High-replication assessment of the effects of poor water quality on South Florida coral health and susceptibility to Stony Coral Tissue Loss Disease

STAR system setup for demonstration during an outreach event in Miami.

High-replication assessment of the effects of poor water quality on South Florida coral health and susceptibility to Stony Coral Tissue Loss Disease

Final Report

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June 2024

Completed in Fulfillment of PO C1E079 for

Florida Department of Environmental Protection
Coral Protection and Restoration Program
8000 N Ocean Dr.
Dania Beach, FL 33004

This report should be cited as follows:
Palacio-Castro, AM, Studivan MS, Enochs IC. 2024. High-replication assessment of the effects of poor water quality on South Florida coral health and susceptibility to Stony Coral Tissue Loss Disease. Florida DEP. Miami, FL. 24 Pages.

This report was funded through a contract agreement from the Florida Department of Environmental Protection's (DEP) Coral Protection and Restoration Program. The views, statements, findings, conclusions, and recommendations expressed herein are those of the author(s) and do not necessarily reflect the views of the State of Florida or any of its subagencies.

Acknowledgments

We would like to thank Dr. Chris Kelble, Tyler Christian, and Ian Smith from NOAA/AOML for their assistance in processing the nutrient samples. We would like to thank Kenzie Cooke, Ashley Stevens and Emilia Silverberg who were instrumental in maintaining the experiment.

Management Summary

Poor water quality is one of the most prevalent stressors on coral reefs, especially in South Florida. Understanding how specific nutrient sources (e.g., NO_3, NH_4, and PO_4), nutrient combinations, and nutrient concentrations affect corals has been challenging, partially due to the complexity of the experimental designs needed to test a high number of treatments and treatment levels. Here, we modified and expanded our recently developed Sequential Treatment Application Robot (STAR) system, in order to allow precise multi-nutrient dosing into individual coral vessels. The dosing system was first tested for nutrient stability without corals (n=572 nutrient samples), and then in an experiment that measured the impacts of multiple nutrient combinations on the threatened staghorn coral, *Acropora cervicornis* (n=563 nutrient samples). These tests included a control treatment (C) where the vessels were only dosed with artificial seawater, as well as a combination of five elevated nutrient treatments: elevated NO_3 only (NO_3), elevated NH_4 only, elevated PO_4 only (PO_4), elevated NO_3 and PO_4 (NO_3 + PO_4) and elevated NH_4 and PO_4 (NH_4+PO_4). The elevated nutrient concentrations targeted a +10 µM increase in the NO_3 and NH_4 concentrations, and +2 µM increase in the PO_4 concentration. Data on the photochemical efficiency (*Fv/Fm*) and buoyant weight of the corals were collected before and after exposure to the nutrient treatments to test the effects of the treatments on coral performance. Future work will statistically test the impacts of these treatments on coral growth and *Fv/Fm.*

Executive Summary

Poor water quality is a major stressor on coral reefs, especially in South Florida. Understanding the effects of specific nutrient sources (e.g., NO_3, NH_4, and PO_4), nutrient combinations, and concentrations on corals has been challenging due to the complexity of required experimental designs. To address this, we modified and expanded the Sequential Treatment Application Robot (STAR) system to enable continuous and precise multi-nutrient dosing into individual coral vessels. Enhancements included modifying the LabVIEW program for precise control over multiple dose sources, improving the stir plate system, constructing new aluminum tank stands for stability, and designing larger coral vessels to buffer nutrient concentration changes. The system was first tested for nutrient stability without corals (n=572 nutrient samples), leading to modifications such as using artificial seawater instead of RO water in the nutrient dosing solutions to improve nutrient mixing in the vessels. This troubleshooting phase successfully achieved target NH_4 and PO_4 concentrations, although maintaining NO_3 levels was challenging due to NO_3 reduction to NO_2.

We then conducted an experiment to test the impacts of multiple nutrient combinations on the threatened staghorn coral, *Acropora cervicornis*. Twelve fragments from each of six *A. cervicornis* genotypes were allocated to 72 vessels across six nutrient treatments, including a control (C) with only artificial seawater and five elevated nutrient treatments: elevated NO_3 only (NO_3), elevated NH_4 only (NH_4), elevated PO_4 only (PO_4), elevated NO_3 and PO4 (NO_3 + PO_4), and elevated NH_4 and PO_4 (NH_4 + PO_4). The elevated nutrient concentrations targeted a +10 µM increase in NO_3 and NH_4 and a +2 µM increase in PO_4. A total of 563 nutrient samples were collected during this phase. Upon introducing corals, NH_4 and NO_3 concentrations declined across all nitrogen-involved treatments, attributed to nutrient uptake by corals and single-celled diatoms. Data on the photochemical efficiency (*Fv /Fm*) and buoyant weight of the corals were collected before and after nutrient exposure to assess treatment effects on coral performance. Future work will statistically analyze the impacts of these treatments on coral growth and *Fv /Fm*.

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List of Acronyms

STAR - Sequential Treatment Application Robot System
NOAA/AOML - National Oceanic and Atmospheric Administration Atlantic Oceanographic and Meteorological Laboratory
UM/AOML - University of Miami Atlantic Oceanographic and Meteorological Laboratory
ERL - Experimental Reef Laboratory
DEP - Department of Environmental Protection
RO - Reverse Osmosis

1. BACKGROUND

Coral reefs are among the most diverse and productive ecosystems on Earth, providing critical ecological services and supporting a vast array of marine life (Reaka-Kudla et al. 1996). However, they are increasingly threatened by a variety of stressors, both local and global. Poor water quality is one of the most prevalent stressors in South Florida reefs where excessive nutrient input, particularly nitrogen and phosphorus, can affect coral physiology, leading to detrimental consequences for coral health and reef ecosystems (Thanopoulou et al. 2022).

Elevated nutrient concentrations can lead to various adverse effects on coral health, such as reduced growth and survivorship, shifts in the composition of coral-associated Symbiodiniaceae and prokaryotic communities, increased vulnerability to additional stressors, and changes in the skeletal composition (Voss and Richardson 2006; Godinot et al. 2011; Palacio-Castro et al. 2021, 2022; Klinges et al. 2022). The impacts of nutrient pollution, however, can vary based on its source (Burkepile et al. 2019; Fernandes de Barros Marangoni et al. 2020), concentration (D'Angelo et al. 2014), relative ratio of nitrogen to phosphorus (Wiedenmann et al. 2013; Morris et al. 2019), and the sensitivity of coral species (Shantz and Burkepile 2014; Palacio-Castro et al. 2021).

Among Caribbean species, nutrient enrichment has been shown to be especially harmful for the threatened staghorn coral, *Acropora cervicornis,* which can experience reduced coral calcification rates (Renegar and Riegl 2005) and higher susceptibility to additional stressors such as high temperatures (Palacio-Castro et al. 2021). Understanding the impacts of nutrient inputs in South Florida reefs, especially on vulnerable and ecologically relevant corals, such as ESA-listed species, is vital for developing effective management strategies to mitigate nutrient pollution and enhance coral reef resilience in the face of ongoing environmental changes.

To inform water quality standards, a systematic assessment of the direct impacts of dissolved inorganic nitrogen (nitrate, nitrite, and ammonium) and dissolved inorganic phosphorus (phosphate) at different concentrations and combinations is necessary. Additionally, prior research indicates that nutrient pollution exacerbates the response to other stressors (Voss and Richardson 2006; Higuchi et al. 2015; Palacio-Castro et al. 2022), making it crucial to develop experimental setups that can incorporate additional simultaneous treatments such as exposure to disease or heat stress.

In this project, we expanded and modified the Sequential Treatment Application Robot (STAR) system (Enochs et al. 2024). The STAR system previously developed with the generous support of FDEP to test the effects of elevated temperature and ammonium dosing on stony coral tissue loss disease (SCTLD) transmission rates. The new modifications incorporated in this project allowed investigating the impacts of multi-nutrient enrichment on the health and physiology of six *A. cervicornis* genotypes used in South Florida coral restoration. These modifications provide an unprecedented resource for further water quality testing, critical for threshold identification, should additional

years be funded. Coral data under different water quality treatments, collected during the validation of this system, will inform ecologically relevant management targets.

2. METHODS

A detailed description of the original Sequential Treatment Application Robot (STAR) system was published earlier this year (Enochs et al. 2024) and a publication describing the effects of elevated temperature and ammonium dosing has been submitted for publication (Palacio-Castro et al. *submitted*). The previous system allowed the dosing of large water volumes from two independent water sources using peristaltic pumps, and a precise nutrient dosing from one source using a syringe pump. Further development and buildout of the STAR system were carried out in this funding cycle to (1) expand the STAR system to eight additional tanks, and (2) allow testing three nutrient sources and thus the examination of multi-nutrient interactions. These modifications provide an unprecedented resource for further water quality testing, critical for threshold identification.

2.1. Modifications to and operation of the STAR system

New dosing boxes: Two dosing boxes were built in-house, including a multichannel syringe pump (Kloehn v6, Norgren) to dose small volumes of any solution (e.g., nutrients) and two brushless peristaltic pumps (A201BX, Anko) to dose bigger volumes (e.g., to provide seawater to the experimental units) (Figure 1).

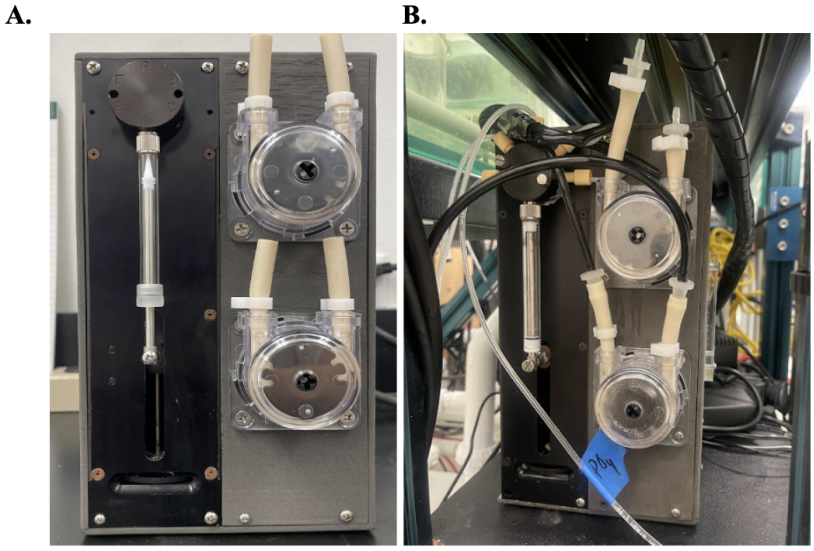


Figure 1: *New dosing boxes.* ***A.*** *Dosing box after being built.* ***B.*** *Dosing box connected to one seawater source (bottom right pump) and multiple nutrient sources (top left) being used during the experiment.*

LabVIEW program: The STAR system LabVIEW program has undergone multiple changes to allow for the addition of multiple nutrient sources into the research vessels with minimal contamination (Figures 2 and 3). The most notorious change is that the user now defines every movement performed by the syringe pump and valve for a given position, where many of these movements were pre-programmed in the previous iteration. This allows for a larger degree of control over how the dosing boxes behave, including more complex activities like dosing from several different valve ports in a given position. There have also been quality improvements that improve the efficiency of the robot movement paths.

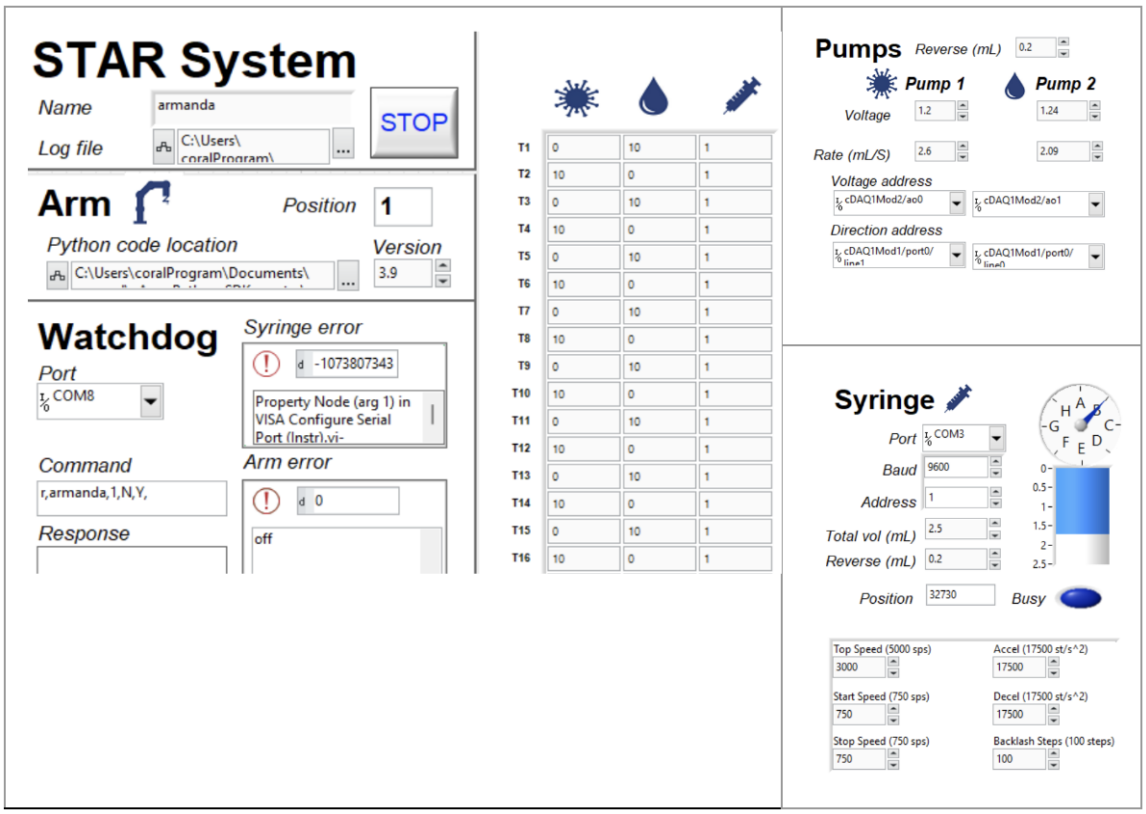


Figure 2: *Changes performed in the LabVIEW program that controls the dosing system - Initial graphical interface for the dosing system.*

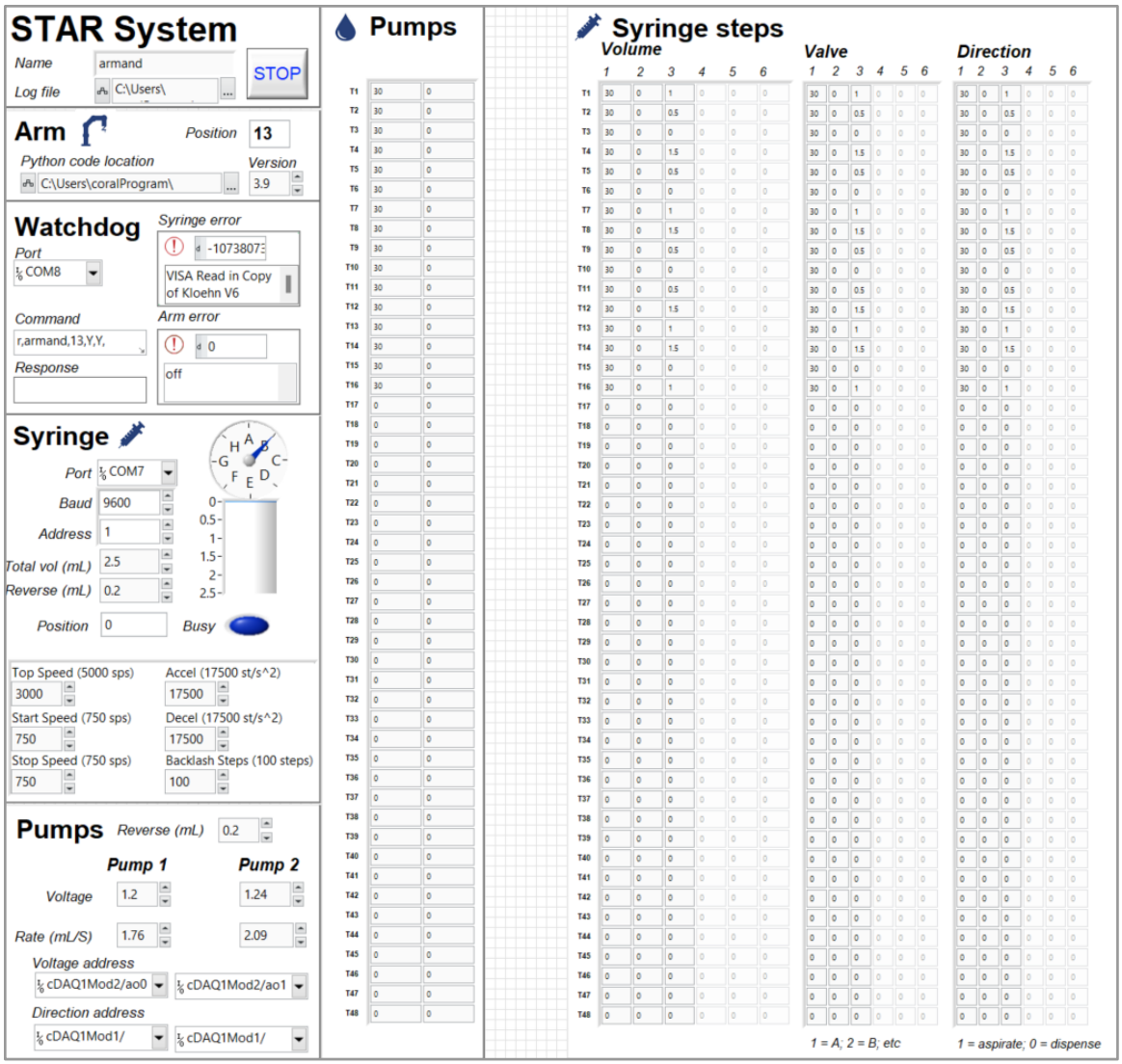


Figure 3: *Changes performed in the LabVIEW program that controls the dosing system - Modified graphical interface for the dosing system allowing complex dosing from several different valve ports.*

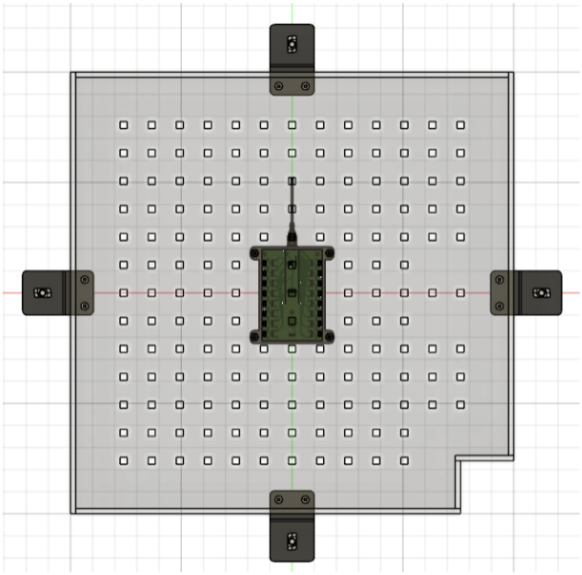
Tank Stands: New tank stands were constructed from aluminum extrusion which allows for sturdy hardware-based connections to the tank stands (Figure 4). This change also allows the tank stands to be affixed to the robot table to increase the stability of the system and enhance the connections of peripheral systems and devices like the stir plate system.

A. B.

Figure 4: *Changes performed in the tank stands to allow more stability in the arm system.* ***A.*** *Assembled aluminum stands for the new systems.* ***B.*** *Aluminum stands installed in the Experimental Reef Lab holding a glass tank, the new stir plates and the new coral vessels.*

Stir plates: The stir plates have been totally redesigned from the previous iteration. The stir plate housing is now able to be screwed into place below the aquarium for a more stable connection (Figure 5). The 3D printed bases of the stir motor units are redesigned to clip to the bottom of the stir plate housing, which has a series of mounting holes cut into the bottom. This allows for greater freedom in the position and size of the vessels in the aquarium. The beakers and beaker racks from the previous iteration are being replaced with custom vessels with magnets inlays on their bases. A magnetic registering system on each stir motor unit interacts with the magnets on the vessels and ensures that the research vessel in the aquarium remains directly above the stir motor unit. The controllers for the stir system have been paired down to remove the OLED screen and IR communication and are secured to the bottom of the stir plate housing, which greatly reduces the amount of wire needed.

A.



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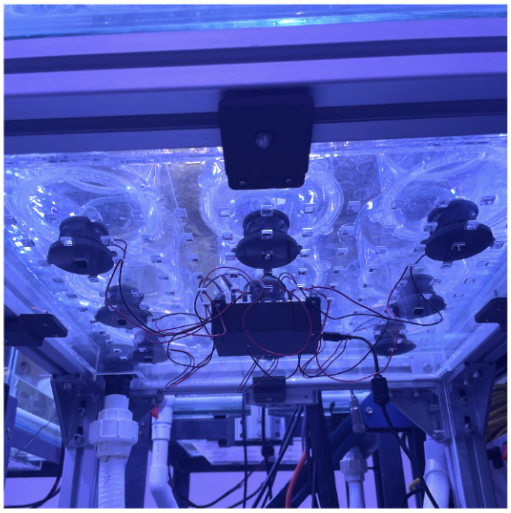

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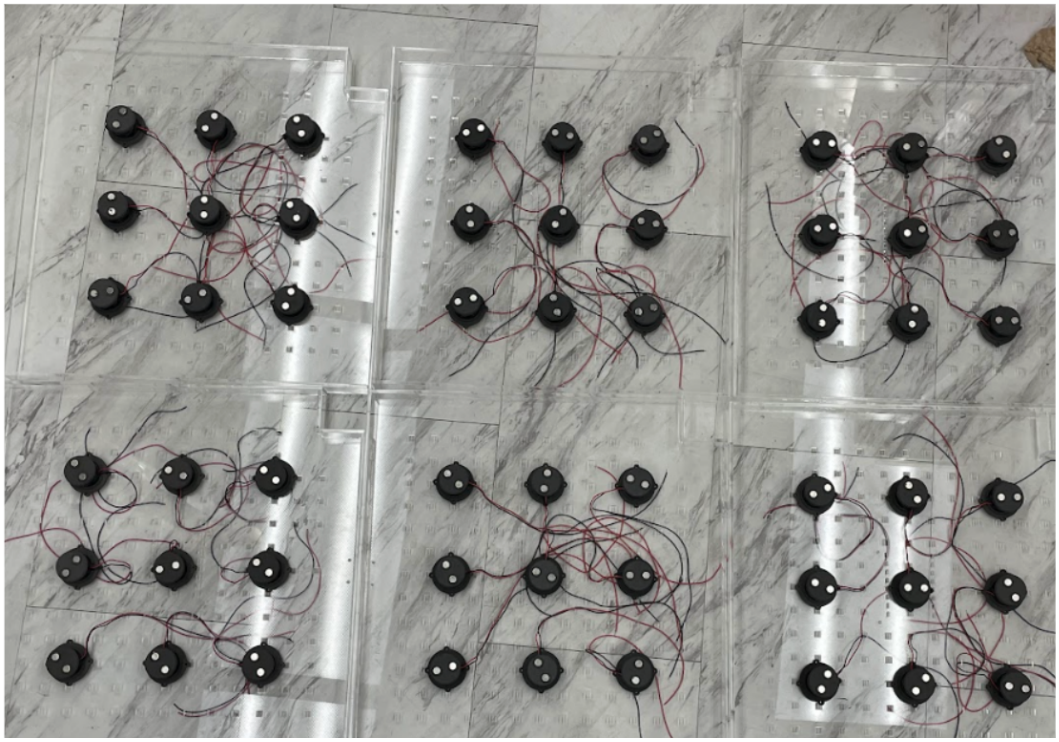

Figure 5: *New stir plates controlling the flow system - **A.** New acrylic stir plate housing design with attached controller and improved mounting system. **B.** New stir plate attached to the aluminum stands showing the mounting system. **C.** Pictures of six of the eight stir plates built (two more were already installed and were being tested).*

New coral vessels: The volume of the vessels has increased from 600 mL in the previous STAR system to 1 L in the new system to reduce the effect of the biological activity on the chemistry of the seawater in the vessel. A bigger water volume allowed us to buffer changes in nutrient concentration as a result of nutrient uptake by the coral biomass. This, however, reduced the number of vessels that can fit in a tank from 16 in the previous STAR system to nine in this new buildout. The modularity of the system allows to switch between configurations and adapt the stir plates to different vessel sizes and arrangements.

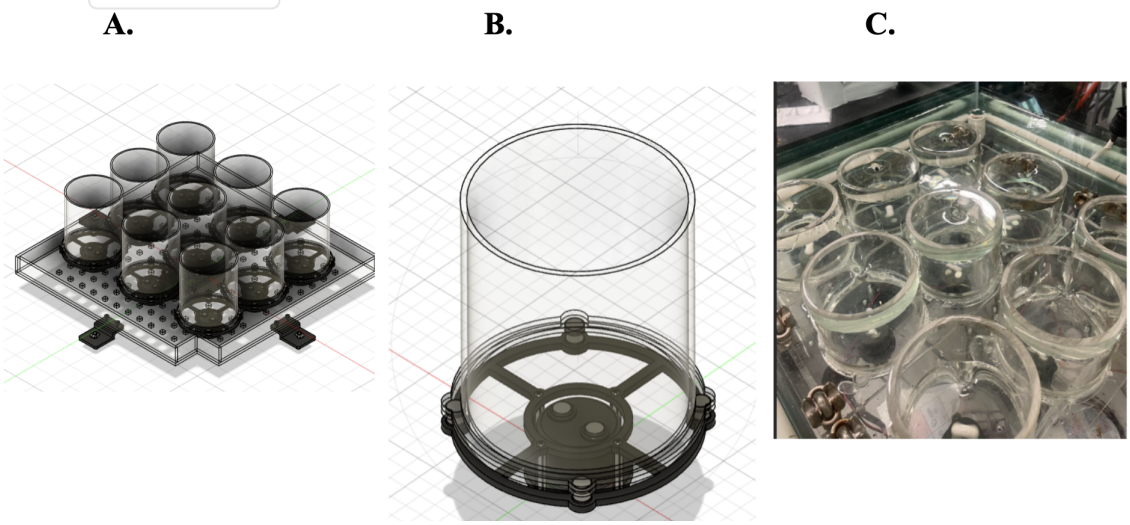

Figure 6: *Experimental vessels - **A.** Image of the final design with the dosing vessels in place. **B.** New coral vessels designed for bigger water volume and more stability in the nutrient concentrations. **C.** Image of the vessels being used in the experiment.*

2.2. Experiment conducted

Experimental Coral Collection and Maintenance: On April 6, 2024, 16 fragments from six *Acropora cervicornis* genotypes were collected from the Key Biscayne "Rescue a Reef" nursery. The fragments were tagged and allowed to recover for a month (until May 3rd) in a glass tank at the University of Miami Rosenstiel School's Experimental Reef Lab. Temperature was initially set to 25 °C to match the temperature at the nursery and ramped up to 26°C over two days (0.5°C per day).

Multi-nutrient STAR dosing set up: On May 2, 2024, 72 independent vessels were evenly allocated to six different nutrient treatments: Control (C), elevated NO_3 only (NO_3), elevated NH_4 only, elevated PO_4 only (PO_4), elevated NO_3 and PO_4 (NO_3 + PO_4) and elevated NH_4 and PO_4 (NH_4+PO_4). The elevated nutrient concentrations targeted a +10 µM increase in the NO_3 and NH_4 concentrations, and +2 µM increase in the PO_4 concentration. In order to accomplish this, all nutrient treatments were dosed with 30 mL of artificial seawater every 45 minutes. Two water vats (750 L each) were used to mix and supply artificial seawater to the system. At a given time one vat was providing water to the

experiment, while the second vat was getting RO water and mixing it with instant ocean sea salt to a salinity of 35 ppt. Corals under elevated NO_3 and NH_4 were additionally dosed 1 mL dose of the respective stock solution (either NH_4Cl or $NaNO_3$) at a concentration of 500 µM. Finally, corals under elevated PO_4 were dosed with 0.5 mL of a Na_3PO_4 stock solution at a concentration of 200 µM. All tanks were maintained at 26°C for the duration of the experiment.

Nutrient concentrations before introducing corals to the vessels were monitored from May 2 to May 14 (Figure 7). First, the initial STAR setup was allowed to run from May 2nd to May 5th, and the nutrient concentrations were monitored (Figures 8-11). Based on the initial nutrient results, modifications to the dosing were introduced from May 6 to 13 to troubleshoot and achieve the nutrient targets. One of the changes consisted of adjusting the concentration of the stock solutions based on the nutrient values measured since these were initially found to be slightly under the target. The other change was to prepare the stock solutions with artificial seawater instead of Reverse Osmosis (RO) water, in order to increase the stock density, thus facilitating mixing in the vessel. A total of 572 water samples were collected during this period, including samples from each individual vessel subjected to the nutrient treatments, blanks (RO water and low nutrients seawater collected offshore), artificial seawater, and stock solutions.

Figure 7: *Modified STAR system running without corals to test the stability of the dosing system.*

Physiological data to test the effects of nutrient enrichment on *A. cervicornis:*
On May 14, 2024, 12 fragments from each of the six *A. cervicornis* genotypes (N= 72; Table 1) were evenly allocated to the nutrient treatments. Photochemical efficiency (*Fv /Fm*) and buoyant weight data were collected for each fragment before and after exposure

to the nutrient treatments to test the effects of elevated nutrients on coral performance. *Fv /Fm* was measured on April 30, and May 13 (before nutrient addition), and May 30, 2024 (after two and a half weeks in nutrient treatments). *Fv /Fm* was acquired using a Maxi Imaging-PAM fluorometer (I-PAM, Walz) after dark-adapting the corals for 30 minutes. Buoyant weight data was collected April 30, May 13 and May 29, 2024. A total of 563 water samples for nutrient analysis were collected during this period, including samples from each individual vessel subjected to the nutrient treatments and housing the corals, blanks, artificial seawater, and stock solutions.

Table 1: *Genotype and treatment allocation during the nutrient experiment.*

Genotype	Control	NH4	NH4+PO4	NO3	NO3+PO4	PO4	Grand Total
BC8A	2	2	2	2	2	2	**12**
Cheetos B	2	2	2	2	2	2	**12**
Cooper	2	2	2	2	2	2	**12**
MBC	2	2	2	2	2	2	**12**
MBD	2	2	2	2	2	2	**12**
SIE	2	2	2	2	2	2	**12**
Grand Total	**12**	**12**	**12**	**12**	**12**	**12**	**72**

Nutrient analysis: Water samples were collected from each vessel 3-5 times a week and frozen upon collection. An RO blank was included in each batch of samples collected, as well as a sample of the artificial seawater being dosed into the coral vessels. The stock solutions were sampled when new batches of solutions were made. However, in order to process the stock solution samples they have to be diluted to be in the range of detection (NH_4 and NO_3 had a 1/50 dilution to target a final 10 µM; and PO_4 had a 1/100 dilution to target 2 µM). All samples were analyzed for NO_2, NO_3, NH_4 and PO_4 using an AA3 Analytical Analyzer (Seal) and included runs of low nutrients seawater collected offshore as a control. The instrument was calibrated before each run following the Standard Operating Procedure for the Calibration of the AA3 Nutrient Analyzer protocol using standard solutions (AOML Ecosystem Assessment Laboratory 2024).

3. RESULTS

3.1. Ammonium (NH_4)

The first two batches of NH_4 stock solution were approximately 30% (~7 µM) under the target concentration (10 µM after a 1/50 dilution from the stock target 500 µM) (Figure 8;

pink triangles in the top panel). The subsequent stocks were adjusted based on this calculation. However, one problem to exactly achieve the NH_4 stock concentrations using artificial seawater was that the instant ocean salts contained NH_4 in variable levels (Figure 8; gray squares in the top panel), therefore slightly elevating NH_4 in the stocks made with artificial seawater. Low nutrient seawater from offshore and RO always have low NH_4 values (<0.5) except for one RO value On May 21 that revealed contamination of the sample. During the troubleshooting phase without corals, we were able to achieve the NH_4 target concentrations (~10 µM) in the NH_4 and NH_4+PO_4 experimental vessels after adjusting the stock solutions concentration and making the stock denser by using artificial seawater (Figure 8; bottom panel). However, NH_4 concentrations were slightly higher than 10 µM since the artificial water itself contained 2-3 µM of NH_4.

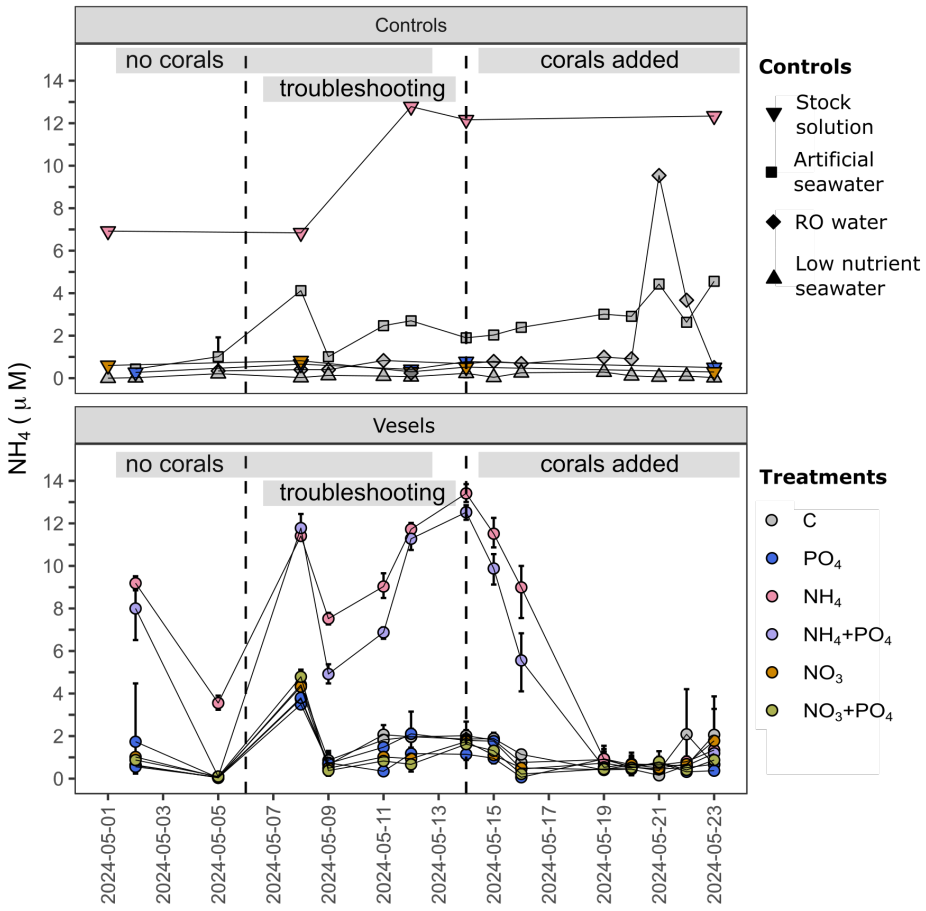

Figure 8: *Ammonium concentrations during the experiment. Top panel: control samples. Lower panel: experimental samples in the STAR system vessels. The vertical dashed lines show the days when changes were made to the system such as adjusting stock concentrations or adding the experimental corals.*

After introducing corals in the experimental vessels, NH_4 concentrations in the NH_4 and NH_4+PO_4 treatments quickly declined and became no different than in any of the other nutrient treatments (Figure 8; bottom panel). However, the stock solution concentration

revealed that the vessels were being constantly dosed with NH_4 at the target levels (Figure 8; top panel).

NH_4 concentrations in the C, NO_3, PO_4 and NO_3 + PO_4 treatments during the testing period without corals followed the NH_4 concentrations in the artificial seawater being dosed at that moment, including a high concentration peak on May 8th. After the addition of corals to the vessels, NH_4 declined in these treatments as well, even when the artificial seawater contained 2-3 µM of NH_4.

3.2. Nitrate (NO_3) and nitrite (NO_2)

The first two batches of NO_3 stock solution were approximately 20% (8-9 µM) under the target concentration (target = 10 µM after a 1/50 dilution from the stock target 500 µM) (Figure 9; orange triangles in the top panel). The subsequent stocks were adjusted based on this calculation. The artificial seawater contained more stable and lower concentrations of NO_3 (1-2 µM) compared to those of NH_4. Low-nutrient seawater from offshore and RO always have NO_3 values close to 0.

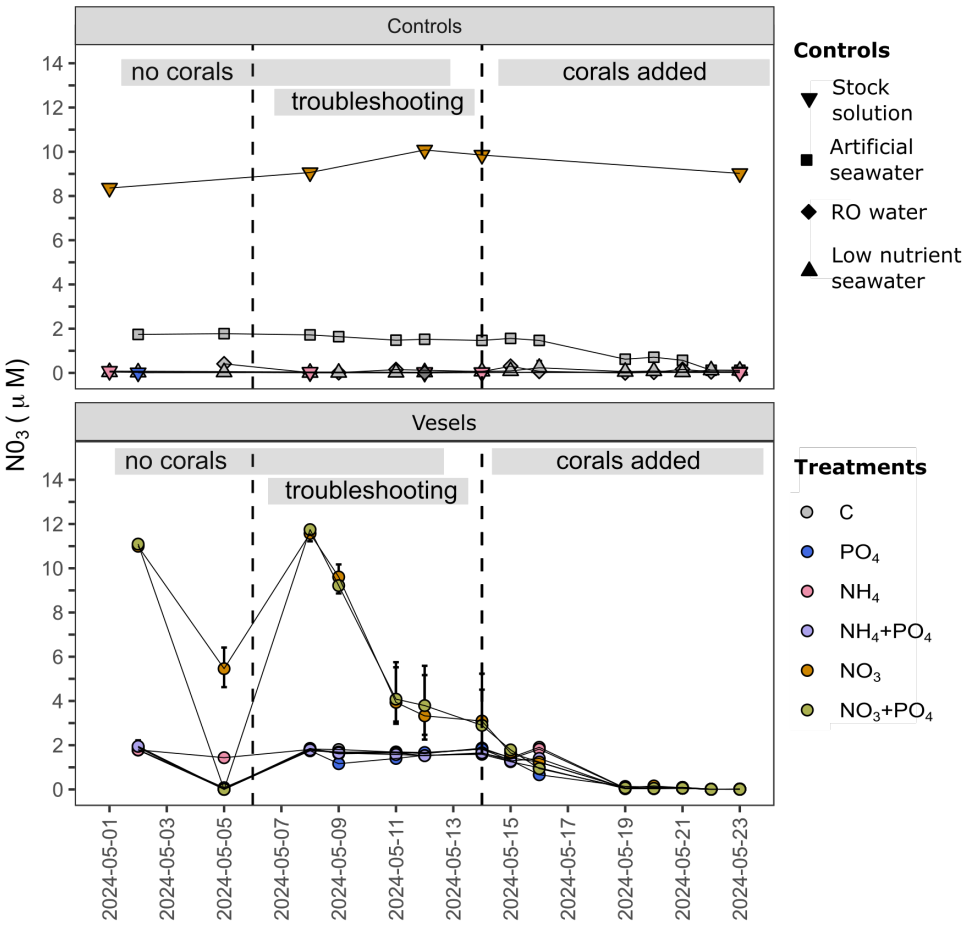

Figure 9: *Nitrate concentrations during the experiment. Top panel: control samples. Lower panel: experimental samples in the STAR system vessels. The vertical dashed lines*

show the days when changes were made to the system such as adjusting stock concentrations or adding the experimental corals

During the troubleshooting phase without corals, we were not able to achieve the NO_3 target concentrations (~10 µM) in the NO_3 and NO_3 +PO_4 experimental vessels, even after verifying the correct stock solutions concentration (Figure 9; bottom panel). A further examination of the NO_2 concentrations showed increasing NO_2 values in the NO_3 and NO_3 + PO_4 treatments over time (Figure 10), indicating that NO_3 was being reduced to NO_2 in the experimental vessels when there were no corals added. However, this was not detected on the NO_3 stock solution (Figures 9-10).

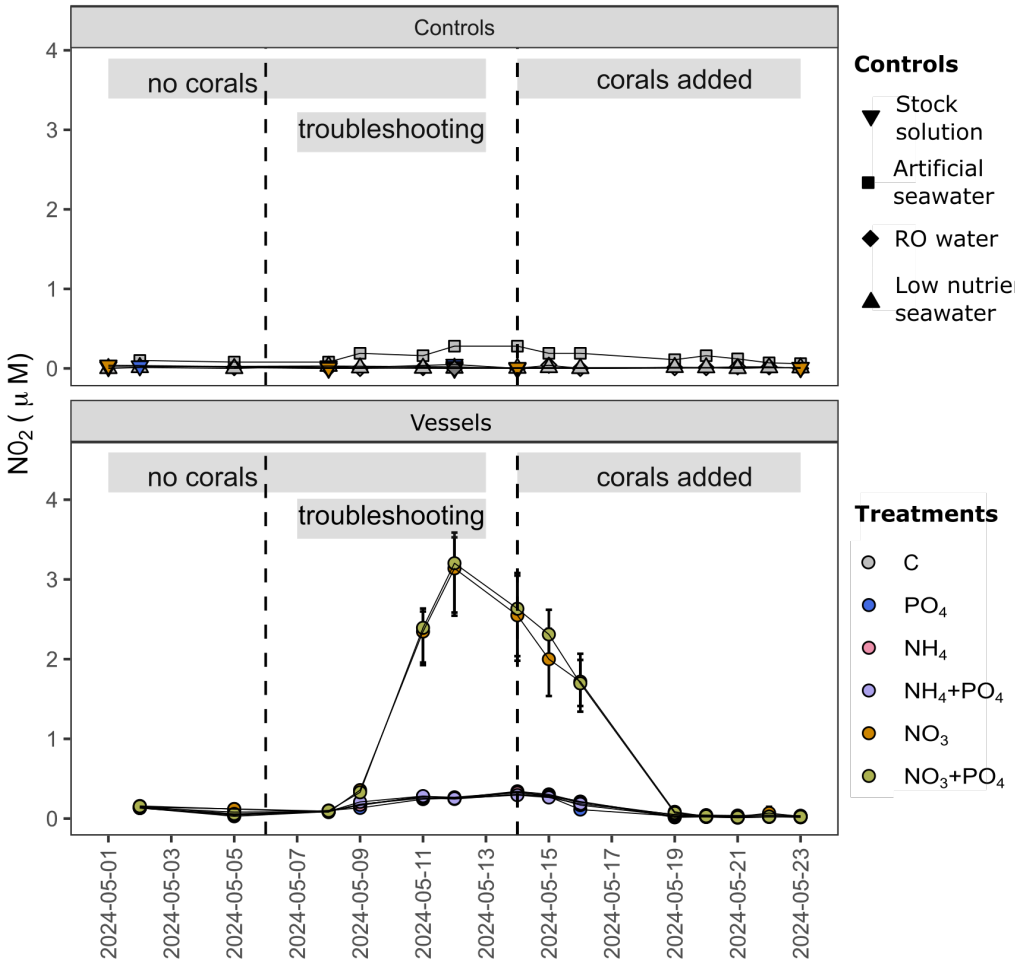

Figure 10: *Nitrite concentrations during the experiment. Top panel: control samples. Lower panel: experimental samples in the STAR system vessels. The vertical dashed lines show the days when changes were made to the system such as adjusting stock concentrations or adding the experimental corals.*

After introducing corals in the experimental vessels, NO_3 concentrations in the NO_3 and NO_3+PO_4 treatments quickly declined to non-detectable (Figure 9; bottom panel), regardless of the constant dosing with NO_3 stock solution (Figure 9; top panel).

NO_3 and NO_2 concentrations in the C, NH_4, PO_4 and NH_4 + PO_4 treatments during the testing period without corals followed the concentrations in the artificial seawater being dosed at the moment (1-2 µM for NO_3 and close to 0 for NO_2). However, NO_3 and NO_2 concentrations reached values under 0.02 µM in all nutrient treatments after the addition of corals to the vessels (Figures 9-10).

3.3. Phosphate (PO_4)

The PO_4 stock solutions were always close to the target concentrations and therefore were not adjusted (~2 µM after a 1/100 dilution from the stock target 200 µM) (Figure 11; blue triangles in the top panel). Although the artificial seawater had PO_4 values below 0.3 µM for most of the experiment, this concentration increased during the last week of the experiment to ~2 µM (Figure 11; gray squares in the top panel). Since the PO_4 concentration in the RO water used to make the artificial seawater was maintained near cero, we presume that the increasing PO_4 in the artificial seawater came from a new batch of sea salts with higher PO_4. Low-nutrient seawater from offshore and RO always have low PO_4 values (<0.3).

During the troubleshooting phase without corals, we achieved elevated PO_4 concentrations of ~1.5 µM in the PO_4, NO_3+ PO_4 and NH_4+PO_4 experimental vessels. These PO_4 concentrations were relatively stable even after introducing corals in the experimental vessels (Figure 11; bottom panel). However, on May 20th one of the lines dosing PO_4 was clogged resulting in smaller PO_4 doses and then declining levels in some of the vessels (Figure 11; bottom panel).

PO_4 concentrations in the C, NO_3, and NH_4 treatments during the testing period without corals were low (<0.2 µM) and followed the PO_4 concentrations in the artificial seawater being dosed at the moment. However, after May 20th, PO_4 concentrations in these treatments showed an increasing trend due to higher PO_4 concentrations in the artificial seawater being dosed in the experiment.

1.1. Coral performance

Data on coral photochemical efficiency (*Fv/Fm*) and calcification rates (buoyant weight) was collected and it is attached as part of the deliverables but have yet to be analyzed. Future work on these data will determine if the nutrient dosing affected the performance of *A. cervicornis.*

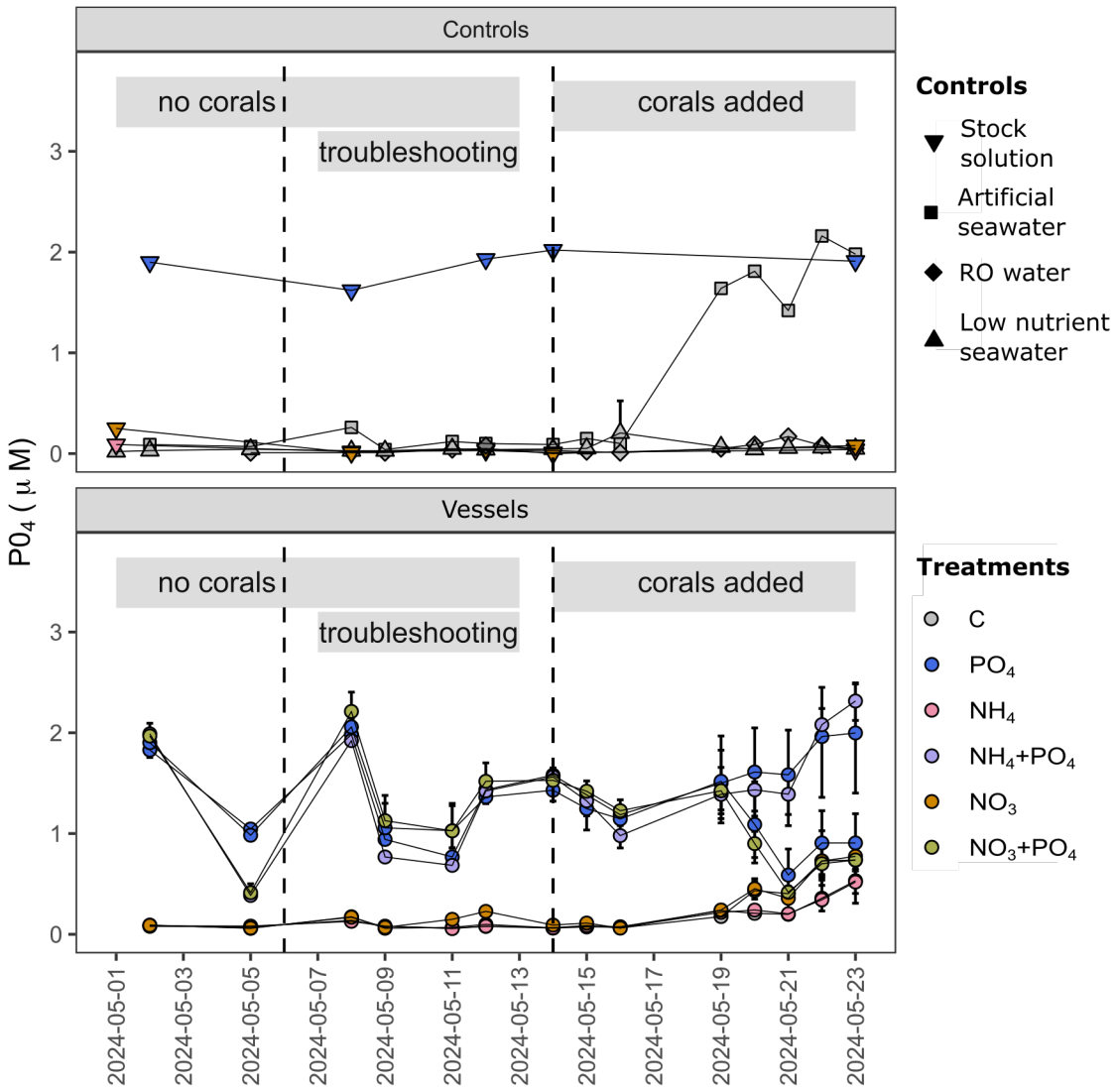

Figure 11: *Phosphate concentrations during the experiment. Top panel: control samples. Lower panel: experimental samples in the STAR system vessels. The vertical dashed lines show the days when changes were made to the system such as adjusting stock concentrations or adding the experimental corals.*

2. Discussion and Management Recommendations

The expansion and modification of the Sequential Treatment Application Robot (STAR) system have enabled a comprehensive assessment of nutrient pollution impacts on coral health. In this experiment we specifically targeted the endangered staghorn coral, *Acropora cervicornis,* but the same system can be implemented for other coral species or sessile organisms (e.g., sponges). The systematic testing of multiple nutrient sources

(NO_3, NH_4, PO_4) and their combinations can provide significant insights into the complex interactions between nutrient enrichment and coral physiology.

The initial phase of the experiment focused on ensuring nutrient stability without the presence of corals to fine-tune the system settings necessary for precise dosing. During this phase, we encountered challenges in achieving the correct concentrations for the stock solutions. It is likely that the NH_4Cl or $NaNO_3$ and $Na3PO_4$ salts absorbed moisture from the humid lab environment, which affected their weight and, consequently, the molarity of the solutions prepared using these salts. To address this issue, we prepared solutions targeting concentrations of 1M in RO water and stored them under refrigeration. These solutions were then measured for their actual nutrient concentrations, and once confirmed, they were used to create the stock solutions for the STAR system at the right concentrations for dosing.

During the testing phase without corals, we also adjusted the salinity of the stock solutions dosed by the STAR system. Initially, these stock solutions were prepared using RO water, but nutrient analysis in the vessels revealed declining nutrient concentrations over time. We hypothesized that the lower density of the RO water-based stocks hindered their mixing with the higher-density seawater in the vessels. Despite the constant mixing provided by the stir bars in each vessel, it was likely that most of the nutrients did not fully integrate before overflowing from the vessels. To address this issue, we prepared the stock solutions using artificial seawater and increased their salinity by 0.5 ppt above that of the artificial seawater. This adjustment facilitated nutrient sinking and better mixing of the stocks in the seawater. These changes resulted in more stable nutrient levels in the vessels for NH_4 and PO_4.

NO_3 concentrations, however, were influenced by factors beyond dosing during the period without corals. Over time, NO_3 levels declined while NO_2 levels increased, indicating NO_3 reduction in the vessels. To investigate this, a vessel designated for NO_3 was removed from the tank and left on the countertop to observe if NO_3 reduction occurred under these conditions as well. The results showed that these vessels maintained the target NO_3 concentrations, suggesting that NO_3 reduction was enhanced by the tank conditions (such as higher temperature, light, or water circulation). Based on this, additional modifications might be needed to achieve NO_3 target concentrations under the experimental conditions required to maintain coral health (including specific temperature and light conditions). Future testing can explore adjustments to NO_3 dosing, potentially shifting from more frequent partial water changes/nutrient dosing (e.g., 30 mL every 45 minutes in this experiment) to less frequent full water changes/nutrient dosing (e.g., 1 L). This approach might prove more effective in achieving NO_3 targets.

Upon introducing corals to the experimental vessels, a significant decline in NH_4 and NO_3 concentrations was observed across all treatments involving nitrogen sources (Figures 8-11). While nutrient uptake by the corals partially explains this decrease, the emergence of a brown biofilm in the vessels suggests that diatoms introduced with the corals were also consuming nutrients. These biofilms were more evident in the NO_3, NO_3 + PO_4, NH_4 and NH_4+PO_4 treatments, but less prevalent in the control (C) or PO_4

treatments (Figure 12). PO_4 concentrations remained relatively stable during the experimental phase, indicating that nitrogen was still the limiting nutrient in the vessels.

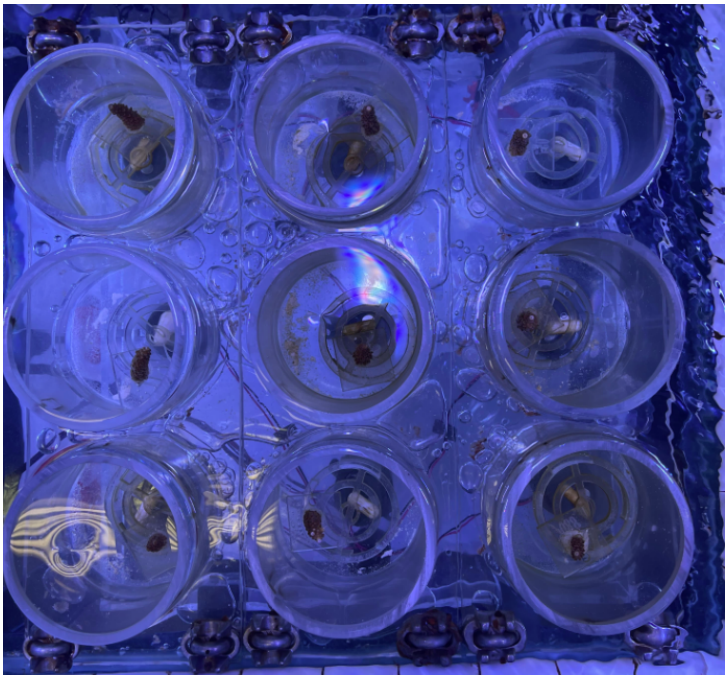

Figure 12: *Representative picture of the differences in diatoms buildup among the experimental vessels.*

Addressing nutrient uptake by microorganisms such as bacteria and diatoms is a significant challenge, as they are difficult to control once introduced in the system, especially if they benefit from nutrient dosing. Prior to setting up the experiment, we thoroughly disinfected all components, including the vats, pipes, tanks, and vessels, which proved effective during the initial phase without corals, as no biofilm formation was observed, and nutrients were stable. Similarly, before introducing the corals to the vessels, they were meticulously cleaned and rinsed with artificial seawater. Despite these precautions, microscopic diatoms likely persisted attached to the corals and were introduced into the vessels.

Two approaches can be taken to mitigate the effects of nutrient uptake by these microorganisms. First, frequent vessel replacement can help maintain lower algae biomass and reduce nutrient uptake. Second, performing full water replacements in the vessels instead of partial dosing (30 mL in this experiment) can expose the corals to the target concentrations more effectively. This method might result in nutrient concentration pulses, where the target is achieved initially and then declines until the next full water exchange. However, it can ensure that the corals are exposed to the desired nutrient levels at least temporarily.

Finally, while data on coral photochemical efficiency (*Fv /Fm*) and calcification rates (buoyant weight) have been collected, the analysis is pending. This analysis will be

completed in the upcoming months to determine how individual and combined nutrient dosing affected *A. cervicornis* physiological performance. The data from this study will provide a robust foundation for future research.

Future applications of the modified STAR system can expand our knowledge about the ecological effects of nutrient inputs to the reefs. Some of the potential applications include (1) **determining nutrient concentrations threshold** at what nutrient inputs produce adverse effects on coral health, (2) **comparing nutrient susceptibility** among species and life stages to determine which organisms are most vulnerable to nutrient pollution and (3) developing **multi stressor assessments** that incorporate additional stressors, such as thermal stress and disease exposure, into future experimental designs to better simulate real-world conditions and inform comprehensive management strategies.

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