4-27-2023

# Sublethal Effects of Petroleum Hydrocarbon Exposure on Tissue Regeneration in Four Atlantic Coral Species

Dawn Bickham
*Nova Southeastern University*

## NSUWorks Citation

Dawn Bickham. 2023. *Sublethal Effects of Petroleum Hydrocarbon Exposure on Tissue Regeneration in Four Atlantic Coral Species.* Capstone. Nova Southeastern University. Retrieved from NSUWorks, . (136) https://nsuworks.nova.edu/hcas_etd_all/136.

# Capstone of
# Dawn Bickham

Submitted in Partial Fulfillment of the Requirements for the Degree of

## Master of Science
## Biological Sciences

Nova Southeastern University
Halmos College of Arts and Sciences

April 2023

Approved:
Capstone Committee

Committee Chair: Dr. Abigail Renegar

Committee Member: Dr. Emily Schmitt

NOVA SOUTHEATERN UNIVERSITY

HALMOS COLLEGE OF ARTS AND SCIENCES

# SUBLETHAL EFFECTS OF PETROLEUM HYDROCARBON EXPOSURE ON TISSUE REGENERATION IN FOUR ATLANTIC CORAL SPECIES

By

Dawn Bickham

Submitted to the Faculty of
Halmos College of Arts and Sciences
in partial fulfillment of the requirements for
the degree of Master of Science with a specialty in:

Biological Science

Nova Southeastern University

Date 26 May 2023

ABSTRACT

Even though coral reefs are very important ecologically and economically, these ecosystems are sensitive to changes in the environment and susceptible to damage from human activities, such as oil spills. The risk of accidents in marine environments and consequently on coral reefs has increased because of the widespread usage of petroleum products. Current approaches to remediate oil contamination in marine ecosystems allow the application of chemical dispersants. However, these methods may pose a greater threat to coral reefs than the oil they are intended to mitigate. This project studied the impacts of hydrocarbon exposure on wound healing rate to assess the relative effects of this environmental stressor on coral health and the potential for recovery from physical damage. To determine how exposure to 1-methylnaphthalene (1MN), Phenanthrene (PHE), oil (WAF), and dispersed oil (CEWAF) affects wound healing, we measured tissue regeneration rates in four species of Atlantic corals. This study hypothesized that exposure to these substances would inhibit tissue regeneration. Phenanthrene exposure had the greatest effect on *P. astreoides*, which had the lowest mean healing rate and percent healed at 28 days post exposure. Healing rates for P. astreoides exposed to 1MN, and PHE were 2.8 times slower than corals exposed to chemically dispersed oil (CEWAF) and oil itself (WAF). There was a lower regeneration rate in *P. astreoides* exposed to phenanthrene was also influenced by the growth of algae. In addition to lesion-specific variables known to influence wound regeneration, hydrocarbon exposure can also affect the healing rates of corals, as shown by the results. This information can be utilized by oil spill managers to help improve nearshore coral reef spill response and mitigation.

*Keywords*: Coral, Tissue Regeneration, Crude Oil, Wound repair

Contents

## LIST OF FIGURES

## INTRODUCTION

### *Coral reefs*

Corals support an environment rich in ecological diversity and are essential to global ocean health. Healthy coral reefs have substantial environmental and economic significance; these ecosystems provide food, coastal protection, and $375 billion annually worldwide (U.N. Environment, ISU, ICRI, & Trucost 2018). Coral reefs support an estimated 25% of all marine life, including sponges, bivalves, crustaceans, corals, and many different fish species (Knowlton et al., 2010). Scleractinian corals provide the reef framework and complex habitats for the numerous fish and invertebrate species to thrive. However, coral reefs are increasingly affected by natural and anthropogenic stressors., Diseases, predation, algae overgrowth, sedimentation, and physical damage, are a few examples of the acute and chronic stresses that can cause tissue lesions in coral (Hughes et al., 2003).

### *Petroleum*

Petroleum is a naturally existing mixture of carbon and hydrogen compounds in liquid, gaseous, or solid forms. Hydrocarbons have complex molecular structures, each with unique physical and chemical attributes, toxicity, and biodegradability. There are four classes of hydrocarbons based on their molecular structure: Paraffinic (alkanes), alkenes, cycloalkanes, and aromatics (Adipah, 2018). Alkanes are the simplest form, containing only single-bonded carbon and no other elements. Like all other hydrocarbons, Alkanes are valuable fuel sources and form a considerable portion of most oils. Their toxicity is usually low, and they break down easily in the environment.

Unsaturated hydrocarbon compounds called alkenes have one or more double bonds between their carbon atoms. They are typically nonexistent or present in very modest amounts within the oil, but they are prominent in refined products like gasoline (Berkowitz, 1998). Cycloalkanes, also known as naphthene's, are monocyclic saturated hydrocarbons that make up the second largest fraction of most oils. Aromatics are cyclic unsaturated hydrocarbons that contain one or more aromatic rings. Polycyclic aromatic hydrocarbons (PAHs) have more than one aromatic ring. Because of PAH's possible acute toxicity, bioaccumulation, and variable biodegradation rates, they are a primary environmental concern.

The liquid form of petroleum, crude oil, is the most important fossil fuel. After extraction, it is refined to make gasoline, diesel, jet, heating fuel, lubricating oils, asphalt, and downstream products used to make solvents, plastics, detergents, and other essential oils products. Rapid global economic activity has increased demand for petroleum-based products; expanded oil production, processing, and transportation activities have thus increased the risk of oil spills, even with oil spill prevention protocols (Fernandes, Carmo, de Jesus, Soriano, Santos, 2022). Furthermore, as the risk of spills rises, concerns have arisen about the environmental behavior and toxicity of different oils.

Oil spilled in the sea undergoes several possible transformations, including evaporation, aerosolization, photooxidation, mixing, emulsification, diffusion, partial water dissolution, spreading, transport, biodegradation, aggregation, adhesion, and sedimentation (National Academies of Sciences & Medicine, 2020). The significance of a spill event depends on several factors, including the physical and chemical characteristics, composition, and volume of oil spilled, atmospheric, and oceanographic conditions, particulate and organic matter in suspension, nutrient availability in the water, and, most importantly, the type of spill (superficial or subsea).

### ***Oil spills and remediation***

Accidents involving oil or its byproducts are possible at any stage of the oil production process, including drilling, refining, transportation, distribution, and usage. With the widespread need for petroleum products, the production process results in frequent spill events (Fingas & Brown, 2018). The majority of oil spill events occur in oceans. Unfortunately, the primary transportation pathways for oil are close to coral reefs. The amount of oil that naturally enters the waters is estimated to be about 700 million liters per year (Kleindienst et al, 2015). Anthropogenic oil spills into marine environments are estimated to amount to more than 120 million liters annually (Kleindienst et al, 2015).

In 1979, 3.4 million barrels of crude oil were spilled during the subsea blowout of the Ixtoc-I well in the Gulf of Mexico. Oil covered the seafloor, coral sites, and seagrass (Soto, Botello, Licea-Durán, Lizárraga-Partida, & Yáñez-Arancibia, 2014). The 1986 Galeta oil spill is the largest spill which directly impacted a shallow reef environment, spilling at least 8 million liters of medium-weight crude oil released from a ruptured storage tank (Jackson et al., 1989). The explosion on the Deepwater Horizon platform in the Gulf of Mexico in April 2010 caused an oil spill that resulted in a leak of approximately 1 million liters per day, making it the worst environmental disaster in U.S. history (Beyer, Trannum, Bakke, Hodson, & Collier, 2016). One study on the impact of this spill found complete destruction of deep-water coral colonies southwest of the site nearly at 1.6 km deep. These corals are vital habitats for marine life.

More than 4500 tons of crude oil leaked from the Greek oil tanker Bouboulina in 2019, contaminating more than 1000 locations over 11 Brazilian coastal states. The oil reached vital coral reefs, including two of the largest protected coral reef areas in the South Atlantic (Soares et al.). The Abrolhos Marine National Park contains the largest and most diverse coral reefs in the South Atlantic, with many species specific to these locations and found naturally nowhere else

(Werner, Pinto, Dutra, & Pereira, 2000). In a recent example of oil-related impacts, the MV Wakashio ran aground on a coral reef in the Indian Ocean near Mauritius, home to world-renowned coral reefs and has areas designated as wildlife sanctuaries. (Florisson et al., 2020). The area also contains wetlands designated as a site of international importance. While the grounding event caused significant physical damage to the reef structure, the carrier also contained 4,000 tons of fuel oil; more than 1,180 tons of this oil leaked from the fuel tanks into the surrounding waters (Regan, 2020). The ship broke into two pieces, and environmentalists are concerned about the increasing ecological threats and subsequent impacts on the country's ecosystem.

Despite the dangers of an oil spill on coral reefs, there is no practical, long-term, and scalable approach to preserving or cleaning up reefs after exposure. Three types of emergency response strategies are used: physical/mechanical methods, chemical methods, and biological methods to reduce oil contamination. The most common method that can negatively affect coral is the application of dispersants for chemical remediation. The dispersant is a mixture of surfactants and solvents that break up oil into tiny droplets that are then distributed into the water column and subjected to waves, wind, and currents, allowing expedited natural remediation. Dispersants, however, have been found in studies to be potentially more toxic to corals than the oil itself, not only because of the latter's toxicity but also due to the resulting rise in hydrocarbon concentration within the water column. (DeLeo, Ruiz-Ramos, Baums, & Cordes, 2016; Silva et al., 2021). Due to the possible negative effect of dispersants on corals, the deployment of dispersants in emergency response plans is typically avoided near shallow reefs. In some extreme instances however (e.g., deep-waater-well blowout), dispersants are useful for oil spill control or combustibility risk reduction (National Academies of Sciences, 2020).

### *Affects of oil on coral*

Among the most vulnerable marine organisms to anthropogenic oil exposure are the shallow and deep-sea sessile benthic invertebrates which cannot escape exposure; thus, a greater understanding of potential effects on coral life processes is crucial. Numerous studies have assessed the biological and ecological effects of oil exposure on coral reef species (Cook & Knap, 1983; Domart-Coulon et al., 2006; Guzmán & Holst, 1993; Guzmán, Jackson, & Weil, 1991; Hartmann et al., 2015; Jackson et al., 1989; Loya & Rinkevich, 1980; Negri & Heyward, 2000; Shafir, Van Rijn, & Rinkevich, 2007). These effects include lethal and sublethal responses, including lower fertility, decreased larval survivorship and settlement, reduced growth, and higher rates of tissue damage (Firman, 1995).

Several studies have also assessed the toxicity of chemically dispersed oil to various coral species (Cook & Knap, 1983; May et al., 2020; Shafir et al., 2007). These studies established lethal and sub-lethal responses such as loss of symbionts, tissue death, impaired larval behavior, and decreased reproductive activity.

### *Wound healing in corals*

The ability and rate at which a coral can recover from a wound are valuable indicators of the overall condition of the coral (Fisher et al., 2007). The capability of the coral to regenerate is its primary defense against external pathogens (Hughes T, 1999). Adult corals are sessile and are damaged easily by storms, predation, disease, poor fishing habits, and anthropogenic change (Henry & Hart, 2005). If the coral cannot heal efficiently, the colony is susceptible to algae growth, pathogens, or boring organisms that can lead to mortality (Highsmith, 1982). During normal healing conditions, healthy corals can regenerate tissue quickly and low healing rates indicate reduced coral health or stress from environmental factors (Lester & Bak, 1985).

Environmental factors that impact coral community health, such as flow rates, sedimentation, U.V. exposure, and temperature influence the rate of wound repair. (Franklin, Jokiel, & Donahue, 2013; Henry & Hart, 2005; Williams, Gove, Eynaud, Zgliczynski, & Sandin, 2015). Studies have shown increased growth and lesion recovery rates at sites with higher flow rates and decreased recovery rates with other environmental factors, including nutrients and acidification (Carpenter et al., 2008; Sabine, Smith, Williams, & Brandt, 2015). Increased acidification and coral bleaching reduce the energy available for tissue growth, impacting colony survival and tissue regeneration (Grottoli et al., 2014; Meesters & Bak, 1993; Schoepf, Stat, Falter, & McCulloch, 2015).

Wounding healing rates can also be affected by colony factors, including colony size, morphology, and the size and shape of the wound (Cróquer, Villamizar, & Noriega, 2002; Darling, Alvarez‐Filip, Oliver, McClanahan, & Côté, 2012; Fisher, Fauth, Hallock, & Woodley, 2007; Henry & Hart, 2005). In contrast to the overall growth rate, tissue regeneration rates associated with wound healing happen more rapidly in larger colonies (Edmunds & Elahi, 2007; Henry & Hart, 2005).

Regeneration is the healing response, fully restoring damaged tissue and its function (Kramarsky-Winter & Loya, 2000). The tissue healing process begins with the production of a tissue layer growing from the edges of a wound, and this thin layer of epithelial tissue covers the exposed skeleton. Once the skeleton is covered, new tissue forms polyps and surrounding tissues (Meesters & Bak, 1993). The final regeneration stage is the return of zooxanthellae and pigment to the coral (Meesters & Bak, 1993). Regeneration rates are species-specific and influenced by injury characteristics, but the regeneration process follows similar patterns (Meesters, Noordeloos, & Bak, 1994).

The proximity of coral reefs to coastal areas with deep-water ports and a high volume of marine traffic or to oceanic zones near shipping lanes or oil exploration areas, means that these coral reefs are at risk of both physical damage from marine vessels (e.g., groundings or anchoring) and exposure to acute and chronic exposure to petroleum (e.g., spills or runoff). Despite this prominent co-occurrence of risk factors, only one study to date has examined the effects of oil exposure on tissue regeneration in corals and found that a 96-hour exposure to crude oil caused a significant decrease in tissue regeneration rate in all concentrations compared to controls in *Pocillopora damicornis* (May et al., 2020). This study thus sought to address this key knowledge gap by examining the impact of acute exposure to constituent hydrocarbons, oil, or dispersed oil on the wound healing rate of four Atlantic scleractinian coral species.

## METHODOLOGY

### ***Subject species***

Four shallow-water coral species, *Porites astreoides, Siderastrea siderea, Stephanocoenia intersepta,* and *Solenastrea bournoni*, were chosen for this study due to their ecological significance in Caribbean reef ecosystems (Dodge et al. 1984, Griffin et al. 2012, Schopmeyer et al. 2017). Three colonies of each species were collected from shallow nearshore reefs in Broward County, Florida, and transported to the laboratory. Colonies were cut with a wet bandsaw into 4 $cm^2$ fragments appropriate for use in exposures and placed in a 300-gallon indoor recirculating coral husbandry system for recovery and acclimation to laboratory conditions.

### ***Experimental approach***

Each experiment's specific design and conduct are described in detail by Turner (2020). A separate experiment was conducted for each test hydrocarbon and test species thus a total of nine experiments were conducted. Each experiment utilized six treatments, including a negative (seawater) control and five hydrocarbon concentrations, with four replicate dosing chambers per treatment (Figure 1) and three corals per replicate.

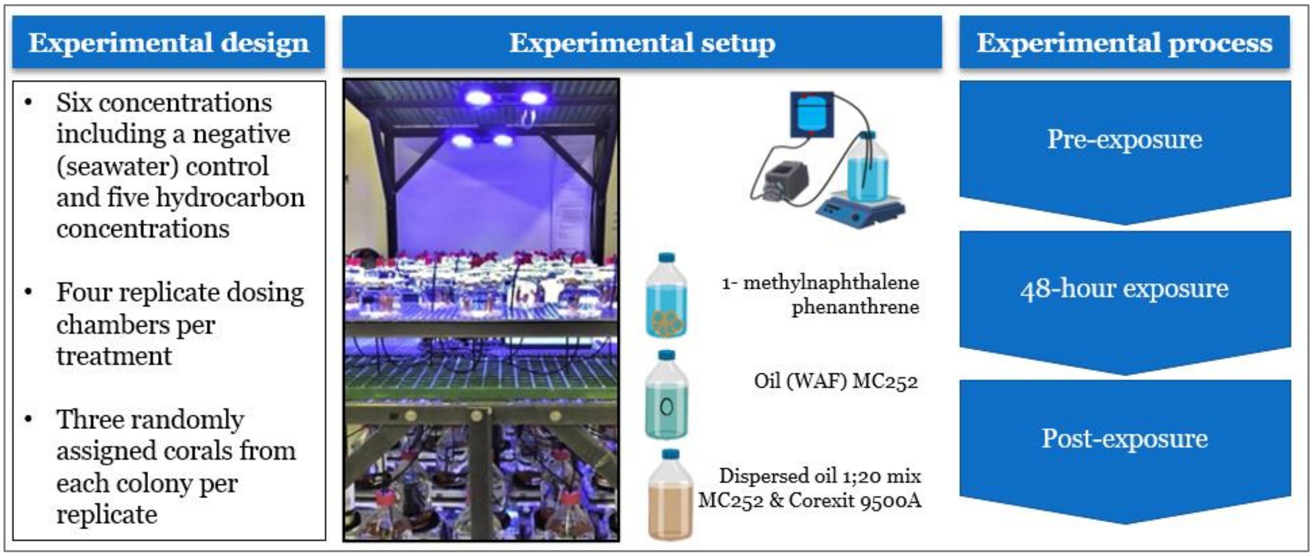

Figure 1. Experimental approach for all experiments. Created with BioRender.com

Experiments with single hydrocarbons 1-methylnaphthalene (1-MN) and phenanthrene (PHE) utilized a passive dosing system which employs chemically loaded O- rings to deliver a specific hydrocarbon concentration. To generate exposures to oil water accommodated fraction (WAF), silicone tubing was filled with a specific volume of MC252 oil, and the tubing was coiled and suspended fully submerged in the dosing vessel, where vigorous mixing ensured dissolution of soluble oil components into the exposure media. To generate exposures to dispersed oil, or chemically enhanced water accommodated fraction (CEWAF) predetermined quantities of oil injected into the mixing vessel was delivered by a flow-through system with a 1:20 mix of Corexit 9500A dispersant and MC252 oil.

Each experiment included a 2-week pre-exposure period, a 48-hour exposure period, and a 4-week post-exposure period. The pre-exposure period allowed corals time to recover and heal from the fragmentation process. Corals were then exposed to hydrocarbons (as briefly described above) for 48 hours.

### *Wound Regeneration*

After the 48-hour exposures, surviving corals were wounded using a Dremel with stone tip, creating approximately four $mm^2$ medial wounds. Corals were then photographed to document initial wound size and placed back in the laboratory coral husbandry system for monitoring over the 4-week post-exposure recovery period. Fragments were photographed for assessment of wound healing rate at 7-, 14-, and 28-days post-exposure. The image processing program Image J (Rasband, 1997-2018) was then used to measure wound size and healing rate. First, each image was calibrated. The pixel units were calibrated to the centimeter ruler in each photo. The total area of the wound was determined by tracing the perimeter of the fragment. The initial, interval, and final wound areas were measured by tracing the wound edges at each time point (Figure 2). The wound healing rate (WHR) was calculated using the equation: $[(Ai-Af)/d]$, where Ai is the initial wound area, Af is the non-healed wound area, and d is the number of elapsed days. The WHR is expressed in $mm^2\ d^{-1}$ and the percent tissue regeneration was determined from the difference between each time point. The mean % healed was calculated from 4 replicates in each concentration at Day 28 post exposure. The mean healing rate was calculated from 4 replicates in each concentration at each time point.

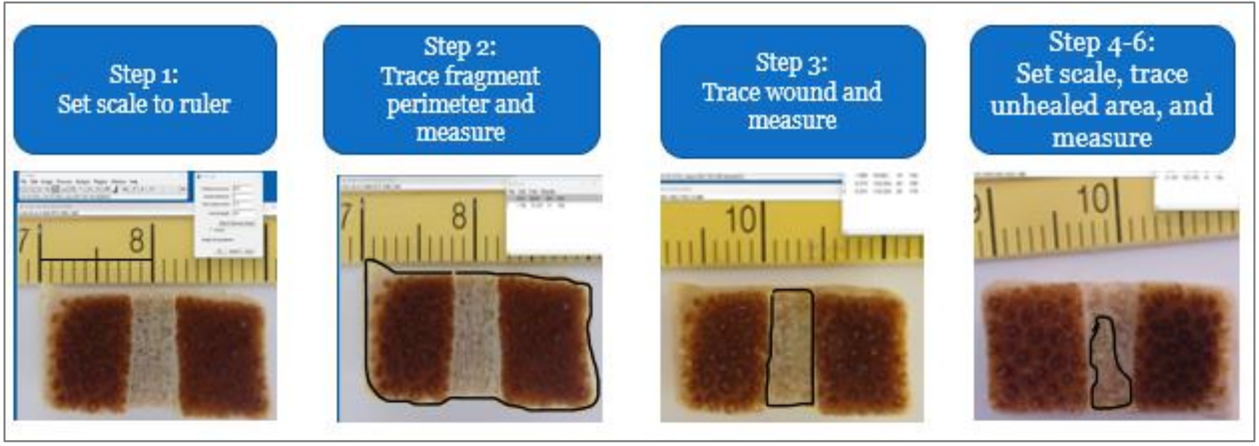

Figure 2. Method for image analysis using ImageJ (Rasband, 1997-2018) Created with BioRender.com

*Statistical Analysis*

Data were analyzed using the statistical software GraphPad Prism (Version 9.5.1). Data were tested for normality (Shapiro-Wilk test) and homogeneity of variance (Bartlett's test), and non-parametric tests were used when data did not meet assumptions of normality. One-way ANOVA (α=0.05) was used to compare the mean % healed between concentrations at 28D post exposure. Where appropriate, post-hoc analysis (Dunnett's) was used to compare the mean % healed between each hydrocarbon test concentration and the control. A repeated measures ANOVA was used to test if time and/or concentration had a significant effect on mean healing rate. Where appropriate, post-hoc analysis (Tukey's HSD) was used.

## RESULTS

### ***Wound regeneration***

The corals healed following previously documented studies detailing the regeneration process for these corals' studies (Meesters and Bak, 1993; Bak and Steward-Van Es, 1980, Meesters et al., 1992, Thornton et al., 2002). Even though all the corals began the healing process in the same manner, not all species healed entirely. Figure 3 shows the healing process for a control fragment for *S. intersepta* in the 1 – methylnaphthalene test.

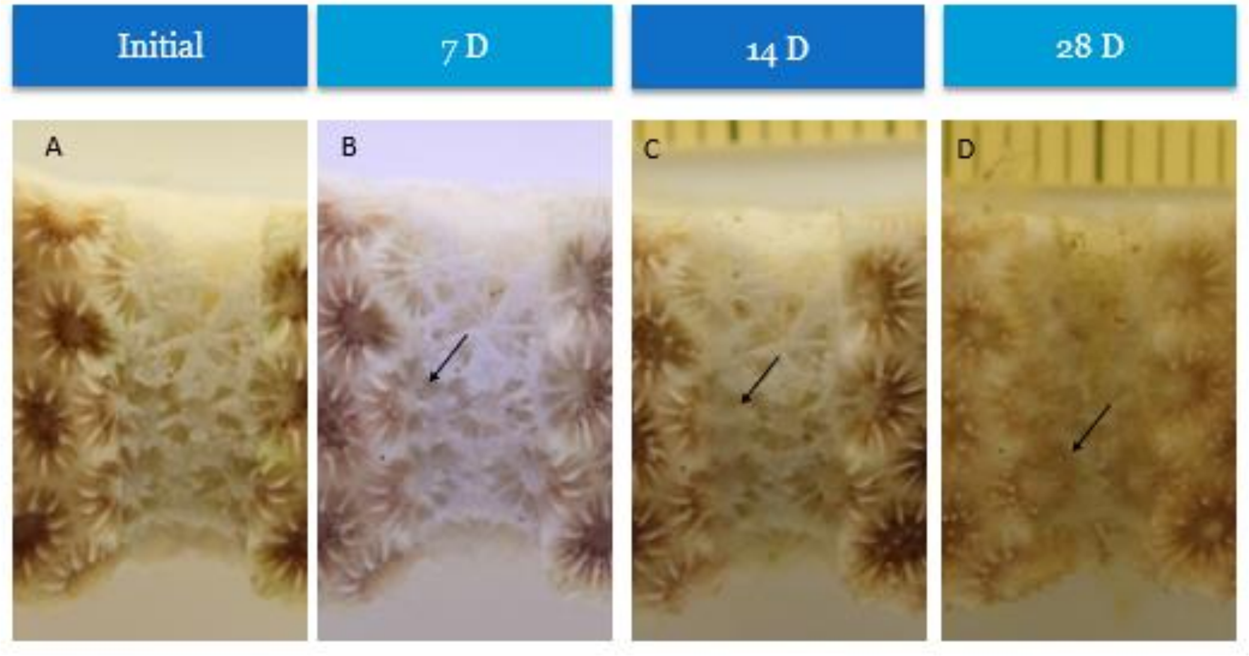

Figure 3. Wound regeneration process in *Stephanocoenia intersepta* exposed to 1- methylnaphthalene. A) shows the initial wounding after the 48-hour exposure. B) At Day 7, the edges of the wound are covered. C) Opaque tissue continues to slowly cover the wound site from the outside to the inside, toward the center of the damage. D) New corallites developed, followed by the return of pigmentation.

### *1-methylnaphthalene*

#### Mean percent healed.

Exposure to 1 –methylnaphthalene resulted in a variable species-specific response (Figure 4). *Porites astreoides* and *S. siderea* had similar overall percent healed of 31% and 26%, respectively, after 28 days post-exposure. *Solenastrea bournoni* and *S. intersepta* had a higher overall mean percent healed of 73% and 86%, respectively. No significant difference in the mean percent healed between concentrations was found at 28 days post-exposure (One-way ANOVA, $p > .05$**) for** any species. For *P. astreoides* and *S. siderea,* no dose response was seen. *Solenastrea bournoni* showed a dose response, where the mean percent healed decreased as concentration increased, although this was not significant. By the conclusion of this study, *S. intersepta* was the only species to have complete regeneration, where 25% of the fragments fully healed by 28 days post-exposure.

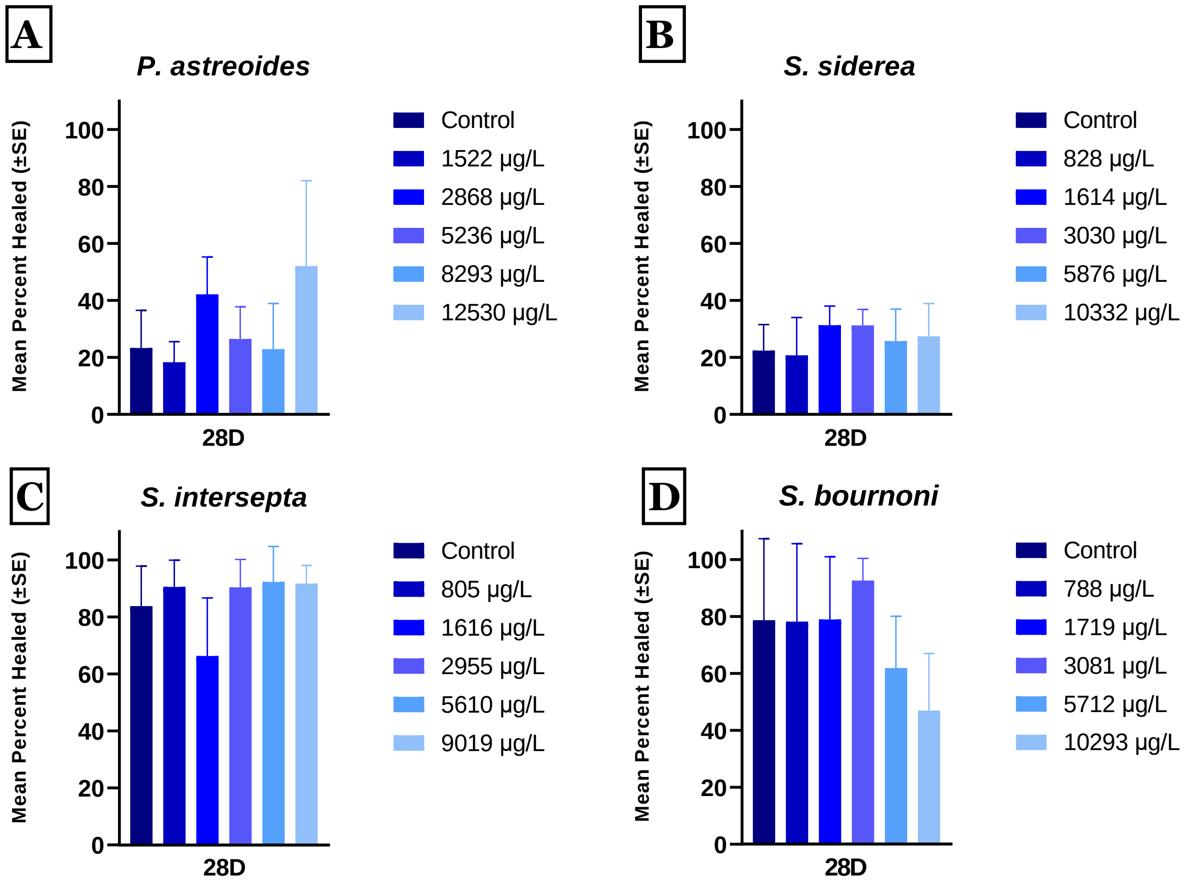

Figure 4. A) *P. astreoides* mean percent healed at 28 days post exposure to 1 – methylnaphthalene. B) *S. siderea* mean percent healed at 28 days post exposure to 1 – methylnaphthalene. C.) *S. intersepta* mean percent healed at 28 days post exposure to 1 – methylnaphthalene. D.) *S. bournoni* mean percent healed at 28 days post exposure to 1 – methylnaphthalene.

### Mean healing rate.

The mean healing rate at each time point per 1-methylnaphthalene concentration varies by species ranging between .337 $mm^2$ - .641 $mm^2$ (Figure 5), although no significant effect was found between time and healing rate or concentration and healing rate (Repeated measures Two-way ANOVA, $p > .05$). *Solenastrea bournoni* showed some dose response although not significant, with the mean healing rate generally decreasing as concentration increases. *Stephanocoenia* *intersepta* showed a stimulatory dose response, with the mean healing rate increasing as concentration increased, although this was not significant (One-way ANOVA, p > .05).

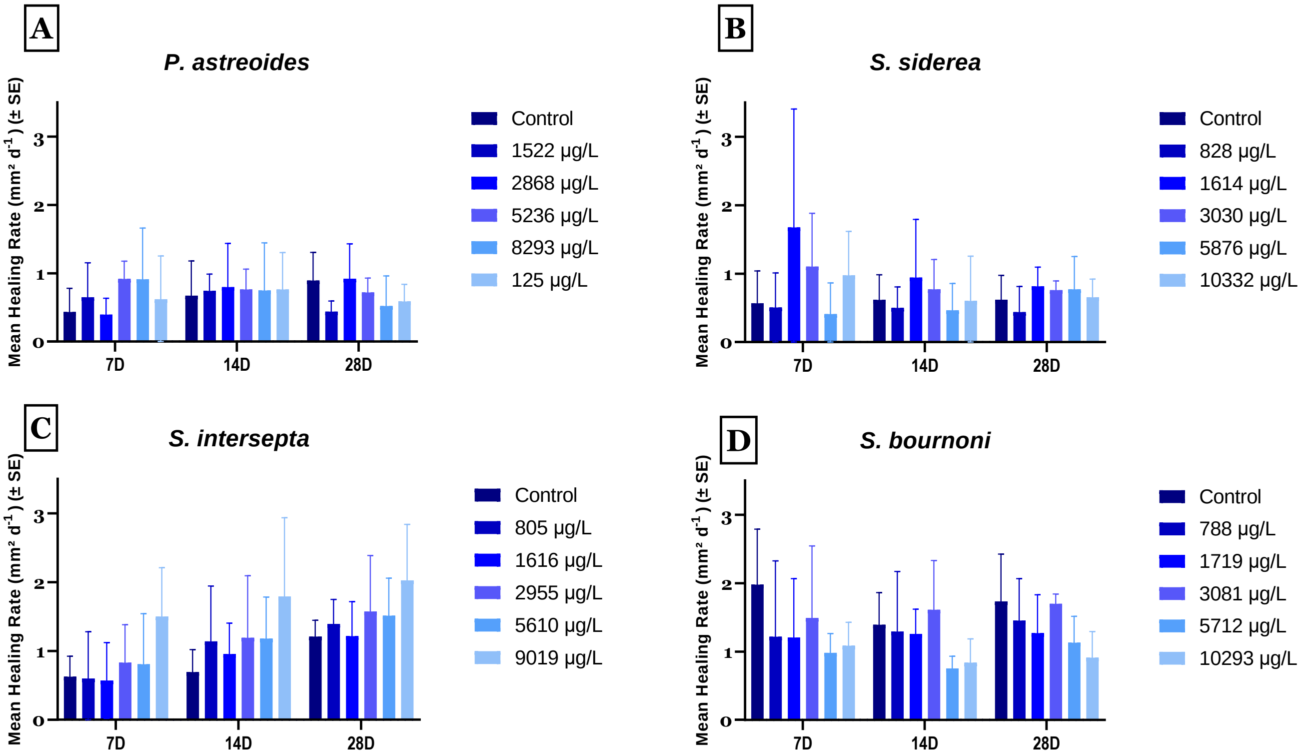

Figure 5.A) *P. astreoides* mean healing rate at initial, interval and final measurements for 1 – methylnaphthalene. B) *S. siderea* mean healing rate at initial, interval and final measurements for 1 – methylnaphthalene. C.) *S. intersepta* mean healing rate at initial, interval and final measurements for 1 – methylnaphthalene. D.) *S. bournoni* mean healing rate at initial, interval and final measurements for 1 – methylnaphthalene.

## *Phenanthrene*

### Mean percent healed.

Exposure to phenanthrene resulted in a less varied response between species compared to 1-methylnaphthalene (Figure 6). *P. astreoides, S. siderea, and S. intersepta* had overall percent healed of 17%, 36%, and 42% respectively, after 28 days post-exposure. No significant difference in the mean percent healed between concentrations was found at 28 days post-exposure (One-way ANOVA, p > .05) for any species. *P. astreoides* showed a dose response, where the mean percent healed decreased as concentration increased, although this was not significant (One-way ANOVA, p > .05). No dose response was observed for *S. siderea* and *S. intersepta*.

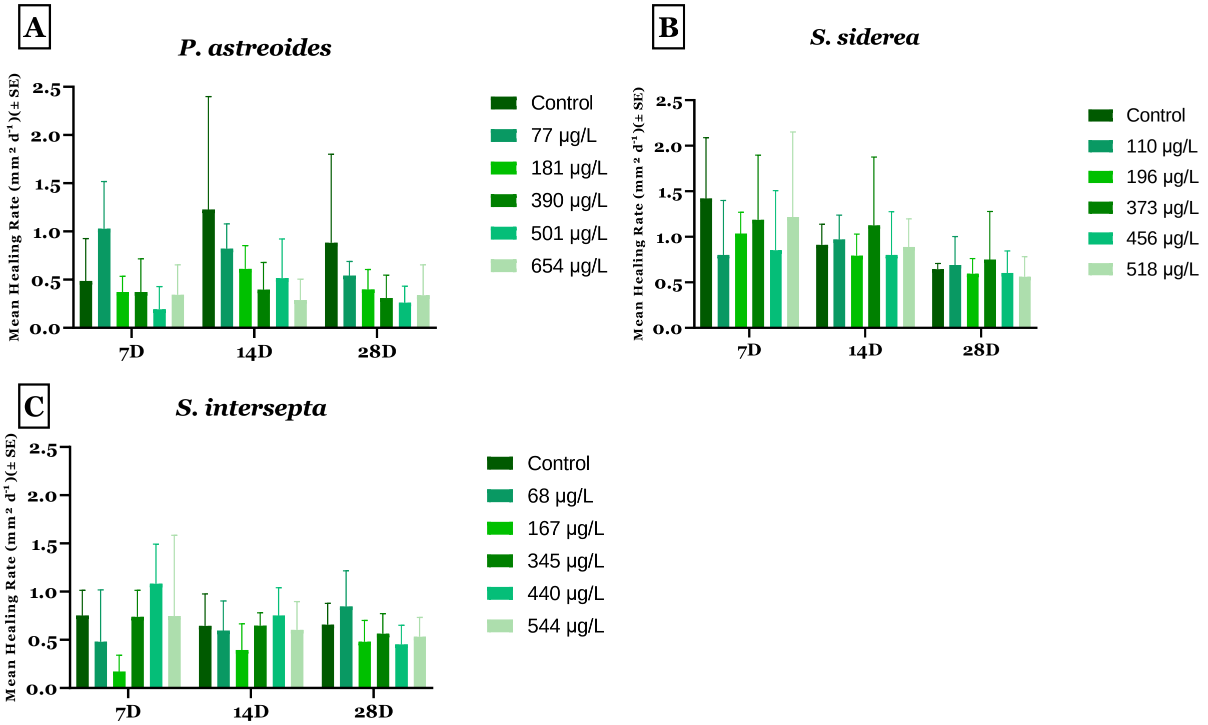

Figure 6. A) *P. astreoides* mean percent healed at 28 days post exposure to phenanthrene. B) *S. siderea* mean percent healed at 28 days post exposure to phenanthrene. C.) *S. intersepta* mean percent healed at 28 days post exposure to phenanthrene.

### Mean healing rate.

A dose response for mean healing rate was observed for both *Siderastrea siderea* and *P. astreoides,* where the mean healing rate generally decreased as phenanthrene concentration increased at 28 days post exposure, although the effect **of** was not significant (One-way ANOVA, p > .05) (Figure 7). No dose response was observed for *S. intersepta*. A significant effect of time on mean healing rate was observed for *S. siderea* [F (1.152, 19.59) = 9.024, p = 0.0055], although post-hoc analysis was unable to resolve the specific differences between concentrations. For *P. astreoides* and *S. intersepta* no significance effect was found (Repeated measures Two-way ANOVA, p > .05).

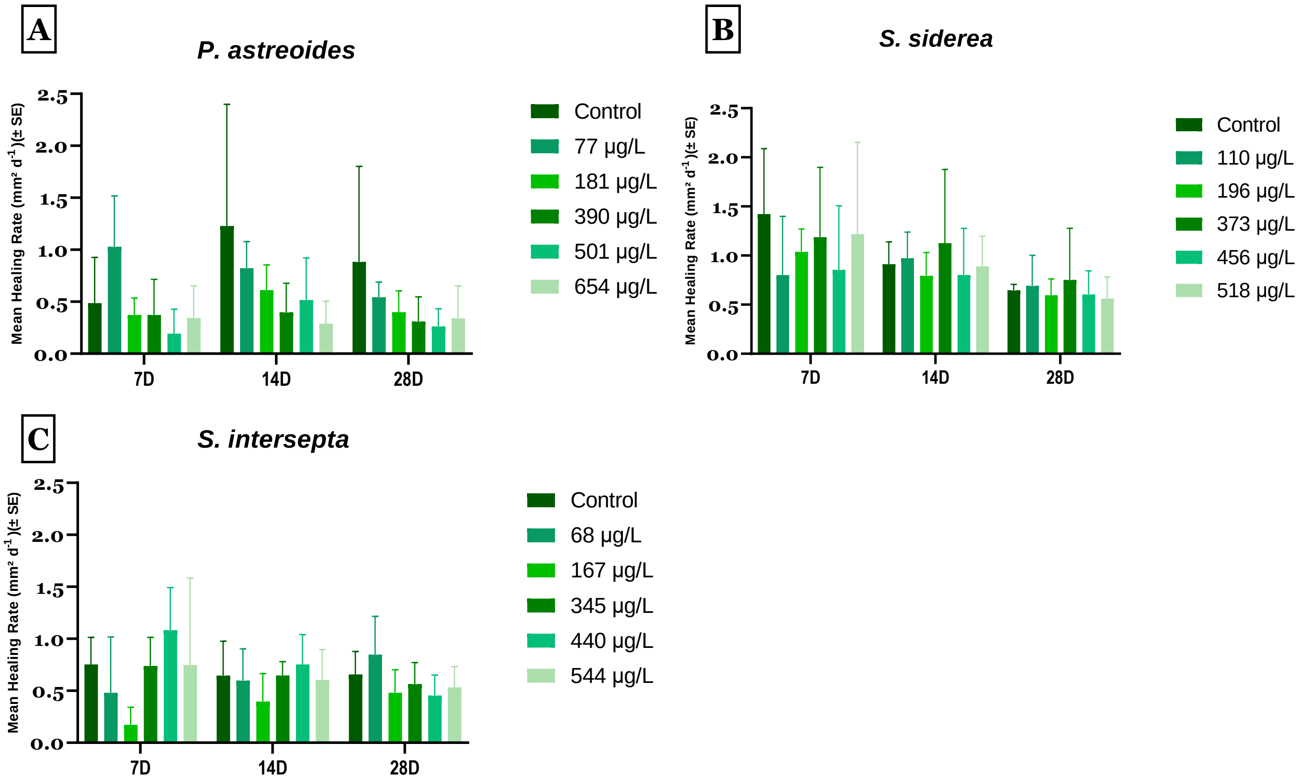

Figure 7. A) *P. astreoides* mean healing rate at initial, interval and final measurements for phenanthrene. B) *S. siderea* mean healing rate at initial, interval and final measurements for phenanthrene.. C.) *S. intersepta* mean healing rate at initial, interval and final measurements for phenanthrene.

### *Oil (WAF) and Dispersed oil (CEWAF)*

### Mean percent healed.

*Porites. astreoides* was the only species tested with WAF and CEWAF. For WAF, this species had an overall mean percent healed of 89% at 28 days post exposure (Figure 8), and 91.6% of the coral fragments had completely healed. A hormetic-type dose response was observed for the WAF exposure, where the mean percent healed was higher at low and intermediate concentrations and lower at high concentrations, although this was not significant.

For CEWAF, the overall mean percent healed was 66% at 28 days post-exposure (Figure 8). No significant difference in mean percent healed was found between concentrations at 28 days post-exposure for either the WAF or CEWAF exposure (One-way ANOVA, p > .05).

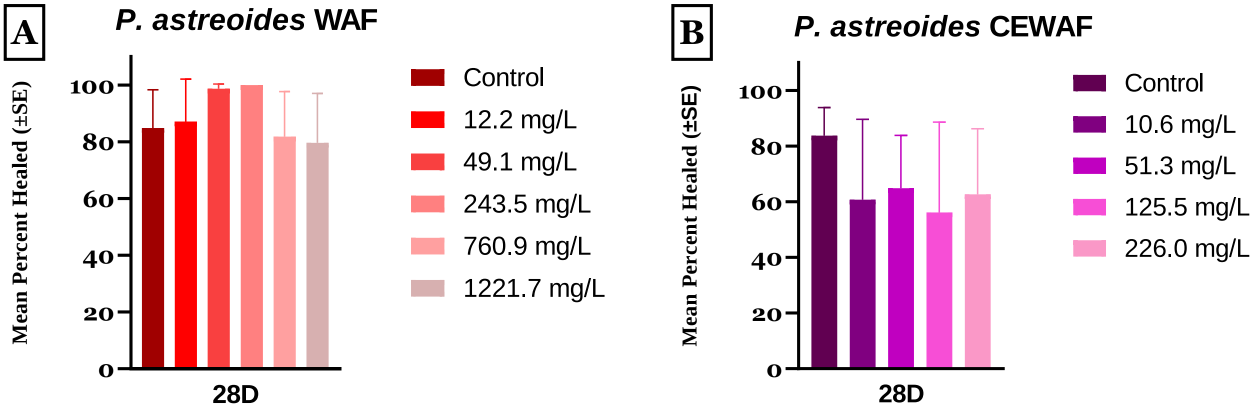

Figure 8. Mean percent healed for *Porites astreoides.* A) Water accommodated fraction. B) Chemically enhanced water accommodated fraction.

**Mean healing rate.**

A non-significant hormetic-type dose response for mean healing rate in *P. astreoides* was observed for WAF at all three time points post-exposure (ANOVA, p > .05) (Figure 9). No significant effect of time on mean healing rate was found for the WAF exposure (Repeated measures Two-way ANOVA, p > .05). For CEWAF, the exposure resulted in time having a very significant effect on mean healing rate (F (1.152, 19.59) = 9.024, [p = 0.0032]), however post-hoc analysis was unable to resolve specific differences.

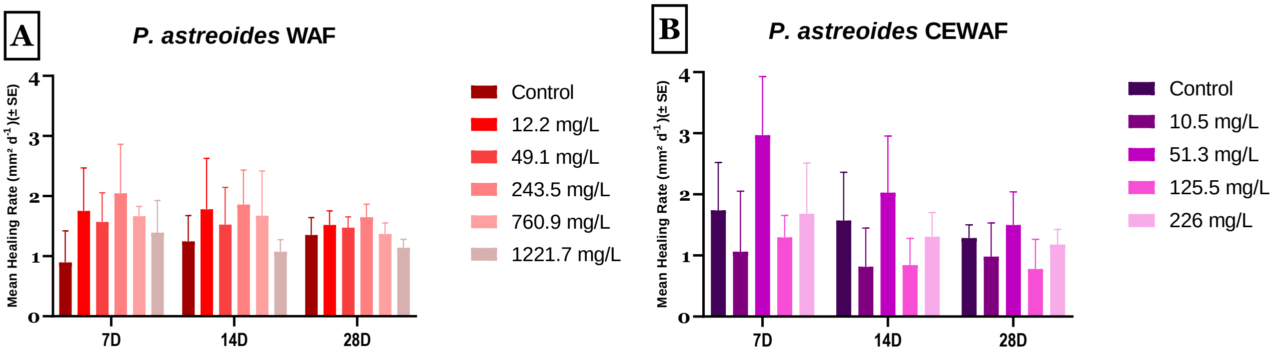

Figure 9. Mean wound healing rate for *Porites astreoides.* A) Water accommodated fraction. B) Chemically enhanced water accommodated fraction.

## DISCUSSION

This study examined potential differences in healing rate after short term- exposure to 1-methylnaphthalene, phenanthrene, oil, and dispersed oil in four species of Atlantic shallow-water corals. Interest in the impacts of petroleum on corals has increased since the Deepwater Horizon event, but little is known of the interaction between hydrocarbon exposure and recovery in corals in terms of wound regeneration (but see Guzmán, Burns, & Jackson, 1994; May et al., 2020; Renegar, Blackwelder, & Moulding, 2008). Several studies have assessed the effect of a variety of stressors on wound healing in corals (Counsell, Johnston, & Sale, 2019; Edmunds & Yarid, 2017; Henry & Hart, 2005; Traylor-Knowles, 2016), but only one study to date has evaluated hydrocarbons (May et al. 2020). In this study, coral fragments were wounded to represent natural (e.g., fish predation, hurricanes, disease) or anthropogenic (e.g., groundings, human interactions) physical breakage or injuries, then the effect of hydrocarbon exposure on the ability of coral tissue to regenerate was assessed.

### *Wound characteristics*

Some studies have suggested correlations between lesion recovery rates and wound characteristics such as injury shape and size, lesion, perimeter, and colony size. The mean rate of healing observed during the initial 7-day period fell within the range of previous studies (Meesters and Bak, 1993; Bak and Steward-Van Es, 1980, Meesters et al., 1992, Thornton et al., 2002). Although many authors reported a decrease in recovery rates with time some saw different trends due to wound variations (e.g., Bak and Steward-Van Es, 1980; Meesters et al., 1997; Cróquer et al., 2002). This reason could explain some of the variation in responses. For example, *S. intersepta* exposed to 1 - methylnaphthalene had an increase in healing rate over time, where the healing rate of *P. astreoides* exposed to 1- methylnaphthalenes remained relatively consistent at each timepoint. However, some of the *P. astreoides* lesions are not as rough or crude as in *S. intersepta*. Also *S. intersepta* lesions still had some remaining tissue close to the wound surface compared to *P. astreoides*. This could allow for an increased healing rate for *S. intersepta*. These observed differences could result from variations in the methods used in our study compared to other methods to cause these wounds. Wound depth and size were found to have a negative effect on regeneration as documented for the species *Porites spp* (van Woesik, 1998). Many previous studies used other methods, such as hand chisels, wet saws, or stainless-steel cutters. Lesions for this study were made using a stone bit on a Dremel, which may have impacted the size and uniformity of the wounds. Although this study did not address how lesion size affects healing rates, the sizes of our lesions were reasonably consistent for this study. This could allow for differences in healing rates between experiments.

### ***Colony variation***

Although the mean percent healed in all experiments were within the same range previously reported for these species, mean healing rates in the 1–methylnaphthalene exposures were generally double those in the phenanthrene exposures (excluding *S. siderea*), the mean healing rate doubled for *P. astreoides* again in the WAF and CEWAF experiments. Throughout all these tests, we have observed high variability in wound regeneration rates not directly related to hydrocarbon exposure, which suggests that other factors or interactions are potentially impacting tissue regeneration, including variation between colonies within a species, such as was observed in the *P. astreoides* CEWAF experiment. (Figure 10) shows two fragments from two different colonies exposed to the same concentration but with contrasting results. Visually you can see differences within the colony's pigmentation; the Colony 1 fragment has different pigmentation than Colony 2, this is usually due to between colony symbiont variation. Colony 2 appears to have some level of bleaching response to this hydrocarbon concentration the loss of symbiont as indicated by the lighter color.

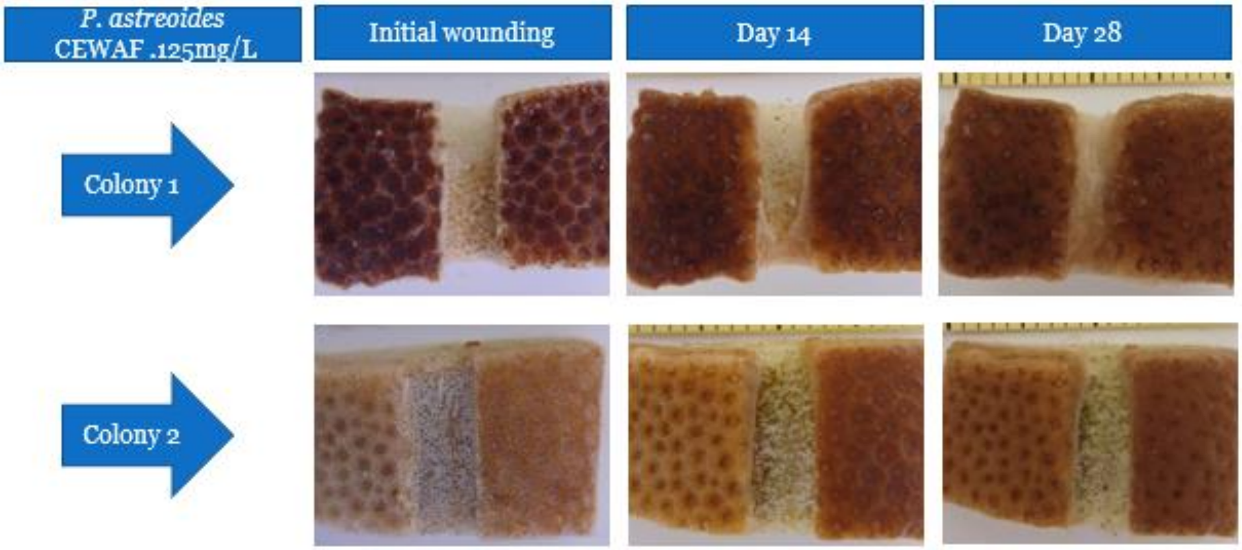

Figure 10. Colony variation can be observed in the *Porites astreoides* CEWAF experiment.

***Algae colonization***

For the first seven days, the mean healing rates for all three species exposed to phenanthrene were similar to rates previously reported in other studies (Meesters and Bak, 1993; Bak and Steward-Van Es, 1980, Meesters et al., 1992, Thornton et al., 2002). *Porites astreoides* showed a dose response, where the mean percent healed decreased as concentration increased when exposed to phenanthrene compared to other species. This concentration effect could be increased by a mixture of green algae and red filamentous algae found around the fragment and in the wound in 17 of the 24 corals by 14 days post exposure. In some instances, the tissue regeneration appeared hindered or even halted by macroalgae that had settled in the lesion. Nineteen of the 24 wounded fragments were unable to heal fully, possibly due to algae colonization of the wound, although 3 of the 24 fragments were able to heal completely despite the presence of algae. Of the fragments that healed completely, two were from the control group and one was from the highest exposure of 2 mg/L. Previous research confirmed that unhealed lesions are vulnerable to colonization by macroalgae and cyanobacteria (Bak, 1980). Due to the inhibitory effects of algae on the healing process, this experimental exposure was the only one where post-exposure mortality was observed.

### ***Coral mortality***

Three of the unhealed fragments had an increase lesion size due to tissue death, with loss of the entire fragment (total mortality) for two of these fragments. Although algae contributed to the decreased healing rate for *P. astreoides*, *S. siderea* healed with no algae interference while recovering at the same time in the same tanks. The initial wounded fragment had retracted tissue and skeleton exposed at the end of the 48-hour exposure. At 7 days post-exposure, there was a continuation of tissue loss and the start of green algae growth on the non-wounded skeleton portion of the fragment. At 14- and 28-days post-exposure, the same pattern with tissue loss and green tint covering the wound area indicating algae growth. This observation does not confirm if the algae caused mortality, or if the mortality allowed the algae to colonize.

### ***Molecular changes***

Molecular changes have significant effects on regeneration also, and key information on these changes can be assessed due to advancements in sequencing technology. An increasing amount of academic research focuses on studying gene expression as a tool for verifying molecular changes. Gene expression is the process by which the information encoded in a gene is turned into a function, and plasticity in this process is a crucial mechanism by which corals adapt to variations in the environment.(Dang, 2021) compared gene expression in *Acropora cervicornis* exposed to 1–methylnaphthalene and phenanthrene and found that phenanthrene had a more significant effect on gene regulation (Turner, 2020). Although coral species react in different ways to hydrocarbon exposures, *S. siderea* was also found to have a similar relative sensitivity to these hydrocarbons (Turner 2020). After exposure, phenanthrene produced approximately 3500 upregulated or downregulated genes compared to only 804 for 1–methylnaphthalene in *Acropora cervicornis*. This may explain why the phenanthrene healing rate was less than 1–methylnaphthalene even within the same species. The specific genes targeted by the Turner (2020) study are responsible for transmembrane transport, protein phosphorylation, and oxidation-reduction processes. Many of these genes make up the corals "chemical defensome"; the primary purpose of the defensome is to enable an organism to protect itself against hazardous chemicals such as hydrocarbons. While transcriptomics has also shed light on the mechanisms that corals use to respond to stress, gene expression and cellular activity are not necessarily correlated.

## CONCLUSION

This research aimed to examine if exposure to hydrocarbons affected the wound regeneration rate in 4 Atlantic shallow-water coral species. Corals react in a variety of ways to hydrocarbon exposures, with some species- and hydrocarbon-specificity. The species in this study were shown to have a variety of species-specific physical reactions to exposure to petroleum hydrocarbons, with single hydrocarbons having a bigger impact on both healing rate and percent healed than oil and dispersed oil. This work shows that short-term exposure to certain hydrocarbons does affect the overall wound healing of these four coral species. Although statistically there was very little significance found, this is likely due to the small sample size; a larger sample size would likely show a greater significance between concentration and exposure effect. Future studies should also assess co-stressors such as UV light exposure, predation, and sedimentation which are environmentally relevant for effective oil spill response.

## ACKNOWLEDGMENTS

I would like to thank my main advisor Dr. Abigail Renegar for all her patience and advice. and Dr. Emily Schmitt Lavin for stepping in on short notice for Dr. Patricia Blackwelder. Their invaluable guidance and knowledge during this process made it a success. Thank you to Nicholas Turner, Eileen Whitemiller, Kyle Pisano, Edward Young, Katrina Smith and Matt Rojano who were crucial members during these experiments. In addition, I would like to thank my family and friends who supported me on this journey.

## REFERENCES

Adipah, Sylvia. "Introduction of Petroleum Hydrocarbons Contaminants and Its Human Effects." 2018.

Aurand, Don, Robert Pond, Gina Coelho, Mark Cunningham, Amy Cocanaur, and Leigh Stevens. "The Use of Consensus Ecological Risk Assessments to Evaluate Oil Spill Response Options: Lessons Learned from Workshops in Nine Different Locations." Paper presented at the International Oil Spill Conference, 2005.

Bak, R. P., & Steward-Van Es, Y. "Regeneration of Superficial Damage in the Scleractinian Corals *Agaricia Agaricites F. Purpurea* and *Porites Astreoides*." In *Bulletin of Marine Science*, 883-87, 1980.

Bera, Gopal, Thomas Parkerton, Aaron Redman, Nicholas R Turner, D Abigail Renegar, Jose L Sericano, and Anthony H Knap. "Passive Dosing Yields Dissolved Aqueous Exposures of Crude Oil Comparable to the Croserf (Chemical Response to Oil Spill: Ecological Effects Research Forum) Water Accommodated Fraction Method." *Environmental toxicology and chemistry* 37, no. 11 (2018): 2810-19.

Beyer, Jonny, Hilde C. Trannum, Torgeir Bakke, Peter V. Hodson, and Tracy K. Collier. "Environmental Effects of the Deepwater Horizon Oil Spill: A Review." *Marine Pollution Bulletin* 110, no. 1 (2016/09/15/ 2016): 28-51.

Butler, Josh D, Thomas F Parkerton, Daniel J Letinski, Gail E Bragin, Mark A Lampi, and Keith R Cooper. "A Novel Passive Dosing System for Determining the Toxicity of Phenanthrene to Early Life Stages of Zebrafish." *Science of the Total Environment* 463 (2013): 952-58.

Cameron, Caitlin M, and Peter J Edmunds. "Effects of Simulated Fish Predation on Small Colonies of Massive *Porites Spp*. And *Pocillopora Meandrina*." *Marine Ecology Progress Series* 508 (2014): 139-48.

Carpenter, Kent E, Muhammad Abrar, Greta Aeby, Richard B Aronson, Stuart Banks, Andrew Bruckner, Angel Chiriboga, et al. "One-Third of Reef-Building Corals Face Elevated Extinction Risk from Climate Change and Local Impacts." *Science* 321, no. 5888 (2008): 560-63.

Cook, CB, and AH Knap. "Effects of Crude Oil and Chemical Dispersant on Photosynthesis in the Brain Coral *Diploria Strigosa*." *Marine Biology* 78 (1983): 21-27.

Counsell, Chelsie WW, Erika C Johnston, and Tayler L Sale. "Colony Size and Depth Affect Wound Repair in a Branching Coral." *Marine Biology* 166 (2019): 1-12.

Cróquer, Aldo, Estrella Villamizar, and Nicida Noriega. "Environmental Factors Affecting Tissue Regeneration of the Reef-Building Coral *Montastraea Annularis* (Faviidae) at Los Roques National Park, Venezuela." *Revista de biología tropical* 50, no. 3-4 (2002): 1055-65.

Dang, Z., Y. Jia, Y. Tian, J. Li, Y. Zhang, L. Huang, C. Liang*, et al.* "Transcriptome-Wide Gene Expression Plasticity in *Stipa Grandis* in Response to Grazing Intensity Differences." *Int J Mol Sci* 22, no. 21 (Nov 2, 2021). https://doi.org/10.3390/ijms222111882.

Darling, Emily S, Lorenzo Alvarez-Filip, Thomas A Oliver, Timothy R McClanahan, and Isabelle M Côté. "Evaluating Life-History Strategies of Reef Corals from Species Traits." *Ecology Letters* 15, no. 12 (2012): 1378-86.

DeLeo, Danielle M, Dannise V Ruiz-Ramos, Iliana B Baums, and Erik E Cordes. "Response of Deep-Water Corals to Oil and Chemical Dispersant Exposure." *Deep Sea Research Part II: Topical Studies in Oceanography* 129 (2016): 137-47.

Dodge, Richard E., Sheila C. Wyers, H. R. Frith, Anthony H. Knap, S. R. Smith, and T. D. Sleeter. "The Effects of Oil and Oil Dispersants on the Skeletal Growth of the Hermatypic Coral *Diploria Strigosa*." *Coral Reefs* 3, no. 4 (1984/12/01 1984): 191-98. https://doi.org/10.1007/bf00288254. https://doi.org/10.1007/BF00288254.

Domart-Coulon, Isabelle J, Nikki Traylor-Knowles, Esther Peters, David Elbert, Craig A Downs, Kathy Price, Joanne Stubbs*, et al.* "Comprehensive Characterization of Skeletal Tissue Growth Anomalies of the Finger Coral *Porites Compressa*." *Coral Reefs* 25 (2006): 531-43.

Drury, Crawford, Stephanie Schopmeyer, Elizabeth Goergen, Erich Bartels, Ken Nedimyer, Meaghan Johnson, Kerry Maxwell*, et al.* "Genomic Patterns in *Acropora Cervicornis* Show Extensive Population Structure and Variable Genetic Diversity." *Ecology and evolution* 7, no. 16 (2017): 6188-200.

Edmunds, Peter J, and Alex Yarid. "The Effects of Ocean Acidification on Wound Repair in the Coral *Porites Spp*." *Journal of Experimental Marine Biology and Ecology* 486 (2017): 98-104.

Fingas, Merv, and Carl E. Brown. "A Review of Oil Spill Remote Sensing." *Sensors* 18, no. 1

(2018): 91. https://www.mdpi.com/1424-8220/18/1/91.

Firman, Julia Christine. *Chronic Toxicity of Pesticides to Reef-Building Corals: Physiological, Biochemical, Cellular and Developmental Effects.* University of Miami, 1995.

Fischer, Fabian, Leonard Böhm, Sebastian Höss, Christel Möhlenkamp, Evelyn Claus, Rolf-Alexander Düring, and Sabine Schäfer. "Passive Dosing in Chronic Toxicity Tests with the Nematode *Caenorhabditis Elegans*." *Environmental Science & Technology* 50, no. 17 (2016/09/06 2016): 9708-16. https://doi.org/10.1021/acs.est.6b02956. https://doi.org/10.1021/acs.est.6b02956.

Fisher, Elizabeth M, John E Fauth, Pamela Hallock, and Cheryl M Woodley. "Lesion Regeneration Rates in Reef-Building Corals *Montastraea Spp*. As Indicators of Colony Condition." *Marine Ecology Progress Series* 339 (2007): 61-71.

Florisson, James H, Andrew J Rowland, Euan S Harvey, Mathew B Allen, Stephanie L Watts, and Benjamin J Saunders. "King Reef: An Australian First in Repurposing Oil and Gas Infrastructure to Benefit Regional Communities." *The APPEA Journal* 60, no. 2 (2020): 435-39.

Franklin, Erik C, Paul L Jokiel, and Megan J Donahue. "Predictive Modeling of Coral Distribution and Abundance in the Hawaiian Islands." *Marine Ecology Progress Series* 481 (2013): 121-32.

French-McCay, Deborah P. "Development and Application of an Oil Toxicity and Exposure Model, Oiltoxex." *Environmental Toxicology and Chemistry: An International Journal* 21, no. 10 (2002): 2080-94.

Goldstone, JV, A Hamdoun, BJ Cole, M Howard-Ashby, DW Nebert, M Scally, M Dean*, et al.* "The Chemical Defensome: Environmental Sensing and Response Genes in the *Strongylocentrotus Purpuratus* Genome." *Developmental biology* 300, no. 1 (2006): 366-84.

Griffin, Sean, H Spathias, T Moore, I Baums, and B Griffin. "Scaling up *Acropora* Nurseries in the Caribbean and Improving Techniques." Paper presented at the Proceedings of the 12th International Coral Reef Symposium, 2012.

Grottoli, Andréa G, Mark E Warner, Stephen J Levas, Matthew D Aschaffenburg, Verena Schoepf, Michael McGinley, Justin Baumann, and Yohei Matsui. "The Cumulative Impact of Annual Coral Bleaching Can Turn Some Coral Species Winners into Losers."

*Global Change Biology* 20, no. 12 (2014): 3823-33.

Guzmán, Héctor M, Kathryn A Burns, and Jeremy BC Jackson. "Injury, Regeneration and Growth of Caribbean Reef Corals after a Major Oil Spill in Panama." *Marine ecology progress series* (1994): 231-41.

Guzmán, Héctor M, and Irene Holst. "Effects of Chronic Oil-Sediment Pollution on the Reproduction of the Caribbean Reef Coral *Siderastrea Siderea*." *Marine Pollution Bulletin* 26, no. 5 (1993): 276-82.

Guzmán, Héctor M, Jeremy BC Jackson, and Ernesto Weil. "Short-Term Ecological Consequences of a Major Oil Spill on Panamanian Subtidal Reef Corals." *Coral reefs* 10 (1991): 1-12.

Hackerott, Serena, Harmony A Martell, and Jose M Eirin-Lopez. "Coral Environmental Memory: Causes, Mechanisms, and Consequences for Future Reefs." *Trends in Ecology & Evolution* 36, no. 11 (2021): 1011-23.

Hartmann, Aaron C, Stuart A Sandin, Valérie F Chamberland, Kristen L Marhaver, Jasper M de Goeij, and Mark JA Vermeij. "Crude Oil Contamination Interrupts Settlement of Coral Larvae after Direct Exposure Ends." *Marine Ecology Progress Series* 536 (2015): 163-73.

Henry, Lea-Anne, and Michael Hart. "Regeneration from Injury and Resource Allocation in Sponges and Corals–a Review." *International Review of Hydrobiology: A Journal Covering all Aspects of Limnology and Marine Biology* 90, no. 2 (2005): 125-58.

Highsmith, Raymond C. "Reproduction by Fragmentation in Corals." *Marine ecology progress series. Oldendorf* 7, no. 2 (1982): 207-26.

Hilker, Monika, Jens Schwachtje, Margarete Baier, Salma Balazadeh, Isabel Bäurle, Sven Geiselhardt, Dirk K Hincha*, et al.* "Priming and Memory of Stress Responses in Organisms Lacking a Nervous System." *Biological Reviews* 91, no. 4 (2016): 1118-33.

Hughes, Terry P, Andrew H Baird, David R Bellwood, Margaret Card, Sean R Connolly, Carl Folke, Richard Grosberg*, et al.* "Climate Change, Human Impacts, and the Resilience of Coral Reefs." *science* 301, no. 5635 (2003): 929-33.

Jackson, Jeremy BC, John D Cubit, Brian D Keller, Victoria Batista, Kathryn Burns, Hugh M Caffey, Roy L Caldwell, et al. "Ecological Effects of a Major Oil Spill on Panamanian Coastal Marine Communities." *Science* 243, no. 4887 (1989): 37-44.

Kleindienst, Sara, Michael Seidel, Kai Ziervogel, Sharon Grim, Kathy Loftis, Sarah Harrison, Sairah Y. Malkin et al. "Chemical dispersants can suppress the activity of natural oil-degrading microorganisms." *Proceedings of the National Academy of Sciences* 112, no. 48 (2015): 14900-14905.

Knowlton, Nancy, Russell E Brainard, Rebecca Fisher, Megan Moews, Laetitia Plaisance, and M Julian Caley. "Coral Reef Biodiversity." *Life in the world's oceans: diversity distribution and abundance* (2010): 65-74.

Kramarsky-Winter, E, and Y Loya. "Tissue Regeneration in the Coral *Fungia Granulosa*: The Effect of Extrinsic and Intrinsic Factors." *Marine Biology* 137 (2000): 867-73.

Lester, RT, and RPM Bak. "Effects of Environment on Regeneration Rate of Tissue Lesions in the Reef Coral *Montastrea Annularis* (Scleractinia)." *Marine Ecology Progress Series* (1985): 183-85.

Loya, Y, and B Rinkevich. "Effects of Oil Pollution on Coral Reef Communities." *Mar. Ecol. Prog. Ser* 3, no. 16 (1980): 180.

May, Lisa A., Athena R. Burnett, Carl V. Miller, Emily Pisarski, Laura F. Webster, Zachary J. Moffitt, Paul Pennington*, et al.* "Effect of Louisiana Sweet Crude Oil on a Pacific Coral, *Pocillopora Damicornis*." *Aquatic Toxicology* 222 (2020/05/01/ 2020): 105454.

Meesters, Erik H, and Rolf PM Bak. "Effects of Coral Bleaching on Tissue Regeneration Potential and Colony Survival." *Marine Ecology Progress Series* (1993): 189-98.

Meesters, Erik H, Marco Noordeloos, and Rolf PM Bak. "Damage and Regeneration: Links to Growth in the Reef-Building Coral *Montastrea Annularis*." *Marine Ecology Progress Series* (1994): 119-28.

National Academies of Sciences, Engineering, and Medicine. *The Use of Dispersants in Marine Oil Spill Response.* Washington, DC: The National Academies Press, 2020. doi:10.17226/25161.

Negri, Andrew P, and Andrew J Heyward. "Inhibition of Fertilization and Larval Metamorphosis of the Coral *Acropora Millepora* (Ehrenberg, 1834) by Petroleum Products." *Marine Pollution Bulletin* 41, no. 7-12 (2000): 420-27.

Parkerton, Thomas F, Daniel J Letinski, Eric J Febbo, Josh D Butler, Cary A Sutherland, Gail E Bragin, Bryan M Hedgpeth*, et al.* "Assessing Toxicity of Hydrophobic Aliphatic and Monoaromatic Hydrocarbons at the Solubility Limit Using Novel Dosing Methods."

*Chemosphere* 265 (2021): 129174.

Purvis, Chelsea. "Coastal State Jurisdiction under Unclos: The Shen Neng 1 Grounding on the Great Barrier Reef." *Yale J. Int'l L.* 36 (2011): 207.

Rajendran, Sankaran, Ponnumony Vethamony, Fadhil N Sadooni, Hamad Al-Saad Al-Kuwari, Jassim A Al-Khayat, Vashist O Seegobin, Himanshu Govil, and Sobhi Nasir. "Detection of Wakashio Oil Spill Off Mauritius Using Sentinel-1 and 2 Data: Capability of Sensors, Image Transformation Methods and Mapping." *Environmental Pollution* 274 (2021): 116618.

Imagej Version 1.53t. U. S. National Institutes of Health, Bethesda, Maryland, USA.

Renegar, D., P. L. Blackwelder, and Alison Moulding. "Coral Ultrastructural Response to Elevated Pco2 and Nutrients During Tissue Repair and Regeneration. In Proceedings of the 11th International Coral Reef Symposium, Ft." *Lauderdale, Florida, 7–11 July* 2008 (01/01 2008): 1320-24.

Renegar, D., and Nicholas Turner. "Species Sensitivity Assessment of Five Atlantic Scleractinian Coral Species to 1-Methylnaphthalene." *Scientific Reports* 11 (01/12 2021). https://doi.org/10.1038/s41598-020-80055-0.

Renegar, D Abigail, and Nicholas R Turner. "Species Sensitivity Assessment of Five Atlantic Scleractinian Coral Species to 1-Methylnaphthalene." *Scientific reports* 11, no. 1 (2021): 529.

Renegar, D. Abigail, Nicholas R. Turner, Gopal Bera, Eileen G. Whitemiller, Bernhard M. Riegl, José L. Sericano, and Anthony Knap. "Comparative Toxicity of Hydrocarbons for Evaluation of *Lysmata Boggessi* as an Experimental Proxy for Deep-Water Column Micronekton." *Toxicology Reports* 9 (2022/01/01/ 2022): 656-62.

Renegar, D Abigail, Nicholas R Turner, Bernhard M Riegl, Richard E Dodge, Anthony Knap, and Paul Schuler. "Quantifying Hydrocarbon Toxicity to Shallow-Water Corals: Range Finding Exposure." Paper presented at the Gulf of Mexico Oil Spill and Ecosystem Science Conference, Houston, TX, 2015.

Renegar, D. Abigail, Nicholas R. Turner, Bernhard M. Riegl, Richard E. Dodge, Anthony H. Knap, and Paul A. Schuler. "Acute and subacute toxicity of the polycyclic aromatic hydrocarbon 1-methylnaphthalene to the shallow-water coral *Porites divaricata*: Application of a novel exposure protocol." *Environmental toxicology and chemistry* 36, no. 1 (2017): 212-219.

Renegar, Dorothy-Ellen A, Patricia Blackwelder, JD Miller, DJ Gochfeld, and Alison L Moulding. "Ultrastructural and Histological Analysis of Dark Spot Syndrome in *Siderastrea Siderea* and *Agaricia Agaricites*."  (2008).

Roberts, Callum M, Colin J McClean, John EN Veron, Julie P Hawkins, Gerald R Allen, Don E McAllister, Cristina G Mittermeier*, et al.* "Marine Biodiversity Hotspots and Conservation Priorities for Tropical Reefs." *Science* 295, no. 5558 (2002): 1280-84.

Sabine, Alexis M, Tyler B Smith, Dana E Williams, and Marilyn E Brandt. "Environmental Conditions Influence Tissue Regeneration Rates in Scleractinian Corals." *Marine pollution bulletin* 95, no. 1 (2015): 253-64.

Scarlett, Alan G., Robert K. Nelson, Marthe Monique Gagnon, Alex I. Holman, Christopher M. Reddy, Paul A. Sutton, and Kliti Grice. "MV Wakashio grounding incident in Mauritius 2020: The world's first major spillage of Very Low Sulfur Fuel Oil." *Marine Pollution Bulletin* 171 (2021): 112917.

Schoepf, Verena, Michael Stat, James L Falter, and Malcolm T McCulloch. "Limits to the Thermal Tolerance of Corals Adapted to a Highly Fluctuating, Naturally Extreme Temperature Environment." *Scientific reports* 5, no. 1 (2015): 17639.

Shafir, Shai, Jaap Van Rijn, and Baruch Rinkevich. "Short and Long Term Toxicity of Crude Oil and Oil Dispersants to Two Representative Coral Species." *Environmental science & technology* 41, no. 15 (2007): 5571-74.

Silva, Denise P., Helena D. M. Villela, Henrique F. Santos, Gustavo A. S. Duarte, José Roberto Ribeiro, Angela M. Ghizelini, Caren L. S. Vilela*, et al.* "Multi-Domain Probiotic Consortium as an Alternative to Chemical Remediation of Oil Spills at Coral Reefs and Adjacent Sites." *Microbiome* 9, no. 1 (2021/05/21 2021): 118.

Soares, Marcelo Oliveira, Carlos Eduardo Peres Teixeira, Luis Ernesto Arruda Bezerra, Emanuelle Fontenele Rabelo, Italo Braga Castro, and Rivelino Martins Cavalcante. "The most extensive oil spill registered in tropical oceans (Brazil): the balance sheet of a disaster." *Environmental Science and Pollution Research* 29, no. 13 (2022): 19869-19877.

Soto, Luis A, Alfonso V Botello, Sergio Licea-Durán, Marcial L Lizárraga-Partida, and Alejandro Yáñez-Arancibia. "The Environmental Legacy of the Ixtoc-I Oil Spill in

Campeche Sound, Southwestern Gulf of Mexico." *Frontiers in Marine Science* 1 (2014): 57.

Torda, Gergely, Jennifer M Donelson, Manuel Aranda, Daniel J Barshis, Line Bay, Michael L Berumen, David G Bourne*, et al.* "Rapid Adaptive Responses to Climate Change in Corals." *Nature Climate Change* 7, no. 9 (2017): 627-36.

Traylor-Knowles, Nikki. "Distinctive Wound-Healing Characteristics in the Corals *Pocillopora Damicornis* and *Acropora Hyacinthus* Found in Two Different Temperature Regimes." *Marine biology* 163 (2016): 1-6.

Turner, N. R. Understanding the Toxicity of Single Hydrocarbons, Oil, and Dispersed Oil: A Species Sensitivity Assessment for Five Atlantic Coral Species. 2020. Dissertation. Halmos College of Natural Sciences and Oceanography, Nova Southeastern Universtiy.

Turner, N. R., Parkerton, T. F., & Renegar, D. A. (2021). Toxicity of two representative petroleum hydrocarbons, toluene and phenanthrene, to five Atlantic coral species. *Marine Pollution Bulletin*, *169*, 112560.

Turner, Nicholas R, and D Abigail Renegar. "Petroleum Hydrocarbon Toxicity to Corals: A Review." *Marine Pollution Bulletin* 119, no. 2 (2017): 1-16.

van Dam, Joost W, Andrew P Negri, Sven Uthicke, and Jochen F Mueller. "Chemical Pollution on Coral Reefs: Exposure and Ecological Effects." *Ecological impacts of toxic chemicals* 9 (2011): 187-211.

Van Veghel, Manfred LJ, and Rolf PM Bak. "Reproductive Characteristics of the Polymorphic Caribbean Reef Building Coral *Montastrea Annularis*. Iii. Reproduction in Damaged and Regenerating Colonies." *Marine Ecology Progress Series* (1994): 229-33.

van Woesik, R. "Lesion Healing on Massive *Porites Spp*. Corals." *Marine Ecology Progress Series* 164 (1998): 213-20. http://www.jstor.org/stable/24825538.

Werner, Timothy B, Luis Paulo Pinto, Guilherme Fraga Dutra, and Paulo Gustavo Do Prado Pereira. "Abrolhos 2000: Conserving the Southern Atlantic's Richest Coastal Biodiversity into the Next Century." *Coastal Management* 28, no. 1 (2000): 99-108.

Williams, Gareth J, Jamison M Gove, Yoan Eynaud, Brian J Zgliczynski, and Stuart A Sandin. "Local Human Impacts Decouple Natural Biophysical Relationships on Pacific Coral Reefs." *Ecography* 38, no. 8 (2015): 751-61.