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Elevated temperature decreases stony coral tissue loss disease transmission, with little effect of nutrients

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Stony coral tissue loss disease (SCTLD) is the deadliest scleractinian coral disease reported, causing significant coral loss in the Western Atlantic reefs. Environmental conditions are known to influence disease dynamics, but determining the specific conditions that exacerbate SCTLD remains challenging. We developed a robotic multi-stressor system to study the effects of temperature and ammonium on SCTLD transmission. For a month, coral fragments were preconditioned to two temperatures (28 °C and 31 °C) and nutrient treatments (with and without ammonium dosing), and subsequently exposed to SCTLD. Environmental treatments only caused modest effects in the corals (based on calcification, photochemical efficiency, and symbiotic algal communities). However, SCTLD incidence was strongly reduced at higher temperature (17% at 31 °C compared to 70% at 28 °C), contrasting with other coral diseases that typically worsen with increased heat. Disease management approaches may involve concentrating SCTLD treatment efforts during warmer periods when reduced incidence might enhance treatment efficacy.

Keywords Coral disease, High temperature, Nutrients, Multiple stressors, Disease transmission, *Orbicella faveolata*

The Tropical Western Atlantic is currently experiencing a multi-year disease outbreak of stony coral tissue loss disease (SCTLD). This disease has caused substantial mortality of at least 22 reef-building coral species, reshaping the coral community composition and pushing susceptible species at risk of local extinction[1–4]. This disease was first observed in late 2014 near Virginia Key, Florida, and has since spread throughout the Western Atlantic, likely becoming the most lethal coral disease recorded[5]. Efforts to control SCTLD spread have been ineffective since marine pathogens can potentially travel vast distances via marine currents and ballast water[6,7]. Despite multiple efforts to identify the causative agent(s), the pathogen or microbial consortium that causes SCTLD remains unknown[8–10], hindering the development of disease treatments that target specific pathogens.

Evidence from other coral diseases suggests that environmental factors can substantially influence disease transmission, host susceptibility, and pathogen virulence[11,12]. Among myriad environmental factors affecting coral health, the effects of elevated nutrients and temperature on disease dynamics have received particular attention[13]. Elevated nutrients have been found to increase lesion progression and disease prevalence in black band and yellow band diseases[14,15]. Similarly, ocean warming has been linked with higher coral diseases prevalence[2,16,17], and faster white plague progression[18]. Moreover, heat-induced coral bleaching can cause loss of disease resistance in corals previously characterized as white-band resistant[19].

Several potential mechanisms have been proposed to explain how environmental factors influence coral disease activity. These include include impairing coral immunity[20], altering the composition of corals' associated microbial communities (e.g., prokaryotes, viruses, and algal symbionts)[21], and increasing the virulence of opportunistic microbial taxa that can become pathogenic under specific conditions[22–24]. Elevated nutrients and temperature, specifically, are known to disrupt coral-associated microbial communities, promoting the proliferation of opportunistic microbes[21,25] and reducing coral health and survivorship[26].

Identifying the specific environmental conditions that shape SCTLD dynamics is critical for informing management strategies. Yet, the influence of environmental factors on SCTLD is still unclear. For example,

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ex-situ nitrate enrichment increased tissue loss in a coral species (*Montastraea cavernosa*) without causing photochemical stress (reduced photochemical efficiency or F_v/F_m). However, the opposite was found for another coral (*Siderastrea siderea*) where nitrate reduced F_v/F_m but did not impact SCTLD progression[27]. A field study found no correlation between SCTLD prevalence or progression and nutrient enrichment when comparing nutrient-enriched vs. control sites, although nutrient concentrations of these sites were not significantly different, making larger inferences challenging[28]. Similar to nutrients, correlative assessments of the impacts of temperature on SCTLD have been difficult to interpret or conflicting. While some field studies found a negative correlation between heat stress and disease activity, particularly during coral bleaching[29–31], others have found no relationship between high or low temperatures and SCTLD[1,4,32,33]. An ex-situ study found faster SCTLD progression rates in one coral species (*Colpophyllia natans*) when maintained at high temperature, but not in another (*Montastraea cavernosa*)[34]. Statistical modeling of disease surveys over time, on the other hand, revealed a positive correlation between the total number of SCTLD infections and temperature stress (over the prior 90 days), inlet flow (over the prior 7 days), and total rainfall (over the prior 90 days), suggesting that heat and human pollution might exacerbate SCTLD transmission and/or prevalence[35].

The interpretation of field data is further complicated by the co-occurrence of temporal variation in multiple conditions, such as simultaneous (e.g., seasonal) changes in temperature, light, pH, nutrients, salinity, etc., which hampers the attribution of a given disease response to a specific environmental parameter[36]. This context, makes ex-situ multi-factorial approaches essential for evaluating the impacts of co-varying environmental conditions on the SCTLD outbreak, as well as the impact of potential environmental management interventions, such as improving water quality, to reduce disease transmission and progression.

Here, we examined the effects of temperature and ammonium enrichment on the transmission of SCTLD in the endangered mountainous star coral, *Orbicella faveolata*. We hypothesized that exposure to higher temperatures and ammonium levels would increase coral susceptibility to infection. Specifically, we tested whether summertime temperatures (31 °C) relative to spring/fall temperatures (28 °C), and the presence versus absence of ammonium enrichment, would increase SCTLD transmission rates.

To address the logistical challenges of conducting multi-factorial disease experiments, including the need for adequate replication and full independence in disease exposure, we developed a novel robotic system: the Sequential Treatment Application Robot (STAR)[37]. The STAR system continuously delivered precise doses of ammonium, disease-exposed seawater, and control-exposed seawater to independent, temperature-controlled beakers, each holding a single experimental coral fragment (Fig. 1). For 31 days, all fragments (N = 80) were maintained under one of four environmental treatments: 28 °C without nutrient dosing (LN + 28), 31 °C without nutrient dosing (LN + 31), 28 °C with nutrient dosing (HN + 28), or 31 °C with nutrient dosing (HN + 31). Afterward, the fragments were exposed to waterborne SCTLD for 20 days while maintaining their respective temperature and nutrient treatments. Our results highlight a strong effect of temperature on SCTLD transmission, with mild effects of ammonium exposure.

Results

Coral's physiological response to environmental treatments

The photochemical efficiency (F_v/F_m) and calcification rates of the fragments after 14 and 31 days of the temperature and ammonium treatments were used as a proxy for the effects of the environmental conditions on coral health, which could potentially increase disease susceptibility. Both temperature and ammonium

Fig. 1. Panoramic view of the STAR system. The picture shows the two robot arms dosing the experimental corals with artificial seawater (previously exposed to healthy or diseased coral colonies) and ammonium.

treatments (Linear mixed model [LMM] $p < 0.001$; Table S1) resulted in statistically different F_v/F_m, but the magnitude of these differences was small and did not reflect photochemical stress in any treatment (Fig. 2A). Within each ammonium treatment (i.e., LN + 28 vs. LN + 31C, and HN + 28 vs. HN + 31), corals maintained at 28 °C had ~ 3% higher F_v/F_m values compared to corals at 31 °C (Tukey HSD $p < 0.05$). Similarly, within each temperature, corals exposed to HN presented ~ 1% higher F_v/F_m compared to LN (Tukey HSD $p < 0.05$). These F_v/F_m differences were consistent over time, with no significant effect of time point (LMM $p > 0.05$; Fig. 2A).

Coral calcification rates were affected by nutrient treatment ($p < 0.01$) and time point (i.e., Day 14 vs. Day 31, LMM $p < 0.001$), but not temperature (LMM $p > 0.05$; Tables S3). On Day 14, corals in HN had ~ 22% lower calcification compared to corals in LN, and by Day 31 this difference reached ~ 33% (Tukey HSD $p < 0.05$; Fig. 2B; Table S4). However, the main difference in calcification was the overall decline from Day 14 to Day 31 (Fig. 2B), potentially because the fragments were not fed during the experiment period to avoid introducing additional nutrients.

Overall, there were no signs of acute stress (e.g., coral bleaching, paling, or tissue loss) in any of the fragments during exposure to the environmental treatments.

Symbiodiniaceae algal community response to the environmental treatments

Symbiodiniaceae communities are known to respond to both temperature and nutrients[38,39], and different algal communities can influence SCTLD susceptibility[40]. We used the symbiont to host (S/H) cell ratio, a metric of relative algal symbiont abundance[41], to assess changes in the algal abundance (number of Symbiodiniaceae algal cells per coral cell) or identity (proportion of each Symbiodiniaceae genus in the algal community) due to the environmental treatments.

After fourteen days of environmental treatments, ammonium and temperature had marginal effects on the composition of the algal community (Fig. 3). Algal abundance was on average ~ 18% higher in the HN corals compared to LN (Fig. 3A; LMM $p < 0.001$), with no effect of temperature (LMM $p > 0.05$). However, the biggest differences in symbiont abundance were observed among experimental colonies, with one colony (colony A) presenting 2–3 times lower S/H cell ratios compared to the other two (colonies B and C; Fig. 3A).

Fragments from all experimental colonies were dominated by algal symbionts in the genus *Durusdinium* (likely thermotolerant *D. trenchii*), which accounted for more than 99% of the community across colonies and treatments (Fig. 3B; Tables S6–S7). However, background *Symbiodinium* and *Breviolum* were detected in some fragments at abundances < 1%. Fragments could not be sampled again at Day 31 due to logistical constraints.

Disease transmission by environmental treatment

Following 31 days of exposure to the environmental treatments, we transitioned the STAR system from dosing control seawater to dosing to disease-exposed seawater to the majority of fragments (n = 66). To confirm that emerging disease signs were exclusively the result of waterborne SCTLD, we maintained a subset of corals from each colony and environmental treatment as disease controls (n = 14). These continued to receive healthy-exposed seawater until the end of the experiment and did not develop disease lesions.

Corals exposed to SCTLD had a lower probability of developing lesions under elevated temperature (30% ± 8 CI at 31 °C compared to 83% ± 9 CI at 28 °C; Log-rank $p < 0.001$), regardless of the ammonium treatment (Fig. 4A). The first disease lesion was recorded in HN + 28 after 12 days of initiating disease exposure (Day 44

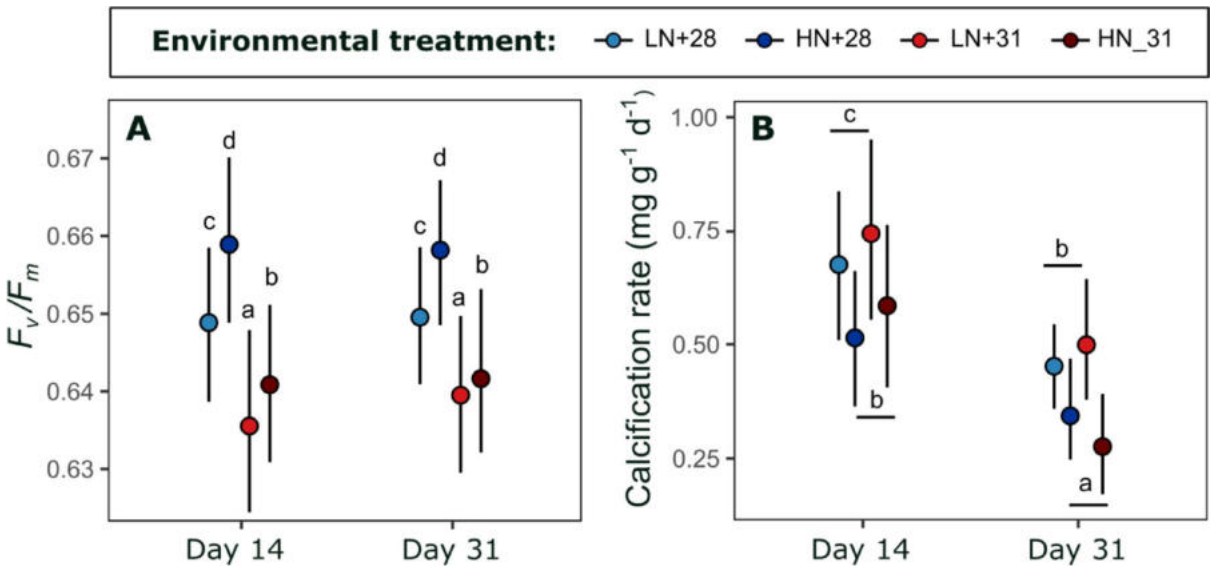

Fig. 2. Physiological response of *O. faveolata* to the environmental treatments (mean ± 95% CI) before disease exposure. (**A**) Photochemical efficiency of the algal symbionts (F_v/F_m). (**B**) Calcification rates (mg g^{-1} d^{-1}). The colors represent the combined temperature and nutrient treatments. The letters represent post hoc Tukey HSD groups. Linear model outputs are shown in Tables S1 and S3 and Tukey HSD tests in Tables S2 and S4.

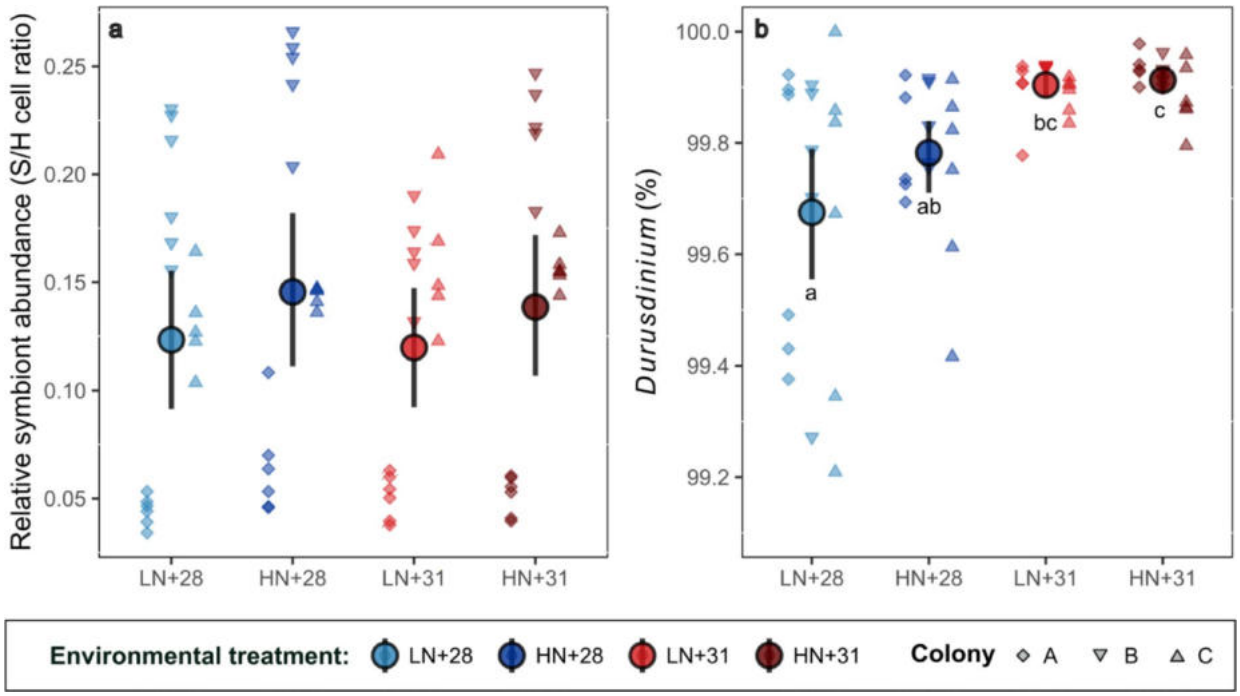

Fig. 3. Symbiodiniaceae algal communities after 14 days of exposure to the environmental treatments. (**A**) Total Symbiodiniaceae to host cell ratio (mean S/H ± 95% CI). Algal abundance was higher for corals in HN compared to LN, but colony had the biggest effect (Table S5). (**B**) Percentage of *Durusdinium* (± 95% CI). The colors represent the combined temperature and nutrient treatments. The shapes represent the different coral colonies. Letters in panel B represent post hoc Tukey HSD groups (Table S7).

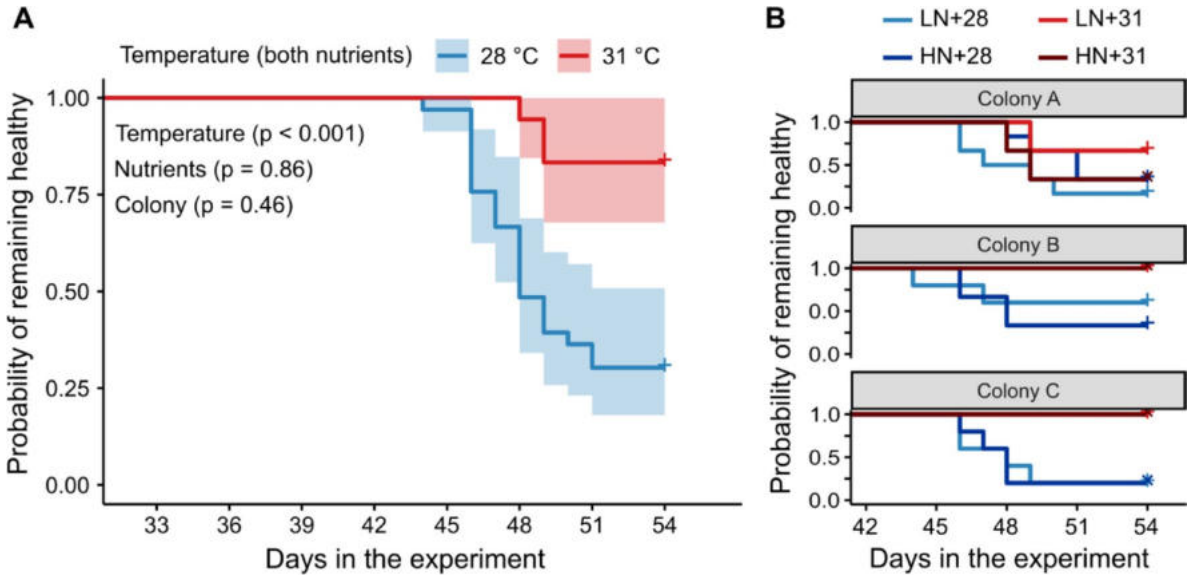

Fig. 4. Probability of *O. faveolata* remaining healthy (with no lesions) after exposure to SCTLD. (**A**) Mean (± 95% CI) probabilities by temperature treatments (nutrients and colonies pooled by temperature). The number of fragments exposed to SCTLD represents the number of corals that remained healthy on a given day and were available to become diseased. (**B**) Probabilities parsed by coral colony, nutrients, and disease treatments.

of the experiment; Fig. 4). Two days later, more corals developed lesions in both low temperature treatments (HN + 28 and LN + 28), and this continued until Days 50–51 of the experiment. Corals under high temperature (HN + 31 and LN + 31) took longer to show signs of disease, and lesions were only reported over two days (Days 48–49). Although coral colony did not have a significant effect on disease transmission ($p = 0.46$), one colony (A)

showed similar disease incidence in all four environmental treatments, while two colonies did not develop any lesions in either of the two high-temperature treatments (LN + 31 or HN + 31; Fig. 4B).

The Cox-hazard ratio (HR), an indicator of the relative risk of developing a lesion in the treatments, showed that corals exposed to 31 °C had a significantly lower risk of getting disease (HR = 0.15) relative to corals at 28 °C ($p = 0.002$; Fig. S4).

Discussion

Our findings about the temperature effects on SCTLD transmission carry important implications for the prospective trajectory of this disease outbreak. Contrary to our original hypothesis, which predicted that higher temperatures would increase coral susceptibility to SCTLD, our results revealed a significant reduction in SCTLD transmission at elevated temperatures typical of the peaks of the summer season. In contrast, we found no evidence to support a significant role of nitrogen availability, specifically ammonium enrichment, in modulating disease transmission. These outcomes offer valuable guidance for coral reef managers in shaping their SCTLD monitoring and intervention plans. A strategic approach may involve concentrating treatment efforts during periods of heightened temperatures, optimizing resource allocation when fewer active disease lesions are present, and thereby potentially enhancing treatment efficacy[31]. This can be accompanied by constant monitoring once the temperature starts decreasing again to treat the remaining active lesions before cooler temperatures accelerate SCTLD activity. However, a pivotal question concerning the future of Caribbean reefs centers on how SCTLD dynamics may evolve following extreme warming and bleaching events.

The observed SCTLD transmission reduction at higher temperature contrasts with other coral diseases such as white plague, white syndrome, and white band disease, which are typically exacerbated under warmer conditions and thermal stress[17–19]. Currently, the specific mechanisms conferring SCTLD protection under high temperatures remain unidentified, but working hypotheses include potential changes in coral microbial communities (algae, prokaryotes, fungi, and viruses) that may influence infection risk[42], the existence of a more rapid and robust immune response by corals at higher temperatures, and temperature-induced alterations in pathogen growth or virulence[43,44].

Changes in corals' Symbiodiniaceae algal communities, in particular, might play a key role in SCTLD susceptibility. Some evidence has suggested that potential SCTLD pathogens could first target the endosymbiotic algae (41) and corals hosting algae in the genus *Breviolum* seem to be the most susceptible to SCTLD, followed by corals hosting *Cladocopium* and *Durusdinium*, and finally *Symbiodinium*[1,40]. Thus, more abundant algal communities or those composed of susceptible algae species might increase SCTLD risk. Because elevated temperatures can both decrease symbiont abundance and favor the dominance of *Durusdinium* over more SCTLD-susceptible algae like *Breviolum*[45,46], thermal exposure could theoretically induce changes in the algal community that reduce SCTLD susceptibility. However, in our study, all coral fragments had similar algal abundances and were consistently dominated by *Durusdinium*, suggesting that other temperature-dependent mechanisms were responsible for the observed reduction in SCTLD transmission at elevated temperatures.

Alternatively, lower SCTLD transmission at 31 °C might be linked to the effects of high temperature on the coral host immunity. Exposure to heat, for example, can induce the upregulation of innate immune genes within the coral host[47,48]; and recent evidence suggests that certain immune responses, such as phagocytic activity, increase under elevated temperature independently of the coral-algal symbiosis state[49]. However, it remains unclear how heat, and the associated upregulation of these immune responses, could reduce coral susceptibility to SCTLD while potentially increasing susceptibility to other diseases. Temperature-driven changes in pathogen virulence or the composition of the prokaryotic communities could also contribute to SCTLD dynamics. Exposure to elevated temperatures is a major cause of shifts in the composition of the microbial communities of multiple coral species[25,50–52] and can modulate the antagonistic interactions between members of the coral microbiomes[53]. Unfortunately, the role of temperature on SCTLD pathogen abundance or virulence remains unexplored as the causative agent of the disease has yet to be identified.

It is important to acknowledge that in our experiment, the temperature-dependent transmission was not consistent for one colony, which had similar disease incidence under all the environmental treatments. Interestingly, this colony also presented a lower S/H cell ratio compared to the other two colonies studied, suggesting potential differences in the genetic makeup of either the *O. faveolata* colony or the *D. trenchii* hosted. These differences can affect the copy number of the genes used to calculate S/H as well as the colony responses to the environmental conditions and disease. This result underscores the potential variability in how distinct coral species, and even colonies within a species, can respond to SCTLD-environment interactions. Future research should test if temperature has a consistent effect on mitigating SCTLD across multiple coral species, genotypes, and their respective symbiotic communities[54].

Considering that climate change will continue to increase ocean temperatures, as well as the frequency and intensity of heat stress events on coral reefs, it is crucial to examine how a broader spectrum of temperatures affects SCTLD activity. This should include differentiating the short- and mid-term effects of normal (e.g., seasonal) warming from those of acute heat stress. In our experiment, warmer conditions reduced SCTLD transmission at temperatures that did not cause acute physiological stress (i.e., no significant effects on coral calcification and only a ~ 3% reduction in F_v/F_m). However, field reports of reduced SCTLD activity during warmer periods often include coral bleaching events[29–31]. Based on this, it seems plausible that varying levels of heat, whether or not they cause bleaching, could temporarily reduce SCTLD activity.

However, it is unclear if bleached and unbleached corals have different susceptibilities to SCTLD once temperatures decrease[19,55–57]. Historical monitoring of the initial SCTLD outbreak indicated a correlation with bleaching, or more specifically with bleaching recovery, with disease prevalence rising as coral colonies regained normal pigmentation[5]. Similarly, statistical modeling suggested a positive correlation between temperature stress and total number of SCTLD infections, peaking approximately three months post-heat stress events[35].

Contrastingly, a study showed that SCTLD incidence was reduced during a bleaching event in the summer of 2018 and remained lower during the following year, suggesting that in this case bleaching did not exacerbate disease activity after the water cooled down[30].

Untangling the interactions among seasonal temperature variation, heat stress, and bleaching on SCTLD dynamics has become critical as the Caribbean and Western Atlantic recover from an unprecedented mass bleaching event[58]. Based on the Coral Reef Watch products, during the boreal summer of 2023, reefs in the Florida Keys, Caribbean islands, Central and South America experienced heat stress levels (15–20 Degree Heating Weeks), which doubled and almost tripled any stress recorded in these locations since the beginning of the satellite temperature monitoring in 1985[58,59]. Teams of scientists and managers have reported widespread bleaching, coral mortality, and reduced SCTLD incidence in these reefs. Continued monitoring as the temperatures decline and surviving corals recover will help determine if this mass bleaching event might have mitigated or exacerbated SCTLD incidence across the Caribbean and Western Atlantic regions.

Similar to previous field results[28], we found no correlation between nutrient dosing and SCTLD dynamics. This lack of effect, however, needs to be interpreted with caution. First, other nutrient sources (e.g., NO_3 and PO_4), concentrations, and coral species should be tested before ruling out the impact of elevated nutrients on SCTLD. For example, elevated NO_3 increased SCTLD progression rates in at least one coral species[27]. Second, waterborne transmission experiments that use disease donor colonies tend to increase the nutrient levels in the water due to tissue sloughing and the breakdown of organic nitrogen in it[60], making it difficult to maintain disease treatments under low nutrient levels. Since we currently lack an isolated pathogen to conduct transmission tests, other disease transmission methods such as SCTLD-exposed sediments[61], which do not directly involve the donor colonies, could be more suitable to test the effects of nutrients on SCTLD dynamics.

It is important to consider that although ammonium treatment did not affect SCTLD transmission, nutrient dosing did cause mild physiological effects on the corals, slightly increasing F_v/F_m and symbiont abundance, and more importantly, reducing calcification rates by 22–33%. These holobiont responses to elevated nitrogen underscore the complexity of nutrient-environment interactions in the coral-algae symbiosis and overall holobiont health[50,62,63]. Elevated nitrogen availability can increase Symbiodiniaceae abundance[64,65], potentially leading to reduced carbon translocation from the algae to the coral host and limiting the energy available for calcification[66–68]. Notably, *O. faveolata* calcification was impaired under ammonium dosing, even though our dosing regimen did not achieve consistently high nutrient concentrations. These findings underline the potential repercussions of undetectable nitrogen input on reefs, given that negative impacts can occur even when measured concentrations are not detected as elevated due to rapid nutrient uptake by corals, plankton, and other reef organisms[69].

Coral reefs are increasingly exposed to physicochemical and biotic stressors, including warming, acidification, nutrient pollution, deoxygenation, overfishing, and infectious diseases[18,70,71]. Extensive literature has documented interactions among the effects of two stressors on corals' physiological and ecological responses[15,72–74]. However, studies involving more than two stressors are limited, partially because the logistical and statistical challenges associated with multivariate studies escalate with the addition of treatments and treatment levels[75]. This represents a critical gap for coral reef conservation since environmental changes often correlate in coastal ecosystems[36], resulting in different impacts from those expected from the isolated stressors[76,77].

The intricate interplay between environmental stressors and coral susceptibility to infectious diseases accentuates the importance of an interdisciplinary approach to comprehensively address the challenges posed by compounding threats. Our capacity to manipulate multiple conditions in a controlled environment is pivotal for elucidating the causal relationships between individual and combined environmental conditions, shedding light on field observations and monitoring efforts. The STAR system allowed us to bridge this knowledge gap, enabling a high-replication multi-stressor test designed to understand the interactions between ammonium, temperature, and SCTLD transmission[37]. Although the combined high temperature and ammonium did not show synergistic effects on disease transmission, this treatment did have the lowest calcification rates for the coral after 31 days. STAR system's versatility allows the incorporation of numerous additional stressors, such as ocean acidification, deoxygenation, sedimentation, and various nutrient sources, and thus exploring additional interactions. Similarly, the system can be employed to test disease treatments and interventions, such as unsupervised constant application of precise doses of different probiotics.

Methods

Experimental setup and STAR system

Our experimental setup consisted of 80 beakers (600 mL) distributed among six independent experimental tanks. From these, three tanks were maintained at 28 °C and three at 31 °C (Fig. 5). Temperature in each tank was measured every 5 min with a high-accuracy RTD sensor (TTD25C, ProSense). Based on the reading, the temperature was manipulated to stay within the target values with a 300 W aquarium heater (TH300, Finnex) and a titanium chiller coil (Hotspot Energy) as described in[78]. Each beaker was semi-submerged in the tanks for temperature control (13–14 beakers per tank) and acted as an independent sample vessel subjected to a combination of temperature, nutrients, and disease treatments (Fig. 5). We placed the beakers within each tank so that their tops were above the water bath level, thereby facilitating unidirectional outward flow from the beaker and eliminating the potential for cross-contamination (Fig. 1). A stir bar inside each beaker ensured gas exchange, water flow, and nutrient mixing in each beaker. Two additional tanks (donor tanks) were maintained at 28 °C and served as upstream water reservoirs for the application of the disease treatments. These held either diseased or healthy coral colonies acting as the source of disease-exposed seawater and control-exposed seawater (Figs. 1, 5).

Ammonium and disease treatments were applied to the beakers using two STAR systems[37]. Briefly, two robotic arms (xArm 6, Ufactory) (Figs. 5, S1) were each affixed to linear tracks that provided 700 mm of travel

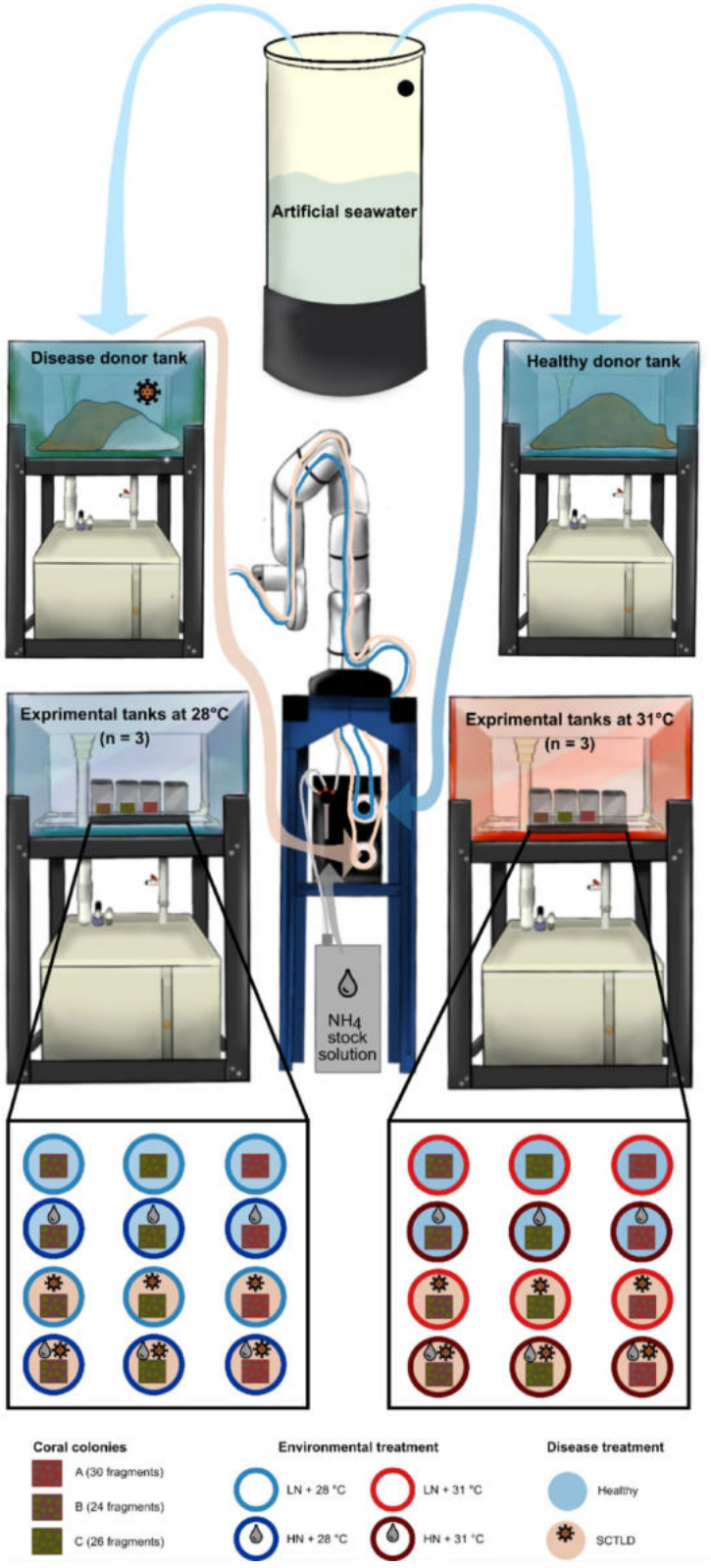

Fig. 5. Diagram of the experimental setup showing the water flow between the tanks and the experimental combinations: Top—artificial seawater flow into the healthy and disease donor tanks, followed by flow into the dosing boxes attached to the robot arms. Bottom—All the possible combinations of treatments and beaker content in a given tank. The diagram represents all possible treatments, but not the replication. For replication see Table 1. Detailed STAR system parts are shown in Figure S1. Treatment and coral colony position in the tanks were randomized but are shown here in order for easier visualization.

between clusters of three experimental tanks (40 beakers per each of two STAR systems). A custom-built dosing box was connected to each arm. The boxes included a syringe pump (2.5 mL syringe, Kloehn v6, Norgren) for dosing ammonium (NH_4) and two brushless peristaltic pumps (A201BX, Anko) for separately dosing either healthy or disease-inoculated seawater (Figs. 5, S1). All pumps were connected to tubing that was routed from the healthy and disease tanks, or the nutrient stock reservoir, along the arm and track, to a custom-built end effector that applied treatments to each beaker through three pipette tips. Each robotic arm cycled between beaker positions across three water bath tanks per system, applying treatment volumes preprogrammed in a graphical interface (Fig. S2). Data on the arm movement and status were logged and uploaded online using a cellular-enabled watchdog device, which produced relevant analytics for dosing performance assessment.

Artificial seawater was used for the experiment to avoid unwanted contamination from nutrients or possible pathogenic microorganisms present in the natural seawater. We mixed reverse osmosis (RO) water and Instant Ocean sea salt in two 750 L vats targeting a 35 ppt salinity. Then, we supplied this artificial seawater to the control and disease donor tanks, from where it was then applied to the beakers by the STAR systems. While one vat was providing seawater to the system, the second one was mixing the new batch of water.

Temperature treatments

On day one of the experiment (May 2, 2023), we randomly and equally allocated the *O. faveolata* fragments from three colonies ($N = 80$; 24–30 per colony) to the experimental beakers, half at 28 °C and half at 31 °C (Fig. 6). These temperature levels represent typical spring/fall (28 °C) and peak of the summer (31 °C) conditions in the South Florida region[79], where the experimental corals were collected, and both remain below the established bleaching threshold for the area[59]. Whereas corals in the 28 °C treatment were directly transferred to beakers at temperature, corals in the 31 °C treatment were brought from 28 °C to the target by increasing the temperature 0.4 °C twice a day for four days (Fig. 6A). Unfortunately, on Day 32, the temperature sensor in one of the tanks assigned to 31 °C failed, and the associated corals could not be used during the second disease exposure (Table 1).

Nutrient treatments

We started nutrient dosing on Day 1 of the experiment, evenly allocating the two nutrient treatments among temperatures and coral colonies (Fig. 6B, Table 1). During the exposure to the environmental treatments (Phase 1: Days 1–31), the seawater provided to all beakers was sourced from the "control" tank containing a healthy *O. faveolata* colony. When a robot arm reached a beaker, the STAR system dosed it with 60 mL of control seawater if the beaker was assigned to low ammonium (LN), and with 60 mL of control seawater and 0.5 mL of an NH_4Cl solution (600 µM) if assigned to high ammonium (HN). This dosing protocol targeted a replenishment concentration of 5 µM ammonium, consistent with levels previously observed on reefs in the region during nutrient input events[80]. For this phase of the experiment, we set the seawater pump rates to dose at 2.6 mL s^{-1}, which resulted in an average dose duration of ~ 39 s per beaker (Table 2). The total time for a STAR cycle between all associated beakers was ~ 31 min, providing more than four full water turnovers per day in all beakers, and a total addition of ~ 14 µmol NH_4 per day in beakers receiving nutrients (Table 2).

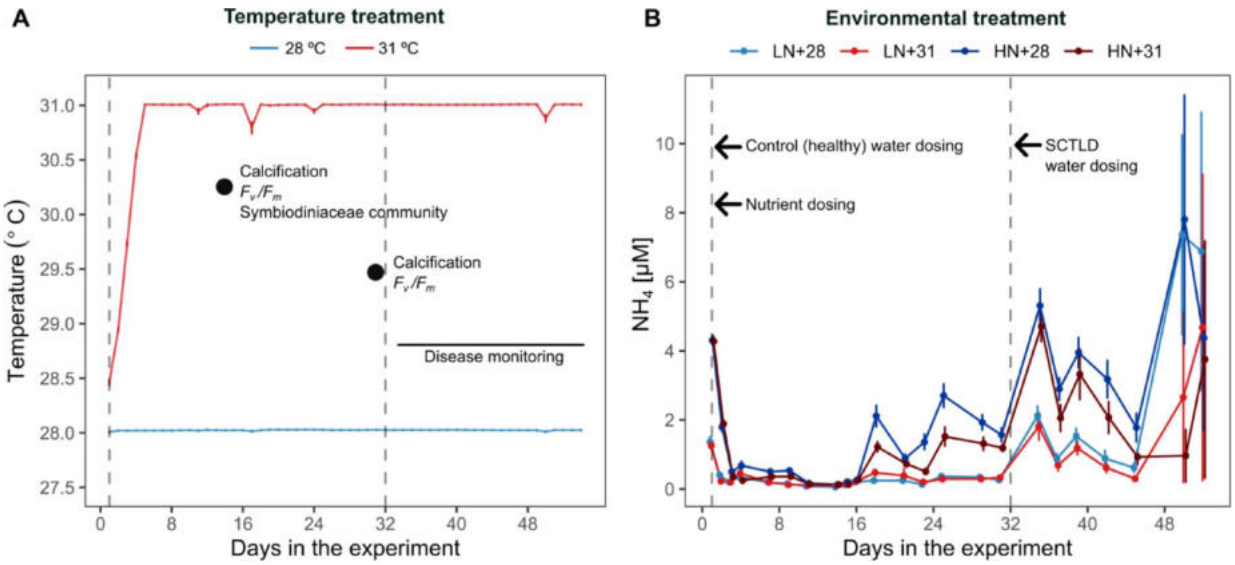

Fig. 6. Experimental timeline and environmental conditions. (**A**) Temperature in the experimental tanks holding the beakers (mean ± se). The black dots and horizontal line denote the days when experimental data was collected. (**B**) Ammonium (NH_4) concentration in the experimental beakers (mean ± se). The colors represent the environmental treatment assigned to the beakers as a combination of nutrient and temperature treatments. The vertical lines and black arrows demarcate the days when dosing with nutrients, control seawater and disease-exposed seawater were initiated.

Coral colony	Disease treatment	Environmental treatment				Total
		28 °C + HN	28 °C + LN	31 °C + HN	31 °C + LN	
Colony A	Control	1	2	1	2 (1)	6 (5)
Colony B	Control	1	1	0	1	3
Colony C	Control	1	1	2	1	5
Total healthy exposed water		**3**	**4**	**3**	**4 (3)**	**14 (13)**
Colony A	SCTLD	6	6	6 (3)	6 (3)	24 (18)
Colony B	SCTLD	5	6	5 (3)	5 (3)	21 (17)
Colony C	SCTLD	5	5	6 (3)	5 (3)	21 (16)
Total SCTLD exposed water		**16**	**17**	**17 (9)**	**16 (9)**	**66 (51)**
TOTAL		**19**	**21**	**20 (12)**	**20 (12)**	**80 (64)**

Table 1. Summary of the experimental replicates per combined temperature, nutrients, and disease treatments. Numbers in parentheses reflect the final experimental numbers after one of the tanks set at 31 °C was removed from the experiment. Bottom rows show the replication for beakers that did not host experimental fragments. Total number of fragments per treatment are in bold.

Phase	STAR system	Mean dose duration (s beaker^{-1})	Mean round duration (min)	Doses day^{-1}	Vol water (mL day^{-1})	Full water changes day^{-1}	Mean NH4 added (µmol day^{-1})
1	A	38.7	31.0	46.5	2789.8	4.6	13.9
1	B	39.1	31.3	46.0	2761.3	4.6	13.8
2	A	61.4	49.2	29.3	1757.9	2.9	13.2
2	B	61.7	49.3	29.2	1751.1	2.9	13.1

Table 2. Statistics of the STAR systems while exposing the corals to environmental treatments only (Phase 1) and environmental and disease treatments (Phase 2). The STAR system relates to the two robot arms (A and B) and their associated dosing boxes.

We collected nutrient samples (30–40 mL) from all 80 beakers two to five times per week to monitor ammonium levels. We refrigerated the samples upon collection (-20 °C) and analyzed them within four days. Ammonium concentrations were measured using an AA3 Analytical Analyzer (Seal). The instrument was calibrated before each run following the standard procedures for the calibration[81].

Despite precise nutrient dosing, measured ammonium concentrations were variable in the experiment potentially due to (1) early nutrient uptake in the HN beakers, and (2) NH_4 spikes caused by tissue release associated with disease lesions (Fig. 6B). Before disease exposure, corals in the LN treatment had NH_4 concentrations below 0.5 µM (mean = 0.24 µM ± 0.40 *sd*). However, LN beakers experienced a spike in NH_4, after the introduction of the disease donors to the experiment (NH_4 = 2.44 µM ± 1.27) and a second spike after Day 44 when the fragments started showing tissue loss. In the HN treatments, target NH_4 concentrations were achieved on Day 1 of the experiment (4.29 µM ± 0.70), but quickly declined on days 2–3 and stayed low until day 14 (NH_4 < 0.4 µM), suggesting strong nutrient uptake (Fig. 6B). However, days 15 to 35 showed an increasing NH_4 trend in the HN treatments.

Disease treatments

We collected three disease donors (two *O. faveolata* colonies and one of *C. natans;* Fig. S3) on May 30, 2023, from Marker 48-6 off Marathon (24.68510, − 81.04293) and transported them to the Experimental Reef Lab. The colonies were maintained in the disease donor tank until disease inoculation started. On June 2, 2023 (Day 32 of the experiment; Fig. 6), we initiated exposure to disease-inoculated water for the majority of the corals (n = 66; Table 1). For these, we reprogrammed the STAR dosing settings to source seawater from the disease donor tank instead of the control tank. A small subset of corals per environmental treatment were maintained as controls (n = 14; Table 1). These continued to receive seawater sourced from the tank holding a healthy colony, thus ensuring the detection of possible tissue loss occurring for reasons other than exposure to the disease donor colonies (e.g., temperature stress, contamination among beakers, or external pathogens). The temperature and nutrient treatments were maintained during the disease exposure phase.

During the disease exposure phase, seawater pump speed was slowed down to 1.4 mL s^{-1} to reduce the risk of cross-contamination and splashing. This resulted in an average dose duration of ~ 61 s per beaker (Table 2). The duration of a robot arm round increased to ~ 49 min, resulting in ~ 2.9 full-volume seawater exchanges per day per beaker. To maintain a similar daily nutrient addition under slower dosing rates, the nutrient dose volume was increased from 0.5 to 0.75 mL, resulting in a total addition of ~ 13 µmol NH_4 per day in the HN beakers (Table 2).

Physiological responses to environmental treatments

The photochemical efficiency (F_v/F_m) and calcification rates of the fragments were measured on days 14 and 31 of the experiment (Fig. 6). These nonconsumptive metrics were used as a proxy for the effects of the environmental conditions on coral health. F_v/F_m was measured at ~ 6 pm local time using a Maxi Imaging-PAM fluorometer (I-PAM, Walz) after dark-adapting the corals for 30 min. Calcification rates were estimated using the buoyant weight technique[82]. Buoyant weight data were used to calculate dry skeletal weight following[83]. Calcification rates (mg g^{-1} d^{-1}) were calculated using the change in mass per day between measurement time points, normalized to the initial weight of the fragment for that interval[84]. F_v/F_m and calcification data were not collected after disease exposure in order to reduce the risks of cross contamination associated with the fragments handling.

Symbiodiniaceae community composition

On Day 14, we collected small samples (~ two polyps) from each experimental fragment and preserved in 1 mL of DNA/RNA shield (Zymo Research). We used ZymoBIOMICS DNA/RNA Miniprep Kits (Zymo Research) to extract total genomic DNA following manufacturer instructions. We analyzed the DNA samples with quantitative PCR (qPCR) using SYBR Green master mix assays on a StepOnePlus Real-Time PCR Instrument (Applied Biosystems). The qPCR assays targeted the actin gene in the algal symbiont genera *Symbiodinium*[85], *Breviolum*[46], and *Durusdinium*[86]; and a single copy gene in *O. faveolata* (SC_Ofav;[46]). We discarded the assay for the algal genus *Cladocopium*[86], another common algal symbiont in scleractinian corals, after not detecting this taxon in a subset of samples ($n = 22$) across colonies and treatments. We run the amplifications in duplicate reactions per target, including two negative controls (non-template reactions) per assay. Each reaction included 5 µL of SYBR Green master mix, 3.4 µL of molecular grade water, 0.3 µL of forward and reverse primers at 10uM concentration, and 1 µL of DNA template.

We used the qPCR cycle thresholds (CT) associated with a ΔRn threshold set to 0.2 to calculate genus-specific symbiont-to-host cell ratios (e.g., *Symbiodinium*/Host [A/H], *Breviolum* to Host [B/H], and *Durusdinium* to Host [D/H])[41,86] using the *StepOneR* repository for R[87]. This package computes the genus-specific ratios with the formula $2^{\text{CT host} - \text{CT symbiont}}$ using the averaged CTs among the two technical replicates per coral fragment and qPCR target. The values are corrected for target ploidy (coral host = 2, Symbiodiniaceae = 1), DNA extraction efficiency (coral host = 0.982 and Symbiodiniaceae = 0.813;[86]), and actin copy number (*Symbiodinium* = 1, *Breviolum* = *1,* and *Durusdinium* = 3;[46]). We applied quality filters to discard data from amplifications in which one of the two technical replicates did not amplify, and samples in which the CT standard deviation between technical replicates exceeded 1.5. The total S/H cell ratio was calculated as the sum of all genus ratios (S/H = A/H + B/H + D/H). The percentage of an algal genus in each sample was calculated by dividing the genus host cell ratio by the total S/H cell ratio [e.g., *Durusdinium* proportion = (D/H)/(S/H)*100].

Due to logistical constraints we were unable to collect additional samples at Day 31, therefore Symbiodiniaceae abundance and community composition may have changed during the period after sampling and before disease exposure (Days 15–31) and thus the data on the symbiont community should be interpreted with this caveat.

Disease transmission

We collected daily observations and top-down photos during the disease exposure phase (Days 32–51; Fig. 6) to identify the onset and track the progression of disease lesions. Observations and treatments stopped on Day 51 after three consecutive days with no new lesions. Disease incidence was calculated for the corals exposed to SCTLD in each environmental treatment (ammonium and temperature) using the Kaplan–Meier estimate[88] with the R packages *survival* 2.38[89] and *survminer* 0.4.6[90]. The Kaplan–Meier estimate calculates the probability of a fragment getting diseased in a given environmental treatment based on the number of fragments observed to develop an active lesion on a given day and the total number of fragments available in that treatment.

Statistical analyses

We assessed the statistical differences in the response parameters (F_v/F_m and calcification rates, total symbiont abundance (S/H), and relative percentages of each algae genus) among the environmental treatments using linear mixed models (LMM) that included nutrient, and temperature treatment as fixed effects, and coral colony as a random effect. For F_v/F_m and calcification rates, we also included time points as a fixed factor to compare the values measured on days 14 and 31 of the experiment. Time point was treated as a fixed factor to compare coral responses at two discrete sampling times under the environmental treatments. This approach allowed us to assess both the main effects and interactions of environmental factors across time points. We ran all LMMs with the *lme4* package v1.1–31[91] for R v3.4.3[92] and Tukey's HSD pairwise comparisons for significant factors with *emmeans* v1.1.3[93] with an alpha value of 0.05. S/H ratios were $\log_{10}$ transformed before running the model to reduce the skewness of the data.

We compared the probabilities of disease transmission among treatments using log-rank tests. Additionally, we used a Cox proportional model to estimate the relative hazard ratio (HR) per treatment, with the "hazard" being developing a disease lesion. The HR is calculated as the ratio of (chance of a lesion occurring in a treatment)/(chance of a lesion occurring in the treatment of reference). In this study, it represented how many times the risk of developing a lesion increases or decreases in the 28 °C treatment compared to 31 °C.

Data availability

All data needed to evaluate the conclusions in the paper are present in the paper, the Supplementary Materials, and in the Zenodo repository https://doi.org/10.5281/zenodo.12730208.[94].

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Author contributions

AMPC, MS, and IE designed the experiments. AMPC, NS, KC, and ZZ performed the research and data collection. ZZ and TG processed and analyzed the symbiont community samples. TC and CK processed and analyzed the nutrient samples. AMPC conducted the statistical analyses and wrote the first version of the manuscript. All authors contributed to the final article and approved the submitted version.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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