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Coral restoration in a stressful environment: Disease, bleaching, and dysbiosis in *Acropora aspera* in Guam, Micronesia

Highlights

- Infectious disease is a challenge to conservation of highly clonal coral species
- Restoration must consider microbiome dynamics to reduce stress in transplanted corals
- Coral exposure to intermediate conditions may reduce stress at the recipient site
- Restoration success relies on site selection and water quality management

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In brief

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Coral restoration in a stressful environment: Disease, bleaching, and dysbiosis in *Acropora aspera* in Guam, Micronesia

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SUMMARY

Despite advances in coral restoration science, challenges imposed by rapid environmental change impede progress. Here, we report mortality from disease and bleaching in an introduced nursery-reared population of the staghorn coral *Acropora aspera,* in Guam, Micronesia. We present disease progression, incidence, synergies between stressors, and response of the coral microbiome. Microbiome composition in nursery vs. outplanted corals indicated dysbiosis induced by the transition to poorer water quality. However, among outplants, there were no differences between diseased tissues, visually healthy tissues on the same infected colony and tissues from non-infected colonies, suggesting that outplanting into a stressful environment may have compromised coral immune response, increasing susceptibility to disease and bleaching. Our study highlights that outplanting is inherently physically stressful, thus underscoring the need for understanding the microbiome's role in the coral transplantation stress response. We suggest workflows to minimize stress and improve restoration in the face of environmental challenges.

INTRODUCTION

As coral reef decline continues worldwide, coral restoration must develop rapidly as a science to provide substantial benefits as a conservation and management option.[1] While significant progress has been made to develop and test methods,[2–4] define and quantify "success",[5] incorporate genetic diversity as a success metric,[6] and facilitate climate resilience,[7,8] the shifting baseline caused by rapid environmental change continues to challenge restoration efforts. The increasing intensification of tropical storms,[9] warming waters, and disease prevalence[10] has made these shifting baselines more evident in conservation efforts and continues to undermine progress with restoration. The process of restoring coral can expose colonies to multiple shifts in their environmental contexts, particularly if a nursery phase is included. One of the most targeted genera for restoration, *Acropora* spp., are broadcast spawners that must acquire their endosymbionts (e.g., *Symbiodiniaceae* algae and bacteria) horizontally at a very early juvenile stage from their surrounding environment.[11] While this attribute generally confers high symbiotic flexibility in responding to environmental change,[12,13] these generalist corals are stress sensitive and often described as ecological 'losers'.[14,15] A symbiotic flexibility can also compound stress in outplanted or source colonies if the recipient site is characterized by environmental conditions that are an extreme contrast to their previous site or beyond eco-physiological limits.[16] Impacts of stress exposure on the coral holobiont include heightened susceptibility to infections[17,18] or bleaching,[19] which can also interact synergistically to increase mortality.[20] An effective management strategy to reduce the risk of infectious disease in coral restoration requires a multi-faceted approach to understanding how stress manifests from outplanting through the post-outplant acclimation period[21,22] and developing protocols to reduce the risk of disease exposure and spread among outplants.

Unlike bleaching, infectious diseases are a direct cause of the disruption of the integrity of a coral holobiont's microbiome, i.e., microbiome dysbiosis as a result of an external influence.[22,23] Disease outbreaks in key community structurers have increased in severity and frequency, e.g., among corals (stony coral tissue loss disease[24,25]; sea stars (sea star wasting disease[26]) and seagrasses (seagrass wasting disease[23]), a trend often linked with accelerated environmental deterioration.[27–31] This association highlights the urgent need for improved disease management and risk reduction in conservation and restoration. Climate change impacts to nearshore communities are often exacerbated by local stressors and it is often beyond the capacity of restoration efforts to address these issues, as they require considerable political collaboration and coordination.[32] Thus, managing disease and stress in a restoration project is most likely to be accomplished by an increased understanding of species-specific resilience and trade-offs to known stressors, and close matching of species requirements with outplant site characteristics.

In Guam, White syndromes (WS) are the most prevalent diseases impacting corals[33] and is a term applied to a group of acute tissue loss diseases with a broad host range. Causal agents have not been identified to date,[34,35] though bacteria attributed to *Vibrio* spp. have been implicated in certain outbreaks.[36,37] The onset, pattern, and rate of tissue loss may vary widely among host coral taxa and the diseases may manifest as acute outbreaks or chronic infections. Among Guam staghorn *Acropora*, WS prevalence is generally low though it often appears as an acute outbreak, during or after summer bleaching events.[38] Lesion onset begins at the base of a branch, progressing upward at a mean rate of 15 mm/week, and circumscribing a branch (Raymundo, unpublished data). Total colony mortality can result, which can have a greater impact on highly clonal thicket-forming species such as staghorns.

Coral restoration in Guam was initiated in 2015 in response to repeated mortality events beginning in 2013. From 2013 to 2017, multiple bleaching events, extreme low tide exposures, and disease outbreaks resulted in island-wide coral mortality and decline.[39,40] Among the worst-impacted taxa were staghorn *Acropora*, which suffered an estimated 59% decline between 2013 and 2021.[40,41] Limited to the shallow reef flats in Guam, these communities exist near their upper thermal limits and are known to have low resistance to bleaching and high susceptibility to tissue loss diseases.[42–44] This sensitivity to environmental stressors is also reflected in their life history strategies; as 'r-selected' species, they much energy in fast, expansive growth and high reproductivity capacity.[45,46] At present, five of the eight described morphospecies (i.e., species identified by morphological characteristics) from Guam are limited to a single wild population and are thus at a high risk of local extinction,[41] making them a critical focus of Guam's initial restoration efforts.[42] All eight species are currently grown in two nurseries: Piti Bomb Holes Marine Preserve (13°28′16.33″N, 144°42′06.38″E), and Cocos Lagoon (13°15′29.77″N, 144°39′39.27″E) (Figure 1A).[41] Each nursery consists of 12 midwater PVC "trees", housing 144 fragments suspended on monofilament which are regularly pruned and outplanted into selected sites supporting existing staghorn populations.

Here, we report a mortality event within an outplanted population of the staghorn coral *Acropora aspera*. Recent bleaching events have reduced *A. aspera* to a single remaining wild population, comprising a 4-ha thicket on the Achang Reef Flat (Figure 1B) with an estimated mean live coral cover of 38.6%.[41] Fragments from this population were collected and established in two ocean nurseries in 2017, and outplanting was initiated in 2019 to re-establish populations near the nursery sites (Figure 1A).[41] A third site, Tumon Bay Marine Preserve, was selected for outplanting as it is an iconic site supporting an extensive population of a congener, *Acropora pulchra.* In February 2022, nursery-grown fragments were introduced into four plots within Tumon Bay (Figure 1C). Outplants showed 100% survival, positive growth, and absence of disease or bleaching for the first three months. However, during the fourth month post-outplant (May), a disease and bleaching event developed coinciding with an algal bloom and seasonal summer elevated sea surface temperatures. As the entire population of outplants was mapped and georeferenced, it provided a unique opportunity to investigate the dynamic nature of this event quantitatively, look for evidence of resistance to disease or bleaching among individual fragments, and document disease incidence and potential recovery within our outplants. This paper reports our investigative process to document these stress impacts on our restoration effort and discusses the implications of infectious disease and stress events on restoration success.

RESULTS

Event timeline, environmental stressors, and disease characterization

We present a timeline of events leading to complete mortality of our outplants over a 3.5-month period in Figures 2 and 3. Outplanting occurred during the cool, dry season (mean temperature: 28.5°C); May marked the beginning of the hot summer season. We recorded significant variation in mean monthly temperatures across this period (ANOVA: $F = 0.495$; $p < 0.001$), notably temperatures were above 32°C May through July, corresponding to afternoon extreme low tides (Figures 2 and S1). *Enterococcus* concentrations spiked twice during this period, reaching colony counts of up to 600/100mL, indicating high nutrient levels and sewage influence (Figure 2A). Extreme negative tides occurred for one week each month during afternoon heat stress periods, resulting in stagnant water, but fragments remained submerged and were not exposed to air (Figure 2B). We observed algal blooms, characterized by greatly reduced visibility, pea-green water color, smell of decomposition, and skin irritation on 5/25 and 7/6; the second event was reported by Guam Environmental Protection Agency and the local press on 7/12 as a red tide event (https://www.guampdn.com/eedition/page-a1/page_3da89a69-5a08-502e-a8d0407c01aa52d7.html).

Lesions were first noted on 33 of the 162 fragments, translating to 19.4% ± 6.4% (mean ± SD) prevalence among outplants, and representing a 9-fold increase over that reported in initial surveys to characterize baseline disease prevalence on Guam (2%.[33]) Lesions appeared as white, exposed skeleton initiating at the base of a colony, progressing upward, and circumscribing the branch; characteristics of previously described *Acropora* white syndrome[38] (Figure 3A). The absence of recruiting turf algae onto exposed coral skeleton indicated tissue loss was rapid and had initiated within the previous three to four days. As there were no observations of predators such as *Drupella* spp. or Crown-of-Thorns seastars (*Acanthaster* cf. *solaris*) within our study area, we concluded that this constituted a disease outbreak. Tagged lesions exhibited a mean progression rate of 1.9 ± 1.6 mm/day but showed a pattern of initially high tissue loss rate, followed by a rapid slowing or cessation in progression; a pattern observed in previous outbreaks in wild populations (Raymundo long-term monitoring program, unpublished data).

We observed a steady increase in stress severity throughout the event, initiating with disease lesions by 5/25 (stress severity mean ± SD: 1.2 ± 0.9) and compounded by the onset of bleaching by 6/8 (stress severity mean ± SD: 2.0 ± 0.8), progressing to severe bleaching by 7/1 (stress severity mean ± SD: 4.5 ± 0.9) (Figures 3A and 4A). New infections also

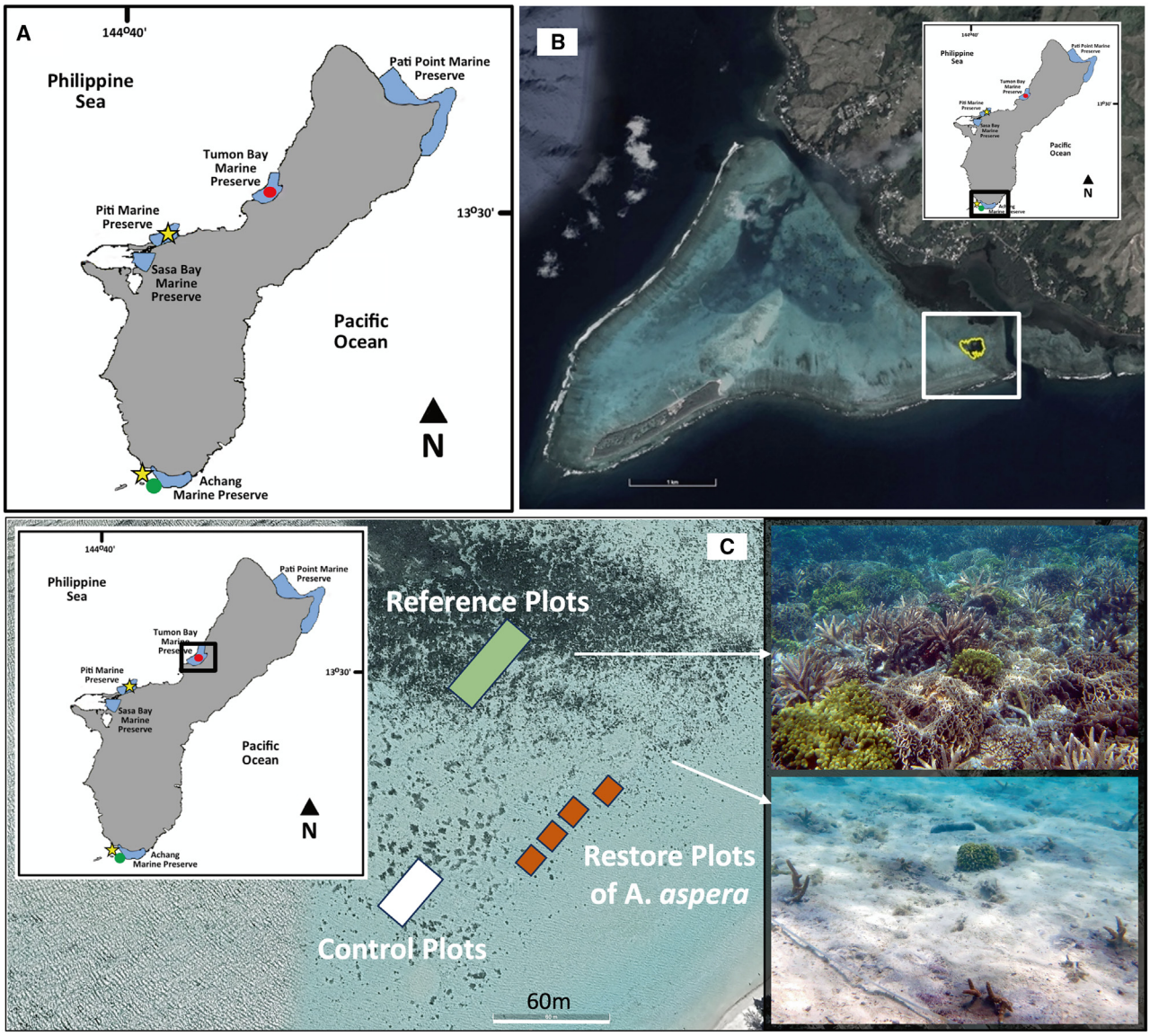

Figure 1. Map of the study area

(A) Map of Guam highlighting Guam's Marine Preserves (blue areas), locations of coral nursery sites (yellow stars), outplant site (red dot), and the wild population (green dot); (B) location of the remaining wild population of *Acropora aspera;* and (C) the outplant site within Tumon Bay Marine Preserve. Control plots refer to unrestored substrate; Reference plots refer to high coral cover areas. Photo credit: D. Burdick and M. Andersen.

declined following a timing similar to that of lesion progression rate but accelerated soon after, possibly exacerbated by bleaching stress (Figures 3B and 3C). This pattern was consistent with our calculations of WS resistance probability, which declined to 65% (±5%) by 7/1, dropping to 46% (±6%) six days later (7/07), and declined to 10% (±8%) thirteen days later (7/20) (Figure 4B). Exacerbated by increasing bleaching severity and an increase in water bacterial load (Figure 2B), the overall outplant corals had a survival probability of 77% (±6%) by 7/20 but 0% (±0%) by 8/5 (Figure 4C). During this period, water temperature continued to rise (Figure S1), a second algal bloom occurred (Figures 2A and 3), negative afternoon tides resulted in reduced water flow and flushing, disease incidence spiked, and overall mortality rose sharply by 7/7 (Figures 3B and 3C). By 8/5, all fragments showed complete mortality.

Symbiotic communities associated with healthy and diseased *A. aspera* coral tissue

Associated symbiotic community composition

A rapid screening of *Symbiodiniaceae* communities via a random assessment of ITS2 sequences in some WS-infected colonies (visually healthy regions vs. diseased regions) vs. apparently not-infected control colonies (Table 1) showed that

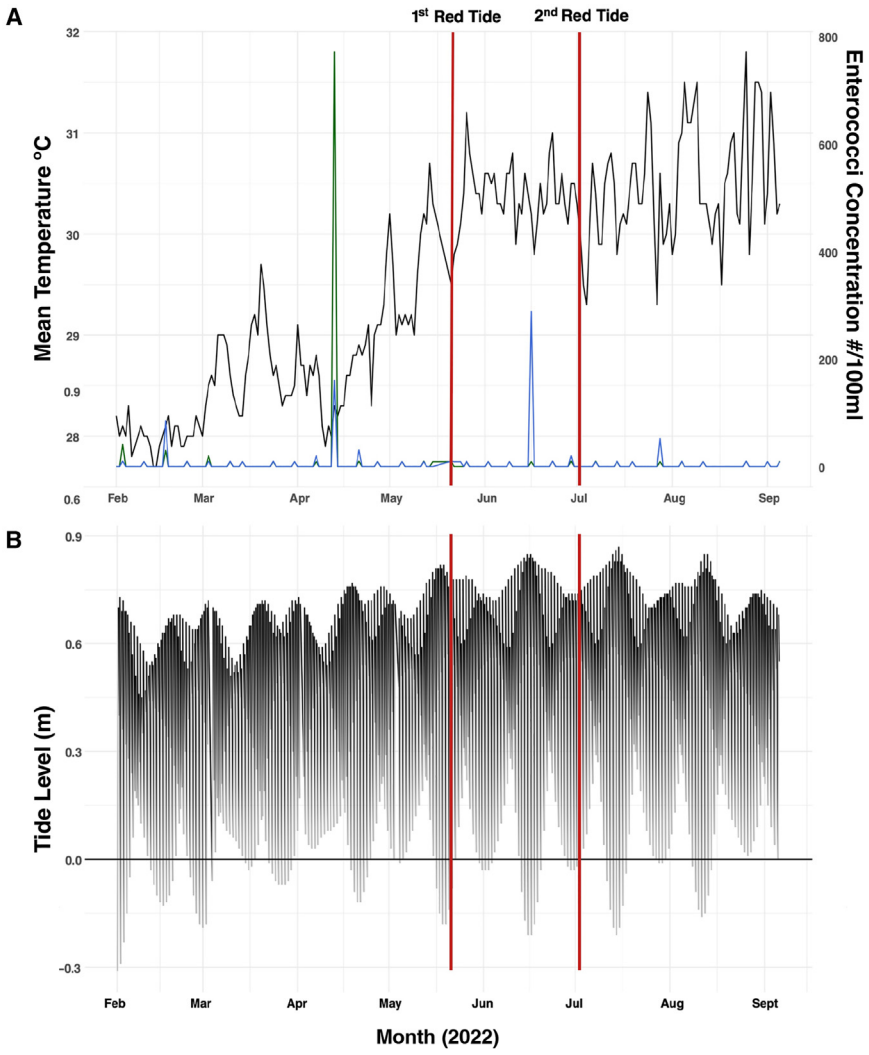

Figure 2. Environmental parameters during the outbreak

Temperature, *Enterococcus* concentrations, and tidal level variation in Tumon Bay, Guam spanning the outplanting and monitoring period (Feb. 2022 - Sept. 2022).

(A) Daily mean temperature (black line) vs. *Enterococcus* concentrations at two sites along the shoreline of the Bay (Outplant site at Matapang Beach: Blue line; 800m south at Ypao Beach: green line (https://www.epa.gov/waterdata/water-quality-data).

(B) Tide data recorded by NOAA (https://tidesandcurrents.noaa.gov) highlighting the tidal variability throughout this study period. Red lines indicate timing of algal blooms corresponding to reported red tide events.

A. aspera algal symbionts were composed of *Cladocopium* under two distinct ITS2 profiles belonging to C40 radiation, regardless of disease state (Figure S2). Bacterial communities were dominated by Proteobacteria (~60%), Bacteroidota (~20–35%), and to a lesser extent, Cyanobacteria (<10%) and Firmicutes (<5%) (Figure 5B). There were no significant differences in bacterial alpha diversity between healthy [VH and control] and diseased [WS] outplants from May and nursery corals (ANOVAs and pairwise comparisons among the five groups 'HealthTime' shown in Figure 5C: df = 4, $p > 0.05$) for Observed, Shannon, and InvSimpson indices. However, the coral outplant bacterial alpha diversity from June was significantly higher than that from May and those from nursery corals for the Shannon index (ANOVA: df = 4, F = 3.025; $p = 0.045$) but not for the observed or InvSimpson indices (Figure 5C). However, the beta diversity of microbiomes (Figure 5D) significantly differed between outplants and nursery fragments (PERMANOVA: df = 1, F = 2.99, $p < 0.001$) as well as between sample periods on outplants corals (PERMANOVA: df = 1, F = 9.33, $p = 0.001$), possibly influenced by the outplanting process, followed by the onset of the WS outbreak (May) and subsequent bleaching (June). However, bacterial community compositions associated with outplanted corals did not differ between tissues from visually healthy regions [VH on WS-infected corals and on non-visually WS-infected control corals] and diseased regions [WS] at any of the sampling months (Figure 5D; PERMANOVA: F = 0.55; $p = 0.8$). June samples (stressed by both bleaching and WS) were characterized by a more dispersed and distinct community compared to May samples (WS initiation) which were characterized by a more homogeneous community (pairwise tests: F = 5.87, adjusted $p = 0.003$). We observed a more homogeneous community among nursery fragments, but the communities were significantly distinct from both outplanting time periods and health states (pairwise comparison with: May: F = 4.81 and adjusted $p = 0.027$ or June F = 2.6 and adjusted $p = 0.006$).

Fine-scale variations of A. aspera *bacterial communities*

Bacterial community dissimilarity between May and June (SIMPER analyses) was significantly driven by the absence of ASV2-*Flavobacteriales* (*Cryomorphaceae* family) and ASV1-*Pseudomonales* (*Litoricolaceae* family) in June samples, with dissimilarity contributions among populations of ~12–13% (Figure 6A, Tables S1 and S2). With a smaller contribution (2.2–3.5%) to the temporal dissimilarity between bacterial communities, a drop of ASV5-*Rhodobacterales* (*Rhodobacteraceae* family) and ASV8-*Flavobacteriales* (*Flavobacteriaceae* family) in June was also noted (Figure 5A and Table 1). In the same way, with contributions <1%, a decrease of some Proteobacteria (Gamma: ASV20 and ASV26 and Alpha: ASV12) and *Desulfobacterota* (ASV16; *Desulfovibrionaceae* family) between May and June significantly impacted the dissimilarity (Table S2). Conversely in June, while no specific bacterial strains contributed to the

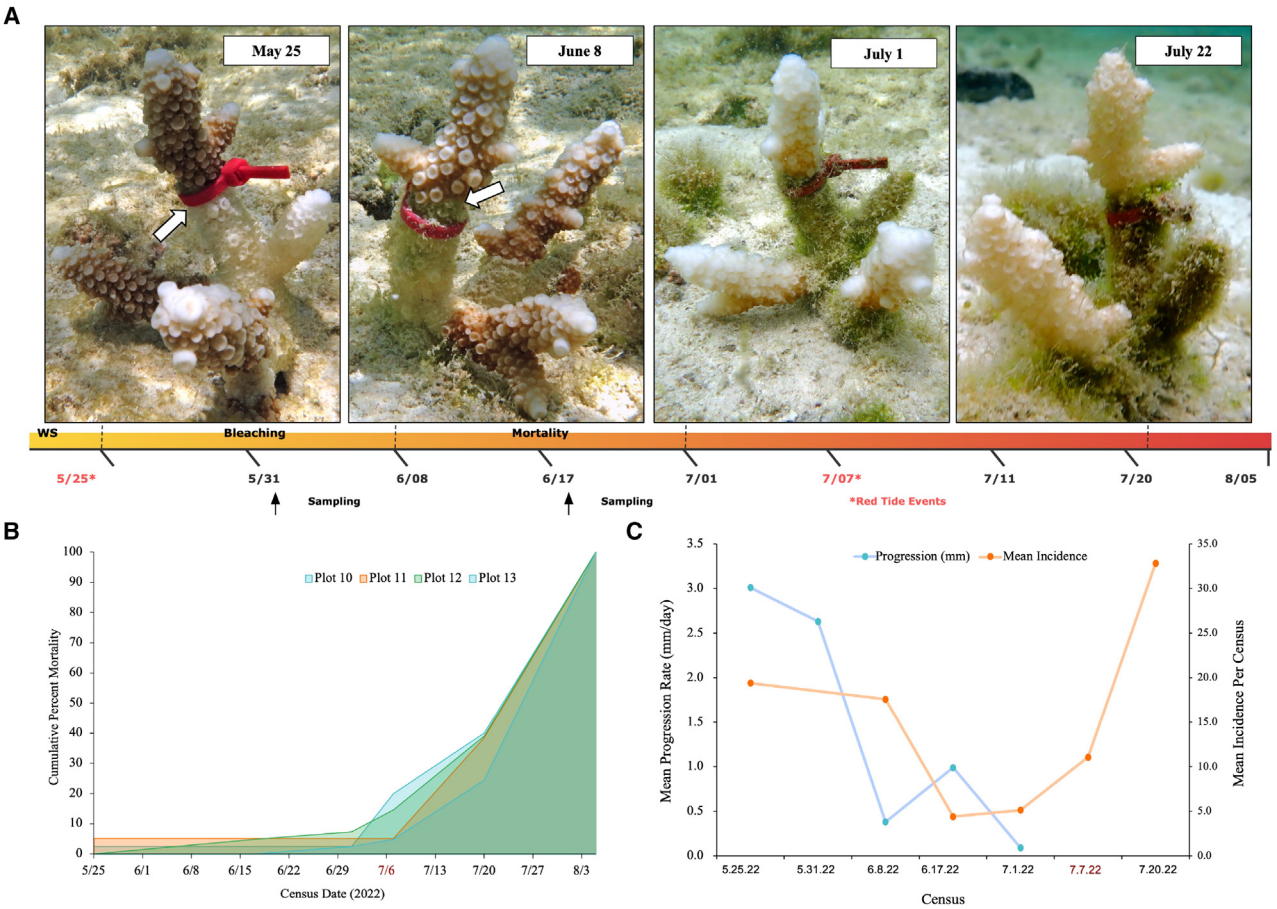

Figure 3. Temporal dynamics of disease and bleaching events

(A) Temporal dynamics of disease and bleaching in Colony 38, Restore Plot 11, showing census dates and timing of red tides; (B) Cumulative mortality over time, showing an exponential increase from compounded disease and bleaching; (C) Comparative tissue loss progression rate vs. disease incidence across the census period. Photo credit: L. Raymundo.

overall structure of bacterial communities, several occasional records of new bacteria strains belonging to different functional groups were observed (Figures 5A and 6): e.g., *Comamonadaceae* (ASV20), *Vibrionaceae* (e.g., ASV4 and ASV9), *Pseudomonadaceae* (e.g., ASV6 and ASV8) or *Rhodobacteraceae* (e.g., ASV13). When comparing communities from outplanted corals with those from nursery corals, we observed a bacterial community dissimilarity significantly driven by several bacterial groups (Figure 5B) that contributed from 4.3% to 0.2% (Table S2), such as: Proteobacteria-*Endozoicomonadaceae* (e.g., ASV27, ASV88), Cyanobacteria (ASV14, ASV22) *or Myxococcota-Myxococcales* (ASV21).

DISCUSSION

In this case study, we report restoration challenges encountered in our first attempt to introduce a key habitat structuring staghorn species threatened with local extinction in Guam, *A. aspera*, into a marine protected area that currently supports extensive stands of a closely related staghorn species, *A. pulchra*. Initial responses of *A. aspera* to this site were favorable, suggesting that reintroduction might be successful. However, three months post-outplant, our fragments developed rapid tissue loss lesions characteristic of *Acropora* white syndrome (WS) simultaneously with an algal bloom that impacted water quality. As a rapid response to investigate the trajectory and potential etiology of the WS disease outbreak, we initiated an investigation that included both ecological and microbial sampling over time. Subsequent to the onset of the disease outbreak, we observed bleaching that progressed from slight paling to severe bleaching within two weeks. The timing of bleaching and its absence in *A. pulchra* stands on the same reef (noted during surveillance snorkels around our study area) suggested that bleaching may have been exacerbated by the presence of disease; a response documented by Muller et al.[20] in a close Western Atlantic relative, *Acropora cervicornis*. This series of physiological disturbances resulted in complete mortality of our reintroduced colonies. We posit that this devastating result was triggered by compounded environmental stressors that included summer extreme low tides and warming temperatures, exacerbated by algal blooms and high bacterial loads. While the recipient site, Tumon Bay, supports a healthy and vital reef community, we

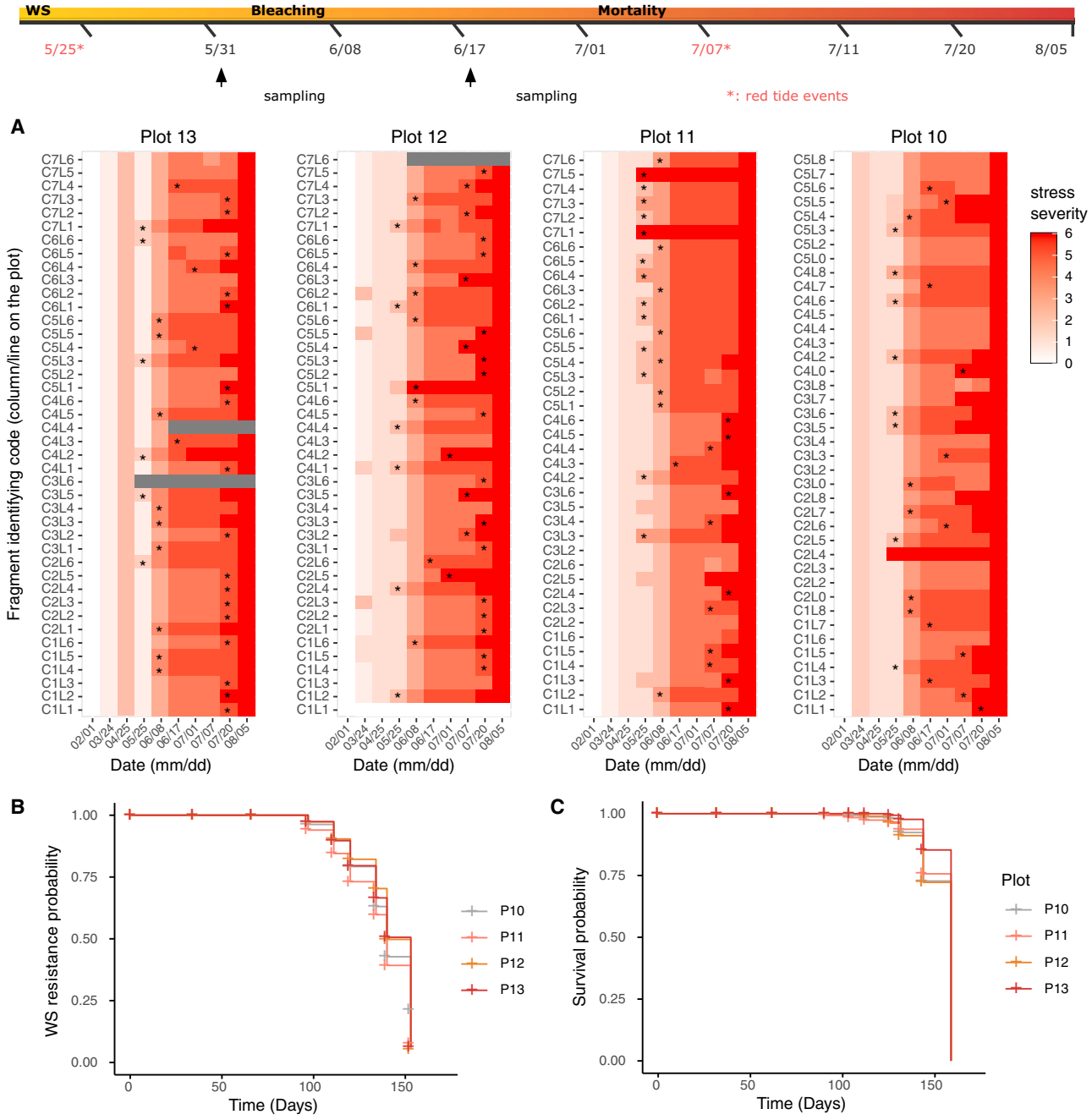

Figure 4. Ecological temporal monitoring of outplanted *A. aspera*

(A) Heatmap expressing stress severity (0 = healthy; 1–5 = diseased and/or bleached; and 6 = dead) experienced by outplants (one row = one colony) over time, calculated per plot. (*) indicates appearance of new WS lesion. Gray bars indicate colonies lost during the monitoring.

(B) Probability of WS resistance; and (C) probability of survival of outplants across the four plots over time.

speculate that the poor performance of our outplants was significantly impacted by the instability and shifts in their microbiome that may have compromised their immune function. Our results suggest this instability may have initiated in the coral nursery phase (the source of our outplants) and offer a potential explanation for observed 'transplantation stress'.[18] This highlights the importance of recipient site acclimation as a key event in restoration success and considers the role of the microbiome in this acclimation process.

While it is reasonable to assume that organisms are not equally resistant to all potential stressors, growing evidence suggests that multiple stressors acting simultaneously can have synergistic effects on organisms, leading to much greater impacts to health. Knowlton et al.[42] noted a prolonged die-off of staghorn

Table 1. Sampling for microbiome analyses table

Conditions	Sampling time	Healthy (Control)	Healthy on WS infected corals (VH)	Diseased (WS)	Colony	Plot	ITS2
Outplanted	May-22		1	1	C6L1	12	x
			1	1	C3L4	13	x
			1	1	C5L3	13	x
			1	1	C6L5	13	x
Outplanted	Jun-22		1	1	C6L4	11	x
		1			C3L4*	12	x
			1	1	C6L4	12	NA
			1	1	C3L3	13	x
		1			C5L2*	13	NA
			1	1	C5L3	13	x
Nursery	Jun-23	5					

Acropora after a devastating hurricane, postulating that corallivore populations spiked after the storm, causing persistent mortality among corals. In our surveys to document surviving populations after repeated bleaching events, we noted that at least two populations surveyed during the bleaching year of 2017 had completely died out by 2020 despite the absence of bleaching after 2017.[41] We speculate that disease events may have wiped out these remaining populations after they had survived multiple bleaching years. Muller et al.[20] suggested a potential mechanism for this observation: normally white band disease (WBD)-resistant *A. cervicornis* outplants became more susceptible to WBD after they had bleached. However, a trade-off between bleaching vs. disease resistance was not demonstrated, illustrating that the severe disease impact observed was not *Symbiodiniaceae*-related. Bleaching and microbial diseases can thus work synergistically; stress caused by one can decrease the host coral's capacity to resist the other when subsequently exposed.[47,48]

As a complex meta-organism, the coral holobiont's response to environmental stress is controlled by a tight association between the host and its mutualistic assemblage of diverse functional groups of microorganisms. The total mortality we observed among our 162 outplants suggests a lack of stress-resistant genotypes in the hologenome of our outplanted population, though our initial objective at the onset of the outbreak was to track all colonies for potential differential resistance. A recent population genetic analysis of the remaining *A. aspera* wild population (our original fragment source) indicated high clonality (Combosch and Torrado, unpublished data), thus precluding the likelihood of genetically-based differential stress resistance within the population. The lack of genetic diversity is well-recognized as a factor promoting disease spread in agriculture[49,50] but has also recently been addressed in the coral restoration literature.[51] Brown et al.[52] report one of the first studies testing the role of genetic diversity in population-level disease resistance in a closely related species, *A. cervicornis;* higher genetic diversity increased disease resistance among nursery-reared colonies. This poses a challenge to the conservation of *A. aspera* in Guam: what is the best restoration approach with a species reduced to a single highly clonal population? Assisted sexual propagation not being an option, our current strategy is to plant populations in multiple sites with differing environmental attributes to determine optimal site preferences, and we are also exploring establishing multi-species plots; i.e., polyculture.

The *Symbiodiniaceae* algal community is established as an essential part of the coral holobiont resistance and innate immune system.[53–55] We did not observe a difference in algal community composition between diseased vs. visually healthy tissue parts of the corals vs. visually healthy corals. It was represented in all samples by two closed ITS2 profiles belonging to genus *Cladocopium* C40 radiation. This high fidelity with *Cladocopium* and the absence of the stress-tolerant genus *Durusdinium* has been observed for several congeneric staghorn species (e.g., *A. pulchra, A. virgata*) in different reefs along Guam's coastline[56] (Andersen et al., unpublished data; Rouzé et al., unpublished data), which may help to explain the high predisposition for disease, low tolerance to bleaching, and lack of differential responses to these two stressors within the population.[37,53] However, the evidence of an association between *Symbiodiniaceae* species identity and coral host disease susceptibility is still not well studied and requires further investigation.[20,57,58]

Similarly, the composition of bacterial communities between apparently healthy and diseased tissues of outplanted corals did not present evidence for: (1) distinct microbiomes as previously reported for other *Acropora* species[22,59–62] or (2) specific pathogens (often belonging to the genus *Vibrio*)[36,37,63,64] as causative agents of the WS outbreak. What we did observe was a clear shift to dysbiosis in the bacterial community structure between May (initiation of WS), and June (WS plus bleaching) in both visually disease-free (though bleaching) and diseased tissues. This supports the findings of Pantos and Bythell[59] of a colony-wide impact of disease in White Band Disease-infected *A. palmata;* bacterial communities differed distinctly between healthy tissues on diseased colonies vs. those from healthy colonies physically distant from diseased colonies. The high clonality and small population size of our outplants may have further influenced the lack of distinction between healthy and diseased microbiomes from within this population. The loss of dominant bacteria from Pseudomonales (ASV1-*Litoricolaceae*) and Flavobacteriales (ASV2-*Cryomorphaceae*, ASV8-*Flavobacteriaceae*) in June communities

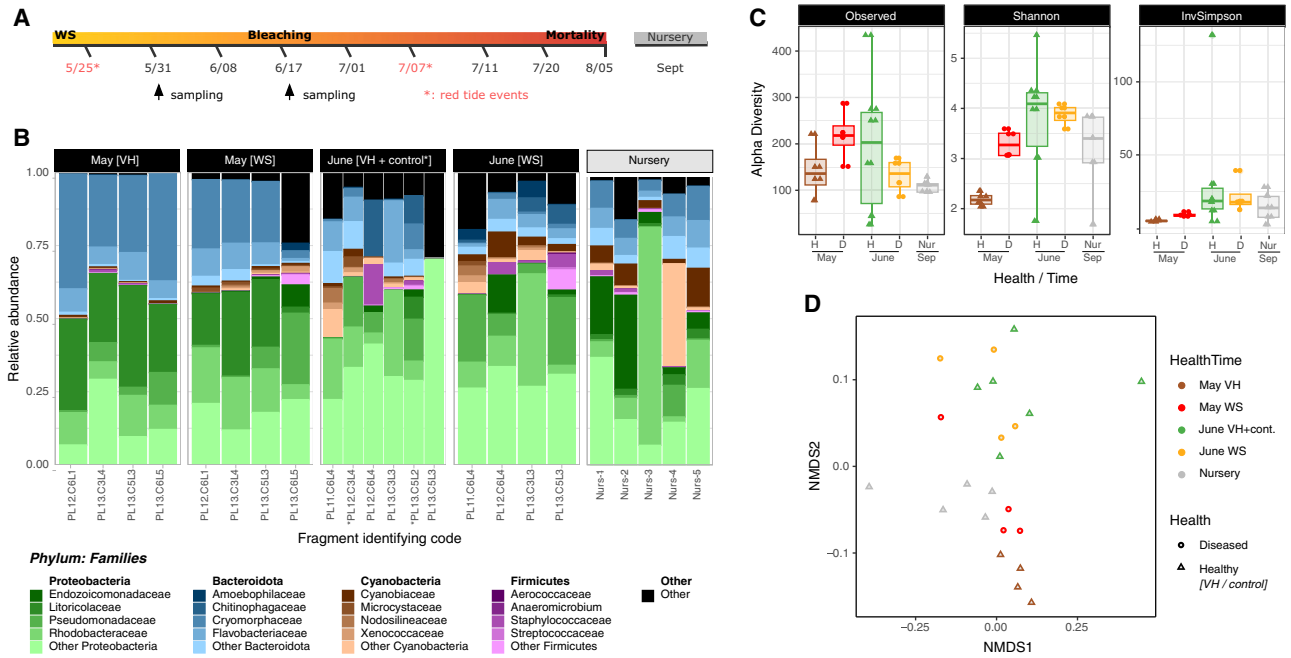

Figure 5. Bacterial community composition of outplanted *A. aspera*

Diversity of bacterial communities in healthy [VH on WS-infected colonies and control on apparently non WS-infected colonies] vs. diseased tissue samples on the same WS-infected colonies [WS] sampled in May and June, and in healthy corals from the nursery [Nur.] as a reference for the pre-outplanted community (timeline detailed in A) (B) Relative proportion of top 4 phyla/families; (C) Alpha diversity with indices of Shannon and InvSimpson; box plots indicate min and max values; median value, and upper and lower quartile values; and (D) Beta diversity NMDS of relative abundances constructed using UniFrac weight distances. For outplanted corals: Healthy include VH = visually healthy tissue on WS-infected colonies + control = healthy tissue on non WS-infected colonies (n = 2 in June 2022); D = tissue on diseased region on WS-infected colonies; "05" = May samples; "06" = June samples. For Nursery corals: reference of source colonies pre-outplanting.

suggest our corals were experiencing two consecutive events of stress (disease onset and bleaching onset), as both groups of bacteria have been previously described with particular role in coral health: Pseudomonales belong to a dominant group present in healthy but not bleached corals,[65,66] while Flavobacteriales have been reported to be involved in other coral diseases such as WBD.[67] This loss most likely promoted a new ecological niche favorable for opportunistic bacteria, explaining the increase in community diversity characteristic of diseased states.[17,35,36,68] Interestingly, Desulfovibrionales, a group of bacteria implicated in Black Band Disease pathogenesis, through the production and accumulation of sulfide[69,70] was also detected at low relative abundance in May when the WS outbreak started. These results support the hypothesis of the complex and poorly understood etiology of White Syndromes, influenced by abiotic stressors.[71] We conclude that the mass mortality event we documented was the consequence of a stress phase from outplanting, and synergisms between multiple stressors: anthropogenically high nutrient, bacterial loads within Tumon Bay,[28,72] repeated algal blooms, and seasonal sea surface warming. These acted in concert to trigger dysbiosis, increasing the susceptibility to opportunistic bacterial invasion. As the summer progressed, we observed other WS hotspots within the Bay affecting closely-related *A. pulchra* colonies, suggesting that the prolonged period of multiple stressors was impacting resident communities as well and that a waterborne pathogen may be involved.

While we have no pre-outbreak microbiome samples from the original outplanted corals, distinct community compositions were detected between nursery corals and outplanted corals (Figure 5B). Interestingly, even with a limited sample size (n = 5), the bacterial communities of nursery corals showed a high alpha diversity and heterogeneity and were not characterized by a strong core microbiome signature similar to those detected in wild populations for other congeneric staghorn *Acropora* species. Within conspecific (*A. aspera*: Anthony et al., unpublished data) and congeneric healthy wild populations investigated in their natural reef conditions, *Endozoicomonas* is a dominant component, consistent with what has been found in other species (*A. pulchra*[73]; *A. cf. muricata*: Andersen et al., unpublished data); in *A. aspera* outplants, we found this in very low relative abundances (~4%; Figure 5B). Our understanding of the role of this dominant genus in coral health and nutrition regulation is growing[74–76] and there is evidence that this genus codiversifies with its hosts.[77] While *Endozoicomonas* are characterized by intraspecific variability in their capacity to respond to changes in local conditions,[76,78] they often exhibit dramatic reductions in relative abundance in stressed, bleached or diseased corals.[79,80] We hypothesize that transfer of *A. aspera* fragments from the wild population to the coral nursery may have triggered an as-of-yet undescribed shift in their microbiome. This could have been caused by the change from a close association with the limestone substrate to the coral nursery trees where they

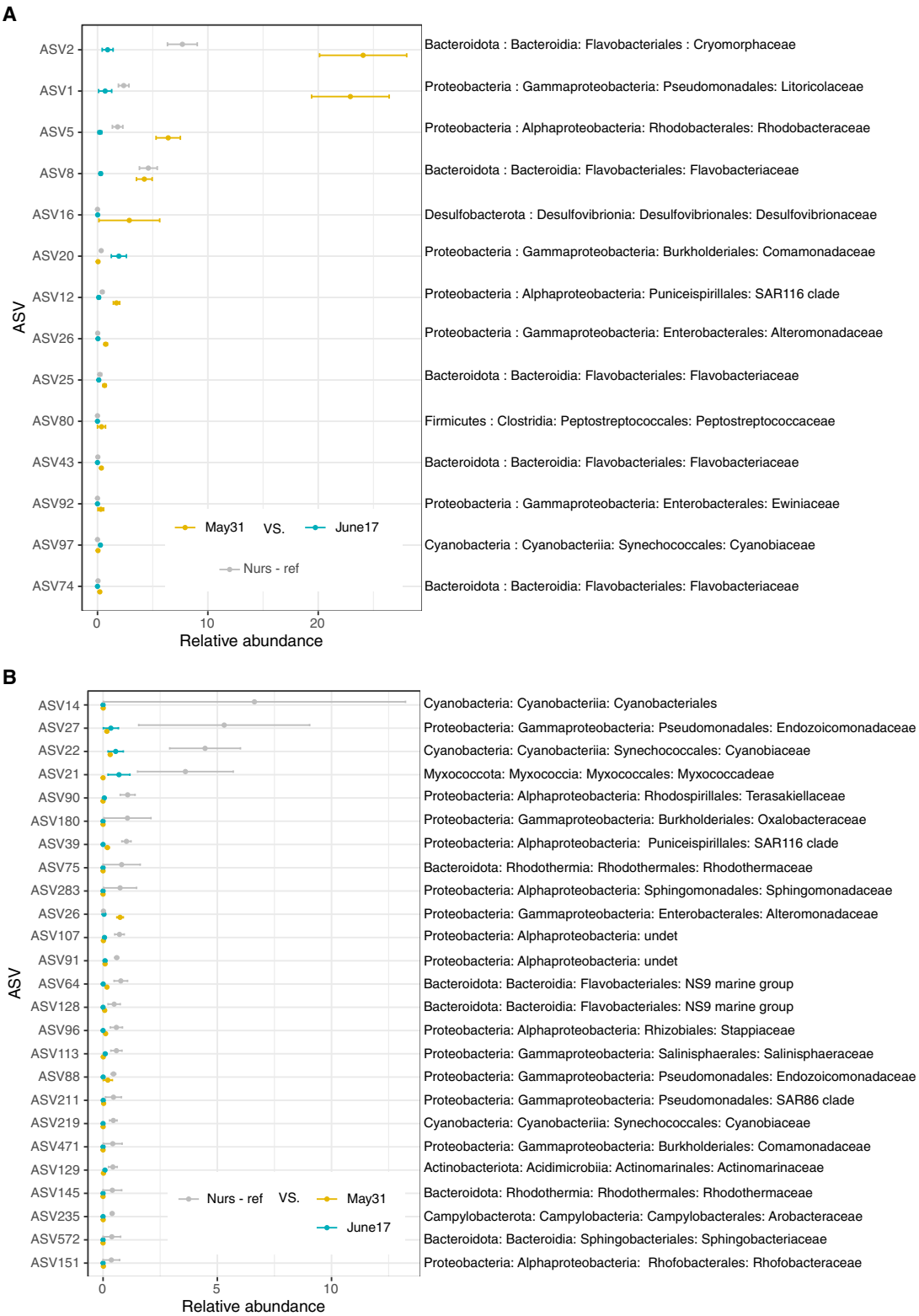

Figure 6. Major bacterial ASVs responsible for community dissimilarity

ASVs were selected based on SIMPER analyses: (A) from outplanted corals of May (WS appearance) vs. June (WS and bleaching) with nursery shown for reference and (B) from nursery corals reference vs. outplanted corals from May or June 2022.

are suspended in the water column.[81] This change in their environment could conceivably alter the core microbiome, potentially with the loss of dominant *Endozoicomonas*. Instability in holobiont microbial communities could, in theory, be caused by an extreme and sudden environmental change (thermal, nutrient, pH, light irradiance),[81,82] resulting in stress to the host. Recent work has documented distinct free-living microbial communities between the coral's ecosphere and the seawater column over the reef.[77,83,84] Thus, while this is a novel application within the field of coral restoration, the phenomenon is supported by recent studies. Strudwick et al.[85,86] reported a shift over time in bacterial communities associated with *Acropora millepora* in response to the transfer from the source site to a nursery. However, the same study reported no bacterial community shift in *Pocillopora verrucosa*, possibly a consequence of the distinct microbiomes transfer mode of *Acropora* corals (horizontal, from the water column, leading to high flexibility) vs. *Pocillopora* (maternal, leading to high co-evolution and low flexibility).[85,87] Together, these results highlight the importance of understanding the changes that coral holobionts experience during both nursery culture and introduction to restoration sites and suggest areas of future research to improve the success of sustainable coral restoration.

While the science of coral restoration is developing rapidly, the consideration of infectious disease in this context is relatively unexplored. This is a critical gap which must be addressed, and lessons learned from aquaculture may provide a starting point. Aquacultured organisms are frequently grown in high densities with low genetic diversity. This can increase susceptibility to – and transmission of – infectious disease (reviewed in Moriarty et al.[88]). Transport of cultured organisms provides opportunities for introduction or transfer of pathogens as well. Thus, quarantine practices have been developed to prevent the transfer of disease within cultured populations and between cultured and wild populations.[89,90] In the present study, we considered whether our nursery fragments may have had latent disease that was not visually observable when they were outplanted. However, we saw neither bleaching nor disease in the wild population of *A. aspera*, in our outplanted populations in other locations, or in our nurseries simultaneous with what we observed in Tumon Bay. However, as we mention in a previous paragraph, a white syndrome outbreak did occur within *A. pulchra*, at multiple sites within the Bay during our observed outbreak, though no bleaching was observed. Thus, we ruled out the possibility that outplanted fragments were latently diseased when introduced.

Considerations for improved restoration and conservation approaches

In applying our observations on shifts in the microbiome communities between nursery and outplanted environments, we identify an objective for improving restoration science: determining how we might facilitate preserving a healthy microbiome in outplanted corals. While transplantation stress is a commonly observed phenomenon, visually observed in corals by paling or bleaching, depressed growth rates, increased disease, and tissue loss, the physiological basis for this response is poorly understood. It is reasonable to speculate that a loss of bacterial species integral to coral health, with a shift to potentially opportunistic species, may factor heavily in this response. In this study, we noted a significant difference between nursery (a water column environment) and outplant (a substrate environment) microbiomes and highlighted the low abundances of the beneficial *Endozoicomonas* bacteria in both treatments. While it is not clear why wild *A. aspera* is dominated by *Endozoicomonas* bacteria (which may function as congeners), we would still expect a core microbiome and homogeneity among colonies within nursery treatments.[85,86] This heterogeneity highlights a potentially unstable microbiome, despite >2 years of acclimation in the nursery environment, and raises concerns about the physiological constraints of culturing corals in the water column (e.g., changes in energetic allocation strategies). Therefore, adding a transitional step between nursery and outplanting for thicket-forming coral such as *Acropora* may be beneficial. We propose moving outplants from midwater trees to near reef substrate within the nursery prior to recipient site outplanting, while sampling midwater and substrates for their bacterial communities. This period may prove necessary to recovering key bacteria for microbiome homeostasis and promoting immune functions observed in adjacent healthy colonies. Alternatively, testing the effect on microbiome communities of nursery structures positioned closer to the substrate may prove useful for morphologies that grow as discrete colonies attached to hard substrate. Both of these concepts require additional testing and development prior to introducing them as best practices for nursery culture. This highlights the need to better monitor and investigate environmental conditions and related microbiome function and dynamics through the whole restoration process from wild to nursery to targeted outplant site.

Data on the population structure of the surviving wild population were not available when we initially outplanted, though we have now confirmed that the remaining wild population of *A. aspera* is almost clonal, with little genetic structure (Combosch and Torrado in prep). Hence, while we initially hoped to observe differential responses to disease and bleaching, suggesting the presence of multiple genotypes of differing resilience,[51] we had no genetic diversity within the population. While we thus have few options to increase local genetic diversity for this species, our options for the persistence of *A. aspera* in the Mariana Islands may rest with efforts to establish populations in more habitats and determine the most beneficial sites, to reduce local extinction risk.

Options to manage a disease outbreak within a limited introduced population must also be developed. We initially considered culling diseased colonies, though we suspected a waterborne pathogen based on the pattern of disease spread in one plot, and the fact that no diseased colonies were in physical contact. Previous outbreaks of WS revealed that progression often stops prior to whole colony mortality (Raymundo and Lozada, unpublished data 2007). Previous observations of WS outbreaks in staghorn *Acropora* indicate that it is relatively short-lived and thus, may not kill larger colonies. Use of probiotics, such as the introduction of beneficial species such as *Endozoicomonas*, may be possibility with high-value and vulnerable species in an early stage of introduction,[91,92] but tools for their application need development. Antibiotic "band aids" may be applied to individual lesions and may be a strategy for *ex-situ*-cultured corals

at risk but the introduction of antibiotics into wild sites is controversial at best.[93,94]

Restoration efforts often cannot be carried out in the most pristine sites; Guam, for instance, does not house any coastline area that can be considered truly unimpacted by human activity. Given the seasonal water quality issues driving coral decline in Tumon Bay, the current restoration challenge is to determine which staghorn species may be resilient to summer conditions and whether these conditions vary within the Bay, identifying areas that may be more suitable for introduction. While addressing the issue of warming sea surface temperatures is beyond the capacity of the local government, managing inputs that reduce water quality is within local control and should be addressed, as this has implications for both marine ecosystems and human health, i.e., the One Health concept.[95,96] Ultimately, restoration success will be limited if political will for tackling anthropogenic inputs is lacking. An increasingly common thread among tropical island nations is that the popularity and success of the tourism industry is intrinsically linked with coral reef health, yet there is a lack of commitment to ensuring the health and continuity of these systems.

In conclusion, our study illustrates the importance of considering risks of infectious disease in restoration efforts and suggests new perspectives to be explored to improve the performance of coral outplants. Failure to consider the impacts of stress will lower the success of restoration and speaks directly to the importance of thoughtful site selection. The nature of the coral immunodefense response to stress has made much progress in recent decades. Now it is time to apply this knowledge to the science of restoration to improve the performance of corals introduced for the goal of conservation and management.

Limitations of the study

Ideally, when examining shifts or differences in the microbiome, we would have sampled from the wild population and the outplanted population on the first day of outplanting as well. Logistically, however, this was not feasible. As disease and bleaching progressed, it was challenging for field assistants to distinguish between WS and bleaching, which may have resulted in underestimates of the number of diseased colonies counted during later censuses. LR and HR were the most practiced at field assessments but could not census all plots in a single survey.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to, and will be fulfilled by the lead contact, Laurie Raymundo (lraymundo@triton.uog.edu).

Materials availability

Materials reported in this paper may be provided by the lead contact, Laurie Raymundo (lraymundo@triton.uog.edu) upon request. The study did not generate any new unique reagents.

Data and code availability

- Sequence data and metadata dataset are available on GenBank.
- Code required to reproduce the data analysis is available from the lead contact upon request.
- Any additional information or data required to reanalyse the data reported in this paper is available from the lead contact upon request.

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AUTHOR CONTRIBUTIONS

Conceptualization: L.J.R. and H.R.; methodology: L.J.R. and H.R.; data collection: L.J.R., M.A., and H.R.; data analysis: L.J.R., M.A., and H.R.; visualization: M.A. and H.R.; writing: L.J.R., H.R., and M.A.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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REFERENCES

1. Boström-Einarsson, L., Babcock, R.C., Bayraktarov, E., Ceccarelli, D., Cook, N., Ferse, S.C.A., Hancock, B., Harrison, P., Hein, M., Shaver, E., et al. (2020). Coral restoration – A systematic review of current methods, successes, failures and future directions. PLoS One *15*, e0226631.
2. Unsworth, J.D., Hesley, D., D'Alessandro, M., and Lirman, D. (2021). Outplanting optimized: developing a more efficient coral attachment technique using Portland cement. Restor. Ecol. *29*, e13299. https://doi.org/10.1111/rec.13299.
3. Banaszak, A.T., Marhaver, K.L., Miller, M.W., Hartmann, A.C., Albright, R., Hagedorn, M., Harrison, P.L., Latijnhouwers, K.R.W., Mendoza Quiroz, S., Pizarro, V., and Chamberland, V.F. (2023). Applying coral breeding to reef restoration: best practices, knowledge gaps, and priority actions in a rapidly-evolving field. Restor. Ecol. *31*, e13913. https://doi.org/10.1111/rec.13913.
4. Yin, A.S., Yang, C., and Martell, H.A. (2023). The influence of fragment type, time, and coral host genotype on early growth of nursery reared *Acropora cervicornis*. Restor. Ecol. *31*, e13879.
5. Voolstra, C.R., Suggett, D.J., Peixoto, R.S., Parkinson, J.E., Quigley, K.M., Silveira, C.B., Sweet, M., Muller, E.M., Barshis, D.J., Bourne,

D.G., and Aranda, M. (2021). Extending the natural adaptive capacity of coral holobionts. Nat. Rev. Earth Environ. *2*, 747–762.

6. Baums, I.B. (2008). A restoration genetics guide for coral reef conservation. Mol. Ecol. *17*, 2796–2811.
7. van Oppen, M.J.H., Oliver, J.K., Putnam, H.M., and Gates, R.D. (2015). Building coral reef resilience through assisted evolution. Proc. Natl. Acad. Sci. USA *112*, 2307–2313.
8. Shaver, E.C., McLeod, E., Hein, M.Y., Palumbi, S.R., Quigley, K., Vardi, T., Mumby, P.J., Smith, D., Montoya-Maya, P., Muller, E.M., et al. (2022). A roadmap to integrating resilience into the practice of coral reef restoration. Glob. Change Biol. *28*, 4751–4764.
9. Knutson, T., Camargo, S.J., Chan, J.C.L., Emanuel, K., Ho, C.-H., Kossin, J., Mohapatra, M., Satoh, M., Sugi, M., Walsh, K., and Wu, L. (2020). Tropical cyclones and climate change assessment: Part II: Projected response to anthropogenic warming. Bull. Am. Meteorol. Soc. *101*, E303–E322.
10. Burke, S., Pottier, P., Lagisz, M., Macartney, E.L., Ainsworth, T., Drobniak, S.M., and Nakagawa, S. (2023). The impact of rising temperatures on the prevalence of coral diseases and its predictability: A global meta-analysis. Ecol. Lett. *26*, 1466–1481.
11. Baird, A.H., Guest, J.R., and Willis, B.L. (2009). Systematic and biogeographical patterns in the reproductive biology of scleractinian corals. Annu. Rev. Ecol. Evol. Syst. *40*, 551–571.
12. Douglas, A.E., and Werren, J.H. (2016). Holes in the hologenome: Why host-microbe symbioses are not holobionts. mBio *7*, e02099.
13. Ziegler, M., Grupstra, C.G.B., Barreto, M.M., Eaton, M., BaOmar, J., Zubier, K., Al-Sofyani, A., Turki, A.J., Ormond, R., and Voolstra, C.R. (2019). Coral bacterial community structure responds to environmental change in a host-specific manner. Nat. Commun. *10*, 3092.
14. Loya, Y., Sakai, K., Yamazato, K., Nakano, Y., Sambali, H., and van Woesik, R. (2001). Coral bleaching: the winners and the losers. Ecol. Lett. *4*, 122–131.
15. Hoegh-Guldberg, O., and Salvat, B. (1995). Periodic mass-bleaching and elevated sea temperatures:bleaching of outer reef slope communities in Moorea, French Polynesia. Mar. Ecol. Prog. Ser. *121*, 181–190.
16. Casey, J.M., Connolly, S.R., and Ainsworth, T.D. (2015). Coral transplantation triggers shift in microbiome and promotion of coral disease associated potential pathogens. Sci. Rep. *5*, 11903.
17. Harvell, D., Jordán-Dahlgren, E., Merkel, S., Rosenberg, E., Raymundo, L., Smith, G., Weil, E., and Willis, B. (2007). Coral disease, environmental drivers, and the balance between coral and microbial associates. Oceanography (Wash., D.C.) *20*, 172–195.
18. Forrester, G.E., Maynard, A., Schofield, S., and Taylor, K. (2012). Evaluating causes of transplant stress in fragments of Acropora palmata used for coral reef restoration. Bull. Mar. Sci. *88*, 1099–1113.
19. Wiedenmann, J., D'Angelo, C., Smith, E.G., Hunt, A.N., Legiret, F.-E., and Achterberg, E.P. (2012). Nutrient enrichment can increase the susceptibility of reef corals to bleaching. Nat. Clim. Change *3*, 160–164.
20. Muller, E.M., Bartels, E., and Baums, I.B. (2018). Bleaching causes loss of disease resistance within the threatened coral species Acropora cervicornis. Elife *7*, e35066.
21. Meade, L. (2022). Comparing post-outplant stress responses of seven staghorn Acropora coral species in Guam (University of Groningen), p. 22, M.S. Thesis.
22. Pollock, F.J., Lamb, J.B., van de Water, J.A.J.M., Smith, H.A., Schaffelke, B., Willis, B.L., and Bourne, D.G. (2019). Reduced diversity and stability of coral-associated bacterial communities and suppressed immune function precedes disease onset in corals. R. Soc. Open Sci. *6*, 190355.
23. Vega Thurber, R., Mydlarz, L.D., Brandt, M., Harvell, D., Weil, E., Raymundo, L., Willis, B.L., Langevin, S., Tracy, A.M., Littman, R., et al. (2020). Deciphering Coral Disease Dynamics: Integrating Host, Microbiome, and the Changing Environment. Front. Ecol. Evol. *8*, 575927. https://doi.org/10.3389/fevo.2020.575927.
24. Estrada-Saldívar, N., Molina-Hernández, A., Pérez-Cervantes, E., Medellín-Maldonado, F., González-Barrios, F.J., and Alvarez-Filip, L. (2020). Reef-scale impacts of the stony coral tissue loss disease outbreak. Coral Reefs *39*, 861–866.
25. Muller, E.M., Sartor, C., Alcaraz, N.I., and van Woesik, R. (2020). Spatial epidemiology of the stony-coral-tissue-loss disease in Florida. Front. Mar. Sci. *7*, 163. https://doi.org/10.3389/fmars.2020.00163.
26. Miner, C.M., Burnaford, J.L., Ambrose, R.F., Antrim, L., Bohlmann, H., Blanchette, C.A., Engle, J.M., Fradkin, S.C., Gaddam, R., Harley, C.D.G., et al. (2018). Large-scale impacts of sea star wasting disease (SSWD) on intertidal sea stars and implications for recovery. PLoS One *13*, e0192870.
27. Jones, R.J., Bowyer, J., Hoegh-Guldberg, O., and Blackall, L.L. (2004). Dynamics of a temperature-related coral disease outbreak. Mar. Ecol. Prog. Ser. *281*, 63–77.
28. Redding, J.E., Myers-Miller, R.L., Baker, D.M., Fogel, M., Raymundo, L.J., and Kim, K. (2013). Link between sewage-derived nitrogen pollution and coral disease severity in Guam. Mar. Pollut. Bull. *73*, 57–63.
29. Vega Thurber, R.L., Burkepile, D.E., Fuchs, C., Shantz, A.A., McMinds, R., and Zaneveld, J.R. (2014). Chronic nutrient enrichment increases prevalence and severity of coral disease and bleaching. Glob. Change Biol. *20*, 544–554.
30. Pollock, F.J., Lamb, J.B., Field, S.N., Heron, S.F., Schaffelke, B., Shedrawi, G., Bourne, D.G., and Willis, B.L. (2014). Sediment and turbidity associated with offshore dredging increase coral disease prevalence on nearby reefs. PLoS One *9*, e102498.
31. Aeby, G.S., Howells, E., Work, T., Abrego, D., Williams, G.J., Wedding, L.M., Caldwell, J.M., Moritsch, M., and Burt, J.A. (2020). Localized outbreaks of coral disease on Arabian reefs are linked to extreme temperatures and environmental stressors. Coral Reefs *39*, 829–846.
32. Houk, P., Comeros-Raynal, M., Lawrence, A., Sudek, M., Vaeoso, M., McGuire, K., and Regis, J. (2020). Nutrient thresholds to protect water quality and coral reefs. Mar. Pollut. Bull. *159*, 111451.
33. Myers, R.L., and Raymundo, L.J. (2009). Coral disease in Micronesian reefs: a link between disease prevalence and host abundance. Dis. Aquat. Org. *87*, 97–104.
34. Roff, G., Kvennefors, E.C.E., Fine, M., Ortiz, J., Davy, J.E., and Hoegh-Guldberg, O. (2011). The ecology of "Acroporid white syndrome". PLoS One *6*, e26829.
35. Ainsworth, T.D., Kvennefors, E.C., Blackall, L.L., Fine, M., and Hoegh-Guldberg, O. (2007). Disease and cell death in white syndrome of Acroporid corals on the Great Barrier Reef. Mar. Biol. *151*, 19–29.
36. Sussman, M., Willis, B.L., Victor, S., and Bourne, D.G. (2008). Coral pathogens identified for White Syndrome (WS) epizootics in the Indo-Pacific. PLoS One *3*, e2393.
37. Rouzé, H., Lecellier, G., Saulnier, D., and Berteaux-Lecellier, V. (2016). Symbiodinium clades A and D differentially predispose Acropora cytherea to disease and Vibrio spp. colonization. Ecol. Evol. *6*, 560–572.
38. Greene, A., Donahue, M.J., Caldwell, J.M., Heron, S.F., Geiger, E., and Raymundo, L.J. (2020). Coral disease time series highlight size-dependent risk and other drivers of White Syndrome in a multi-species model. Front. Mar. Sci. *7*. https://doi.org/10.3389/fmars.2020.601469.
39. Raymundo, L.J., Burdick, D., Lapacek, V.A., Miller, R., and Brown, V. (2017). Anomalous temperatures and extreme tides: Guam staghorn Acropora succumb to a double threat. Mar. Ecol. Prog. Ser. *564*, 47–55.
40. Raymundo, L.J., Burdick, D., Hoot, W.C., Miller, R.M., Brown, V., Reynolds, T., Gault, J., Idechong, J., Fifer, J., and Williams, A. (2019). Successive bleaching events cause mass coral mortality in Guam, Micronesia. Coral Reefs *38*, 677–700.
41. Raymundo, L., Andersen, M., Moreland-Ochoa, C., Castro, A., Lock, C., Burns, N., Combosch, D., and Burdick, D. (2022). Conservation and Active Restoration of Guam's Staghorn Acropora Corals (Marine Laboratory (University of Guam).

42. Knowlton, N., Lang, J.C., Christine Rooney, M., and Clifford, P. (1981). Evidence for delayed mortality in hurricane-damaged Jamaican staghorn corals. Nature *294*, 251–252.

43. Howells, E.J., Vaughan, G.O., Work, T.M., Burt, J.A., and Abrego, D. (2020). Annual outbreaks of coral disease coincide with extreme seasonal warming. Coral Reefs *39*, 771–781.

44. Berg, J.T., David, C.M., Gabriel, M.M., and Bentlage, B. (2020). Fluorescence signatures of persistent photosystem damage in the staghorn coral Acropora cf. pulchra (Anthozoa: Scleractinia) during bleaching and recovery. Mar. Biol. Res. *16*, 643–655.

45. Darling, E.S., Alvarez-Filip, L., Oliver, T.A., McClanahan, T.R., Côté, I.M., and Bellwood, D. (2012). Evaluating life-history strategies of reef corals from species traits. Ecol. Lett. *15*, 1378–1386.

46. Carlot, J., Rouzé, H., Barneche, D.R., Mercière, A., Espiau, B., Cardini, U., Brandl, S.J., Casey, J.M., Pérez-Rosales, G., Adjeroud, M., et al. (2022). Scaling up calcification, respiration, and photosynthesis rates of six prominent coral taxa. Ecol. Evol. *12*, e8613.

47. Rosenberg, E., Koren, O., Reshef, L., Efrony, R., and Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. Nat. Rev. Microbiol. *5*, 355–362.

48. DeFilippo, L.B., McManus, L.C., Schindler, D.E., Pinsky, M.L., Colton, M.A., Fox, H.E., Tekwa, E.W., Palumbi, S.R., Essington, T.E., and Webster, M.M. (2022). Assessing the potential for demographic restoration and assisted evolution to build climate resilience in coral reefs. Ecol. Appl. *32*, e2650.

49. Zhu, Y., Chen, H., Fan, J., Wang, Y., Li, Y., Chen, J., Fan, J., Yang, S., Hu, L., Leung, H., et al. (2000). Genetic diversity and disease control in rice. Nature *406*, 718–722.

50. Altermatt, F., and Ebert, D. (2008). Genetic diversity of Daphnia magna populations enhances resistance to parasites. Ecol. Lett. *11*, 918–928.

51. Baums, I.B., Baker, A.C., Davies, S.W., Grottoli, A.G., Kenkel, C.D., Kitchen, S.A., Kuffner, I.B., LaJeunesse, T.C., Matz, M.V., Miller, M.W., et al. (2019). Considerations for maximizing the adaptive potential of restored coral populations in the western Atlantic. Ecol. Appl. *29*, e01978.

52. Brown, A.L., Anastasiou, D.-E., Schul, M., MacVittie, S., Spiers, L.J., Meyer, J.L., Manfrino, C., and Frazer, T.K. (2022). Mixtures of genotypes increase disease resistance in a coral nursery. Sci. Rep. *12*, 19286.

53. Stat, M., Morris, E., and Gates, R.D. (2008). Functional diversity in coral–dinoflagellate symbiosis. Proc. Natl. Acad. Sci. USA *105*, 9256–9261.

54. Lesser, M.P., Stat, M., and Gates, R.D. (2013). The endosymbiotic dinoflagellates (Symbiodinium sp.) of corals are parasites and mutualists. Coral Reefs *32*, 603–611.

55. Traylor-Knowles, N., Baker, A.C., Beavers, K.M., Garg, N., Guyon, J.R., Hawthorn, A., MacKnight, N.J., Medina, M., Mydlarz, L.D., Peters, E.C., et al. (2022). Advances in coral immunity 'omics in response to disease outbreaks. Front. Mar. Sci. *9*, 952199. https://doi.org/10.3389/fmars.2022.952199.

56. Anthony, C.J., Lock, C., Taylor, B.M., and Bentlage, B. (2023). Cellular plasticity facilitates phenotypic change in a dominant coral's Symbiodiniaceae assemblage. Front. Ecol. Evol. *11*. https://doi.org/10.3389/fevo.2023.1288596.

57. Correa, A.M.S., Brandt, M.E., Smith, T.B., Thornhill, D.J., and Baker, A.C. (2009). Symbiodinium associations with diseased and healthy scleractinian corals. Coral Reefs *28*, 437–448.

58. Dennison, C., Karp, R., Weiler, B., Goncalves, A., del Campo, J., Rosales, S., and Baker, A.C. (2021). The role of algal symbionts (genus Breviolum) in the susceptibility of corals to Stony Coral Tissue Loss Disease in South Florida. Florida Department of Environmental Protection Technical Report, 23.

59. Pantos, O., and Bythell, J.C. (2006). Bacterial community structure associated with white band disease in the elkhorn coral Acropora palmata determined using culture-independent 16S rRNA techniques. Dis. Aquat. Org. *69*, 79–88.

60. Pollock, F.J., Wada, N., Torda, G., Willis, B.L., and Bourne, D.G. (2017). White syndrome-affected corals have a distinct microbiome at disease lesion fronts. Appl. Environ. Microbiol. *83*, e02799-16. https://doi.org/10.1128/AEM.02799-16.

61. Rosales, S.M., Miller, M.W., Williams, D.E., Traylor-Knowles, N., Young, B., and Serrano, X.M. (2019). Microbiome differences in disease-resistant vs. susceptible Acropora corals subjected to disease challenge assays. Sci. Rep. *9*, 18279.

62. Schul, M.D., Anastasious, D.-E., Spiers, L.J., Meyer, J.L., Frazer, T.K., and Brown, A.L. (2023). Concordance of microbial and visual health indicators of white-band disease in nursery reared Caribbean coral Acropora cervicornis. PeerJ *11*, e15170.

63. Luna, G.M., Bongiorni, L., Gili, C., Biavasco, F., and Danovaro, R. (2010). Vibrio harveyi as a causative agent of the White Syndrome in tropical stony corals: Vibrio pathogens of stony corals. Environ. Microbiol. Rep. *2*, 120–127.

64. Ushijima, B., Smith, A., Aeby, G.S., and Callahan, S.M. (2012). Vibrio owensii induces the tissue loss disease Montipora white syndrome in the Hawaiian reef coral Montipora capitata. PLoS One *7*, e46717.

65. Sun, X., Xu, Z., Xie, J., Hesselberg-Thomsen, V., Tan, T., Zheng, D., Strube, M.L., Dragoš, A., Shen, Q., Zhang, R., and Kovács, Á.T. (2022). Bacillus velezensis stimulates resident rhizosphere Pseudomonas stutzeri for plant health through metabolic interactions. ISME J. *16*, 774–787.

66. Chavanich, S., Kusdianto, H., Kullapanich, C., Jandang, S., Wongsawaeng, D., Ouazzani, J., Viyakarn, V., and Somboonna, N. (2022). Microbiomes of healthy and bleached corals during a 2016 thermal bleaching event in the Andaman Sea of Thailand. Front. Mar. Sci. *9*, 763421. https://doi.org/10.3389/fmars.2022.763421.

67. Gignoux-Wolfsohn, S.A., and Vollmer, S.V. (2015). Identification of candidate coral pathogens on white band disease-infected staghorn coral. PLoS One *10*, e0134416.

68. Klinges, G., Maher, R.L., Vega Thurber, R.L., and Muller, E.M. (2020). Parasitic "Candidatus Aquarickettsia rohweri" is a marker of disease susceptibility in Acropora cervicornis but is lost during thermal stress. Environ. Microbiol. *22*, 5341–5355.

69. Viehman, S., Mills, D.K., Meichel, G.W., and Richardson, L.L. (2006). Culture and identification of Desulfovibrio spp. from corals infected by black band disease on Dominican and Florida Keys reefs. Dis. Aquat. Org. *69*, 119–127.

70. Jompa, J., and Tahir, A. (2021). Transmission of Desulfovibrio salexigens DSM2638 bacteria in Pachyseris involuta-infection rate and changes in coral morphology at different temperatures. IOP Conf. Ser. Earth Environ. Sci. *763*, 012055.

71. Bourne, D.G., Ainsworth, T.D., Pollock, F.J., and Willis, B.L. (2015). Towards a better understanding of white syndromes and their causes on Indo-Pacific coral reefs. Coral Reefs *34*, 233–242.

72. Page, C.E., Leggat, W., Egan, S., and Ainsworth, T.D. (2023). A coral disease outbreak highlights vulnerability of remote high-latitude lagoons to global and local stressors. iScience *26*, 106205.

73. Miller, T.C., and Bentlage, B. (2024). Seasonal dynamics and environmental drivers of tissue and mucus microbiomes in the staghorn coral Acropora pulchra. PeerJ *12*, e17421.

74. Neave, M.J., Michell, C.T., Apprill, A., and Voolstra, C.R. (2017). Endozoicomonas genomes reveal functional adaptation and plasticity in bacterial strains symbiotically associated with diverse marine hosts. Sci. Rep. *7*, 40579.

75. van Oppen, M.J.H., and Blackall, L.L. (2019). Coral microbiome dynamics, functions and design in a changing world. Nat. Rev. Microbiol. *17*, 557–567.

76. Tandon, K., Chiou, Y.-J., Yu, S.-P., Hsieh, H.J., Lu, C.-Y., Hsu, M.-T., Chiang, P.-W., Chen, H.-J., Wada, N., and Tang, S.-L. (2022). Microbiome restructuring: Dominant coral bacterium Endozoicomonas

species respond differentially to environmental changes. mSystems *7*, e0035922.

77. Hochart, C., Paoli, L., Ruscheweyh, H.-J., Salazar, G., Boissin, E., Romac, S., Poulain, J., Bourdin, G., Iwankow, G., Moulin, C., et al. (2023). Ecology of Endozoicomonadaceae in three coral genera across the Pacific Ocean. Nat. Commun. *14*, 3037.
78. Shiu, J.-H., Keshavmurthy, S., Chiang, P.-W., Chen, H.-J., Lou, S.-P., Tseng, C.-H., Justin Hsieh, H., Allen Chen, C., and Tang, S.-L. (2017). Dynamics of coral-associated bacterial communities acclimated to temperature stress based on recent thermal history. Sci. Rep. *7*, 14933.
79. McDevitt-Irwin, J.M., Baum, J.K., Garren, M., and Vega Thurber, R.L. (2017). Responses of coral-associated bacterial communities to local and global stressors. Front. Mar. Sci. *4*, 262. https://doi.org/10.3389/fmars.2017.00262.
80. Keller-Costa, T., Lago-Lestón, A., Saraiva, J.P., Toscan, R., Silva, S.G., Gonçalves, J., Cox, C.J., Kyrpides, N., Nunes da Rocha, U., and Costa, R. (2021). Metagenomic insights into the taxonomy, function, and dysbiosis of prokaryotic communities in octocorals. Microbiome *9*, 72. https://doi.org/10.1186/s40168-021-01031-y10.21203/rs.3.rs-968300/v1.
81. Zaneveld, J.R., McMinds, R., and Vega Thurber, R. (2017). Stress and stability: applying the Anna Karenina principle to animal microbiomes. Nat. Microbiol. *2*, 17121.
82. Vega Thurber, R., Willner-Hall, D., Rodriguez-Mueller, B., Desnues, C., Edwards, R.A., Angly, F., Dinsdale, E., Kelly, L., and Rohwer, F. (2009). Metagenomic analysis of stressed coral holobionts. Environ. Microbiol. *11*, 2148–2163.
83. Ochsenkühn, M.A., Schmitt-Kopplin, P., Harir, M., and Amin, S.A. (2018). Coral metabolite gradients affect microbial community structures and act as a disease cue. Commun. Biol. *1*, 184.
84. Weber, L., Gonzalez-Díaz, P., Armenteros, M., and Apprill, A. (2019). The coral ecosphere: A unique coral reef habitat that fosters coral–microbial interactions. Limnol. Oceanogr. *64*, 2373–2388.
85. Strudwick, P., Seymour, J., Camp, E.F., Edmondson, J., Haydon, T., Howlett, L., Le Reun, N., Siboni, N., and Suggett, D.J. (2022). Impacts of nursery-based propagation and out-planting on coral-associated bacterial communities. Coral Reefs *41*, 95–112.
86. Strudwick, P., Seymour, J., Camp, E.F., Roper, C., Edmondson, J., Howlett, L., and Suggett, D.J. (2023). Bacterial communities associated with corals out-planted on the Great Barrier Reef are inherently dynamic over space and time. Mar. Biol. *170*, 85.
87. Rouzé, H., Lecellier, G., Pochon, X., Torda, G., and Berteaux-Lecellier, V. (2019). Unique quantitative Symbiodiniaceae signature of coral colonies revealed through spatio-temporal survey in Moorea. Sci. Rep. *9*, 7921.
88. Moriarty, T., Leggat, W., Huggett, M.J., and Ainsworth, T.D. (2020). Coral Disease Causes, Consequences, and Risk within Coral Restoration. Trends. Microbiol. *28*, 793–807.
89. Mayfield, A.B., Tsai, S., and Lin, C. (2019). The Coral Hospital. Biopreserv. Biobanking *17*, 355–369.
90. Glidden, C.K., Field, L.C., Bachhuber, S., Hennessey, S.M., Cates, R., Cohen, L., Crockett, E., Degnin, M., Feezell, M.K., Fulton-Bennett, H.K., et al. (2022). Strategies for managing marine disease. Ecol. Appl. *32*, e2643.
91. Ritchie, K.B. (2006). Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar. Ecol. Prog. Ser. *322*, 1–14.
92. Shnit-Orland, M., and Kushmaro, A. (2009). Coral mucus-associated bacteria: a possible first line of defense: Coral mucus-associated bacteria. FEMS. Microbiol. Ecol. *67*, 371–380.
93. Neely, K.L., Macaulay, K.A., Hower, E.K., and Dobler, M.A. (2020). Effectiveness of topical antibiotics in treating corals affected by Stony Coral Tissue Loss Disease. PeerJ *8*, e9289.
94. Shilling, E.N., Combs, I.R., and Voss, J.D. (2021). Assessing the effectiveness of two intervention methods for stony coral tissue loss disease on Montastraea cavernosa. Sci. Rep. *11*, 8566. https://doi.org/10.1038/s41598-021-86926-4.
95. Lamb, J.B., van de Water, J.A.J.M., Bourne, D.G., Altier, C., Hein, M.Y., Fiorenza, E.A., Abu, N., Jompa, J., and Harvell, C.D. (2017). Seagrass ecosystems reduce exposure to bacterial pathogens of humans, fishes, and invertebrates. Science *355*, 731–733.
96. Sweet, M., Burian, A., and Bulling, M. (2021). Corals as canaries in the coalmine: Towards the incorporation of marine ecosystems into the "One Health" concept. J. Invertebr. Pathol. *186*, 107538.
97. Parada, A.E., Needham, D.M., and Fuhrman, J.A. (2016). Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. *18*, 1403–1414.
98. Apprill, A., McNally, S., Parsons, R., and Weber, L. (2015). Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. *75*, 129–137.
99. Denton, G.R.W., Concepcion, L.P., Wood, H.R., and Denton, C.M.S. (2005). Nutrient status of Tumon Bay in relation to intertidal blooms of the filamentous green alga, Enteromorpha clathrata. Technical Report.
100. Denton, G.R.W., and Sian–Denton, C. (2015). Unsightly algal blooms in Tumon Bay, Guam's premier tourist location: possible connection to hotel landscaping activities. Interdiscip. Environ. Rev. *9*, 94–106.
101. Matson, E.A. (1991). Water chemistry and hydrology of the" Blood of San Vitores", a Micronesian red tide. Micronesica *24*, 95–108.
102. Siebeck, U.E., Marshall, N.J., Klüter, A., and Hoegh-Guldberg, O. (2006). Monitoring coral bleaching using a colour reference card. Coral Reefs *25*, 453–460.
103. Work, T.M., and Aeby, G.S. (2011). Pathology of tissue loss (white syndrome) in Acropora sp. corals from the Central Pacific. J. Invertebr. Pathol. *107*, 127–131.
104. Work, T.M., and Aeby, G.S. (2006). Systematically describing gross lesions in corals. Dis. Aquat. Org. *70*, 155–160.
105. Raymundo, L.J., Couch, C.S., Bruckner, A.W., Harvell, C.D., Work, T.M., Weil, E., Woodley, C.M., Jordan-Dahlgren, E., Willis, B.L., Sato, Y., et al. (2008). In Coral Disease Handbook Guidelines for Assessment, Monitoring & Management, L.J. Raymundo, ed. (CS Couch and DC Harvell (Coral Reef Targeted Research)).
106. Hume, B.C.C., D'Angelo, C., Smith, E.G., Stevens, J.R., Burt, J., and Wiedenmann, J. (2015). Symbiodinium thermophilum sp. nov., a thermotolerant symbiotic alga prevalent in corals of the world's hottest sea, the Persian/Arabian Gulf. Sci. Rep. *5*, 8562.
107. Comeau, A.M., Douglas, G.M., and Langille, M.G.I. (2017). Microbiome Helper: a Custom and Streamlined Workflow for Microbiome Research. mSystems *2*, e00127-16. https://doi.org/10.1128/mSystems.00127-16.
108. Therneau, T.J. (2015). A package for survival analysis in R. https://github.com/therneau/survival.
109. Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., and Holmes, S.P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods *13*, 581–583.
110. Kenkel, C.D., and Bay, L.K. (2018). Exploring mechanisms that affect coral cooperation: symbiont transmission mode, cell density and community composition. PeerJ *6*, e6047.
111. Hume, B.C.C., Smith, E.G., Ziegler, M., Warrington, H.J.M., Burt, J.A., LaJeunesse, T.C., Wiedenmann, J., and Voolstra, C.R. (2019). SymPortal: A novel analytical framework and platform for coral algal symbiont next-generation sequencing ITS2 profiling. Mol. Ecol. Resour. *19*, 1063–1080.
112. Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic. Acids. Res. *41*, D590–D596.
113. Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'hara, R.B., and Oksanen, M. (2013). Package 'vegan'. Community ecology package, version 2, pp. 1–295.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Acropora aspera	Piti coral nursery (Guam, Micronesia)	Marine Laboratory, Guam, permit no. SC-MPA-20-009 (Dept. of Agr. Div. of Aquatic & Wildlife Resources)
Critical commercial assays		
Zymo BIOMICS DNA Miniprep kit	ZYMO	Cat#D4300
TaKaRa Ex Taq DNA Polymerase Hot Start	Takara Bio. USA Inc.	Cat#RR006B
GeneJet purification kit	Life Technologies	Cat#K0702
Deposited data		
Raw 16S amplicon sequence data	This study	Bioproject: PRJNA1200717
Raw ITS2 amplicon sequence data	This study	Symportal.org
Oligonucleotides		
Hyb515F	Modified from Parada et al.[97]	TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNN NNGTGYCAGCMGCCGCGGTAA
Hyb806R	Modified from Apprill et al.[98]	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGN NNNGGACTACNVGGGTWTCTAAT
Software and algorithms		
R v4.2.2	R Core Team	https://www.r-project.org

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The restoration efforts on Guam currently prioritize vulnerable populations of staghorn *Acropora*, renowned for their rapid growth rates that aid in recovery after stress events. These corals are ecologically significant as they serve as architects of complex habitats, essential fish habitat, and buffers against coastal waves. The branching species are hermaphroditic and reproduce through broadcast spawning. This study focused on *Acropora aspera*, one of the most at-risk coral species in Guam, whose genetic diversity is restricted to a single population on the island.

METHOD DETAILS

Study site

Tumon Bay Marine Preserve (13°30′34.77″N, 144.°47′52.87″E) (Figure 1C) is a 1.52 km^2 shallow (0.5m - 2m depth) embayment on the central-western coast of Guam, bordered by a line of hotels known as "Hotel Row" and the site of intense tourism development. Stormwater runoff and nutrient input are poorly managed, resulting in periods of poor water quality.[43,44,99,100] Red tide blooms of *Scrippsiella*, *Peridinium*, or *Gymnodinium* spp. have been recorded within the Bay for centuries; these events are locally referred to as the "Blood of Sanvitores", after a priest executed in 1672.[101] The Bay is also influenced by freshwater karst seeps delivering nitrogen, phosphorus, and iron.[99,101] Its declaration in 1997 as a Marine Preserve allowed recovery of coral and fish communities despite intense coastal development for tourism; these communities remain today and are highly prized for their recreational value and cultural fishing traditions. Existing communities exhibit bleaching and increased disease prevalence within the summer months (Raymundo, Long Term Monitoring Program, unpublished data), but show recovery as water cools. Strong support for restoration activities is present among the Bay's stakeholders and thus it was included as a recipient site for Guam's restoration activities.

In February 2022, 162 *Acropora aspera* fragments cultured in the Piti Bomb Holes *in situ* nursery (Figure 1A) were pruned to ~12 cm diameter (longest axis) and prepared for outplanting. In contrast to Tumon Bay, the Piti Marine Preserve is less impacted by intense human activity, located along a coastal road but with significantly less shoreline development. The Preserve is a large embayment featuring numerous karst sinkholes; depth ranges from 1.5m to 6m, and the deep reef crest allows for continual mixing with oceanic water throughout the year. The nursery is located in one of the sinkholes, allowing protection during intense storms. Prepared fragments were transported immediately and outplanted into Tumon Bay, onto four 16-m^2 plots (Restore Plots 10-13) of weathered limestone pavement covered with a thin layer of sand supporting scattered colonies of *Acropora pulchra* and *Porites* spp. Plots were at 1.5 m depth and housed between 39 and 42 fragments per plot, arranged in a grid with a spacing of 30 cm between fragments. All fragments were visually assessed monthly for skeletal growth onto the substrate and health, noting predation scars, disease lesions, discoloration, partial mortality, or bleaching using the Coral Health Chart[102] (a semi-quantitative estimate of endosymbiont density).

At the first observation of acute tissue loss, plots were revisited approximately every two weeks until total mortality was observed (Figure 2).

White syndrome (WS) and bleaching tracking

On May 25, 2022, monitoring revealed the presence of acute tissue loss lesions characteristic of *Acropora* White Syndrome[103] on 33 of our outplants (Figure 2A). This constituted a prevalence of 20.4% across our four plots; more than twice that of our original baseline surveys (mean prevalence: 8.9% across 15 surveyed reefs).[33] The high prevalence of lesions suggested an outbreak may be initiating and prompted increased monitoring to track the rate of tissue loss, the appearance of new cases (incidence), recovery or mortality, and sampling for bacterial profiling to document possible microbiome dysbiosis. As the disease outbreak occurred in early summer, we predicted bleaching might occur and developed a protocol for distinguishing between progressing disease and bleaching. All fragments were examined using a hand-held Bausch & Lomb® 5X magnifying lens; lesions were scored as disease where tissue was completely absent and tissue loss was acute and progressing, and surrounding tissue was present.[104,105] Bleaching was scored where tissue was pale to white, but present; in later stages of the outbreak, bleaching mortality was scored as tissue loss within a patch of severely bleached tissue. Tracking the disease outbreak involved visual inspection of all colonies at each census, and photographic monitoring of n=6 lesions/plot tagged with a ziptie at the progressing front of the lesion, to record spreading of tissue loss beyond the ziptie (Figure 2A).[105] Progression rate was analyzed using Image J® software on a subset of n=13 lesions that were clear and measurable over time and utilized only the first six weeks of monitoring (5/25 – 7/7); after this point, progression had either stopped or slowed to a point where measuring accuracy was compromised. Incidence was calculated as: (number of new cases/number of living fragments) x 100. For analysis, mortality and White Syndrome were assessed using a binary system, with mortality scored as 0 (=alive) or 1 (=dead) and WS was scored as either present (=1; progressing tissue loss) or absent (=0; either absence of disease or cessation of progression). Bleaching severity was scored using a Coral Health Watch card, for both upper and lower branch surfaces. The mean of upper and lower branch scores was calculated to obtain a total colony score, using the following: 0 = full pigmentation (=D5 on coral health chart); 1 = slightly pale (=D4); 2 = distinctly pale (=D3); 3 = mostly white (=D2); 4 = completely white (=D1); 5 = bleaching and white syndrome partial mortality; 6 = full loss of tissue (i.e., full mortality).

Environmental data

Sea surface temperature was recorded using an Onset® HOBO tidbit data logger deployed on the reef flat adjacent to the outplant site, set to record hourly from January 2022 to September 30, 2022. Mean ± SD were calculated weekly prior and during the period of the outbreak. *Enterococcus* colony count data, an indicator of sewage contamination and a proxy for bacterial load, were obtained from the Guam Environmental Protection Agency water portal (https://www.epa.gov/waterdata/water-quality-data). Daily sea level data was obtained from the National Oceanic and Atmospheric Administration, using the NOAA NOS CO-OPs data API (https://tidesandcurrents.noaa.gov/stationhome.html?id=1630000).

Sample collection

To profile the bacterial community associated with outplanted *A. aspera* at the observed onset of the WS event, tissue samples were collected from WS-infected colonies at both the "visually healthy region (VH)" and the "diseased region (WS)" as well as on the rare healthy colonies not presenting WS symptoms (control) (Sampling for Microbiome Analyses Table). All samples were identified across plots and a haphazardly-selected subset of eight colonies were sampled on May 31, 2022 (WS: n=4, VH: n=4), six days after initial observations of disease. These were sampled *in situ* by gently scraping tissue off the skeleton using a sterile syringe, sampling both healthy areas and diseased areas of the same coral. A second sampling took place on June 17, 2022 (WS: n=4, VH: n=4), using a bone cutter to remove a 5 cm section of "healthy" tissue and a 5 cm section that included the lesion margin and tissue adjacent to the lesion margin. Additional samples were taken from two colonies identified as visually non-WS-infected to serve as microbial control samples (control: n=2). As additional reference points, samples were also collected from source *A. aspera* colonies reared in the Piti Nursery on September, 2022, from which outplant fragments were taken (Nursery: n=5). All samples were immediately preserved in DNA/RNA shield buffer (ZYMO 5ml tubes) and stored at -80°C at the University of Guam Marine Laboratory (UOGML) until further molecular analyses.

Associated symbiotic communities: Bacterial and Symbiodiniaceae metabarcoding

DNA was extracted from each healthy and diseased fragment using the ZymoBIOMICS DNA Miniprep Kit (Zymo) following the protocol with minor modifications. Specifically, we added a step of three cryo-shock cycles (1 d at -80°C, followed by 1 d at room temperature ~25°C), followed by mechanical treatments using the Biospec Mini-BeadBeater-16 (5 cycles: maximum speed during 1 min with pause time of 2 min). For the bacterial communities, a 16S library was generated by amplifying the hypervariable V4 region of the 16S rRNA genes, amplified using modified bacteria-specific primers 515f[97] and 806r[98] at the UOGML. The 16S library was sequenced using the MiSeq Illumina platform at the University of Perpignan (France). For the Symbiodiniaceae communities, amplification and sequencing were carried out at the Integrated Microbiome Resource (Canada; www.imr.bio) using specific primers SYM_VAR_5.8S and SYM_VAR_REV[106] using the MiSeq Illumina platform under conditions previously described.[107]

QUANTIFICATION AND STATISTICAL ANALYSES

White syndrome prevalence was calculated as no. fragments infected per plot/total number of fragments per plot x 100; mean prevalence was calculated as the mean across the four plots. To quantify the severity of cumulative stress of simultaneous disease and bleaching, the severity of stress visually observed on coral outplants was estimated by adding the scores assessed for survival, presence/absence of active lesions, and coral bleaching severity, as described in section symbiotic communities associated with healthy and diseased *A. aspera* coral tissue. Total coral stress severity scores thus ranged from 0 for good health [i.e. 0 (tissue death) + 0 (bleaching) + 0 (WS lesions)] to 6 for colonies experiencing complete mortality [i.e. 1 (tissue death) + 5 (bleaching severity: full mortality) + 0 (WS lesions)]. In addition probabilities of WS resistance and survival were evaluated using the Kaplan-Meier method with *Survival* package in R.[108]

Symbiotic communities were analyzed in R (v. 4.3.2.) as follows: sequence data were processed following the standard pipeline of the DADA2 package (version 1.12.1)[109] and Kenkel's script[110] for bacterial and Symbiodiniaceae communities, respectively. A total of 1,368,373 (23 samples) for 16S and 360,465 (10 samples) for ITS2 high-quality reads (an average of 62,000 reads per sample) were obtained after removing poor quality reads, chimeras, and singletons. The Amplicon Sequence Variants (ASVs) were first phylogenetically assigned against the most up-to-date SymPortal (access date: June 16, 2023)[111] databases for *Symbiodiniaceae* and the Silva database v.138[112] for the bacterial communities. Alpha and Beta diversity measures and the Bray-Curtis similarity index were computed based on rarefied reads (90% of the abundance of the sample with less reads). In addition, NMDS plots for bacteria were created based on weighted unifrac distance using *Vegan* package.[113] Patterns identified from NMDS plots were further analyzed and tested for significance with a PERMANOVA from the ''adonis'' function in the *Vegan* package. Bacterial relative abundances that were most dissimilar between sampling periods in May vs. June (beginning and mid-outbreak) vs. September (nursery reference samples) were identified with a similar percentage analysis (SIMPER) using the *Vegan* package.

Raw 16S sequencing datasets have been deposited in the NCBI Sequence Read Archive under NCBI's BioProject (PRJNA1200717). In addition to the DADA2 analysis of the Symbiodiniaceae, demultiplexed forward and reverse fastq.gz Illumina sequencing files were also submitted to SymPortal.org in order to predict ITS2 type profiles from specific sets of defined intragenomic ITS2 sequence variants (access date of the SymPortal database: June 16, 2023) following the method described in Hume et al.[111]