# Defining suspended sediment tolerance and warming thresholds for Caribbean Endangered Species Act (ESA) and/or Stony Coral Tissue Loss Disease (SCTLD) susceptible corals


Visual Comparison of the four turbidity (NTU) treatments experimental *Orbicella faveolata* were exposed to during turbidity threshold determination experiments.


# Defining suspended sediment tolerance and warming thresholds for Caribbean Endangered Species Act (ESA) and/or Stony Coral Tissue Loss Disease (SCTLD) susceptible corals

Final Report

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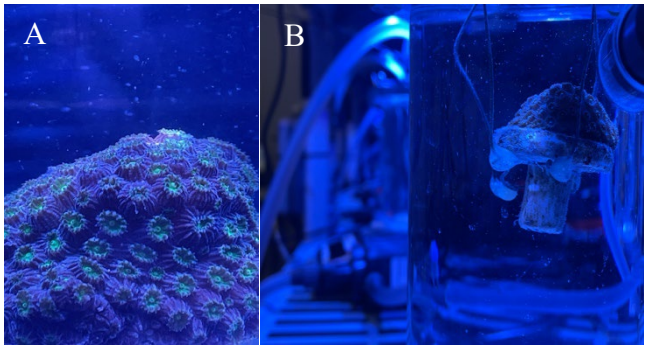
## MANAGEMENT SUMMARY

The study found that the physiological and metabolic response of *Orbicella faveolata* (OFAV) to increased turbidity was highly variable with metabolism (oxygen usage or production) having the highest capacity for showing real time changes in the physiology of the corals. Higher NTUs caused higher variation in both metabolism and growth of OFAV, but when including elevated temperature, the effect of elevated NTU was overshadowed by the negative effects of temperature. Trends in the data show that OFAV might have some adaptive capacity to acute stress, or rather that OFAV has a longer response time to acute stress. As time increases from exposure genotypic variation is evident in the lag time responses with some genotypes lacking the capacity to recover after exposure, and other genotypes fully recovering if not recovering to a level higher than pre-exposure.

Photo-biological changes were evident across all treatments with the interaction between elevated NTU and elevated temperature having the greatest negative affect on symbiont density, chlorophyll concentration, and protein concentration. Interestingly, symbiont density increased at all levels of NTU and temperature stress, but only at 50 NTU and elevated temperature was there a positive increase in chlorophyll when standardized to symbiont density (increase chlorophyll per symbiont).

In summary, the study provides valuable insights into the response of *Orbicella falveotata* to acute stressors. It underscores the importance of conducting research comparing various treatment, considering genetic variability, recognizing the metabolic impacts of acute exposure, understanding the delayed effects of stressors on coral physiology, comparing various sediment types from various location, increasing exposure lengths, 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.




A: *Orbicella faveolata* sitting in chamber during acclimation before any sediment was added to the system. B: *Orbicella faveolata* hanging in a chamber inside of a custom basket to hold itself during exposure treatments.

## EXECUTIVE SUMMARY

Coral reefs play a vital role in marine tourism, food production, coastal protection, and as habitats for numerous marine species. With about half of the global population living near coastlines, monitoring and managing human impacts on these ecosystems, particularly turbidity from activities like dredging, is crucial. Elevated turbidity levels can significantly stress and reduce coral health, with specific thresholds varying by species and region. Research has shown that corals, such as those in the genus *Orbicella*, are highly sensitive to changes in water clarity. Turbidity levels above 10 NTU in Florida and 30-40 NTU in other regions have been linked to coral stress and mortality. The decline in *Orbicella* corals in the Caribbean, primarily due to bleaching and disease, is a critical concern. Managing local stressors like turbidity is essential for their recovery. The study aimed to: 1) identify suspended sediment thresholds for *Orbicella faveolata* under short-term exposure; 2) examine the combined effects of turbidity and temperature on its metabolism, health, and calcification; and 3) assess the interactions between turbidity and temperature. The methodology involved using fine-grain sediment from a dredge site in Port Everglades, Florida, and employing acute intermittent flow respirometry to expose *O. faveolata* to elevated turbidity and temperature independently and together across two phases for a 72-hour exposure. Measurements focused on metabolism, photosynthetic efficiency, calcification, and other biological responses. Findings indicated that 29 and 50 NTU turbidity levels significantly reduced *O. faveolata* oxygen production, mainly affecting photosynthesis during daylight. Elevated temperatures had a more dramatic effect on oxygen production and photosynthesis. No significant differences were observed in photosynthetic efficiency, though elevated temperatures showed negative trends. Visual trends in calcification indicated potential acclimation and stress responses over time, suggesting the need for longer exposure studies. Chlorophyll and protein concentrations were not significantly affected by turbidity or temperature, indicating slower response rates compared to photosynthesis and respiration. The study concluded that coral metabolism is highly sensitive to environmental stressors, making it a useful indicator for future studies. Other measured responses were not impacted by short-term (72h) exposure to fine-grain sediment, suggesting potential latent effects of treatment. Genotypic variation was observed across all treatments, highlighting the importance of considering genetic diversity in future research. For future studies, testing different types of sediment, specific to various sites and ports, is recommended to better understand the impacts on coral health. Assessing indirect impacts and conducting longer exposure periods ranging from 72 hours to 4 weeks will provide more comprehensive insights. Increasing genotype replication and including more coral species in the studies will enhance the understanding of species-specific and genotype-specific responses to environmental stressors. Defining biologically relevant turbidity benchmarks is essential for managing human activities and mitigating climate change impacts on coral reefs. This study underscores the importance of ongoing research and adaptive management strategies to protect and sustain coral reef ecosystems amidst growing environmental pressures.

**ACKNOWLEDGMENTS**

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Furthermore, we would like to express our gratitude to the team from the Florida Department of Environmental Protection for their invaluable project feedback and assistance in shaping our experimental design. Their expertise and guidance were instrumental in ensuring the scientific rigor of our study.

A special thanks to Ken Nedimeyer and his team at Reef Renewal for collecting and providing us with the OFAV nursery corals used throughout this project. His commitment to coral conservation and Caribbean coral reef rehabilitation is inspiring and the timeline of this project would have been very different without his and his team's help during these experimental phases.

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## 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, such as dredging (Good & Bahr, 2021; Miller et al., 2016), so monitoring anthropogenic effects on coastal systems is very important.

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). 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 & Van 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 et al., 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., 2014; 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 enables 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.

### 1.1 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 contribute to the generation of turbid conditions in marine ecosystems (Tuttle & Donahue, 2022). Dredging, however, is the human activity that most directly increases local turbidity for multiple days at a time. There is substantial evidence that turbidity indirectly affects important benthic fauna, such as corals, primarily by decreasing the amount of light available for photosynthesis (Bessell-Browne et al., 2017; Erftemeijer et al., 2012; Fisher et al., n.d.; N. P. Jones et al., 2023) and acting as a vector for diseases (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 (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.

### 1.2 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 (Erftemeijer et al., 2012). While *Orbicella faveolata* (OFAV) sometimes shows higher growth (Carricart-Ganivet, 2004) or calcification in more turbid environments (Manzello, 2015), OFAV abundance is reduced on highly turbid reefs (Jordán-Garza et al., 2017). 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 surfaces and equipment, and clog filters, pumps, and tubing (Sa'ad et al., 2021). 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 the individual and synergistic effects of turbidity and sedimentation on corals and their associated ecosystems.

There are four main groups of sediment that make up the seabed. 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 when chemical reactions in seawater lead to the precipitation of solid particles. Cosmogenous sediments originate from extraterrestrial sources such as meteors. Chemical composition, grain size, and other characteristics of sediments may vary. 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-Scharrón, 2022), particularly when the impacts of dredging are combined with the likely impact of rising sea surface temperatures. Cunning et al. (2019) observed coral mortality following port dredging in Miami, Florida, but did not mention any specific impacts on OFAV. Additionally, Evans et al. (2020) observed turbidity to reduce *Acroporid* recovery from bleaching in Northern Australia. Conversely, Oxenford and Valles (2016) found a turbid water mass to reduce mortality rates of all corals, including those in the genus *Orbicella,* during a marine heatwave in Barbados but did not directly quantify the turbidity levels responsible for this effect. Clearly, further research is needed to investigate any species-specific positive or negative effects of turbidity on OFAV and its ability to withstand climate change. This experiment investigated the threshold turbidity level for adverse impacts on OFAV and the combined impacts of turbidity and thermal stress on this 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 threatened coral species, such as OFAV*,* respond to different levels of increased turbidity. Corals in the genus *Orbicella* are the dominant reef builders in most of the Caribbean (Pandolfi & Budd, 2008) and have been since at least the Pleistocene (Jackson, 1992) and possibly as early as the Pliocene in some locations (Goreau, 1959). It is essential that adverse human impacts on corals in this genus be reduced to avoid causing the most drastic alteration to Caribbean reef communities in at least ten thousand years and possibly in as many as five million years.

The abundance of corals in the genus *Orbicella* in the Caribbean is declining (Edmunds, 2015), with losses primarily attributed to mass bleaching events and disease (Bruckner, 2012). While the decline of this genus has not been as drastic as that of *Acropora* (Muller et al., 2014) it may recover even more slowly due to lower sexual recruitment and asexual recruitment by fragmentation (Bruckner, 2012). While the threat that climate change poses to OFAV is not locally manageable, reducing turbidity by all causes, including dredging, has been suggested as an early stage in the recovery plan for this species (NOAA Fisheries Protected Resources, 2015). This study aims to address this critical knowledge gap by focusing on the threshold turbidity level that causes negative effects on OFAV and whether threshold turbidities have additive, synergistic, or antagonistic interactions with the negative impacts of climate change on this species.

To achieve this, the study used fine-grain terrigenous sediment collected from a dredge site in Port Everglades, Florida. We employ acute intermittent flow respirometry exposures, which involve subjecting OFAV to short-term intervals (72 h) of elevated turbidity levels, elevated temperatures, or a combination of the two factors. By closely monitoring and analyzing the corals' responses in

the chambers and subsequent recoveries or lack thereof after they are removed from chambers, this study aims to gain insights into the immediate impacts of acute turbidity increase on the physiological and ecological aspects of OFAV.

Research questions:

1. What are the suspended sediment thresholds for acute exposure (72 h) for OFAV
2. What is the effect of fine grain suspended sediment and temperature on the metabolism, health, and growth of OFAV
3. How does suspended sediment affect the metabolic response of OFAV under ambient and elevated temperatures, and what are the implications for coral health and growth?
4. When does OFAV begin to display physiological responses to sub-lethal stress under various turbidities and targeted temperatures, and how does this contribute to defining a biologically relevant benchmark of turbidity?

The objectives of this project 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 NTU) at which corals begin to display physiological responses to sub-lethal stress.
4. Examine the effect of fine-grain suspended sediment and temperature on coral metabolism, health, and growth.
5. Conduct acute sediment exposure experiments to produce coral metabolic response curves under both ambient and elevated temperatures.
6. Define a biologically relevant benchmark of turbidity measured in NTU at which corals begin to display physiological responses to sub-lethal stress under targeted temperatures.

## 2. MATERIALS AND METHODS

### 2.1 Coral Acquisition and Maintenance

*Orbicella faveolata* (OFAV) were received in four shipments from Reef Renewal (Tavernier, FL) on August 2nd, 16th, and 31st, 2023, and February 19th, 2024. Each coral was individually wrapped in wet bubble wrap, then in wet paper towels, and placed inside a plastic bag. These bags were placed inside a cooler with cooling packs and temperature loggers to monitor the temperature during shipping. All shipments were sent overnight from Reef Renewal to Texas A&M University-Corpus Christi. Upon arrival, the corals were kept in a quarantine tank for 3-5 weeks to check for pests and monitor their health and recovery. After pest removal and acclimation, the OFAV fragments were transferred to the main holding tank. Photos documenting the corals' condition were taken upon arrival and used to track their recovery progress. The holding tank (350 L) recirculated artificial seawater (Red Sea Salt, Red Sea Fish, Tel Aviv, Israel). Water quality was tested twice weekly, followed by a 25% water change. Target water parameters were chosen to mimic the natural environment of OFAV: ammonia (0ppm), nitrite (0ppm), nitrate (0-20ppm), phosphate (0-0.3ppm), temperature (26.0°C), salinity (35 ppt), pH (8.1), total alkalinity (2500 µmol $Kg^{-1}$), calcium (390-420ppm), and magnesium (1250-1350ppm) (Enochs et al., 2018).

Artificial lighting is supplied by four 180W LED fixtures (Model MAD180, Wattshine, China) set to 12 hours of illumination per day from 8 AM to 8 PM. At the base of the coral fragments, light measured 150-200PAR. Nutrient levels were monitored using a colorimetry kit (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.

**2.2 Experimental Approach**

To determine the turbidity threshold of OFAV, corals were exposed to several turbidity levels, including 0, 15, 29, and 50 NTU. The experimental designs for Phases 1 and 2 (Table 1 and Table 2, respectively) were determined prior to experimentation. The designs considered the target treatments, coral genotype, individual fragments, racks, individual chambers, and the experiment. Each individual coral was given an ID and referenced as such 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. For each experimental week, Day 1 was allocated to pre-exposure measurements, including Pulse Amplitude Modulated-fluorometry (PAM), buoyant weight, and photographs; Days 2 – 4 were data collection, and Day 5 was used for post-treatment measurements, including PAM, buoyant weight, photographs, and subsampling each individual for destructive endpoint analysis (Figure 1). An individual from each genotype was selected to undergo destructive endpoint analysis to act as a baseline for later comparison to determine the holobiont response of corals exposed to sediment. These corals were chosen based on how well they represented the genotype according to calcification rates ($G_{net}$) and PAM readings (Fv/Fm) across the genotype.

*Day 1*: 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 PAM measurements at 10 AM. 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. Following photosynthetic assessments, corals were placed in respiration chambers at 8 PM to allow for chamber acclimation overnight. In elevated temperature exposures, chamber temperatures were raised from 27ºC (ambient) to 28ºC to begin acclimation overnight. Oxygen sensors in each chamber setup were calibrated to 0% and 100% oxygen.

*Days 2 – 4*: The 72-hour sediment exposure period began at 7 AM on Day 2 and ended at 9 AM on Day 4. Temperature in elevated temperature sumps was raised from 28ºC to 29ºC at 6 AM and 29ºC to 30ºC at 7 AM. For each treatment that included sediment, the corresponding sumps were dosed according to the target NTU for each treatment (Table 3). During the exposures, total alkalinity (TA) and turbidity samples were collected every four hours (i.e., 8 AM, 12 PM, and 4 PM) for each chamber system. TA samples were collected from the sumps 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 borosilicate glass bottles, stored in a water bath at 25˚C, and analyzed within

two hours of collection. These samples were used to monitor the TA of the water and changes in alkalinity that occurred during the incubation due to calcification or dissolution of 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 AM collection until 10 PM. The parameters were salinity, temperature, dissolved oxygen (DO mg $L^{-1}$), and percent dissolved oxygen (DO%) using a YSI multi-parameter 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*: Final sump parameters (turbidity, DO%, and salinity) were taken at approximately 8:30 AM. The corals were then removed from the chamber systems at 9:30 AM and dark acclimated (20 mins) before PAM measurements were performed at 10 AM. Then, their post-exposure photographs were taken, and buoyant weights were recorded. A subsample of each coral was removed using a coral diamond saw (Gryphon AquaSaw XL C-40 CR, Gryphon) and stored in an -80°C freezer for destructive endpoint analysis, while the remaining half was returned to the holding tank for recovery. This same process was repeated for each trial with each experimental coral fragment.

*Post-Experimental Recovery*: PAM, buoyant weight, and wet weight were measured, followed by a top-down and a side profile photograph. Each experimental coral was subsampled using a diamond bandsaw (Gryphon AquaSaw XL C-40 CR, Gryphon), after which the other half was returned to the holding tank. Recovery measurements were taken per fragment every month post-experiment to monitor changes in photosynthetic efficiency (Fv/Fm) and determine the period of recovery for each treatment group.

Table 1. Experimental design for turbidity threshold determination experimental trials for OFAV.

| Experiment | Rack | Chamber | Genotype* | ID* | Treatment (NTU)* |
|---|---|---|---|---|---|
| 1 | A | 1 | 67 | 1 | 50 |
| 1 | A | 2 | 67 | 2 | 29 |
| 1 | A | 3 | 67 | 3 | 15 |
| 1 | A | 4 | 67 | 5 | 0 |
| 1 | B | 5 | 80 | 2 | 29 |
| 1 | B | 6 | 80 | 3 | 50 |
| 1 | B | 7 | 80 | 4 | 15 |
| 1 | B | 8 | 80 | 5 | 0 |
| 2 | A | 1 | 84 | 2 | 50 |
| 2 | A | 2 | 84 | 4 | 29 |
| 2 | A | 3 | 84 | 6 | 15 |
| 2 | A | 4 | 84 | 7 | 0 |
| 2 | B | 5 | 90 | 2 | 29 |
| 2 | B | 6 | 90 | 5 | 50 |
| 2 | B | 7 | 90 | 8 | 15 |
| 2 | B | 8 | 90 | 10 | 0 |
| 3 | A | 1 | 97 | 1 | 50 |
| 3 | A | 2 | 97 | 3 | 29 |
| 3 | A | 3 | 97 | 10 | 15 |
| 3 | A | 4 | 97 | 7 | 0 |
| 3 | B | 5 | 101 | 8 | 29 |
| 3 | B | 6 | 101 | 10 | 50 |
| 3 | B | 7 | 101 | 11 | 15 |
| 3 | B | 8 | 101 | 14 | 0 |
| 4 | A | 1 | 50 | 1 | 50 |
| 4 | A | 2 | 50 | 2 | 29 |
| 4 | A | 3 | 50 | 3 | 15 |
| 4 | A | 4 | 50 | 5 | 0 |
| 4 | B | 5 | 60 | 2 | 29 |
| 4 | B | 6 | 60 | 4 | 50 |
| 4 | B | 7 | 60 | 5 | 15 |
| 4 | B | 8 | 60 | 6 | 0 |

Table 2. Experimental design for multi-stressor trials for OFAV.

| Experiment | Rack | Chamber | Genotype | ID* | Treatment (ºC x NTU)* |
|---|---|---|---|---|---|
| 5 | A | 1 | 67 | 6 | 30ºC x 29 NTU |
| 5 | A | 2 | 67 | 9 | 30ºC x 0 NTU |
| 5 | A | 3 | 80 | 6 | 30ºC x 29 NTU |
| 5 | A | 4 | 80 | 10 | 30ºC x 0 NTU |
| 5 | B | 5 | 67 | 8 | 27ºC x 29 NTU |
| 5 | B | 6 | 67 | 7 | 27ºC x 0 NTU |
| 5 | B | 7 | 80 | 7 | 27ºC x 29 NTU |
| 5 | B | 8 | 80 | 12 | 27ºC x 0 NTU |
| 6 | A | 1 | 84 | 1 | 30ºC x 29 NTU |
| 6 | A | 2 | 84 | 9 | 30ºC x 0 NTU |
| 6 | A | 3 | 90 | 3 | 30ºC x 29 NTU |
| 6 | A | 4 | 90 | 6 | 30ºC x 0 NTU |
| 6 | B | 5 | 84 | 8 | 27ºC x 29 NTU |
| 6 | B | 6 | 84 | 5 | 27ºC x 0 NTU |
| 6 | B | 7 | 90 | 4 | 27ºC x 29 NTU |
| 6 | B | 8 | 90 | 7 | 27ºC x 0 NTU |
| 7 | A | 1 | 50 | 7 | 30ºC x 29 NTU |
| 7 | A | 2 | 50 | 9 | 30ºC x 0 NTU |
| 7 | A | 3 | 60 | 7 | 30ºC x 29 NTU |
| 7 | A | 4 | 60 | 9 | 30ºC x 0 NTU |
| 7 | B | 5 | 50 | 8 | 27ºC x 29 NTU |
| 7 | B | 6 | 50 | 10 | 27ºC x 0 NTU |
| 7 | B | 7 | 60 | 8 | 27ºC x 29 NTU |
| 7 | B | 8 | 60 | 1 | 27ºC x 0 NTU |
| 8 | A | 1 | 67 | 14 | 30ºC x 29 NTU |
| 8 | A | 2 | 67 | 18 | 30ºC x 0 NTU |
| 8 | A | 3 | 80 | 22 | 30ºC x 29 NTU |
| 8 | A | 4 | 80 | 16 | 30ºC x 0 NTU |
| 8 | B | 5 | 67 | 14 | 27ºC x 29 NTU |
| 8 | B | 6 | 67 | 18 | 27ºC x 0 NTU |
| 8 | B | 7 | 80 | 13 | 27ºC x 29 NTU |
| 8 | B | 8 | 80 | 16 | 27ºC x 0 NTU |

## 2.3 Timeline

Turbidity threshold experiments were conducted November 6th – December 8th, 2023, with a quality assurance experiment conducted April 8th - April 12th, 2024, to compare treatment manipulation methods. Multi-stressor experiments were conducted January 22nd, 2024 – February 16th, 2024, with a quality assurance experiment conducted April 1st – April 5th, 2024, to compare treatment manipulation methods. All experimental weeks consisted of 5 days with 3 days (72 hours) of treatment exposure.

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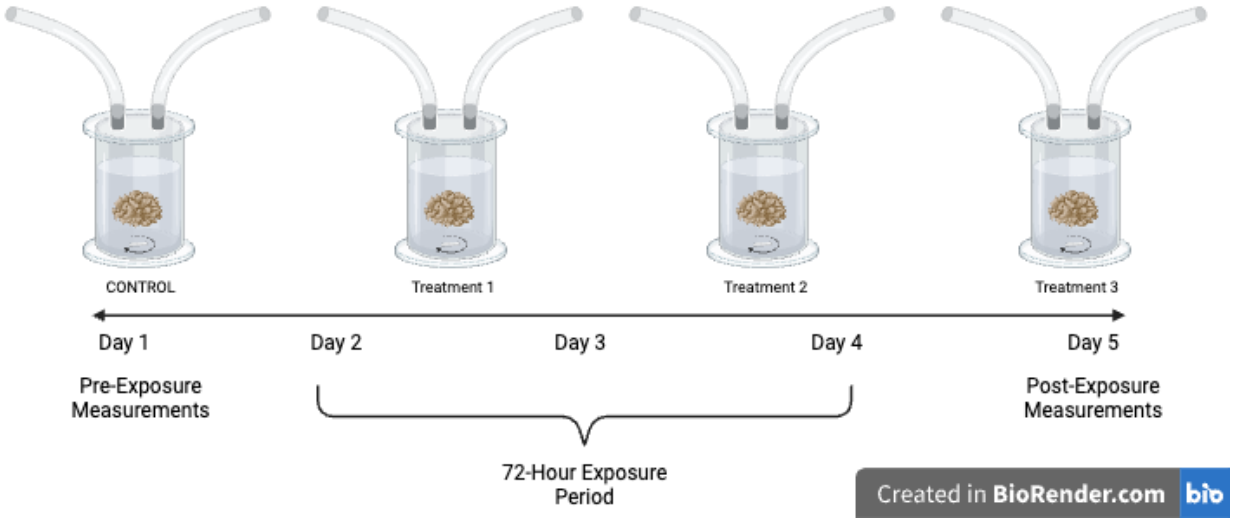


Figure 1. Experimental trial procedure for turbidity threshold and multi-stressor experiments for *Orbicella faveolata*. Trials consisted of 24 hours of pre-exposure measurements, 72 hours of exposure, and 24 hours of post-exposure measurements. The figure was created using BioRender.com.

## 2.4 Sediment Characterization

Sediment was placed in a drying oven at 80°C for 24 hours to ensure it was dry before grinding. Fifty grams of sediment was added to a vial and ground using a cryomill (6875 Freezer/Mill, Cole-Parmer, Vernon Hills, IL). After grinding sediment was stored in a glass jar until composition analysis. Three ceramic crucibles were labeled and weighed (grams). Pre-grinded sediment was homogenized and ~10g was added to each pre-weighed crucible. Sediment was burned at 100°C for 10 hours, 500°C for 12 hours, and 1,000°C for 2 hours. Following each burning, the sediment was cooled and weighed to determine the weight of material removed (i.e., moisture, organic material, and carbonate, terrigenous). For sediment grain size analysis, the sediment obtained from Fort Lauderdale, FL provided by the Florida Department of Environmental Protection was thawed and homogenized prior to wet sieving. Once the sediment had been thoroughly mixed, three samples of approximately 30 g of sediment were wet sieved through a series of sieves (63µm, 500µm, and 63µm respectively). 15mL of bulk ground sediment was then digested with 30% $H_2O_2$ in 50mL falcon tubes for one month by gradually adding 1mL of $H_2O_2$ to the sample over the time period until 15mL of total sample was reached and no more visual bubbling occurred. Digested sediment samples were then analyzed using a particle size analyzer (Various models).

## 2.5 Treatment Manipulation

A standardized turbidity curve (g • $mL^{-1}$) was generated to determine the proper dosage of sediment (g) to seawater (mL) to produce the desired turbidity. Various masses of sediment (g) were

repeatedly added to 1000 mL of seawater until the desired data amount was collected. The turbidity of the sample was determined by averaging three readings of two duplicate turbidity vials (6 total readings). The amount of sediment ($g \cdot mL^{-1}$) and the resulting turbidity (NTU) of each sample was recorded and plotted on a graph. A trendline was created based on the points ($R^2 = 0.9959$). Using the equation ($y = mx + b$) where y is the desired NTU (15, 29, 50) and x is the grams of sediment needed to dose a volume of artificial seawater (mL). Based on this equation, the amount of sediment required to dose each treatment was determined (Table 3). The target NTU concentrations were (15 ± 10 NTU, 29 ± 10 NTU, and 50 ± 10 NTU). An initial dose of pre-measured sediment was added to the sump prior to beginning an experiment and suspended with electric overhead stirrers set to 300 RPM (Lab Fish). Sump NTU levels were monitored every two hours and dosed hourly over the 72-hour experiment. Auto Dosing Pumps (Jecod Co., Ltd) fed by a stock container of suspended sediment in seawater (55g in $5000mL^{-1}$) were used to maintain the NTU and regulate the dose (mL) each sump received every hour. Dose volumes (mL) 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, and or a water change was done until the NTU dropped back into the target range. If NTU decreased below the set range (<10 NTU), dosing was increased. To manipulate temperature, a 200-watt titanium aquarium heater (Hyggar, Hong Kong, China) was placed in the water baths containing the sumps for high-temperature treatments. An infrared thermometer gun (Hyper Tough Model 1504V, Walmart Inc.) was used to monitor the temperature of the chamber before and after each flush, and the water temperature of the sump was recorded prior to every collection period using a multi-probe water quality sonde (YSI ProQuatro, Xylem Inc., Yellow Springs, Ohio). During all multi-stressor exposure periods, a wireless temperature logger (HOBO Pendant Data Logger, Onset Computer Corporation) was placed in each sump to monitor temperature changes over the 72-hour experimental period. A wireless temperature logger was also placed in multiple chambers to determine water temperature fluctuation over a single incubation period. All treatments were subjected to five-liter water changes once a day to control algal blooms caused by recirculating untreated sediment through a closed respirometry system. All water changes were completed using prepared NTU stocks in large drums. For all high-temperature treatments, stock water was heated to the target temperature, and all NTU stocks were dosed to the target NTU before changing any sump water.

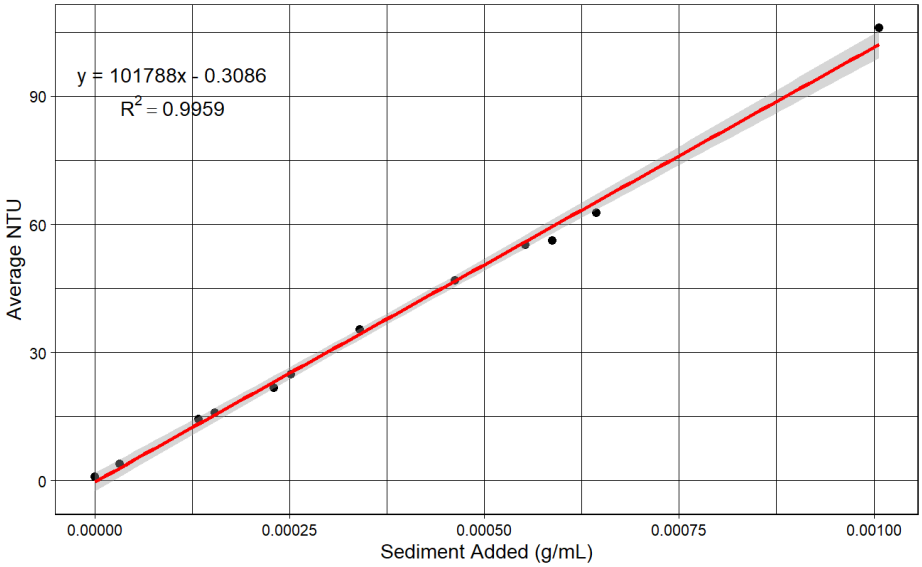


Figure 2. The turbidity equation was calculated from grams of sediment added (g • $mL^{-1}$) to each beaker and the resulting NTU after mixing (R2 = 0.9959).

## 2.6 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 10 mm tubing and a second pump line containing an oxygen sensor (Witrox 4, Loligo Systems, Viborg, Denmark) via 8 mm tubing (Figure X). 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 resuspend, settling within the chamber. Corals were placed on a wire pedestal at a height in the chamber 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 mmol of photons $m^{-2}$ $s^{-1}$). Intake and outflow flush tubing were secured deep in the sump water using suction cups to prevent air from disturbing the respirometry readings. The sumps consisted of a twenty-liter clear cylindrical container placed in a water bath. Each water bath was outfitted with a digital thermometer for temperature control (Figure 3 AM). Two adjacent sumps fit in each water bath, allowing two systems to be placed on each rack. The room temperature was consistently 26˚C. An overhead stirrer was placed in each sump to disturb settling sediment, and an air stone was placed near the surface to supply oxygen without allowing bubbles to enter the chamber system. Air was supplied to each rack by a 4-channel air pump (95 air pump 4-way, Fedour).

Figure 3. Respirometry setup: A full sump and chamber are set up without coral (Right), and a full chamber is set up with coral exposed to treatment after a flush period (left).

**2.7 Turbidity Threshold Determination experiments**

Acute turbidity exposures were conducted using untreated sediment collected from Port Everglades Miami, FL, and dry shipped to the Bahr Marine Ecology Lab at TAMU-CC, where it was held at -20ºC until the time of use. Untreated sediment was dried at 80°C in the drying oven (Model DX302C, Yomato Scientific America Inc., Santa Clara, CA) and ground into powder using a cryogenic grinder (Freezer Mill 6875, SPEX SamplePrep, Metuchen, New Jersey). Milled sediment was then held at -20ºC until the time of use. Unfiltered, milled sediment was used to dose the sumps at 7:00 AM on Day 2 of each experimental week. The total grams of milled sediment were determined using the methods outlined in the turbidity manipulation section and added directly to the sump. The sediment was allowed to settle to reduce the introduction of larger, heavier particles into the chamber before stirring commenced with electric overhead stirrers (Lab Fish).

**2.8 Photosynthetic Efficiency**

PAM measurements were obtained (DIVING-PAM 2.0, Heinz Walz GmbH, Effeltrich, Germany), which is used to measure changes in algal symbiont activity and photosynthetic efficiency. Corals were assessed with PAM before going into the chambers, after the conclusion of an experiment, and periodically post-experiment to assess recovery. Corals were dark acclimated for at least 20 minutes before PAM measurements were taken. Whole OFAV fragments were measured twice with PAM, and halved recovery fragments were measured once. The PAR sensor was situated 5-10mm from the surface of the coral using a marked sensor cap and was not moved while a measurement was being taken. All PAM parameters were recorded (Fv/Fm, (Y)NPQ, ETRm, and alpha); however, only Fv/Fm was reported, as this is the general value for photosynthetic efficiency, or “health” of algal symbionts (Ralph et al., 2015).

## 2.9 Calcification

The total alkalinity (TA) anomaly technique (Kinsey, 1978; Smith & Kinsey, 1978) was used to determine the net calcification rates of corals over the course of the experiment. Water samples were collected from the sump and chamber in 150 mL borosilicate glass bottles. The initial TA was collected from the sump prior to the flush. The final TA was collected from the water exiting the chamber during the flush. After collection, samples were placed in a water bath at room temperature (25ºC) and then weighed out on a scale (VWR-224AC) and run on a Metrohm Compact Sample Changer and EcoTitrator. Duplicates (w/ in 5 µmol) were run for each chamber sample and then averaged together. A pH benchtop (Thermoscientific) was used to verify the pH of each sample. Net calcification ($G_{net}$) in µmol $CaCO_3$ • g $bwt^{-1}$ • $h^{-1}$ were calculated from changes in TA (ΔTA) based on the following equation (McNicholl & Koch, 2021).

$$Gnet = -0.5\rho_w \frac{\Delta TA \cdot v}{(BW \cdot 1.54) \cdot t}$$

## 2.10 Post-experimental processing

After the experiment, various biological analyses were conducted on the coral fragments. These included measuring the concentration of total protein and chlorophyll (*a*, $C^2$, total), determining the abundance of symbiotic algae (*Symbiodinium spp.*), assessing the bulk skeletal density, and calculating the surface area of each halved 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 (20 mL) was then sonicated for twenty seconds using a sonicator ultrasonic processor (Qsonica, LLC). The sonicated slurry was divided into separate sample sets for protein, symbiont, and chlorophyll analysis. This was done by using a vortex mixer (Four E's Scientific) and a centrifuge (VWR International, LLC. Radnor, PA). The abundance of algal symbionts (*Symbiodinium spp.*) cells was determined by counting them using a hemocytometer (Bright-Line, Hausser 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 (10% bleach) and then dried for four hours at 60˚C using the Drying Oven DX302C (Yomato Scientific America Inc., Santa Clara, CA). Following this, the coral skeletons were weighed using a VWR-4002B2 balance (VWR International, Radnor, PA). The skeletal density of each coral fragment was determined by dividing the dry mass of the coral, and the volume found using water displacement. Three-dimensional scans of the coral skeletons were generated and edited using the Einscan-SE 3D Scanner (Hangzhou Shining 3D Tech Co., LTD., Hangzhou, China) and MeshLab software (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 areas. This allowed for the determination experiments of the total abundance and concentration of symbionts, chlorophyll, and protein in the corals' tissue.

## 2.11 Statistical Approach

Shapiro-Wilks tests for normality were conducted on each treatment level for every response variable to assess normality. Following the testing hypothesis framework ($H_o$ = Response variable is normally distributed, $H_A$ = Response variable is not normally distributed). Two-way ANOVAs

were used to compare the results of the turbidity x temperature experiment that met the requisite assumptions of normality and homogeneity of variances. All non-normally distributed response variables were tested against single level variables using the Kruskal-Wallis ranked test all of which were conducted in R using the *stats* package (Hollander et al., 2015). All non-normally distributed response variables were tested against two variables using a ranked two-way ANOVA. Any significant results from non-parametric testing were followed by Dunn's rank-sum test (Dunn, 1964) using the R package *dunn.test* (Dinno, 2015) using the Holm's alpha adjustment method (Holm, 1979) to adjust for significance inflation. All statistical analysis and graphing were completed in R and RStudio (R Core Team, 2021).

## 3. RESULTS

### 3.1 Sediment Characterization

The sediment received from Fort Lauderdale, FL was primarily comprised of terrigenous sediment (average: 65.315%) followed by Carbonate substrate (average: 31.069%), Organic material (average: 3.134%), and moisture (average: 0.480%). The pre-milling grain size composition was coarse sand (67.43%), with the second largest portion being medium sand (25.25%) followed by small sand or silt (7.32%). Post-milled grain size composition was 54.86% heavy sediment (250µm - 1000µm), 24.92% small grain sand (63µm - 125µm), and 20.22% silt (<63µm).

### 3.2 Experimental Treatments

Turbidity threshold experiments: The control treatments had readings between 0 and a maximum of 4.45 NTU every time readings were taken. Turbidity readings in 15 NTU treatments were between 5 and 15 NTU 83% of the time. Turbidity readings in 29 NTU treatments were between 19 and 39 NTU 61% of the time. Finally, turbidity readings in 50 NTU treatments were between 40 and 60 NTU 46% of the time. All mean treatment NTUs were within 2 NTUs of desired levels, showing relatively low standard error. A Kruskal-Wallis test had a p-value less than $2.2x10^{-16}$, confirming a significant difference between treatments, and a Dunn's test showed that all experimental turbidity treatments had turbidities significantly different from each other (p-value less than $10^{-5}$ across all comparisons between treatments; Figure 4).

Multi-stressor Experiments: Turbidity in 0 NTU treatments was below 10 NTU 100% of the time, and turbidity in 29 NTU treatments was between 19 and 39 NTU 75% of the time. All mean treatment NTUs were within 2 NTUs of desired levels, showing relatively low standard error. A ranked 2-factor ANOVA indicated that high turbidity treatments did not differ significantly from each other (p-value> 0.05) but did differ significantly from low turbidity treatments (p-value <0.05). A significant difference between turbidities in Control and High Temp treatments was detected (p-value < 0.05); however, both had mean turbidities between 1 and 2 NTU. This difference may be statistically significant but have no effect on coral physiological metrics based on the results of the first phase of this experiment. Temperature treatments were always within 3 ºC of their desired temperatures of 27ºC and 30ºC, respectively, and showed very little overlap between treatments. A ranked 2-factor ANOVA showed that high-temperature treatments differed significantly from ambient temperature treatments with no significant differences related to turbidity (Figure 5).

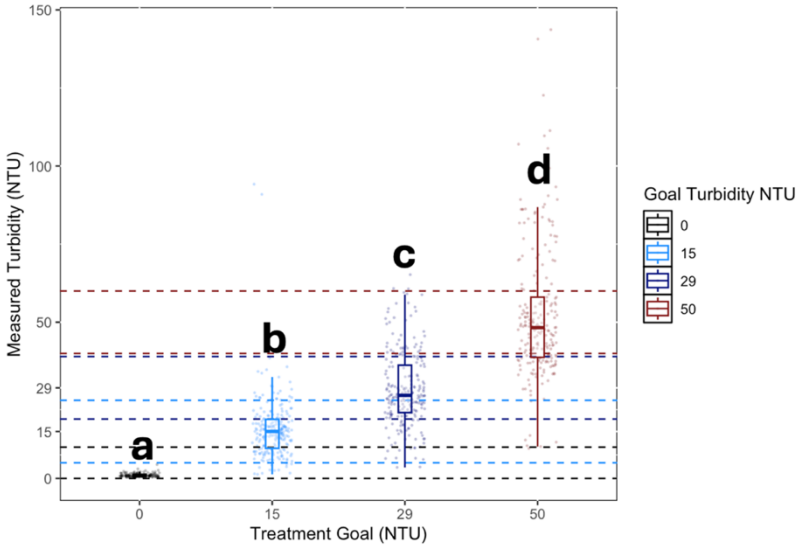


Figure 4. Measured NTU plotted against goal NTU for turbidity threshold determination experiments. Color denote treatment where black is control, light blue is 15 NTU, dark blue is 29 NTU, and red is 50 NTU. Dotted line colors correspond to their respective treatments and show the minimum and maximum ranges of goal NTUs (i.e. goal NTU ± 10) and the letters signify statistical differences between treatment groups (i.e. is treatment groups share the same letter, they are statistically similar).

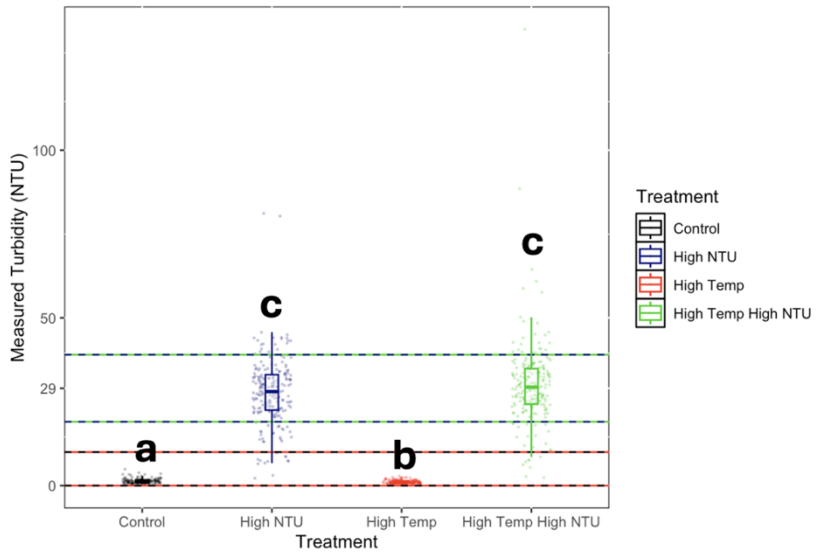


Figure 5. Measured turbidity plotted against treatment level colored by treatment where black is control, blue is high NTUx27ºC, red is 30ºC x 0 NTU, and green is 30ºC x 29 NTU. Dotted lines are colored corresponding to treatment levels and denote the minimum and maximum NTU range for each treatment (i.e. goal NTU ± 10). Letters denote significance between treatment levels (i.e. treatments that share the same letter did not differ significantly).

### 3.3 Respirometry

Turbidity threshold determination experiments: Metabolic oxygen was not normally distributed within or across NTU treatments (0 NTU, 15 NTU, 29 NTU, 50 NTU; p-value << 0.05). Kruskal-Wallis ranked test was highly significant between treatments, $X^2(N = 2{,}56, df = 3) = 69.78$, p-value = $4.76 \times 10^{-15}$. Dunn's test for multiple comparisons with Holm's alpha adjustment (Table 4) was

significant for treatment comparisons between 29 NTU – 0 NTU, 50 NTU – 0 NTU, 50 NTU – 15 NTU, and 50 NTU – 29 NTU, suggesting that turbidities of 15 NTU are too low to significantly affect OFAV photosynthesis and respiration, but values of 29 or higher adversely affect this metric.

Multi-stressor experiments: A Two-way analysis of variance was conducted on integrated $MO_2$ values following the first 12 hours of exposure for OFAV. Metabolic activity was significantly affected by temperature but not turbidity nor the interaction between the two suggesting elevated temperature has a greater effect on OFAV opposed to elevated stress caused by turbidity.

Table 3. Non-parametric test of significance for $MO_2$ for turbidity threshold determination experiments levels. Treatment levels were 0 NTU, 15 NTU, 29 NTU, and 50 NTU. Asterisks (*) denote a significant difference between treatment levels.

| | Degrees of Freedom | Sum of Squares | Mean of Squares | F-Value | P-Value |
|---|---|---|---|---|---|
| Treatment | 1 | 0.669 | 0.6989 | 2.062 | 0.161 |
| Residuals | 30 | 10.170 | 0.3390 | | |

Table 4. Two-way ranked analysis of variance conducted on metabolic oxygen ($MO_2$) during multi-stressor exposure experiments with OFAV where treatment levels were 27ºC x 0 NTU, 30ºC x 0 NTU, 27ºC x 29 NTU, and 30ºC x 29 NTU.

| | Degrees of Freedom | Sum of Squares | Mean of Squares | F-Value | P-Value |
|---|---|---|---|---|---|
| NTU | 1 | 0.167 | 0.1669 | 0.349 | 0.5569 |
| Temperature | 1 | 2.804 | 2.8036 | 5.858 | 0.0222* |
| NTU x Temperature | 1 | 0.001 | 0.0008 | 0.002 | 0.9676 |
| Residuals | 28 | 13.402 | 0.4786 | | |
| | | | | | |

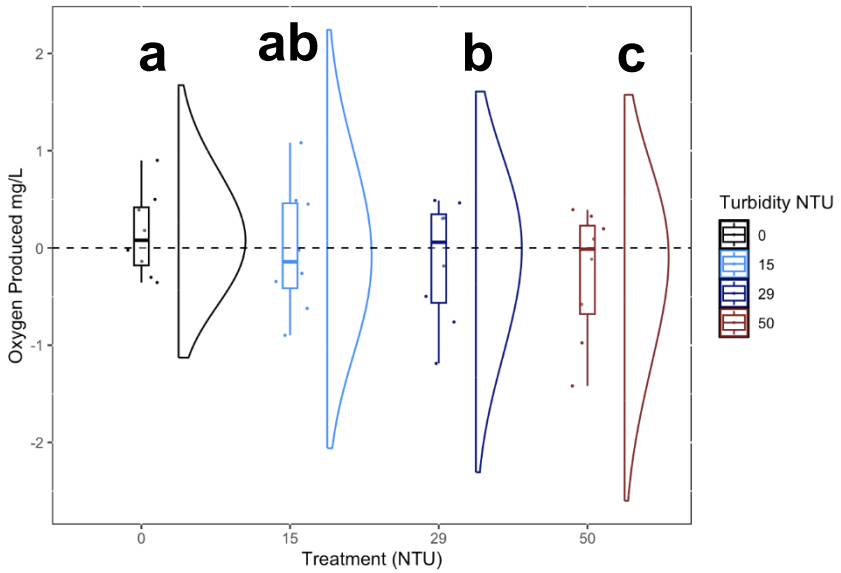


Figure 6. Integrated oxygen production from hour 13 to hour 60 of the experiment. Negative values indicate net respiration and positive values indicate net photosynthesis. Colors denote the treatment: Control (black, 0 NTU), 15 NTU (blue), 29 NTU (purple), 50 NTU (red). Points indicate data values, boxplot bars indicate the median, boxes indicate the interquartile range, and whiskers indicate the smallest value within 1.5 times the interquartile range below the 25th percentile and the largest value within 1.5 times the interquartile range above the 75th percentile. Violin plots indicate probability density for each treatment based on observed values.

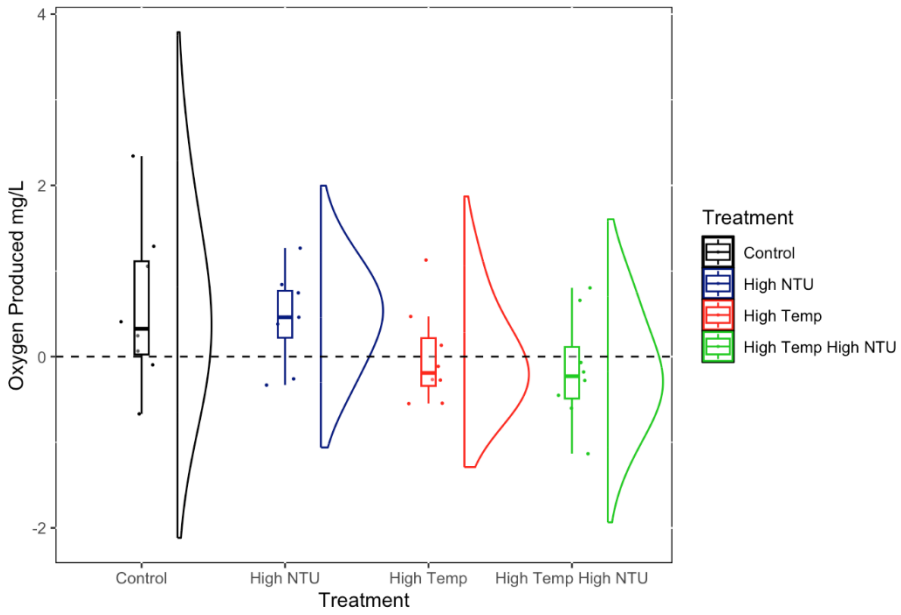


Figure 7. Integrated oxygen production from hour 13 to hour 60 of the experiment. Negative values indicate net respiration and positive values indicate net photosynthesis. Colors denote the treatment: Control (black, 0 NTU), High NTU (blue 29 NTU, 27ºC), High Temp (red 0 NTU, 30ºC), High Temp High NTU (green 29 NTU, 30ºC). Points indicate data values, boxplot bars indicate the median, boxes indicate the interquartile range, and whiskers indicate the smallest value within 1.5 times the interquartile range below the 25th percentile and the largest value within 1.5 times the interquartile range above the 75th percentile. Violin plots indicate probability density for each treatment based on observed values.

### 3.4 Calcification

Turbidity threshold determination experiments: Net calcification ($G_{net}$) was not normally distributed within or across NTU treatments (0 NTU, 15 NTU, 29 NTU, 50 NTU; p-value << 0.05). Kruskal-Wallis ranked test was not significant between treatments, $X^2(N = 2,250, df = 3) = 0.456$, p-value = 0.928. Therefore, the tested NTU levels had no observable impact on calcification during the experimental period.

Multi-stressor experiments: Net calcification ($G_{net}$) was not normally distributed within or across multi-stressor treatment levels (27ºC x 0 NTU, 30ºC x 0 NTU, 27ºC x 29 NTU, and 30ºC x 29 NTU). A two-way ranked analysis of variance was conducted on $G_{net}$ between treatment levels with no significant differences (p-value(s) > 0.05). Therefore, acute exposure to elevated temperature and turbidity did not significantly affect the growth of OFAV.

Table 5. Two-way ranked analysis of variance conducted on calcification ($G_{net}$) during multi-stressor exposure experiments with OFAV where treatment levels were 27ºC x 0 NTU, 30ºC x 0 NTU, 27ºC x 29 NTU, and 30ºC x 29 NTU.

| | Degrees of Freedom | Sum of Squares | Mean of Squares | F-value | P-value |
|---|---|---|---|---|---|
| NTU | 1 | 871 | 871 | 0.080 | 0.777 |
| Temperature | 1 | 23749 | 23749 | 2.189 | 0.140 |
| NTU x Temperature | 1 | 1742 | 1742 | 0.161 | 0.689 |
| Residuals | 356 | 3861605 | 10847 | | |

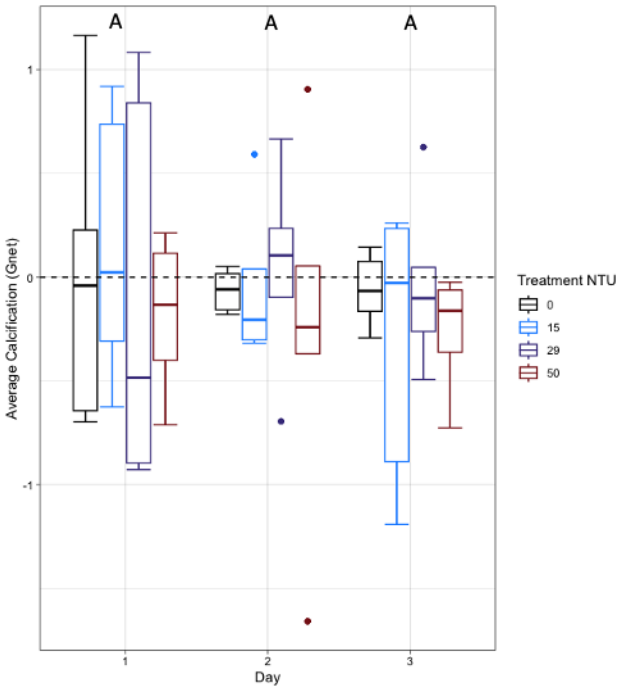


Figure 8. Net calcification (Gnet = µmol CaCO3 g • bwt-1 • h-1) plotted for each day during the 72-hour turbidity threshold exposure period. Colors denote the treatment: Control (black, 0NTU), 15 NTU (blue), 29 NTU (purple), 50NTU (red) with letters denoting a significant difference (i.e. treatments sharing the same letters do not differ significantly). The black dotted line denotes a null response.

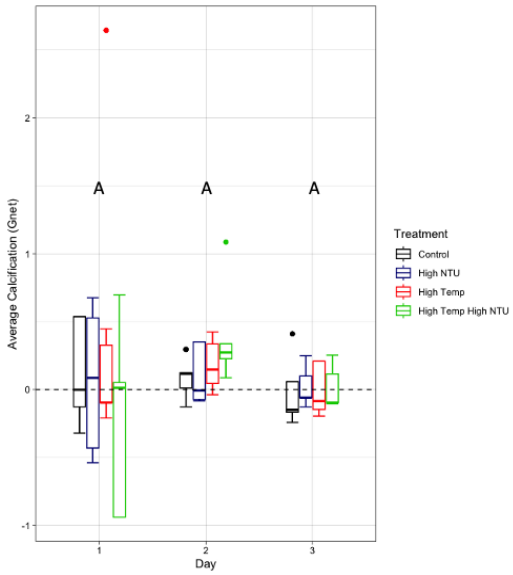


Figure 9. Net calcification ($G_{net}$ = µmol $CaCO_3$ g • *bwt*$^{-1}$ • h$^{-1}$) plotted for each day during the 72-hour multi-stressor exposure period. Colors denote the treatment: Control (black, 0 NTU x 27ºC), High Temperature (red, 0 NTU x 30ºC), High Turbidity (purple, 29 NTU x 27ºC), and High Temperature x High NTU (green, 29 NTU x 30ºC) with letters denoting a significant difference (i.e. treatments sharing the same letters do not differ significantly). The black dotted line denotes a null response.

### 3.5 Photosynthetic efficiency

Turbidity threshold determination experiments: All treatment levels for turbidity exposure (0 NTU, 15 NTU, 29 NTU, and 50 NTU) were normally distributed (p-value > 0.05). A one-way ANOVA was used to assess significant differences in ΔFv/Fm between treatments, with no significance found (Table 6); therefore, no statistical effects of turbidity were seen in the changes of photosynthetic efficiency for each genotype exposed.

Multi-stressor experiments: All treatment levels for multi-stressor treatments were normally distributed within and across treatment levels (p-value > 0.05). A two-way ANOVA was conducted to assess for treatment level differences before and after exposure ΔFv/Fm. A secondary test of marginal means (Esarey et al., 2017) was used to test for significant interactions between treatment levels (p-value(s) > 0.05). Therefore, no differences in the change of photosynthetic efficiency (ΔFv/Fm) were seen, and there is no evidence that a single level of temperature or turbidity measured during the exposure was acting synergistically or antagonistically on photosynthetic efficiency.

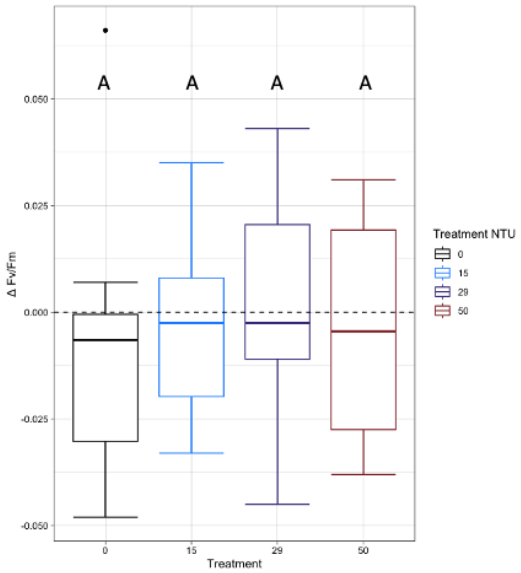


Figure 10. Change in photosynthetic efficiency (Fv/Fm) across turbidity threshold treatments. Treatment levels are 0 NTU, 15 NTU, 29 NTU, and 50 NTU.

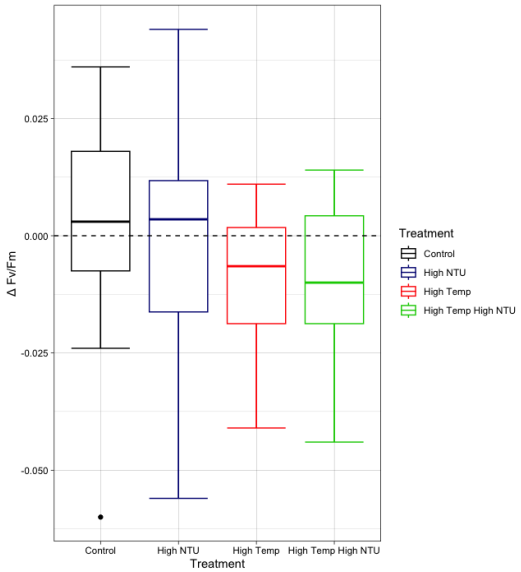


Figure 11. Change in photosynthetic efficiency (Fv/Fm) across multi-stressor treatments. Treatment levels are Control, High NTU, High Temp, and High Temp x High NTU.

### 3.6 Destructive Endpoint

Symbiont density:

Turbidity threshold determination experiments: A Kruskall-Wallis test showed no significant effect of turbidity treatments on symbiont density ($p > 0.05$) and plotting turbidities does not indicate a positive or negative trend with increasing turbidities. Multi-stressor experiments: A ranked 2-factor ANOVA showed no significant effect of turbidity, temperature, or interactions between the two factors on symbiont density ($p > 0.05$).

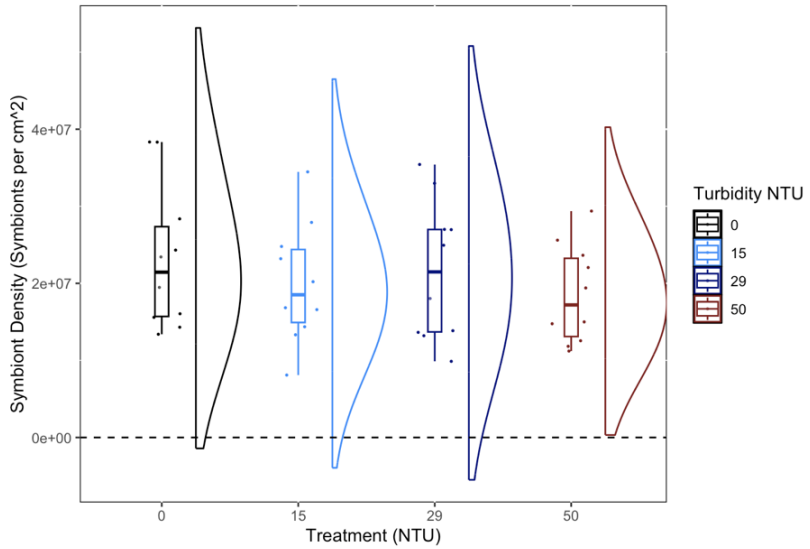


Figure 7. Symbiont density (symbionts/cm$^2$) plotted against target NTU. Symbionts were counted after acute exposure to turbidity threshold treatment where black is control, blue is 15 NTU, purple is 29 NTU, and red is 50 NTU.

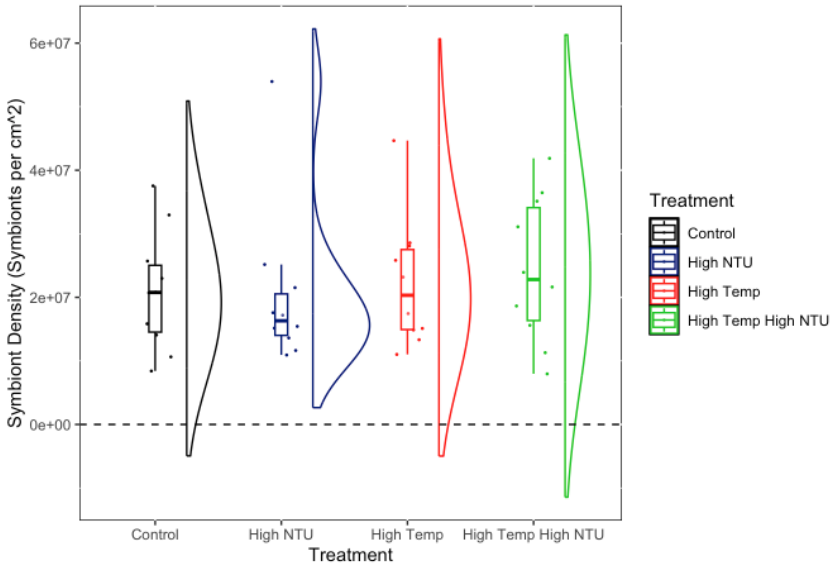


Figure 8. Symbiont density (symbionts/cm2) plotted against target treatments. Symbionts were counted after acute exposure to multi-stressor treatments where black is control, blue is 27ºC x 29 NTU, red is 30ºC x 0 NTU, and green is 30ºC x 29 NTU.

<u>Chlorophyll concentration (chl a, c2, total chl):</u>

Turbidity threshold determination experiments: A Kruskall-Wallis test showed no significant effect of treatment ($p > 0.05$) on Chlorophyll *a* concentrations, and plotting chlorophyll *a* values showed no indication of a trend across treatments.

Multi-stressor experiments. A Kruskall-Wallis showed no significant effect of treatment on chlorophyll $c_2$ concentrations ($p > 0.05$) and plotting concentrations by turbidity treatment gave no indication of a trend (Figure 12). The ratio of total chlorophyll per cm2 to symbiont density per cm2 across turbidity threshold treatments. Treatment levels are 0 NTU, 15 NTU, 29 NTU, and 50 NTU.

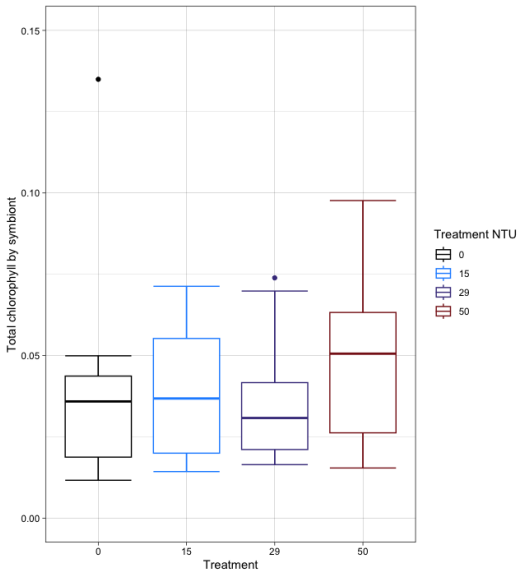


Figure 9. The ratio of total chlorophyll per cm2 to symbiont density per cm2 across turbidity threshold treatments. Treatment levels are 0 NTU, 15 NTU, 29 NTU, and 50 NTU.

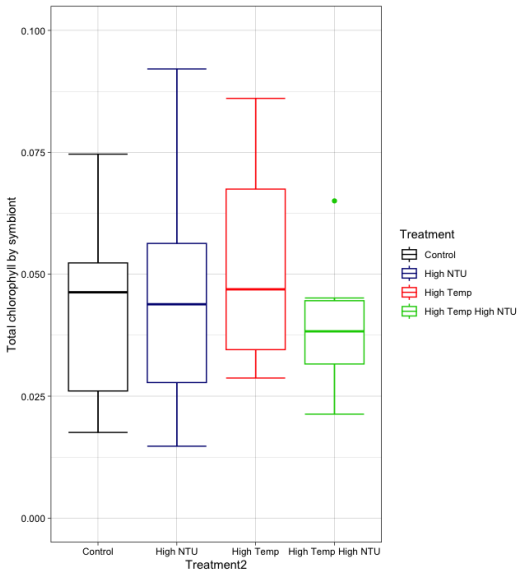


Figure 10. The ratio of total chlorophyll per cm2 to symbiont density per cm2 across multi-stressor treatments. Treatment levels are Control, High NTU, High Temp, and High Temp x High NTU.

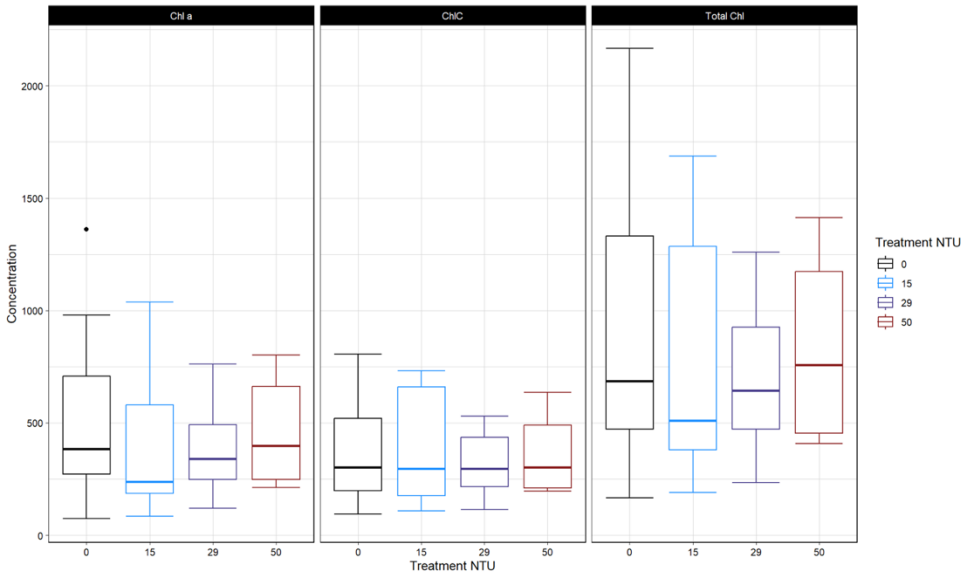


Figure 11. Chlorophyll a, c2, and total concentration standardized to surface area and averaged within treatment. Treatment levels are 0 NTU, 15 NTU, 29 NTU, and 50 NTU.

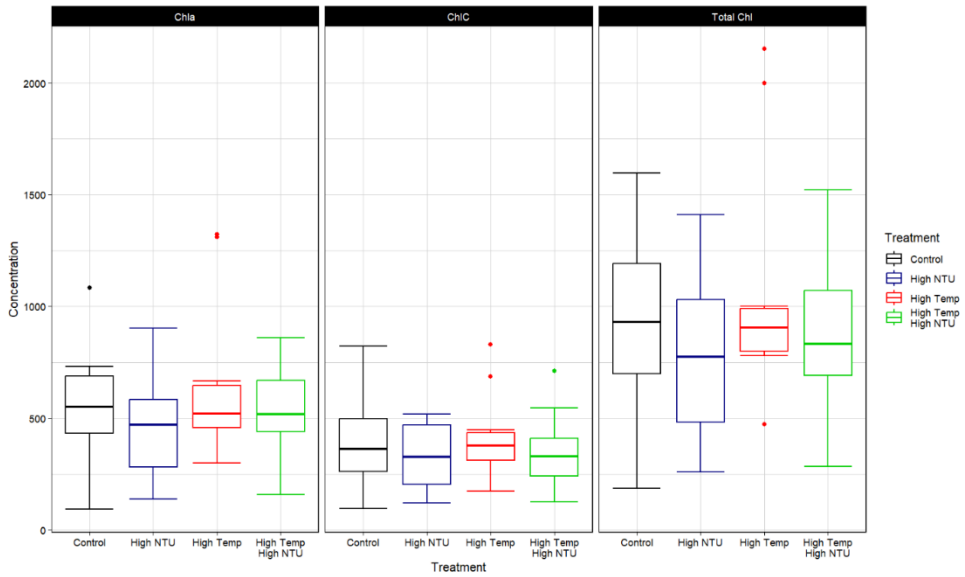


Figure 12. Chlorophyll a, c2, and total concentration standardized to surface area and averaged within treatment. Treatment levels are Control, High NTU, High Temp, and High Temp x High NTU.

<u>Protein Concentration:</u>

Turbidity threshold determination experiments: A one-way ANOVA showed no significant effect ($p > 0.05$) of turbidity treatments on protein concentrations in OFAV tissue.

Multi-stressor experiments: A two-way ANOVA showed no significant effect of turbidity, temperature, or an interaction between turbidity and temperature ($p > 0.05$ for all) on OFAV protein concentrations.

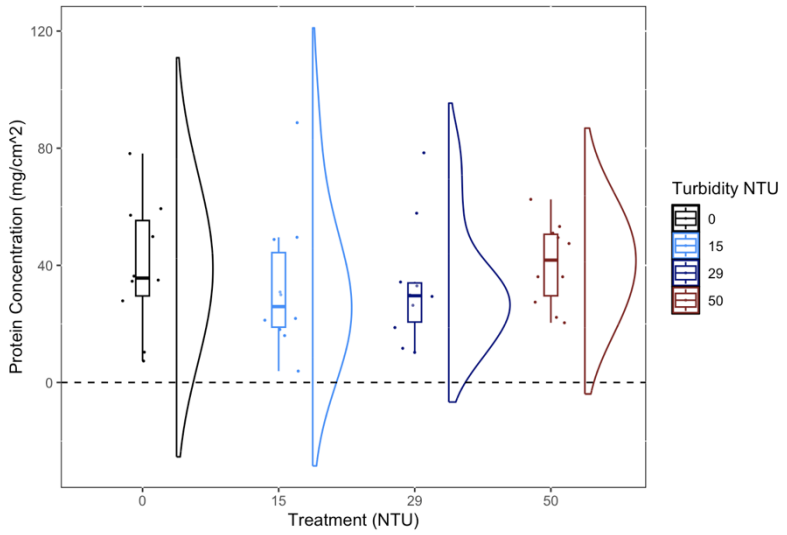


Figure 13. Protein concentration measured for OFAV after treatment exposure during turbidity threshold experiments where black is control, blue is 15 NTU, purple is 29 NTU, and red is 50 NTU.

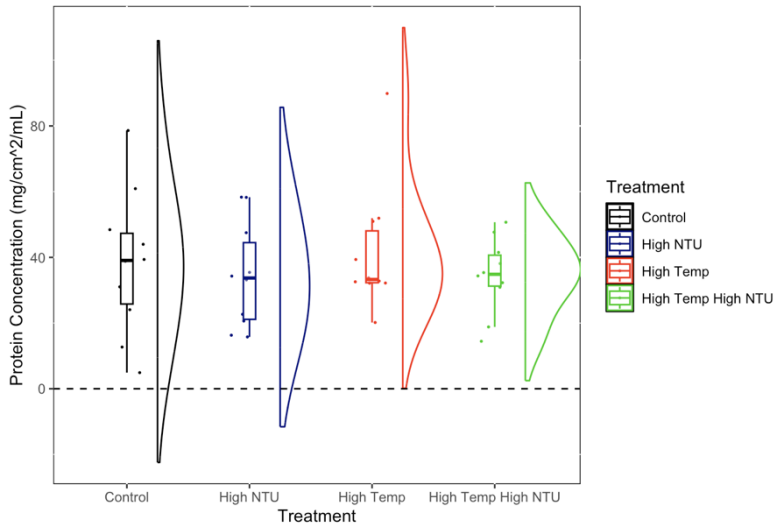


Figure 14. Protein concentration measured for OFAV after treatment exposure during turbidity threshold experiments where black is control, blue is 15 NTU, purple is 29 NTU, and red is 50 NTU.

Recovery:

Turbidity threshold determination experiments: Recovery varied within genotypes with an initial decrease in Fv/Fm after exposure. On average genotypes recovered by the 4th measurement indicated by meeting or exceeding the Pre-Ex (pre-exposure) Fv/Fm value for each genotype. Individual responses varied within and across recovery measurements leading to a grouped average to be taken for each genotype.

Multi-stressor experiments: Average recovery by genotype varied within and across treatments with an initial decrease in Fv/Fm post-exposure. A steady increase in Fv/Fm can be seen after recovery measurement 0 but is then followed by a delayed negative response by measurement 3. After which, a dramatic decrease in Fv/Fm can be seen by recovery measure 4. Individual

responses varied within and across recovery measurements leading to a grouped average to be taken for each genotype.

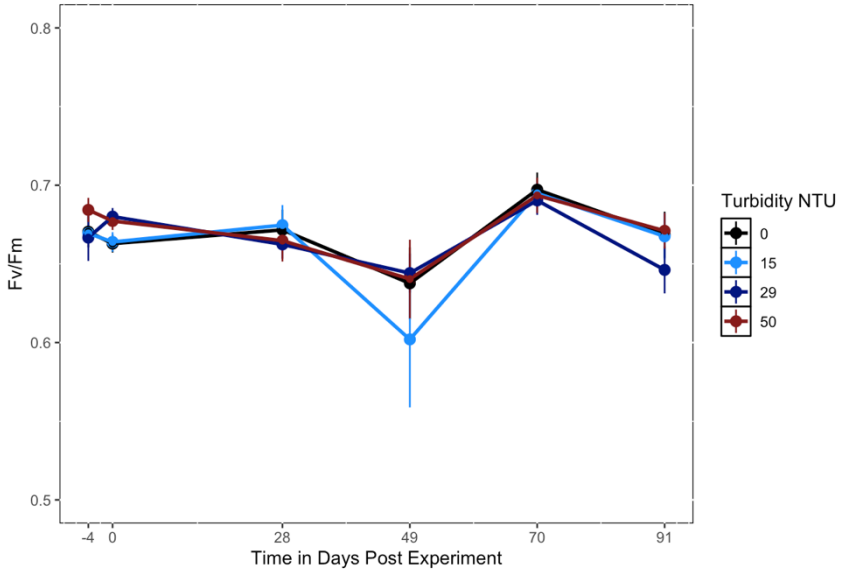


Figure 15. Variable fluorescence divided by maximum fluorescence (Fv/Fm) for corals in different experimental treatments by experimental recovery phase. Initial measurement is indicated by "-4". Higher Fv/Fm values generally indicate less-stressed photosynthetic organisms. Colors denote the treatment: Control (black, 0NTU), 15 NTU (blue), 29 NTU (purple), 50NTU (red).

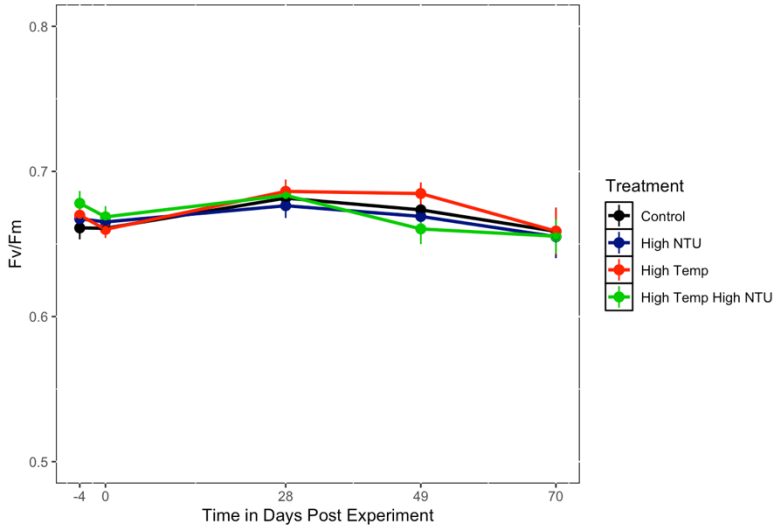


Figure 16. Variable fluorescence divided by maximum fluorescence (Fv/Fm) for corals in different experimental treatments by experimental recovery phase. Initial measurement is indicated by "-4". Higher Fv/Fm values generally indicate less-stressed photosynthetic organisms. Colors denote treatment levels where black is control, blue is 27ºC x 29 NTU, red is 30ºC x 0 NTU, and green is 30ºC x 29 NTU.

## 4. DISCUSSION

The project aimed to examine the impact of fine-grain suspended sediment on the metabolism, health, and growth of OFAV corals. It sought to define a biologically relevant benchmark of turbidity, measured in NTU, at which these corals begin to display physiological responses to sub-lethal stress. Additionally, the project investigated the combined effects of fine-grain suspended sediment and temperature on OFAV coral metabolism, health, and growth, which is needed to determine how turbidity benchmarks may need to be adjusted during marine heatwaves.

Short-term exposure (72 hours) to fine-grain sediment and elevated temperatures had minimal to no effect on the calcification, photosynthetic efficiency, and symbiont characteristics of OFAV. However, the metabolism of OFAV was significantly impacted by temperature, highlighting metabolism as a particularly sensitive biological response to environmental stress.

### 4.1 Respirometry

While the turbidity threshold experiment did not see a significant difference between 0 and 15 NTU, it did see a significant negative effect of turbidities of 29 and 50 on OFAV oxygen production (Table 4) and this appears to be driven mostly by reduced photosynthesis during daylight hours (Figure 12). Conversely in the turbidity x temperature experiments, no significant effect of turbidity on oxygen production was detected at ambient temperature, but a significant effect of turbidity was detected at elevated temperatures (Table 5). The impacts of temperature on oxygen production appeared to be more dramatic than the impacts of turbidity (Figure 15) and driven more by photosynthesis during the day (Figure 14) than respiration at night (Figure 13). This may indicate a greater negative impact of elevated temperatures on the photosymbionts than on OFAV corals themselves and suggest that while turbidity levels of 29 NTU may not cause much stress to corals at ambient temperatures they can worsen the effects of elevated temperatures, which suggests that climate change may make turbidity reductions an increasingly critical priority.

### 4.2 Photosynthetic Efficiency

No statistically significant differences in the effects of turbidity or turbidity x temperature treatments were detected in coral Fv/Fm measurements, although in the turbidity x temperature experiment, both elevated temperature treatments showed mean negative changes in Fv/Fm from pre-to-post-experimental conditions. A previous study on *A. cervicornis* in the same conditions found that Fv/Fm increased with NTU in response to decreased light availability. Therefore, larger OFAV samples or a longer experimental duration may be necessary to see a significant change in this variable under these conditions due to its morphology and biological traits. The observed significant difference in *A. cervicornis*, but not OFAV may suggest that ACER is a species less adapted to tolerate stress and more adapted to recover quickly once a stressor is no longer present whereas OFAV is more adapted to persist under stressful conditions (Grime, 1977). Further study is desirable to determine the mechanism for this.

### 4.3 Calcification

Calcification data collection begins on Day 2 of the experiment but will be referenced as Day 1 to indicate that it was the first 24 hours of exposure, Day 2 is hours 25-48 of exposure, and Day 3 is hours 49-72. No significant differences were found between turbidity threshold treatments; however, visual trends do appear in Figure 6 when compared to the control. The control (0 NTU)

appears to acclimate on Day 1 and then stay between –0.5 and 0.5 on Days 3 and 4. Similarly, corals exposed to turbidity (15, 29, 50 NTU) appear to acclimate on Day 1 and then recover within the treatments on Day 2. On Day 3, there appears to be a decrease in calcification for corals exposed to 15, 29, and 50 NTU, which could be the corals experiencing the full effect of the treatment. For the multi-stressor experiment, no significant differences were determined between stressors. However, Figure 7 shows trends similar to those seen in Figure 6. Corals from all four exposures (Control, High Temperature, High Turbidity, and High Temperature x High NTU) appear to acclimate to the chamber and treatment on Day 1, recover within the treatment on Day 2, and then experience a decrease in calcification on Day 3 after the exposure has fully taken effect. Trends between both experiments indicate that OFAV is not an ideal species to use for short-term exposure experiments, as it takes two days (48 hours) to see the full effect of the treatments/exposures on calcification rates. A longer exposure period might be necessary to see the full effect turbidity treatments and exposures have on coral calcification rates to accurately determine the impacts of dredging on the growth of the species.

**4.4 Destructive Endpoint**

No significant effects of different turbidity levels or turbidity x temperature treatments were observed of OFAV chlorophyll or protein concentrations. This may suggest that these indicators respond more slowly than changes in photosynthesis and respiration to environmental stress. This suggests that laboratory respirometry studies may be especially useful as early indicators of conditions that corals find stressful without having to wait long enough to observe changes to physiological processes that respond more slowly. Longer term studies may also be useful to determine how long corals can continue to function under altered photosynthesis and respiration without it affecting these other variables and if this effect varies by coral species.

Turbidity threshold determination experiments - ΔFv/Fm:

There were no statistical differences between the treatments and the change in Fv/Fm for Turbidity threshold determination experiments, which is shown in Figure 5. This could suggest that acute exposure of turbidity alone at 50NTU or less does not affect the photosynthetic efficiency for OFAV, or it could take greater than 72 hours for changes in photosynthetic processes to occur.

Multi-stressor experiments - ΔFv/Fm:

Figure 5 shows the ΔFv/Fm pooled across trials for each treatment. While there were again no statistical differences in ΔFv/Fm for Multi-stressor experiments, there are some differences between treatments. The elevated NTU treatment mean is similar to the control treatment mean, while elevated Temperature and Temperature + NTU treatment means are negative and noticeably lower than the controls, showing a decrease in photosynthetic efficiency. This could indicate that sudden elevated temperature (27ºC to 30ºC) sustained for 72 hours has a negative impact on the photosynthetic efficiency of OFAV. Though, there is not enough significance to suggest there are any interacting effects between elevated NTU and temperature.

Recovery Fv/Fm:

No statistically significant effect of turbidity treatments or turbidity x temperature exposures was detected on Fv/Fm recovery. A notable reduction in Fv/Fm, however, was observed in all turbidity x temperature treatments at recovery measurement 4 for Multi-stressor experiments trials, likely as a result of an unintentional holding aquarium temperature spike from 27ºC to 29ºC over the

course of 1-2 hours and remained elevated for at least 12 hours. This further suggests that sudden thermal increases may be more stressful to OFAV than more gradual ones to the same temperature.

## 5. CONCLUSION AND FUTURE DIRECTIONS

This project underscores the critical importance of defining biological benchmarks for coral health and that not all biological benchmarks respond to stressors equally quickly. Through this study, coral metabolism was found to be highly sensitive to environmental stressors and provided real time responses to acute stressor treatments, which may make it especially useful for future studies of other Caribbean coral species. While not significant, many of the other biological benchmarks (Fv/Fm, calcification, and destructive-endpoint samples) showed relative trends in response to acute stressor exposure, but no definitive thresholds for future management plans. Contrary to these findings, recovery and the measures of this species resilience to acute stress does offer a baseline understanding that morphology and species specific response rates might necessitate a longer exposure period to reflect real work conditions during high turbid and high temperature stress events. Longer term studies would also provide information on how long corals can tolerate stress that alters photosynthesis and respiration before showing signs of other physiological alteration that may take longer to recover as stressors are removed. Working with additional species of coral could also provide insight on how physiological differences between different species could lead to ecological changes on reefs in terms of species abundance.

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