Intraspecific variation in coral reproductive output and symbiont community structure following a thermal bleaching event

by

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Abstract

Marine heatwaves and associated bleaching events are projected to increase in frequency and severity in the future, imperiling charismatic ecosystems such as coral reefs. Although mass bleaching events are often accompanied by widespread coral mortality, there is intraspecific variation in bleaching susceptibility, leading to a mosaic of responses where corals may bleach and die, bleach and recover, or resist bleaching altogether. Because the ecological persistence of coral reefs hinges on the survivors of environmental stress, understanding the sublethal effects of bleaching and the mechanisms that promote resilience or resistance are crucial for both projecting coral populations into the future and developing effective management or intervention tools. This thesis examines natural intraspecific variation in bleaching susceptibility in the Indo-Pacific reef-building coral *Acropora hyacinthus* during and after the 2019 thermal anomaly in Mo'orea, French Polynesia, with particular emphasis on coral reproductive output and microalgal endosymbiont community structure.

In Chapter 1, I employed reproductive histology and energetic assays to relate coral host energy reserves to gamete quantity and quality in resistant and recovered colonies. I found that, despite healthy appearances in all individuals five months after the bleaching event, recovered colonies harbored diminished energy reserves compared to resistant conspecifics and exhibited compound effects of stress on reproduction: they displayed not only a lower probability of containing gametes, but also lower fecundity per polyp. These results illustrate energy allocation strategies among physiological processes and indicate that bleaching imposes a constraint on concurrent stress recovery and gamete production, with the decreased reproductive capacity of bleaching survivors possibly restricting overall reef resilience.

In Chapter 2, I utilized ITS2 amplicon sequencing to assess spatial and temporal flexibility in Symbiodiniaceae community structure and their relationship to the host heat stress response during and after the thermal anomaly. I found that there was substantial flexibility in coral-microalgal associations across the reefscape, but that symbiont community composition was strongly linked to both reef zone and holobiont affinity for resistance or recovery, signifying a role of local environmental conditions in structuring symbiont communities and thus heat stress response. Although no temporal shifts in symbiont assemblages were observed, comparisons with previous studies suggest thermal stress may have induced a switch to novel coral-microalgal combinations. Further, I identified symbiont types that are potentially diagnostic of bleaching susceptibility and could be developed into biomarkers or used to enhance coral thermal tolerance.

My thesis provides fundamental yet critical insight into the complex dynamics of coral recovery after thermal stress. Together, the results highlight the presence of intraspecific responses to bleaching and underscore the importance of accounting for multiple trajectories of individual species when projecting population recovery after disturbance.

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Introduction

Coral reefs in a rapidly changing ocean

Since the Industrial Revolution, atmospheric levels of carbon dioxide have soared by almost 70%, with no evidence for significant decreases in emission rate in the near future (Monastersky 2013). Consequently, average global temperature is projected to rise between 0.3 °C and 4.8 °C by the end of the 21st century, in addition to the 1 °C warming already realized since the mid-1700s. The oceans, which act as a sink for excess heat, are expected to increase between 0.2 °C and 3.5 °C by 2100, but local and regional increases and short-term anomalies may be even greater (IPCC 2019). Rising temperatures induce a variety of consequences that span multiple scales, from landscape-level impacts, such as alteration of oceanic currents (IPCC 2019), to community-level, such as shifts in the relative abundance of macroalgae in coastal ecosystems (Norström et al. 2009), down to molecular-level, including an increased production of harmful reactive oxygen species in marine biota (Hansel and Diaz 2021). As the impacts of global climate change cascade through the oceans, one of the most imperiled ecosystems is coral reefs.

Tropical scleractinian corals are ecosystem engineers responsible for building the complex, three-dimensional framework of coral reefs (Moberg and Folke 1999). Although coral reefs cover a mere 0.1% of the ocean floor and occur in nutrient-poor waters, they are staggeringly biodiverse: estimations suggest that they support about 25% of all marine species and may house upwards of 2.4 million species (Reaka-Kudla 1997; Knowlton et al. 2010; Mora et al. 2011). By maintaining such high levels of diversity, coral reefs are able to supply a myriad of ecosystem services, upon which human societies are heavily reliant (Chapin et al. 1997; Moberg and Folke 1999). Over one billion people worldwide depend on coral reefs as a source of food, income, coastal protection, and cultural value (Brander and van Beukering 2013; O'Mahoney et al. 2017; Nazarnia et al. 2020;

Grilli et al. 2021). However, coral reefs are under threat, as they face a range of local and global stressors unequivocally linked to anthropogenic activities (Riegl et al. 2009; Rockström et al. 2009; Smith et al. 2016).

Rapid increases in sea surface warming, human development and pollution, and overfishing have emerged as the three greatest threats to coral reef ecosystem structure, biodiversity, and function (Graham and Nash 2013; Hughes et al. 2017). Of these, temperature stress is the most well-studied and widespread (Heron et al. 2016). Thus far, 71% of subtropical and tropical reefs have experienced warming between 0.25°C and 0.75°C (Hughes et al. 2017). All organisms have a range of environmental conditions in which their performance is maximized and outside of which their tolerance and fitness decline (Hofmann and Todgham 2010). Tropical corals are particularly vulnerable to increases in ocean temperatures because they already live close to their upper physiological thermal limits (Lough et al. 2018). For corals, and other symbiotic cnidarians, under thermal stress, extension beyond this upper limit leads to physiological failure that may manifest as bleaching.

During bleaching, endosymbiotic dinoflagellates are expelled from their coral host into the surrounding seawater, resulting in a paling of the coral tissue to reveal the white skeleton underneath (Hoegh-Guldberg 1999). The first observation of coral bleaching occurred in the late 1920s on the Great Barrier Reef and was attributed to warm sea temperatures (Yonge and Nicholls 1931), but it did not become a common occurrence until the 1980s (Glynn 1991; Hughes et al. 2018). Since then, the risk of moderate to severe bleaching has increased by about 3.9% annually and is predicted to intensify in both frequency and severity (Hoegh-Guldberg 1999; Hughes et al. 2018). Although several abiotic factors can cause bleaching, including salinity changes, sedimentation, and high irradiation (Lesser 2011), elevated seawater temperatures are the primary

agent, particularly as it relates to climate change (Brown 1997). Acute or prolonged thermal stress of only 1 °C above the average temperature can be sufficient to trigger a bleaching response (Ainsworth and Gates 2016).

The consequences of coral bleaching can be severe, both for the coral host itself and the reef ecosystem as a whole. At the colony level, bleached corals are nutritionally compromised due to reduced photosynthetic efficiency from the loss of symbiont densities or function (Warner et al. 1996; Baird and Marshall 2002). They can also exhibit decreased growth, calcification rates, reproductive success, and recruitment rates (Ward and Harrison 2000; Carilli et al. 2009; D'Olivo and McCulloch 2017; Hughes et al. 2019). Bleaching can also have interactive effects with other stressors, such as disease, excess nutrients, acidification, and sedimentation. Combinations of these effects can be antagonistic, thus reducing the impacts of one stressor, but may alternatively be additive or synergistic, contributing to higher net negative effects on coral holobionts (Anthony et al. 2008; Carilli et al. 2009; Darling et al. 2010; Ban et al. 2014; Pendleton et al. 2016; Brodnicke et al. 2019). Because corals are foundational species for coral reefs, these consequences cause bottom-up ecological cascades. Bleaching can lead to phase shifts in which the reef becomes dominated by organisms more tolerant to thermal stress, like macroalgae or non-scleractinian anthozoans (Norström et al. 2009), rather than reef-building corals, resulting in an overall loss of reef structure and ecosystem function (Takeshita et al. 2016).

Post-bleaching drivers of coral recovery

Coral adults are sessile and thus cannot relocate when confronted with unfavorable environmental conditions (e.g., prolonged heat stress); instead, they must rely on other mechanisms to modulate the impacts of high temperatures, or else suffer potential dieback.

Sustained high temperatures in conjunction with bleaching are likely to result in coral mortality. However, if the temperature returns to normal within a sufficiently short period of time, endosymbiotic microalgae may repopulate the coral tissues, restore photosynthate translocation, and facilitate recovery (Stat and Gates 2011). Coral holobiont recovery, the recovery of the coral animal and its associated microbes, is a complex process mediated by several factors including host energy reserves, microalgal associations/interactions, heterotrophic feeding ability, epigenetic mechanisms, and transcriptional responses (Grottoli et al. 2006; Rodrigues and Grottoli 2007; Baker et al. 2008; Thomas et al. 2019; Hackerott et al. 2021). In this thesis, I will specifically focus on (1) host energy reserves and (2) microalgal associations, following a mass bleaching event. The former will be investigated in the context of host reproductive output, a crucial component for overall reef ecosystem recovery following disturbance.

Host energy reserves, reproduction, and their relationship

Corals are considered to be mixotrophic, obtaining energy through both host heterotrophy and symbiont autotrophy (although the relative contribution of these trophic strategies differs by species and location) (Radice et al. 2019). In healthy corals, the photosynthetic microalgae living in the coral tissues can provide up to 95% of the host's daily metabolic needs through the translocation of photosynthetically fixed organic compounds (Muscatine et al. 1981; Grottoli et al. 2006; Rädecker et al. 2015). Excess fixed carbon is stored in the host tissue as lipids, proteins, and carbohydrates (Grottoli et al. 2004). In bleached corals, however, decreases in algal symbiont densities result in a net loss of photosynthetic function and there is a considerable reduction in the amount of carbon provided to the host (Rädecker et al. 2021). To compensate for the energetic deficit, corals can catabolize their stored energy reserves (Grottoli et al. 2006; Schoepf et al. 2015).

Breaking down energy reserves can support corals for several months as they recover from bleaching stress; for instance, after 1.5 and 11 months of bleaching recovery, bleached *Porites astreoides* protein concentrations were 52% and 54% lower, respectively, compared to unbleached controls (Schoepf et al. 2015). *Montipora capitata,* on the other hand, does not heavily rely on energy reserves for bleaching recovery, emphasizing the species-specific nature of recovery strategies (Grottoli et al. 2004). Although the use of energy reserves can be a major contributor to coral holobiont stress recovery, it may incur a cost to reproduction.

The idea that organisms face trade-offs is a central tenet of physiological and evolutionary ecology (Tilman 2000). A classic example considers the trade-off between the size and number of eggs a fish can produce in one clutch: energetic limitations force the mother to "choose" whether to produce many small eggs, or fewer larger eggs. Energy or resources available to an organism are often limited and must be divided between various processes essential for survival and reproduction (Antonovics 1980). Corals that favor a strategy of consuming their energy reserves to survive bleaching conditions may then be expected to have less available for use in reproduction. Energy reserves play an essential role in determining coral reproductive output, as they are provisioned into developing gametes (Szmant and Gassman 1990; Michalek-Wagner and Willis 2001a). For example, in soft corals, individuals with larger energy reserves were found to contribute more proteins and lipids to developing gametes and thus produce larger oocytes (Michalek-Wagner and Willis 2001a). This relationship is particularly important in broadcast spawning coral species that produce lecithotrophic larvae, because the energetic contributions from the eggs serve as the primary energy source for planulae during dispersal (Harii et al. 2007). Reproductive trade-offs are well-documented in nature, but poorly characterized in corals (Stearns 1989; Fisch et al. 2019), particularly as they relate to bleaching. If bleaching forces a coral to tap

into its stored energy reserves, decreased energy reserves could result in smaller oocytes, fewer oocytes, or low larval survival (Jones and Berkelmans 2011; Graham et al. 2013), all of which may hamper reef recovery dynamics. The reproductive capacity of dominant reef building corals is of paramount importance for coral reef survival and recovery. Corals can reproduce sexually and asexually, but sexual reproduction is perhaps more important, as it both generates genetic diversity through recombination that may be beneficial in stressful environments and provides critical stock for degraded reefs (Harrison and Wallace 1990; Hughes et al. 2008; Edmunds 2018).

Symbiodiniaceae, their role in bleaching resilience, and conservation applications

The success of coral reefs in oligotrophic tropical waters is largely due to their symbiotic relationship with endosymbiotic dinoflagellates from the family Symbiodiniaceae (Muscatine and Porter 1977). The family Symbiodiniaceae consists of nine lineages, A-I, and its members associate with a suite of marine invertebrates, including corals, jellyfish, sponges, giant clams, and foraminiferans (LaJeunesse et al. 2018). Scleractinian corals, however, are only known to form symbioses with *Symbiodinium*, *Breviolum*, *Cladocopium*, *Durusdinium*, *Fugacium,* and *Gerakladium*, formerly clades A, B, C, D, F, and G, respectively (Coffroth and Santos 2005; LaJeunesse et al. 2018). Of these, *Cladocopium* is the most commonly found symbiont in Indo-Pacific corals (Baker 2003). Within a single genus, there is considerable genetic, ecological, and physiological diversity, and these factors influence their affinity for forming symbioses with certain coral hosts. Coral-associated Symbiodiniaceae communities, the total assemblages of all symbiont types present in a coral host, can consist of one or multiple symbiont types (Thornhill et al. 2009; Silverstein et al. 2012), but analyses using molecular markers, particularly the first and second internal transcribed spacer (ITS1 and ITS2, respectively) regions, indicate that corals are

generally dominated by a single Symbiodiniaceae type, with any other types present at background levels (Baker 2003; Ulstrup and Van Oppen 2003; Thomas et al. 2014).

Corals can broadly be grouped into two groups with respect to their endosymbiont communities: generalists and specialists (Putnam et al. 2012). Genera defined as generalists are flexible and can associate with an array of Symbiodiniaceae taxa. Specialists, on the other hand, show low flexibility and are observed to associate with only a few Symbiodiniaceae taxa (Putnam et al. 2012). (Note that symbionts can also be classified as generalists or specialists: generalists are found in many hosts, whereas specialists are restricted to a certain region or host taxon. Most symbionts are generalists (Baker 2003)). Symbiotic flexibility has been hypothesized to contribute to corals' ability to adapt to climate change, as greater opportunity to associate with diverse Symbiodiniaceae can result in adopting phenotypes that are locally adapted to specific habitats, and contribute to resilience in changing environments (Nyström 2006). This may be advantageous under stable conditions, but when confronted with prolonged stressful conditions, such as high temperatures or low pH, this pattern does not always hold. Instead, generalist coral species often exhibit higher environmental sensitivity, evidenced by more extensive bleaching and mortality, than specialist coral species (Hoegh Guldberg and Salvat 1995; Fabricius et al. 2011; van Woesik et al. 2011; Putnam et al. 2012). Competitive interactions between multiple symbiont types may destabilize and impair the symbiosis, leading to fitness consequences (Miller 2007; Kenkel and Bay 2018; McIlroy et al. 2020). However, symbiont specificity is not absolute: one study detected multiple Symbiodiniaceae types in 26 species of corals considered to be specialists (Silverstein et al. 2012). This highlights the complexity of coral-algal symbioses and the need for more studies across a range of spatial and temporal scales in many coral species.

The discovery that many coral species can associate with multiple Symbiodiniaceae taxa prompted the introduction of the adaptive-bleaching hypothesis, which posits that the proportional abundance of microalgal endosymbionts within the coral tissues can shift during or after bleaching to favor types that are more tolerant to thermal stress (Buddemeier and Fautin 1993). This shift can occur through "shuffling" of symbiont taxa already present in the coral host or uptake of symbiont cells in the surrounding environment ("switching") (Kinzie et al. 2001; Berkelmans and van Oppen 2006; Jones et al. 2008; Cunning et al. 2015; Boulotte et al. 2016). For example, an increase in the proportional abundance of *Durusdinium* symbionts has been implicated in higher coral host heat tolerance and bleaching resistance. In the Great Barrier Reef, *Acropora millepora* from a cool offshore reef hosted *Cladocopium* type C2, but after experimental transplantation to a warmer inshore reef, they recovered from bleaching with *Durusdinium* and exhibited a 1.0-1.5 °C increase in thermal tolerance compared to control corals that were not transplanted (Berkelmans and van Oppen 2006). In Panama, a survey of ecologically dominant corals found that colonies containing *Durusdinium* resisted bleaching during the 1997 El Niño-Southern Oscillation, while those dominated by *Cladocopium* bleached severely. Upon resurveying the reef three years later, the percentage of colonies containing *Durusdinium* had increased from 43% to 63% (Baker et al. 2004). Despite the compelling evidence that symbiont shuffling or switching may be an adaptive measure to survive bleaching events, there are also many instances of no alteration in dominant symbiont type as a response to thermal stress or bleaching (Goulet 2006; Sampayo et al. 2008; Thornhill et al. 2009; Smith et al. 2017). Therefore, assessing coral microalgal assemblages and their capacity to exhibit temporal changes is a crucial component in understanding the coral holobiont's ability to adapt and/or acclimate to increasing ocean temperatures.

Because the sensitivity and resiliency of reef-building corals to temperature stress is tightly linked to their associations with Symbiodiniaceae, considerable effort has been made to investigate symbiont communities' application to coral reef conservation, restoration, and management (van Oppen et al. 2015; Quigley et al. 2018; Caruso et al. 2021). Many approaches take advantage of naturally occurring variation within a population to select coral individuals with heat-tolerant symbionts for nursery rearing and subsequent outplanting (Morikawa and Palumbi 2019). Although this can be effective in promoting reef resilience, the current rapid pace of temperature increases necessitates the implementation of further interventions, such as assisted evolution, which can involve active manipulations of coral symbiont communities (Anthony et al. 2017; Suggett and van Oppen 2022). These techniques are in their infancy, but already show some promise. For example, inoculation of conspecific corals with distinct symbiont types or from disparate thermal environments produced holobionts with different thermal tolerances (Mieog et al. 2009; Howells et al. 2012). Several studies have also shown that symbiont thermal tolerance can be augmented in culture through natural selection via thermal, radiation, or chemical stress (van Oppen et al. 2015; Chakravarti et al. 2017; Buerger et al. 2020), and that this change can occur relatively quickly (within 2.5 years) (Chakravarti et al. 2017). When reintroduced to host larvae, corals exhibited an increased bleaching threshold (Buerger et al. 2020). Further, Symbiodiniaceae probiotics may be administered to promote bleaching resilience: when experimentally bleached *Acropora millepora* fragments were subjected to *Cladocopium goreaui* probiotics, they recovered significantly more quickly than control fragments, likely due to heterotrophic nutritional supplementation (Morgans et al. 2020). Although there are limitations and challenges involved in manipulating symbioses, including physiological trade-offs that may result from new partnerships, host-symbiont genotypic compatibility, ethical questions, and

scalability (Quigley et al. 2018; Epstein et al. 2019; Parkinson et al. 2019), the rapid rate of adaptive evolution in Symbiodiniaceae makes them an attractive avenue for potential use in restoration (Quigley et al. 2018). However, the long-term stability of these methods is unknown and future work will have to investigate their application in a wide range of locations and coral taxa to assess their feasibility (Epstein et al. 2019).

Study system and thesis goals

Mo'orea, French Polynesia is a small volcanic island located in the Southern Pacific Ocean. Since 2004, the coral reef complex that surrounds the 60 km island perimeter has served as the site of a Long-Term Ecological Research (LTER) program, providing scientists with an unparalleled opportunity to study the biological and physical processes that shape coral reef communities. Shortly after the establishment of the program, Mo'orea was impacted by a series of natural disturbances, including a crown-of-thorns sea star outbreak in 2007 and Cyclone Oli in 2010, allowing researchers to explore the mechanisms involved in resilience after disturbance. Coral cover on the forereef was drastically reduced as a consequence of these events, but recovered quickly in some areas due to high coral recruitment (Holbrook et al. 2018).

In the austral summer of 2019, another acute disturbance struck the island. From December 2018 to May 2019, Mo'orea experienced a prolonged marine heatwave in which sea surface temperatures remained above the 29 °C thermal stress accumulation threshold for several months (Pratchett et al. 2013). This thermal anomaly was one of the most intense occurrences recorded for the island in the last 30 years and resulted in extensive mass bleaching and coral mortality (Speare et al. 2021). Upwards of 100% of *Acropora* colonies at some sites bleached or died (Speare et al. 2021). Despite this widespread bleaching response, some colonies resisted bleaching entirely,

while others began showing signs of recovery by August 2019, suggesting that these corals harbor mechanisms to persist and recover following extreme thermal stress. The two heat stress responses, resistance and recovery, exemplify coral plasticity to environmental disturbances and provide a natural experiment that can be harnessed to investigate questions about these mechanisms and their effects.

Acropora is one of the most abundant genera of reef-building corals in the Indo-Pacific Ocean (Veron 2000). Coral reefs in Mo'orea are dominated by *Pocillopora* and *Acropora,* in the latter particularly the cosmopolitan species *Acropora hyacinthus* (Gleason 1993). Branching, fast-growing coral species, like *A. hyacinthus*, are disproportionately vulnerable to and affected by thermal and bleaching stress (Gleason 1993; McClanahan et al. 2004; Putnam et al. 2012). Because of their importance as reef framework builders, any factor that results in declines in their growth, health, or abundance as a result of climate change-induced bleaching may lead to a loss of structural complexity and/or biodiversity, and hinder overall ecosystem recovery between disturbances (Alvarez-Filip et al. 2009). Therefore, the survival and recovery strategies *A. hyacinthus* adopted during the 2019 mass bleaching event are of significant importance for the future of coral reef ecosystems, not only in Mo'orea, but also in the greater Indo-Pacific basin.

My thesis aims to (1) investigate the potential for reproductive trade-offs associated with host energetics and (2) assess the role of Symbiodiniaceae community composition in host resistance and recovery from heat stress in *Acropora hyacinthus* following the 2019 thermal bleaching event in Mo'orea. A recent study estimated that the bleaching event decreased *Acropora spp.* fecundity by 64% (Speare et al. 2021), but no research has specifically investigated this question in *A. hyacinthus* or in the two disparate heat stress responses, and few studies illustrate the link between host energetic reserves and reproduction. Another recent study demonstrated that

in 2013 the majority of *A. hyacinthus* colonies in the backreef and forereef were dominated by *Cladocopium,* with a small proportion being dominated by *Symbiodinium* (Kriefall et al. 2022), but no study has assessed the extent to which the relative abundances of Symbiodiniaceae genera were perturbed by mass bleaching, their spatial and temporal flexibility, and their relationship to the host heat stress response. Understanding sublethal effects of bleaching and mechanisms that promote resistance or resilience in corals, and how they impact reproductive output, is imperative to better predict the net population and ecosystem level effects of rising ocean temperatures, especially as bleaching is predicted to become more frequent in the future, with less time between consecutive bleaching events (Hughes et al. 2018).

Chapter 1

Energetic and reproductive costs of coral recovery in divergent bleaching responses

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Introduction

Coral reefs worldwide face unprecedented levels of stress caused by anthropogenic climate change. Elevated sea surface temperatures that trigger mass bleaching events are widely regarded as the greatest threat to coral reefs because they cause substantial coral mortality and threaten the persistence of corals as ecologically relevant framework builders (Alvarez-Filip et al. 2009; Hughes et al. 2017). Coral bleaching events are projected to increase in both frequency and severity in the near future (Hughes et al. 2018); hence, there is an urgent need to understand the consequences of recovery from climate change-induced temperature stress on coral physiology and reproduction in order to more accurately predict future population and community dynamics. Thermal stress is a major contributor to declines in coral cover (Halpern et al. 2008) and accordingly many studies on the impacts of coral bleaching have focused on mortality (Loya et al. 2001; Anthony et al. 2009a; Depczynski et al. 2013). However, sublethal effects, particularly on reproduction, may play an important role in overall reef recovery following bleaching events because surviving colonies will populate the next generation of coral recruits (Edmunds 2018; Richmond et al. 2018). Further, as the incidence of marine heat anomalies increases globally (Hughes et al. 2018; Oliver et al. 2021), colonies that survive mass bleaching events may experience sublethal bleaching multiple times within their lifespan, warranting additional study on the sublethal impacts of bleaching on corals and reef resilience.

Reproduction, and ultimately fitness, is fundamentally influenced by the energetic condition of the coral holobiont (Rinkevich 1989; Lesser 2013). The majority of corals' daily

energy requirements are met using photosynthetically fixed compounds translocated from their endosymbiotic microalgae (Muscatine et al. 1981). During bleaching, this symbiotic relationship destabilizes, resulting in a considerable reduction in the amount of carbon provided to the host (Rodrigues and Grottoli 2007; Rädecker et al. 2021). To compensate for the energetic deficit, corals must either increase heterotrophic feeding or catabolize stored energy reserves to meet their metabolic needs (Grottoli et al. 2006; Schoepf et al. 2015). Under prolonged stress conditions, such as a severely bleached state, there are finite resources available that the coral must allocate to physiological processes such as tissue maintenance, defense, and reproduction (Leuzinger et al. 2012). Energy would likely be allocated towards one of these strategies that facilitate colony recovery (i.e., heterotrophic feeding or consuming energy reserves), rather than to non-essential life functions; this potentially limits energy diverted towards gamete production (Oren et al. 2001; Fisch et al. 2019).

Bleaching can induce profound negative effects on coral reproductive output, some of which may persist for multiple spawning seasons (Ward et al. 2002; Levitan et al. 2014; Johnston et al. 2020). Bleaching events can lead to reductions in the percent of colonies that spawn within a population, and colonies that do spawn produce fewer gametes (Ward et al. 2002). Heat stress has been linked to decreased energy reserves and consequently reduced fecundity and size of lipid-rich, energetically costly oocytes (Szmant and Gassman 1990; Jones and Berkelmans 2011; Figueiredo et al. 2012). Colonies that undergo bleaching also display smaller spermary size and abundance and impaired sperm motility, although these negative effects on sperm persist for a shorter duration than those on oocytes (Hagedorn et al. 2016; Johnston et al. 2020). However, the reproductive costs associated with coral bleaching are species-specific and related to the severity of the heat stress, highlighting the need for further investigation into reproductive output following

bleaching across many species and locations (Michalek-Wagner and Willis 2001b; Howells et al. 2016; Godoy et al. 2021). What is not entirely reconciled is to what degree the bleaching response, as opposed to the heat stress itself, is responsible for these reproductive effects, and whether differential intraspecific bleaching responses have reproductive – and ultimately demographic – implications.

Here, we examined the impact of thermal bleaching stress on stored energy reserves and reproductive output, two parameters which are critical for coral community recovery, in the tabular coral *Acropora hyacinthus,* one of the key reef-builders in the Indo-Pacific Ocean (Veron 2000). From December 2018 to May 2019, the island of Mo'orea, French Polynesia experienced a massive heat anomaly in which sea surface temperatures were sustained above 29 °C, the noted thermal stress accumulation threshold for corals in Mo'orea (Pratchett et al. 2013), for a total of 115 days over a period of 139 days (Figure 1a). The heatwave resulted in one of the most severe mass bleaching events ever recorded for the island. At the most highly impacted sites, >80% of *Acropora spp.* colonies were bleached or dead in July 2019 (Speare et al. 2021). Despite widespread coral bleaching and mortality, recovery following the bleaching event was observed (Figure 1b). There was also colony-level variability in the prevalence and severity of bleaching, including individuals that never showed any visual signs of bleaching ('resistant' colonies, Figure 1c). In contrast, some colonies that were severely bleached in May showed visual signs of symbiont recovery by August and full recovery by October 2019 ('recovered' colonies, Figure 1d). These two types of colonies (resistant vs. recovered) provide a natural experiment to better understand the reproductive consequences of bleaching in *Acropora hyacinthus* colonies showing different heat stress responses. Specifically, we postulated that (a) resistant colonies would have higher stored energy reserves than colonies that bleached and later recovered, (b) resistant colonies

would be more likely to harbor developing gametes, (c) oocyte production would be more negatively impacted in colonies with prior bleaching, and (d) resistant colonies would produce more oocytes per polyp than recovered colonies. Mo'orean reefs have a history of recovery from disturbance: while coral reef community recovery is a function of multiple processes including coral recruitment, growth, survival of recruits, and regrowth of surviving colonies (Holbrook et al. 2018), an important step in assessing possible resilience is examining reproductive potential (i.e., the ability of a colony to generate reproductive output) of surviving colonies, which we consider in this study.

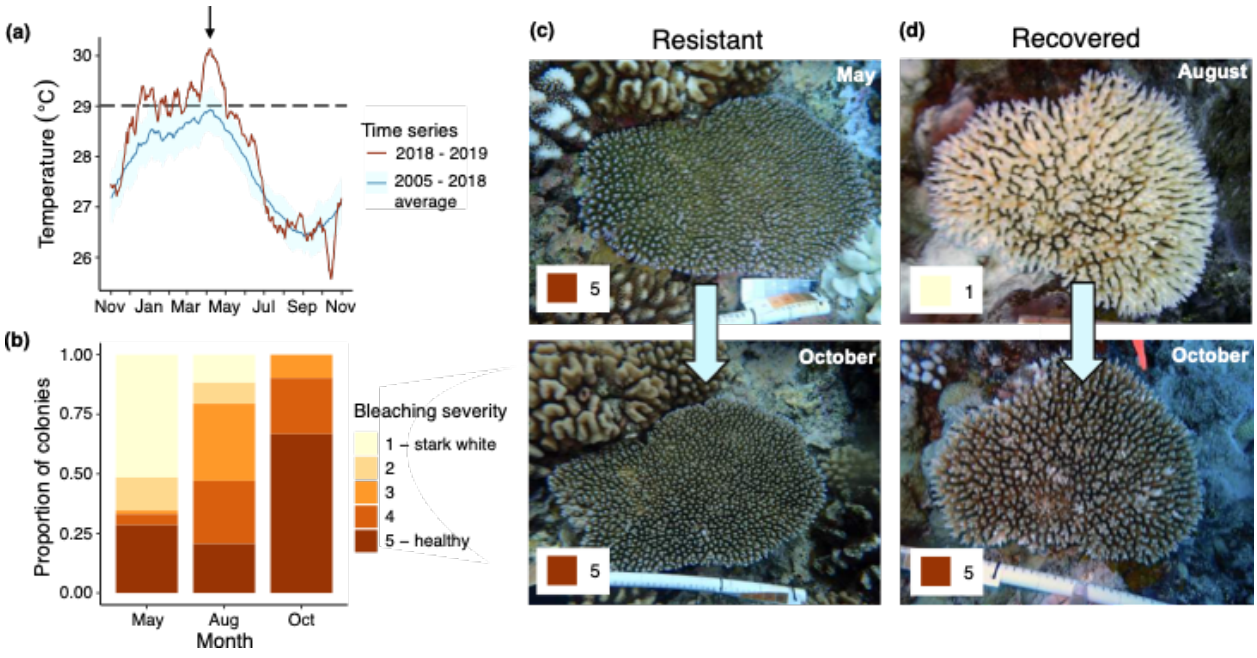


Figure 1. The 2019 bleaching event in Mo'orea, French Polynesia. **(a)** Average sea surface temperatures over 13 years (blue line) and observed sea surface temperature from November 2018 – October 2019 (red line) at all LTER sites in Mo'orea. Dashed black line indicates the bleaching threshold for corals in Mo'orea. Heat anomaly and related bleaching event indicated with black arrow. **(b)** Proportion of surveyed colonies displaying various bleaching severities. Colonies were surveyed at the height of the bleaching event in May (N = 131), after three months of recovery in August (N = 34), and after five months of recovery in October (N = 51). **(c)** A colony resistant to bleaching photographed in May and October with associated bleaching scores. **(d)** A colony that bleached during the bleaching event in May and showed signs of recovery in August, and later recovered by October with associated bleaching scores.

Methods

Study site and sampling scheme during and after mass bleaching *in situ*

Mo'orea, French Polynesia is a volcanic island with a ~60 km perimeter in the South Pacific Ocean. The island is surrounded by a barrier reef system with lagoons up to 1.3 km in width, surrounded by a reef crest and forereef habitat. The forereef, where we conducted our study, maintained ~47% live coral cover (mean of six outer reef sites at 10m depth) as of January 2019 (Edmunds 2020). We conducted our study at one site located on the north shore of Mo'orea (17.4731° S, 149.8177° W; Figure S1). Individual *Acropora hyacinthus* coral colonies were observed, photographed, and tagged between 5-14 m depth on the forereef in May 2019, at the height of the mass bleaching event. At ~14 m, 59/111 tagged colonies were observed in a severe state of bleaching and 52/111 were partially bleached, while at ~5 m 16/52 colonies were severely bleached, with the remaining 36/52 maintaining a visibly healthy state. By August 2019, all the previously tagged colonies at ~14 m depth had died. Although mortality at ~14 m was high, in August, 25 additional (previously untagged) colonies were observed to be visibly recovering from bleaching (Figure 1b). These recovering colonies were photographed and individually tagged. We note that survey timing during extended thermal stress events, such as the one investigated here, can influence perceptions of coral susceptibility to bleaching (Claar and Baum 2019). However, because of the high prevalence of bleaching at this site and depth during May (53.2% severely bleached and 46.8% partially bleached), we are confident that these previously untagged colonies were bleached during the bleaching event. In October 2019, 30 and 28 previously tagged colonies at ~5 and 14 m, respectively, were found, photographed, and sampled via SCUBA for physiological metrics and/or reproductive histology (see Table S1 for full sample details). For all corals sampled, bleaching severity and colony area were determined using standardized

photographs. Each colony was assigned a score from 1-5 according to the bleaching severity the colony experienced, with a 1 indicating stark white bleaching and a 5 indicating no visible bleaching (Figure 1b). Colony area was estimated by tracing the outline of each colony and calculating the planar surface area using ImageJ (Schneider et al. 2012).

Water temperature data (Figure 1a) were collected as part of the Mo'orea Coral Reef Long Term Ecological Research (MCR LTER) time series data collection (Leichter et al. 2020). Data were collected at six MCR LTER sites at 10 m depth on the forereef using bottom-mounted thermistors (Seabird SBE 39) that recorded the water temperature every 20 minutes. To evaluate long-term temperature trends on the outer reef we considered data through October 31, 2018. We first calculated the average temperature at each site for each day of the time series. We then used the daily site average to calculate the average water temperature across all sites ± one standard deviation for each day in a 365-day year. We used the same approach to calculate the average daily temperature for late 2018 – 2019 using data from November 1, 2018 – October 31, 2019.

Physiological condition of corals following mass bleaching

In tagged coral colonies that were resistant to or recovered from bleaching, the energetic condition of the host was assessed ($N_{resistant} = 12$, $N_{recovered} = 20$). One small branch (~2-4 cm length) was sampled from each colony and airbrushed in filtered seawater to remove coral tissue and algal cells (blastate) from the skeleton. The blastate was homogenized and 200 µL was collected and preserved in Z-fix (10% zinc formalin) for algal symbiont counts. The remaining blastate was centrifuged at 2,000 g for 2 minutes to separate the host tissue from endosymbiont cells. Host tissue slurry was preserved at -20 °C until further processing. Microalgal endosymbiont density was quantified using a hemocytometer (Hausser Scientific, Horsham, PA) under an Olympus BH-

2 microscope. Total host protein content was quantified using a Bradford assay (Bradford 1976) with bovine-serum albumin (BSA) as a standard (Pierce Coomassie Plus Assay Kit, Thermo Fisher Scientific). Briefly, host tissue homogenate was diluted 10x and triplicate 100 µL aliquots were loaded onto a 96-well plate and mixed with 200 µL of Bradford reagent. After a 10-minute incubation period at room temperature, absorbance at 595 nm was recorded with a microplate reader (BioTek PowerWave XS). Sample protein concentrations were calculated using a standard curve of BSA ranging from 0 to 120 µg/mL. Total host carbohydrate content was quantified using a modified phenol-sulfuric acid method (Dubois et al. 1955; Masuko et al. 2005). Triplicate 50 µL aliquots of host tissue homogenate were loaded onto a 96-well plate and mixed with 150 µL of concentrated sulfuric acid immediately followed by 30 µL of 5% phenol. Samples were incubated at 90 °C for 5 minutes and then allowed to cool. Absorbance was measured at 490 nm with a microplate reader and sample carbohydrate concentrations were determined using a standard curve of dextrose ranging from 0 to 3500 µg/mL. All physiological metrics were standardized to coral skeleton surface area following the paraffin wax-dipping technique (Stimson and Kinzie 1991).

Reproductive histology

Small fragments from tagged colonies were sampled by hand via SCUBA ($N_{resistant}$ = 26, $N_{recovered}$ = 21) in October 2019, the start of the typical spawning season for *A. hyacinthus* (Carroll et al. 2006). For each colony, the selected fragment was sampled 5-10 cm from the colony edge, and branch tips and colony edges were avoided. Samples were immediately preserved in Z-fix for 24 hours and then stored in 100% ethanol until histological processing. Samples were decalcified with a 1% EDTA decalcifier solution for 48-72 hours and stored in 70% ethanol until processing on a Leica ASP6025 tissue processer. Paraffinized tissue was embedded in wax blocks (Leica

EG1150H embedding machine) and then allowed to cool in a freezer 24 hours prior to sectioning. Blocks were serially sectioned at 5 μm thickness on a Leica RM2125RTS microtome every 300 μm, which corresponds to the average oocyte diameter. Sections were arranged on microscope slides and stained using a modified Heidenhain's aniline blue stain on a Leica ST5020 multistainer.

Histological sections were analyzed for measurements of reproductive effort: (1) presence/absence of male and female gametes, (2) diameter of oocytes, and (3) relative fecundity, detailed below. Gametes (oocytes and spermatocytes) were staged from I-V following the classification of Szmant-Froelich et al. (Szmant-Froelich et al. 1985). Slides were examined using an Olympus BX41 microscope with an Olympus SC180 camera attachment. Measurements were made using ImageJ (Schneider et al. 2012). Oocyte diameter was determined by averaging the longest and shortest axis of each oocyte. A total of 25 oocytes were measured from each colony. In fragments containing fewer than 25 oocytes, the maximum number of oocytes observed was used (Table S1). Only oocytes with a visible nucleus were measured to ensure no oocytes were counted more than once and that the maximum diameter was measured.

Due to the small size of the fragments and polyps, as a proxy for fecundity, three polyps were randomly selected on the middle slide from each individual. When there was an even number of slides, the first of the two middle slides was used. Because only one slide from each individual was examined, there was no risk of double-counting oocytes, so the number of both nucleated and non-nucleated oocytes was counted in each of the randomly selected polyps. These counts were averaged to produce the average number of oocytes per polyp for each individual as a measure of relative fecundity. It should be noted that this relative estimate is lower than true fecundity.

Statistical analyses

All statistical analyses were implemented in R (V. 4.0.3). To determine how symbiont density and protein and carbohydrate content were impacted by bleaching history, a categorical linear regression was used. Bleaching history was defined as being either 'resistant' or 'recovered'. Bleaching history was highly collinear with depth. Although depth was non-significant in all models, we chose to include it to control for the effects of depth on heat stress response. A mixed-effects model was employed to examine whether oocyte diameter was influenced by bleaching history, with fixed effects of bleaching history and depth and a random effect of colony identity to account for repeated measures. A linear regression was performed to assess the relationship between colony area and relative fecundity for each heat stress response.

The remainder of statistical analyses on the histological measurements were performed using generalized linear models (GLMs). To determine if recovered and resistant colonies differed in displayed oocyte and spermatocyte stages, log-linear models with a Poisson distribution were used with gamete stage, bleaching history, and depth as fixed effects, an interaction effect between gamete stage and bleaching history, and a random effect of colony identity. Oocyte and spermatocyte stages were analyzed separately. To determine if bleaching history affected the probability a colony contained gametes, a logistic regression was used with the binomial response variable being whether the gamete of interest was observed in the colony. Depth was included as a fixed effect and the presence of oocytes and spermatocytes were analyzed separately. A Poisson regression was utilized to examine how relative fecundity differed between bleaching histories and depth. We also used a Poisson regression to determine the effect of bleaching history and colony size on relative fecundity. The interaction term between colony size and bleaching history was

found to be non-significant and was removed from the model. All model outputs and results are listed in Table S2.

Results

Energetic condition of resistant and recovered corals differed after recovery

Five months after the peak of the thermal anomaly and bleaching event, *Acropora hyacinthus* colonies in the field were categorized as dead, recovered, or resistant to bleaching (Figure 1, Table S1). Endosymbiont density did not differ significantly between resistant and recovered colonies (p = 0.96; Figure 2a), which were visually healthy (Fig 1c, d). Energetic condition was assayed in these colonies; total protein concentration and total carbohydrate concentration differed significantly between resistant and recovered corals. Resistant colonies had, on average, 123.09 µg/cm^2 higher protein concentrations compared to resilient corals (46.55 – 199.63, 95% CI, p = 0.0026; Figure 2b). They also had 250.48 µg/cm^2 higher carbohydrate concentrations (8.07 – 492.90, 95% CI, p = 0.043; Figure 2c).

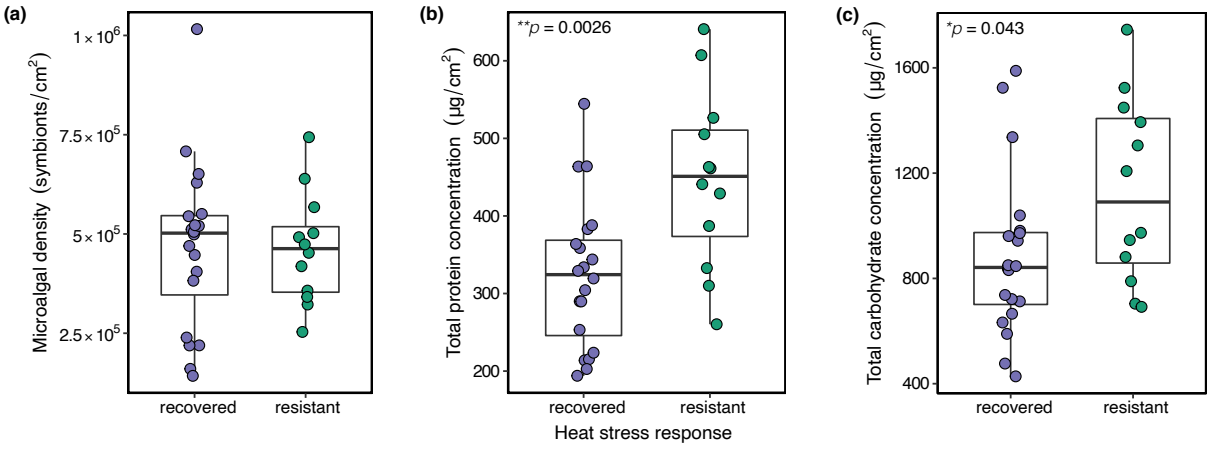


Figure 2. Endosymbiont density and energetic condition of recovered and resilient corals five months after the mass bleaching event. **(a)** Microalgal counts normalized to host surface area. **(b)** Total protein content normalized to host tissue surface area. **(c)** Total carbohydrate content normalized to host tissue surface area. Each data point represents a single colony.

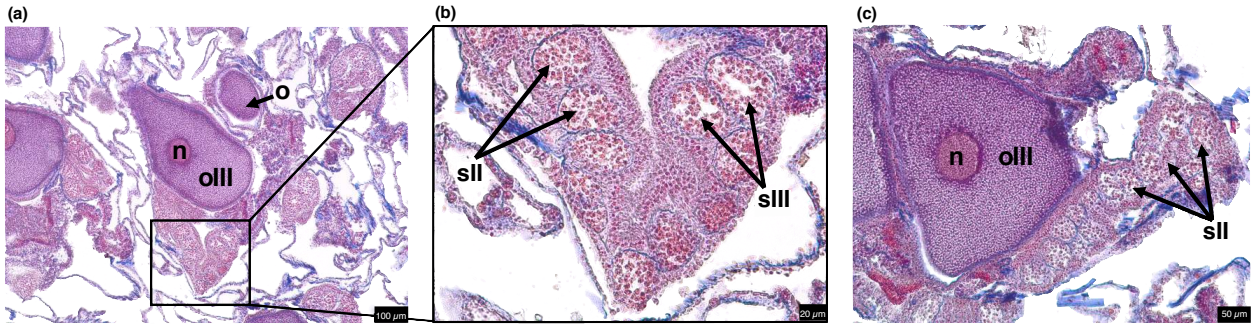


Figure 3. Histological sections of gravid A. hyacinthus. Scale bar represents **(a)** 100 µm, **(b)** 20 µm, and **(c)** 50 µm, respectively. n, nucleus; o, oocyte; oIII, oocyte stage III; sII, spermary stage II; sIII, spermary stage III.

Bleaching resistant corals were more likely to harbor mature gametes compared to recovered corals

To investigate long-term impacts on fitness between the two heat stress responses, we performed reproductive histology on colonies collected in October 2019 (Figure 3), which falls within the typical spawning season for *Acropora hyacinthus* (Carroll et al. 2006). We observed a strong difference in reproductive potential between resistant and recovered coral colonies (Figure 4). A total of 24 out of 26 (92.31%) resistant colonies contained gametes, while only 8 out of 21 (38.10%) recovered colonies contained gametes. Spermatocytes were observed in 24 out of 26 (92.31%) resistant colonies, but only 8 out of 21 (38.10%) recovered colonies (Figure 4a). Oocytes were observed in 24 out of 26 (92.31%) resistant colonies and in 7 out of 21 (33.33%) recovered colonies (Figure 4b). The probability of a colony containing spermatocytes or oocytes varied significantly with heat stress response. Resistant colonies were 36.00 times more likely to contain both spermatocytes and oocytes compared to recovered corals (3.13 – 978.89, 95% CI, $p = 0.0089$; Figure 4c).

Gamete stage and oocyte diameter did not differ between resistant and recovered corals

Among colonies containing spermatocytes, there was no significant difference in observed spermatocyte stages between recovered and resistant corals (p = 0.84). The majority of spermatocytes (686/723, 94.88%) were documented in stage II, with resistant and recovered colonies displaying 98.28% (514/523) and 86% (172/200) of spermatocytes in stage II. The remaining spermatocytes were observed to be in stage III.

Among colonies containing oocytes, there was no significant difference in oocyte size between resistant and recovered corals (p = 0.81; Figure S2). The average oocyte diameter for resistant colonies (N = 437) was 312.03 µm, and 309.75 µm for recovered colonies (N = 94). There was no significant difference in oocyte stages between the two heat stress responses (p = 0.99). The majority of oocytes (525/537, 97.77%) were observed in stage III, with resistant and recovered colonies displaying 97.75% (434/444) and 97.85% (91/93) of oocytes in stage III, respectively. The remaining oocytes were observed to be in stage IV.

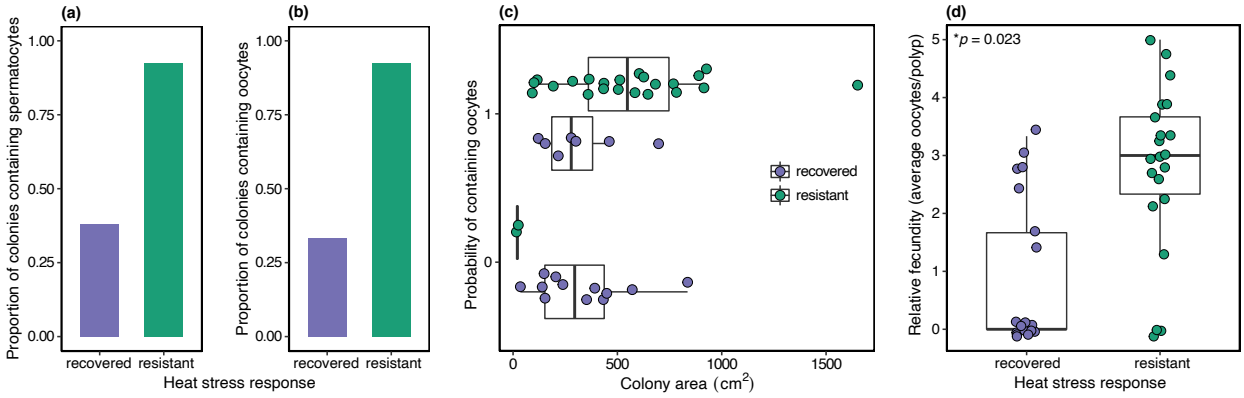


Figure 4. Differences in reproductive output between resistant and recovered coral colonies. Proportion of recovered and resistant colonies containing **(a)** spermatocytes and **(b)** oocytes. **(c)** Probability of containing oocytes for recovered and resistant colonies over the range of colony area. 0 means a colony did not contain oocytes. 1 means a colony contained oocytes. **(d)** Relative fecundity in recovered and resistant colonies. Each data point represents one colony.

Relative fecundity was higher in resistant corals

To further understand the influence of the observed heat stress responses on fitness, we measured relative fecundity for each colony. On average, resistant colonies exhibited a relative fecundity of 2.78 (± 1.45, SD) oocytes per polyp, while recovered colonies exhibited a much lower relative fecundity of only 0.81 (± 1.24, SD) oocytes per polyp. Resistant corals produced 4.17 times as many oocytes compared to recovered corals (1.47 – 18.94, 95% CI, $p = 0.023$, Figure 4d). Although colony area was associated with higher relative fecundity, this overall trend was not significant ($p = 0.11$, Figure S3). For resistant colonies, colony area had a marginally significant positive correlation with relative fecundity ($r^2 = 0.18$, $p = 0.052$). For recovered colonies, colony area and fecundity showed no significant relationship ($r^2 = 9.43 \times 10^{-6}$, $p = 0.99$).

Discussion

Energy reserves represent an important currency for physiological performance, including processes such as growth, maintenance, and reproduction, all of which are tightly intertwined with organism survival and fitness (Leuzinger et al. 2012). Following severe stress events, individuals may allocate these energy reserves towards recovery rather than reproduction, delaying population recovery after disturbances with high mortality (Tsounis et al. 2012; Edmunds 2018). Here, we examined the energetic condition and reproductive effort in *Acropora hyacinthus* individuals showing heat stress responses of resistance and recovery. Despite both responses maintaining healthy appearances and high endosymbiont densities five months post-bleaching (Figure 2a), our findings reveal divergent physiological responses that were not visually detectable during the October field surveys (Figure 2b, c). Bleaching stress also resulted in compounding impacts on reproduction in recovered colonies. Not only were recovered colonies less likely to contain

gametes than their resistant counterparts, but they also exhibited lower fecundity per polyp (Figure 4). Taken together, our study suggests that bleaching imposes an energetic constraint on both stress recovery and gamete production in *A. hyacinthus*, reflecting energy allocation strategies among processes, which may hamper reef recovery from disturbance.

Following the 2019 bleaching event in Mo'orea, visual colony recovery was a poor indicator of the coral animal's energetic state. This is consistent with previously published studies which show quick, short-term recovery of photopigmentation or endosymbiont concentrations, but much longer recovery times for host energy reserves (Rodrigues and Grottoli 2007; Schoepf et al. 2015; Wall et al. 2019). For example, an experimental bleaching study in *Porites compressa* found that although healthy pigmentation returned within 1.5 months of recovery, energy reserves (i.e., protein, carbohydrate, and lipid concentrations) remained depressed until 8 months of recovery (Rodrigues and Grottoli 2007). We observed complete visual and endosymbiont recovery of bleached colonies within five months of the bleaching event (Figure 2a); however, their energy reserves were still depleted. Protein and carbohydrate levels were 27.5% and 22.1% lower, respectively, than resistant colonies (Figure 2b, c). This indicates that previously bleached colonies are catabolizing protein and carbohydrate reserves during or after the bleaching event, and demonstrates that lipid reserves are not the sole metabolite pool drawn from during stress recovery, as is commonly assumed for corals (Lesser 2013; Jung et al. 2021). Although endosymbiont populations had evidently recolonized the coral tissues following bleaching, the translocation of photosynthates from symbiont to host may not have been fully restored (Tremblay et al. 2016), forcing the coral to catabolize energy reserves to fulfill its metabolic requirements. Past studies have shown that it can take more than 11 months of recovery to reestablish pre-bleaching levels of nutrient transfer from symbionts to the coral host and to replenish host energy reserves (Baumann

et al. 2014; Schoepf et al. 2015). It is currently unknown how long it takes *A. hyacinthus* to accumulate lost energy reserves after bleaching, but our study clearly shows that it requires greater than five months. With the threat of annual bleaching looming in the future (Hughes et al. 2018), failure to fully recover energy reserves within a year could compromise corals' ability to effectively cope with further stressors (Anthony et al. 2009b; Grottoli et al. 2014), leading to reduced reproductive output (Ward et al. 2002; Fisch et al. 2019; Johnston et al. 2020).

Energy reserves, particularly lipids, play an essential role in determining coral reproductive output and larval survival (Graham et al. 2013; Fisch et al. 2019). Lipids compose up to 86% of oocyte biomass in broadcast spawners (Figueiredo et al. 2012), such as *A. hyacinthus*. During gametogenesis, stored energy reserves are provisioned to the developing gametes (Szmant and Gassman 1990; Michalek-Wagner and Willis 2001a) and they serve as the primary energy source for planulae during dispersal (Harii et al. 2007). The gametogenic cycle for *A. hyacinthus* lasts for approximately nine months. Oogenesis initiates about six months prior to spermatogenesis and both male and female gametes reach maturity synchronically (Wallace 1985). Spawning for this species in Mo'orea typically occurs in October to November (Carroll et al. 2006), meaning that oogenesis for the colonies in our study likely initiated around January 2019, one month after the onset of the thermal anomaly and four months before bleaching was first observed in April. Thus, corals underwent much of gametogenesis under thermal stress, with the bleaching event starting around month four of oogenesis and two months before the beginning of spermatogenesis. Because energy reserves are consumed as a recovery response to bleaching stress (Schoepf et al. 2015), this incurs a significant cost to resources that would normally be allocated to gametogenesis (Michalek-Wagner and Willis 2001a; Howells et al. 2016), which could explain the decrease in fecundity and gamete production we observed in colonies that bleached but later recovered (Figure 4). The

importance of energy reserves for oocyte production is well-known (Ziegler and Ibrahim 2001; Jones and Berkelmans 2011; Baliña et al. 2018), but no such link has been established for sperm in corals. Our study provides evidence that depleted energy reserves may hinder both oogenesis and spermatogenesis, a process generally relegated as energetically inexpensive.

Oocyte size is a common metric used to assess the quality of reproductive output because of its positive relationship with fertilization success, postzygotic survival, and maternal investment (Levitan 2006; Caballes et al. 2016). Oocyte size is an indicator of maternal condition; individuals with larger energy reserves are able to provision more proteins and lipids to their oocytes and thus produce larger oocyte sizes (Michalek-Wagner and Willis 2001a; Caballes et al. 2016). A decrease in oocyte size is often observed as a consequence of severe stresses such as coral bleaching (Ward et al. 2002; Johnston et al. 2020; Godoy et al. 2021), yet we found remarkably similar distributions and averages of oocyte sizes between resistant and recovered colonies (Figure S2), despite clear differences in gamete presence. A study of *Acropora millepora* demonstrated that under thermal stress, colonies maintained oocyte sizes across bleaching phenotypes, but produced fewer oocytes compared to when they reproduced under non-bleached conditions (Jones and Berkelmans 2011). Because oocyte size is correlated with maternally provisioned lipids, this response is hypothesized to ensure adequate energy is available for all of the now limited number of oocytes to survive through settlement (Jones and Berkelmans 2011). Since we observe a similar phenomenon to this study, it is possible that the strategy of producing fewer high quality oocytes over more low quality oocytes is specific to *Acropora,* as opposed to other coral genera where this strategy has not been observed (Ward et al. 2002; Johnston et al. 2020; Godoy et al. 2021). In *A. hyacinthus,* bleaching stress appears to induce energetic constraints on reproduction, resulting in hosts provisioning a baseline level of nutrients into oocytes, with the number of oocytes being limited by the energetic

costs of recovery. Oocyte size estimates in this study were notably smaller than in some previous studies (Madin et al. 2017; Foster and Gilmour 2020), which is likely attributable to our methodology, as histological processing is known to cause coral oocyte tissue to shrink up to 30% in comparison to dissected oocytes (Harriott 1983). Furthermore, we collected samples in October, the beginning of the spawning season (Carroll et al. 2006), where we observed most oocytes in stage III, indicative that they had not yet reached their mature size (Vargas-Ángel et al. 2006). However, we cannot definitively rule out bleaching-induced discrepancies in oocyte size. Future *in situ* surveys of *A. hyacinthus* reproductive traits will help elucidate the drivers of oocyte size observed during the 2019 bleaching event.

Colony size influences both bleaching susceptibility and fecundity (Hall and Hughes 1996; Brandt 2009; Sakai et al. 2019), but the size-dependent effects of bleaching on reproductive output are only recently coming to light (Johnston et al. 2020). In contrast to previous research which demonstrated that larger colonies were less likely to have reduced reproductive fitness following bleaching (Johnston et al. 2020), we found that colony size was not a significant factor in determining reproductive output in recovered and resistant colonies (Figure 4c, S3). For resistant colonies, there was a marginally significant effect of increasing polyp fecundity with increasing colony size, as expected in corals (Nozawa and Lin 2014), but this relationship was not present for recovered colonies, likely because the majority of polyps measured contained no oocytes, regardless of colony area (Figure S3). Coral reproductive maturity depends on both colony age and size (Álvarez-Noriega et al. 2016). *Acropora hyacinthus* reaches reproductive maturity around four to five years of age, which corresponds to a minimum colony diameter of about 7 cm (Wallace 1985). All colonies we measured were larger than this threshold, except for two (one recovered and one resistant): these may not have been reproductively mature at the time of sampling, as

neither were observed to contain gametes. However, colonies lacking oocytes were not limited to small, and thus possibly immature, colonies, and virtually all colonies that produced no oocytes underwent bleaching. Therefore, the trends in reproductive output we observed likely represent a true biological signature of bleaching, not an artifact of colony size or age. Additionally, we documented substantial overlap in colony size range between the resistant and recovered colonies, but none of the recovered corals were very large colonies (> 1,000 cm^2) (Figure S3). During the 2019 bleaching event, mortality for *Acropora* colonies was size-dependent and larger individuals (≥ 30 cm diameter) were more likely to die as a result of bleaching stress (Loya et al. 2001; Bena and Van Woesik 2004; Shenkar et al. 2005; Speare et al. 2021). Large colonies contribute a disproportionate amount of reproductive material compared to small colonies due to their higher per polyp fecundity and larger surface area (Hall and Hughes 1996; Nozawa and Lin 2014). Thus, the loss of large colonies has serious consequences for the overall population reproductive output, reducing recruitment and delaying coral community recovery (Hughes et al. 2019).

Intraspecific bleaching severity and recovery can vary across different habitats and are shaped by environmental factors such as light intensity, water flow, and water temperature (McClanahan et al. 2005; Hoogenboom et al. 2017; Schoepf et al. 2020). We recorded local-scale heterogeneity in heat stress response, partitioned by depth. Coral communities at the shallower (~5 m) and deeper (~14 m) depths both experienced bleaching during the 2019 thermal anomaly, but bleaching and subsequent mortality of *A. hyacinthus* were much more extensive at deeper depths. Furthermore, colonies resistant to bleaching were only observed at the shallower depths, while recovered colonies were seen at both depths. The spatially variable bleaching patterns we documented are consistent with previous studies (Golbuu et al. 2007; van Woesik et al. 2012); for example, differential bleaching susceptibilities associated with depth were reported in Mo'orea

during the 1994, 2002, and 2007 bleaching events, with coral assemblages displaying less severe bleaching at 6 m depth than at 12 m and 18 m (Penin et al. 2007, 2013). Together, our observations indicate a higher thermotolerance in corals from shallower depths at this location. Both depths likely experienced similar heat stress exposures during the bleaching event, which suggests that the variation in bleaching response is, at least in part, driven by local environmental conditions, particularly higher light intensity and/or greater daily temperature fluctuations at shallow depths (Brown et al. 2002; Schoepf et al. 2020). These differences in habitat microenvironments may have conditioned colonies at ~5 m to be more robust to extreme heat stress than deeper colonies through long-term acclimatization or local adaptation (Kenkel et al. 2013; Jung et al. 2021). However, we acknowledge that our study only examined one reef during the 2019 bleaching event and depth-associated bleaching patterns may have varied across the island. Avoiding the energetic cost of bleaching allows resistant corals to provide critical gamete stocks for stress-tolerant populations, promoting multigenerational resilience to a rapidly changing climate.

Temperature exerts a strong influence on coral gametogenic and spawning cycles (Vargas-Ángel et al. 2006). Recent thermal events have underscored the fact that bleaching and heat stress can depress coral reproduction. For example, the 2015 bleaching event in Hawai'i was followed by a reduction in spermary and oocyte production for multiple reproductive seasons in *Pocillopora meandrina* (Johnston et al. 2020). On the Great Barrier Reef, bleaching in 2016 and 2017 resulted in an 89% decline in recruitment (Hughes et al. 2019). Similar patterns have been documented elsewhere, including the Persian Gulf, where consecutive mass bleaching preceded a 58% decrease in settlement (Burt and Bauman 2020). Bleaching stress acts on populations by first removing individuals due to mortality and then impeding reproductive success in survivors through reductions in oocyte size, fecundity, and settlement. Because the success of surviving colonies can

influence reef recovery trajectories, understanding the reproductive and energetic ramifications of bleaching on surviving colonies is crucial. Our study adds to the growing body of literature demonstrating the deleterious impacts of thermal stress and bleaching on coral physiology and reproduction, and is the first, to our knowledge, to utilize reproductive histology to investigate these questions in *A. hyacinthus*. We identified *A. hyacinthus* colonies displaying one of two distinct temperature stress responses and, by combining analyses at the cell, polyp, colony, and site levels, we confirm that bleaching impairs reproduction and is related to the energetic state of the coral during the reproductive season immediately following the 2019 mass bleaching event in Mo'orea. Both phenotypes appeared visually recovered within five months after bleaching, but previously bleached colonies harbored diminished energy reserves coupled with significantly reduced gamete production and fecundity compared to colonies with no history of bleaching. Our results emphasize the importance of considering the invisible, sublethal effects of thermal anomalies in assessing reef health. Further, we likely underestimate the consequences of this bleaching event on coral reproduction since we did not evaluate other potential impacts such as perturbed spawning synchrony (Shlesinger and Loya 2019), larval mortality (Edmunds et al. 2001), or suppressed settlement and recruitment (Hughes et al. 2019; Burt and Bauman 2020). Because these processes can be highly variable on spatiotemporal scales (Holbrook et al. 2018; Bouwmeester et al. 2021; Edmunds 2021) and our study focused on a single reef, assessing the possibility of hampered reef recovery dynamics as a result of bleaching will require extensive data collection in addition to the data presented herein. Corals exhibit intraspecific variation in response to extreme thermal stress, as we show, which can be harnessed for investigating community resilience dynamics. As coral bleaching and other anthropogenic disturbances increase in

magnitude and frequency (Hughes et al. 2017), more than ever there is a critical need to understand inter- and intraspecific variation in recovery and reproductive success.

Supplementary Materials

Table S1. Spreadsheet detailing bleaching response and sampling details for all colonies in the study. Note that the majority of colonies were not sampled for both energetic and reproductive measurements. Cells filled with 'NA' means either the colony was not photographed or was not sampled for the particular metric of that column. This spreadsheet is included as separate excel file that can be found at https://github.com/sarahleinbach/thesis_documents.

Table S2. Model results for all statistical tests. Results of: **(a)** categorical linear regression testing the effect of heat stress response on physiological metrics; **(b)** logistic regression testing whether heat stress response affected gamete presence; **(c)** log-linear model testing for differences in gamete stage between resistant and recovered colonies; **(d)** mixed-effects model testing the impact of heat stress response on oocyte size; **(e and f)** Poisson regressions testing for differences in relative fecundity between heat stress responses. Asterisks denote $p < 0.05$ significance.

(a)

Test	Fixed factor	d.f.	t-value	*p*-value
Symbiont density	Heat stress response	30	-0.052	0.959
Protein content	Heat stress response	30	3.284	0.0026*
Carbohydrate content	Heat stress response	30	2.11	0.0433*

(b)

Test	Fixed factor	d.f.	z-value	*p*-value
Oocyte presence	Heat stress response	44	2.617	0.00887*
	Depth	44	0.390	0.69621
Spermatocyte presence	Heat stress response	44	2.617	0.00887*
	Depth	44	0.591	0.55454

(c)

Test	Fixed factor	z-value	*p*-value
Oocyte stage	Heat stress response	-0.013	0.98928
	Depth	-1.681	0.09270
Spermatocyte stage	Heat stress response	0.208	0.835
	Depth	0.000	1.000

(d)

Test	Fixed factor	t-value	*p*-value
Oocyte size	Heat stress response	-0.244539	0.8087
	Depth	-0.748876	0.4607

(e)

Test	Fixed factor	d.f.	z-value	*p*-value
Fecundity	Heat stress response	39	2.279	0.0227*
	Depth	39	0.352	0.7247
Fecundity over size	Heat stress response	38	3.535	0.000408*
	Colony size	38	1.606	0.108231

(f)

Test	Fixed factor	r^2	d.f.	t-value	*p*-value
Fecundity, resistant	Colony size	0.1849	19	2.076	0.05167
Fecundity, recovered	Colony size	9.43×10^{-6}	18	-0.013	0.9897

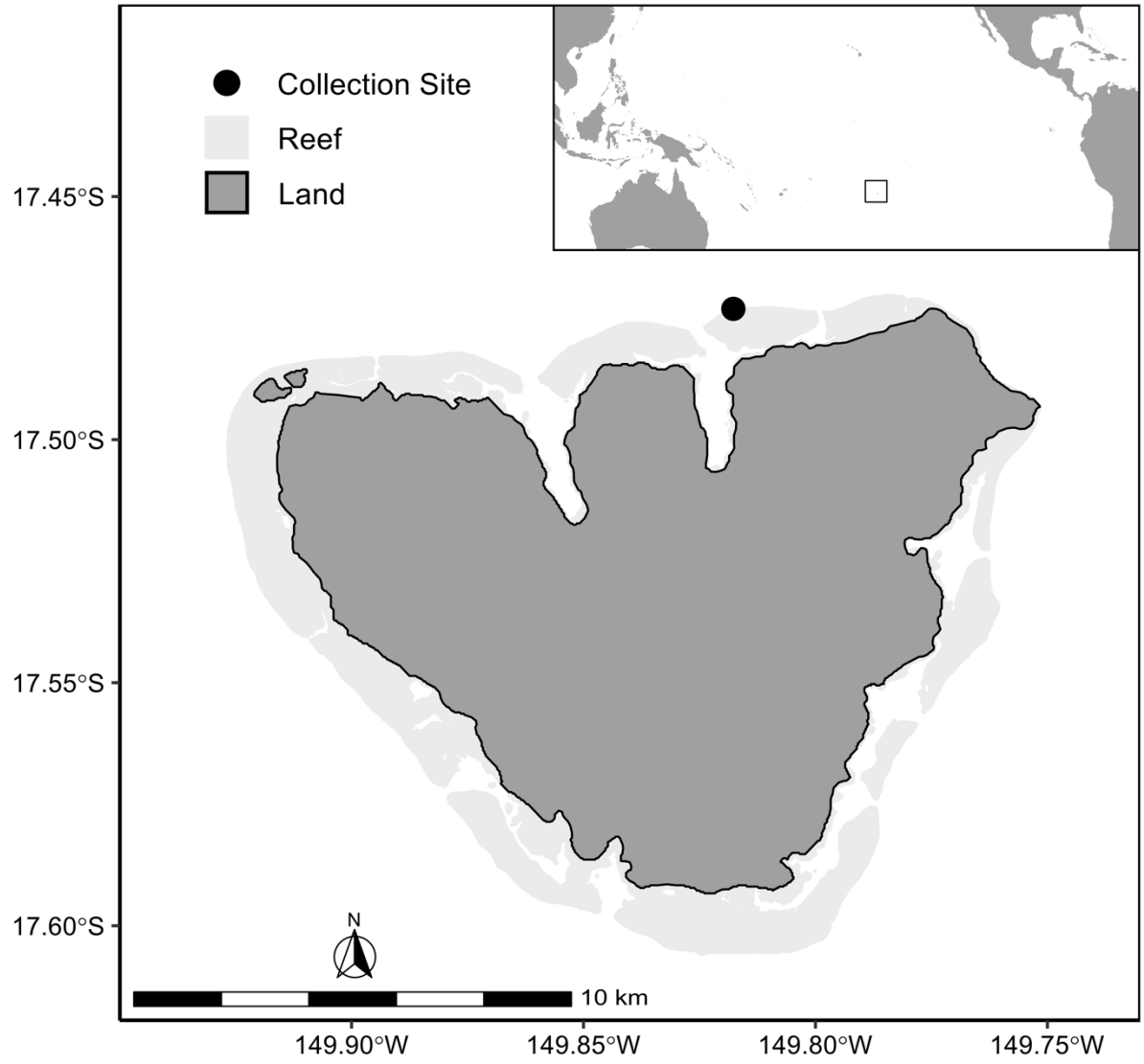


Figure S1. Map of Mo'orea, French Polynesia showing the sampling site along the north shore (17.4731° S, 149.8177° W). Island shapefile adapted from OpenStreetMap. Map data copyrighted OpenStreetMap contributors and available from https://www.openstreetmap.org.

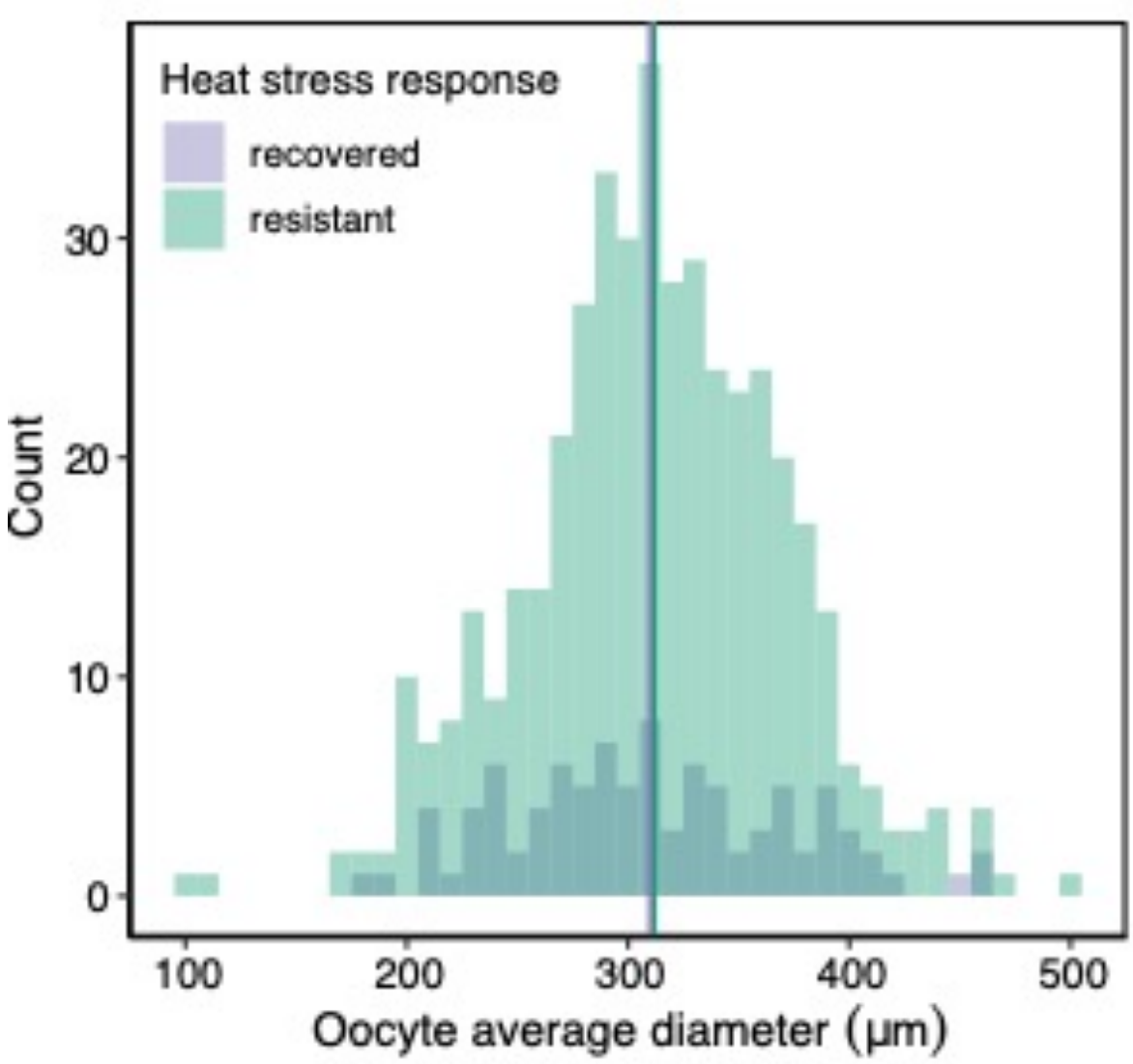


Figure S2. Distribution of oocyte diameters in recovered (N = 94 oocytes) and resistant (N = 437 oocytes) colonies. Central vertical lines represent the average oocyte diameters.

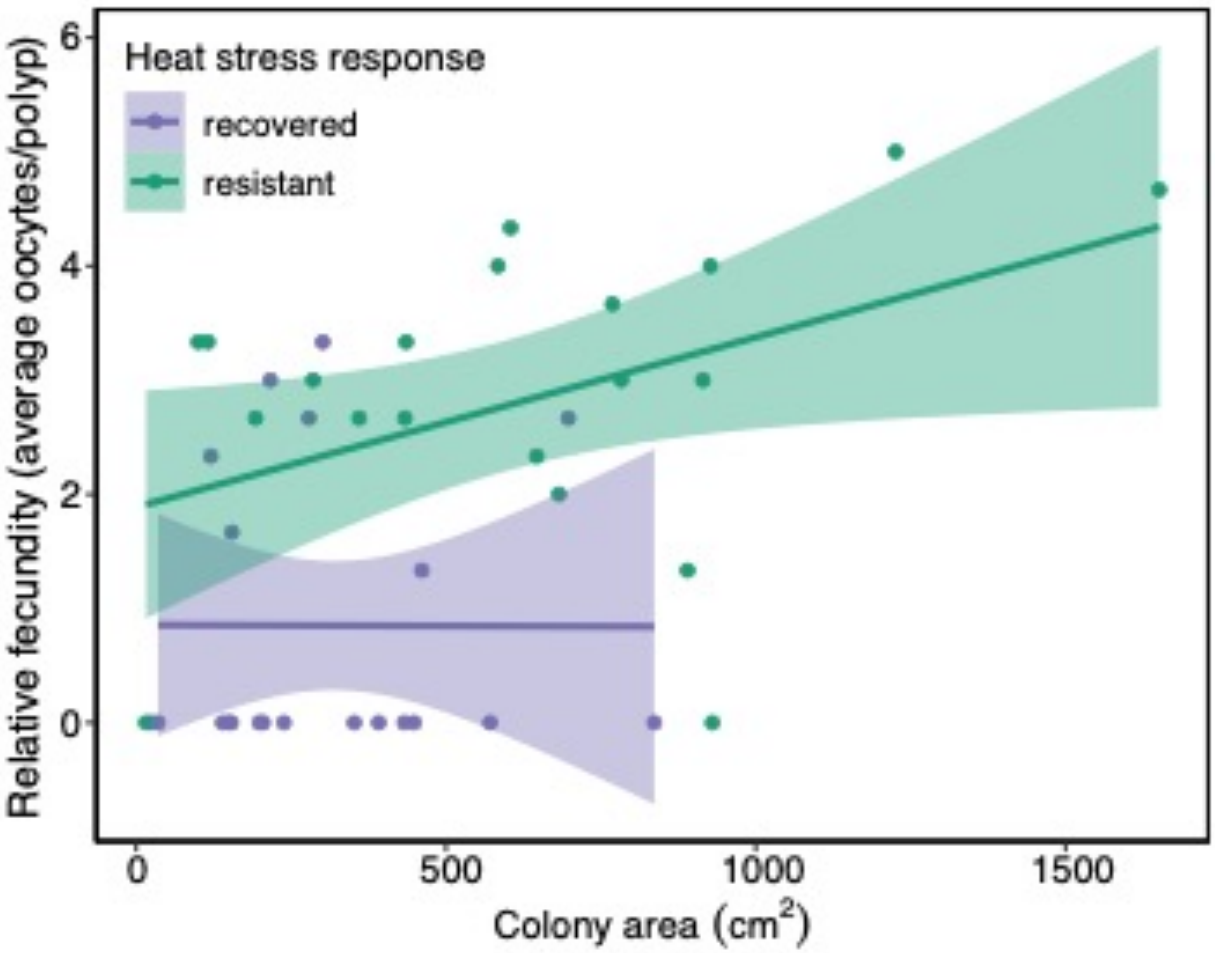


Figure S3. Relative fecundity in recovered and resistant corals across colony size. Each point represents one colony. Shaded areas are 95% confidence intervals.

Chapter 2

Reef habitats structure symbiotic microalgal assemblages and contribute to differential heat stress responses in a hermatypic coral

Introduction

Coral microbial symbionts play an integral role in the health, survival, and ecological functioning of their hosts (Bourne et al. 2016; Hernandez-Agreda et al. 2017). Endosymbiotic dinoflagellate microalgae from the family Symbiodiniaceae are obligate mutualists to coral holobionts; the coral host depends on Symbiodiniaceae to translocate photosynthetically fixed carbon compounds, providing the majority of the coral's daily energetic demands (Muscatine et al. 1981). In exchange, Symbiodiniaceae benefit by gaining access to inorganic nutrients released through the host's metabolic wastes (Rädecker et al. 2015). This tightly regulated nutrient exchange between the coral host and algal symbiont underpins the evolutionary success of coral reef ecosystems and has allowed them to thrive in oligotrophic waters for the last 160 million years (Muscatine and Porter 1977; LaJeunesse et al. 2018). However, corals already live close to their upper thermal physiological limits and recent anomalously warm seawater temperatures, or marine heatwaves (Oliver et al. 2021), resulting from anthropogenic climate change can disrupt their symbiotic relationship and lead to coral bleaching (Hoegh-Guldberg and Smith 1989; Warner et al. 1999). Coral bleaching is a state of depressed health characterized by host energetic starvation through loss of Symbiodiniaceae density (Lough et al. 2018; Rädecker et al. 2021). Given that projections indicate an increased incidence of marine heatwaves and associated coral bleaching in the next century (Hughes et al. 2018), there is an urgent need to understand coral-algal symbiosis dynamics, especially how these symbiotic relationships may be altered in situ on the reef during or after bleaching events.

Diverse Symbiodiniaceae taxa are known to routinely associate with coral hosts (LaJeunesse et al. 2018). These symbiont taxa exhibit varying tolerances to environmental stressors and, as such, may modulate the coral host's physiology and phenotype (Rowan 2004; Berkelmans and van Oppen 2006; Cunning et al. 2015; Rädecker et al. 2021). For instance, *Durusdinium* may confer some degree of thermal bleaching resistance to their hosts; corals housing *Durusdinium* display higher heat tolerance than other genera (Berkelmans and van Oppen 2006; LaJeunesse et al. 2014; Cunning et al. 2016). Although symbiont identity is a major component influencing holobiont stress response, corals may harbor a single Symbiodiniaceae type or multiple taxa simultaneously (Thornhill et al. 2009; Silverstein et al. 2012), and emerging evidence points to symbiont type abundance and diversity as additional important factors (Cunning and Baker 2013; Kenkel and Bay 2018; Claar et al. 2020a; Howe-Kerr et al. 2020). An increase in algal symbiont alpha diversity has been implicated in poor host performance under stressful conditions, including elevated temperature, carbon dioxide level, and nutrient runoff (Claar et al. 2020a; Howe-Kerr et al. 2020). Therefore, a clear connection exists between symbiont community composition and corals' responses to changing ocean conditions and it must be considered when evaluating holobiont stress tolerance.

The modification of Symbiodiniaceae communities has been proposed as a potential mechanism to bolster corals' resiliency to environmental stressors, including temperature-induced bleaching (Buddemeier and Fautin 1993; Kinzie et al. 2001). According to this idea, the proportional abundance of microalgal endosymbiont taxa can shift, either through "shuffling" of taxa already present within the coral tissues or uptake of exogenous symbiont cells, to favor types that are better able to tolerate current levels of warming (Kinzie et al. 2001; Berkelmans and van Oppen 2006; Jones et al. 2008; Cunning et al. 2015; Boulotte et al. 2016). Shuffling, which

involves an increase in the relative abundance of background Symbiodiniaceae taxa, has been demonstrated as being a particularly important mechanism for increasing holobiont heat tolerance (Baker 2003; Berkelmans and van Oppen 2006; Bay et al. 2016). For example, colonies of *Acropora millepora* in the Great Barrier Reef that transitioned from *Cladocopium*- to *Durusdinium*-dominated increased their thermal tolerance threshold by 1.0-1.5 °C and were subsequently afforded an increased capacity to recover from bleaching (Berkelmans and van Oppen 2006; Jones et al. 2008). Despite the potential of shuffling or switching to enhance holobiont thermal tolerance, this is not a ubiquitous response. (Stat et al. 2009; Thornhill et al. 2009; Smith et al. 2017). In fact, there are more examples of coral colonies not altering their dominant symbiont type in response to thermal stress, suggesting a strong role of host-symbiont genotype compatibility and calling into question how ecologically relevant alteration of Symbiodiniaceae assemblages within a colony is in situ (Goulet 2006; Sampayo et al. 2008; Thornhill et al. 2009; LaJeunesse et al. 2010; Smith et al. 2017). Hence, assessing microalgal assemblages within coral colonies and their potential to change through time is an essential facet in understanding the coral holobiont's capability to adapt and/or acclimatize in the face of climate change.

From December 2018 to May 2019, the island of Mo'orea, French Polynesia suffered a prolonged marine heatwave (Figure 1b) accompanied by widespread coral bleaching and mortality (Speare et al. 2021). However, we observed marked colony-level differences in bleaching severity (Figure 1c), with some individuals entirely avoiding bleaching ('resistant' colonies, Figure 1d) and others bleaching but later recovering after the thermal stress subsided ('recovered' colonies, Figure 1e). Here, we leverage this natural bleaching event to compare Symbiodiniaceae communities between the two forenamed coral heat stress responses and across distinct reef habitats of the

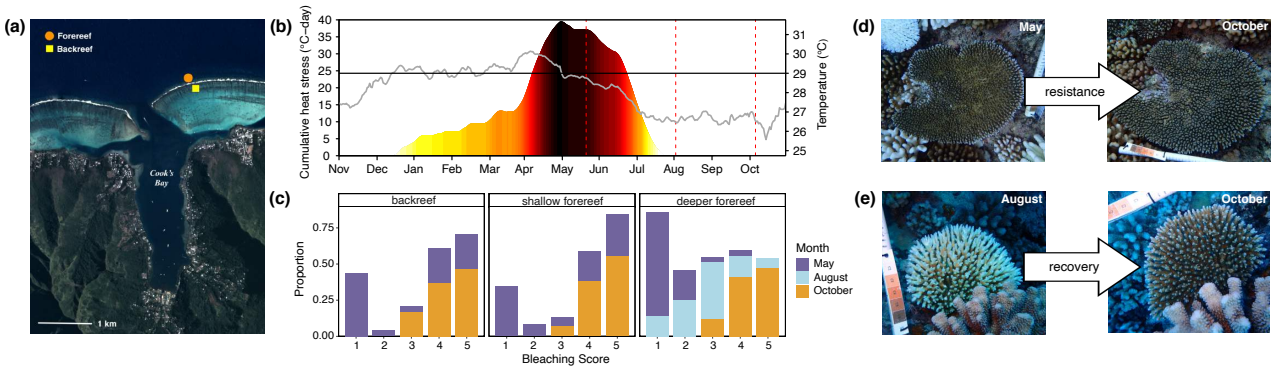


Figure 1. Thermal stress and associated bleaching event in 2019 on the island of Mo'orea, French Polynesia. **(a)** Map of sampling locations in forereef and backreef habitats along the north shore of Mo'orea. Satellite imagery from Allen Coral Atlas. **(b)** Observed sea surface temperatures from November 2018 to November 2019 (gray line) at all Mo'orea LTER sites and bleaching threshold for corals in Mo'orea (black line; right axis). Vertical red dashed lines indicate sampling timepoints (May, August, and October 2019). Color gradient from yellow to black illustrates increasing cumulative heat stress (left axis) around the island. **(c)** Bleaching severity in *Acropora hyacinthus* observed across reef zones. A bleaching score of 1 indicates stark white bleaching and a 5 indicates dark pigmentation. **(d)** A colony resistant to bleaching in the shallow forereef photographed in May and October. **(e)** A colony in the deeper forereef that experienced bleaching, photographed in August with signs of symbiont recovery and October with full symbiont recovery.

tabular coral *Acropora hyacinthus,* a key reef-builder in the Indo-Pacific Ocean that is also highly sensitive to climate change (Veron 2000; Baird and Marshall 2002). Using amplicon sequencing of the second internal transcribed spacer (ITS2) marker in tandem with the SymPortal framework (Hume et al. 2019), we aimed to identify possible microalgal drivers for the observed variability in coral heat tolerance, including spatial and temporal changes in Symbiodiniaceae taxa. Specifically, we predicted that a) coral symbiont communities would differ across reef zones, b) bleached and healthy corals within a reef zone would host different symbiont taxa, c) resistant and recovered colonies would harbor distinct symbiont types, with resistant colonies hosting thermally tolerant taxa, and d) symbiont composition within recovered colonies would shift after the bleaching event. Associations between coral host and symbiont types can exhibit significant variation in response to abiotic factors and this partnership may be constrained by selective

environmental variables, such as temperature and light intensity, that vary over small spatial scales (Iglesias-Prieto et al. 2004; van Oppen et al. 2018; Dubé et al. 2021; Kriefall et al. 2022). Addressing spatial and temporal differences in *A. hyacinthus* Symbiodiniaceae assemblages within colonies displaying divergent responses to a thermal anomaly will elucidate possible acclimatization mechanisms that coral "winners" adopt when confronted with extreme heat stress.

Methods

Study site and sample collection

Mo'orea, French Polynesia is a small volcanic island in the Southern Pacific Ocean surrounded by a barrier reef system. Water temperature data (Figure 1b) were collected at six sites on the outer reef as a component of the Mo'orea Coral Reef Long Term Ecological Research (MCR LTER) core time series data collection (Leichter et al. 2020). Bottom-mounted thermistors (Seabird SBE 39) attached at 10 m depth at each site recorded water temperatures at 20 min intervals. Cumulative heat stress (in °C-days) was calculated as a 12-week running sum of all daily temperatures from November 1, 2018 to October 31, 2019 exceeding 29 °C, the maximum monthly mean (MMM) and a noted bleaching threshold for corals in Mo'orea (Pratchett et al. 2013).

In May 2019, during the height of the bleaching event, roving SCUBA diver surveys at one site on the north shore of Mo'orea (17.4731° S, 149.8176 °W (forereef) and 14.4751° S, 149.8170° W (backreef); Figure 1a) identified bleached and healthy *Acropora hyacinthus* coral colonies in three reef zones: backreef (~3 m depth), shallow forereef (~5 m depth), and deeper forereef (~14 m depth). Individual colonies were photographed, and small branches (~2-4 cm length) were collected and preserved in 100% ethanol from all *A. hyacinthus* colonies encountered for genetic analysis. Colonies from the shallow and deeper forereef habitats were tagged for future sampling.

By August 2019, all the previously tagged bleached colonies in the deeper forereef had died. Despite this high mortality, August surveys, which occurred outside the period of accumulated thermal stress (Figure 1b), identified 16 previously bleached, untagged colonies that were observed to be visibly recovering from bleaching (Figure 1e). These colonies were photographed, tagged, and sampled. Due to the high prevalence of bleaching in May (53.2% severely bleached and 46.8% partially bleached for this site and depth (Leinbach et al. 2021)), we maintain that these previously untagged colonies were bleached during the bleaching event. In October, tagged colonies at both forereef depths were resampled and photographed. Untagged colonies in the backreef were also sampled and photographed. Bleaching severity and colony size for all sampled corals were determined using standardized photographs taken during the surveys. Each colony was assigned an integer score from 1 to 5 based on a visual evaluation of bleaching severity, where 1 indicates stark white bleaching and 5 indicates no bleaching with dark pigmentation (Figure 1c). The outline of each coral colony was traced in ImageJ to calculate planar surface area as an estimate of colony size (Schneider et al. 2012).

DNA extraction and ITS2 amplicon sequencing

Genomic DNA was extracted from samples ($N_{May} = 59$, $N_{August} = 16$, $N_{October} = 59$; see Table S1 for more detailed sample sizes) using a sodium dodecyl sulfate (SDS) digestion protocol (Lundgren et al. 2013). For each sample, preserved coral tissue was scraped off the skeleton with a razor, transferred into 750 µL of extraction buffer (1 M Tris pH 9, 0.5 M EDTA, 10% SDS, 5 M NaCl), and incubated overnight at 65 °C. Proteins were removed from chilled extracts using 5 M potassium acetate. DNA was precipitated with 100% isopropanol, washed in 70% ethanol, and resuspended in 10 mM Tris pH 9. Following extraction, all samples were cleaned with the Zymo

Research Genomic DNA Clean and Concentrator-10 kit. DNA concentrations were quantified using the dsDNA Broad-Range Qubit assay (Invitrogen, ThermoFisher Scientific) and DNA quality was assessed with gel electrophoresis. For each sample, 900 ng of DNA was sent to the Georgia Genomics and Bioinformatics Core at the University of Georgia for sequencing. ITS2 amplicon libraries were generated using the Symbiodiniaceae-specific primers SYM_VAR_5.8S2 (5'-GAATTGCAGAACTCCGTGAACC-3') and SYM_VAR_REV (5'-CGGGTTCWCTTGTYTGACTTCATGC-3') (Hume et al. 2018) and sequenced on the Illumina MiSeq platform with 250 bp paired-end reads.

Amplicon sequencing analysis

To characterize Symbiodiniaceae taxa, raw reads from each sample (i.e., paired forward and reverse demultiplexed fastq.gz files) were submitted directly to the analytical framework SymPortal (https://symportal.org). SymPortal operates on the principle that, within a single coral sample, a group of ITS2 sequences that always co-occur represent one Symbiodiniaceae genotype. First, sequences were subjected to SymPortal's standardized quality control pipeline, including removal of PCR duplicates, implemented with mothur 1.39.5 (Schloss et al. 2009), the BLAST+ suite of executables (Camacho et al. 2009), minimum entropy decomposition (Eren et al. 2015), and custom Python functions designed to minimize the number of non-Symbiodiniaceae sequences incorporated into the dataset (Hume et al. 2019). Then, SymPortal algorithmically searched for re-occurring sets of ITS2 sequences, called defining intragenomic variants (DIVs). The presence and abundance of DIVs in each sample were compared to samples already in the SymPortal database and used to define ITS2 type profiles, which are representative of putative Symbiodiniaceae taxa. In this way, SymPortal is able to differentiate between intra- and intergenomic sources of variation

without using additional genetic markers (Hume et al. 2019). The final outputs from SymPortal used in downstream statistical analyses included files of ITS2 type profile sequence abundances for all coral samples and files separated by major Symbiodiniaceae clade of principal coordinate analysis (PCoA) coordinates conducted on Bray-Curtis indices.

Statistical analyses

Statistical analyses of Symbiodiniaceae alpha and beta diversity were conducted on SymPortal outputs in R Version 4.0.3. ITS2 type profile reads were normalized using trimmed mean of M-values (TMM) in the package edgeR (Robinson and Oshlack 2010) to account for differences in sequencing depth. The 21 resultant ITS2 type profiles from SymPortal were then collapsed into seven more conservative groupings based on PCoAs of their Bray-Curtis indices (Figure S1). We chose to further collapse the type profiles so as not to inadvertently overestimate Symbiodiniaceae diversity. The groupings were determined based on their distribution along PC1 because this axis explained the majority of variation. All subsequent analyses were conducted on the collapsed ITS2 type profiles.

Alpha diversity was measured by calculating ITS2 type profile richness for each sample. Generalized linear mixed-effects models were employed to examine the effects of heat stress response, reef zone, colony health (defined as “bleached” or “healthy”), and month on richness, with a random effect of colony identity included. A linear mixed-effects model was used to analyze the relationship between richness and colony size. Poisson regressions were also utilized to investigate richness differences over time in colonies that were sampled over multiple months.

Multivariate statistics were used to examine beta diversity, specifically community structure, defined as the relative abundance of sequencing reads for each collapsed ITS2 type

profile. Permutational analyses of variance (PERMANOVAs), via the *adonis* function in the package vegan (Okansen et al. 2020), were utilized to explore how coral heat stress response, reef zone, colony health, and month impacted symbiont community structure. Additional iterations of these analyses were conducted on single colonies that were sampled over multiple timepoints (referred to as "paired colonies") to assess any changes in Symbiodiniaceae community within an individual over time. Pairwise comparisons were performed using the function *pairwiseAdonis* after any significant PERMANOVA results. Multivariate dispersion was quantified (function *betadisper*) using Bray-Curtis dissimilarities for community structure data. Differences in dispersion (i.e., if *betadisper* is significant) between samples can confound PERMANOVA results, resulting in a false positive when comparing symbiont communities. To ensure significant PERMANOVA results indicated true community differences, a bootstrapped sensitivity analysis was executed on any PERMANOVA results that showed significant heterogeneity of dispersion (Claar et al. 2020b). PERMANOVA is largely unaffected by heterogenous dispersion if sample sizes in the groups being tested are balanced (Anderson and Walsh 2013). Therefore, a subsample from the larger group was randomly selected to match the sample size of the smaller group (e.g., for analysis of the effect of heat stress response on ITS2 type profiles, original $N_{resistant} = 42$ and $N_{recovered} = 36$, subsampled to $N = 36$). The sensitivity analysis was bootstrapped 100 times for each test and if the results for all iterations were statistically significant ($p < 0.05$), we concluded that the tests were robust to an unbalanced design and that the original significant PERMANOVA results genuinely indicated significant community differences. All tests passed the sensitivity test.

Community structure data was visualized with non-metric multidimensional scaling (NMDS), using Bray-Curtis dissimilarities. Venn diagrams were created with the Eulerr package (Larsson 2018) to visualize shared Symbiodiniaceae ITS2 type profiles between heat stress

responses, reef zones, and colony health statuses. All model outputs and results are listed in Table S2.

Results

Here we aimed to characterize spatial and temporal differences in Symbiodiniaceae assemblages within *Acropora hyacinthus* colonies that responded differently to an extreme thermal anomaly using the ITS2 region. Amplicon sequencing of 134 samples from 110 individual *A. hyacinthus* colonies yielded 12,491,776 sequencing reads, 8,379,219 of which passed the quality filtering in SymPortal (67.08%) (Table S1). In total, we detected 21 ITS2 type profiles within our samples, which we further collapsed into seven more conservative type profiles (Figure S1). These ITS2 type profiles included representatives from three Symbiodiniaceae genera: *Symbiodinium*, *Cladocopium*, and *Durusdinium*.

Symbiont communities varied across reef zones

Coral colonies in the backreef, shallow forereef, and deeper forereef were characterized by distinctive symbiont communities ($p = 0.001$; Figure 2b). Backreef colonies hosted highly variable symbiont associations, with many colonies (32/44, 72.72%) hosting more than one ITS2 type profile (Figure 2a,d), often at high abundances. Furthermore, several backreef colonies (14/44, 31.82%) contained symbiont representatives from all three Symbiodiniaceae genera. A smaller proportion of colonies (9/44, 20.45%) associated with multiple ITS2 type profiles within the same genus. Backreef coral colonies exhibited significantly higher alpha diversity than both the shallow and deeper forereef habitats ($p_{shallow} = 0.0054$, $p_{deeper} = 0.0014$; Figure 2a; Table S3a). There was no significant difference in alpha diversity between the two forereef depths ($p = 0.84$) and the

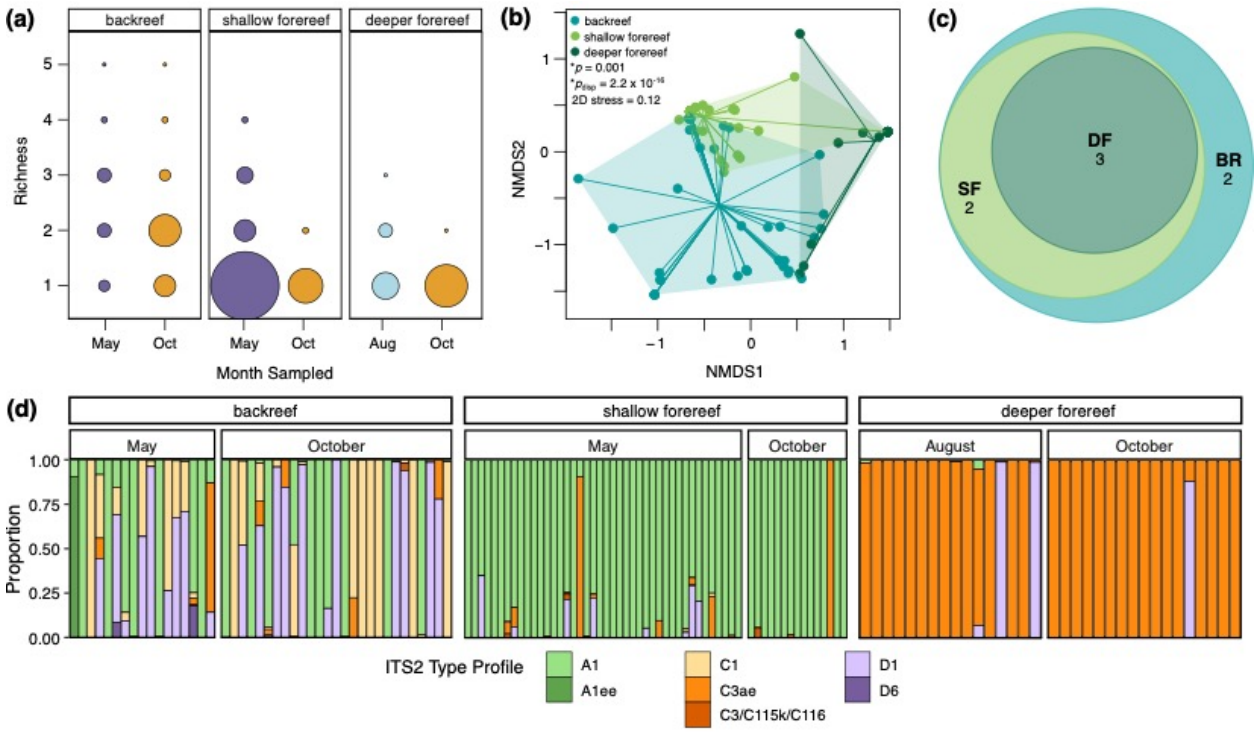


Figure 2. Symbiodiniaceae communities across three reef zones: backreef (N = 44), shallow forereef (N = 57), and deeper forereef (N = 33). **(a)** ITS2 type profile richness per colony across reef zones and sampling timepoints. Size of each circle is proportional to the number of colonies housing a given number of ITS2 type profiles. **(b)** Non-metric multidimensional scaling (nMDS) of symbiont community structure based on ITS2 type profiles. Statistical significance for PERMANOVA (p) and multivariate dispersion (p_{dis}) is denoted with an asterisk. **(c)** Venn diagram of the number of ITS2 type profiles present in colonies found in each reef zone. **(d)** Normalized relative proportion of ITS2 type profiles from colonies across backreef, shallow forereef, and deeper forereef habitats over the sampling timepoints.

majority of forereef colonies housed only one ITS2 type profile, although mixed communities were observed (18/57, 31.58% in the shallow forereef; 7/33, 21.21% in the deeper forereef) (Figure 2a; Table S3a). Coral colonies in the shallow forereef were dominated by *Symbiodinium* ITS2 type profile A1. However, 2/57 (3.51%) colonies were dominated by *Cladocopium* ITS2 type profile C3ae and 8/57 (14.04%) colonies contained observable levels of *Durusdinium* ITS2 type profile D1. Mixed-genera symbiont assemblages were observed in 17/57 (29.83%) samples and four of these housed multiple ITS2 type profiles from the same genera. In the deeper forereef,

Cladocopium ITS2 type profile C3ae was the predominant type in 30/33 (90.91%) colonies, with the remaining three samples hosting a *Durusdinium* ITS2 type profile D1 majority (Figure 2d). Five of the *Cladocopium*-dominated colonies in August (5/16, 31.25%) supported mixed-genera symbiont assemblages and one colony housed Symbiodiniaceae from all three observed genera (Figure 2d).

We identified a trend that as water depth decreases, the number of ITS2 type profiles found amongst *A. hyacinthus* colonies increased. The three ITS2 type profiles found in colonies on the deeper forereef (A1, C3ae, D1) were also found in colonies in the shallow forereef and backreef (Figure 2c). All ITS2 type profiles recorded in colonies on the shallow forereef (deeper forereef profiles plus C1 and C3/C115k/C116) were also found in backreef colonies (Figure 2c). The backreef contained colonies housing two unique ITS2 type profiles (A1ee, D6) (Figure 2c).

Within each reef zone, we observed no significant shifts in Symbiodiniaceae community composition over time ($p_{backreef}$ = 0.85, $p_{shallow}$ = 0.52, p_{deeper} = 0.23; Figure 2d; Table S3a). Additionally, during each sampling timepoint, symbiont community structure in corals across reef zones remained significantly different from each other (p_{May} = 0.001, Figure S2a; $p_{October}$ = 0.001, Figure S2b). Although colony size differed significantly between reef zones – backreef colonies were significantly smaller than forereef colonies from both depths, and deeper forereef colonies were significantly smaller than shallow forereef colonies (Table S2q) – it did not influence ITS2 type profile richness (p = 0.91, Figure S3).

Relationship between symbiont assemblages and coral health depended on reef zone

Overall, in coral colonies sampled at the height of the thermal anomaly in May, there was no significant difference in symbiont community structure between healthy and bleached colonies

(p = 0.18; Figure 3b). However, we found divergent patterns when considering the backreef and shallow forereef separately. In the backreef, healthy and bleached colonies did not support significantly different symbiont communities (p = 0.81; Figure 3d, S4b). Healthy backreef corals generally contained fewer profiles than bleached colonies, but this trend was not significant (p = 0.25; Figure 3a, Table S3b) and the majority of colonies (13/17, 76.47%) housed more than one ITS2 type profile (Figure 3a, 3d). Conversely, in the shallow forereef, healthy and bleached corals' symbiont communities were significantly different (p = 0.001, Figure 3d, S4a). Healthy shallow forereef colonies also supported significantly fewer ITS2 type profiles compared to bleached conspecifics (p = 0.00085; Figure 3a, Table S3b). All healthy corals in the shallow forereef were dominated by *Symbiodinium* ITS2 type profile A1, whereas bleached corals, although they still tended to affiliate primarily with type profile A1, also contained *Durusdinium* type profile D1 and *Cladocopium* type profiles at lower abundance (Figure 3d).

Healthy and bleached colonies from both the backreef and shallow forereef in May showed substantial overlap in the number of ITS2 type profiles present (5/7, 71.43%) (Figure S4c). However, there was one unique type profile found only in healthy colonies (A1ee) and one unique type profile found only in bleached colonies (D6) (Figure S4c). Among backreef colonies, three ITS2 type profiles were shared between healthy and bleached individuals (A1, C1, D1) (Figure S4d), one ITS2 type profile was unique to healthy colonies (A1ee), and three were unique to bleached colonies (C3ae, C3/C115k/C116, D6) (Figure S4d). On the shallow forereef, all three profiles recorded in healthy colonies (A1, C3ae, C3/C115k/C116) were also found in bleached colonies; bleached colonies additionally housed two unique ITS2 type profiles (C1, D1) (Figure 3c).

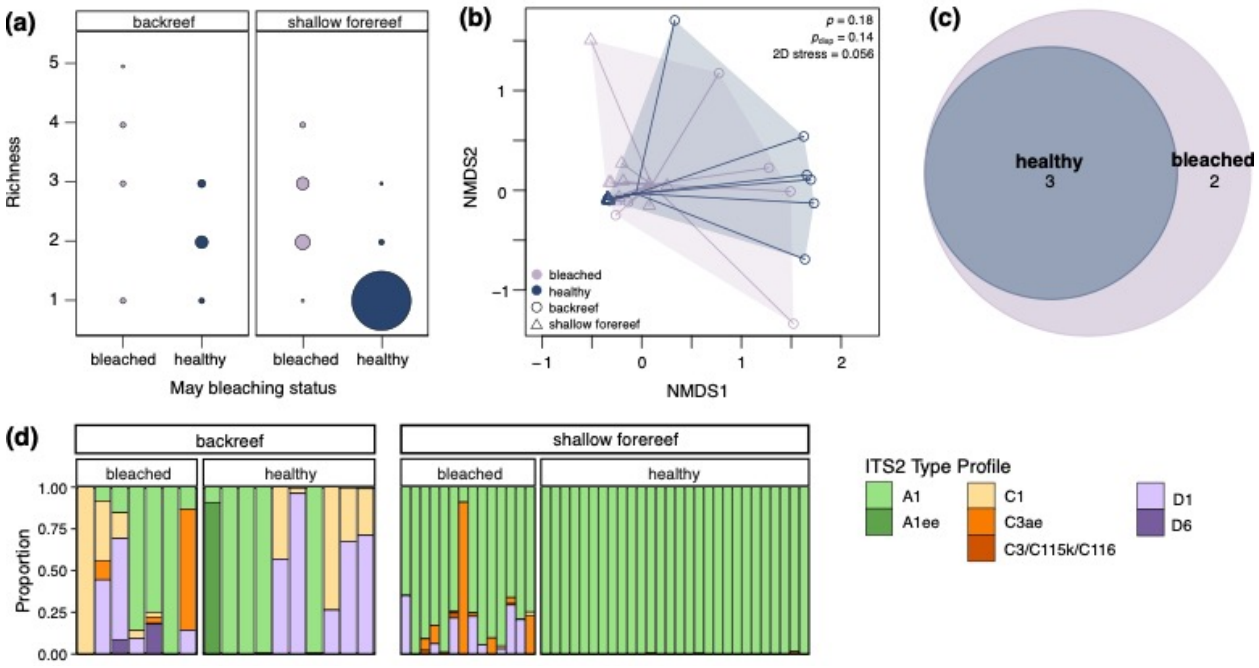


Figure 3. Symbiodiniaceae communities in healthy and bleached colonies sampled during May in the backreef ($N_{bleached}$ = 7 , $N_{healthy}$ = 10) and shallow forereef ($N_{bleached}$ = 14, $N_{healthy}$ = 28). **(a)** ITS2 type profile richness per colony for healthy and bleached colonies found in two reef zones. Size of each circle is proportional to the number of colonies housing a given number of ITS2 type profiles. **(b)** Non-metric multidimensional scaling (nMDS) of symbiont community structure based on ITS2 type profiles. Statistical significance for PERMANOVA (p) and multivariate dispersion (p_{dis}) is denoted with an asterisk. **(c)** Venn diagram of the number of ITS2 type profiles present in healthy and bleached colonies from the shallow forereef in May. **(d)** Normalized relative proportion of ITS2 type profiles from bleached and healthy colonies in the backreef and shallow forereef habitats.

Symbiont communities differed between coral heat stress responses

Our data revealed significant differences in symbiont community composition and structure between the two observed heat stress responses resistance and recovery (p = 0.001; Figure 4b). Resistant colonies were consistently dominated by *Symbiodinium* ITS2 type profile A1, whereas recovered corals displayed more flexibility in symbiont association. The majority of samples were dominated by *Cladocopium* ITS2 type profile C3ae (32/36, 88.89%), but some colonies were found to instead associate predominantly with *Symbiodinium* A1 (1/36, 2.78%) or

Durusdinium D1 (3/36, 8.33%) type profiles (Figure 4d). There was no significant difference in alpha diversity between heat stress responses (p = 0.20) and 82.05% of colonies (64/78) housed only one ITS2 type profile (Figure 4a; Table S3c). A small portion of samples (14/78, 17.95%) supported more than one ITS2 type profile, but these tended to occur at low abundances.

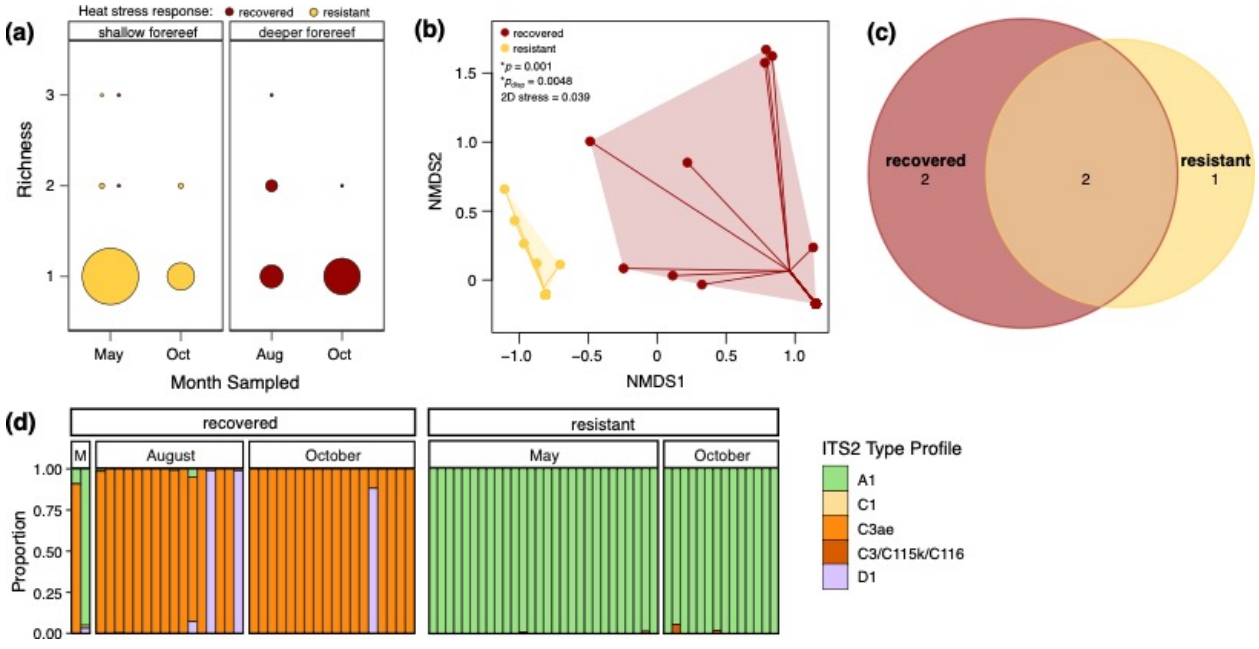


Figure 4. Symbiodiniaceae communities in recovered (N = 36) and resistant (N = 42) colonies. **(a)** ITS2 type profile richness per colony across reef zones and sampling timepoints. Size of each circle is proportional to the number of colonies housing a given number of ITS2 type profiles. **(b)** Non-metric multidimensional scaling (nMDS) of symbiont community structure based on ITS2 type profiles. Statistical significance for PERMANOVA (p) and multivariate dispersion (p_{dis}) is denoted with an asterisk. **(c)** Venn diagram of the number of ITS2 type profiles present in resistant and recovered colonies. **(d)** Normalized relative proportion of ITS2 type profiles from resistant and recovered colonies over the sampling timepoints. "M" represents May.

Although there was overlap in the ITS2 type profiles hosted by resistant and recovered colonies (A1, C3ae), recovered colonies hosted two unique ITS2 type profiles (C1, D1) and resistant colonies hosted one unique type profile (C3/C115k/C116) (Figure 4c). Generally,

overlapping ITS2 type profiles were dominant in one heat stress response and found in low abundance in the other.

In tagged resistant and recovered colonies, we found stable symbiont associations over time. There was no significant change in symbiont community structure within an individual across the sampling timepoints ($p_{recovered} = 0.71$, $p_{resistant} = 0.48$; Figure 5; Table S3d). Except for one individual (colony ID 324, Figure 5a), all colonies were dominated by the same ITS2 type profile in both of their respective sampling timepoints. However, in a small number of colonies (7/25, 28.00%) there were noticeable changes in background ITS2 type profile abundance; among these colonies hosting more than one ITS2 type profile, the proportional abundance of *Cladocopium* taxa increased in the second sampling timepoint.

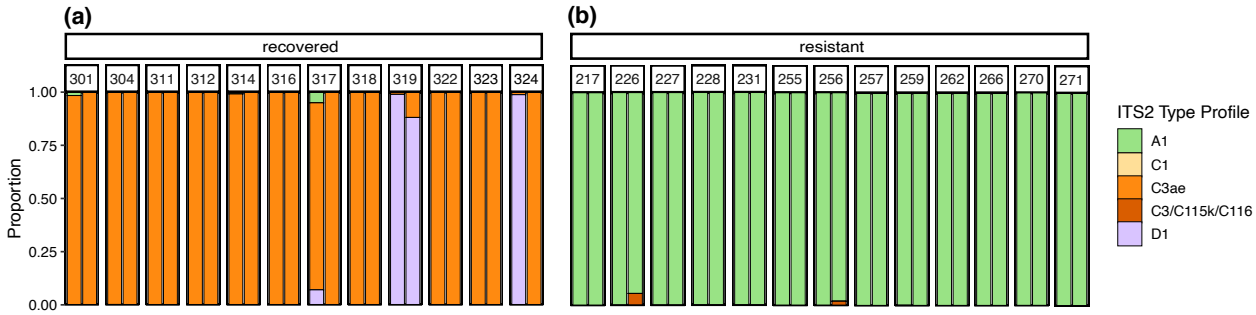


Figure 5. Symbiodiniaceae communities in paired recovered (N = 12 pairs) and resistant (N = 13 pairs) colonies. Normalized relative proportion of ITS2 type profiles from **(a)** paired recovered and **(b)** paired resistant colonies. For each recovered individual, the first and second columns represent the symbiont community during the August and October sampling timepoint, respectively. For each resistant individual, the first and second columns represent the symbiont community during the May and October sampling timepoint, respectively.

Discussion

In this study, we examined Symbiodiniaceae assemblages in *Acropora hyacinthus* colonies displaying two distinct heat stress responses and assessed the temporal stability of these

relationships, including how they differ across reef zones, in the context of a mass bleaching event. Previous studies have classified *Acropora* species as flexible with respect to Symbiodiniaceae associations (i.e., as symbiont generalists) (van Oppen et al. 2001; Putnam et al. 2012; Kriefall et al. 2022). Our results support this notion, as we found *Acropora hyacinthus* in Mo'orea harbored microalgal endosymbionts from multiple ITS2 type profiles distributed across three genera, *Symbiodinium, Cladocopium,* and *Durusdinium.* We also found that some colonies were capable of establishing symbiosis with multiple genera simultaneously. Although we observed coral host-Symbiodiniaceae flexibility within a reefscape, our findings reveal that dominant Symbiodiniaceae type profile was strongly linked to both reef zone and holobiont propensity for thermal resistance or recovery. Further, there was covariance between these different responses to the heat anomaly and the reef zone in which the colonies were found; resistant colonies were exclusively found in the shallow forereef, while recovered colonies were almost exclusively found in the deeper forereef. Taken together, this suggests a prominent role of local environmental conditions in structuring *A. hyacinthus* symbiont communities and, in turn, host response to heat stress.

Intraspecific coral holobiont responses to heat stress can vary across reef habitats and are influenced by abiotic factors including light intensity, water temperature and temperature variability, and water flow (McClanahan et al. 2005; Hoogenboom et al. 2017; Schoepf et al. 2020). We documented divergent patterns of bleaching severity and recovery in *A. hyacinthus*, with colonies residing in the deeper forereef exhibiting more extensive bleaching and mortality than those in the backreef and shallow forereef (Figure 1c). Observations of previous bleaching events in Mo'orea noted similar spatially heterogenous bleaching susceptibilities. For example, during the 1994, 2002, and 2007 bleaching events, coral assemblages at shallow depths displayed less severe bleaching than those at deeper depths (Penin et al. 2007, 2013). These results were

ascribed to an interplay between hydrodynamic conditions and differential acclimatization and/or adaptation of coral-algal symbioses, but they were not specifically investigated (Penin et al. 2007, 2013). We extend these conclusions by demonstrating that *A. hyacinthus* colonies hosted distinct symbiont communities within each reef zone (Figure 2). *Acropora hyacinthus* is a broadcast spawning coral that acquires its symbionts via horizontal transmission from the surrounding water column, and thus may be able to form symbioses with the endosymbionts best adapted to local environmental conditions (Buddemeier and Fautin 1993; Van Oppen et al. 2001; Quigley et al. 2017). Symbiont zonation (i.e., coral hosts associating with different endosymbiont lineages over their bathymetric distribution) is a common feature in broadcast spawning corals (Bongaerts et al. 2015) and, because Symbiodiniaceae types can be physiologically distinct, it enables the coral holobiont to survive over a gradient of environmental conditions, in particular light, temperature, and nutrient availability, that can vary over small spatial scales (Iglesias-Prieto et al. 2004; Rowan 2004; Frade et al. 2008; Dubé et al. 2021; Kriefall et al. 2022).

Selective abiotic factors may influence the free-living Symbiodiniaceae communities present in each reef zone and, consequently, coral in hospite microalgal assemblages. The deeper forereef (~14 m depth) receives the lowest irradiance out of all three reef habitats we sampled (Dubé et al. 2021), and colonies located there were predominantly associated with *Cladocopium* C3ae (Figure 2d). *Cladocopium* has been documented as being more photosynthetically efficient than members of other Symbiodiniaceae genera and is, as a result, frequently observed in symbiosis with coral colonies at depth, where there is less light (Cooper et al. 2011; Eckert et al. 2020; Wall et al. 2020). Alternatively, the shallow forereef (~5 m depth) is characterized by a higher irradiance (Dubé et al. 2021), and we observed that the vast majority of colonies there were dominated by *Symbiodinium* A1. *Symbiodinium* is capable of several photoprotective mechanisms,

including the production of UV-adsorbing mycosporine-like amino acids (MAAs) and upregulation of alternative photosynthetic electron pathways (Banaszak et al. 2000; Reynolds et al. 2008), explaining why *Symbiodinium* is chiefly found in coral hosts in shallow waters where light levels are high (Rowan and Knowlton 1995; LaJeunesse 2002; Reynolds et al. 2008). Backreef environments are characterized by high irradiance, temperature, temperature fluctuation, and nitrogen concentration (Kriefall et al. 2022) and, as such, they present the most stressful conditions for corals. A prior investigation of *A. hyacinthus* in Mo'orea before the 2019 thermal anomaly found that the backreef constrained Symbiodiniaceae diversity in comparison to the forereef (Kriefall et al. 2022), whereas we observed the opposite trend, suggesting that the heat stress and concordant bleaching event may have disrupted coral-algal associations (discussed further below). We found significantly higher alpha diversity in colonies on the backreef compared to both forereef depths (Figure 2a) and highly variable symbiont community compositions (Figure 2d). Such symbiont variation could be a form of bet-hedging, allowing corals to exploit different physiological traits of multiple symbiont taxa and thus enhance survival in the dynamic backreef environment (Loram et al. 2007; Torda et al. 2017). Because backreef colonies were significantly smaller than those on the forereef (Figure S3, Table S2), it is possible that they were younger and had not yet finished the winnowing process to establish a dominant symbiont type. Although some colonies were below the accepted size threshold for maturity in *A. hyacinthus* (~7 cm diameter) (Wallace 1985), we found no correlation between colony size and number of ITS2 type profiles (Figure S3), indicating that the increased alpha diversity of backreef colonies is related to some other factor, such as environmental conditions, not an artifact of coral life stage.

Symbiont zonation, in addition to reflecting environmental constraints, is also connected to genetic structuring of the host (Bongaerts et al. 2010; Frade et al. 2010; Brazeau et al. 2013).

The reef-wide patterns of Symbiodiniaceae community diversity and composition we observed may thus reflect underlying host differentiation, which we did not specifically address in this study. Kriefall et al. however found high gene flow between reef zones and no evidence for genetic structuring in *A. hyacinthus* in Mo'orea (Kriefall et al. 2022). They also found no evidence for host genetic variation correlating with symbiont associations. Cryptic species of *A. hyacinthus* have also been uncovered throughout the Pacific Ocean, including in Samoa, Palau, Australia, and Japan (Ladner and Palumbi 2012; Fifer et al. 2022). Although no such finding has yet come to light in Mo'orea, island-wide genotyping efforts will investigate this possibility further. If there is indeed no genetic partitioning within our study system, this points to an underlying environmental driver of symbiont structuring and suggests that the distinct symbiont communities we observed between heat stress responses partitioned by depth and reef zone may be an emergent property of reef-wide distribution of symbionts that are adapted to their environment.

At the height of the bleaching event in May 2019, all colonies within a reef zone hypothetically experienced similar thermal stress, yet we observed significantly different Symbiodiniaceae community structures between healthy and bleached *A. hyacinthus* colonies on the shallow forereef (Figure 3d, S4a), suggesting that factors beyond the prevailing reef zone conditions mediate the distribution of in hospite symbiont communities. Bleached colonies harbored higher alpha diversity of ITS2 type profiles compared to healthy conspecifics and housed symbiont communities that more closely resembled the variable mixed assemblages found in backreef colonies (Figure 3a, 3d). This pattern could potentially be explained by the recently proposed Anna Karenina principle (AKP), which posits that stressors induce stochastic changes in microbial community composition, leading to microbiomes of dysbiotic individuals exhibiting higher dispersion than those in healthy individuals (Zaneveld et al. 2017). Bleached backreef

colonies also tended to host more ITS2 type profiles than healthy backreef colonies (Figure 3a), but we did not detect significant differences in symbiont diversity or structure between them. This may be due to the decreased statistical power resulting from a smaller sample size for backreef colonies compared to shallow forereef colonies; thus the AKP may be operating in the backreef as well as the shallow forereef and could, to some degree, explain differences in symbiont communities in our study compared to those documented by Kriefall et al. (Kriefall et al. 2022). The thermal anomaly and subsequent bleaching in 2019 may have impaired host mechanisms to regulate and constrain symbiont assemblages (Moeller and Peay 2016; Zaneveld et al. 2017; Howe-Kerr et al. 2020), leading to increased variation and diversity in symbiont communities in bleached individuals. Alternatively, antagonistic interactions between diverse symbionts could have destabilized the coral-algal symbiosis and led to the observed bleaching (Miller 2007; Kenkel and Bay 2018; McIlroy et al. 2020), although our study is unable to disentangle whether the observed symbiont community variability is the result of thermal stress or the cause of differential bleaching phenotypes within a reef zone. Because we observed both bleached and healthy individuals within a reef zone, this points to additional factors influencing heat stress response and symbiont community composition, such as host microhabitat occupation, host genotype, or differential gene regulatory pathways involved in thermal physiology and symbiosis (Ganot et al. 2011; Barshis et al. 2013; Hoogenboom et al. 2017; Kavousi et al. 2020; Dilworth et al. 2021). Nonetheless, our results contribute to the expanding number of studies illustrating the AKP in the context of endosymbiotic dinoflagellates (Claar et al. 2020b, 2020a; Howe-Kerr et al. 2020).

Projections for the survival of future coral reefs often hinge upon colonies harboring *Durusdinium* symbionts, which contribute to holobiont tolerance to thermal stress (Berkelmans and van Oppen 2006). However, our study adds to the growing body of literature demonstrating

that enhanced bleaching tolerance is not universally associated with *Durusdinium* (Abrego et al. 2008; Howe-Kerr et al. 2020; Howells et al. 2020). We found that colonies resistant to bleaching in the shallow forereef were invariably dominated by ITS2 type profile *Symbiodinium* A1 (Figure 4d). *Symbiodinium* A1 produces UV-protective MAAs and low amounts of hydrogen peroxide, a causative agent of coral bleaching (Lesser 2011), at elevated temperatures (Banaszak et al. 2000; Suggett et al. 2008), which could potentially contribute to the lack of bleaching we observed in the resistant colonies living on the shallow forereef. This is an interesting finding considering that *Symbiodinium* A1 is better adapted for a free-living lifestyle and generally enters into symbiosis with corals opportunistically, and thus resembles parasitism rather than mutualism (Stat et al. 2008). In previous experimental work that manipulated coral-algal combinations in *Acropora millepora,* colonies hosting *Symbiodinium* A1 had the lowest thermotolerance and fitness (Mieog et al. 2009). Conversely, *Symbiodinium* A1-A1v was the dominant symbiont type profile in *Acropora pulchra* thriving in a thermally extreme lagoon in New Caledonia (Camp et al. 2020) and *Porites divaricata* in the Caribbean hosting *Symbiodinium* were able to swiftly acclimate to repeat bleaching (Grottoli et al. 2014). These contrasting reports indicate that the physiological costs and benefits of hosting *Symbiodinium* could be species- and/or location-specific and highlight the complexity of coral-algae symbiotic relationships, especially as they relate to thermal tolerance.

Investigations into the physiological ramifications of hosting certain symbiont genera often target differences in corals hosting *Durusdinium* and *Cladocopium*, but seldom focus on *Symbiodinium.* Corals hosting *Durusdinium,* for example, are well-documented as being stress-tolerant (Rowan 2004; Berkelmans and van Oppen 2006; Silverstein et al. 2015; Bay et al. 2016), but they exhibit physiological trade-offs including reduced host carbon acquisition and energy

reserves (Cantin et al. 2009; Jones and Berkelmans 2011; Sproles et al. 2020), which can lead to decreased holobiont growth and reproduction (Mieog et al. 2009; Jones and Berkelmans 2011). Similarly, *Symbiodinium* exhibits lower net translocation of carbon to its coral host compared to *Cladocopium* – a central line of evidence supporting the hypothesis that symbiosis with *Symbiodinium* borders on parasitism (Stat et al. 2008; Baker et al. 2018). Leinbach et al. 2021 reported decreased energy reserves in coral colonies recovered from bleaching compared to resistant colonies, which we show here predominantly hosted *Cladocopium* C3ae and *Symbiodinium* A1, respectively (Leinbach et al. 2021). This indicates that perhaps *Symbiodinium* does not always impose energetic constraints on their hosts and may do so only under certain conditions. Although we acknowledge the disparity in energy reserves may be a function of a factor other than dominant symbiont identity, such as depth or heterotrophic feeding capacity (Houlbrèque and Ferrier-Pagès 2009), we recommend that future studies further investigate the physiological impacts of hosting *Symbiodinium* and its potential role in bleaching resistance, since our study suggests they may be dominant in some corals and play a role in thermal tolerance.

Restructuring of symbiont communities or shifts in dominant symbiont type have been observed as a response to heat stress and proposed as a mechanism of host plasticity and adaptation (Buddemeier and Fautin 1993; Jones et al. 2008; Grottoli et al. 2014; Cunning et al. 2015). However, we observed no significant temporal changes in symbiont community composition within colonies that varied in their heat stress responses or reef zone locations during or after the thermal anomaly (Figure 2, 4, 5). Although other studies have similarly reported temporally stable symbiont assemblages in *A. hyacinthus* after bleaching (Thomas et al. 2019), our study cannot definitely rule out the possibility that corals hosted different Symbiodiniaceae types before the bleaching event, as we did not monitor *A. hyacinthus* prior to the peak of thermal stress. Earlier

surveys in Mo'orea conducted in 2013 found the majority of *A. hyacinthus* colonies in the backreef and forereef were dominated by *Cladocopium* type profiles, with only a very small proportion of colonies dominated by *Symbiodinium* (Kriefall et al. 2022). This is in stark contrast to our results, which found colonies in the deeper forereef alone dominated by *Cladocopium*; backreef colonies were instead found to host highly variable symbiont communities and shallow forereef colonies hosted *Symbiodinium* A1, suggesting that the thermal anomaly may have induced changes in symbiont communities. The increased prevalence of corals hosting *Symbiodinium* in the shallow forereef corals could be due to opportunistic proliferation and/or the selective loss of *Cladocopium* during heat stress. Although we did not observe bleaching in the resistant forereef colonies, it is possible that a shift may have occurred without visible bleaching or before the sampling timepoint in May (Thornhill et al. 2006; LaJeunesse et al. 2009). *Symbiodinium* is commonly identified in corals recovering from bleaching or thermal stress (Toller et al. 2001), and thus the resistant colonies we observed in the field could have potentially experienced bleaching outside the scope of our sampling regime.

Interestingly, among the coral individuals sampled over multiple timepoints (paired colonies; Figure 5), recovered and resistant colonies hosting more than one ITS2 type profile displayed a decrease in the proportion of *Durusdinium* D1 and *Symbiodinium* A1, respectively, over time, concordant with an increase in the relative abundance of *Cladocopium* type profiles. This could indicate both the preference of *A. hyacinthus* in Mo'orea to associate with *Cladocopium* and the transient nature of symbiotic associations with stress-tolerant opportunistic genera such as *Durusdinium* and *Symbiodinium* (Stat et al. 2008; LaJeunesse et al. 2009; Kriefall et al. 2022). It also reflects the energetic expense associated with hosting *Durusdinium* symbionts, particularly at depth since those colonies were located in the deeper forereef (~ 14 m). Future in situ surveys of

bleaching severity and molecular analyses of symbiont communities will elucidate the extent to which these assemblages are persistent symbiotic relationships, rather than ephemeral consequences of heat stress.

Acropora spp. are universally considered to be thermally sensitive relative to other coral genera (Loya et al. 2001; Putnam et al. 2012); however, we identified "winners" in this "loser" taxon, individuals able to resist or recover from heat stress incurred by the 2019 thermal anomaly. Intraspecific variation in bleaching susceptibility and coral-algal symbioses underpin coral adaptive potential and surviving individuals can be utilized for intervention methods, such as assisted evolution, used in reef conservation, restoration, and management (Quigley et al. 2018; Suggett and van Oppen 2022). Understanding the spatial and temporal dynamics of coral-Symbiodiniaceae associations during and after bleaching events is crucial in identifying host-symbiont pairs that are both more tolerant to projected temperature changes and well-suited to their surrounding reef habitat. Our work contributes to this active area of research by demonstrating flexibility of *Acropora hyacinthus* microalgal symbiont associations across a reefscape, but fidelity within a reef zone, indicating that these associations are at least partly influenced by local environmental conditions and ultimately contribute to response to heat stress. We also documented no significant temporal shifts in symbiont assemblages, including no reversion back to pre-bleaching communities (Kriefall et al. 2022), despite the cessation of thermal stress for several months; this could potentially point to novel coral-algal relationships being maintained after thermal stress, but future surveys will explore this possibility further. At the site level, we observed a considerable pool of diverse Symbiodiniaceae associating with *A. hyacinthus* colonies, including observations of symbioses with multiple Symbiodiniaceae taxa simultaneously. This suggests that is may be possible to enhance *A. hyacinthus* thermal tolerance,

within the constraints of host-symbiont genotype compatibility, through the manipulation of symbiont types or diversity, where types could be selected based on holobiont thermal tolerance observed in each reef zone. Moreover, we identified ITS2 type profiles that were specifically associated with resistant and recovered colonies, and healthy and bleached colonies: for example, all healthy colonies in the backreef were uniquely associated with ITS2 type profile A1ee. These symbiont associations may be diagnostic of heat stress response or bleaching susceptibility and could conceivably be developed into biomarkers for coral resilience or employed to augment thermal tolerance. Although our study was limited to one site in Mo'orea, it provides fundamental yet critical insight into natural symbiont dynamics in the field. In hospite symbiont assemblages are shaped by a complex interplay between prevailing local environmental conditions, acute and chronic stressors, symbiont physiology and interactions, and host factors. Future work should investigate levels of symbiont flexibility and fidelity over larger spatial and temporal scales, and further characterize symbiont physiology and its relationship to thermal tolerance, to provide a more nuanced view of host-symbiont combinations and their ability to withstand environmental perturbations, such as bleaching. The extent to which humans are able to manipulate specific symbiont types or assemblages represents a valuable path of future inquiry and action for coral reef management efforts, with the goal of promoting resistance and resilience to the anticipated impacts of anthropogenic global change.

Supplementary Materials

Table S1. Spreadsheet detailing heat stress response, reef zone, sampling details, and sequencing outputs for all coral colonies in the study. Colonies with an 'unknown' response were either not tagged and tracked through time or could not be located in subsequent surveys. Cells filled with 'NA' means either the colony was not photographed or was not sampled for the particular metric of that column. Cells filled with 'dns' indicated that a sample was collected for that timepoint, but it failed to sequence. This spreadsheet is included as separate excel file that can be found at https://github.com/sarahleinbach/thesis_documents.

Table S2. Model results for all statistical tests. **(a-g)** Permutational analyses of variance (PERMANOVAs) and associated pairwise comparisons testing for differences in symbiont community structure. **(h-q)** Linear regression models and associated pairwise comparisons testing for differences in ITS2 type profile richness. Specific model used is included in parentheses. Asterisks denote $p < 0.05$ significance and daggers indicate that a pairwise comparison was conducted. Abbreviations: DF, degrees of freedom; SS, sum of squares; MS, mean square; GLMER, generalized linear mixed-effects model; GLM, generalized linear model; LME, linear mixed-effects model.

(a) Change over time within one reef zone

Test	**Fixed Factor**	**DF**	**SS**	**MS**	**F**	***p*-value**
Backreef	Month	1	0.0862	0.0862	0.2679	0.846
Shallow forereef	Month	1	0.02665	0.026647	0.7389	0.523
Deeper forereef	Month	1	0.1163	0.1163	1.1268	0.228

(b) Comparisons of reef zones at different timepoints

Test	**Fixed Factor**	**DF**	**SS**	**MS**	**F**	***p*-value**
May	Reef zone	1	2.5858	2.5858	25.322	0.001*
October†	Reef zone	2	9.8282	4.9141	26.43	0.001*

(c) Pairwise comparison of above

Test	**DF**	**SS**	**F**	***p*-value**
Backreef vs. shallow forereef	1	2.59899	10.7380	3.0×10^{-4}*
Backreef vs. deeper forereef	1	5.881	26.06135	1.0×10^{-4}*
Shallow forereef vs. deeper forereef	1	6.623156	119.37516	1.0×10^{-4}*

(d) Effect of reef zone overall

Test	**Fixed Factor**	**DF**	**SS**	**MS**	**F**	***p*-value**
Reef zone†	Reef zone	2	21.510	10.7551	74.425	0.001*

(e) Pairwise comparison of above

Test	**DF**	**SS**	**F**	***p*-value**
Backreef vs. shallow forereef	1	6.120533	38.80446	1.0×10^{-4}*
Backreef vs. deeper forereef	1	9.4639	41.96208	1.0×10^{-4}*
Shallow forereef vs. deeper forereef	1	17.06083	281.6116	1.0×10^{-4}*

(f) Based on coral health

Test	**Factor**	**DF**	**SS**	**MS**	**F**	***p*-value**
All May	Bleaching status	1	0.1610	0.16101	1.5717	0.182
	Reef zone	1	2.5089	2.50890	24.4916	0.001*
May shallow forereef	Bleaching status	1	0.15693	0.156933	6.9908	0.001*
May backreef	Bleaching status	1	0.1082	0.10824	0.3486	0.807

(g) Based on heat stress response and paired colonies over time

Test	Fixed Factor	DF	SS	MS	F	*p*-value
Heat stress response	Heat stress response	1	16.008	16.008	292.048	0.001*
	Depth	1	0.6557	0.6557	11.963	0.001*
Paired resistant	Month	1	5.5×10^{-5}	5.5×10^{-5}	1.6291	0.481
Paired recovered	Month	1	0.1752	0.1752	0.4558	0.706

(h) Change over time within one reef zone (GLM)

Test	Factor	z-value	*p*-value
Backreef	Month	-1.292	0.1962
Shallow forereef	Month	-1.315	0.188
Deeper forereef	Month	-0.773	0.439

(i) Comparison of reef zones at different time points (GLM)

Test	Factor	z-value	*p*-value
May	Reef zone	-3.338	8.45×10^{-4}*
October†	Reef zone	See below	See below

(j) Pairwise comparison for above

Test	z-value	*p*-value
Backreef vs. shallow forereef	-2.251	0.0244*
Backreef vs. deeper forereef	-2.552	0.0107*
Shallow forereef vs. deeper forereef	0.201	0.8406

(k) Effect of reef zone overall (GLMER)

Test	Factor	z-value	*p*-value
Reef zone†	Reef zone	See below	See below

(l) Pairwise comparison for above

Test	z-value	*p*-value
Backreef vs. shallow forereef	-2.782	0.00541*
Backreef vs. deeper forereef	-3.198	0.00139*
Shallow forereef vs. deeper forereef	0.960	0.33706

(m) Based on coral health (GLM)

Test	Factor	z-value	*p*-value
All May	Bleaching status	-3.333	8.59×10^{-4}*
	Reef zone	-1.879	0.06020
May shallow forereef	Bleaching status	-3.338	8.45×10^{-4}*
May backreef	Bleaching status	-1.156	0.248

(n) Based on heat stress response (GLMER) and paired colonies over time (GLM)

Test	Factor	z-value	*p*-value
Heat stress response	Heat stress response	-1.292	0.1962
	Depth	-1.089	0.2761
Paired resistant	Month	0.186	0.853
Paired recovered	Month	-1.054	0.2917

(o) Effect of colony size on richness taking reef zone into account (LME)

Test	Factor	DF	t-value	*p*-value
Colony size	Colony size	19	-2.8637	0.911
	Reef zone*	105	See below	See below

(p) Pairwise comparison for above

Test	DF	t-value	*p*-value
Backreef vs. shallow forereef	105	-2.8637	0.0051*
Backreef vs. deeper forereef	105	-4.1056	1.0×10^{-4}*
Shallow forereef vs. deeper forereef	105	-1.2799	0.2034

(q) Comparison of colony sizes between reef zones (LME)

Test	DF	t-value	*p*-value
Backreef vs. shallow forereef	105	7.93614	1.0×10^{-5}*
Backreef vs. deeper forereef	105	2.42017	0.0172*
Shallow forereef vs. deeper forereef	105	-3.89662	2.0×10^{-4}*

Table S3. Average (and standard deviation (SD)) number of collapsed ITS2 type profiles in **(a)** each reef zone over time, **(b)** bleached and healthy colonies in the backreef and shallow forereef in May, **(c)** the two observed heat stress responses, and **(d)** paired resistant and recovered colonies over time.

(a)

Reef zone	Month	Average	SD
Backreef	May	2.471	1.179
	October	2.111	1.050
Shallow forereef	May	1.619	0.909
	October	1.133	0.352
Deeper forereef	August	1.438	0.629
	October	1.059	0.243

(b)

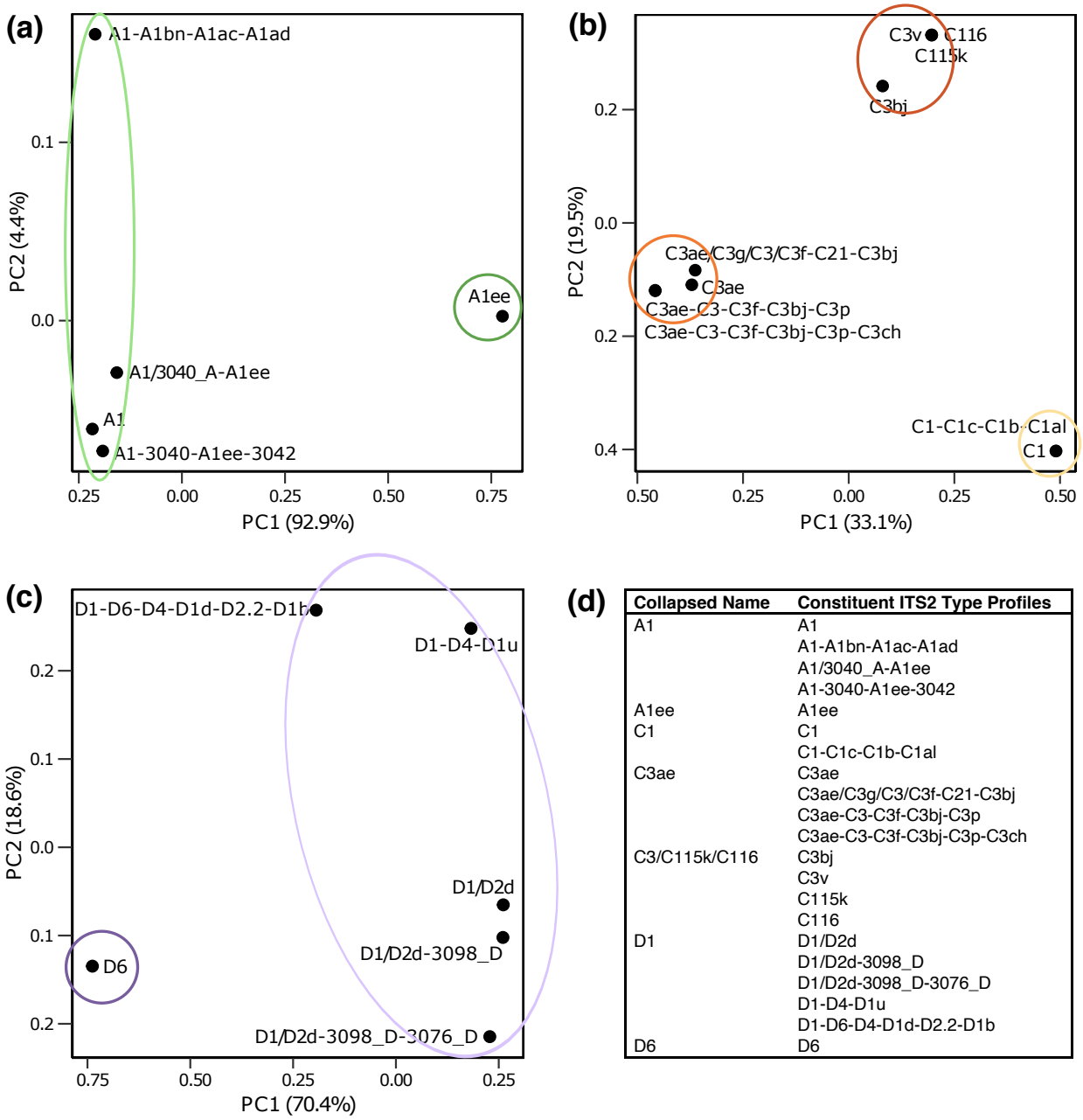
Reef zone	Bleaching status	Average	SD
Backreef	Bleached	3.000	1.528
	Healthy	2.100	0.738
Shallow forereef	Bleached	2.571	0.852
	Healthy	1.143	0.448

(c)

Heat stress response	Average	SD
Resistant	1.143	0.427
Recovered	1.306	0.577

(d)

Heat stress response	Month	Average	SD
Paired resistant	May	1.077	0.277
	October	1.154	0.376
Paired recovered	August	1.583	0.669
	October	1.083	0.289



(d)

Collapsed Name	Constituent ITS2 Type Profiles
A1	A1
	A1-A1bn-A1ac-A1ad
	A1/3040_A-A1ee
	A1-3040-A1ee-3042
A1ee	A1ee
C1	C1
	C1-C1c-C1b-C1al
C3ae	C3ae
	C3ae/C3g/C3/C3f-C21-C3bj
	C3ae-C3-C3f-C3bj-C3p
	C3ae-C3-C3f-C3bj-C3p-C3ch
C3/C115k/C116	C3bj
	C3v
	C115k
	C116
D1	D1/D2d
	D1/D2d-3098_D
	D1/D2d-3098_D-3076_D
	D1-D4-D1u
	D1-D6-D4-D1d-D2.2-D1b
D6	D6

Figure S1. Collapsed Symbiodiniaceae ITS2 type profiles. Principle coordinate analysis (PCoA) of Bray-Curtis distances provided by SymPortal between **(a)** *Symbiodinium*, **(b)** *Cladocopium*, and **(c)** *Durusdinium* ITS2 type profiles. Circles/ellipses are drawn around profiles that were combined based on similarity. Note that the precise size and location of these circles were not determined computationally; they are just designed to represent the profiles being grouped together. **(d)** List of newly collapsed ITS2 type profiles and their constituent ITS2 type profiles. Collapsed type profile names are derived from the dominant defining intragenomic variant (DIV) in each constituent ITS2 type profile, with slashes separating DIVs from other genotypes.

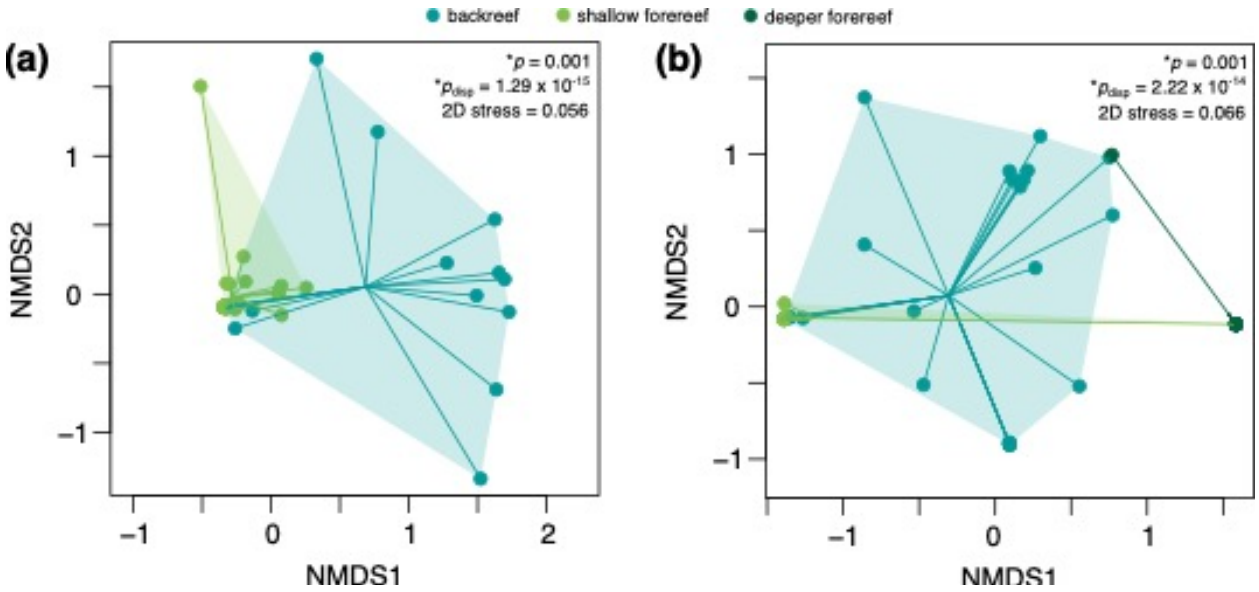


Figure S2. Symbiodiniaceae community structure across reef zones. nMDS of symbiont community structure based on ITS2 type profiles across reef zones in **(a)** May and **(b)** October. Note that the deeper forereef was not sampled in May and is thus not represented in the graph. Statistical significance for PERMANOVA (p) and multivariate dispersion (p_{dis}) is denoted with an asterisk.

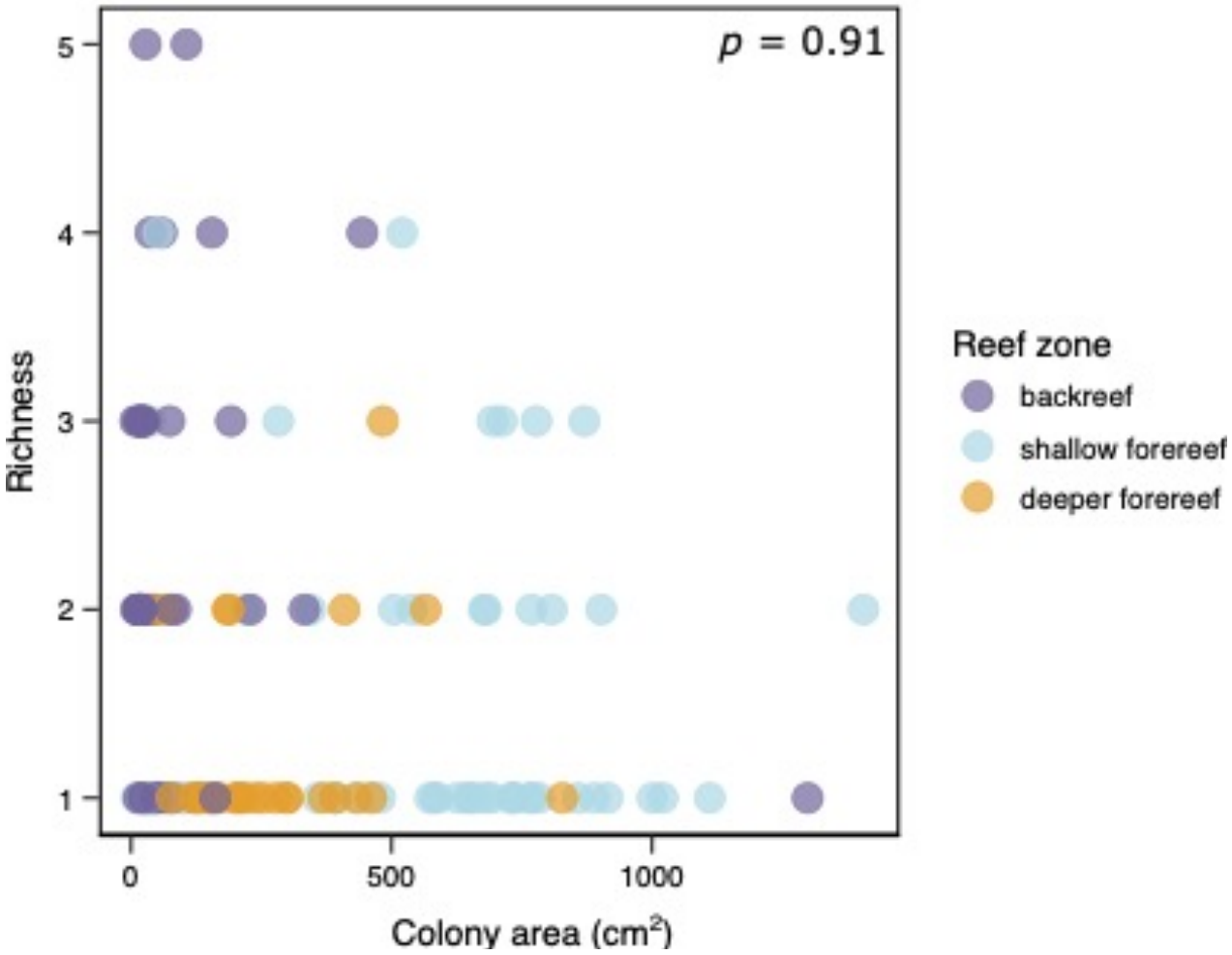


Figure S3. ITS2 type profile richness across colony sizes compared between reef zones. Each point represents one colony.

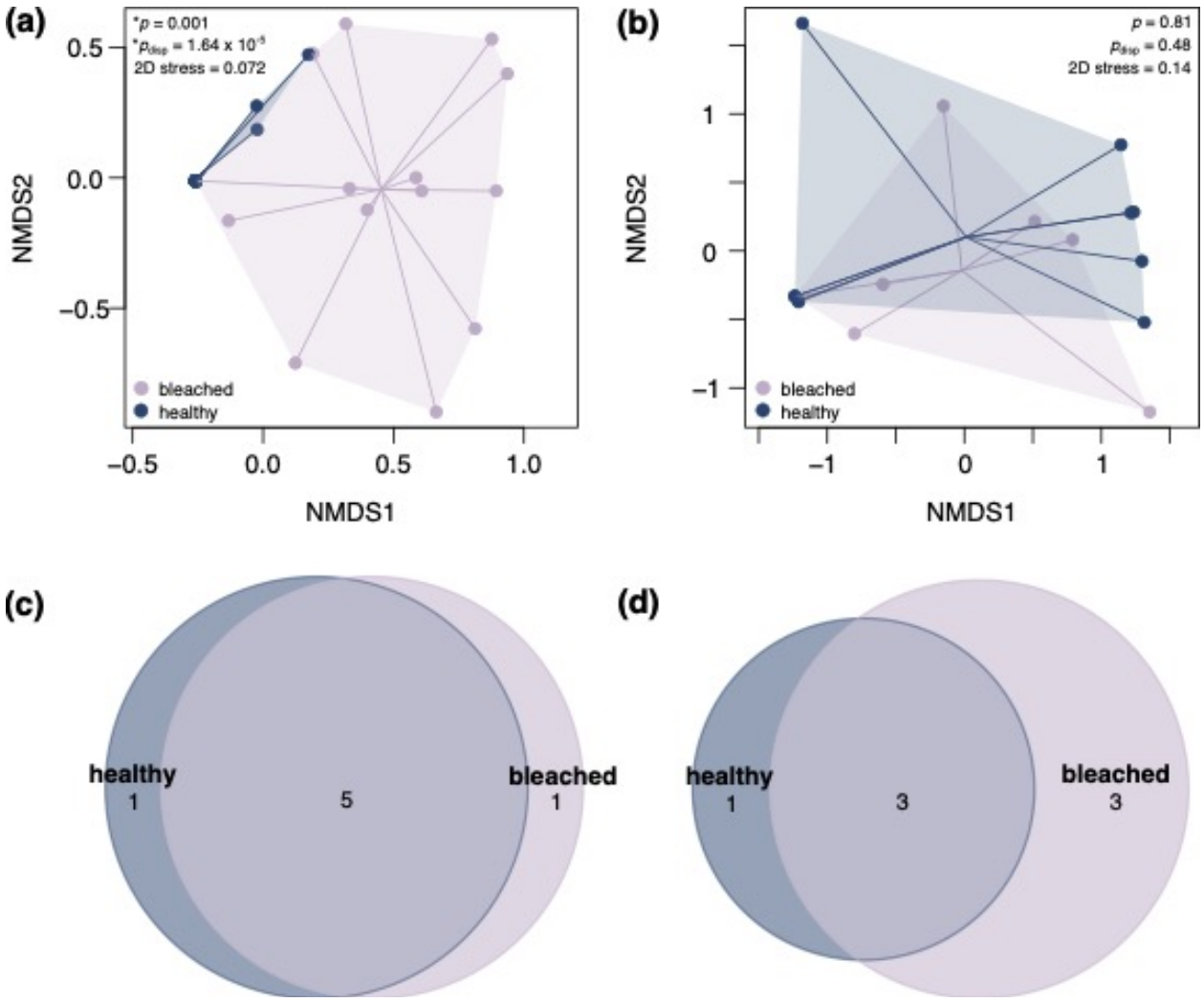


Figure S4. Symbiodiniaceae communities in healthy and bleached colonies sampled during May in the backreef and shallow forereef. nMDS of symbiont community structure based on ITS2 type profiles from colonies in the **(a)** shallow forereef and **(b)** backreef. Statistical significance for PERMANOVA (p) and multivariate dispersion (p_{dis}) is denoted with an asterisk. Venn diagram of the number of ITS2 type profiles present in healthy and bleached colonies from **(c)** all May forereef and backreef colonies combined and **(d)** the backreef in May.

Conclusions

Sea surface temperatures are rising dramatically and are projected to have cascading impacts on marine ecosystems. Thermal stress and associated mass bleaching represent an existential threat to coral reefs worldwide. Despite the dire negative consequences, there exists intraspecific variation in bleaching susceptibility and severity, even within a site, suggesting some individuals may be able to persist when confronted with extreme thermal stress and potentially replenish degraded reefs. In this thesis, I considered the subtle, sublethal effects of survival that can only be discerned by probing deeply into the coral holobiont, which offers insight into the mechanisms corals utilize to survive severe heat stress and the capacity of survivors to maintain populations after disturbance with high mortality. I harnessed natural intraspecific variation in coral response to a thermal anomaly in the field and integrated traditional techniques, including ecological field surveys and histology, with molecular methods to investigate two potential pathways for bleaching resilience in *Acropora hyacinthus*, a hermatypic coral species regarded to be highly susceptible to global change.

In Chapter 1, I combined energetic assays with reproductive histological techniques to illustrate the link between coral host energetic state and potential reproductive output in resistant and recovered coral colonies. Despite both appearing healthy five months after the bleaching event, my findings revealed that colonies that resisted bleaching harbored greater stored energy reserves and had higher reproductive potential than colonies that bleached and later recovered, indicating that bleaching inflicts an energetic constraint on the simultaneous re-accumulation of energy reserves and the production of gametes. This work is one of only a few studies to examine both oocytes and spermatocytes and advances our understanding of gametogenesis and nutrient provisioning in tropical corals.

In Chapter 2, I employed next-generation sequencing of the ITS2 region to evaluate spatial and temporal differences in symbiont assemblage alpha and beta diversity within resistant and recovered coral colonies. I showed that *A. hyacinthus* is flexible in its symbiont associations, but that these associations are strongly connected to both the observed host heat stress response and the reef zone in which the colonies resided, suggesting a prominent role of local environmental factors in structuring in hospite symbiont communities and, consequently, host thermal tolerance. Despite no observation of temporal changes throughout the study period, I detected the presence of novel coral-algal partnerships in comparison to previous studies, indicating that thermal stress may have facilitated a shift. Although *Acropora hyacinthus* is a symbiont generalist and thus sensitive to thermal stress, this flexibility appears to have allowed for diverse associations across reef zones and enabled them to persist through the thermal anomaly in different environments.

Given the integral role of endosymbiont identity in shaping coral thermal tolerance, the distinct symbiont communities I documented in Chapter 2 likely contributed to differences in coral holobiont responses to the marine heatwave, potentially leading to the disparate reproductive and host energy reserve patterns from Chapter 1. While there are known energetic constraints of hosting certain symbiont types, particularly *Durusdinium* and *Symbiodinium*, the latter of which was omnipresent in resistant colonies with comparatively high energy reserves and reproductive output, in the current era of global change, it may be beneficial to host them, even if ephemerally. With corals facing repeated thermal anomalies, hosting alternative taxa that bolster holobiont thermal tolerance may outweigh the energetic consequences of hosting *Durusdinium* and *Symbiodinium,* but future work should consider the long-term legacy effects of hosting these taxa in the Anthropocene.

Taken together, the results from these two chapters provide a nuanced view of the complex recovery dynamics of reef-building corals, highlighting the variable nature of recovery after extreme disturbances and the sublethal consequences of surviving thermal stress. Changes in energetics, reproduction, and symbiotic associations following severe heat stress are invisible yet critically important shifts in coral holobiont phenotypes that will influence the demographics of coral populations into the future. Identifying and understanding the mechanisms underlying thermal tolerance and recovery not only helps better predict natural ecosystem responses to climate change, but also may aid in selecting individuals for use in restoration projects aimed at enhancing the resilience of vulnerable populations in the face of anthropogenic bleaching events.

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