

Acute Exposure of *Acropora cervicornis* to Port Everglade Sediment



Control

15 NTU

29 NTU

50 NTU



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Final Report

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MANAGEMENT SUMMARY:

The study found that the physical aspects of turbidity, specifically light reduction, had minimal impacts on the biology and physiology of *Acropora cervicornis*. However, it highlighted the need for further research using uncleaned sediment to better understand the true impact on coral health. Differences were observed in the responses of different genotypes of corals. Genotype 014 showed a decrease in calcification, while genotype 020 displayed increased photosynthetic efficiency, indicating varying levels of resilience to turbidity-induced stress. This highlights the significance of genetic variability in determining coral response. After three days of exposure to elevated turbidity levels, an increase in coral respiration was observed. This suggests that prolonged exposure to turbidity can affect the metabolic activity of corals.

Photo-physiological changes were detected in the corals, indicating that the impacts of turbidity on coral health may be delayed and not immediately apparent. Conducting recovery assessments is crucial to understanding the short-term and potential long-term effects of acute turbidity on corals. The study emphasized the need for more replication of data to ensure the reliability of the findings. Further research is necessary to gather comprehensive evidence on the effects of turbidity on coral health.

In summary, the study provides valuable insights into the response of *Acropora cervicornis* to acute turbidity exposures. It underscores the importance of conducting research using uncleaned sediment, considering genetic variability, recognizing the metabolic impacts of prolonged exposure, understanding the delayed effects of turbidity on coral physiology, and increasing replication in future studies. These findings contribute to our understanding of how turbidity affects coral reef ecosystems and can inform management and conservation efforts for these vulnerable marine organisms.

EXECUTIVE SUMMARY (NO MORE THAN A PAGE)

Coral reefs play a critical role in marine ecosystems by forming intricate structures along the coastlines that are capable of dissipating wave energy. This barrier not only provides a safe habitat for many marine species but also protects the beaches and human infrastructure from destructive storms and erosion. Despite the robust nature of the coral structures, the organisms themselves are highly sensitive to changes in their environment, predation, and disease which have led to massive global population declines. Anthropogenic activities, such as dredging and beach renourishment, cause large fluctuations in the water quality of coastlines and recent studies have observed that the sediment suspended in the water by these activities is detrimental to coral health. However, the biologically relevant turbidity benchmark of coral reefs has not yet been established.

This study examined the impacts of acute turbidity exposures on *Acropora cervicornis* health. Sediment obtained from a dredging site from the Port Everglades region was milled, cleaned, and suspended in artificial seawater to create turbidity treatments (0 NTU, 15 NTU, 29 NTU, and 50 NTU). The exposure duration was seventy-two hours using a custom-made intermittent-flow respirometry setup. Prior to the exposure, various coral measurements were taken, including individual photographs, buoyant weight, coral volume displacement, and coral wet weight. Dark-acclimated pulse amplitude modulation (PAM) measurements were conducted using a Diving PAM 2.0 device to evaluate the photosynthetic efficiency of coral symbionts. During the 72-hour exposure period, turbidity and total alkalinity (TA) samples were collected every four hours to monitor treatment stability and assess changes in water alkalinity due to coral calcification or dissolution. Turbidity samples were analyzed using a portable turbidimeter, while TA samples were processed within two hours of collection. Additionally, sump parameters such as salinity, temperature, dissolved oxygen (DO), and percent dissolved oxygen (DO%) were recorded throughout the exposure. On the final day of the exposure, post-exposure measurements were taken, including dark-acclimated PAM measurements, photographs, and buoyant weights of the corals. Fragments of each coral were obtained and stored for further analysis. Biological assessments included measuring total protein and chlorophyll concentrations, determining symbiotic algae abundance, assessing bulk skeletal density, and calculating surface area. It's important to note that recovery assessments were not conducted in this study. The same set of procedures was repeated for each trial using new coral fragments.

Preliminary analysis of the physiological and endosymbiont health responses suggested that *A. cervicornis* may be capable of coping with high sediment conditions for a limited amount of time. However, the coral % air saturation curves displayed a steady decrease over the three-day exposure and a positive correlation with turbidity concentration. This indicated that the coral was becoming increasingly stressed as the experiment progressed and that turbidity may affect coral metabolism.

However, it should be noted that this sediment is not entirely representative of what the corals would be exposed to in their natural habitat. Biological components of the natural (uncleaned) sediment are likely to play a large role in the health response of the corals. Therefore, future research must be done on the impact of unclean sediment.

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2. INTRODUCTION

Coral reefs play a multifaceted role in supporting various industries and providing essential ecosystem services worldwide. They are crucial for marine tourism, food production, and coastal protection and serve as habitats for countless marine species (Knowlton et al., 2010). It is estimated that about half of the global human population lives within 200km of coastlines (Kummu et al., 2016). Approximately 22% of reefs are negatively impacted by local stressors alone, such as dredging (Good & Bahr, 2021; Miller et al., 2016), so it is very important to monitor anthropogenic effects on coastal systems.

One crucial ecosystem service provided by coral reefs is shoreline protection. These intricate underwater structures act as natural barriers, buffering the coastlines against the destructive forces of waves and storms. Coral reefs help to dissipate wave energy and reduce the impact of coastal erosion, safeguarding nearby communities and infrastructure (Elliff & Silva, 2017). However, despite their ecological significance, corals are highly sensitive organisms. They are susceptible to environmental changes and disturbances caused by human activities (Good & Bahr, 2021; Kummu et al., 2016; Nalley et al., 2021). Activities such as beach nourishment and coastal dredging can significantly increase the turbidity of the water surrounding coral reefs. Elevated turbidity levels can interfere with water quality and coral health, as they rely on sunlight for photosynthesis and prefer clear, nutrient-poor waters (Miller et al., 2016; Walker et al., 2012).

Corals can be highly sensitive to changes in water clarity (Mumby & Woesik, 2014), and elevated turbidity levels can impede their ability to thrive and survive. Research has identified specific turbidity levels that can have detrimental effects on coral health. For example, in Florida, research has indicated that when turbidity levels rise above ten nephelometric turbidity units (NTU), it can lead to coral mortality (Miller et al., 2016; Walker et al., 2012). Similarly, in other coral reef regions, turbidity levels above 30-40 NTU have been associated with significant coral stress and reduced coral cover (Fabricius, 2005). It is important to note that these turbidity benchmarks can vary depending on the coral species and local environmental conditions. Some corals are more tolerant of turbidity than others, while certain species are extremely sensitive and may experience negative impacts even at lower turbidity levels (Duckworth et al., 2017; Piniak, 2007; Weber & Fabricius, 2006). Understanding these biologically relevant benchmarks is critical for managing human activities, such as dredging and beach nourishment, that can contribute to increased turbidity. By implementing measures to minimize turbidity and maintain water clarity below these critical benchmarks, we can help safeguard coral health and ensure the long-term survival of these vital and fragile ecosystems.

Additionally, corals are highly susceptible to diseases and population decline, particularly when exposed to stressful conditions such as turbidity (Gilmour, 1999; Pollock et al., 2016; Studivan et al., 2022). It is, therefore, of utmost importance to conduct research that goes beyond merely characterizing the effect of suspended sediment on coral health and instead provides a biologically relevant benchmark for coral well-being under turbid conditions. This research would enable us to gain a comprehensive understanding of the impacts of turbidity on coral reefs and develop effective mitigation strategies.

By investing in research that addresses the specific vulnerabilities of corals to turbidity-related stress, we can strive to protect and conserve these invaluable ecosystems. This knowledge will

contribute to the long-term viability of coral reefs and ensure their ability to provide essential ecosystem services. Safeguarding coral health not only benefits the diverse marine life that depends on coral reefs but also supports industries such as marine tourism, food production, and coastal protection. By taking proactive steps to mitigate the impacts of turbidity and maintain optimal conditions for corals, we can secure the continued provision of these ecosystem services for future generations.

2.1. Marine sediment and their effects on coral reef environments:

Various factors, such as sediment in runoff, wind and wave action in shallow water, intense storm activity, and phytoplankton blooms, can naturally contribute to the generation of turbid conditions in marine ecosystems (Hubeny, 2012; Tuttle & Donahue 2022). However, anthropogenic activity, such as dredging, can increase turbidity for multiple days in the local water column. There is substantial evidence that turbidity indirectly affects important benthic fauna such as corals, primarily by decreasing the amount of light available for photosynthesis (Bessel-Browne et al., 2017; Erftemeijer et al., 2012; Fisher et al., 2019; Jones et al., 2020) and acting as a vector for diseases (Pollock et al., 2016; Studivan et al., 2022). Chronic turbidity in systems with historically clear water has been specifically shown to reduce coral fertilization, larval survival, and larval settlement (Gilmour, 1999), induce stress responses and bleaching, decrease growth, and cause partial colony mortality for both juvenile and adult scleractinian corals (Jones et al., 2020; Tuttle & Donahue, 2022). Because dredging can contribute to the decrease in wild coral health, studies aimed toward understanding how important and endangered coral species respond to specific doses of increased turbidity levels are necessary to properly manage coastal environments.

Sedimentation impacts extend beyond corals, affecting the entire reef ecosystem. The presence of sediment can alter the composition and structure of marine habitats, impacting the marine organisms that rely on these habitats for shelter, feeding, and reproduction. Excessive sedimentation leads to significant shifts in community dynamics and a decrease in overall biodiversity (Lunt et al., 2020; Pollock et al., 2014). Therefore, mitigating the effects of sedimentation is essential not only for preserving coral health but also for maintaining the overall resilience and functionality of the entire reef ecosystem.

2.2. Sedimentation vs. turbidity

Sedimentation is the process of suspended solid particles depositing out of a fluid medium. While acute sedimentation exposures (of less than <24 hours) may not have a significant effect on the metabolism of coral (Bahr et al., 2020), chronic sedimentation has been shown to induce stress responses and decrease growth and recruitment rates in some coral species, and even induce mortality (Abdel-Salam & Porter, 1988; Erftemeijer et al., 2012). Branching forms of species like *Acropora cervicornis* and *A. palmata* may be better adapted to dealing with sedimentation than plating species (Ashey et al. 2023), but chronic exposure to high turbidity may still pose a threat. Turbidity is a measurement of reduced light attenuation caused by suspended particles in a water body and is notoriously difficult to study ex-situ because of the tendency of sediment to settle out of the water column, weather away at surfaces, and clog water equipment (Tahir et al. 2019). Identifying the difference between sedimentation (the settlement of solid particles onto corals and their environment) and turbidity (increased cloudiness in water caused by suspended particles) is crucial to understanding their individual and synergistic effects on corals and their associated ecosystems.

There are four main groups of sediment that make up marine benthos. Lithogenous sediments, also called terrigenous sediments, originate from pre-existing rock structures. This type of sediment typically comes from land masses via runoff. Biogenous sediments are composed of organic matter, usually decomposed organisms in aquatic habitats. Hydrogenous sediments are formed by sedimentation, where solid particles are suspended in water deposits. Cosmogenous sediments originate from extraterrestrial sources. Characteristics of sediment may vary, such as chemical composition and grain size (Webb, 2021). The sediment resuspended at dredging sites is usually a mix of fine-grain terrigenous sediment and biogenous sediment, where internal (organic) material from coastal ecosystems mixes with external (land-based) sediment from runoff.

There is a severe lack of studies focusing on terrigenous sediment resuspended by dredging and their impacts on Caribbean reefs specifically (Rogers & Ramos-Scharron, 2022). Evidence shows that chronic industrial sedimentation can significantly decrease growth rates of *A. cervicornis*, the Caribbean staghorn coral (Crabbe & Carlin, 2007), but the effects of specific levels of acute elevated turbidity on the species are not well understood. Therefore, this experiment aims to study the effects of acute turbidity increase on this Caribbean species.

To properly manage coastal environments and mitigate the negative effects of turbidity on corals, it is crucial to conduct detailed studies that investigate how important and endangered coral species, such as *A. cervicornis*, respond to different levels of increased turbidity. The staghorn coral is a dominant reef-building coral in tropical reefs, including Florida's Coral Reef, where it plays a vital role in providing shoreline protection from increasingly intense storms (National Marine Fisheries Service, 2015).

Historically, the staghorn coral population has faced significant challenges, with population decline reaching as high as 97% in the 1970s and 1980s. The loss was primarily attributed to the devastating impact of multiple coral diseases, including White Pox and White Band (National Marine Fisheries Service, 2015). Although the staghorn coral has shown signs of recovery, ongoing threats such as rising ocean temperatures, ocean acidification, and sedimentation resulting from coastal development continue to reduce recovery. Sedimentation, including the fine-grain terrigenous sediment resuspended by dredging activities, is recognized as one of the potential threats to the recovery of the staghorn coral by the National Marine Fisheries Service (2015).

The effects of chronic industrial sedimentation on *A. cervicornis* have been documented, showing a significant decrease in growth rates (Crabbe & Carlin, 2007). However, the specific effects of acute elevated turbidity at different levels on the species are not well understood. Therefore, this study aims to address this critical knowledge gap by focusing on the effects of acute turbidity increases on Caribbean coral species like *A. cervicornis*.

To achieve this, the study will utilize fine-grain terrigenous sediment collected from a dredge site in Port Everglades, Florida. The researchers will employ acute intermittent-flow respirometry exposures, which involve subjecting *Acropora cervicornis* to short-term intervals of elevated turbidity levels. By closely monitoring and analyzing the corals' responses, the study aims to gain insights into the immediate impacts of acute turbidity increase on the physiological and ecological aspects of *A. cervicornis*. The main objectives are to:

- 1. Conduct acute sediment exposure experiments to produce coral metabolic response curves.**
- 2. Examine the effect of fine-grain suspended sediment on the metabolism, health, and growth of selected Florida coral species.**
- 3. Define a biologically relevant benchmark of turbidity (measured in Nephelometric Turbidity Units [NTU]) at which corals begin to display physiological responses to sub-lethal stress.**

The results of this study will contribute to a more comprehensive understanding of the effects of turbidity on the staghorn coral, shedding light on its tolerance thresholds and potential mechanisms of response. This knowledge will be instrumental in developing targeted management strategies and guidelines to minimize the negative impacts of dredging and other activities that induce turbidity in coastal environments. Ultimately, by protecting and conserving *A. cervicornis* and its habitats, we can ensure the long-term survival and resilience of this important and endangered species for future generations.

3. MATERIALS AND METHODS

3.1. Coral Acquisition and Maintenance

Coral fragments of four different genotypes (CU-020, CU-060, CU-002, and CU-014) were collected directly from an *in-situ* nursery and dry-shipped through priority mail to the Bahr Marine Ecology Lab at Texas A&M University-Corpus Christi by Mote Marine Lab (Summerland Key, FL). Once received, the corals were immediately placed in the holding mesocosm to acclimate for three weeks.

The holding mesocosm (350 L) was created with artificial seawater (Red Sea Salt, Red Sea Fish, Tel Aviv, Israel). Water quality was tested weekly, followed by a 25% volume water change. Target water parameters were chosen to mimic the natural environment of *A. cervicornis*: ammonia (0ppm), nitrite (0ppm), nitrate (0-20ppm), phosphate (0-0.3ppm), temperature (25.0°C), salinity (35ppt), pH (8.1), total alkalinity (2500 $\mu\text{mol Kg}^{-1}$), calcium (390-420ppm), and magnesium (1250-1350ppm) (Enochs et al., 2018). Artificial lighting was supplied by four 180W LED fixtures (Model MAD180, Wattshine, China) set to 12 hours of illumination per day from 8:00 to 20:00. At the base of the coral fragments, light measured 150-200PAR. Nutrient levels were monitored using colorimetry (API Saltwater Master Kit, Mars Fishcare, Chalfont, PA and Magnesium, Calcium, Phosphate Profi Test, Salifert, Holland). Weekly measures of pH (Orion Star A111, Thermo Scientific, Waltham, MA) and alkalinity (Eco Titrator, Metrohm AG, Herisau, Switzerland) were also measured to ensure tank health. In addition to water changes, Soda Ash, Calcium Chloride, and Magnesium Mix (Bulk Reef Supply, Golden Valley, MN) doses were supplied as directed by the manufacturer to maintain the water quality. Corals were fed daily with Tropic Marin +NP and a Seachem Phytoplankton/Polyp Lab ReefRoid mix every two days.

3.2. Experimental Design

The experimental design (Table 1) was created prior to the start of the experiments. The design takes into account the target NTU treatments, coral genotype, individual fragments, racks, individual chambers, and the experiment. An identifier is also included to aid in future processing and labeling. Each variable was randomized to prevent bias from affecting the results of the experiment and to prevent confounding factors that may be caused by the chamber or location from affecting the results.

Table 1: The experimental design, including the coral genotype and individual fragments to be put on each rack and in each treatment.

Exp Date	Experiment	Rack	Chamber	Genotype	Fragment	Treatment (NTU)	Identifier
3-Apr	1	A	1	CU-002	5	50	1-002-5-50
	1	A	2	CU-002	4	29	1-002-4-29
	1	A	3	CU-002	6	15	1-002-6-15
	1	A	4	CU-002	9	0	1-002-9-0
	1	B	5	CU-014	2	29	1-014-2-29
	1	B	6	CU-014	1	50	1-014-1-50
	1	B	7	CU-014	6	15	1-014-6-15
	1	B	8	CU-014	10	0	1-014-10-0
10-Apr	2	A	1	CU-020	2	50	2-020-2-50
	2	A	2	CU-020	4	29	2-020-4-29
	2	A	3	CU-020	6	15	2-020-6-15
	2	A	4	CU-020	1	0	2-020-1-0
	2	B	5	CU-060	9	29	2-060-9-29
	2	B	6	CU-060	7	50	2-060-7-50
	2	B	7	CU-060	10	15	2-060-10-15
	2	B	8	CU-060	4	0	2-060-4-0
17-Apr	3	A	1	CU-060	8	50	3-060-8-50
	3	A	2	CU-060	1	29	3-060-1-29
	3	A	3	CU-060	2	15	3-060-2-15
	3	A	4	CU-060	6	0	3-060-6-0
	3	B	5	CU-002	10	29	3-002-10-29
	3	B	6	CU-002	3	50	3-002-3-50
	3	B	7	CU-002	7	15	3-002-7-15
	3	B	8	CU-002	1	0	3-002-1-0
24-Apr	4	A	1	CU-014	8	50	4-014-8-50
	4	A	2	CU-014	7	29	4-014-7-29
	4	A	3	CU-014	3	15	4-014-3-15
	4	A	4	CU-014	5	0	4-014-5-0
	4	B	5	CU-020	8	29	4-020-8-29
	4	B	6	CU-020	9	50	4-020-9-50
	4	B	7	CU-020	7	15	4-020-7-15
	4	B	8	CU-020	10	0	4-020-10-0

3.3. Timeline and Overview

The experiment occurred over a period of four weeks from April 3-28th, 2023, with each week being a separate trial. Corals underwent pre- and post-procedure tests explained below on Days 1 and 5 of each trial, respectively, and the exposure period occurred for seventy-two hours over Days 2, 3, and 4 (Figure 1).

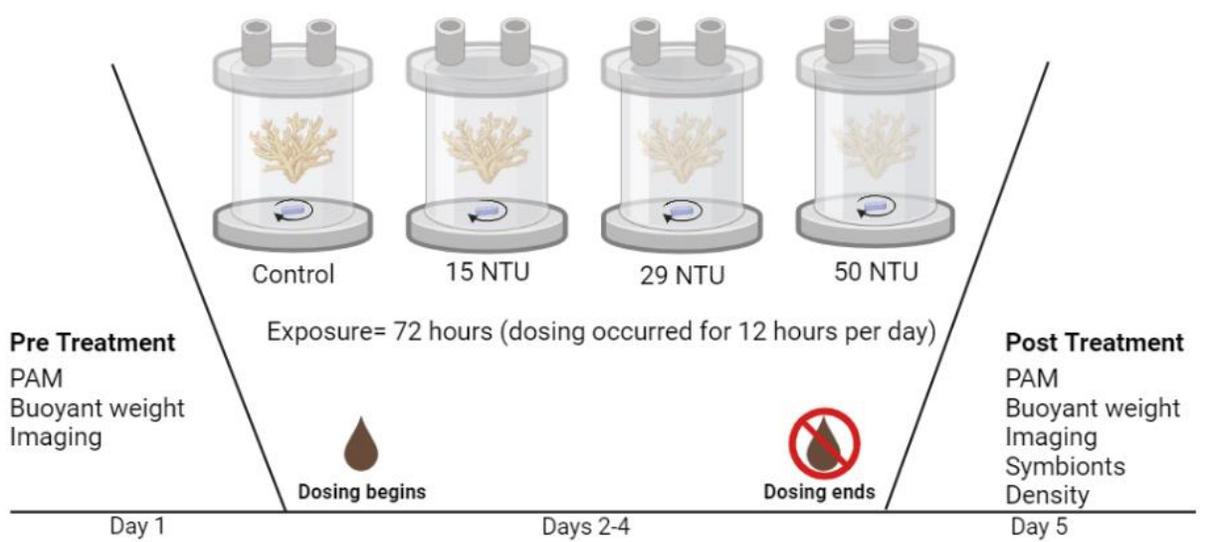


Figure 1: The timeline in which the trials occurred. This figure was generated with BioRender Software.

The corals were removed from the chambers at 09:30 on Day 5 to complete the exposure cycle and begin post-exposure testing. There were two replicate racks of four target treatments: 50 NTU, 29 NTU, 15 NTU, and 0 NTU (control). For each trial, two coral genotypes were randomly assigned to the racks, and the individual coral fragments were randomly assigned to an NTU treatment.

3.3.1. Respirometry Chamber System:

Custom-made 500 mL, 7 cm x 13 cm, cylindrical respirometry chambers (Loligo Systems, Viborg, Denmark) were connected to a 20 L sump via 10mm tubing and a second pump line containing an oxygen sensor (Witrox 4, Loligo Systems, Viborg, Denmark) via 8mm tubing (Figure 2).

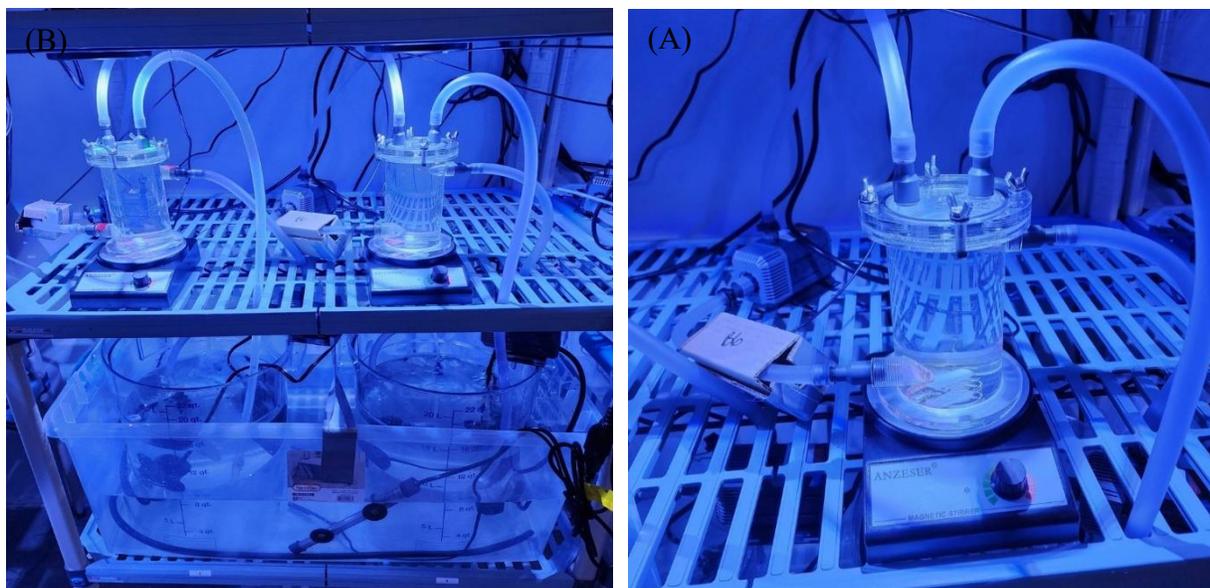


Figure 2: Full chamber (A) and sump setup (B)

External pumps with dimensions 5.7 x 7.9 x 3.7 inches (Eheim Universal 300 Pump, Eheim GmbH & Co.KG, Deizisau, Germany) were used for both the respirometry and sump flush connections. The chambers rested on a stir plate so that a stir bar could physically disturb sediment settling within the chamber. Corals, when they were in the chamber, were placed on a wire pedestal at a height so that the stir bar did not directly disturb the individual and to reduce sediment buildup on the coral plug. A single LED light (A80 Tuna Blue, Kessil, Richmon, CA) was suspended above each chamber to supply light to the corals ($150 - 200 \mu\text{mol of photons m}^{-2} \text{s}^{-1}$). Intake and outtake flush tubing were secured deep in the sump water using suction cups to prevent air from disturbing the respirometry process. The sumps themselves consisted of a 20L clear cylindrical container placed in a water bath, each with a digital thermometer, for temperature control (Figure 2). Two adjacent sumps fit in each temperature tub, making for two tubs in a replicate rack. The room temperature was consistently 21.1°C . In the sump were two pumps (Maxijet 400, Marineland Spectrum Brands Pet, LLC., Blacksburg, VA) aimed at the bottom of the sump to disturb settling sediment, and an air stone placed near the surface to supply oxygen without bubbles entering the chamber system. Air was supplied to all racks by a single 4-channel air pump (95 air pump 4-way, Fedour,) with each channel split into two stones for adjacent sumps.

Turbidity in the sump was monitored and dosed hourly. A high NTU stock solution was used to maintain the correct NTU within each sump. The target NTU concentrations were (15 ± 10 NTU, 29 ± 10 NTU, and 50 ± 10 NTU). Sump NTU levels were monitored every two hours and dosed hourly for twelve hours from 8:00 to 20:00 during the exposures. Dose volumes differed in response to the sediment uptake by the individual respiratory chamber systems. If NTU concentrations within the sumps exceeded the desired range (>10 NTU), all dosing was halted until the NTU dropped back into the target range. If NTU decreased below the set range, sediment was added directly to the sump until it reached the target value.

3.3.2. Exposure Timeline (Figure 1):

Day 1 consisted of pre-procedure tests. For each coral participating in the trial, an individual photograph, the buoyant weight, coral volume displacement, and coral wet weight were taken, as well as dark-acclimated pulse amplitude modulation (PAM) measurements at 10:00 prior to the start of each exposure. PAM measurements were obtained using a Diving PAM 2.0 (Heinz Walz GmbH, Effeltrich, Germany). This technique is used to measure the photosynthetic efficiency of coral symbionts and detect the impacts of treatments on photosynthesis. Corals were dark acclimated for at least 20 minutes before PAM measurements were taken. Witrox oxygen sensors were calibrated using two-point calibration (0% and 100%) in air-saturated and sodium sulfate before every trial, and background oxygen was recorded afterward to account for bacterial oxygen consumption. Witrox system was programmed to flush the chambers every 30 minutes for 120 seconds with sump water for the duration of the exposure. The chambers were allowed to flush for 15 seconds before sample collection occurred to avoid collecting water from the tubing rather than the chamber.

On Day 2, the seventy-two-hour exposure period began for the corals. The corals were placed in the chambers at approximately 08:00. During the exposures, total alkalinity (TA) and turbidity samples were collected every four hours (i.e., 08:00, 12:00, 16:00, and 20:00) for each chamber system. TA samples were collected from the sump before the target flush sequence and from the outtake tubing at the start of the flush sequence. The water samples were collected in 150 mL airtight borosilicate glass bottles, stored in a water bath at 25°C, and analyzed within 2 hours of collection. All samples were processed within two hours of the collection since sediment has negligible effects on TA within that time frame. The purpose of collecting these samples was to monitor the TA levels in the water and the changes in alkalinity due to calcification or dissolution by the corals.

Turbidity samples were collected during the flush sequence in 50 mL falcon tubes and analyzed using a turbidimeter (HACH 2100Q Portable Turbidimeter, HACH Company, Loveland, CO) to monitor treatment stability. Parameters of the sump were recorded before the first flush sequence on Day 2 and every two hours from the 8:00 collection until 22:00.

The parameters measured were salinity, temperature, dissolved oxygen (DO mg L⁻¹), and percent dissolved oxygen (DO%) using a YSI multiparameter meter (Pro DSS, YSI Inc., Yellow Springs, OH). This monitoring was done to assess the water conditions entering the chamber. TA, turbidity, and sump parameters were recorded similarly throughout the exposure.

Day 5 concluded the exposure period. Final sump parameters (TA, turbidity, DO%, and salinity) were taken at approximately 8:30. The corals were then removed from the chamber systems at 9:30 and dark acclimated (20 mins), and PAM measurements were performed at 10:00. Then, their post-exposure photographs were taken, and buoyant weights recorded. Half of each individual was fragged off using sterilized coral shears. The top half of every fragment was stored in an -80°C freezer, while the bottom half was placed back in the holding mesocosm to recover. Recovery assessments were not conducted. This same process was repeated for each trial with new coral fragments.

3.4. Post-experimental processing

After the experiment, various biological analyses were conducted on the coral fragments. This included measuring the concentration of total protein and chlorophyll (a , c_2 , total), determining the abundance of symbiotic algae (zooxanthellae), assessing the bulk skeletal density, and calculating the surface area of each coral fragment. To begin, the coral tissue was removed using an airbrush and phosphate buffer solution (PBS) using a Paasche Airbrush Co. (Kenosha, WI). The resulting mixture was then homogenized for thirty seconds using the Tissue Master 125 from Omni International (Kennesaw, GA). The homogenized slurry was divided into separate sample sets for protein, zooxanthellae, and chlorophyll analysis. This was done by using a vortex mixer from Four E's Scientific and a centrifuge from VWR International, LLC. (Radnor, PA). The abundance of algal symbionts (zooxanthellae) cells was determined by counting them using a hemocytometer (Bright-Line, Hauser Scientific, Horsham, PA) and a microscope at 10X magnification (ICC50W, Leica Microsystems Inc., Deerfield IL). Protein and chlorophyll absorbance was measured using a spectrophotometer (Spectromax M3, Molecular Devices, LLC., San Jose, CA), with PBS and 100% acetone as a blank, respectfully. Next, the coral skeletons were bleached and weighed while wet using the VWR-4002B2 balance from VWR International (Radnor, PA). The skeletal density of each coral fragment was determined by calculating the mass of the coral and the total water displacement. The coral skeletons were then dried for four hours at 60°C using the Drying Oven DX302C from Yomato Scientific America Inc. (Santa Clara, CA). Three-dimensional scans of the coral skeletons were generated and edited using the Einscan-SE 3D Scanner from Hangzhou Shining 3D Tech Co., LTD. (Hangzhou, China) and MeshLab software from the National Research Council and Institute of Information Sciences and Technology (Pisa, Italy). These scans were used to calculate the total surface area of each coral fragment. Finally, all the biological results obtained for the individual coral fragments were standardized to their respective surface area. This allowed for the determination of the total abundance of zooxanthellae, chlorophyll, and protein in the coral tissue.

3.5. Statistical Approach

All data were analyzed using JMP Pro 16 (The SAS Institute, Cary, NC). Analysis of Variance (ANOVA) was used in conjunction with Tukey Honest Significant Differences (HSD) to determine the significance of differences seen in the data. The normality of the data was confirmed using a Shapiro Wilks W test. Equal variance was tested using O'Brien, Brown-Forsyth, and Barlette testing. Data that was not normal was analyzed with Kruskal Wallis and Wilcoxon paired tests. The same level of significance was used for all statistical tests performed ($\alpha=0.05$). In cases where the explanatory variable was continuous rather than categorical, regression was used to determine if the treatments had any effect.

4. RESULTS AND DISCUSSION

4.1. Data Preparation and Preliminary Analysis

Preliminary assessment of the turbidity treatments revealed that there were significant differences in the treatment NTUs between trials ($\alpha= 0.05$) and significant differences within treatment replicates within trials ($\alpha=0.05$) (Table 2). This indicated that the treatment replicates were not

true. Therefore, the use of ANOVA on the categorical treatments would not be an accurate assessment of the data. While the exclusion of outliers (trials in which the treatment mean is outside of ± 10 NTU) may aid in more consistent treatment replication, it would result in losses of valuable data. In order to prevent such a loss, treatments were reconsidered to be continuous variables rather than categorical variables. This enabled linear regression analysis, which uses both ANOVA and correlation to be performed.

Table 2: Summary of statistical testing performed on the turbidity treatment data. All data was used for the analysis of statistical testing performed on the turbidity treatment data. All data used for the analysis of turbidity treatments is not normalized.

Data Analyzed	Anova (Parametric)	Normality (Shapiro Wilks)	Variance (O'Brian)	Kruskal Wallis (Nonparametric)	Pairwise Analysis
Turbidity All 4 Exps by Chamber	x ^z	x	x	x	Tukey HSD
Turbidity Exp 1 by Chamber	x	x	x	x	Tukey HSD
Turbidity Exp 2 by Chamber	x	x	x	x	Tukey HSD ^{*y}
Turbidity Exp 3 by Chamber	x	x	x	x	Tukey HSD
Turbidity Exp 4 by Chamber	x	x	x	x	Tukey HSD*

z "x" indicates significance ($\alpha=0.05$)

y "*" indicated treatment replicates were not significantly different from each other.

The environmental conditions within the chamber and sump systems were monitored continuously (HOBO) and intermittently (all other parameters). All parameter data were averaged across the respective trials to show the optimal conditions for *A. cervicornis* were maintained throughout the experiment (Table 3). Lighting was measured once over a two-hour period after corals were removed from the system to provide insight into light conditions within the chambers (Table 4). It is important to note that the light conditions are not representative of what the corals received during the exposures since no sediment dosing was carried out on day 4. Hence, the turbidity levels were likely to be much lower on day four than they were during the exposure periods.

Table 3: Average weekly chamber and sump conditions over the exposure period.

	Sump	Turbidity NTU	YSI Temp (° C)	HOBO Temp (° C)	Salinity (ppt)	Dissolved O2 %	FTA (mol kg-1)
TRIAL 1							
	1	43.66	24.9	25.3	35.5	97.3	2771.3
	2	24.25	25.1	25.6	35.5	95.8	2636.0
	3	10.12	24.9	25.5	35.2	97.9	2487.4
	4	0.98	24.9	25.5	35.5	99.9	2461.3
	5	25.91	24.9	25.2	35.6	99.1	2589.9
	6	32.25	24.9	25.3	34.8	99.0	2632.2
	7	13.17	24.6	25.2	35.6	99.6	2976.9
	8	0.93	24.7	25.3	35.4	100.0	2437.0
TRIAL 2							
	1	42.83	24.8	25.2	35.3	97.2	2667.9
	2	22.31	24.9	25.4	35.4	98.7	2564.4
	3	11.09	24.9	25.4	35.4	99.2	2487.4
	4	0.98	24.8	25.4	35.3	99.4	2461.3
	5	26.66	24.8	25.3	35.5	100.0	2544.3
	6	40.99	25.0	25.5	35.4	99.5	2550.7
	7	9.43	24.7	25.3	35.3	99.4	2976.9
	8	0.83	24.7	25.3	35.4	100.3	2569.7
TRIAL 3							
	1	50.79	24.9	25.3	35.4	99.1	2613.1
	2	26.83	25.0	25.5	35.4	99.9	2588.1
	3	14.28	25.1	25.6	35.5	100.0	2521.3
	4	0.83	24.9	25.5	35.6	100.0	2462.2
	5	30.42	25.0	25.5	35.4	100.3	2501.8
	6	41.36	24.9	25.4	35.4	99.0	2605.8
	7	13.26	24.8	25.4	35.5	99.4	2483.6
	8	0.71	24.8	25.4	35.6	100.2	2451.3
TRIAL 4							
	1	50.99	25.0	25.4	35.5	99.0	2523.1
	2	28.88	25.2	25.7	35.4	99.7	2503.6
	3	14.50	25.0	25.5	35.4	99.6	2461.1
	4	0.91	25.0	25.6	35.5	100.0	2365.4
	5	31.67	25.1	25.6	35.4	100.1	2468.7
	6	48.18	25.1	25.6	35.5	100.0	2570.5
	7	14.34	24.9	25.4	34.5	99.9	2421.4
	8	0.81	24.7	25.3	35.3	99.9	2372.8

Table 4: Light conditions within the individual chambers and treatments post-trial. Light readings are not correspondent to the NTU target conditions.

Chamber	Turbidity (NTU)	Temp (°C)	Light (lux)	PAR ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)
1	53.2	25.9	3155.5	64.6
2	18.3	25.9	4681.1	95.2
3	4.1	25.8	8091.2	163.6
4	0.9	25.9	10026.2	202.5
5	27.0	26.0	6088.7	123.4
6	32.4	25.8	4268.9	86.9
7	5.7	25.6	7418.6	150.1
8	0.9	25.5	7525.0	152.3

4.2. Coral health response by controls

Coral health was assessed by looking only at the 0 NTU controls using genotype as the explanatory variable (Table 5). By assessing the controls, we can easily determine whether any one genotype was significantly healthier/sicker than the rest, regardless of the treatment. If they are significantly different, blocking by them would be necessary to prevent genotype from affecting the turbidity results. It also provides us with baseline information about the health of each genotype.

Table 5: Summary of all statistical tests performed on the biological responses of the genotype controls.

Data	Anova (Parametric)	Normality (Shapiro Wilks)	Variance (O'Brien)	Kruskal Wallis (nonparametric)	Notes
Protein	0 ^z	0	x ^y	0	
Zoox	0	x	x	0	
Chl a ^w	x	x	x	0	Genotypes 14, 20, and 60 differ (anova)
Chl c ₂	0	x	x	0	
Chl a & c ₂ ^v	x	x	x	0	Genotypes 14 and 60 differ (anova).
Alpha	0	x	x	0	
Fv/Fm	0	x	x	0	
ETRmax	0	x	x	0	
Y(NPQ)	0	0	x	0	
Calcification	0	0	0	0	

z "0" indicates non significant difference ($\alpha=0.05$).

y "x" indicates significance ($\alpha=0.05$).

w,v Anova and Kruskal wallis testing contradict each other.

Analysis of the symbionts showed that there were no significant differences in the density of zooxanthellae between the coral genotypes (Kruskal-Wallis, $p = 0.1038$). This indicated that the genotype did not significantly affect the density of symbionts present in the coral tissue. The range

in symbiont densities of the controls was about 9.3×10^6 to about 26.8×10^6 cells cm^{-2} . The trial had no effect on symbiont density within the controls (ANOVA, $p = 0.1388$).

The chlorophyll concentrations were analyzed by comparing chlorophyll *a*, chlorophyll *c*₂, and total chlorophyll (chlorophyll *a* + *c*₂). Analysis of chlorophyll by genotype revealed that the chlorophyll concentration in genotype CU-014 was higher (11.36 ± 0.70) than those found in CU-020 (6.51 ± 0.70) and CU-060 (6.15 ± 0.70), which had the lowest average concentrations. However, the differences were not significant (Kruskal-Wallis, $p = 0.1038$). The concentration of chlorophyll *a* in CU-002 was not significantly different from any genotype. The average chlorophyll *c*₂ concentrations ranged from CU-060 ($3.22 \pm 0.54 \text{ nm cm}^{-2}$) to CU-014 ($5.67 \pm 0.54 \text{ nm cm}^{-2}$) in CU-014 but were not significantly different (Kruskal-Wallis, $p = 0.16$). The total chlorophyll CU-060 was 45% less than that found in CU-014. All other genotypes had similar average total chlorophyll concentrations to both CU-060 and CU-014.

Protein concentrations did not significantly differ between the treatments and had an average of 0.74 mg cm^{-2} .

For PAM measures, variables Alpha, Fv/FM, ETRmax, and Y(NPQ) were analyzed based on the percent change of pre-exposure (Day 1) and post-exposure (Day 5) of the same coral. The average value for alpha ranged from 8.24 in CU-002 to 24.4 in CU-060 but was not significantly different. All genotypes produced similar values of Fv/FM, which averaged of 3.99. Similarly to Alpha and Fv/FM, ETR max and Y(NPQ) did not vary significantly between the genotypes.

4.3. Biological responses to turbidity

The average biological responses for each chamber to show the variation in responses by the NTU treatment are outlined in Table 6.

Table 6: Average biological responses standardized to the surface area of the individual coral frags.

Trial No.	Chamber No.	Turbidity	Symbiotic Abundance	Chlorophyll <i>a</i>	Chlorophyll <i>c</i> ₂	Chlorophyll (<i>a</i> + <i>c</i> ₂)	Protein	% O ₂ sat hr ⁻¹
		NTU	(cells·cm ⁻²)	(ug·cm ⁻²)	(ug·cm ⁻²)	(ug·cm ⁻²)	(mg·cm ⁻²)	
Trial 1	1	43.66	2.37E+07	15.33	8.10	23.43	0.57	-6.21
	2	24.25	1.61E+07	8.38	3.71	12.09	0.67	-7.23
	3	10.12	1.45E+07	8.62	3.52	12.14	0.59	-13.77
	4	0.98	2.15E+07	9.71	5.28	14.99	0.59	-11.12
	5	25.91	2.67E+07	11.14	4.23	15.36	0.65	-4.06
	6	32.25	1.50E+07	10.08	4.33	14.42	0.63	-8.74
	7	13.17	2.54E+07	10.69	4.49	15.19	0.58	-6.74
	8	0.93	2.68E+07	11.48	5.38	16.85	0.69	-8.53
Trial 2	1	42.83	1.06E+07	3.77	3.20	6.97	0.64	-9.79
	2	22.31	9.24E+06	6.69	2.80	9.49	0.67	-7.91
	3	11.09	1.33E+07	4.50	2.08	6.58	0.57	-4.97
	4	0.98	1.42E+07	6.92	3.90	10.82	0.61	-11.86
	5	26.66	2.38E+07	12.12	7.60	19.72	0.66	-0.52*
	6	40.99	1.87E+07	7.26	3.26	10.52	0.51	-8.33
	7	9.43	2.02E+07	6.38	3.68	10.07	0.59	-7.00
	8	0.83	2.04E+07	5.62	3.02	8.63	0.62	-6.91
Trial 3	1	50.79	1.33E+07	6.87	4.13	11.00	0.52	-6.46
	2	26.83	2.61E+07	9.84	5.35	15.19	0.63	-5.69
	3	14.28	1.50E+07	5.26	2.56	7.82	0.56	-8.80
	4	0.83	1.44E+07	6.68	3.43	10.11	0.62	-8.32
	5	30.42	2.19E+07	6.02	2.93	8.95	0.64	-3.90
	6	41.36	1.89E+07	6.23	3.05	9.29	0.63	-4.82
	7	13.26	1.91E+07	7.00	4.04	11.04	0.69	-3.24
	8	0.71	1.52E+07	7.27	3.94	11.21	0.75	-4.61 ^{*z}
Trial 4	1	50.99	1.26E+07	7.52	4.12	11.64	0.59	-10.90
	2	28.88	1.23E+07	13.50	7.78	21.28	0.71	-5.39
	3	14.50	1.74E+07	12.33	7.19	19.53	0.63	-0.89
	4	0.91	1.64E+07	11.25	5.97	17.23	0.69	-8.32
	5	31.67	1.08E+07	4.72	2.59	7.31	0.65	-3.90
	6	48.18	7.33E+06	9.36	4.62	13.98	0.78	-4.82
	7	14.34	9.24E+06	6.80	3.61	10.41	1.14	-3.24
	8	0.81	9.28E+06	6.09	5.44	11.53	1.05	-4.61

z "*" r² decreased from >0.90 to >0.80

The biological responses of the corals to the turbidity levels were analyzed with regression analysis. The statistical significance of the biological response data is summarized in Table 6. All PAM measurements were assessed, but only Fv/Fm is of interest in this study (Table 7).

Table 7: Average percent change in PAM responses by chamber and average turbidity.

Trial No.	Chamber No.	Turbidity (NTU)	Alpha	Fv/FM	ETRmax	Y(NPQ)
1	1	43.7	13.5	5.2	9.0	-3.6
	2	24.3	16.8	4.3	-5.6	-14.1
	3	10.1	23.9	4.8	29.9	-33.4
	4	1.0	6.2	1.7	-12.0	-42.1
	5	25.9	17.7	3.4	2.3	-3.1
	6	32.2	15.2	7.3	-7.6	-13.4
	7	13.2	-13.6	0.5	-27.7	-1.5
	8	0.9	0.8	3.9	-10.9	37.2
2	1	42.8	41.2	10.3	-16.0	-50.5
	2	22.3	60.1	16.9	-8.6	-58.2
	3	11.1	34.8	7.3	19.3	-27.5
	4	1.0	-0.2	3.5	-7.7	-20.3
	5	26.7	24.6	4.3	15.6	-54.4
	6	41.0	35.2	7.3	15.1	-47.4
	7	9.4	39.7	2.5	47.8	-38.5
	8	0.8	15.6	1.8	0.2	-24.8
3	1	50.8	28.4	4.9	-21.4	-46.9
	2	26.8	16.9	3.4	7.3	-40.2
	3	14.3	19.5	3.7	-9.2	-41.6
	4	0.8	33.2	7.5	-26.7	-56.4
	5	30.4	11.6	4.8	14.1	-40.1
	6	41.4	17.2	7.4	-9.4	-34.3
	7	13.3	16.9	5.8	-8.4	-45.2
	8	0.7	10.3	3.3	3.7	-30.2
4	1	51.0	9.0	9.2	-8.2	-31.7
	2	28.9	29.2	7.2	12.7	-29.7
	3	14.5	15.6	2.1	14.6	-10.8
	4	0.9	25.0	3.4	7.6	-33.8
	5	31.7	76.5	11.3	4.2	-40.6
	6	48.2	47.3	10.5	61.9	-64.7
	7	14.3	51.8	4.0	-7.3	-38.2
	8	0.8	30.4	6.7	-4.7	-43.9

Table 8: Summary of the statistical testing used to determine the biological responses of the coral by trial and by genotype.

Data	Symbiont Measures ^z					Physiological Measures				
	Symbiotic Cells	Chl <i>a</i>	Chl <i>c</i> ₂	Chl <i>a</i> + <i>c</i> ₂	Protein	Alpha	Fv/FM	ETRmax	Y(NPQ)	Calcification (Day 3 ΔTA)
Trial	0 ^y	0	0	0	0	0	X ^x	0	0	0
Genotype 2	0	0	0	0	0	0	X	0	X	0
Genotype 14	0	0	0	0	0	0	0	0	0	0
Genotype 20	0	0	0	0	0	0	0	0	0	0
Genotype 60	0	0	0	0	0	0	0	0	0	0

^z Symbiont measures were all standardized by the surface area of the coral

^y "0" nonsignificant differences ($\alpha=0.05$)

^x "X" significant differences ($\alpha=0.05$)

4.3.1. Across Genotypes

The biological responses of the corals to the turbidity levels were analyzed with regression analysis. Regressions were initially performed blocking by trial to reduce the effect of initial coral health on the biological responses. Further investigation revealed blocking on trial had no change on the results of the other biological response variable. The analysis of symbiont density did not show any correlation between the abundance of cells per cm² of coral to the concentration of NTU (ANOVA, $p = 0.44$). The protein content of the coral fragments after the exposure averaged 0.66 mg cm⁻². The regression of protein concentration by NTU concentration did not show a significant difference in the concentration of protein per cm² of coral across trials (ANOVA, $p = 0.22$). Chlorophyll *a*, *c*₂, and total chlorophyll (*a* + *c*₂) showed no significant correlation to turbidity concentration across trials (ANOVA, $p \geq 0.75$). The percent change in PAM parameters alpha, Y(NPQ), and ETRmax showed no significant correlations to turbidity across all trials (ANOVA, $p \geq 0.19$). Fv/Fm values exhibited a positive, weak correlation to turbidity with a slope of 3.689 and an R² of 0.21 (ANOVA, $p = 0.0087$).

4.3.2. Within Genotype

The biological responses described in the previous section were also analyzed using linear regression within each genotype to identify if the genotype played a role in physiological stress response to turbidity. Similar to the analysis across trials, protein and chlorophyll concentration, symbiont density, and ETRmax and alpha showed no significance within each of the four genotypes (ANOVA, $p > 0.05$). Fv/Fm showed significance in only CU-002 with a positive correlation to increasing turbidity. Additionally, CU-002 expressed a significant positive correlation (ANOVA, $p = 0.0495$, R² = 0.50) of Y(NPQ) to turbidity. These regressions are shown in Figure 3. None of the other genotypes showed any significant correlation between these PAM parameters and turbidity levels (ANOVA, $p > 0.05$).

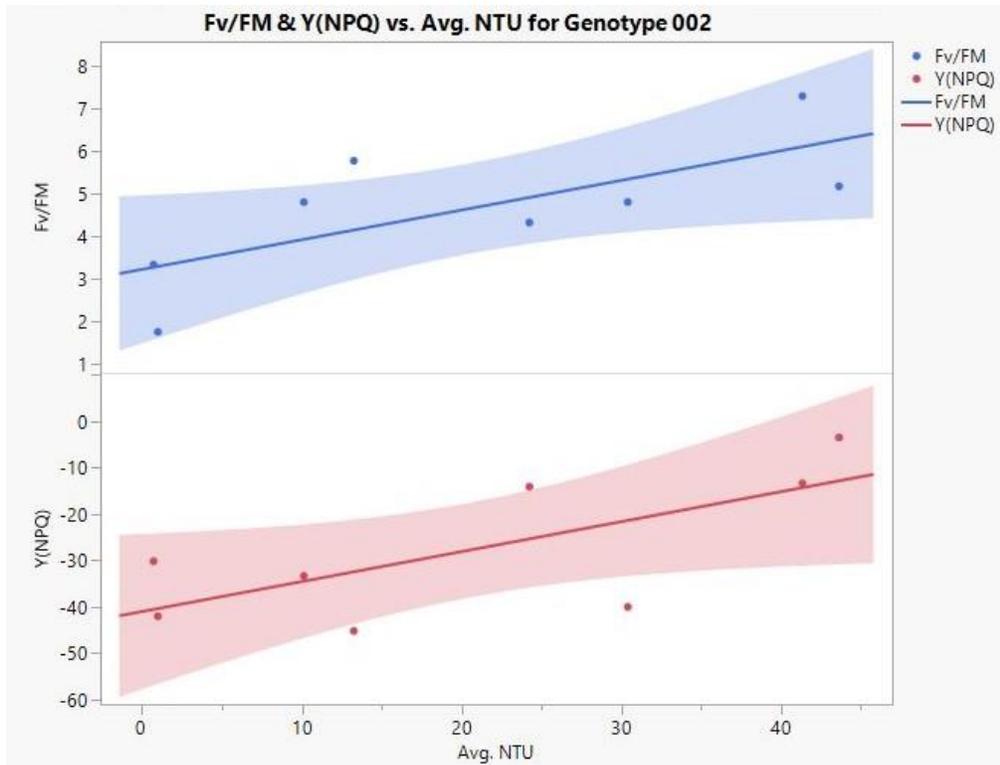


Figure 3: F_v/F_M and $Y(NPQ)$ vs. Average NTU for Genotype 002. F_v/F_M is shown in blue, while $Y(NPQ)$ is shown in red. The red and blue lines represent the significant statistical regression for their respective set of data.

4.4. Calcification response

Total alkalinity (TA) is influenced by bicarbonate and carbonate ion concentrations, as well as various minor compounds (Gómez Batista et al., 2020; Wolf-Gladrow et al., 2007). In the calcification analysis, the focus was on examining changes in alkalinity. Calcification involves the consumption of carbonate or bicarbonate ions through a reversible reaction:



For every mole of CaCO_3 produced, calcification consumes 2 moles of HCO_3^- , resulting in a decrease of 2 moles in total alkalinity (TA) (Reaction Eq 1). The rate of net calcification (gross calcification - dissolution) can be determined by measuring TA before and after incubating an organism or a community. However, it should be noted that this method assumes that calcification is the sole biological process influencing TA (Smith & Key, 1975). To calculate the change in alkalinity, the following formula was used:

$$\Delta TA = TA_S - TA_C \text{ (Eq 2)}$$

Here, ΔTA represents the overall change in the sump over a 30-minute period, TA_S denotes the total alkalinity at the sump, and TA_C refers to the alkalinity of the chamber. In this case, $-\Delta TA$ indicates the dissolution of CaCO_3 into the water, and $+\Delta TA$ indicates calcification or the creation of CaCO_3 skeleton. The ΔTA of each chamber over time for each exposure (Figure 4).

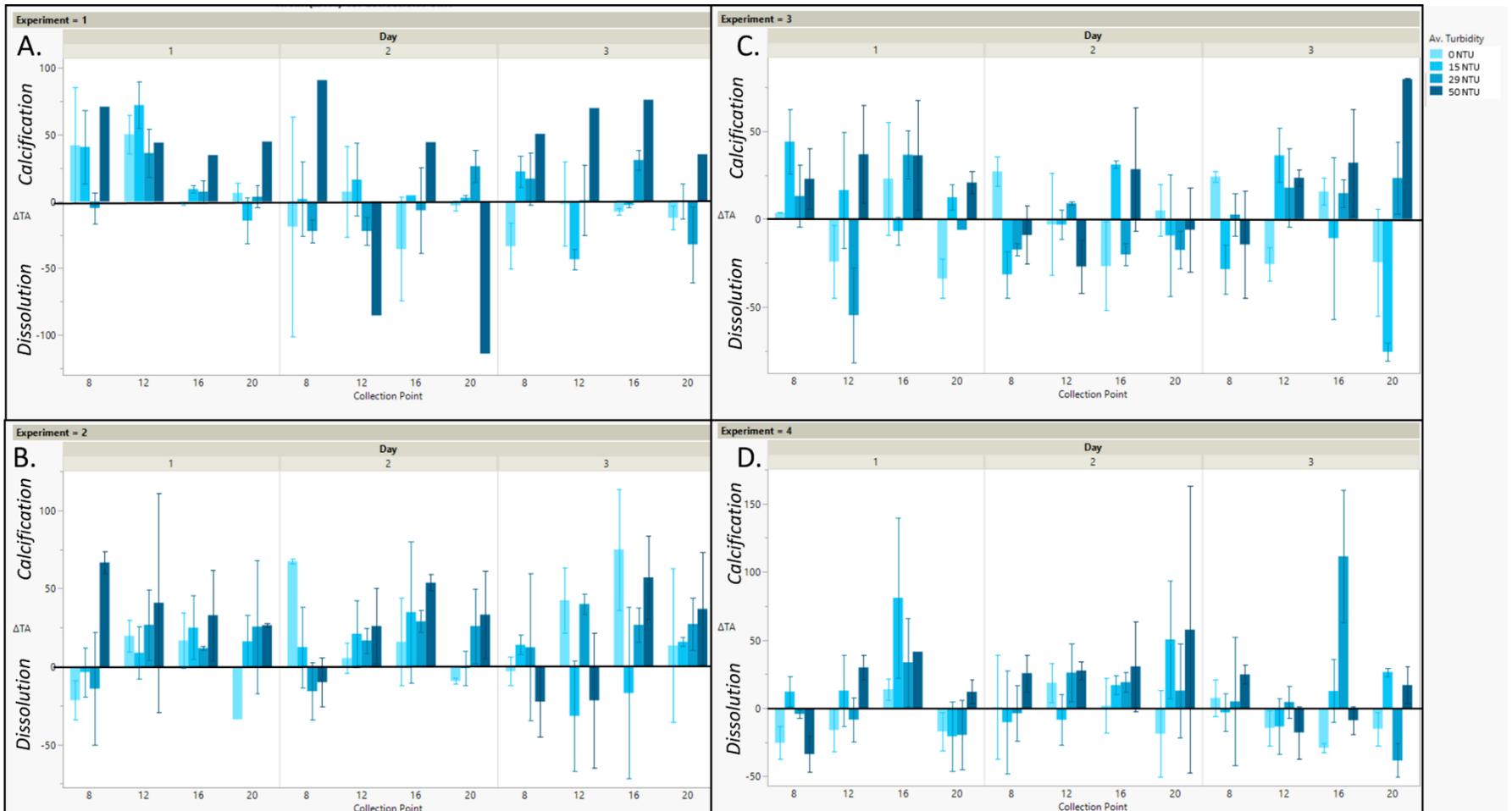


Figure 4 : ΔTA vs. Collection Point over each Experiment. The color of the bars represents the target turbidity treatment. Above the black line at $0\Delta TA$ indicates calcification, and below the black line indicates dissolution of the coral skeleton. Experiment 1 is Graph A, Experiment 2 is Graph B, Experiment 3 is Graph C, and Experiment 4 is Graph D.

In this analysis, the ΔTA values at the 12:00 collection on Days 1 and 3 were selected for further examination. This choice aimed to compare the peak calcification points at the beginning and end of the exposure, allowing for an assessment of any changes in calcification due to water turbidity. The ΔTA values were analyzed across all trials as well as within each genotype.

4.4.1. Regression comparing day 3 to NTU

A linear regression model was generated using ΔTA of only day 3 across trials and within each genotype to compare ΔTA results to the average NTU. The trial was blocked in order to prevent possible response variation caused by differences in coral health. Regression results showed that ΔTA in response to NTU across all trials and within all genotypes was not significant.

4.4.2. ANOVA Comparing Day 1 and Day 3 ΔTA

ANOVA was performed within each genotype and across all trials to compare the difference from day 1 to day 3 ΔTA . Across all genotypes, there was no significant difference between day 1 and day 3 ΔTA . Genotypes CU-002, CU-020, and CU-060 also showed no significant difference in ΔTA between day 1 to day 3 and ΔTA . The ANOVA of Genotype CU-014, however, expressed a significant decrease in ΔTA on day 3 compared to day 1 with a $p = 0.004$ ($F < 0.05$). The data was normal (Shapiro-Wilks, $p = 0.1146$, $\alpha = 0.05$) and had equal variance (O'Brien, $p = 0.1033$, $\alpha = 0.05$), fulfilling the assumptions of an ANOVA test. The ΔTA of Genotype CU-014 is expressed in Figure 5.

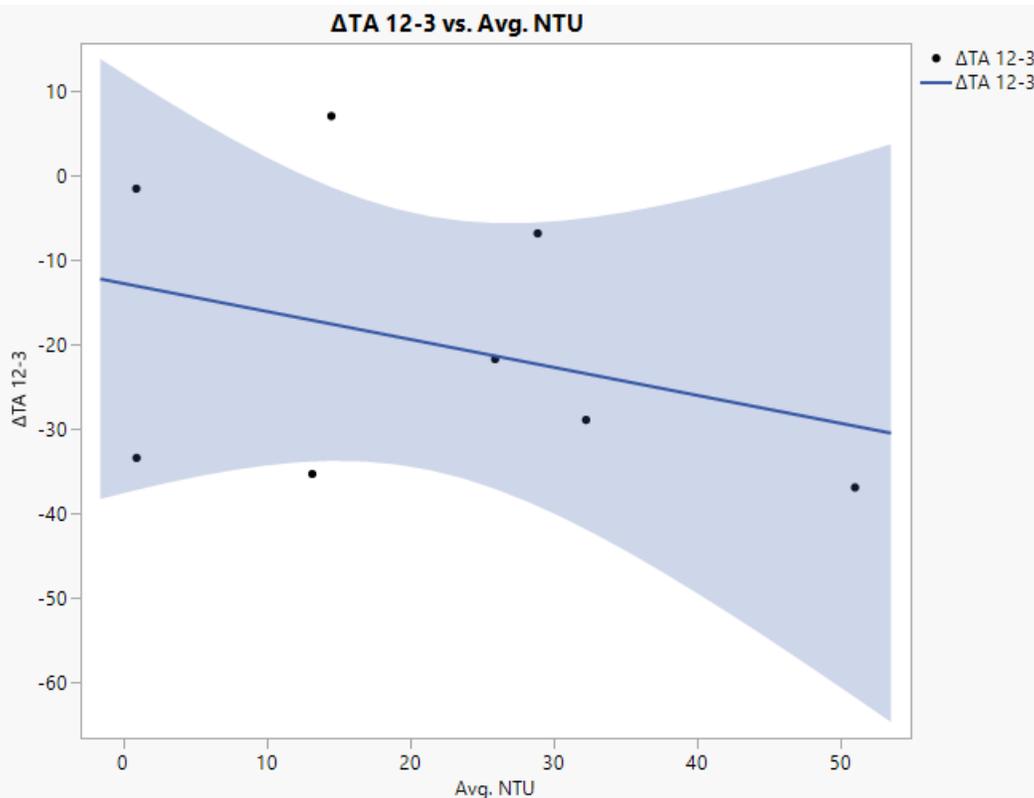


Figure 5: TA from days 1 to 3 of 12:00 Collection for Genotype CU-014.

4.5. Respiration Response

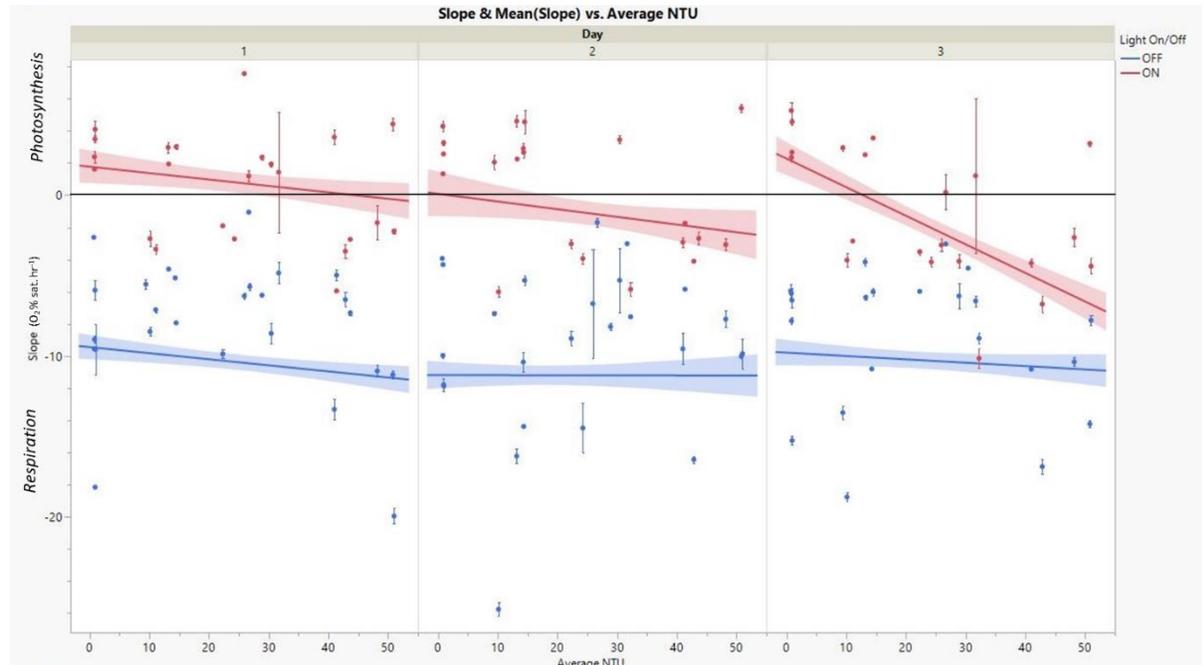


Figure 6: Slopes of oxygen saturation % ($\pm SE$) plotted against average NTU separated by each day of the trials. Red indicates the lights were on and photosynthesis was occurring, while blue indicates the lights were off and respiration was occurring. A dotted line was added at 0 slopes (neutral change in oxygen saturation).

When analyzing the respiration data, the slope of oxygen saturation (O_2 % sat hr^{-1}) was compared across treatments. AutoResp gives one average slope of oxygen consumption along with an R^2 value for each flush cycle. All slopes that were not associated with an $R^2 > 0.90$ were removed, except for chamber 5 in trial 2 and chamber 8 in trial 3, where we included values with an $R^2 > 0.80$ as those chambers did not maintain an $R^2 > 0.9$ for any flush cycles. Fig 6 displays the slopes against the average NTU of each chamber during each trial, split into each day. Red represents when the lights were on during the experiment and indicates photosynthesis would be occurring, while blue represents lights off/nighttime where solely respiration would be occurring. The rate of respiration seems unchanged throughout the three days and across the gradient of turbidity, but there is a trend seen with respiration while the lights are on (photosynthesis producing oxygen). On days 1 and 2, the mean oxygen saturation hovers around and just below 0, meaning there is no net oxygen production or consumption, with a slightly lower average rate of saturation towards the higher NTU levels. On day 3 during the day, the slopes of the control group are relatively unchanged, while the slopes in the higher NTU treatments drastically drop, signified by the red line of best fit on day 3 ($R^2 = 0.37$). This indicates that the higher NTU treatments experienced inhibited photosynthesis, but not until day 3 of the exposure. Since the slopes during the day are trending downward, we can expect a further decrease in the rate of photosynthesis with a longer exposure period.

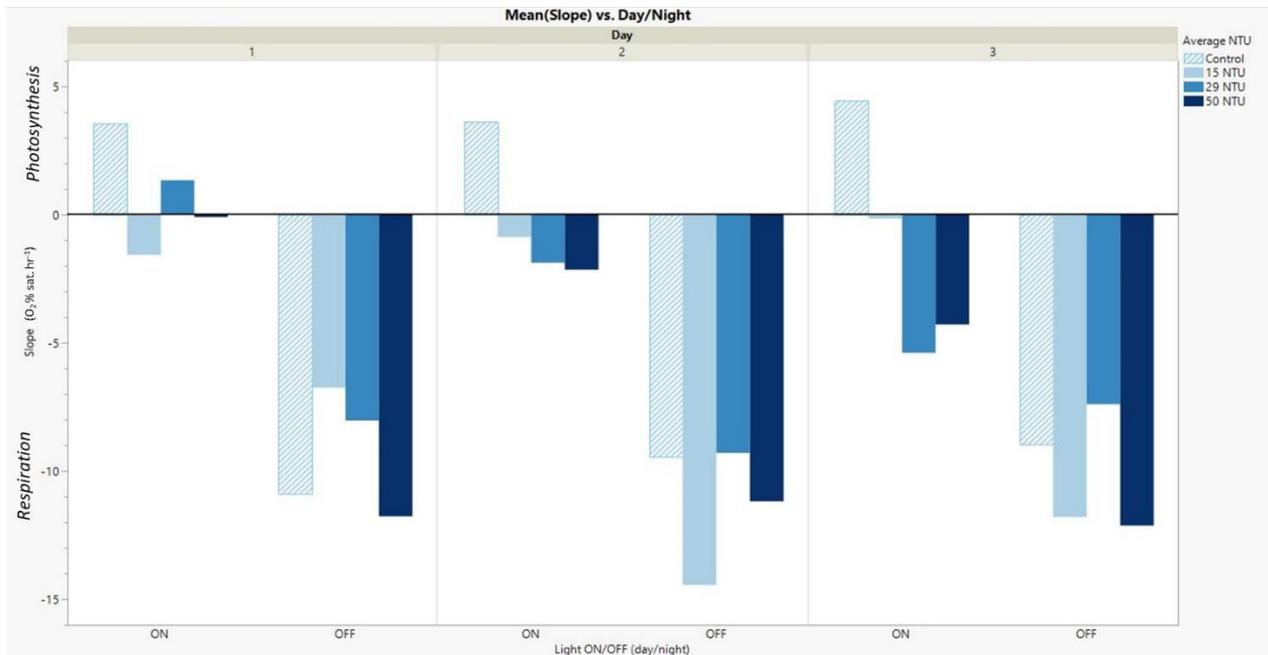


Figure 7: Slopes of oxygen saturation % (\pm SE) plotted by day/night cycle (lights on/off) separated by each day of the trials. The shade of blue represents the treatment, with darker blue indicating a higher NTU treatment.

Figure 7 reorients the data to plot the average slopes of oxygen saturation against the light cycle and separated by NTU treatment. The control group is the only group, besides day 1 of 29 NTU, that had any positive average slope in oxygen saturation with the lights on, where a positive slope indicates oxygen production from photosynthesis. This could mean that the turbidity treatments were not allowing the coral to photosynthesize and that they were relying solely on respiration, which consumes oxygen, causing a more negative slope of oxygen saturation.

5. DISCUSSION

Turbidity can have several effects on corals and their surrounding environment. One of the primary influences on corals is the reduction of light availability. High turbidity reduces the penetration of sunlight into the water, leading to reduced light availability for the coral symbiotic algae. Reduced light availability can result in decreased photosynthetic activity and, consequently, reduced coral growth and calcification rates (Erfteimeijer et al., 2012; Jones et al., 2020). Our results showed an increase in Fv/Fm in coral symbionts as NTU increased, indicating a response to decreased light availability. Coral symbionts needed to increase their photoreceptive efficiency to maintain photosynthetic output in greater light attenuation. This aligns with previous studies that show Fv/Fm is the first variable to respond under altered environmental conditions (Philipp & Fabricius 2003; Piniak, 2007).

Turbid waters can carry suspended particles that may contain nutrients or organic matter. While some nutrients can be beneficial for corals, excessive nutrient loading due to turbidity can lead to an imbalance in the nutrient dynamics of coral reef ecosystems and promote the growth of algae, which can outcompete corals for resources and compromise their health. (Lunt et al. 2020; Pollock et al. 2014). This study did not focus on the effects of sediment-based nutrient composition, but it

is important to note that there was increased bacterial growth in some chambers with higher NTU treatments.

High turbidity often indicates an increased sediment load in the water. Sediments can settle on coral surfaces, smothering the coral tissue and interfering with feeding, respiration, and light capture. Excessive sedimentation can cause stress, tissue damage, and even mortality in corals. (Abdel-Salam & Porter, 1988; Erftemeijer et al., 2012; Jones et al., 2020) The shape and growth form of corals can play a role in their response to turbidity. Branching corals, such as *Acropora* species, generally have a more open and branching structure that allows water flow and potential sediment flushing. This morphology can help reduce sediment deposition and maintain better access to light (Ashley et al., 2023; Duckworth et al., 2017). Considering the branching morphology of the *A. cervicornis* fragments, we did not observe sedimentation directly on the coral individuals. Sedimentation may not be a critical stressor for this species, but others with a mountainous or tabling morphology may be more greatly affected by sedimentation following increased turbidity.

Corals may undergo symbiont shuffling or switching in response to turbidity. Some species of zooxanthellae are better adapted to low-light conditions, and corals may acquire these types of symbionts to enhance their photosynthetic efficiency in turbid waters (Sawall et al., 2014). Corals may also retract their polyps and reduce their surface area exposed to the water column as a response to stressors (Gladfeiter 1982). This experiment, however, did not consider the complexity of symbionts that can inhabit this species. This experiment showed varying trends of changes in symbionts; however, these data were not significant.

Turbidity can influence water quality parameters, such as dissolved oxygen levels, TA, temperature, and pH (United States Environmental Protection Agency, 2021). Changes in these factors can have direct or indirect impacts on coral health and physiological processes. While we noticed elevated TA in chambers with higher NTU treatments at the beginning of each exposure, there was no analysis of the sediment properties and their effects on seawater composition. We aimed to keep temperature, pH, dissolved oxygen, and salinity stable in each chamber system. Further work will investigate the influence of turbidity on alkalinity concentrations.

This study aimed to quantify the effects of increasing turbidity on the respiration, photosynthetic efficiency, and physiological health of fragments of *A. cervicornis*. The health of the coral was inspected by analyzing symbiont densities, chlorophyll and protein concentrations, and calcification. This experiment saw no significant difference in symbiont density, protein or chlorophyll concentration, photosynthetic efficiency, or calcification between treatments, with only a few differences in PAM measurements (photosynthetic efficiency) between genotypes. Although there was a lack of biological and physiological responses seen as a result of increasing turbidity in this experiment, some interesting trends can be observed in the data and possibly further investigated with a longer exposure period.

Based on the findings of the study, the physical aspects of turbidity, specifically light reduction, had minimal impacts on the biology and physiology of the tested *A. cervicornis* corals. However, it was noted that further research using uncleaned sediment is necessary to better understand the true impact on coral health.

The study observed differences in responses across genotypes, particularly in terms of calcification response and resilience. Genotype 014 showed a decrease in calcification, while Genotype 020 exhibited increased photosynthetic efficiency, indicating some level of resilience. These findings highlight the importance of genetic variability in determining the response of corals to turbidity-induced stress. Furthermore, the study revealed an increase in coral respiration after three days of exposure to turbidity. This suggests that prolonged exposure to elevated turbidity levels can have an impact on the metabolic activity of corals. Photo-physiological changes were detected in the corals, indicating that the impacts of turbidity on coral health may be delayed and not immediately apparent. Therefore, conducting recovery assessments is crucial to fully understand the short-term and potential long-term effects of acute elevated turbidity on corals. The study also highlighted the need for more replication of data to ensure the robustness and reliability of the findings. Additional research efforts are necessary to gather more comprehensive and conclusive evidence regarding the effects of turbidity on coral health.

Overall, the study provides valuable insights into the response of *A. cervicornis* to acute turbidity exposures. It emphasizes the need for further investigation using uncleaned sediment, the importance of genetic variability, the metabolic impacts of prolonged exposure, the delayed effects of turbidity on coral physiology, and the necessity for increased replication in future studies. These findings contribute to our understanding of how turbidity affects coral reef ecosystems and can inform management and conservation efforts for these vulnerable marine organisms.

5.1. Coral Response Sensitivity

Research that investigated the effect of increased turbidity on the rate of respiration and photosynthesis in two South Florida reef species saw a decrease in the photosynthesis/respiration ratio in their corals starting on day 3 in *Meandrina meandrites* and day 2 in *Dichacaenia stokesii* (Telesnicki & Goldberg, 1995). This reflects that our results showed a trend towards a decreasing rate of photosynthesis starting on day 3. This could indicate that stony coral may take a few days to exhibit a stress response in rates of photosynthesis and respiration. Telesnicki & Goldberg (1995) also showed the photosynthesis/respiration ratio, after an initial decrease, held constant through the 21-day exposure.

Other studies have observed coral stress responses occurring after longer stress exposure periods. In a study that tested heat-stress training on *A. cervicornis*, laboratory control corals (exposed to no stress training and acclimated in the lab) started showing signs of bleaching and rapid tissue loss an average of 6.4 days after the start of heat stress assays. Corals were subjected to heat stress assays of 32°C for two weeks. A bleaching response was seen after about a week in most of the control corals, and a decrease in photosynthetic efficiency (Fv/Fm) was recorded after week 2 of the heat exposure (DeMerlis et al., 2022). Another study showed specific genets of *Acropora cervicornis* did not display any change in microbiome diversity in response to acute exposure to nutrient enrichment, but after six weeks of exposure, they displayed significant decreases in microbial diversity of the microbiome (Klinges et al., 2023). Our experiment only took place over three days, so it is possible with a longer exposure time, more stress responses in the *A. cervicornis* could be observed.

Understanding the timing and nature of coral stress responses is crucial for accurately assessing their health and well-being. Research has shown that pigmentation and omics (genomic,

transcriptomic, proteomic) are highly sensitive and can respond to acute stress treatments, but other responses may require prolonged exposure to stress or exhibit delayed effects. The process of calcification, which involves the deposition of skeletal material, may be sustained under stress due to the coral's ability to utilize alternative energy sources, such as lipid reserves (Grottoli et al., 2021). Therefore, assessing the coral's energy acquisition and lipid reserves may provide a more comprehensive understanding of their stress tolerance.

It is important to note that the study did observe an impact on the metabolic response of the corals after prolonged exposure, specifically on day 3. This indicates that there may be short-term and delayed effects of acute elevated turbidity on the corals. To gain a complete understanding of these impacts, it would be valuable to include a recovery assessment to evaluate how the corals respond and potentially recover from turbidity-induced stress.

By considering both short-term and long-term responses, as well as potential recovery processes, we can enhance our understanding of the acute and delayed effects of increased turbidity on corals. This knowledge will contribute to the development of effective management strategies and conservation efforts aimed at minimizing the negative impacts of dredging and other turbidity-inducing activities on coral reef ecosystems.

5.2. *Acropora cervicornis*

Many scleractinian corals are known to be sensitive to shifts in environmental conditions, and the Caribbean species *Acropora cervicornis* is no exception. Listed on the IUCN Red List as critically endangered, they have experienced high mortality since the 1970s due to multiple biological and environmental factors (Crabbe et al., 2022), including disease outbreaks, predation increase, and bleaching. Climate disturbances such as high winds and intense storms alter the salinity of the water and cause physical damage to reef structures. There is substantial evidence that the species requires long periods void of environmental and biological disturbance to express natural group recovery as a species (Goergen et al., 2019).

Individual *A. cervicornis* fragments exhibit rapid physiological responses to acute environmental changes. In previous experiments, tissue loss and mortality were observed within a day of extreme hypoxic treatment (Johnson et al. 2021). Transcriptomic expression varies in response to the time of day and light intensity at the base, mid-branch, and tips of colonies (Ashey et al., 2023; Hemond & Vollmer, 2015). Enochs et al. (2014) observed that the photochemical efficiency (Fv/Fm) of *A. cervicornis* increased in low light over a treatment period of 9 days. Increased Fv/Fm was seen in Genotype 014 of our experiment. It is important to note that the corals in our holding tank experienced PAR readings around 300-400, but the chamber PAR readings were closer to 150-200. This difference may have induced a light response, considering that the coral can dynamically respond to changes in light.

Acropora cervicornis varies greatly in response to temperature stress between genetically different individuals (DeMerlis et al., 2022; Yetsko et al., 2020). Elevated temperatures cause increased respiration, a decrease in net photosynthesis, and decreased ability to repair tissue abrasion. Exposure to elevated nutrients in conjunction with elevated temperature greatly increased mortality even among individuals that are relatively heat-resistant (Palacio-Castro et al., 2021). Nutrients were not a factor studied in this experiment, but temperature was controlled for, with all

treatments at 25°C. The coral in this experiment were acclimated to 25°C in the holding tank similarly to other studies (Paradis et al., 2019), but other studies on *A. cervicornis* use an ambient temperature of 27°C (Johnson et al., 2021) or 28°C (DeMerlis et al., 2022; Enochs et al., 2014; Yetsko et al., 2020). This difference in holding environmental conditions could have induced a response in the individuals before the trials began, and there is still ongoing debate on the optimal temperature for *A. cervicornis*. It is difficult to pinpoint an optimal temperature for this species primarily because average temperature in the Caribbean varies annually (Goergen et al., 2019).

The studies mentioned above indicate that *A. cervicornis* responses to environmental disturbances of concern may be greatly dependent on both genetic expression and light availability (DeMerlis et al., 2022; Yetsko et al., 2020). The species overall quickly responds to environmental and biological changes and can require long periods of time to fully recover.

6. FUTURE STUDIES AND STEPS

As previously mentioned, branching corals such as *Acropora palmata* and *Acropora cervicornis* respond differently to sediment deposition compared to bouldering and flat corals. Corals with fast-growing, branching skeletons collect less sediment on their branches in turbid waters, while bouldering scleractinians have trouble removing piles of sediment that settle on top of them, limiting exposure to light (Ashey et al., 2023; Rogers, 1990). Since these two phenotypic types of coral respond differently to sediment deposition, it should be investigated if they respond differently to chronic turbidity and extended exposure to suspended sediments in the water column. Star corals, such as *Orbicella faveolata* and *Montastrea cavernosa*, and brain corals, such as *Diploria labyrinthiformis* and *Pseudodiploria strigosa/clivosa*, are other species of concern that could be susceptible to inhibited functioning while exposed to high turbidity. These stony corals are also highly or likely susceptible to Stony Coral Tissue Loss Disease (SCTLD) and could become more vulnerable to infection with compounding stress factors (Florida Keys National Marine Sanctuary, 2018).

In addition to studying corals of different morphologies, the effect of different types of sediment should also be investigated. To fully understand the scope of the impact anthropogenic activities such as dredging can have, we must also study pollutants that could be contaminating the sediment. As sediment is stirred up and becomes suspended, the particles may also carry any chemical pollutants they've accumulated into the water column, such as mercury or other industrial waste products (Cantillo et al., 1999). The combined stress of chemical pollution and depressed photosynthetic capability due to increased turbidity could have compounding effects on a coral's overall health (Bessell-Browne et al., 2017; Nalley et al., 2023)

It would also be more indicative of the natural environment to study increasing turbidity in combination with increasing temperatures and pH. As ocean temperatures rise and become more acidic, this combined stress with chronic high turbidity in areas of industrial construction could have compounding detrimental effects on the corals' physiological processes and health. Additional stress can leave corals increasingly vulnerable to disease if their immune system is compromised or overworked. Studies should be done to further tease out the most accurate response of threatened coral exhibits in turbid conditions.

A study using longer-term exposure is necessary, as some corals aren't impacted by acute sedimentation stress (Bahr et al., 2020). Long-term dredging projects might cause sediment to be continuously suspended for multiple days or weeks, so to anticipate the effect on local wild coral, the study should be replicated with an exposure time as long as possible. Acute short-term exposure may not cause any physiological response until a certain tolerance level is reached. Teasing out different species' exact tolerance levels is imperative to planning ahead and preserving the coral populations that still thrive in South Florida.

In conclusion, with more studies, we can promote the adoption of environmentally responsible dredging practices and implement effective strategies to protect and restore coral reefs. By prioritizing the integration of sediment management into dredging operations, we can ensure the long-term health and sustainability of both sediments and coral ecosystems, safeguarding their ecological functions and supporting the livelihoods of communities dependent on these resources.

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