPA maximum allowable concentrations in seawater, indicating the need for review of these management threshold values based on new coral sensitivity data.

- Establishing the level of sediment and reef substrate contamination of key contaminant groups at potential outplant sites would allow identification of ideal sites for outplanting, and the protection and prioritization of high-quality sites which are more likely to support successful recruitment.
- Effects on early coral life stages are expected to be more conservative than effects on adults; therefore thresholds of effect based on coral larval exposures should be preferentially employed for the revision of EPA water quality standards and State of Florida sediment thresholds of effect which will improve restoration outcomes.

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