

RESEARCH ARTICLE

Genotypes of *Acropora cervicornis* in Florida show resistance to either elevated nutrients or disease, but not both in combination

Ana M. Palacio-Castro^{1,2*}, Danielle Kroesche^{3,4}, Ian C. Enochs², Chris Kelble², Ian Smith^{1,2}, Andrew C. Baker³, Stephanie M. Rosales^{1,2}

1 Cooperative Institute for Marine and Atmospheric Studies, University of Miami, Miami, Florida, United States of America, **2** Atlantic Oceanographic and Meteorological Laboratory, NOAA, Miami, Florida, United States of America, **3** Department of Marine Biology and Ecology, University of Miami, Miami, Florida, United States of America, **4** NOVA Southeastern University, Davie, Florida, United States of America

* apalacio@earth.miami.edu



Abstract

Coral restoration programs are expanding to revive coral populations and ecosystem services, but local and global stressors still threaten coral survival. In the Caribbean, the ESA-listed staghorn coral *Acropora cervicornis* has experienced profound declines due to multiple stressors, including disease and nutrient pollution. We studied the impact of these two stressors on ten *A. cervicornis* genotypes for which disease susceptibility was previously ranked in a disease transmission experiment. Results showed that elevated ammonium, disease, and their combination negatively affected *A. cervicornis* survivorship, with variable susceptibility among genotypes. Three genotypes were susceptible to elevated ammonium alone and experienced mortality in up to 80% of their fragments. Exposure to a disease homogenate under ambient ammonium caused mortality in 100% of the fragments in four coral genotypes, intermediate mortality in five (33–66% of their fragments), and no mortality in one genotype. However, all genotypes experienced mortality (30–100% of their fragments) when exposed to both elevated ammonium and disease. Despite the detrimental effects of ammonium on coral survivorship, corals under elevated ammonium presented higher photochemical efficiency (F_v/F_m) of the algal symbionts. Disease susceptibility did not align with the genotypic ranking established in a previous study, suggesting that, while genotypes may vary in their disease resistance, rankings may change due to environmental factors or disease type. Regardless of individual susceptibility, our results suggest that water quality improvement is necessary for increasing *A. cervicornis* survivorship.

Introduction

Coral diseases are responsible for significant declines in coral populations, particularly in the Caribbean [1–6], which comprises just 8% of the world's coral reefs by area, but reports over 70% of all coral diseases [7]. A variety of taxa, including bacteria, fungi, viruses, and protozoa have been associated with coral diseases [8–11], but despite extensive efforts to identify the causative agents, pathogen identification for most coral diseases and syndromes remains elusive [12,13].

OPEN ACCESS

Citation: Palacio-Castro AM, Kroesche D, Enochs IC, Kelble C, Smith I, Baker AC, et al. (2025) Genotypes of *Acropora cervicornis* in Florida show resistance to either elevated nutrients or disease, but not both in combination. PLoS ONE 20(3): e0320378. <https://doi.org/10.1371/journal.pone.0320378>

Editor: Satheesh Sathianeson, King Abdulaziz University, SAUDI ARABIA

Received: May 29, 2024

Accepted: February 17, 2025

Published: March 26, 2025

Copyright: © . This is an open access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the [Creative Commons CC0](https://creativecommons.org/licenses/by/4.0/) public domain dedication.

Data availability statement: All data and code for the statistical analysis are available on the Zenodo archive: Palacio-Castro AM. Workflow for: Assessing *Acropora cervicornis* genotype resistance to elevated ammonium and disease. Zenodo; 2024. doi: <https://doi.org/10.5281/ZENODO.10989808>.

Funding: Funding was provided to AMP-C through the National Academy of Sciences' National Research Council Postdoctoral Fellowship and to CK through the Coral Reef Conservation Program (Grant 31250). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

Acropora cervicornis and *A. palmata* are the only two fast-growing branching scleractinian corals in the Caribbean and western Atlantic region. These species perform unique ecological functions and disproportionately contribute to reef growth, coastline protection, and essential habitat formation [14,15]. Unfortunately, *Acropora* populations have been severely impacted by diseases throughout the Caribbean, with declines of up to 90% between the 1970s and 1990s attributed to white band disease (WBD) [2]. Furthermore, the remaining populations of *Acropora* spp. continue to experience mortality and tissue loss associated with diseases and syndromes [16] as well as heat-induced coral bleaching [17]. Previous research has suggested bacteria in the orders *Vibrionales*, *Rickettsiales*, and *Flavobacteriales* as candidate causative agents for WBD [8,18–20], but a specific pathogen for WBD has not yet been identified.

In response to these population declines, *A. cervicornis* and *A. palmata* became the first coral species designated as threatened under the US Endangered Species Act (ESA) in 2006 [21]. Over the past few decades, numerous coral restoration programs have been established to recover *Acropora* spp. coral cover and restore their ecological function [16,22,23]. However, since reefs still face numerous anthropogenic and natural threats [24,25], restoration initiatives are now evaluating and adopting population enhancement practices, such as selecting stress-resistant corals to improve restoration efficacy and the long-term persistence of the restored populations [26].

Previous studies have documented differences in survivorship and stress tolerance among *A. cervicornis* genotypes [27–31], providing valuable insights into prioritizing the outplanting of resilient corals in environments exposed to specific stressors. However, we still lack a comprehensive characterization of genotype-specific traits, their heritability, and their plasticity under different environmental conditions or multiple stressors [27,32,33]. For instance, while differences in disease resistance have been identified among *A. cervicornis* genotypes [27,28,30], these traits could be compromised under additional stressors such as elevated temperature [27], or elevated nutrients.

In the Caribbean, elevated nutrient levels in coastal reefs are often driven by anthropogenic factors, such as agricultural runoff and inadequate sewage treatment [34–36]. In Southeast Florida and the Florida Keys particularly, dissolved inorganic nitrogen ($\text{DIN} = \text{NH}_4 + \text{NO}_3 + \text{NO}_2$) concentrations typically remain below 0.6 μM but can rise to 2.5–7.5 μM during nutrient input events, often coinciding with the onset of the wet season [36,37]. Elevated nutrient levels have been linked to various negative impacts on coral reefs, including increased macroalgal cover [38] and enhanced reef bioerosion [39]. Furthermore, the disproportionate increase in DIN relative to phosphorus (elevated N:P ratios) has been linked to reduced coral resilience to additional stressors, such as temperature [40].

Nutrient pollution can also interact with a coral's resistance to disease [41–43]. Increased disease progression and prevalence have been reported in other Caribbean corals such as *Siderastrea siderea* [41,42], *Orbicella annularis*, and *O. franksi* [43] when exposed to nutrient enrichment. However, the mechanisms by which nutrients increase disease susceptibility are not fully understood. Evidence suggests that higher disease susceptibility could result from the effects of elevated nutrients on corals' symbiotic communities, rather than the direct effects of nutrients on the coral host [44,45]. For instance, higher densities of algal symbionts (Family Symbiodiniaceae), a common phenomenon in corals exposed to nutrient enrichment [46,47], can depress the expression of coral immune-related genes [44]. Elevated nutrients can also disrupt coral's prokaryotic communities, leading to dysbiosis [45]. In *A. cervicornis*, elevated nutrients seem to marginally affect microbiome composition [29,48], but correlate with an increase in the relative abundance of *Aquarickettsia* [48], a bacterial genus associated with reduced disease resistance [49].

To assess the effects of elevated ammonium (NH_4) on *A. cervicornis* response to disease, we used ten nursery-reared coral genotypes that were previously tested in a disease transmission experiment [28]. These genotypes presented a range of disease transmission risks from 0 to 1, offering a full range of disease susceptibility [28]. Coral survivorship, algal symbiont composition, and photochemical efficiency (F_v/F_m) of the genotypes were compared under a full factorial design, including preconditioning to ambient nutrients followed by exposure to a placebo slurry (Ambient+Placebo), preconditioning to ambient nutrients followed by exposure to a diseased slurry (Ambient+Disease), preconditioning to elevated NH_4 followed by exposure to a placebo slurry (elevated NH_4 + Placebo), and preconditioning to elevated NH_4 followed by exposure to a diseased slurry (elevated NH_4 + Disease). We aimed to determine if the addition of NH_4 changed *A. cervicornis* disease susceptibility and if changes in disease susceptibility were associated with disturbance of the Symbiodiniaceae communities. We identified genotypes resistant to either nutrients or disease but not the combination of the two. However, the disease susceptibility ranking by genotype was different from that previously reported.

Materials and methods

Coral collection and maintenance

Coral fragments from 10 nursery-reared *Acropora cervicornis* genotypes ($n = 240$; 24 per genotype) were donated during the summer of 2020 by three South Florida coral nurseries. Coral fragments from the University of Miami's Rescue a Reef nursery were collected offshore of Key Biscayne, Miami (25.68°N, 80.11°W); fragments from the Coral Restoration Foundation were collected offshore of Tavernier in the Upper Keys (24.99°N, 80.43°W); and fragments from the Florida Fish and Wildlife Conservation Commission were collected offshore of Marathon in the Middle Keys (S1 Fig, S1 Table). All fragments were 5–8 cm long single branches with one apical tip. From these genotypes, four were previously characterized as resistant to disease (Acerv2, Cooper-9, K2, and FM9; risk of disease transmission 0–0.3); one as intermediate (U44; disease transmission 0.39); and five as susceptible (Kelsey-1, Elkhorn, FM6, FM14 and FM19, disease transmission between 0.5 and 1; S1 Table) [28]. After collection at their respective nursery, the corals were transported to the University of Miami Cooperative Institute for Marine and Atmospheric Studies (CIMAS) and the NOAA Atlantic Oceanographic and Meteorological Laboratory (AOML) Experimental Reef Lab (ERL). The fragments were mounted on numbered acrylic tags and acclimated to the lab conditions in eight independent ~180 L flow-through tanks [50] for six weeks. During the acclimation period, all tanks were maintained at 28°C, consistent with the approximate temperature in the nurseries at the time of collection. The temperature in each tank was measured with a high-resolution resistive temperature detector (TTD25C, Pro-Sense) and adjusted to the target levels with a 400 W submersible heater and a solenoid-operated titanium chiller coil [50]. Light was supplied by Hydra 64 HD LED Reef lights under a 12:12 light-dark schedule, which included 1 h “up and dawn” ramp and 10 h at a PAR peak of ~350 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Filtered (1 μm) and UV-treated seawater from Bear Cut in Virginia Key, Florida was supplied at a rate of 200 mL/min. The coral fragments were fed daily with 1.0 g of ReefRoids in separate feeding tanks and transferred back to the experimental tanks after 1 h.

Nutrient treatments

After the acclimation period, all fragments of each genotype were evenly and haphazardly assigned to two nutrient treatments: ambient nutrients (Ambient) or elevated ammonium (NH_4). Each nutrient treatment was replicated in four independent tanks ($n = 3$ fragments per genotype per tank) and maintained under the same temperature, water flow, and light conditions as the acclimation period. For ~1.5 months (47 d), Ambient tanks were maintained

under nutrient levels consistent with the values in Virginia Key, FL, while elevated NH_4 tanks were dosed with NH_4Cl [3 mM] every 15 minutes using peristaltic pumps. The initial NH_4 dose volume was 10 mL of the stock solution, targeting a $\sim 10\ \mu\text{M}$ increase in NH_4 concentration. These values were calculated to account for the dilution of the nutrients resulting from adding new ambient seawater to the tanks (200 mL/min in a total tank volume of 180 L). However, after detecting higher than normal NH_4 concentrations in the incoming seawater from Biscayne Bay, the NH_4 dose volume was lowered to 5 mL of the stock solution, targeting $\sim 5\ \mu\text{M}$ NH_4 increase above ambient values (Fig 1).

Water samples (~ 40 mL) were collected to monitor NH_4 levels in the treatments and immediately refrigerated at 4°C . The elevated NH_4 tanks were sampled daily, but the Ambient tanks were sampled less frequently (~ 2 -3 days, and no samples were collected during weeks 1 and 3 of the experiment). Nutrient concentrations were measured at NOAA-AOML using an AA3 nutrient analyzer (Seal Analytical, Southampton, UK). The instrument was calibrated before each run using standard solutions and procedures [51]. Initially, only NH_4 was monitored, but after high NH_4 concentrations in the source seawater were detected, additional measurements of PO_4 were included (Fig 1).

Photochemical efficiency (F_v/F_m)

The photochemical efficiency of each fragment (F_v/F_m) was measured every week during the nutrient exposure period (days 1 to 47) to assess the effects of elevated NH_4 on algal symbiont community function in *A. cervicornis* [52]. Corals were dark-adapted for 30 minutes and measured using a Maxi Imaging-PAM fluorometer (I-PAM, Walz, Effeltrich, Germany). Monitoring of F_v/F_m stopped once the tissue slurries were applied to the experimental fragments to avoid cross-contamination between the placebo and disease treatments. The effect of nutrients on F_v/F_m was analyzed with a linear mixed model that included nutrient treatment, genotype, and day of the experiment as fixed interacting effects. Coral fragment was included as a random effect to account for repeated measurements of the same fragments during different time points. The models were run with the lme4 1.1-17 package [53] for R 3.4.3 [54] and pairwise (Tukey's HSD) comparisons for significant factors were performed with emmeans 1.1.3 [55] with an alpha value of 0.05.

Symbiodiniaceae community

Three tissue samples per genotype and nutrient treatment were collected on day 47 from a subset of fragments ($n=60$). Small tissue biopsies (~ 4 polyps) were collected using a new razor blade per coral and were preserved in DNA/RNA Shield (Zymo Research). Total genomic DNA was extracted using the MagBead RNA/DNA extraction kit (Zymo Research). The tissue samples were first homogenized in the collection tubes using an MP bead beater for 30 s at 4 m/s and then centrifuged at 10,000g for 60 s. Proteinase K was added for 30 minutes. A total of 200 μL of supernatant was used for extractions using the semi-automatic Kingfisher Flex (Thermofisher) and extracted samples were stored in a -80°C freezer until they were used for quantitative PCR (qPCR) analysis.

The symbiont to host cell ratio (S/H) was used as a proxy to quantify differences in algal symbiont abundance relative to the coral host tissue as a response to the nutrient treatments [56]. S/H ratios were estimated using quantitative PCR (qPCR) assays that target the actin gene in the genus *Symbiodinium*, *Breviolum*, *Cladocopium*, or *Durussidinium* [57,58] and the Calmodulin (CAM) gene in *Acropora* [59]. S/H ratios were calculated using the r package SteponeR [60]. This R package uses the formula $\text{S/H cell ratio} = 2^{\text{Ct}(\text{host}) - \text{Ct}(\text{symbiont})}$, divided by the symbiont to host ploidy ratio (1/2), DNA extraction efficiency ratio (0.828), and target locus

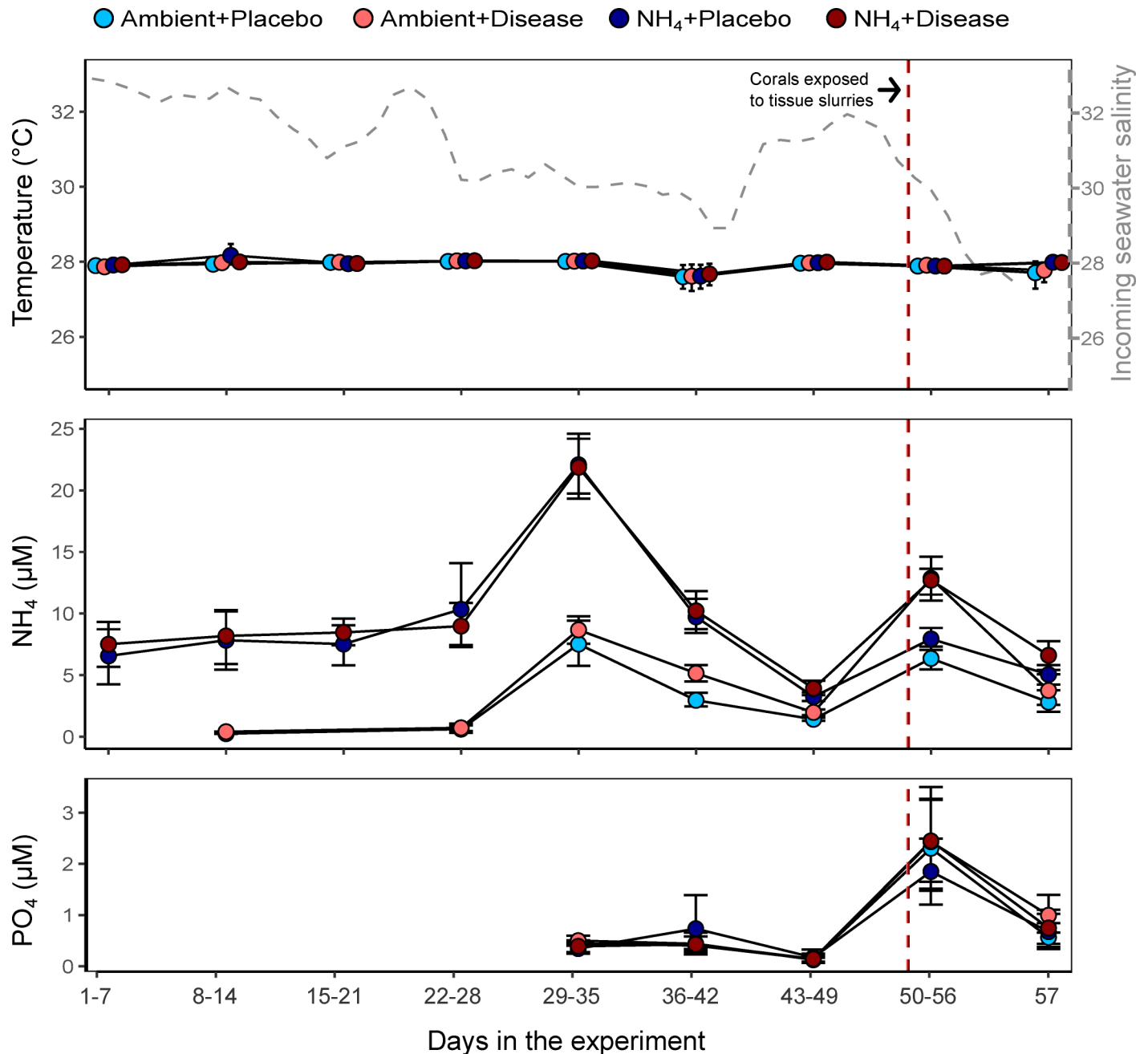


Fig 1. Measured experimental conditions. The colors represent the treatment combination assigned to each tank (nutrients + disease). However, disease was only applied on day 48. The gray line in the top panel represents the salinity of the incoming water from Biscayne Bay. The red vertical line shows the day when the tanks were dosed with tissue slurries. Values are shown as weekly averages for each treatment (7-day mean \pm 95% CI).

<https://doi.org/10.1371/journal.pone.0320378.g001>

copy number ratio (9/1) [56–58]. To quantify the differences in S/H cell ratio by genotype and nutrient treatment the ratios were analyzed with a linear mixed model that included nutrient treatment and genotype as fixed interactive effects, and tank as a random effect. The models were run with the lme4 1.1-17 package. Non-significant factors were dropped with the function 'step' and pairwise (Tukey's HSD) comparisons for significant factors were performed with emmeans 1.1.3 with an alpha value of 0.05.

Disease treatments

After 48 days of exposure to the nutrient treatment, half of each nutrient cohort was exposed to a diseased coral tissue slurry (Disease) and half to a healthy coral tissue slurry (Placebo). This resulted in a multi-factorial experiment with four treatments of combined stressors replicated in two independent tanks: ambient nutrients and placebo (Ambient+Placebo), ambient nutrients and disease (Ambient+Disease), elevated nutrients and placebo (elevated NH_4 +Placebo), and elevated nutrients and disease (elevated NH_4 +Disease).

Tissue slurries were made following guidance from a previous disease exposure study [27]. Briefly, fragments of *A. cervicornis* characterized as “visually healthy” and fragments with lesions indicative of WBD were collected from the University of Miami - Rescue a Reef nursery. The healthy and diseased corals were obtained from two different locations and maintained in separate tanks to confirm the absence of disease signs in the placebo (visually healthy) fragments and active lesion progression in disease fragments (for 3 and 2 days, respectively). The slurries were made using six healthy and seven diseased fragments which were blasted using an airbrush with 0.22 μm filtered and autoclaved seawater. Each slurry was transferred to a Falcon tube containing sterile steel beads, and vortexed for 10 minutes to homogenize the mixture and break up mucus [61].

To facilitate infection, all experimental corals were scraped around their circumference near their base using a new razor blade for each fragment before exposing them to the slurries [27]. All fragments from a tank were then transferred to a closed 21 L glass aquarium containing 14 L of seawater from their respective nutrient treatment. Depending on the treatment, the aquaria were dosed with either 31 mL of placebo or disease slurry. All corals were maintained in the closed aquaria with recirculating pumps for ~ 12 h to facilitate exposure to the pathogen. After this, the corals, along with the water from the 21 L glass aquaria, were returned to their original flowthrough tanks and nutrient treatments, where the appearance of disease lesions was monitored for nine days (until day 57).

Tissue loss and survivorship probabilities

The appearance of lesions or tissue loss was monitored daily to determine the effects of single and combined stressors on the genotypes' survivorship. Mortality was recorded once each coral exhibited tissue loss since the whole fragment usually died within a day of the first appearance of a lesion. Consequently, percentage mortality represents the proportion of fragments that developed lesions and not the percentage of tissue affected in each coral. Monitoring of the fragments was stopped nine days after exposure to the slurries because tissue loss had slowed on day eight and none of the fragments experienced mortality on day nine. Survival probabilities were calculated for each genotype and treatment combo (nutrients x slurry) using the Kaplan-Meier estimate [62] with the R packages survival 2.38 [63] and survminer 0.4.6 [64]. Log-rank tests were used to compare the survival curves for each genotype, treatment, and combined genotype and treatment. All data and code for the statistical analysis are available on Zenodo [65].

Results

Treatment conditions

Temperature was maintained at ~28 °C in all four treatments (Ambient+Placebo = 27.89 ± 0.34 sd, Ambient+Disease = 27.79 ± 0.99 , NH_4 +Placebo = 27.99 ± 0.33 , and NH_4 +Disease = 27.94 ± 0.24 ; Fig 1). During the first four weeks of nutrient treatments, average NH_4 concentration was 0.51 μM (± 0.21 SD) in the ambient tanks, and 8.17 μM (± 1.13) in the NH_4 tanks.

However, in week 5, nutrient concentrations in the incoming seawater spiked to $8.10 \mu\text{M NH}_4$ (± 0.82) in ambient nutrients, and $21.97 \mu\text{M}$ (± 0.14) in elevated NH_4 . This spike (in October 2020) coincided with a period of heavy rain and a mass fish kill in Biscayne Bay [66] and a reduction in the salinity of the incoming water (Fig 1 top panel). After detecting this nutrient spike, nutrient dosing was reduced by half to avoid excessively high nutrient levels in the NH_4 treatments. During weeks 6 and 7, NH_4 concentration in the incoming water started to decline and by week 6 it was $4.06 \mu\text{M}$ (± 1.56) in the ambient tanks and $9.98 \mu\text{M}$ (± 0.35) in the elevated NH_4 . By week 7 it had declined to $1.70 \mu\text{M}$ (± 0.40) in the ambient tanks and 3.59 (± 0.47) in elevated NH_4 (Fig 1). In week 8, the addition of tissue slurries temporarily increased NH_4 and PO_4 , with NH_4 concentrations reaching $7.15 \mu\text{M}$ (± 1.12) in the tanks dosed with placebo and $12.79 \mu\text{M}$ (± 0.11) in the tanks dosed with disease slurry before declining again in week 9 (Fig 1).

Photochemical efficiency (F_v/F_m)

Nutrient treatment ($p < 0.001$, $F_{\text{val}} = 9.07$), genotype ($p < 0.001$, $F_{\text{val}} = 30.08$), and day of the experiment ($p < 0.001$, $F_{\text{val}} = 90.74$) had significant effects on the F_v/F_m (S3 Table). However, these differences were generally small with all corals presenting F_v/F_m values above 0.5. Across nutrient treatments and days, U44 had the highest F_v/F_m compared to the rest of the genotypes (0.606 ± 0.004 , $p < 0.05$), and Kelsey-1 had the lowest (0.520 ± 0.004 ; Fig 2A). Corals exposed to elevated NH_4 presented higher F_v/F_m values compared to corals in Ambient nutrients, but these differences were not significant during the early days of the experiment (days 1–20, Fig 2B). There was no interaction between genotype and nutrient treatment, with all genotypes exhibiting similar responses to the nutrient treatments over time.

Symbiodiniaceae community

All *A. cervicornis* genotypes hosted algal symbionts in the genus *Symbiodinium* (likely *S. fittii*) with no other genera being detected by qPCR. There were no statistical differences among the S/H cell ratios in the corals under elevated NH_4 compared to Ambient nutrients ($p = 0.86$, $F_{\text{val}} = 0.03$; Fig 3; S5 Table). Coral genotype showed a significant effect on S/H model ($p = 0.03$,

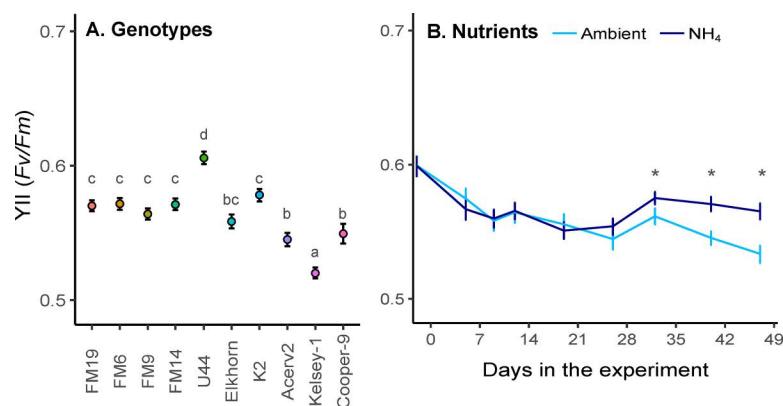


Fig 2. Maximum quantum yield (F_v/F_m ; mean \pm 95% CI) of *A. cervicornis*. (A) F_v/F_m across time points for the ten genotypes used in the experiment. The letters represent the TukeyHSD groups (i.e., F_v/F_m in genotypes with shared letters are not significantly different). Genotypes are arranged by descending survivorship probabilities on the final day of the experiment from left (highest overall survivorship across treatments) to right (lowest overall survivorship). (B) F_v/F_m by nutrient treatment and time points before adding the tissue slurries. The asterisks denote the time points when significant differences occur between the nutrient treatments.

<https://doi.org/10.1371/journal.pone.0320378.g002>

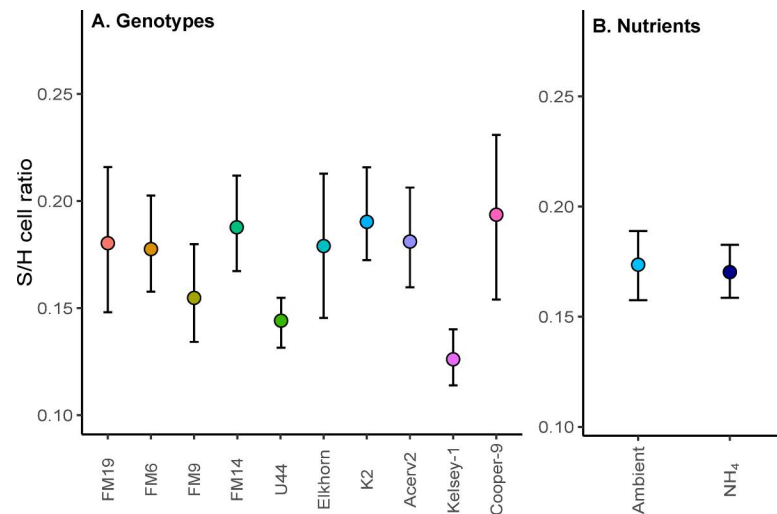


Fig 3. Symbiont to host cell ratio (S/H; mean \pm 95% CI) of *A. cervicornis*. (A) S/H on day 47 for all the different genotypes used in the experiment. Genotypes are arranged by descending survivorship probabilities on the final day of the experiment from left (highest overall survivorship across treatments) to right (lowest overall survivorship). (B) S/H on day 47 by nutrient treatment (all genotypes combined).

<https://doi.org/10.1371/journal.pone.0320378.g003>

F val=2.43), but post-hoc Tukey comparisons were not significant among the genotypes ($p > 0.05$; [S6 Table](#)). The interaction between nutrient treatment and genotype on S/H was also not significant ($p = 0.66$, F val=0.75).

Coral survivorship

There was no mortality across genotypes in the Ambient+Placebo treatment ([Fig 4A](#)). On average, exposure to NH₄+Placebo reduced survivorship probabilities to 83% (log-rank $p < 0.05$), but this reduction in survivorship was observed in only four genotypes ([Fig 4B](#)). From these, three genotypes started experiencing tissue loss before exposure to the placebo slurry (Cooper, day 38; Acerv2, day 41; Kelsey-1, day 43), and their survivorship probabilities dropped below 20% by the end of the experiment (day 56). The remaining nutrient-susceptible genotype (K2) experienced tissue loss after placebo exposure (day 55), and had a higher survivorship probability by the end of the experiment (80%), compared to the other three NH₄ susceptible genotypes ([Fig 4B](#)).

All but one genotype (FM19) experienced tissue loss in the Ambient+Disease treatment after exposure to the disease slurry. By the end of the experiment, survivorship probabilities were reduced to 0% for Cooper-9, Kelsey-1, K-2, and FM14; 33% for Acerv2, Elkhorn, U44, and FM9; and 66% for FM6 ([Fig 4B](#)).

All genotypes, including FM19, experienced tissue loss in the NH₄+Disease treatment either before or after exposure to the disease slurry. However, overall mortality (all genotypes pooled) was not significantly different between the NH₄+Disease and Ambient+Disease ([Fig 4A](#)). Genotypes Cooper, Acerv2, Kelsey-1, and K2 in the NH₄+Disease treatment started experiencing mortality before exposure to the disease slurry, in agreement with their susceptibility to elevated NH₄. The rest of the genotypes only experienced mortality after the addition of the slurry. Survivorship probabilities were reduced to 0% in Cooper-9 and Acerv2, to 16% in Kelsey-1, 33% in Elkhorn, U44 and FM19, 50% in FM6, and 67% in FM14, FM9, and K2 ([Fig 4B](#); [Table 1](#)).

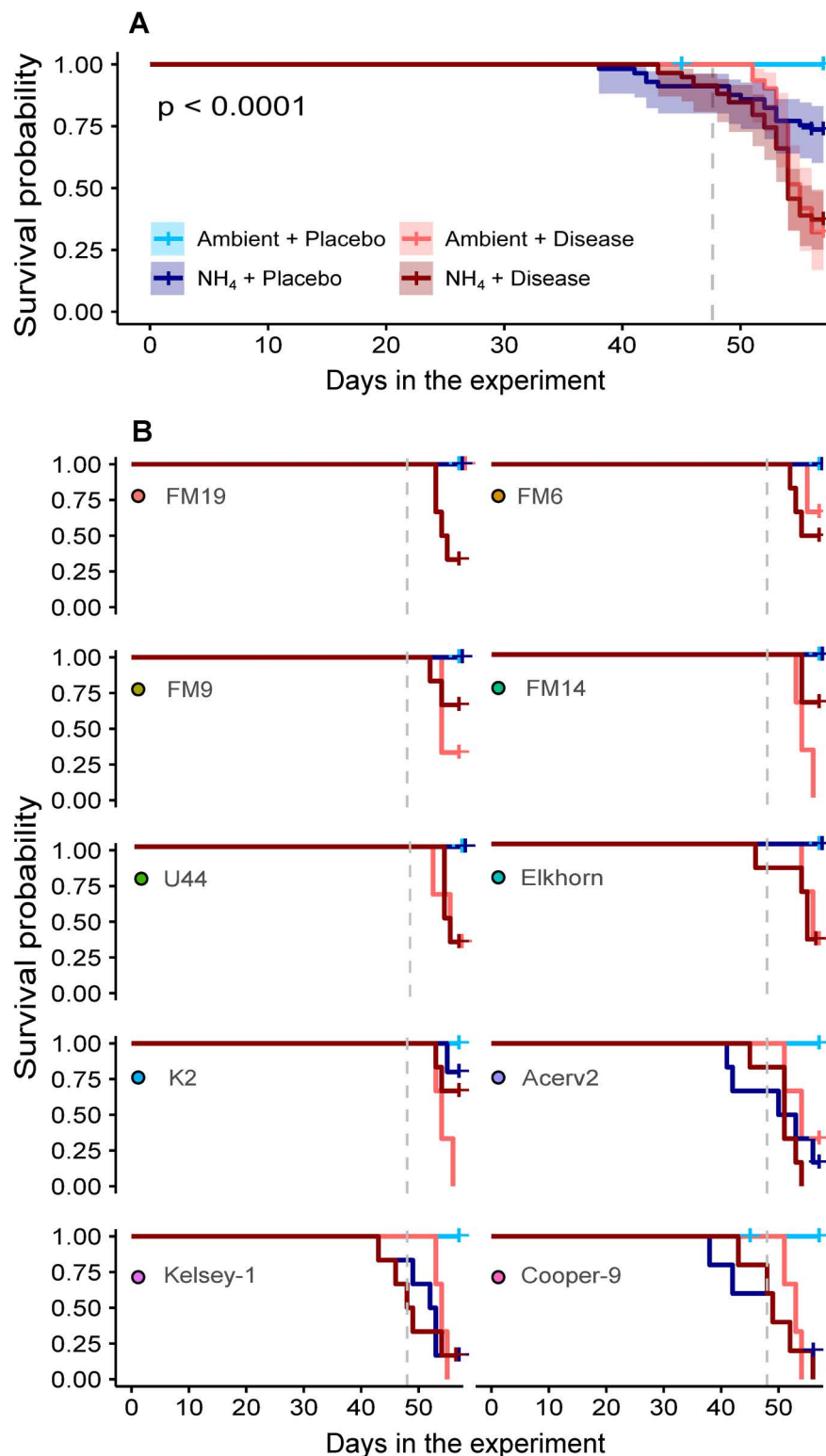


Fig 4. Survival probabilities in *A. cervicornis* exposed to Ambient or elevated NH₄ in combination with exposure to a placebo or disease slurry. (A) Overall survival probabilities by treatment (\pm 95% CI). (B) Survival probabilities by treatment and genotype. The vertical dashed lines represent the day when the corals were exposed to the tissue slurries.

<https://doi.org/10.1371/journal.pone.0320378.g004>

Table 1. Final survival probabilities of *A. cervicornis* genotypes.

Genotype	Treatment	Survivorship	Standard Error	Survivorship in Miller et al. [28]
FM19	Ambient+Disease	1	NA	0.5
	NH ₄ +Placebo	1	NA	
	NH ₄ +Disease	0.33	0.19	
FM6	Ambient+Disease	0.67	0.27	0.35
	NH ₄ +Placebo	1	NA	
	NH ₄ +Disease	0.50	0.20	
FM9	Ambient+Disease	0.33	0.27	1
	NH ₄ +Placebo	1	NA	
	NH ₄ +Disease	0.67	0.19	
FM14	Ambient+Disease	0.00	NA	0.35
	NH ₄ +Placebo	1	NA	
	NH ₄ +Disease	0.67	0.19	
U44	Ambient+Disease	0.33	0.27	0.61
	NH ₄ +Placebo	1	NA	
	NH ₄ +Disease	0.33	0.19	
Elkhorn	Ambient+Disease	0.33	0.27	0
	NH ₄ +Placebo	1	NA	
	NH ₄ +Disease	0.33	0.19	
K2	Ambient+Disease	0.00	NA	0.78
	NH ₄ +Placebo	0.80	0.18	
	NH ₄ +Disease	0.67	0.19	
Acerv2	Ambient+Disease	0.33	0.27	0.7
	NH ₄ +Placebo	0.17	0.15	
	NH ₄ +Disease	0.00	NA	
Kelsey-1	Ambient+Disease	0.00	NA	0.1
	NH ₄ +Placebo	0.17	0.15	
	NH ₄ +Disease	0.17	0.15	
Cooper-9	Ambient+Disease	0.00	NA	0.7
	NH ₄ +Placebo	0.20	0.18	
	NH ₄ +Disease	0.00	NA	

The Survivorship and Standard Error columns represent the survival probabilities at the end of the experiment (day 57) for corals exposed to Ambient+Disease, NH₄+Placebo, and NH₄+Disease treatments. The Survivorship in Miller et al. [28] column represents 1 minus the risk of transmission reported in Miller et al. [28].

<https://doi.org/10.1371/journal.pone.0320378.t001>

Discussion

Identifying coral genotypes resistant to multiple stressors is key for improving restoration success since this can help select genotypes to be outplanted in zones exposed to specific threats [26,67]. Here, we examined the survivorship of ten *A. cervicornis* genotypes previously ranked for disease resistance. These genotypes were evaluated under a disease challenge alone, elevated NH₄ alone, and the combination of these two stressors.

Disease response under ambient nutrients

We found significant differences in disease susceptibility among *A. cervicornis* genotypes after being exposed to ambient nutrients and a disease slurry. Only one genotype was completely resistant to disease (FM19), five genotypes exhibited variable survivorship (33% to 66% of the fragments), while four genotypes had no survivorship (Fig 2). Based on our results, genotype

FM19 may be promising to outplant in areas with above average disease outbreaks. However, for this approach to succeed, it is crucial to verify that the observed resistance of a coral genotype persists over time and across varying environmental conditions (i.e., if it is a fixed effect deriving from, for example, the coral's genetic makeup or its stable association with specific symbiotic communities). In this study, we did not identify differences in the Symbiodiniaceae community that can be associated with disease resistance in the coral genotypes (Fig 3A), but it is possible that more fine differences in the algal community (e.g., to the species or strain level), the prokaryotic community [49], or the coral genetic make-up of the corals [68] could still play a role on *A. cervicornis* survivorship when exposed to disease. Further exploration of the holobiont composition and genetic traits of FM19 can help elucidate the mechanisms of its disease resistance and provide insights into the longevity of its resilience under varying environmental conditions.

To incorporate genotypic information into restoration, it is also essential to ensure that resistance to one stressor does not result in unintended trade-offs that can be detrimental to the long-term sustainability of the coral population (e.g., higher susceptibility to other stressors, lower reproductive output, or reduced growth rates) [69,70]. Here, all coral genotypes exhibited F_v/F_m values characteristic of healthy algal symbiont communities, but other performance metrics besides survivorship were not measured.

Among the corals exposed to ambient nutrients and disease, genotype FM19 had the lowest disease transmission rate in this experiment (transmission rate = 0). However, FM19 resistance may not persist across multiple disease challenges since this genotype was previously characterized as disease-susceptible by Miller et al. [28] (transmission rate = 0.5). Similarly, FM9, Acer 2, Cooper, and K2 were previously among the most disease-resistant genotypes (transmission rate = 0–0.3), but they were among the susceptible genotypes in this study (Fig 4). This variability in resistance across experiments could suggest that some of the disease susceptibility traits in *A. cervicornis* might not be a result of fixed genetic effects but also modulated by the environmental history of the corals, including exposure to additional stressors or suboptimal conditions that can increase disease susceptibility [27,42]. Although in this study all coral fragments were common gardened in the laboratory for six weeks before initiating the experiment, the genotypes came from three different nurseries, one located near Miami, one in the Upper Keys and one in the Middle Keys (S1 Fig), and were therefore previously exposed to different environments [71]. To control for the impacts of environmental history, future studies should use genotypes that have been common-gardened in the same nursery prior to ex-situ experimentation, ensuring they experienced similar conditions in terms of temperature, nutrients, light, access to heterotrophic feeding, etc.

Additionally, it is possible that the pathogens involved in the infections differed between the two experiments; hence, the genotypes might exhibit different resistance levels to different diseases. In our experiment, all corals were exposed to the same disease slurries. In contrast, in Miller et al. [28], disease transmission occurred through direct contact with fragments exhibiting tissue loss. This last method could introduce additional variability in the disease transmission response, as different disease donors might not have the same virulence or could be affected by different pathogens. Indeed, a follow-up microbiome analysis of the Miller et al. [28] samples found that a putative pathogen was only found in 67% of diseased samples [72]. Future experiments could benefit from using isolated pathogens rather than disease homogenates or direct contact with diseased fragments [73]. Testing disease resistance with isolated microorganisms might reduce the variability associated with mixed-pathogen exposures and allow for a more precise evaluation of genotype-pathogen interactions.

Elevated ammonium and *A. cervicornis* performance

The addition of ammonium (NH_4) for over a month had a small and positive effect on the photochemical efficiency (F_v/F_m) of the algal symbionts, no effect on the Symbiodiniaceae abundance (relative to the host cells), but detrimental effects on coral survivorship (Figs 2B and 4), a pattern previously reported for *A. cervicornis* under elevated nutrients [59]. Differences in F_v/F_m and survivorship were observed only after a month of nutrient addition, suggesting that chronic vs. acute nutrient enrichment might have differential effects on *A. cervicornis* performance. For example, significant disruption of the prokaryotic communities in *A. cervicornis* can take up to six weeks of nutrient exposure [74], and changes in growth rates might take up to a month [29,75]. This suggests that *A. cervicornis* might initially buffer the impacts of elevated nutrients, but this initial effect may be lost over the longer term [29,74,75].

Survivorship in the NH_4 +Placebo treatment was reduced in four of ten genotypes (Cooper, Acer2, Kelsey-1, and K2), with mortality observed in approximately 80% of their fragments. This result underscores the potential impact of sustained nutrient enrichment on *A. cervicornis* health, but also the variability of responses among genotypes. Corals are associated with diverse microorganisms that participate in nutrient cycling, and sustained nutrient enrichment in the NH_4 treatments may alter the composition and abundance of members of the holobiont, impacting coral health [29,45]. Previous research also identified performance differences among *A. cervicornis* genotypes exposed to ammonium, with survivorship rates linked to two bacterial taxa (*Midichloriaceae* and *Spirochaetaceae*), suggesting that some coral microbiomes can be more resilient to nutrient stress [29].

During the nutrient spike, ammonium on the ambient treatments reached an average of ~8uM, highlighting the occurrence of high nutrient concentrations in Biscayne Bay that could impact the persistence and recovery of *A. cervicornis* in this area [76,77]. Despite the effects of elevated ammonium on coral survivorship and F_v/F_m , this treatment did not affect the abundance of the Symbiodiniaceae communities (Fig 3). However, these results should be interpreted with caution since our ambient tanks were exposed to a spike in NH_4 a week before collecting the tissue samples, caused by high nutrient levels in the incoming seawater. Furthermore, at the time of tissue collections, ammonium was notably similar between ambient and elevated NH_4 (Fig 1). Unlike the host and prokaryotic community, symbiont densities may respond quickly to nitrogen concentrations [78] and thus these results may be especially sensitive to time of sampling.

Disease response under elevated ammonium

The overall mortality of corals exposed to disease under ambient and elevated NH_4 was similar by the end of the experiment, reaching ~62–68% (Fig 4A). This appears to contrast with previous studies where nutrient enrichment has been found to exacerbate coral diseases [41–43]. However, we found important differences in the response to the combined stressors based on genotype (Fig 4). The only genotype with 100% survivorship under elevated NH_4 alone and disease exposure alone (FM19) experienced mortality in ~67% of its fragments under the combined stressors. In contrast, pre-exposure to elevated NH_4 did not exacerbate mortality in other genotypes, such as FM9, FM14, U44, or Elkhorn. Additionally, nutrient-sensitive genotypes, such as Acer2, Kelsey-1, and Cooper-9 started experiencing mortality in the NH_4 +Disease treatment before exposure to the disease slurry, making it difficult to interpret if the subsequent mortality was the result of one or both stressors combined.

Another challenge to the interpretation of these results is that the Ambient+Disease corals were exposed to two periods of elevated nutrients. One before disease exposure, characterized by elevated NH_4 , and a second one after the application of the tissue slurries, characterized by elevated NH_4 and PO_4 . Both periods of nutrient enrichment coincided with reduced salinity

in the incoming seawater (Fig 1), suggesting that runoff contributed to the nutrient increases. Additionally, the second nutrient spike coincided with the application of the placebo and diseased tissue slurries. Although the coral fragments were initially exposed to the slurries in separate closed aquaria, they were later transferred back to their flowthrough systems along with the water from the closed exposure phase, potentially introducing additional nutrients into the tanks. Based on the flow rates of our systems (~200 mL/min), a full water exchange would occur within 13 hours. To better understand the interactions between elevated nutrients and disease, future studies may benefit from using artificial seawater to minimize natural nutrient variability common in facilities sourcing seawater from coastal environments. Additionally, when feasible, replacing tissue slurries with isolated bacterial cultures as a method for disease exposure [73] may also be preferable to prevent unwanted nutrient enrichment.

Conclusions

Restoration efforts could benefit from identifying coral stocks with greater resistance to stressors such as disease and eutrophication to increase long-term coral survival, and thus restoration efficacy. Here, we found that some *A. cervicornis* genotypes were resistant to elevated NH_4 or disease, but there were no genotypes resistant to both stressors combined. Additionally, our results did not align with previous genotypic rankings of disease susceptibility, suggesting that coral response may be impacted by previous environmental history, and/or that coral genotypes may vary in their susceptibility to different pathogens. Significant coral mortality under elevated ammonium alone, as well as the loss of disease resistance in one genotype exposed to combined nutrients and disease, indicate that improvement of water quality may be necessary to recover and sustain *A. cervicornis* populations.

Supporting information

S1 Fig. Geographical location of the coral nurseries. The gray shapes represent South Florida and the Florida Keys' land. The color scale represents the ocean bathymetry obtained from the Second-generation Louvain la-Neuve Ice-ocean Model (SLIM, <https://www.slim-ocean.be>). The white dots demarcate the nurseries' location. UM: University of Miami - Rescue a Reef, CRF: Coral Restoration Foundation, FWC: Florida Fish and Wildlife Conservation. (DOCX)

S1 Table. Genotypes used in the experiment and summary of their previous disease susceptibility based on Miller et al. (2019).
(DOCX)

S2 Table. Weekly conditions in the experimental treatments.
(DOCX)

S3 Table. *Fv/Fm* model.
(DOCX)

S4 Table. *Fv/Fm* pairwise comparisons among *A. cervicornis* genotypes.
(DOCX)

S5 Table. *Fv/Fm* Pairwise comparisons between the nutrient treatments.
(DOCX)

S6 Table. S/H cell ratio model.
(DOCX)

S7 Table. S/H cell ratio pairwise comparisons among *A. cervicornis* genotypes.
(DOCX)

Acknowledgments

We thank the University of Miami - Rescue a Reef, Florida Fish and Wildlife Conservation, and the Coral Restoration Foundation nurseries, which facilitated access to the corals used in this experiment. We are especially grateful to D. Lirman, M. D'Alessandro, J. Unsworth, K. Maxwell, D. Pharo, K. Smith, and A. Moura, who collected and donated the corals. We thank J. Hendee for his support. The scientific results and conclusions, as well as any opinions expressed herein, are those of the author(s) and do not necessarily reflect the views of NOAA or the Department of Commerce.

Author contributions

Conceptualization: Ana M. Palacio-Castro, Ian C Enochs, Stephanie M. Rosales.

Data curation: Ana M. Palacio-Castro, Danielle Kroesche, Ian Smith.

Formal analysis: Ana M. Palacio-Castro, Danielle Kroesche.

Funding acquisition: Ana M. Palacio-Castro, Ian C Enochs, Chris Kelble, Andrew C. Baker, Stephanie M. Rosales.

Investigation: Ana M. Palacio-Castro, Stephanie M. Rosales.

Methodology: Ana M. Palacio-Castro, Ian C Enochs, Chris Kelble, Ian Smith, Andrew C. Baker, Stephanie M. Rosales.

Project administration: Ana M. Palacio-Castro.

Resources: Ian C Enochs, Chris Kelble.

Supervision: Ana M. Palacio-Castro, Ian C Enochs, Andrew C. Baker, Stephanie M. Rosales.

Visualization: Ana M. Palacio-Castro.

Writing – original draft: Ana M. Palacio-Castro, Stephanie M. Rosales.

Writing – review & editing: Ana M. Palacio-Castro, Danielle Kroesche, Ian C Enochs, Chris Kelble, Andrew C. Baker, Stephanie M. Rosales.

References

- Walton CJ, Hayes NK, Gilliam DS. Impacts of a Regional, Multi-Year, Multi-Species Coral Disease Outbreak in Southeast Florida. *Front Mar Sci*. 2018;5. <https://doi.org/10.3389/fmars.2018.00323>
- Aronson RB, Precht WF. White-band disease and the changing face of Caribbean coral reefs. *Hydrobiologia*. 2001;460:25–38.
- Gardner TA, Côté IM, Gill JA, Grant A, Watkinson AR. Long-term region-wide declines in Caribbean corals. *Science*. 2003;301(5635):958–60. <https://doi.org/10.1126/science.1086050> PMID: [12869698](https://pubmed.ncbi.nlm.nih.gov/12869698/)
- Kuta KG, Richardson LL. Abundance and distribution of black band disease on coral reefs in the northern Florida keys. *Coral Reefs*. 1996;15(4):219–23. <https://doi.org/10.1007/bf01787455>
- Richardson LL, Goldberg WM, Carlton RG, Halas JC. Coral disease outbreak in the Florida Keys: Plague Type II. 1. 1998;187–198.
- Sutherland K, Porter J, Torres C. Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. *Marine Ecology Progress Series*. 2004;266:273–302.
- Harvell D, Jordán-Dahlgren E, Merkel S, Rosenberg E, Raymundo L, Smith G, et al. Coral Disease, Environmental Drivers, and the Balance Between Coral and Microbial Associates. *Oceanog*. 2007;20(1):172–95. <https://doi.org/10.5670/oceanog.2007.91>
- Gignoux-Wolfsohn SA, Vollmer SV. Identification of Candidate Coral Pathogens on White Band Disease-Infected Staghorn Coral. *PLoS One*. 2015;10(8):e0134416. <https://doi.org/10.1371/journal.pone.0134416> PMID: [26241853](https://pubmed.ncbi.nlm.nih.gov/26241853/)

9. Rosales SM, Clark AS, Huebner LK, Ruzicka RR, Muller EM. Rhodobacterales and Rhizobiales Are Associated With Stony Coral Tissue Loss Disease and Its Suspected Sources of Transmission. *Front Microbiol.* 2020;11:681. <https://doi.org/10.3389/fmicb.2020.00681> PMID: [32425901](#)
10. Lawrence SA, Davy JE, Aeby GS, Wilson WH, Davy SK. Quantification of virus-like particles suggests viral infection in corals affected by Porites tissue loss. *Coral Reefs.* 2014;33(3):687–91. <https://doi.org/10.1007/s00338-014-1168-8>
11. Meyer JL, Castellanos-Gell J, Aeby GS, Häse CC, Ushijima B, Paul VJ. Microbial Community Shifts Associated With the Ongoing Stony Coral Tissue Loss Disease Outbreak on the Florida Reef Tract. *Front Microbiol.* 2019;10:2244. <https://doi.org/10.3389/fmicb.2019.02244> PMID: [31608047](#)
12. Richardson LL. Coral diseases: what is really known?. *Trends Ecol Evol.* 1998;13(11):438–43. [https://doi.org/10.1016/s0169-5347\(98\)01460-8](https://doi.org/10.1016/s0169-5347(98)01460-8) PMID: [21238385](#)
13. Vega Thurber R, Mydlarz LD, Brandt M, Harvell D, Weil E, Raymundo L, et al. Deciphering Coral Disease Dynamics: Integrating Host, Microbiome, and the Changing Environment. *Front Ecol Evol.* 2020;8. <https://doi.org/10.3389/fevo.2020.575927>
14. Alvarez-Filip L, Dulvy NK, Gill JA, Côté IM, Watkinson AR. Flattening of Caribbean coral reefs: region-wide declines in architectural complexity. *Proc Biol Sci.* 2009;276(1669):3019–25. <https://doi.org/10.1098/rspb.2009.0339> PMID: [19515663](#)
15. Young CN, Schopmeyer SA, Lirman D. A review of reef restoration and coral propagation using the threatened genus *Acropora* in the Caribbean and Western Atlantic. *Bulletin of Marine Science.* 2012;88:1075–98.
16. Miller MW, Kerr K, Williams DE. Reef-scale trends in Florida *Acropora* spp. abundance and the effects of population enhancement. *PeerJ.* 2016;4:e2523. <https://doi.org/10.7717/peerj.2523> PMID: [27703862](#)
17. NOAA and FWC protocols guide return of temperature-threatened corals to their Mission: Iconic Reefs in-water nurseries. [cited 30 May 2024]. Available from: <https://floridakeys.noaa.gov/news/2023/noaa-fwc-protocols-guide-return-of-corals-to-in-water-nurseries.html>
18. Sweet MJ, Croquer A, Bythell JC. Experimental antibiotic treatment identifies potential pathogens of white band disease in the endangered Caribbean coral *Acropora cervicornis*. *Proc Biol Sci.* 2014;281(1788):20140094. <https://doi.org/10.1098/rspb.2014.0094> PMID: [24943374](#)
19. Gil-Agudelo DL, Smith GW, Weil E. The white band disease type II pathogen in Puerto Rico. *Revista de Biología Tropical.* 2006;54:59–67.
20. Casas V, Kline DI, Wegley L, Yu Y, Breitbart M, Rohwer F. Widespread association of a Rickettsiales-like bacterium with reef-building corals. *Environ Microbiol.* 2004;6(11):1137–48. <https://doi.org/10.1111/j.1462-2920.2004.00647.x> PMID: [15479247](#)
21. Endangered and threatened species: final listing determinations for elkhorn coral and staghorn coral. *Federal Register.* 2006;71:26852–61.
22. Lirman D, Schopmeyer S. Ecological solutions to reef degradation: optimizing coral reef restoration in the Caribbean and Western Atlantic. *PeerJ.* 2016;4:e2597. <https://doi.org/10.7717/peerj.2597> PMID: [27781176](#)
23. Johnson ME, Lustic C, Bartels E. Caribbean *Acropora* restoration guide: best practices for propagation and population enhancement. 2011. Available from: <https://dspace.mote.org/handle/2075/2910>
24. Ware M, Garfield EN, Nedimyer K, Levy J, Kaufman L, Precht W, et al. Survivorship and growth in staghorn coral (*Acropora cervicornis*) outplanting projects in the Florida Keys National Marine Sanctuary. *PLoS One.* 2020;15(5):e0231817. <https://doi.org/10.1371/journal.pone.0231817> PMID: [32374734](#)
25. Miller MW, Lohr KE, Cameron CM, Williams DE, Peters EC. Disease dynamics and potential mitigation among restored and wild staghorn coral, *Acropora cervicornis*. *PeerJ.* 2014;2:e541. <https://doi.org/10.7717/peerj.541> PMID: [25210660](#)
26. National Academies of Sciences, Engineering, and Medicine. A Decision Framework for Interventions to Increase the Persistence and Resilience of Coral Reefs. Washington, DC: The National Academies Press. 2019. <https://doi.org/10.17226/25424>
27. Muller EM, Bartels E, Baums IB. Bleaching causes loss of disease resistance within the threatened coral species *Acropora cervicornis*. *Elife.* 2018;7:e35066. <https://doi.org/10.7554/eLife.35066> PMID: [30203745](#)
28. Miller MW, Colburn PJ, Pontes E, Williams DE, Bright AJ, Serrano XM, et al. Genotypic variation in disease susceptibility among cultured stocks of elkhorn and staghorn corals. *PeerJ.* 2019;7:e6751. <https://doi.org/10.7717/peerj.6751> PMID: [30993053](#)
29. Palacio-Castro AM, Rosales SM, Dennison CE, Baker AC. Microbiome signatures in *Acropora cervicornis* are associated with genotypic resistance to elevated nutrients and heat stress. *Coral Reefs.* 2022;41(5):1389–403. <https://doi.org/10.1007/s00338-022-02289-w>

30. Vollmer SV, Kline DI. Natural disease resistance in threatened staghorn corals. PLoS One. 2008;3(11):e3718. <https://doi.org/10.1371/journal.pone.0003718> PMID: 19005565
31. Cuning R, Parker KE, Johnson-Sapp K, Karp RF, Wen AD, Williamson OM, et al. Census of heat tolerance among Florida's threatened staghorn corals finds resilient individuals throughout existing nursery populations. Proc Biol Sci. 2021;288(1961):20211613. <https://doi.org/10.1098/rspb.2021.1613> PMID: 34666521
32. Drury C, Manzello D, Lirman D. Genotype and local environment dynamically influence growth, disturbance response and survivorship in the threatened coral, *Acropora cervicornis*. PLoS One. 2017;12(3):e0174000. <https://doi.org/10.1371/journal.pone.0174000> PMID: 28319134
33. Randall CJ, van Woessik R. Contemporary white-band disease in Caribbean corals driven by climate change. Nature Clim Change. 2015;5(4):375–9. <https://doi.org/10.1038/nclimate2530>
34. Camacho-Cruz KA, Ortiz-Hernández MC, Carrillo L, Sánchez A. Dissolved inorganic nutrients in a reef lagoon influenced by submarine groundwater discharge in the Mexican Caribbean. Regional Studies in Marine Science. 2024;79:103814.
35. Metcalfe CD, Beddows PA, Bouchot GG, Metcalfe TL, Li H, Van Lavieren H. Contaminants in the coastal karst aquifer system along the Caribbean coast of the Yucatan Peninsula, Mexico. Environ Pollut. 2011;159(4):991–7. <https://doi.org/10.1016/j.envpol.2010.11.031> PMID: 21232837
36. Lapointe B, Barile P, Matzie W. Anthropogenic nutrient enrichment of seagrass and coral reef communities in the Lower Florida Keys: discrimination of local versus regional nitrogen sources. Journal of Experimental Marine Biology and Ecology. 2004;308:23–58.
37. Caccia VG, Boyer JN. Spatial patterning of water quality in Biscayne Bay, Florida as a function of land use and water management. Mar Pollut Bull. 2005;50(11):1416–29. <https://doi.org/10.1016/j.marpolbul.2005.08.002> PMID: 16194552
38. Vermeij MJA, van Moorselaar I, Engelhard S, Hörnlein C, Vonk SM, Visser PM. The effects of nutrient enrichment and herbivore abundance on the ability of turf algae to overgrow coral in the Caribbean. PLoS One. 2010;5(12):e14312. <https://doi.org/10.1371/journal.pone.0014312> PMID: 21179215
39. Prouty NG, Cohen A, Yates KK, Storlazzi CD, Swarzenski PW, White D. Vulnerability of Coral Reefs to Bioerosion From Land-Based Sources of Pollution. JGR Oceans. 2017;122(12):9319–31. <https://doi.org/10.1002/2017jc013264>
40. Wiedenmann J, Angelo C, Smith E, Hunt A, Legiret F-E, Postle A, et al. Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nature Climate Change. 2013;3(2):160–4.
41. Voss JD, Richardson LL. Nutrient enrichment enhances black band disease progression in corals. Coral Reefs. 2006;25(4):569–76. <https://doi.org/10.1007/s00338-006-0131-8>
42. Vega Thurber RL, Burkepile DE, Fuchs C, Shantz AA, McMinds R, Zaneveld JR. Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Glob Chang Biol. 2014;20(2):544–54. <https://doi.org/10.1111/gcb.12450> PMID: 24277207
43. Bruno J, Petes L, Harvell C, Hettinger A. Nutrient enrichment can increase the severity of coral diseases. Ecol Lett. 2003;6(10):1056–61.
44. Fuess LE, Palacio-Castro AM, Butler CC, Baker AC, Mydlarz LD. Increased Algal Symbiont Density Reduces Host Immunity in a Threatened Caribbean Coral Species, *Orbicella faveolata*. Front Ecol Evol. 2020;8. <https://doi.org/10.3389/fevo.2020.572942>
45. Rådecker N, Pogoreutz C, Voolstra CR, Wiedenmann J, Wild C. Nitrogen cycling in corals: the key to understanding holobiont functioning?. Trends Microbiol. 2015;23(8):490–7. <https://doi.org/10.1016/j.tim.2015.03.008> PMID: 25868684
46. Falkowski PG, Dubinsky Z, Muscatine L, McCloskey L. Population Control in Symbiotic Corals. BioScience. 1993;43(9):606–11. <https://doi.org/10.2307/1312147>
47. Muscatine L, Falkowski P, Dubinsky Z, Cook P, McCloskey L. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proceedings of the Royal Society B. 1989;236:311–24.
48. Klimes JG, Patel SH, Duke WC, Muller EM, Vega Thurber RL. Phosphate enrichment induces increased dominance of the parasite *Aquarickettsia* in the coral *Acropora cervicornis*. FEMS Microbiol Ecol. 2022;98(2):fiac013. <https://doi.org/10.1093/femsec/fiac013> PMID: 35157069
49. Klimes G, Maher RL, Vega Thurber RL, Muller EM. Parasitic “*Candidatus Aquarickettsia rohweri*” is a marker of disease susceptibility in *Acropora cervicornis* but is lost during thermal stress. Environ Microbiol. 2020;22(12):5341–55. <https://doi.org/10.1111/1462-2920.15245> PMID: 32975356
50. Enochs I, Manzello D, Jones P, Aguilar C, Cohen K, Valentino L, et al. The influence of diel carbonate chemistry fluctuations on the calcification rate of *Acropora cervicornis* under present day and future acidification conditions. Journal of Experimental Marine Biology and Ecology. 2018;506:135–43.

51. AOML Ecosystem Assessment Laboratory. Standard operating procedure: Calibration of the AA3 nutrient analyzer, revision 5. NOAA Atlantic Oceanographic and Meteorological Laboratory. 2024. <https://doi.org/10.25607/OBP-1986>
52. Warner ME, Fitt WK, Schmidt GW. Damage to photosystem II in symbiotic dinoflagellates: a determinant of coral bleaching. *Proc Natl Acad Sci U S A*. 1999;96(14):8007–12. <https://doi.org/10.1073/pnas.96.14.8007> PMID: 10393938
53. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Statistical Software*. 2015;67(1):51. <https://doi.org/10.18637/jss.v067.i01>
54. R Core Team: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing; 2021. Available from: <https://www.r-project.org>
55. Lenth R, Singmann H, Love J, Buerkner P, Herve M. Emmeans: Estimated marginal means, aka least-squares means R Package Version 1 (2018). 2018.
56. Mieog JC, VAN Oppen MJH, Berkelmans R, Stam WT, Olsen JL. Quantification of algal endosymbionts (*Symbiodinium*) in coral tissue using real-time PCR. *Mol Ecol Resour*. 2009;9(1):74–82. <https://doi.org/10.1111/j.1755-0998.2008.02222.x> PMID: 21564569
57. Cuning R, Baker A. Excess algal symbionts increase the susceptibility of reef corals to bleaching. *Nature Climate Change*. 2013;3(3):259–62.
58. Cuning R, Silverstein RN, Baker AC. Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under environmental change. *Proc Biol Sci*. 2015;282(1809):20141725. <https://doi.org/10.1098/rspb.2014.1725> PMID: 26041354
59. Palacio-Castro AM, Dennison CE, Rosales SM, Baker AC. Variation in susceptibility among three Caribbean coral species and their algal symbionts indicates the threatened staghorn coral, *Acropora cervicornis*, is particularly susceptible to elevated nutrients and heat stress. *Coral Reefs*. 2021;40(5):1601–13. <https://doi.org/10.1007/s00338-021-02159-x>
60. Cuning R. StepOneR: R package for importing qPCR data from StepOne™ Software. 2018. <https://doi.org/10.5281/zenodo.1173321>
61. Rosales S, Palacio-Castro AM. Homogenate of *A. cervicornis* v1 protocol. 2020. <https://doi.org/10.17504/protocols.io.4r3l248xpg1y/v1>
62. Kaplan EL, Meier P. Nonparametric Estimation from Incomplete Observations. *Journal of the American Statistical Association*. 1958;53(282):457–81. <https://doi.org/10.1080/01621459.1958.10501452>
63. Therneau T. A package for survival analysis in S. version 2.38. 2015.
64. Kassambara A, Kosinski M, Przemyslaw B. survminer: Drawing survival curves using “ggplot2” R package version 0.4. 6.999. 2019.
65. Palacio-Castro AM. Workflow for: Assessing *Acropora cervicornis* genotype resistance to elevated ammonium and disease. 2024. <https://doi.org/10.5281/ZENODO.10989808>
66. Miami Waterkeeper. Fish kill in biscayne bay: A report. 2020. <https://d3n8a8pro7vhmx.cloudfront.net/miamiwaterkeeper/pages/5046/attachments/original/1627491061/fish-kill-report.pdf>
67. Caruso C, Hughes K, Drury C. Selecting Heat-Tolerant Corals for Proactive Reef Restoration. *Front Mar Sci*. 2021;8. <https://doi.org/10.3389/fmars.2021.632027>
68. Vollmer SV, Selwyn JD, Despard BA, Roesel CL. Genomic signatures of disease resistance in endangered staghorn corals. *Science*. 2023;381(6665):1451–4. <https://doi.org/10.1126/science.adi3601> PMID: 37769073
69. Jones AM, Berkelmans R. Tradeoffs to Thermal Acclimation: Energetics and Reproduction of a Reef Coral with Heat Tolerant *Symbiodinium* Type-D. *Journal of Marine Biology*. 2011;2011:1–12. <https://doi.org/10.1155/2011/185890>
70. Cornwell B, Armstrong K, Walker NS, Lippert M, Nestor V, Golbuu Y, et al. Widespread variation in heat tolerance and symbiont load are associated with growth tradeoffs in the coral *Acropora hyacinthus* in Palau. *Elife*. 2021;10:e64790. <https://doi.org/10.7554/eLife.64790> PMID: 34387190
71. Palacio-Castro AM, Enochs IC, Besemer N, Boyd A, Jankulak M, Kolodziej G, et al. Coral Reef Carbonate Chemistry Reveals Interannual, Seasonal, and Spatial Impacts on Ocean Acidification Off Florida. *Global Biogeochemical Cycles*. 2023;37(12). <https://doi.org/10.1029/2023gb007789>
72. Rosales SM, Miller MW, Williams DE, Traylor-Knowles N, Young B, Serrano XM. Microbiome differences in disease-resistant vs. susceptible *Acropora* corals subjected to disease challenge assays. *Sci Rep*. 2019;9(1):18279. <https://doi.org/10.1038/s41598-019-54855-y> PMID: 31797896
73. Young BD, Rosales SM, Enochs IC, Kolodziej G, Formel N, Moura A, et al. Different disease inoculations cause common responses of the host immune system and prokaryotic component of the

- microbiome in *Acropora palmata*. PLoS One. 2023;18(5):e0286293. <https://doi.org/10.1371/journal.pone.0286293> PMID: [37228141](https://pubmed.ncbi.nlm.nih.gov/37228141/)
74. Klinges JG, Patel SH, Duke WC, Muller EM, Vega Thurber RL. Microbiomes of a disease-resistant genotype of *Acropora cervicornis* are resistant to acute, but not chronic, nutrient enrichment. Sci Rep. 2023;13(1):3617. <https://doi.org/10.1038/s41598-023-30615-x> PMID: [36869057](https://pubmed.ncbi.nlm.nih.gov/36869057/)
 75. Renegar D, Riegl B. Effect of nutrient enrichment and elevated CO2 partial pressure on growth rate of Atlantic scleractinian coral *Acropora cervicornis*. Marine Ecology Progress Series. 2005;293:69–76.
 76. Jones NP, Ruzicka RR, Colella MA, Pratchett MS, Gilliam DS. Frequent disturbances and chronic pressures constrain stony coral recovery on Florida's Coral Reef. Coral Reefs. 2022;41(6):1665–79. <https://doi.org/10.1007/s00338-022-02313-z>
 77. Jones NP, Gilliam DS. Temperature and local anthropogenic pressures limit stony coral assemblage viability in southeast Florida. Mar Pollut Bull. 2024;200:116098. [https://doi.org/10.1016/j.marpol-bul.2024.116098](https://doi.org/10.1016/j.marpolbul.2024.116098) PMID: [38310721](https://pubmed.ncbi.nlm.nih.gov/38310721/)
 78. Bythell JC. Nutrient uptake in the reef-building coral *Acropora palmata* at natural environmental concentrations. Marine Ecology Progress Series. 1990;68:65–9.