

Persistent changes in coral-microalgal symbioses with climate change-amplified
heat stress

by

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B.A., University of Oxford, United Kingdom, 2016

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We acknowledge and respect the Lək̓ʷəŋən (Songhees and X̱wsep̓səm/
Esquimalt) Peoples on whose territory the university stands, and the Lək̓ʷəŋən and W̱SÁNEĆ
Peoples whose historical relationships with the land continue to this day.

Supervisory Committee

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Abstract

Climate change-amplified marine heatwaves pose the greatest threat globally to the future of coral reefs. The algal symbionts (family Symbiodiniaceae) hosted by corals are intimately linked to host physiology and as such the composition of corals' symbiont assemblages can have dramatic consequences for their survival under stress and resilience through bleaching events. This dissertation addresses persistent changes in symbiont assemblages using empirical datasets from study sites characterising exposure to globally relevant stressors. The island of Kiritimati (central Pacific) harbours reefs across a gradient of local human disturbance and was the epicentre for the 2015-2016 tropical Pacific marine heatwave, whilst the reefs of Palau (Micronesia) have experienced consistent local differences in historical marine heatwave exposure. Prior to analysing datasets from these study sites, I review evidence of the effects of climate change on the interactions between eukaryotic hosts and their microbial symbionts from a range of ecosystems to better inform hypotheses involving coral-Symbiodiniaceae responses: I summarise ongoing shifts in diversity, flexibility, and degree of parasitism in terrestrial and aquatic symbioses and reveal a pervasive lack of longitudinal data tracking long-term symbiotic changes or stability. Then, using ITS2 DNA metabarcoding to characterise Symbiodiniaceae in coral tissue samples from Kiritimati collected from 2014 to 2019 and spanning the 2015-16 marine heatwave, I reveal greater temporal and spatial flexibility in the symbiont taxa hosted by coral species that inherit their symbionts horizontally (from their environment) compared to those that inherit symbionts vertically (from their parents). The heatwave-induced changes detected over 5 years within corals' symbiont assemblages were smaller with increasing human disturbance in corals with horizontal symbiont inheritance and greater in those with vertical symbiont inheritance, exposing contrasting effects of multiple stressor exposure on symbioses

for these two coral life histories. I then report findings from a decade-long time series which revealed factors shaping the recovery trajectories of heatwave-transformed coral-Symbiodiniaceae associations for one coral species on Kiritimati. The extreme intensity of acute heat stress exposure disrupted reversion to pre-heatwave symbiont assemblages, promoting the dominance of stress-tolerant generalist symbionts, with differential local disturbance exposure shaping the succession of recovered assemblages through intermediary symbiont community compositions. Finally, in a coral species with comparatively more specific symbiont associations in Palau, I uncover increased symbiont diversity at fine taxonomic scales associated with increased historical heat stress exposure spanning more than 20 years. Experimental heat exposure revealed that the symbiont taxa driving this increased diversity were linked to low heatwave tolerance in corals from historically more heat-stressed reefs, but not in corals from historically less heat-stressed reefs, reflecting a putative interaction between symbiont function and chronic heat stress. This dissertation addresses uncertainty surrounding the fate of climate change-transformed symbioses by assessing the long-term changes in other symbiotic study systems, the spatial and temporal flexibility in coral-symbiont associations over multiple years, the persistence of heatwave-transformed symbiont assemblages, and the links between chronic historical heat stress, Symbiodiniaceae, and heatwave tolerance. This research has applicability to coral reef conservation through improved understanding of the mechanisms driving natural variation in bleaching resilience and addresses foundational questions in symbiosis ecology under climate change and human disturbance, with relevance to other symbiotic systems.

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Dedication

To my brilliant mum, who was denied her own place at university based on sex yet has been my number one cheerleader throughout my entire academic journey. I don't know any other 65-year-olds that would snorkel with an underwater scooter to take pictures of coral reefs to send me. This degree is part yours- a 'Ph' at least.

Chapter 1 – Introduction

1.1 Tropical coral reefs under climate change

Tropical coral reefs are the most vulnerable marine ecosystem to climate change impacts and are thought to be the ecosystem most at risk of imminent ecological extinction (Cooley et al., 2022). Despite a long paleontological history of steady local degradation (Pandolfi et al., 2003), the first indications of the globally ubiquitous vulnerability of tropical coral reefs came from the first ‘mass-bleaching’ event in recorded history in 1982-83: Anomalously high sea-surface temperatures caused corals of multiple species across large expanses of the Tropical Eastern Pacific to exhibit the ‘bleaching’ response, leading to extensive coral mortality across the region (Glynn & D’Croz, 1990). Coral reefs have since faced four global mass-bleaching events (mass-bleaching recorded simultaneously in multiple ocean basins), with a minimal number of reefs thought to have escaped severe bleaching over the past 40 years (Donner et al., 2017; Hughes et al., 2018; Skirving et al., 2019; Reimer et al., 2024). Meanwhile the frequency, duration and intensity of marine heatwaves, the largest driver of coral bleaching, continue to increase (Hughes et al., 2017; Oliver et al., 2021).

The Cnidarian reef-building corals (Order Scleractinia) that engineer these ecosystems are counted in a select group of taxa with the greatest climate change vulnerability, due to their high exposure to climate impacts, high climate sensitivity and low adaptive capacity (Foden et al., 2013), with bleaching under prolonged exposure to anomalously high temperatures the largest driver of global reef coral mortality (Hughes et al., 2018; Fordyce et al., 2019). Coral bleaching is the apparent whitening of a coral colony as dinoflagellate endosymbionts within the coral gastrodermis are expelled or become degraded (Glynn, 1996; Weis, 2008). These

endosymbionts (Family Symbiodiniaceae; LaJeunesse et al., 2018) are photosynthetic and provide the majority of the fixed-carbon requirements of their coral hosts (Falkowski et al., 1984; Muscatine et al., 1997; Ferrier-Pagès et al., 2003). Reef-building corals owe their evolutionary success to the acquisition of these endosymbionts in the Triassic period (Muscatine & Porter, 1977; Stanley & Swart, 1995), key to resolving ‘Darwin’s Paradox’ of the high productivity of these ecosystems in nutrient-poor tropical waters (Darwin, 1842; Cui et al., 2023). However, this symbiosis is also central to their ecological vulnerability. In other taxa, such as the mycorrhizal fungal symbionts of vascular plants (Kivlin et al., 2013), microbial symbionts may expand the fundamental niches of their eukaryote hosts. In contrast, the obligate vertically-transmitted bacterial symbionts of insect guts (liable to genome deterioration) and photosynthetic microalgae (including Symbiodiniaceae) in particular seem to limit holobiont tolerances to warming temperatures compared to aposymbiotic hosts (Jones et al., 1998; Wernegreen, 2012; Edgar et al., 2013).

Symbioses are sustained and intimate interactions between multiple organisms and are liable to breakdown when the cost of the relationship outweighs its benefit to either partner, at which point ‘dysbiosis’ ensues. In reef-building corals the dysbiosis at the root of heat-induced bleaching has been linked to several non-mutually exclusive mechanisms. One hypothesised mechanism is that Symbiodiniaceae photosynthetic machinery can become damaged under heat stress, and continued exposure to high light intensity causes excess photons that cannot be integrated into photosynthesis to instead be converted into reactive oxygen species (ROS), capable of cell and tissue damage (Iglesias-Prieto et al., 1992; Lesser, 1997). Further expulsion of Symbiodiniaceae can occur through a stress response of the coral immune system to ROS-induced damage (Glynn, 1996; Weis, 2008; Palmer, 2018). This coral-algal dysbiosis may also

be viewed as a deviation from the nitrogen-limited state that facilitates host regulation of Symbiodiniaceae population growth *in hospite*, to a carbon-limited state when symbiont photochemical efficiency is impaired, supported by evidence of increased coral bleaching susceptibility with higher environmental nitrogen availability (Wooldridge, 2009; Vega Thurber et al., 2014; Rådecker et al., 2023). Coral survival without their microalgal symbionts is limited to short periods of time, with the probability of mortality following bleaching dependent on corals' capacity to recover Symbiodiniaceae assemblages (Anthony et al., 2009). Even if Symbiodiniaceae are recovered post-bleaching, the physiological cost born by the coral host can endure for several years, with energetically expensive behaviours such as sexual reproduction slow to return to pre-bleaching rates (Coles & Brown, 2003; Baker et al., 2008; Anthony et al., 2009; Rådecker et al., 2021).

1.2 Symbiodiniaceae diversity and their associations with reef corals

Over the last 2-3 decades, tropical coral ecology has transitioned towards a 'holobiont-view' of bleaching resistance and resilience (considering the role of all associated microbiota in addition to the host organism) (Voolstra et al., 2021). Nevertheless, symbiosis ecology in tropical corals is a younger field than the study of coral reef ecosystems, and as such the effects of climate stressors such as marine heatwaves on symbiont ecology remain comparatively unresolved relative to effects on the wider coral reef ecosystem.

Across the phylogeny of the Symbiodiniaceae Family, considerable phenotypic variation in traits such as coral heat tolerance and bleaching resistance has been linked to the identity of symbionts hosted, often with drastic consequences for coral survival (Berkelmans & van Oppen,

2006; Swain et al., 2017; LaJeunesse et al., 2018). Symbiont assemblages hosted by adult corals commonly resemble the structure of the bacterial biosphere, consisting of several low abundance taxa (together often comprising <1% of the community) in addition to one or a small number of high abundance taxa, with both dominant and low abundance ‘background’ symbionts capable of shaping coral and coral reef function (Silverstein et al., 2012; Boulotte et al., 2016; Jousset et al., 2017; Ziegler et al., 2018a).

The highest richness of single-celled eukaryotic Symbiodiniaceae on tropical coral reefs is found in reef sediment (Cunning et al., 2015; Quigley et al., 2022), where these microalgae exist in their ‘free-living’ motile form. Symbiodiniaceae are hosted within vacuoles (symbiosomes) in the mesodermal cells of a range of invertebrate hosts on tropical reefs as immotile coccoid inhabitants reproducing only asexually (Meyer & Weis, 2012; Bourne et al., 2013). In healthy adult corals, sufficient host-symbiont cophylogeny is required to initiate and maintain symbiosis without host immune rejection (Voolstra et al., 2009; LaJeunesse et al., 2010a; Yamashita et al., 2014). Even in the absence of stress, turnover of Symbiodiniaceae cells within corals is continuous (Yeager et al., 2025), with excess microalgae shed from hosts via polyp mouths. The larvae of most reef coral species settle on the benthos lacking Symbiodiniaceae and subsequently ingest and phagocytose them from the water column (horizontal acquisition), whilst other coral taxa inherit Symbiodiniaceae from parental gametes (vertical transmission) (Baird et al., 2009). Between the two modes of reef-coral sexual reproduction, most that employ broadcast spawning (gametes are released into the water column prior to fertilisation) acquire Symbiodiniaceae horizontally and most brooding spawners (larval development begins whilst still within the parental colony) acquire them vertically, although many exceptions exist (Kerr et al., 2010; Quigley et al., 2018b).

Whilst symbiont communities hosted by some adult corals show year-to-year stability (Thornhill et al., 2006a; Stat et al., 2009a; Pettay et al., 2011; Lewis et al., 2024), others are dynamic in response to environmental disturbance (Baker, 2003; LaJeunesse et al., 2010b; Putnam et al., 2012; Ziegler et al., 2015). Both coral and symbiont taxa show inherent differences in partner specificity (the number of taxa they are known to associate with across their range) and partner flexibility (associating with different partners according to environmental gradients or in response to stressors) (Fabina et al., 2012, 2013). Flexibility in the symbiont taxa hosted by corals has been linked to coral phylogeny, geographic range and reproductive mode, with larger host geographic ranges and horizontal symbiont transmission (as opposed to vertical inheritance of symbionts from parent corals) both associated with increased flexibility (Swain et al., 2018; Zarate et al., 2024). A disturbance event that causes a colony to bleach may precede recovery with different symbiont taxa, typified by increases in the symbiont genus *Durussdinium* during and after bleaching (Baker et al., 2004; Jones et al., 2008; LaJeunesse et al., 2009b; Claar et al., 2020b), attributed to its relatively higher thermotolerance and bleaching-resistance (Silverstein et al., 2017; Cunning et al., 2018). This can occur through the differential loss and proliferation of residual symbionts following bleaching or through exogenous uptake (Boulotte et al., 2016; Scharfenstein et al., 2022; Buzzoni et al., 2023; Johnston et al., 2025), facilitating a putatively adaptive pathway to increased thermotolerance through selection for coral bleaching sensitivity and symbiotic flexibility (as per the ‘Adaptive Bleaching Hypothesis’ of Buddemeier & Fautin, 1993).

Although bleaching may theoretically allow corals to recover a symbiont assemblage better adapted to environmental conditions, the association between coral-Symbiodiniaceae flexibility and holobiont resilience under climate change is disputed, with the sub-lethal

physiological and fitness costs of bleaching likely much greater for coral host than for their endosymbionts (Anthony et al., 2009; Leinbach et al., 2021; Rådecker et al., 2021; Scott et al., 2024). Symbiodiniaceae diversity has been implicated in bolstering holobiont and ecosystem resilience by facilitating flexible associations with functionally distinct symbionts (Ziegler et al., 2018a), yet comparisons across several coral species have revealed correlations between environmental sensitivity higher partner flexibility (Putnam et al., 2012). Understanding how this trait shapes coral resilience is hindered by the very small number of longitudinal datasets that have recorded the changes or persistence of Symbiodiniaceae in coral colonies or on coral reefs over timescales longer than one or two years (Thornhill et al., 2006b; Baker et al., 2013; Edmunds et al., 2014; Palacio-Castro et al., 2023; Lewis et al., 2024). With the barrage of stressors facing tropical corals limiting the survival of many stress-sensitive corals over longer timeframes, tracking the Symbiodiniaceae within individual corals becomes increasingly challenging, yet even long-term symbiont-tracking in coral reefs or populations is currently lacking (Cunning et al., 2024).

Symbiosis researchers, like romantic novelists, tend to focus on the routes by which the partners come together, and to neglect the processes by which partners coexist in the long term. (A.E. Douglas, 2010, The Symbiotic Habit)

1.3 Study locations: Effects of multiple stressors and cumulative heat stress

The world's coral reefs are ubiquitously exposed to some degree of local human disturbance, most notably fishing pressure and water pollution (with sediment or nutrients) (Andreello et al.,

2022). Current human population growth rates are highest in areas adjacent to coral reefs compared to the global average (Sing Wong et al., 2022), hence the effects of climate change superimposed upon local disturbance typifies the pressures currently facing coral reefs. Exposure to nutrient pollution and fishing pressure has been linked to slower rates of disturbance recovery on some coral reefs (Mellin et al., 2016; MacNeil et al., 2019), although protection from local disturbance in the form of Marine Protected Areas has also been linked to higher mortality and slower recovery from bleaching events (Côté & Darling, 2010; Bruno et al., 2018; Bates et al., 2019; Baumann et al., 2021). For example, during the worst recorded global bleaching event on record in 2015-16, the northern Great Barrier Reef, one of the most intensely locally managed reefs in the world, suffered significantly more severe bleaching and mortality compared to central and southern areas (Hughes et al., 2017). Significant interactions, both antagonistic and synergistic (effects smaller or larger than the summed effects of individual stressors, respectively), between multiple stressors have been reported on a variety of coral reefs in the context of coral bleaching (Darling et al., 2010; Ban et al., 2014; Courtial et al., 2017; Donovan et al., 2020; Maucieri et al., 2023). Accounting for exposure to multiple stressors on coral reefs is therefore crucial when considering their responses to anthropogenic change, and more accurately approximates the current state of tropical coral reefs. Currently, uncertainty remains surrounding the effects of putative interactions between multiple stressors, such as marine heatwaves and local disturbance, on coral Symbiodiniaceae assemblages. The tropical coral reefs of Kiritimati and Palau can serve as model systems exemplifying major threats to the world's coral reefs and their effects on coral-Symbiodiniaceae ecology, exposed to chronic human disturbance combined with acute heat stress and experiencing the cumulative effects of historical heat stress, respectively.

The island of Kiritimati (Republic of Kiribati) in the central equatorial Pacific is the world's largest atoll (388 km²) and was the epicentre for the 2015-2016 global coral bleaching event (experiencing heat stress for over 10 months). Kiritimati's resident population (estimated at 6,500 -7,400 between 2015 and 2020 (Kiribati National Statistics Office, 2016, 2021)) is concentrated on the island's northeast lagoon-face, and reefs closer to population centres have consistently been exposed to higher local human disturbance since the turn of the century (Walsh, 2010; Watson et al., 2016). Sites deemed to be exposed to high human disturbance suffer significantly poorer water quality from sedimentation and microbial load together with increased physical damage from dredging and greater fishing pressure (Watson et al., 2016; Baum et al., 2023).

In contrast, the reefs of the Republic of Palau (Micronesia) are significantly more intensely locally managed than those of Kiritimati. Palauan reefs are considered well-protected from local impacts compared to surrounding Micronesian countries; an established and extensive network of Marine Protected Areas surrounding the country and community-led watershed management (limiting nutrient and sediment runoff) confine any direct degradation to coastal lagoons, with only minimal local impacts on fringing reefs (Richmond et al., 2002, 2007; Wabnitz et al., 2018). Palau also suffered milder heat stress compared to Kiritimati during the 2016-17 Pacific heatwave, although mass-bleaching was still reported there in 2017 (following mass-bleaching in 1998 and 2010) (Liu et al., 2014; NOAA Coral Reef Watch, 2018). Reefs surrounding Palau's northern lagoons have consistently been exposed to milder heat stress across mass-bleaching events, most likely owing to frequent flushing of northern lagoons through reef channels, with greater tidal retention recorded in the southern lagoons (Colin, 2009; Barkley et al., 2015; Golbuu et al., 2016).

1.4 Research overview

This dissertation addresses changes in symbiont assemblages using empirical datasets from study sites characterising exposure to globally relevant stressors, including changes likely to shape coral resilience under climate change-amplified heat stress. Research questions were developed to address two poorly understood facets of climate change-driven changes in coral-algal symbioses; the persistence of disturbance-induced changes in Symbiodiniaceae assemblages over multiple years and the interactions between multiple stressors in driving changes in Symbiodiniaceae assemblages. Firstly, I review and synthesise evidence of climate change-induced shifts in host-symbiont ecology across terrestrial and aquatic ecosystems to determine whether climate change-transformed symbioses might persist long-term and to better inform hypothesised coral-Symbiodiniaceae symbiotic shifts (Chapter 2). Subsequently, using data collected on Kiritimati over 5 years, spanning the 2015-16 marine heatwave, I investigate whether corals with horizontal and vertical symbiont inheritance differ in their symbiont temporal or spatial flexibility upon exposure to both acute heat stress and chronic human disturbance (Chapter 3). Then, focusing on one coral species on Kiritimati, I use time series data spanning a decade to ascertain whether exposure to chronic human disturbance affected the post-heatwave rate and trajectory of reversion from heatwave-transformed symbiont assemblages (Chapter 4). Finally, by sampling corals from Palauan reefs that have been exposed to consistently different amounts of heat stress over the last 20 years, I test for corresponding fine-scale differences in Symbiodiniaceae taxa hosted and how this relates to coral experimental heatwave tolerance (Chapter 5). In addressing these questions, this research has applicability to

coral reef conservation by improving understanding of the mechanisms driving natural variation in bleaching resilience and addressing foundational questions in symbiosis ecology under climate change and human disturbance, with wider relevance to other symbiotic systems.

1.5 Research significance

In addition to their inherent value, the ecosystem services provided by coral reefs include food security (coral reefs provide 50-90% of dietary protein for Pacific Island populations), long-standing cultural significance and crucial shoreline protection against storm flooding due to rising sea levels and extreme weather events, the loss of which poses a humanitarian crisis of enormous scale (Beck et al., 2018; Bell et al., 2018). On the frontline are Small Island Developing States (such as Palau and Kiribati), a collection of countries and territories with the highest vulnerability to climate change impact worldwide, largely due to small land area, subsistence fishing and the proximity of their populations to coastlines. In particular, Kiribati ranks among nations with the highest dependency on coral reefs (Cinner et al., 2016), with its entire population within 5km of the coast (Sing Wong et al., 2022). Bleaching-induced coral mortality is directly linked to the loss of structure of coral reef ecosystems from the erosion of dead coral skeletons (Morais et al., 2022).

Advancing understanding of the causes of natural variation in bleaching resistance and resilience is of urgent importance for guiding successful coral reef conservation to secure the persistence of these ecosystems under accelerating climate change. Increased recognition of the ongoing shifts in coral-Symbiodiniaceae interactions may help better predict reefs' bleaching susceptibility, guide local management priorities, inform reef restoration best practices and even

facilitate manipulative interventions into corals' algal symbionts (Cinner et al., 2016; Logan et al., 2021; Voolstra et al., 2021; Shaver et al., 2022), as the suite of anthropogenic stressors imposed on coral reefs intensifies.

Chapter 2 – Strained and shifting symbioses under climate change

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2.1 Introduction

Microbial symbioses are ubiquitous across eukaryotes and exist on a continuum of mutualism to parasitism. Some of the most well-studied symbiotic relationships include mycorrhizal fungi and rhizobial bacteria in the rhizospheres of crop plants, the bacteriomes of insects (especially crop pests and vectors of human disease) and globally threatened reef-building corals. Increasingly, a whole-holobiont approach is being used to address host and symbiont function together as a single unit, with a rise in the use of metagenomics for tackling biological challenges in agriculture, carbon sequestration and clinical medicine (Vega Thurber et al., 2009; Schmieder & Edwards, 2012; Shi et al., 2019; Porter & Sachs, 2020). Indeed, the whole-holobiont may be considered the target of natural selection and the object of conservation initiatives (Roughgarden et al., 2018; Carthey et al., 2020). But despite these intimate associations, microbial symbionts and hosts may have markedly different responses to stressors, including those imposed by climate change. In host-parasite interactions for example, pulse stressors such as heatwaves may induce a physiological response from a host and a simultaneous evolutionary response from associated microbes, due to differences in generation times and adaptive potential (Claar & Wood, 2020). Association with a eukaryotic host may provide a symbiont with nutritional

benefits or a degree of protection from environmental extremes (especially in the case of endosymbionts), and benefits to the host can broadly be categorised as nutritional supplementation, environmental amelioration or defence (through direct mechanisms or via competitive exclusion of pathogens). If the stressors of climate change do indeed generate divergent responses from host and symbiont, the ratio of these symbiotic benefits to the cost of the association may change for either partner, affecting the fundamental nature of the relationship. This may manifest as changes in symbiont density, diversity, and community composition or even as symbiosis breakdown, as reviewed here.

The acute and chronic abiotic stressors driven by global climatic change include, increased temperature variability and unpredictability, rising temperatures (land and sea-surface), increased desertification, increased coastal salinization, increased ocean acidification and decreased oxygen content, increased upper ocean stratification, and increased frequency and intensity of extreme events (including land and marine heatwaves, droughts, extreme rainfall, wildfires) (Pörtner et al., 2019; van Diemen et al., 2019). Most research addressing holobiont responses to climate change has focused solely on exposure to high temperatures, distantly followed by the increasing threat of drought to crops. Different holobionts, mostly under experimental conditions, exhibit diverse responses to abiotic stress (Benard et al., 2020), and the evolutionary consequences of these responses have been comprehensively reviewed (Kiers et al., 2010; Renoz et al., 2019). However, much less attention has been given to the proximal ecological changes to symbioses under climate change, especially across a range of taxa. Here, I review evidence that climate change is affecting ecology of key symbioses. By identifying patterns between different symbiosis study systems, we may better predict and manage the effects of climate change across a wide range of ecosystems.

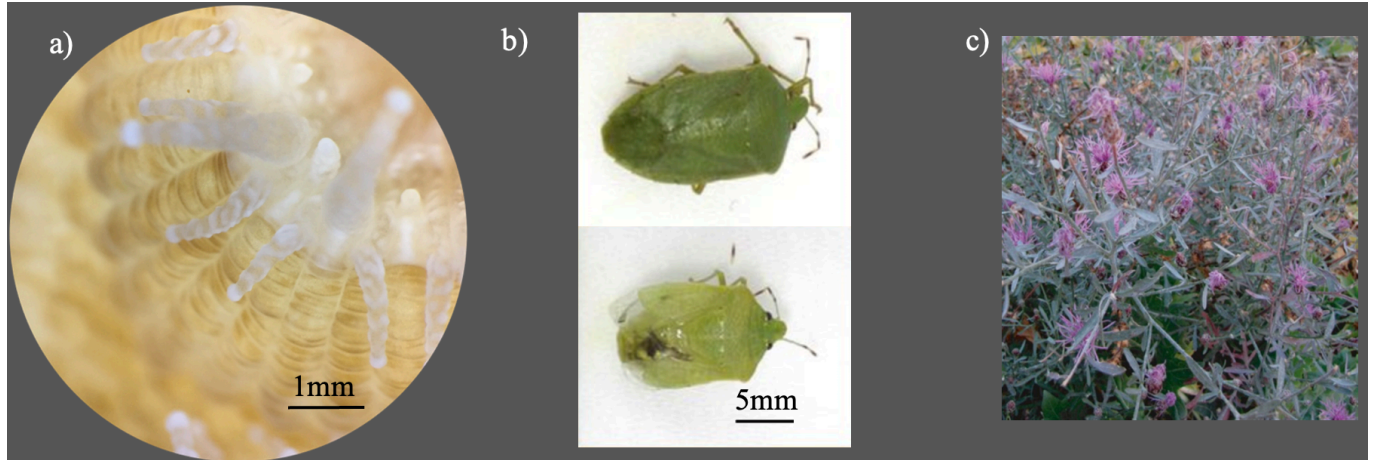


Figure 2.1: Indications of symbiont-mediated responses to climate change. **a)** A polyp of reef-building coral *Montastraea cavernosa* recovers from heat-induced experimental bleaching with genus *Durusdinium* symbionts, seen as brown specks proliferating in bands within the coral mesodermis. Image: D. Buzzoni. **b)** Southern green stinkbugs (*Nezara viridula*) treated with (bottom) and without (top) antibiotics during development. The loss of obligate hindgut bacteria causes hosts to develop into smaller, paler adults, an analogous phenotype to stinkbugs raised at high temperatures (Kikuchi et al., 2016). Image: (Kikuchi et al., 2016). **c)** Range expansions of knapweed (*Centaurea maculosa*) in USA grasslands are thought to be dependent on and facilitated by arbuscular mycorrhizal fungal symbionts (Harner et al., 2010). Image: D. Mummey, in Johnson et al., (2013).

2.2 Symbiont density: winners & losers

Dramatic increases in symbiont density in response to abiotic change may represent either increased symbiotic benefits to both partners or reduced host control over symbiont population growth, yet a collapse of *in hospite* symbiont populations is indicative of the breakdown of a symbiotic relationship. Coral bleaching is a symptom of the dysbiosis between a Cnidarian coral host (most notably reef-building corals, order Scleractinia) and its dinoflagellate symbionts in the family Symbiodiniaceae (Glynn, 1996; LaJeunesse et al., 2018). Coral mortality may follow bleaching due to the high nutritional dependence of corals on their symbionts (Muscatine & Porter, 1977). Coral bleaching is arguably the most ecologically devastating form of dysbiosis

both in terms of the spatial scale of global mass-bleaching events and the extent of ecosystem functional changes associated with the mortality of these foundation coral species (Baker et al., 2008; Hughes et al., 2018; Stuart-Smith et al., 2018). The study of these coral-algal symbioses is therefore of large significance for marine conservation, especially given tropical coral reefs are both the most speciose marine ecosystems and those most vulnerable under climate change (Knowlton et al., 2010; Bindoff et al., 2019; Smale et al., 2019). A dominant theory of the mechanism of stress-induced dysbiosis in corals, among others, is through host and/or symbiont cellular damage from reactive oxygen species (ROS) produced by symbiont photosynthetic impairment under environmental stress (Weis, 2008; Helgoe et al., 2024), such as that caused by temperature, salinity, irradiative stress, or exposure to pollutants. Experimentally, corals with higher symbiont densities have proved more susceptible to bleaching (Cunning & Baker, 2013, 2014).

In addition to corals, Symbiodiniaceae photosymbionts are hosted by other marine invertebrates including the excavating sponge *Cliona orientalis*, providing nutritional benefits to the host through the transfer of photosynthate. *C. orientalis* has been experimentally bleached under future warming and acidification conditions, exhibiting similar decreases in symbiont density and photochemical efficiency to those seen in reef-building corals (Fang et al., 2018). Yet unlike corals, bleaching under warm and acidic conditions did not appear to compromise host health in *C. orientalis*, but caused an increase in sponge growth rate (Fang et al., 2013). This increase in host biomass may be linked to this host's function as a bioeroder, benefitting from reduced metabolic costs of bioerosion under acidified conditions (Fang et al., 2013). Beyond Metazoans, foraminifera hosting either diatom or dinoflagellate photosynthetic symbionts at elevated temperatures show a decrease in photochemical efficiency preceding symbiont loss and

a bleached appearance (Schmidt et al., 2011; Uthicke et al., 2012). Changes over geological time in the carbon isotope ratios in fossilised foraminifera during the Middle Eocene transient warming period may also imply the evolutionary loss (and subsequent regain) of photosynthetic symbioses in response to warming (Edgar et al., 2013). This implies an environmental vulnerability incurred by hosting photosynthetic symbionts, subject to the holobiont's ability to limit, withstand or compensate for photoinhibition under the abiotic stressors of climate change.

The effects of abiotic stress on bacterial symbiont density changes are more varied; perhaps unsurprising given the diversity of bacterial environmental sensitivities and metabolic capabilities. For example, symbioses in rhizobial nitrogen-fixing trees are predicted to thrive with continued warming, mirroring the emergence of nitrogen-fixing angiosperms multiple times over evolutionary history in periods of increased temperature and atmospheric carbon dioxide (Li et al., 2015; Liao et al., 2017). On the other hand, hosts of other bacterial symbionts express a 'bleaching' phenotype with symbiont loss under environmental stress, including many insect hosts with obligate gut bacteriomes (Renoz et al., 2019). For example, raising southern green stinkbugs (*Nezara viridula*) at 2.5 °C above their seasonal maximum temperature causes them to be pale in colour and to host reduced obligate hindgut bacterial densities, with overall reduced fitness. An analogous response is observed in hosts treated with antibiotics during development (Kikuchi et al., 2016). *Wolbachia* bacteria infect a wide range of arthropod and nematode hosts, running the gamut from obligate mutualist to parasite depending on bacterial strain and host species among other factors (Chrostek et al., 2013). The effect of heat stress on *Wolbachia* densities is similarly strain-dependent (Wiwatanaratnabutr & Kittayapong, 2009; Ferguson et al., 2024), which may have been a driver in the global replacement of the previously dominant wMelCS *Wolbachia* strain with the wMel strain in *Drosophila melanogaster* over the course of

the 20th century (Riegler et al., 2005). This wMel strain also exists at lower densities in the fruit fly host which may reduce the cost to the host (Chrostek et al., 2013), and increases host thermal preference by at least 3 °C compared to wMelCS (Truitt et al., 2018). Interactions between bacterial symbionts may also shape the maintenance or breakdown of symbioses under climate change: Most species of aphid harbour *Buchnera* bacteria as obligate symbionts (host depends on the symbiosis for survival) in addition to various facultative (non-obligate) bacterial symbionts. Whilst *Buchnera* densities may decrease with heat stress, infection with certain facultative symbionts aids recovery of *Buchnera* densities through the production and release of heat shock proteins, reducing host reproductive failure under increased temperatures (Chen et al., 2000; Burke et al., 2010; Heyworth et al., 2020).

The broad environmental tolerances of many fungal symbionts may explain comparatively fewer reports of fungal symbiotic breakdown under abiotic stress. Mycorrhizal fungal symbionts of vascular plants are well-documented to buffer their plant hosts against abiotic stress such as drought (Kannadan & Rudgers, 2008) and extended heat stress (Kivlin et al., 2013; Cabral et al., 2016). However, quantifying the effects of climate change on these symbioses is complicated by the largely beneficial effects of increased atmospheric carbon dioxide on vascular plants. When exposed simultaneously to multiple climate change factors for 5 years, infections of grasses with above-ground fungal endophytes increased under elevated carbon dioxide, but exposure to warm temperatures or simulated drought had no significant effect on fungal growth (Brosi et al., 2011). Below-ground mycorrhizae can be categorised as either arbuscular mycorrhizal (AM) or ectomycorrhizal (ECM) symbionts, with these two groups responding differently to changes such as elevated carbon dioxide. Meta-analyses have revealed a consistent positive effect of elevated carbon dioxide on both mycorrhizal fungal growth and

plant host growth, with subtle distinctions between AM and ECM holobionts: The ratio of plant to fungal growth was higher with AM compared to ECM symbionts (Dong et al., 2018). Lower comparative resource allocation to the plant host by ECM symbionts suggests that these symbionts exhibit increased parasitic (more selfish) behaviour with increasing atmospheric CO₂.

2.3 Shifts from mutualism to parasitism

A mutualistic symbiosis depends on both the host and symbiont receiving a gross benefit from the association, yet in some cases the abiotic stress imposed by climate change may disrupt the cost to benefit ratio of symbiosis. For example, researchers compared the fitness of polar chlorophyte algae when algae were free-living and when associated with a fungal host (to form lichen) and found that the largest symbiotic benefit to the algae was protection from freezing, without which lichenization incurred a net fitness loss via carbon transfer to the host. Therefore, with increased polar warming the authors predict that these symbioses will no longer be mutualistic (Fernández-Marín et al., 2020). Similarly, the toxins produced by several defensive symbionts hosted by insects are labile under elevated temperatures, which suggests the anti-herbivore or anti-pathogen benefits provided by these symbionts may reduce as temperatures rise (Oliver & Perlman, 2020). The fungal endophytes associated with grassland plant species seem to become decoupled from plant host metabolism under experimental warming, which may also reflect reduced metabolic benefits to plants under increasing temperatures (Edwards et al., 2025). Conversely, the effects of climate change may alter some parasitic associations to become more mutualistic, such as some endolithic bacteria that colonise the shells of California mussels: As the structural integrity of mussel shells becomes compromised with endolithic infection, shells

also become lighter in colour, increasing solar reflectivity which was associated reduced mussel heatwave mortality (Gehman & Harley, 2019).

Variation in performance of coral-Symbiodiniaceae holobionts hosting different symbiont species may reflect their position on a continuum of symbiont mutualism to parasitism (Lesser et al., 2013), with the genus *Durusdinium* receiving considerable attention in this regard.

Durusdinium has long been implicated in conferring increased bleaching resistance to Scleractinian hosts under high temperature stress compared to some other species (Berkelmans & van Oppen, 2006), and consequently may be found in greater abundance in reef corals during and after bleaching events (LaJeunesse et al., 2009b). Whilst species of *Durusdinium* may incur smaller declines in photochemical efficiency under heat stress compared to other common Caribbean symbionts (Cunning et al., 2018), this may not fully account for the degree of bleaching resistance that they confer. Stress-induced photodamage has been shown to cause less bleaching of *Durusdinium* than expected, revealing a certain ‘tenacious’ quality of these symbionts and putative resistance to expulsion by their hosts (Silverstein et al., 2017). How differences in photochemical performance between *Durusdinium* and homologous symbionts affect host coral metabolism at ambient and at stressful temperatures is disputed: Corals hosting *Durusdinium* have been associated with reduced growth and reproductive output (Jones & Berkelmans, 2010). At elevated (sub-bleaching) temperatures these symbionts have been seen to transfer proportionally less photosynthate to their coral hosts and retain a greater proportion for growth (Pettay et al., 2015; Baker et al., 2018), exposing more parasitic tendencies of this symbiont under stress, although no analogous metabolic costs were detected in other coral taxa (Kemp et al., 2023). Whilst dramatic changes in symbiont density would not be expected at sub-

bleaching temperatures, a lack of dynamic change in symbiont density in response to changing returns may reflect reduced host control in a partnership.

In a model system for Cnidarian symbioses *Cassiopea xamachana*, *Symbiodinium microadriaticum* symbionts can be transmitted both vertically and horizontally at different stages of the host life cycle. Vertically-transmitted symbionts reached higher *in hospite* densities and were associated with increased host growth and reproduction compared to their horizontally-transmitted counterparts of the same species and phylotype (Sachs & Wilcox, 2006). This finding is consistent with the theory that vertically-transmitted symbionts are less inclined to parasitism than horizontally-transmitted symbionts due to the shared risk of reproductive failure (Lesser et al., 2013). However, a contrasting result was observed in a social amoeba which facultatively associates with various bacterial symbionts, when exposed to experimental warming: host reproductive success decreased the most when hosting the ‘most obligate’ bacterial symbiont taxon, the bacterium that benefits the most from the symbiosis and has the longest-standing association with this amoeboid host over evolutionary time (Shu et al., 2020).

Increased genetic diversity of symbionts *in hospite* increases the potential for competitive antagonism between symbionts, representing another risk factor in the descent to symbiont parasitism under environmental stress. In a co-culture of Symbiodiniaceae *Durusdinium trenchii* and *Cladocopium goreau*, elevated temperatures caused metabolic shifts in both species (comparative to monocultures) concurrent with competitive resource acquisition (McIlroy et al., 2020). In contrast, a similar investigation into the two obligate fungal symbionts of the beetle *Dendroctonus ponderosae* in culture revealed that the optimal temperatures of the two fungi differed, resulting in one outcompeting the other at elevated temperatures, yet there were no metabolic shifts observed in co-culture (Moore & Six, 2015). Competitive exclusion of

symbionts under elevated temperatures can increase holobiont fitness when the outcompeted symbiont is parasitic, as seen in cultures of bumblebee microbiomes (Palmer-Young et al., 2018), but the allocation of symbiont resources into competition may manifest in costs to the host. In an experimental comparison of photosynthate transfer to six reef-building corals, those corals hosting more diverse symbiont communities did receive marginally less photosynthate under heat stress (Kenkel & Bay, 2018). In contrast, hosting a diverse symbiont assemblage may provide benefits, such as the increased dietary flexibility afforded to insects with diverse gut microbiomes (Renoz et al., 2019). This could feasibly shape the capacity for climate change-related insect range shifts, as they encounter novel plant hosts.

2.4 Microbiome diversity changes

Research across a range of ecosystems has revealed contrasting effects of environmental disturbance on microbiome diversity. For example, decreased endophyte diversity has been observed upon exposing poplar trees to experimental warming (Bálint et al., 2015), yet meta-analysis has detected increased bacteriome diversity in corals under climate change-induced warming (McDevitt-Irwin et al., 2017). Researchers attributed increased alpha diversity within the bacteriomes of excavating sponges to the rapid growth of new and previously low abundance bacteria under experimental heat stress, whether through reduced host control or through competitive bacterial interactions (Fan et al., 2013). Similarly, exposure of the common Pacific reef-building coral *Pocillopora damicornis* to experimental heat stress caused bacteriome alpha diversity (within coral colonies) to increase by orders of magnitude and also increased the abundance of known bacterial pathogens (Tout et al., 2015). Conversely, Millar and Bennet

(2016) hypothesise that arbuscular mycorrhizal fungal alpha diversity will decrease under the abiotic stressors of climate change through environmental filtering of sensitive taxa.

At macroecological scales, decreases in beta diversity (between individuals) have been observed in a range of habitats subjected to local human disturbance and climate change (Socolar et al., 2016). The ‘Anna Karenina Principle’, when applied to animal microbiomes, predicts that dysbiosis may present as stochastic changes in microbiome composition and increased beta diversity (Zaneveld et al., 2017). Metanalysis of the effects of warming on microbiome community composition has revealed a lack of consistency in the effect of warming on microbial diversity, revealing a higher propensity for warming-induced increases in beta dispersion in microbiomes with lower initial richness (Li et al., 2023). However, the Anna Karenina Principle is supported by observations of increased gut microbiome heterogeneity (beta diversity) between individuals of the common Eurasian lizard (*Zootoca vivipara*) that were reared in warmer microclimates, despite decreased alpha diversity within the microbiomes of individual lizards (Bestion et al., 2017). Similarly, *Aiptasia*, a facultatively-symbiotic Cnidarian model system, exhibited both higher alpha and beta microbiome diversity following experimental heat stress over several years (Ahmed et al., 2019). The bacteriome beta diversity hosted by corals in a population exposed to a heatwave was seen to increase, but this effect was obscured in corals on reefs exposed to high local human disturbance (McDevitt-Irwin et al., 2019), emphasising the importance of considering interactions between multiple stressors on microbiome changes.

2.5 Harnessing symbiont adaptive potential

In addition to the environmental buffering afforded to holobionts by some (predominantly fungal) symbionts under environmental extremes (Benard et al., 2020), microbial symbionts with short generation times and the capacity for horizontal gene transfer may provide hosts with a larger pool of genetic variation for rapid adaptation (van Oppen & Medina, 2020). With a view to engineering climate change tolerance, this capacity for rapid adaptation has been leveraged by experimentally accelerating selection for these symbiont traits, with varying success. Artificially selecting for drought tolerance in fungal endophytes has proved a successful method of inferring these traits to commercially valuable rice varieties (Redman et al., 2011). Similarly, cultures of Symbiodiniaceae algae have shown increased thermal tolerance under artificial selection (Chakravarti & van Oppen, 2018), although uncertainty remains as to whether this tolerance is conferred to invertebrate hosts inoculated with cultured symbionts (Quigley et al., 2021).

The adaptive capacity of microbial symbionts is subject to greater constraints in other symbioses. The obligate bacterial gut symbionts of insects are known to have reduced genome sizes compared to non-obligate or free-living analogues (Renoz et al., 2019). These obligate bacteria are vertically transmitted, creating population bottlenecks with transmission between host generations, driving the accumulation of deleterious mutations and counteradaptations in the reduced genomes of these clonal populations and possibly narrowing symbiont thermal niches (Wernegreen, 2002, 2012; Bennett & Moran, 2015; Corbin et al., 2017).

2.6 New partnerships

The establishment, maintenance and recovery of symbiosis depend on both the availability of partners and their compatibility. Symbioses may be dynamic over a host's lifetime, and as

climate change shifts species' phenologies and spatial distributions, the potential for novel symbiotic interactions arises. The still contested 'Adaptive Bleaching Hypothesis' suggests that bleaching provides an opportunity for individual corals to host different algal symbionts upon bleaching recovery, whether through the differential retention and proliferation of resident symbionts or through the uptake of new taxa, effectively expanding corals' fundamental niches (Buddemeier & Fautin, 1993). Comparatively thermotolerant genus *Durussdinium* symbionts have undergone transient increases in abundance on reefs during and after bleaching events (LaJeunesse et al., 2009b), with more persistent abundances on reefs where *Durussdinium* is thought to have undergone less-recent adaptive radiations (LaJeunesse et al., 2010a). However, evidence of large-scale or long term symbiont shifts with global climate change are currently limited (except see Baker et al.,(2004) and Palacio-Castro et al.,(2023)). A global shift in the dominant strain of *Wolbachia* infecting fruit fly populations towards a comparatively thermotolerant strain occurred over the course of the 20th century, although this cannot definitively be linked to environmental changes (Riegler et al., 2005; Wiwatanaratnabutr & Kittayapong, 2009).

The degree of partner specificity or generalism may itself be a trait that is influenced by climate change as partner availability and suitability shifts. It has been suggested that those ectomycorrhizal symbionts capable of more generalist tree host associations will become more abundant as host ranges shift polewards and novel hosts become available (Markkola et al., 2016). Sampling of the bacterial endosymbiont communities within the leaves of mangrove plants revealed higher similarity between plants from warmer regions, attributed to the dominance of generalist bacterial taxa (Nethmini et al., 2025). On the other hand, Pacific reef-building coral genera associated with higher sensitivity to climate-related stress show greater

propensities for flexibility in their associations with Symbiodiniaceae taxa (Putnam et al., 2012). Host-symbiont coevolution may limit the formation of novel associations when host immune recognition of symbionts shows high specificity. For example, *Aiptasia* anemones experimentally inoculated with heterologous *Durussdinium trenchii* symbionts exhibited an increased immune response and *D. trenchii* reached lower infection densities compared to inoculations with native (homologous) *Symbiodinium linucheae* symbionts (Matthews et al., 2017; Medrano et al., 2019).

2.7 In focus: Symbiosis and immunity in corals

The host immune system is an integral component in the establishment and maintenance of symbioses and may be influenced by the abiotic conditions of a changing climate. Stable symbioses depend on evasion of the host immune system by symbionts, or distinctions made by the host immune system between symbionts and pathogens (Gerardo et al., 2020). Indeed, the maintenance of Cnidarian-Symbiodiniaceae symbioses is facilitated by a combination of host pattern recognition receptors and a degree of host immunosuppression (Mansfield & Gilmore, 2019). Immunosuppression in symbiosis may increase the coral host's susceptibility to pathogenic infections, supported by experimental evidence of decreased expression of key bacterial, viral and fungal immune response genes at higher algal symbiont densities (Fuess et al., 2020). Researchers have suggested that immunological vulnerability peaks upon recovery from bleaching, as energetic reserves have been depleted and the transient immunological protection afforded by low symbiont densities is lost, supported by field reports of higher disease incidence upon bleaching recovery in unbleached compared to bleached corals (Merselis et al.,

2018). This is particularly ominous in light of projections for increased coral pathogen abundance and virulence under climate change (Maynard et al., 2015). This dampening effect of symbiosis on the host's innate immune system is not universal across symbiotic systems. For example, in addition to the benefit of pathogen competitive exclusion by mutualistic microbiomes, *Wolbachia* directly aids mosquito host immunity to parasites by eliciting a priming response, triggering host immune upregulation (Moreira et al., 2009).

2.8 Conclusion

Microbial symbioses are ubiquitous across eukaryotes, ranging from mutualism to parasitism, with many habitat-forming holobionts forming the foundation of entire ecosystems (Tylianakis et al., 2008). However, increasing exposure to climate change-amplified stressors threatens the persistence of ecosystem services provided by microbial symbioses (Kiers et al., 2010). These stressors are known to be responsible for range shifts, local extinctions, and changes to the population structure of eukaryotic hosts (Chen et al., 2011; Wiens, 2016), with similar transformative changes likely for their microbial symbionts (DeAngelis et al., 2015; Zhou et al., 2016). Here, I have revealed commonalities in the responses of a range of microbial symbioses to environmental stress under climate change. Despite the potential for a diverse symbiont assemblage to expand a host's environmental tolerance in some systems, I have described evidence for competitive antagonism between symbiont taxa within a holobiont under environmental stress. Indeed, evidence from a range of natural symbioses suggests a general increased risk of shifts towards parasitism with the incursion of opportunistic symbiont taxa or the disruption of host-provisioning under environmental stress. Susceptibility to invasions of

parasitic or opportunistic symbionts varies with the flexibility of symbiotic associations, which is shaped by life history traits such as mode of symbiont transmission and the obligate or facultative nature of the symbiosis. Based on results from several ecosystems, we may expect increased dominance of more generalist symbiotic partners, as increasingly frequent and severe environmental disturbances facilitate novel host-symbiont interactions, favouring symbiotic flexibility.

Despite the importance of symbiotic interactions for ecosystem function (Tylianakis et al., 2008), multi-year monitoring surveys of symbiont assemblages in natural populations or communities under climate change are rare compared to those of their eukaryotic hosts (Elmendorf et al., 2012; Tanner & Connell, 2022). Notable exceptions to this include comparisons of the *Wolbachia* strains infecting wild *Drosophila melanogaster* populations over 30 years (Riegler et al., 2005) and a decade of monitoring the *D. melanogaster*-derived *Wolbachia* strain that was artificially introduced into wild mosquito populations (Ross et al., 2022). In addition, a 20-year soil warming experiment has already provided some insight into the effects of warming on nutrient transfer between plants and their mycorrhizal symbionts (Qiu et al., 2021; Chari et al., 2024). Currently, the paucity of these longitudinal datasets from natural populations constrains our understanding of the effects of climate change on the changes of stability of microbial symbioses, and the consequences for the ecosystems they support.

Chapter 3 – Exposure to multiple stressors underscores the differences in symbiotic flexibility between coral life histories

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3.1 Abstract

Climate change-amplified marine heatwaves pose the greatest threat globally to the future of coral reefs. Simultaneously, coral reefs are ubiquitously exposed to varying degrees of human disturbance, such as dredging and nutrient pollution. The algal symbionts (family Symbiodiniaceae) hosted by corals are intimately linked to host physiology and as such the composition of corals' symbiont assemblages can have dramatic consequences for their survival under stress. The effect of exposure to multiple stressors (e.g. human disturbance and heatwaves) on coral populations is not well-understood and the effect of multiple stressors on their symbiont assemblages remains even more obscure. The Pacific island of Kiritimati harbours reefs across a gradient of local human disturbance and was the epicentre for the 2015-2016 global coral bleaching event. Here we report symbiont metabarcoding from samples spanning the heatwave collected from 973 tagged colonies of 2 coral species that inherit symbionts vertically (maternally) and 4 that inherit symbionts horizontally (from the environment) at sites around Kiritimati. Before the heatwave, 90% of vertical-inheritance corals and 72% of horizontal-inheritance corals hosted only genus *Cladocopium* symbionts, with those hosting mixed-genus assemblages found mainly at sites exposed to higher human disturbance. After the heatwave, 80% of vertical-inheritance corals still hosted only *Cladocopium*, whilst 62% of horizontal-inheritance corals hosted exclusively genus *Durisdinium*, with mixed-genus assemblages now found at sites exposed to lower human disturbance. Characterising Symbiodiniaceae in a subset of coral colonies sampled before and after the heatwave revealed that 88% of horizontal-inheritance corals underwent a change in the symbiont genera hosted, compared to only 12% of vertical-inheritance corals, with the probability of change decreasing and increasing with higher

human disturbance in horizontal- and vertical-inheritance corals, respectively. The overall increased propensity for heatwave-induced symbiotic change in horizontal-inheritance corals was mirrored at finer taxonomic scales, with changes in the *Cladocopium* taxa hosted spanning larger phylogenetic distances in horizontal-inheritance corals. This natural experiment on a symbiotic model system reveals contrasting interactions between multiple stressors on symbiotic transitions between coral life histories, with implications for predicting and managing coral resilience in an increasingly disturbed ocean.

3.2 Introduction

Climate change-amplified marine heatwaves pose the greatest global threat to the persistence of coral reef ecosystems (Hughes et al., 2018), and simultaneous exposure to local anthropogenic disturbance further reduces their predicted resilience (Setter et al., 2022). However, only about 10 % of published research on coral responses to heat stress has considered the influence of local disturbance (Baum et al., 2023) despite the globally ubiquitous exposure of corals to some degree of local disturbance (González-Barrios et al., 2023). Non-linear or non-additive interactions between heat stress and human disturbance increase the variation in coral reef responses to multiple stressor exposures (Côté et al., 2016; Maucieri et al., 2023). For example, meta-analyses have revealed a range of instances whereby protection from local stressors has decreased coral reef community resilience upon exposure to an acute climate stressor, with prior environmental filtering of climate-sensitive taxa suggested as a mechanism for increased resilience of disturbed communities (Côté & Darling, 2010; Bates et al., 2019). However, the effects of multiple stressors on ecosystems are variable across spatial and biological scales (Simmons et al., 2021), including on coral reefs. Reef-coral communities exposed to more chronic human disturbance suffered smaller reductions in coral cover when exposed to an extreme marine heatwave, suggesting greater resilience, yet at the coral population level heatwave-resilience was seen to decrease with chronic disturbance (Baum et al., 2023). The coral host animal exists in association with a community of microbiota to form individual holobionts (Peixoto et al., 2017) and compared to the uncertainty surrounding the effects of multiple stressors on the coral host, understanding of multiple stressor effects on coral-associated

microbes is even more limited, and likely distinct from responses observed at the scale of the host.

The microalgal symbionts (Family Symbiodiniaceae) that live inside the endodermis of reef-building corals are intimately linked to the holobiont disturbance response (Berkelmans & van Oppen, 2006; Silverstein et al., 2015). Exposure to either chronic human disturbance or acute heat stress has been linked to increased beta-dispersion of coral-associated bacterial assemblages, with an antagonistic interaction between human disturbance and heat stress dampening the size of this response (McDevitt-Irwin et al., 2019). Simultaneous experimental exposure of *Pocillopora meandrina* corals to nutrient pollution and heat stress revealed a similar antagonistic interaction between these two stressors in the magnitude of shift in bacteriome community composition (Maher et al., 2019). Increased beta diversity in the Symbiodiniaceae hosted by multiple reef-building coral species has also been observed under increased chronic human disturbance across (Claar et al., 2020a). In contrast, exposure to severe heat stress or other chronic environmental stressors has resulted in the loss of algal symbiont richness within several coral populations (Kriefall et al., 2022; Quigley et al., 2022; Starko et al., 2023; Leiva et al., 2025), leaving uncertainty surrounding the effects of multiple stressors on the diversity of Symbiodiniaceae within and between coral colonies.

In addition to the generalised effects of disturbance on coral-microalgal symbioses, coral taxa differ in their inherent specificity with respect to microbial associations. Coral species may be described as microbiome ‘conformers’ or microbiome ‘regulators’ based on whether they show high or low flexibility, respectively, in their associated bacteriome in response to disturbance or environmental change (Ziegler et al., 2019). Similarly, some associations between corals and Symbiodiniaceae taxa show close co-phylogeny, a trait often associated with highly

specific symbioses that display minimal flexibility under environmental change (Turnham et al., 2021; Johnston et al., 2022). There is evidence that the mode of algal symbiont inheritance between coral generations shapes whether coral taxa show high or low specificity in their associations with Symbiodiniaceae due to differences in partner co-phylogeny between inheritance modes (Baker, 2003; Swain et al., 2018). More than 70% of reef-building coral species do not inherit Symbiodiniaceae directly from a parent but rather as larvae via exogenous uptake from their environment, employing ‘horizontal’ symbiont inheritance (Hartmann et al., 2017). Coral taxa that do inherit algal symbionts directly from their parents via ‘vertical’ symbiont inheritance, may also uptake additional Symbiodiniaceae horizontally from their environment, but often form highly specific symbioses (Quigley et al., 2018b; Swain et al., 2018). Results from a meta-analysis of the number Symbiodiniaceae taxa associated with corals support the link between horizontal symbiont inheritance and more diverse symbioses (Zarate et al., 2024), as these coral taxa have shown greater flexibility in their Symbiodiniaceae assemblage composition with environmental changes (Quigley et al., 2017; Marzoni et al., 2024). In comparison, the algal symbiont associations of corals with vertical symbiont inheritance may be more strongly influenced by coral host genetic factors than environmental factors (Quigley et al., 2017, 2018a).

Changes in symbiont assemblage composition in corals under chronic or acute stress can involve changes between or within Symbiodiniaceae genera. Levels of solar irradiation and inorganic nitrogen are both known to shape the distribution of symbiont taxa within and between reefs, with several taxa in genus *Symbiodinium* adapted to higher light intensities (Sampayo et al., 2007; Kemp et al., 2015) whilst genus *Durisdinium* symbionts often dominate reefs with increased sedimentation, turbidity and nitrogenous nutrient pollution characteristic of high

human disturbance (Ulstrup & Van Oppen, 2003; LaJeunesse et al., 2010b; Cooper et al., 2011; Poquita-Du et al., 2020; Nitschke et al., 2022). In this study, we use the term ‘spatial flexibility’ to describe the propensity for different corals to host different Symbiodiniaceae assemblages across gradients of environmental variables or chronic disturbance. Coral colonies may recover from bleaching to host different Symbiodiniaceae assemblages to their pre-bleaching symbionts and we use the term ‘temporal flexibility’ to describe coral propensity to undergo changes in the symbionts hosted in response to acute stress. Algal symbionts in the genus *Durisdinium* are known to increase in abundance on reefs following heat-induced coral bleaching (Baker et al., 2004; Palacio-Castro et al., 2023), in addition to their prevalence on chronically disturbed reefs. This has been attributed to relatively higher heat tolerance and bleaching resistance of *Durisdinium* taxa (Berkelmans & van Oppen, 2006; Silverstein et al., 2017; Cunning et al., 2018), although these stress-tolerant generalists in genus *Durisdinium* do not match the high heat tolerance of certain specialised species in genus *Cladocopium* (Hume et al., 2015; LaJeunesse et al., 2018).

The Pacific island of Kiritimati harbours reefs across a gradient of local human disturbance and was the epicentre for the 2015-2016 global coral bleaching event. Here we characterise the Symbiodiniaceae in samples collected from 2 coral species that inherit symbionts vertically and 4 that inherit symbionts horizontally at sites around Kiritimati exposed to different levels of chronic human disturbance, collected before and after exposure to acute heatwave stress. We investigate whether corals with horizontal and vertical symbiont inheritance differ in their spatial and temporal symbiont flexibility, as indicated by changes in Symbiodiniaceae assemblages upon exposure to multiple different stressors. We hypothesise that corals with horizontal symbiont inheritance show comparatively higher spatial symbiont

flexibility between symbiont taxa across a chronic human disturbance gradient, reflecting their tendency to host a greater diversity of algal symbionts. Secondly, we hypothesise that corals with horizontal symbiont inheritance respond to acute heat stress with greater temporal flexibility in the symbionts they host, as they shift to host different symbiont assemblages upon bleaching recovery. Finally, we hypothesise that corals with horizontal and those with vertical symbiont inheritance will host similar symbiont assemblages under exposure to multiple stressors, reflecting the cotolerance of genus *Durussdinium* symbionts to both human disturbance and heat stress. Changes to these symbioses under acute and chronic stress remains a cryptic yet influential factor shaping reef-building coral persistence this century.

3.3 Materials & methods

3.3.1 Exposure to acute and chronic disturbance

Kiritimati Island (Republic of Kiribati, central tropical Pacific) was the epicentre for the 2015-16 marine heatwave, exposed to more than 24 °C-weeks of accumulated heat stress, a metric representing the compounded duration and amplitude of sea surface temperature anomalies (Skirving et al., 2020). Heat stress exposure during this event was highly consistent between reef sites around Kiritimati, with variation in maximum accumulated heat stress estimated to be less than 1.08 °C-weeks (Baum et al., 2023).

Kiritimati's coral reefs are simultaneously exposed to a gradient of local human disturbance. Kiritimati's population of approximately 6,500, distributed mostly along the northeast lagoon-face (Kiribati National Statistics Office, 2016), exposes adjacent reefs to disturbance from wastewater runoff, subsistence-fishing, coastal construction, and dredging

(Figure 3.1a). To quantify these effects, two spatial data layers have been used to calculate a combined metric of chronic human disturbance at each reef site (Table A1): the first used community interview data to create a kernel density function of subsistence-fishing pressure within 10 km (Watson et al., 2016), the second estimates point-source waste-water exposure by generating a geographic buffer based on human population size within 2 km. Natural breakpoints in this combined metric allow sites to be discretely categorised by local disturbance (very low, low, medium, high and very high), which have previously been used to explain variability in many facets of coral reef health and function around Kiritimati (Magel et al., 2019; McDevitt-Irwin et al., 2019; Claar et al., 2020b, 2020c; Baum et al., 2023; Maucieri et al., 2023; Starko et al., 2023). Due to the spread of sample sizes used in this study across coral host species, sites and expeditions, very low and low disturbance categories have been combined, as have high and very high categories, hence sites are described as experiencing low, medium or high human disturbance.

3.3.2 Sampling coral colonies

Across each of 19 sites included in this study, samples from one consistent morphotype of each of 6 coral species were collected. Coral taxonomic classification was assigned based on colony macro-morphology (as described in Baum et al., (2023)). Study species were identified as *Dipsastraea matthai*, *Favites pentagona*, *Hydnophora microconos*, *Platygyra ryukyuensis*, *Pocillopora grandis* and *Porites lobata*. The symbiont transmission mode of each coral species was assigned from Baird et al., (2009) and Hartmann et al., (2017): *Dipsastraea matthai*, *Favites pentagona*, *Hydnophora microconos* and *Platygyra ryukyuensis* reproduce sexually by broadcast spawning, with horizontal symbiont inheritance (larvae take up Symbiodiniaceae from their

immediate environment), whereas *Pocillopora grandis* and *Porites lobata*, are also broadcast spawners but transmit Symbiodiniaceae from parent to larvae in the ova (vertical symbiont inheritance).

Coral tissue samples were collected over the course of 9 expeditions: August 2014, January 2015, May 2015, July 2015, March 2016, November 2016, July 2017, July 2018 and July 2019. Maximum heat-stress accumulation on Kiritimati occurred between December 2015 and January 2016, with the highest categorical Bleaching Alert status (Bleaching Alert Level 5) occurring between November 2015 and February 2016 (Skirving et al., 2020). Expeditions between August 2014 and July 2015 are therefore classed as ‘before peak-heatwave exposure’ (abbreviated to ‘before-heatwave’) and expeditions from March 2016 to July 2019 are classed as ‘after peak-heatwave exposure’ (‘after-heatwave’), (Figure 3.1b). Some individual corals were sampled during multiple expeditions before and/or after peak-heatwave exposure, but a maximum of one sample from each individual from before- and a maximum of one from after-heatwave expeditions were retained for analysis. In these cases, samples collected during expeditions closest to peak heatwave exposure were prioritised (July 2015 > May 2015 > January 2015 > August 2014 and March 2016 > November 2016 > July 2017 > July 2018 > July 2019), (Table A2).

On each expedition, colonies that were at least 20cm in diameter and at least 5m apart were tagged and sampled along 60m fore-reef transects following a 10-12m isobath, and previously tagged colonies were re-sampled. Tissue samples were taken from the central, uppermost surface of colonies using a woodworking chisel, and then stored in individual bags on ice until the end of each day, when $\sim 1\text{cm}^3$ of tissue from each sample was transferred to

microcentrifuge tubes containing a guanidinium isothiocyanate buffer. Samples were stored at 4°C until DNA extraction.

3.3.3 DNA extraction and sequencing

A protocol designed for Symbiodiniaceae was used to extract DNA from coral tissue samples preserved in guanidinium isothiocyanate buffer (Stat et al., 2009b). 200µl subsamples of homogenised tissue/buffer slurry were incubated overnight at -20°C with an equal volume of isopropanol. Precipitated DNA was then collected into a pellet by centrifugation and washed three times with 70% ethanol prior to resuspension in Tris buffer. The internal transcribed spacer 2 (ITS2) ribosomal region was amplified using a two-step PCR. The first round of PCR used forward primer ITS-DINO (Pochon et al., 2001) and reverse primer ITS2Rev2 (Stat et al., 2009b) with standard Illumina adapters to amplify a ~271-303bp amplicon. PCR amplicons were visualised with gel electrophoresis on a 2% agarose gel. Samples that failed to amplify were re-amplified using template DNA that was cleaned using a Genomic DNA Clean & Concentrator kit (Zymo Research) according to the manufacturer's protocol. Negative controls from each DNA extraction and each PCR were used to visually check for contamination using gel electrophoresis. Library preparation followed an adapted Illumina 16S Metagenomics protocol (Illumina, 2015; Pochon et al., 2019; Hume et al., 2020), with two AMPure bead (Beckman Coulter) clean-ups, an indexing PCR using Nextera primers (Illumina) and amplicon normalisation with a PicoGreen dsDNA assay (Invitrogen) prior to pooling. Sequencing was performed across ten runs on the Illumina MiSeq platform (yielding paired-end, 300 base-pair reads), either at the Hawaii Institute of Marine Biology Genetics Core Lab or at the John G. Shedd Aquarium (Chicago) Molecular Ecology Laboratory.

3.3.4 Sequence quality control, clustering and classification

Sequence data were submitted to the remote SymPortal database (Hume et al., 2019), which uses co-occurrence patterns to identify defining intragenomic variants (DIVs) and further clusters these into ITS2 profiles (putative species). To minimise the effect of uneven sample sequencing depths on the detection of low abundance taxa, sample coverage of DIV richness was modelled against sequencing depth using the ‘iNEXT’ package (Hsieh et al., 2024), revealing estimated sample coverage greater than 0.999 at 1000 reads. Hence, only sequence data from samples with >1000 total post-processing Symbiodiniaceae reads were retained, with a mean of 18,563 Symbiodiniaceae reads per retained sample. Sequence variants detected in these retained samples were collapsed by SymPortal into 178 distinct ITS2 profiles; 15 in genus *Symbiodinium* (formerly Clade A), 128 in genus *Cladocopium* (formerly Clade C) and 35 in genus *Durisdinium* (formerly Clade D). Estimated Unifrac distances between ITS2 profiles within each symbiont genus were obtained from SymPortal (based on sequence dissimilarities between profiles’ composite defining intragenomic variants). ITS2 profiles in genus *Cladocopium* were assigned to lineages based on the Unifrac-distance clustering of profiles, supported by their dominant sequence variants (Figure A1).

3.3.5 Statistical analyses

To investigate the spatial flexibility of the Symbiodiniaceae genera hosted by coral taxa under local human disturbance, two metrics were used: The change in Jaccard dissimilarity (presence/absence) in the symbiont genera hosted or change in the number of genera hosted simultaneously, across a gradient of local human disturbance. Symbiodiniaceae genus Jaccard

dissimilarities between corals of each genus within the same human disturbance category were calculated using the ‘vegdist’ function (Oksanen, 2024). To test the effect of symbiont inheritance mode and human disturbance on Jaccard dissimilarities, generalised linear mixed effects models with beta error distributions were fit to samples from before the heatwave and again for samples collected after the heatwave using the glmmTMB package (Brooks et al., 2017). Symbiont inheritance mode (horizontal or vertical), human disturbance (rescaled site-level continuous disturbance estimate) and their interaction, were included as fixed effects, whilst the collection dates and collection sites of each paired sample, along with host genus (nested within inheritance mode, allowing for random slopes and random intercepts) were included as random effects. The same random effects were used in generalised mixed effects linear models (with binomial error distributions) used to assess the relationship between a coral hosting more than symbiont genus simultaneously, symbiont inheritance mode, human disturbance and their interaction, either before or after the heatwave.

To investigate the temporal flexibility of individual coral colonies’ associations with Symbiodiniaceae taxa, a subset of 121 tracked colonies which were sampled both before and after peak-heatwave exposure were used (comprising 242 of the 1,094 samples used in this study). This subset included 15 *Dipsastraea*, 22 *Favites*, 11 *Hydnophora*, 24 *Platygyra*, 48 *Porites* and only 1 *Pocillopora* colony due to minimal heatwave survival of *Pocillopora* corals. Therefore, results of symbiont temporal flexibility of vertical-inheritance corals almost exclusively represent *Porites* corals. Generalised linear mixed effects models with binomial error distributions were used to test the effect of symbiont inheritance mode and human disturbance on the probability of a change in symbiont genera hosted by a tracked coral from before to after peak-heatwave exposure: a binary variable representing a change in the symbiont genus/genera

hosted served as the response variable, with symbiont inheritance mode and rescaled continuous human disturbance as fixed effects, and coral genus (nested within inheritance mode), site, before-heatwave sampling date and after-heatwave sampling date as random effects. Similarly, to test the effect of human disturbance on the probability of a change in the *Cladocopium* ITS2 profile hosted by tracked vertical-inheritance corals, a generalised linear mixed effects model with binomial error distribution was fit, with site, before-heatwave sampling date and after-heatwave sampling date as random effects. To parse out the contributions of individual fixed effects to linear trends from models, the ‘emtrends’ function from the ‘emmeans’ package was used (Lenth, 2022).

3.4 Results

3.4.1 Spatial flexibility in symbiont genera hosted under chronic disturbance

Before peak heatwave exposure, corals showed flexibility in the symbiont genera they hosted when exposed to higher human disturbance exposure: Most corals with vertical inheritance hosted *Cladocopium* only, with the remaining 10% (33/345 corals) hosting mixed *Cladocopium-Durusdinium* or *Cladocopium-Symbiodinium* assemblages, mainly at high human disturbance sites (Figure 3.2). Similarly, 72% (188/260) of horizontal-inheritance corals hosted *Cladocopium* only, with those corals hosting *Durusdinium*-only or *Cladocopium-Durusdinium* assemblages mainly at high human disturbance sites. Dissimilarity in the symbiont genera hosted by corals increased with human disturbance exposure for corals with vertical inheritance ($z = 9.43$, $P < 0.001$) and with horizontal inheritance ($z = 17.88$, $P < 0.001$), although this association was significantly stronger in horizontal-inheritance corals (interaction; $z = 12.89$, $P < 0.001$,

estimated difference between slopes = 0.22). In contrast, the association between symbiont genus dissimilarity and human disturbance was less variable between coral genera within each symbiont inheritance mode (Figure A2; slope standard deviation within symbiont inheritance modes = 0.10). Before peak heatwave exposure, significantly more coral colonies hosted multiple Symbiodiniaceae genera simultaneously with human disturbance exposure (vertical-inheritance corals; $z = 5.02$, $P < 0.001$, horizontal-inheritance corals; $z = 3.22$, $P = 0.001$), with a stronger association in vertical-inheritance corals (interaction; $z = 1.98$, $P = 0.048$).

After peak heatwave exposure, associations between human disturbance exposure and coral-symbiont spatial flexibility had transformed (Figure 3.2). Whilst most (163/204 corals, 80%) vertical-inheritance corals still hosted assemblages consisting only of *Cladocopium*, more corals hosted *Symbiodinium-Cladocopium* assemblages under medium disturbance and *Cladocopium-Durusdinium* or *Durusdinium*-only under high disturbance. In contrast, the horizontal-inheritance corals hosting only *Durusdinium* had increased from 11% (28/260) before to 62% (178/285) after the heatwave, with the remainder hosting mixed *Symbiodinium-Durusdinium* or *Cladocopium-Durusdinium* assemblages mostly under medium or low human disturbance and only 4% (12/285) hosting *Cladocopium* only. After peak heatwave exposure there was no significant effect of human disturbance exposure on the dissimilarity in symbiont genera hosted for vertical-inheritance corals ($z = -0.41$, $P = 0.683$). With the heatwave-induced shift from *Cladocopium*-only to *Durusdinium*-only assemblages in many horizontal-inheritance corals, symbiont dissimilarity decreased with human disturbance exposure post peak heatwave ($z = -4.29$, $P < 0.001$). The association between symbiont dissimilarity and human disturbance differed significantly between vertical- and horizontal-inheritance corals after the heatwave (interaction; $z = -6.71$, $P < 0.001$, estimated difference between slopes = 0.13), with

comparatively modest variability between coral genera within symbiont inheritance modes (Figure A2; slope standard deviation within inheritance modes = 0.08). Associations between human disturbance and hosting multiple symbiont genera were not statistically significant for vertical- ($z = 1.07$, $P = 0.295$) or horizontal-inheritance corals ($z = -1.91$, $P = 0.054$), although this response differed significantly between symbiont inheritance modes (interaction; $z = 2.30$, $P = 0.021$).

3.4.2 Temporal flexibility in symbiont genera hosted through acute disturbance

Pre-to-post heatwave changes in the symbiont genera hosted by the subset of coral colonies that were tracked through the heatwave mirror the trends in symbiont assemblages shown by the full dataset: 100% (26/26) of tracked vertical-inheritance corals (mainly represented by *Porites lobata*) at low disturbance hosted only *Cladocopium* both before and after peak heatwave exposure, whereas 22% (5/23) of corals at medium or high human disturbance switched to hosting mixed *Symbiodinium-Cladocopium* or *Cladocopium-Durusdinium* assemblages (Figure A3). Similarly, changes within tracked horizontal corals mirrored changes observed in coral populations, with 42% (24/57) of individuals under low or medium human disturbance switching from hosting *Cladocopium* to *Durusdinium* and 47% (27/57) switching to or from mixed *Symbiodinium-Cladocopium* or *Cladocopium-Durusdinium* assemblages. In contrast, 27% (4/15) of horizontal corals at high disturbance retained *Durusdinium*-only assemblages from pre-to-post heatwave and an additional 60% (9/15) switched to hosting to *Durusdinium* only. Overall, the probability of a heatwave-induced change in the symbiont genera hosted by these tracked colonies was higher in horizontal transmitters (88%, 63/72) than in vertical (12%, 6/49) transmitters ($z = -5.05$, $P < 0.001$). The association between a change in symbiont genera and

human disturbance exposure was also significantly different between horizontal and vertical corals (Figure 3.3; interaction; $z = -2.73$, $P = 0.006$, estimated difference between slopes = 1.70), tending to decrease in horizontal corals and increase in vertical corals with increasing human disturbance although neither association was significant in isolation (horizontal; $z = -1.76$, $P = 0.079$, vertical; $z = 1.69$, $P = 0.091$). The estimated standard deviation in slopes (probability of change in symbiont genera ~ human disturbance) between coral genera within each mode of symbiont inheritance was negligible (< 0.001), supporting the grouping of coral genera into ‘horizontal’ and ‘vertical’ corals with contrasting responses in their spatial and temporal symbiotic flexibility under multiple stressors (Figure A4).

3.4.3 Temporal flexibility in symbiont species hosted through acute disturbance

Comparing the heatwave-induced changes or persistence of species within Symbiodiniaceae genera revealed differences in temporal flexibility between coral life histories at finer symbiont taxonomic scales. Detections of genus *Symbiodinium* before the heatwave were limited to 9 coral colonies and detections of genus *Durusedinium*, whilst common both before and after heatwave exposure, span a very narrow phylogenetic distance (all *Durusedinium* taxa detected had high sequence similarity) compared to genera *Symbiodinium* and *Cladocopium* (Figure A5). For these reasons, detections of genus *Cladocopium* before and after the heatwave were used to investigate the flexibility of associations of individual corals with specific symbiont taxa (Figure 3.4), based on those tracked corals hosting *Cladocopium* both before and after peak heatwave exposure (18 colonies total of *Dipsastraea*, *Favites* and *Platygyra* and 48 *Porites* colonies). All horizontal-transmitting corals hosted different *Cladocopium* profiles before and after heatwave exposure ($n = 18$), most of which were in different lineages ($n = 17/18$) and the identity of *Cladocopium*

lineages hosted differed between *Dipsastraea*, *Favites* and *Platygyra* corals (Figure A6). In contrast, most vertical-inheritance corals (represented by *Porites* only) associated with the same *Cladocopium* taxa within the C15 lineage before and after the heatwave (52%, $n = 25/48$), and those that associated with a different *Cladocopium* taxon mostly hosted another within the C15 lineage (31%, $n = 15/48$). No tracked horizontal-inheritance coral hosted the same *Cladocopium* taxon before and after the heatwave, hence the probability of *Cladocopium* change could not be fit to a model. However, the probability of a change in the *Cladocopium* profile hosted by tracked vertical corals tended to increase with increasing human disturbance, although this change was not statistically significant ($z = 1.19$, $P = 0.24$).

3.5 Discussion

3.5.1 Symbiotic flexibility differences between coral life histories

We observed more spatial flexibility between Symbiodiniaceae genera with chronic human disturbance exposure and more temporal flexibility in the Symbiodiniaceae taxa hosted with acute heatwave exposure in horizontal- compared to vertical-inheritance coral species, both in accordance with our hypotheses. These results are consistent with the paradigm of symbiosis ecology that vertical transmission promotes inflexible co-evolved partnerships (Douglas, 2010), and with previous results linking corals with horizontal symbiont transmission to a diversity of associated Symbiodiniaceae taxa (Zarate et al., 2024). The greater symbiont flexibility in horizontal- compared to vertical-inheritance corals in response to disturbance exposure was largely driven by shifts from *Cladocopium*-only assemblages towards generalist symbionts in genera *Durusdinium* and *Symbiodinium*. This is consistent with reports that horizontal-

inheritance corals are more likely to associate with generalist symbionts than vertical-inheritance corals (LaJeunesse et al., 2004, 2008; Stat et al., 2008a; Fabina et al., 2012).

The inflexible symbioses reported here between *Porites lobata* and *Cladocopium* C15 symbionts are characteristic of those formed by *Porites* spp. (LaJeunesse et al., 2018; Forsman et al., 2020). However, *Pocillopora* spp. have previously been described as symbiont generalists, based on high heterogeneity of Symbiodiniaceae sequence variants detected in coral colonies of this genus (Putnam et al., 2012). Extensive heatwave-mortality of *Pocillopora grandis* colonies on Kiritimati limited our ability to assess the temporal flexibility of symbioses within colonies, yet our population-level sampling revealed *Cladocopium* associations largely confined to the C42 lineage both before and after heatwave exposure, with additional incursion of *Durussinium* (Figure A2, Figure A5.b). Indeed, more recent sequencing of *Pocillopora* spp. has revealed Symbiodiniaceae associations specific to host phylotypes, rather than reflecting local reef environments as per more symbiotically-flexible coral taxa (Burgess et al., 2021; Johnston et al., 2022; Marzoni et al., 2024).

3.5.2 Multiple stressor responses in corals' Symbiodiniaceae assemblages

Heatwave exposure combined with high human disturbance generated divergent responses in horizontal- and vertical-corals in terms of the symbiont flexibility they displayed (Figure 3.5). The disturbance-induced changes in symbionts hosted by *Porites* coral colonies with exposure to acute heatwave stress combined with higher human disturbance were greater than those seen in colonies in response to heatwave stress without additional human disturbance. In contrast, symbiont changes in horizontal-inheritance coral colonies were greater with exposure to heatwave stress only, compared to combined heatwave and human disturbances. In systems with

unbounded continuous response variables, a response under combined stressors that is smaller than their summed individual effects may indicate an antagonistic interaction between stressors (Côté et al., 2016). However, in this study, symbiont flexibility is bounded by the range of discrete states observed in corals' symbiont assemblages, limiting our interpretation of interactions between heatwave and human disturbance exposure. We instead suggest that the diminished changes observed in horizontal-inheritance corals under multiple stressors reflect a natural ceiling for symbiont assemblage changes in these more symbiotically flexible coral taxa under stress, characterised by the dominance of *Durusdinium*. This supports the characterisation of *Durusdinium* symbionts as stress-tolerant generalists with the potential to dominate marginal coral reef habitats (LaJeunesse et al., 2010b). An analogous shift from typically more host-specific ectomycorrhizal symbionts to more generalist arbuscular mycorrhizal symbionts has recently been detected across plant roots in temperate regions across the world with increasing habitat urbanisation (Radujković et al., 2025).

3.5.3 Implications of symbiotic flexibility for coral resilience

Exposure to chronic human disturbance or acute heatwave stress saw increases in corals hosting symbionts in genera *Symbiodinium* and *Durusdinium* and in those hosting mixed-genus assemblages, around Kiritimati. Multiple genera of Symbiodiniaceae are commonly found cohabiting within juvenile corals (Abrego et al., 2009) or across spatial micro-niches of a colony's morphology (Lewis et al., 2022), although adult corals of a range of taxa simultaneously host additional symbiont genera at very low relative abundances (Silverstein et al., 2012). A change from single- to multi-genus symbiont assemblages, such as that observed on Kiritimati, may normally be hindered by niche pre-exemption by the dominant genus, inhibiting

invasion or out-competition by additional genera when not exposed to significant disturbance (Fukami, 2015; Duijser et al., 2025). The implications for the coral host of an invasion of additional Symbiodiniaceae genera are uncertain, but may increase the risk of antagonistic interference between symbiont taxa (Magnoli & Bever, 2023). Hosting solely Symbiodiniaceae in genera *Symbiodinium* or *Durusdinium* may also incur physiological costs to corals associated with reduced energetic provisioning from these generalist symbionts compared to many host-specific *Cladocopium* taxa, especially under non-stressful conditions (Stat et al., 2008b; Jones & Berkelmans, 2010), although costs may only appear over timescales of several months (Kemp et al., 2023; Matsuda et al., 2023)

Beyond the implications for coral health of hosting particular Symbiodiniaceae taxa, the resilience implications of corals' capacity for symbiont flexibility are disputed. The Adaptive Bleaching Hypothesis of reef coral resilience under environmental stress posits that the loss of algal symbionts from coral tissue via bleaching facilitates the recovery of different symbionts (whether beneficial to the host or not), effectively expanding corals' ecological niche (Buddemeier & Fautin, 1993). However, network analysis of coral-Symbiodiniaceae associations suggests that symbiont transmission mode, shown here to shape the flexibility of symbioses, is not a reliable predictor of coral bleaching resilience (Swain et al., 2021). Indeed, the heatwave survival on Kiritimati of *Pocillopora grandis* and *Porites lobata* was estimated as 2% and 69% respectively, whilst that of horizontally-inheritance corals *Dipsastraea matthai*, *Favites pentagona*, *Hydnophora microconos* and *Platygyra ryukyuensis* ranged from 49-70% (Baum et al., 2023). It has been suggested that flexible coral-microalgal symbioses are indicative of holobiont environmental sensitivity, largely based on the sensitivity of corals in genera *Pocillopora* and *Acropora* compared to more symbiotically-inflexible *Porites* spp. corals

(Putnam et al., 2012). However, these inflexible associations are not universally bleaching-resilient, with fine-scale taxonomic differences in symbiont associations between coral phylotypes driving definitive differences in holobiont bleaching and mortality under heat stress (Sampayo et al., 2008; Johnston et al., 2022; Starko et al., 2023). Indeed, the generally high susceptibility of many *Pocillopora spp.* and *Acropora spp.* corals to heat-induced bleaching and mortality may simply demonstrate the generalised environmental sensitivity of fast-growing branching coral morphologies (Marshall & Baird, 2000; Loya et al., 2001; Baum et al., 2023).

The near-ubiquitous dominance of *Durusedinium* symbionts in Kiritimati's symbiotically flexible corals under the combined pressures of chronic human disturbance and acute heatwave stress calls into question the capacity for any further symbiont flexibility in these corals. The capacity of symbiotically flexible reef corals to recover some of their pre-heatwave heterogeneity in the Symbiodiniaceae hosted, despite ongoing human disturbance, is likely to significantly influence their resilience through subsequent heatwaves (Fabina et al., 2013; Ziegler et al., 2018a; Shan et al., 2025).

3.6 Figures

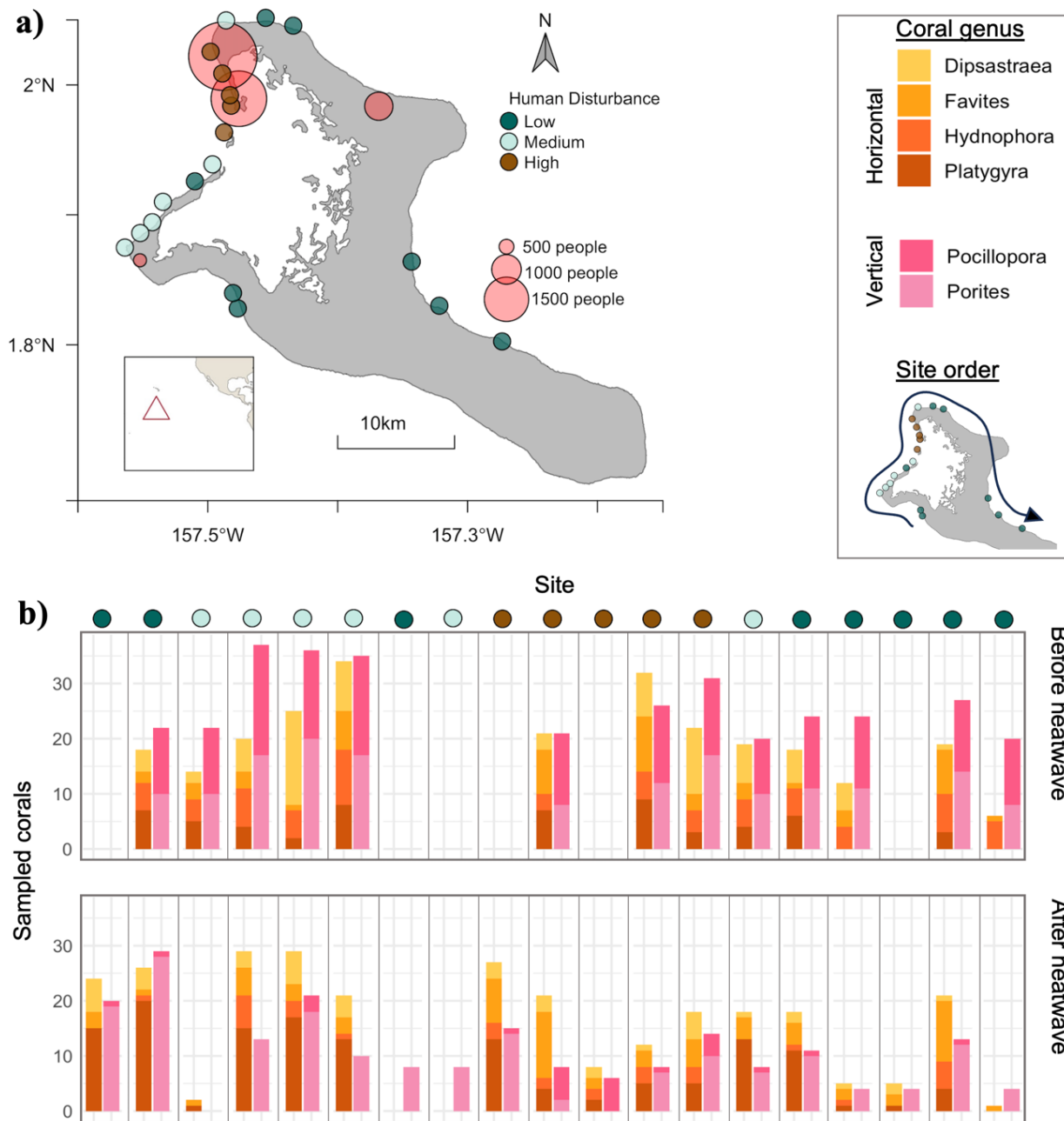


Figure 3.1: Corals of 4 genera with horizontal symbiont inheritance and 2 genera with vertical symbiont inheritance were sampled from 19 sites around Kiritimati Island (Republic of Kiribati) between August 2014 and July 2019. Permanent settlements are shown as red spheres scaled by estimated human population size. **a)** Sampling sites are distributed across a gradient of chronic human disturbance exposure and have hence been categorised as low, medium or high human disturbance. **b)** The number of coral samples from each genus collected before peak-heatwave exposure (August 2014 - July 2015, n = 605) and after (March 2016 – July 2019, n = 489). The 1094 coral samples are shown by sampling site, with sites ordered left to right according to their location moving clockwise around Kiritimati ('site order').

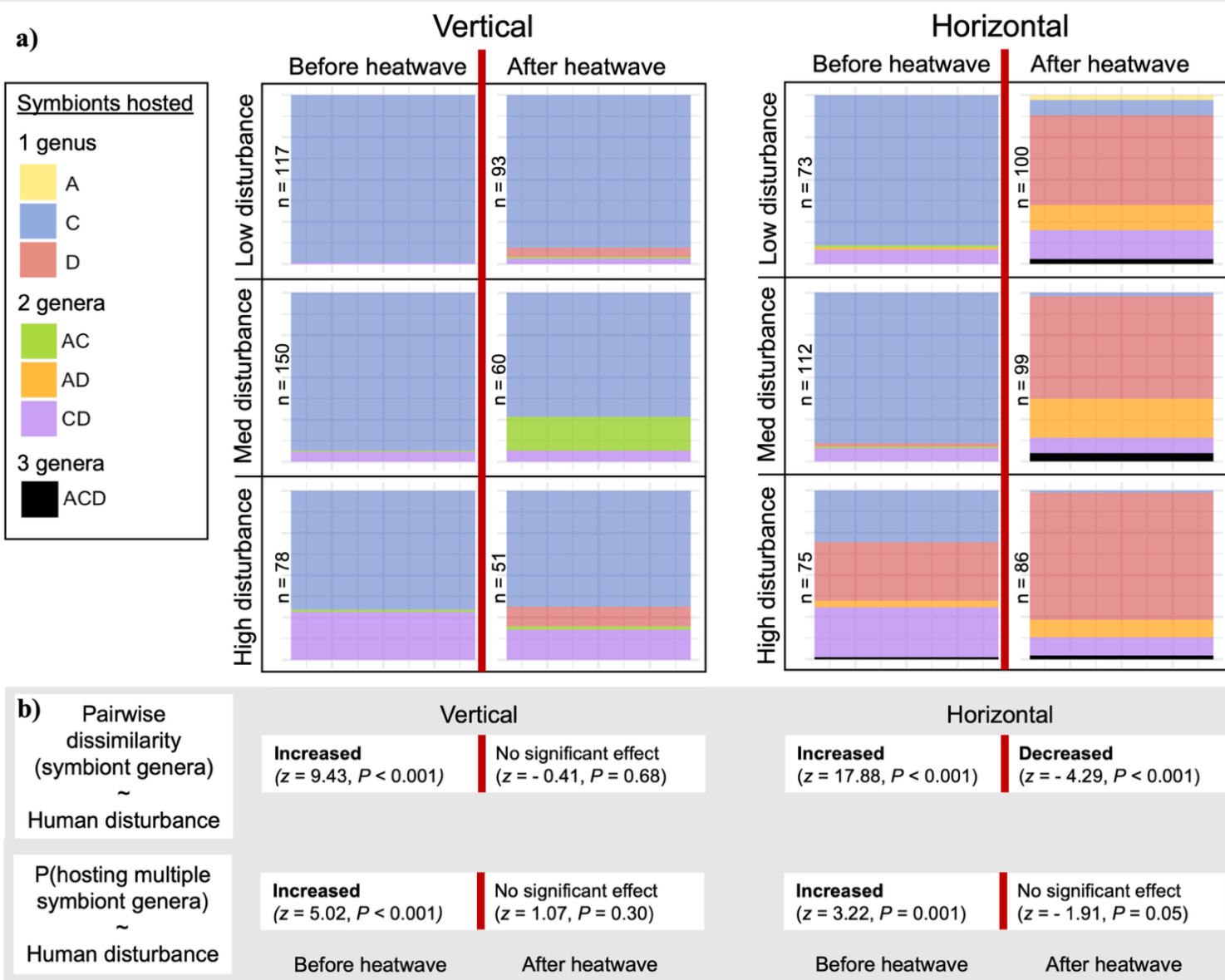


Figure 3.2: Symbiodiniaceae genera detected in corals with **a)** vertical or **b)** horizontal symbiont inheritance, before or after peak heatwave exposure and from sites exposed to low, medium or high human disturbance (1,094 samples from 973 distinct colonies). Coloured areas represent the proportion of corals in each group found to host each combination of symbiont genera *Symbiodinium* ('A'), *Cladocopium* ('C') and *Durussdinium* ('D'). **c)** Associations between increasing continuous human disturbance exposure and two metrics of the beta diversity of corals' symbiont assemblages; pairwise Jaccard dissimilarity in Symbiodiniaceae genera and a binary variable for hosting more than one symbiont genus.

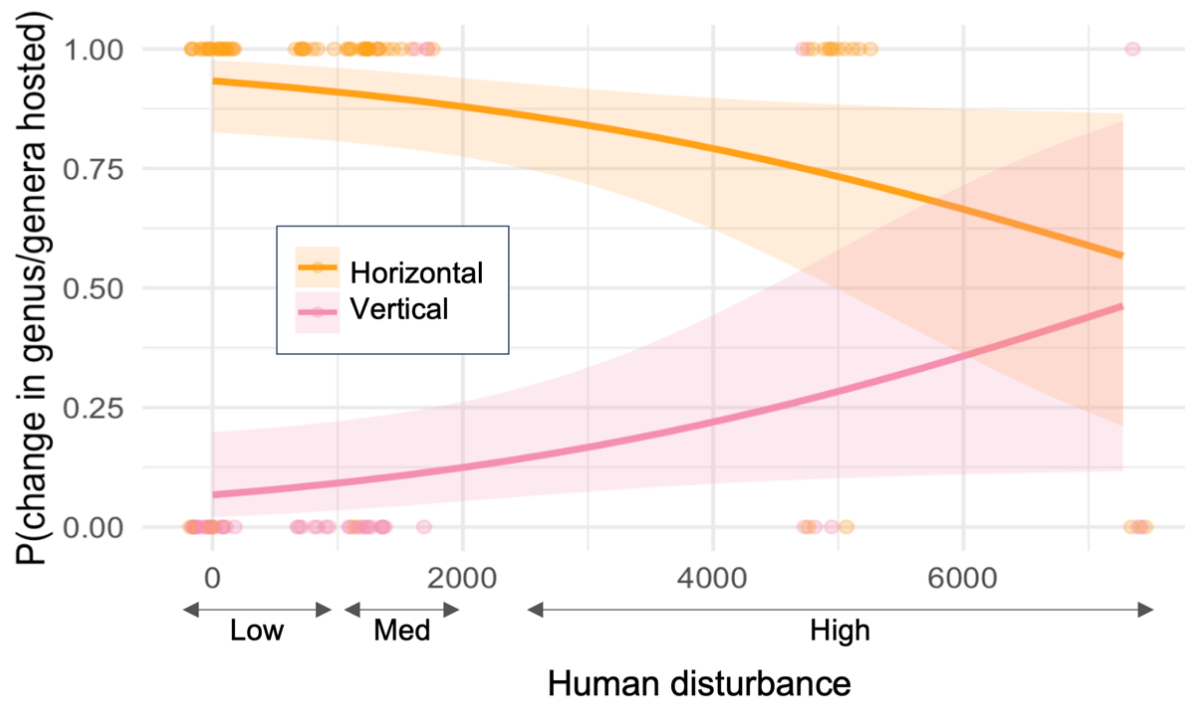


Figure 3.3: Probability of a change in the combination of Symbiodiniaceae genera hosted by a tracked coral from before peak heatwave exposure to after peak heatwave exposure. Points represent a binary variable of change, coloured by symbiont inheritance mode; horizontal (72 corals) or vertical (49 corals). Coloured lines and surrounding shading represent estimated probabilities and 95% confidence intervals of change for each inheritance mode against continuous human disturbance exposure.

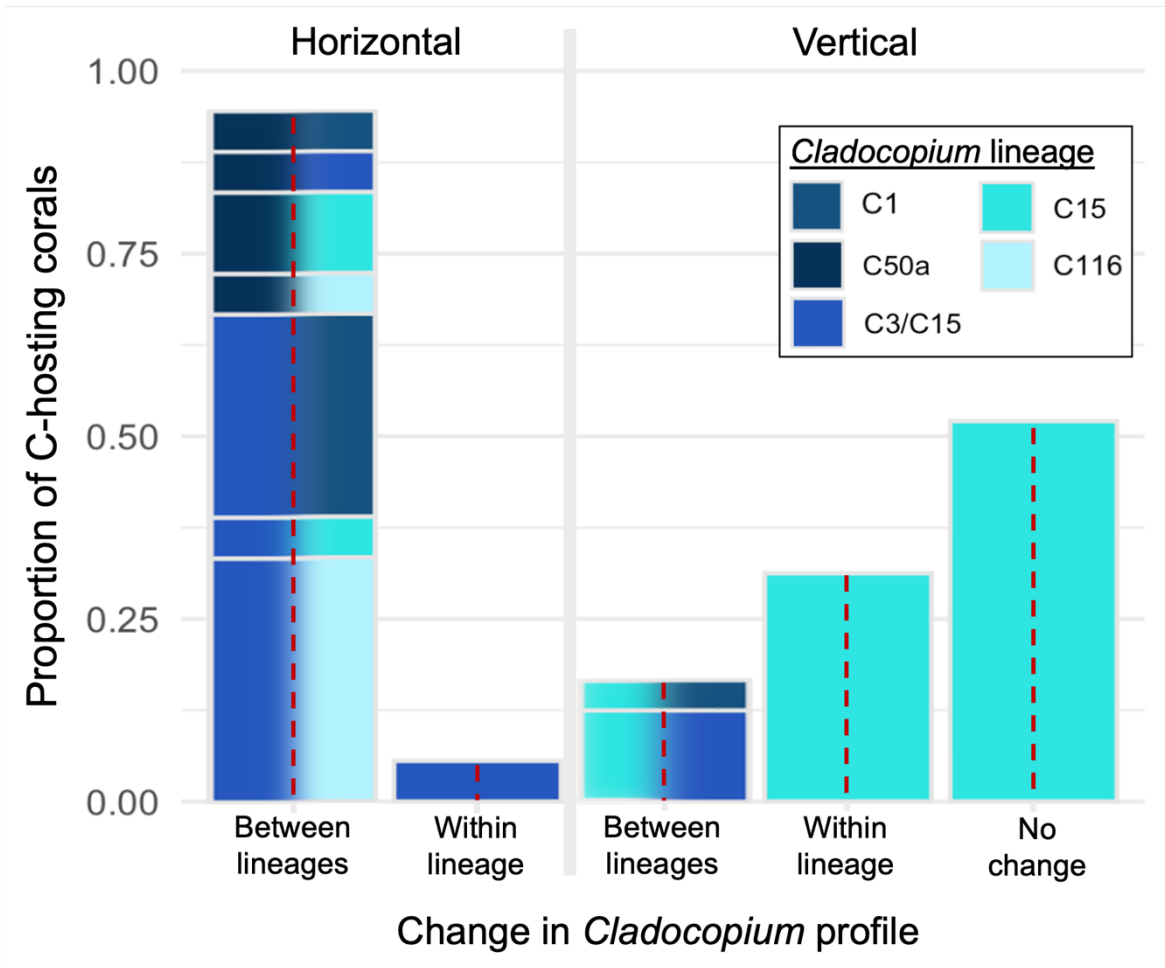


Figure 3.4: Proportions of corals in each mode of symbiont inheritance that underwent pre-to-post heatwave changes in the *Cladocopium* symbionts hosted, from the subset of tracked corals that hosted *Cladocopium* both before and after peak heatwave exposure (n = 18 colonies of horizontal corals from genus *Dipsastraea*, *Favites* or *Platygyra* and n = 48 vertical corals from genus *Porites*). Bar colours represent the *Cladocopium* lineage of the profile hosted by a coral before peak heatwave (left of red-dashed line) to after peak heatwave (right of red-dashed line). Changes between two *Cladocopium* profiles from different lineages ('between lineages') are differentiated from changes between two *Cladocopium* profiles from the same lineage ('within lineage').

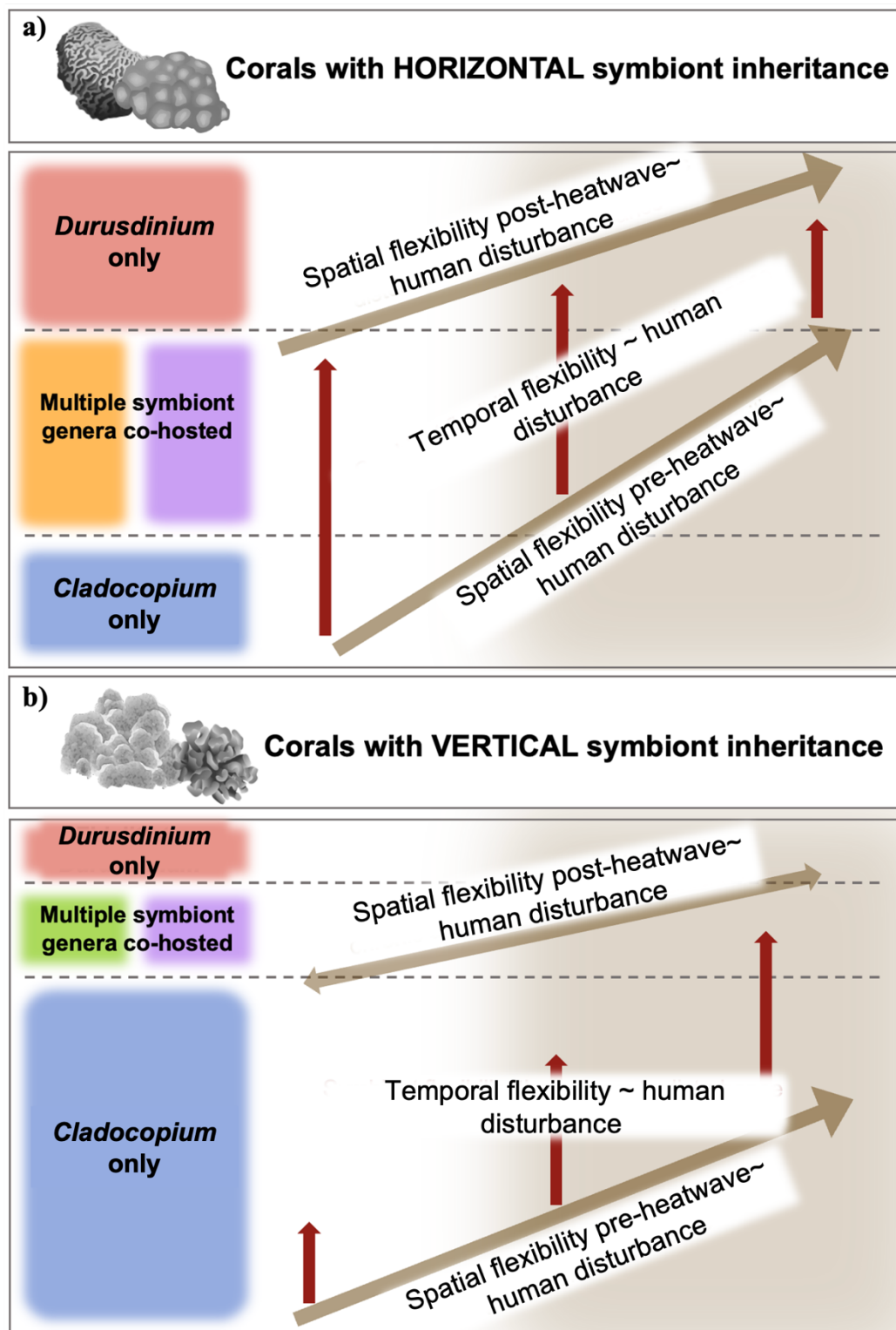


Figure 3.5: Illustration summarising the shifts between symbiont assemblages in corals with horizontal symbiont inheritance and those with vertical symbiont inheritance upon exposure to multiple stressors. *Durusdinium*-only assemblages are indicated by pale red, *Cladocopium*-only by blue, mixed *Cladocopium*-*Durusdinium* by purple, *Durusdinium*-*Symbiodinium* by orange and *Cladocopium*-*Symbiodinium* by green. Spatial flexibility represents the changes in symbiont genus beta diversity hosted by corals across spatial environmental gradients, with the slopes of brown arrows showing the changes between symbiont assemblages with increasing human disturbance (left to right). The height of red arrows represents the magnitude of temporal flexibility

shown by individual corals in terms of changes in symbiont assemblages hosted before and after the heatwave, at different levels of human disturbance exposure (increasing left to right).

Chapter 4 – Multi-year heatwave hangover in coral symbioses

Adapted from “Persistent legacy effects of marine heatwaves on coral symbioses”, manuscript in revision.

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4.1 Abstract

Obligate endosymbioses between eukaryotes and their single-celled inhabitants form the foundations of many ecosystems, yet their long-term stability under climate change remains unresolved. Acute and chronic stressors drive temporal and spatial transformations in symbioses between reef-building corals and Symbiodiniaceae with consequences for coral survival, but whether, and at what rate, they recover following heatwave disruption is largely unknown. The Pacific island of Kiritimati harbours reefs along a gradient of chronic local disturbance and was the epicentre for the extreme 2015-2016 marine heatwave. Here we report ITS2 DNA metabarcoding of symbiont assemblages from 237 colonies (598 samples) of the brain coral *Platygyra ryukyuensis* from 2014 to 2023. Stress-tolerant *Durusdinium* symbionts were hosted mainly by corals under high local disturbance before the heatwave but dominated the assemblages of 92% of corals seven years after the heatwave, reflecting a post-heatwave homogenisation of symbioses. Recovery of previously abundant *Cladocopium* symbionts was severely limited and restricted to taxa totally distinct from their ‘C3’ and ‘C50a’ pre-heatwave congeners. Over the three years immediately following heat stress, half of all tracked corals hosted transient assemblages codominated by *Durusdinium* and previously rare genus *Symbiodinium*, which increased in prevalence especially at sites with low human disturbance and high water clarity. The extended dominance of heatwave-transformed symbiont assemblages foreshadows their persistence spanning successive heat stress events, which together with the disappearance of several *Cladocopium* symbiont species from *P. ryukyuensis* colonies, raises concerns over the resilience of their coral hosts.

4.2 Introduction

Climate change poses a major threat to coral reef ecosystems, with rising temperatures driving increasingly frequent and intense heatwaves (Smith et al., 2023). Marine heatwaves often trigger coral bleaching, during which corals lose many of their symbiotic dinoflagellates (family: Symbiodiniaceae) (Glynn, 1996), re-structuring symbiont assemblages and leading to host mortality when prolonged (Buddemeier & Fautin, 1993). Symbiont identity plays an important role in mediating coral responses to thermal stress, recovery, and long-term resilience (Silverstein et al., 2015). Symbionts in the genus *Durussdinium* are thermotolerant generalists, and often increase in relative abundance in a range of coral host species following coral bleaching events (Baker et al., 2004; Berkelmans & van Oppen, 2006). In contrast the genus *Cladocopium* is comprised of many highly host-specific symbiont species (LaJeunesse et al., 2018). The fitness trade-offs for corals hosting different taxa within the Symbiodiniaceae are responsive to environmental conditions: whilst *Durussdinium* symbionts may resist bleaching under elevated temperatures, their photosynthetic contribution can be comparatively lower under ambient temperatures, with coral traits such as growth and reproductive output suffering the consequences in some coral species (Jones & Berkelmans, 2011; Matsuda et al., 2023), although perhaps not in tropical-Pacific corals which have the longest evolutionary history of coevolved mutualisms with *Durussdinium* (Kemp et al., 2023).

Following acute heat stress and an increase in heat-tolerant symbionts, assemblages are considered liable to replacement by homologous taxa based on putative competitive inferiority and reduced benefits conferred to coral hosts under ambient conditions (Buddemeier & Fautin, 1993; Cunning et al., 2018). However, in natural populations, the few reports of the post-

disturbance persistence of heat-tolerant symbionts have been variable. Intrageneric symbiont changes have been reported to persist for only 2-8 months following heat stress (Sampayo et al., 2008; Thornhill et al., 2009; Lewis et al., 2019), whereas intergeneric replacement, most notably by *Durussdinium*, has been shown to endure for as little as 5 months in some coral species and in others for 2 to 4 years after heat stress (Thornhill et al., 2006b; LaJeunesse et al., 2009b; Elder et al., 2022). There are few other studies that have tracked coral symbioses following an acute disturbance, especially in Pacific coral taxa, leaving the trajectories and drivers of symbiont recovery largely unknown. Moreover, the few long-term studies of coral symbioses in the absence of acute stress have had contrasting results. A 9-year time series from 55 Palauan tracked colonies reported symbiont persistence over time (Lewis et al., 2024), yet a 16-year study of 12 Caribbean colonies found intracolony shifts in the symbiont relative abundances (Edmunds et al., 2014), whilst a 10-year study of haphazardly-selected Kenyan corals finding symbiont dynamism but no directional changes (Baker et al., 2013).

Post-heatwave recovery of coral symbioses may also be influenced by environmental factors. Light intensity has been implicated in symbiont niche-partitioning, as light-tolerant symbionts in the genus *Symbiodinium* are detected in higher abundances at shallow depths, in low-turbidity (high water-clarity) and even on the shallowest, least shaded surfaces of individual colonies (Rowan & Knowlton, 1995; Kemp et al., 2015). In contrast, the proliferation of *Durussdinium* symbionts is seemingly favoured in corals subject to high turbidity and high inorganic nutrient pollution (nitrogen in particular), characteristic of coral reefs exposed to higher anthropogenic disturbance (Claar et al., 2020b; Poquita-Du et al., 2020). It remains unclear if local variation in human disturbance or other environmental factors influences symbiont competitive dynamics during the symbiotic instability that follows coral bleaching.

Here we present one of the longest time-series of Symbiodiniaceae assemblages in tracked coral colonies to date, using a nine-year time series of 598 samples from 237 tracked coral colonies from Kiritimati (Republic of Kiribati) in the central equatorial Pacific. This island was the epicentre for the 2015-2016 global coral bleaching event, experiencing severe heat stress for over 10 months (Liu et al., 2014; Baum et al., 2023) and harbours reefs which have consistently been exposed to a steep spatial gradient of local human disturbance since the turn of the century (Sandin et al., 2008; Walsh, 2010). *Platygyra* brain corals are widely dispersed across Indo-Pacific reefs and demonstrate the symbiotic flexibility typical of the Merulinidae family (Keshavmurthy et al., 2020; Jiang et al., 2021). Local human disturbance exposure strongly influenced symbiont assemblages hosted by Kiritimati's population of *Platygyra ryukyuensis* prior to peak heatwave exposure, with those exposed to higher human disturbance hosting predominantly *Durusdinium* (Claar et al., 2020b). However, by the end of 2016, following this marine heatwave, the entirety of the Kiritimati *P. ryukyuensis* population had shifted to *Durusdinium* (Claar et al., 2020). In this study, we investigate recovery trajectories and rates for coral-Symbiodiniaceae associations following heatwave-induced disruption for coral colonies exposed to distinct levels of underlying chronic local anthropogenic disturbance. We hypothesise that corals will revert to their pre-heatwave Symbiodiniaceae assemblages as the competitive superiority of these symbionts returns following the end of heat stress. We further hypothesise that the post-heatwave trajectory of symbiont succession will be influenced by local environmental variables known to define symbiont niches (such as light), as they compete to recolonise corals recovering from bleaching.

4.3 Materials & methods

4.3.1 Sampling coral-associated Symbiodiniaceae

Coral tissue samples were collected over the course of 10 expeditions: August 2014, January 2015, May 2015, July 2015, March 2016, November 2016, July 2017, July 2018, July 2019 and September 2023. Across each of the twelve sites included in this study, one morphotype of the genus *Platygyra* was universally common, described here as belonging to the species *P.*

ryukyuensis based on key skeletal characteristics (meandroid colonies with short and occasionally monocentric valleys and irregular septo-costae) (Yabe et al., 1936; Veron, 2000) (Figure B1). On each expedition, *P. ryukyuensis* colonies that were at least 20cm in diameter and at least 5m apart were tagged and sampled along 60m fore-reef transects following a 10-12m isobath, and previously tagged colonies were re-sampled (Figure B2). Tissue samples were taken from the central, uppermost surface of colonies using a woodworking chisel, and then stored in individual bags on ice until the end of each day, when $\sim 1\text{cm}^3$ of tissue from each sample was transferred to microcentrifuge tubes containing a guanidinium isothiocyanate buffer. Samples were stored at 4°C until DNA extraction.

4.3.2 Quantifying acute & chronic disturbance exposure

To quantify acute heat stress exposure at the 12 reef sites sampled in this study, satellite-derived sea surface temperature (SST) at $\sim 5\text{km}^2$ resolution was extracted from NOAA's 'CoralTemp' product and used to calculate daily average SST between 1st July 2014 and 1st October 2023 (NOAA Coral Reef Watch, 2019) (Figure 4.1). Degree-Heating-Week (DHW) data was extracted from NOAA's 'Coral Reef Watch' product to indicate accumulated heat stress. The DHW metric represents the compounded duration and amplitude of SST anomalies in $\sim 5\text{km}^2$

pixels over a 12-week rolling window when temperatures exceed the pixel's maximum monthly mean (MMM) by more than 1°C (Skirving et al., 2020). In-situ temperature loggers were also deployed at the 12 reef sites in this study and when compared for 2011 to 2016, revealed close agreement with NOAA's satellite-derived temperature data and consistency in SST variance and SST anomalies (Claar et al., 2019). The 12 reef sites included in this study all experienced severe heat stress during an extreme pan-Pacific marine heatwave in 2015-16, with minimal variation in maximum accumulated heat stress between all 12 reef sites (24.26-25.28 DHW) when evaluated using *in situ* and satellite-based data (Claar et al., 2019).

Kiritimati's population (6,500 - 7,400 during this study (Kiribati National Statistics Office, 2016, 2021)), which is distributed mostly along the northeast lagoon-face, exposes adjacent reefs to disturbance from wastewater runoff, subsistence-fishing, coastal construction, and dredging (Figure 4.1). To quantify these effects, two spatial data layers have been used to calculate a combined metric of chronic human disturbance at each reef site (Table B1): a kernel density function of subsistence-fishing pressure within 10 km based on community interview data (Watson et al., 2016) and estimates of point-source waste-water exposure based on a geographic buffer of human population size within 2 km. Natural breakpoints in this combined metric allow sites to be discretely categorised by local disturbance, which have been used to explain variability in many facets of coral reef health and function around Kiritimati (Magel et al., 2019; McDevitt-Irwin et al., 2019; Claar et al., 2020; Baum et al., 2023).

Several additional environmental factors differ between reef sites (Baum et al., 2023) and were therefore also characterized: reef aspect (windward- or leeward-facing), surface-water productivity, inorganic nitrogen, and water clarity (in-water visibility). The prevailing wind direction from 1979-2009 (Bosserele et al., 2015), was used to categorise sites as windward- or

leeward-facing (Figure 4.1). Persistent and concentrated upwelling off Kiritimati's western shore (Walsh, 2010) was observed using 4 km² resolution daily chlorophyll-a satellite data as a proxy for surface water phytoplankton content and productivity (NOAA CoastWatch Central, 2012) and mean annual chlorophyll-a concentration ($\mu\text{M}/\text{m}^3$) was calculated for each reef site for 2014 to 2023 (Figure 4.1). Site-level visibility was quantified as the furthest point (in metres) at which reef structure was visible along the transect, recorded at depth at the start and end of each 60m sampling transect, three times per expedition. Visibility is inversely proportional to turbidity and is a reliable indicator of the photosynthetically-active radiation reaching the benthos on tropical reefs (Jones et al., 2015); on Kiritimati, visibility is correlated with local human disturbance, with more disturbed sites tending to have lower visibility (Baum et al., 2023). Three surface water samples of 30 ml were collected per site per expedition for inorganic nutrient content analysis and frozen on the day of collection prior to quantification at the Institute of Ocean Sciences (BC, Canada) (McDevitt-Irwin et al., 2019). Combined nitrate and nitrite molar concentrations are taken as a proxy for seawater inorganic nitrogen content. Not all sites were visited on every expedition to collect visibility and inorganic nutrient data due to inclement weather or logistics (Table B2).

4.3.3 Symbiodiniaceae DNA extraction and sequencing

DNA was extracted from coral tissue samples preserved in guanidinium isothiocyanate buffer using a protocol designed for Symbiodiniaceae (Stat et al., 2009b). 200 μl subsamples of homogenised tissue/buffer slurry were incubated overnight at -20°C with an equal volume of isopropanol. Precipitated DNA was then collected into a pellet by centrifugation and washed three times with 70% ethanol prior to resuspension in 50 μl Tris buffer. The internal transcribed

spacer 2 (ITS2) ribosomal DNA was amplified using a two-step PCR for DNA metabarcoding. The first round of PCR used forward primer ITS-DINO (Pochon et al., 2001) and reverse primer ITS2Rev2 (Stat et al., 2009b) with standard Illumina adapters to amplify a ~271-303bp amplicon. PCR amplicons were visualised with gel electrophoresis on a 2% agarose gel. Samples that failed to amplify were re-amplified using template DNA that was cleaned using a Genomic DNA Clean & Concentrator kit (Zymo Research) according to the manufacturer's protocol. Negative controls from each DNA extraction and PCR performed were used to check for contamination using gel electrophoresis. Library preparation followed an adapted Illumina 16S Metagenomics protocol (Illumina, 2015; Hume et al., 2020), with two AMPure bead (Beckman Coulter) clean-ups, and an indexing PCR using Nextera primers (Illumina). Indexed amplicons were normalisation with a PicoGreen dsDNA assay (Invitrogen) prior to pooling sampled for multiplex sequencing. Sequencing was performed across ten runs on the Illumina MiSeq platform (MiSeq Reagent Kit v3 600-cycle), either at the Hawaii Institute of Marine Biology Genetics Core Lab or at the John G. Shedd Aquarium (Chicago) Molecular Ecology Laboratory, producing on average 28,140 raw contigs per sample.

4.3.4 Clustering & classifying Symbiodiniaceae sequence data

ITS2 sequence data were analysed using two workflows to classify distinct taxa: i) SymPortal, which uses co-occurrence patterns of Symbiodiniaceae sequence variants to identify defining intragenomic variants (DIVs) and further clusters these into ITS2 profiles (Hume et al., 2019), and ii) DADA2, which identifies amplicon sequence variants (ASVs) using a denoising algorithm that models sequencing errors (Callahan et al., 2016), (Figure B3). These two approaches of distinguishing intra- and inter-genomic sequence variation from the multi-copy

ITS2 region in Symbiodiniaceae were evaluated in parallel to provide more robust interpretation of sequencing results (Quigley et al., 2022). Sequence data were submitted to the remote SymPortal database (symportal.org). For the DADA2 approach, primer sequences were removed using the ‘cutadapt’ tool, then forward and reverse reads were trimmed by 100bp at the 3’ and by an additional 31bp at the 5’ end of reverse reads based on declines in read quality and to ensure equal amplicon sequence lengths with the SymPortal-based approach. Trimmed reads were then denoised and merged using default parameters for read quality and error rates. Samples with fewer than 500 total post-processing Symbiodiniaceae reads were removed from the analyses. Incidences of ITS2 profiles, DIVs or ASVs comprising <1% of Symbiodiniaceae reads in a sample were also discarded. ASVs were assigned genus-level taxonomy using a naïve Bayesian classifier algorithm, implemented in DADA2, with a minimum bootstrap confidence of 80 using a comprehensive Symbiodiniaceae reference database available within the online data repository (Nitschke, 2020). Separate neighbour-joining trees based on Tamura-Nei distances were built in Geneious Prime (version 2023.2.1) for each symbiont genus, to calculate unifrac similarity between ASVs.

The impact of variation in ITS2 copy number on the quantitative accuracy of relative read abundance (RRA) of symbiont genera (Davies et al., 2023) was accounted for using two approaches. Changes in the numerical and relative read abundance of the three most abundant symbiont genera were investigated in this study and include *Cladocopium* (C), *Durusdinium* (D), and *Symbiodinium* (A). *Symbiodinium* RRA was only interpreted as the relative abundance of this genus in a coral when comparing samples from the same coral colony. In all other cases, interpretation of symbiont relative abundances were limited to background or dominant categorisations, based on the natural bimodality of symbiont RRA observed (Figure B4) using

thresholds for dominant and background in accordance with other studies (Silverstein et al., 2012; Quigley et al., 2014). Samples were designated dominant when only one genus was observed with > 90% RRA and codominant when more than one symbiont genus was detected with RRA between 10% and 90%.

4.3.5 Statistical analyses

To investigate the probability of reversion to pre-heatwave symbiotic associations in the *P. ryukyuensis* population, logistic regressions of the absence or presence of *Durusdinium* (hosted as the dominant or codominant genus) were fit against continuous human disturbance exposure and sample collection date as fixed effects, with coral colony included as a random effect. Unifrac distances, based on sequence similarity between composite sequence variants grouped into ITS2 type profiles by Symportal and ASV sequence alignments, were used to generate pairwise dissimilarities between *Cladocopium* or *Durusdinium* taxa detected in samples before and after the heatwave at the same site. Sites not represented in both 2014-15 and in 2016-23 were not included in pairwise analyses. Unifrac dissimilarities for the genus *Symbiodinium* were not reported because it was rarely detected prior to the heatwave and few corals hosted this genus. The ‘pairwiseAdonis’ wrapper was used to perform pairwise PERMANOVA with sites included as strata (Martinez Arbizu, 2017), to test for significant unifrac dissimilarities within each symbiont genus. A subset of the 24 coral colonies that were sampled both before and after the heatwave were used to assess symbiotic reversion within tracked colonies (4.4.1, 4.4.2; coral colonies indicated in Figure B2).

Sequential sample pairs (363 samples in total) spanning 2016-2017 (either between expeditions 2016a and 2016b, or 2016b and 2017), 2017-2018, 2018-2019 or 2019-2023, were

used to track post-heatwave changes in Symbiodiniaceae assemblages within 107 distinct tracked coral colonies (4.4.3; resampled corals included in post-heatwave analyses are shown in Figure B2). Beta regression models were fit to post-heatwave changes in *Symbiodinium* RRA in re-sampled corals against continuous human disturbance exposure. Corals were grouped by expedition class as described above and the Bonferroni approach was used to correct P-values for multiple-comparisons. The effects of additional environmental variables on changes in *Symbiodinium* RRA in these re-samples were tested by fitting a beta generalised mixed-effects model with ‘glmmTMB’ (Brooks, 2024): site, coral colony and categorical human disturbance exposure were all included as random effects and site-level annual measures of chlorophyll-a, inorganic nitrogen and visibility were averaged from 2016 to 2023 and included as fixed effects along with the windward or leeward aspect of each site.

4.4 Results

4.4.1 Persistent dominance of *Durusdinium* across all reefs

In 2023, more than seven years after the end of the heatwave, all but one *P. ryukyuensis* corals ($n = 144/145$) hosted the symbiont genus *Durusdinium*, in striking contrast to the reef-wide associations observed prior to the event (25 of 54 colonies (46.3%) containing *Durusdinium* at some point) (Figure 4.2a). Almost all colonies sampled in 2023 (91.7%) were classified as *Durusdinium* dominant, while 7.6% of colonies were codominant with another symbiont genus (2.8% *Cladocopium*; 4.8% *Symbiodinium*; Figure 4.2a). Counter to our hypothesis of reversion to *Cladocopium* following a return to non-stressful temperatures, time had no significant effect on the proportion of colonies hosting *Durusdinium* from 2016 onwards ($z = 0.03$, $P > 0.98$). The

effect of human disturbance on the probability of *P. ryukyuensis* corals hosting *Durusdinium*, significant prior to the heatwave ($z = 5.95$, $P < 0.001$), was not apparent post-heatwave ($z = 0.20$, $P > 0.84$; Figure 4.2b), reflecting a homogenisation of symbioses following exposure to chronic human disturbance and acute heatwave stressors.

These enduring population-scale changes in the symbiont assemblages of *P. ryukyuensis* are further supported by the persistence of *Durusdinium* in individually tracked *P. ryukyuensis* colonies that were sampled both before and after the heatwave ($n = 24$ colonies). All but one of these colonies hosted assemblages dominated or codominated by *Durusdinium* post-heatwave, despite most exclusively hosting *Cladocopium* prior to peak heatwave exposure (Figure B3).

4.4.2 No recovery of pre-heatwave *Cladocopium* taxa

In addition to the post-heatwave entrenchment of *Durusdinium*, none of the 16 *Cladocopium* ITS2 profiles found in *P. ryukyuensis* before the heatwave ($n=107$ samples) were observed after the heatwave. The most abundant pre-heatwave *Cladocopium* ITS2 profiles, characterised by the ‘C3’ and ‘C50a’ sequence variants, were entirely replaced by phylogenetically distinct profiles comprised of mainly ‘C15’, ‘C15h’ or ‘C116’ variants, which had not been detected in the *P. ryukyuensis* population prior to the heatwave (Figure 4.3a). The disparity between pre- and post-heatwave *Cladocopium* taxa is further indicated by significant differences in paired unifrac dissimilarities between pre- and post-heatwave samples containing dominant, codominant or background *Cladocopium* hosted by corals at the same site (ASVs: $F = 25.0$, $R^2 = 0.191$, $P < 0.001$, ITS2 profiles: $F = 74.7$, $R^2 = 0.430$, $P < 0.001$). No analogous difference was detected in *Durusdinium* ASVs ($F = 0.621$, $R^2 = 0.002$, $P > 0.24$) or ITS2 profiles ($F = 0.436$, $R^2 = 0.001$, $P > 0.931$) hosted before and after the heatwave.

Of the 24 individually tracked *P. ryukyuensis* colonies, most that hosted *Cladocopium* pre-heatwave hosted *Durusdinium* or *Durusdinium-Symbiodinium* codominant assemblages post-heatwave (Figure 4.3b). The few colonies that hosted *Cladocopium* before and after peak heat stress underwent a shift in symbiont sequence variants matching the changes observed at the population scale (Figure 4.3b), indicating that the phylogenetic shift in *Cladocopium* lineages was not an artefact of sampling different colonies pre- and post-heatwave.

4.4.3 Post-heatwave *Symbiodinium* dynamism

Extensive dynamism in symbiotic assemblages was also observed after the heatwave, driven by the emergent genus *Symbiodinium*. *Symbiodinium* was observed in 43% of all samples (214 of 491, in 113 distinct colonies) collected after the heatwave, contrasting 9.3% of samples (10 of 107, in 8 distinct colonies) before the heatwave (Figure B3). Of the 107 tracked colonies resampled after the heatwave, approximately half (52 colonies) underwent at least one symbiotic state change to or from a *Durusdinium-Symbiodinium* codominant assemblage (Figure 4.4a). This contrasts the overall consistent proportion of colonies codominant for both genera observed during expeditions from 2016-2019 (Figure 4.2a).

Further comparison of colonies resampled across successive post-heatwave expeditions ($n = 107$ corals, 363 samples) identified distinct trends in the change in *Symbiodinium* RRA. Although there was only a marginally significant negative trend in the change in *Symbiodinium* RRA associated with human disturbance exposure over the 8-12 month sampling periods between successive expeditions from March 2016 to July 2019 ($z = 2.64$, $P_{\text{Bonferroni}} = 0.036$), a significant positive relationship between the change in *Symbiodinium* RRA and human disturbance was observed over the longer and later time period between July 2019 and

September 2023 ($z = 2.70$, $P_{\text{Bonferroni}} = 0.028$, Figure 4.4b). This largely reflects differences in the retention of *Symbiodinium* across the disturbance gradient, with high RRA enduring until 2019 at the least disturbed sites when most colonies at medium and high disturbance sites hosted exclusively *Durusdinium* or *Symbiodinium* with low RRA. Subsequently, colonies under lower human disturbance underwent larger declines in *Symbiodinium* RRA from 2019 to 2023 as colonies became dominated by *Durusdinium* (Figure 4.4a).

The dynamism of *Symbiodinium* associations may be attributed to between-site differences in in-water visibility. Although the estimated effects of site-level nitrate & nitrite, chlorophyll-a, and the windward or leeward status of each site on changes in *Symbiodinium* RRA were not significantly different from zero at any point post-heatwave, post-heatwave *Symbiodinium* RRA significantly increased with higher site-level in-water visibility until 2019 ($z = 2.28$, $P < 0.023$), and subsequently decreased with site-level visibility between 2019 and 2023 ($z = 4.00$, $P < 0.001$) (Figure. 4.4c).

4.5 Discussion

Our decade-long survey of coral symbioses reveals enduring transformations of coral-Symbiodiniaceae associations in the wake of an extreme marine heatwave. The heat-tolerant genus *Durusdinium* remained dominant in *P. ryukyuensis* corals across Kiritimati more than seven years after heat-stress exposure, revealing an alternative pathway to long-term symbiotic transformation beyond the cumulative impacts of multiple heatwaves (Quigley et al., 2022; Palacio-Castro et al., 2023). We observed the decimation of a previously diverse community of *Cladocopium* symbionts, expanding upon studies that have reported heatwave-driven turnovers

and losses of *Cladocopium* richness (Quigley et al., 2022; Starko et al., 2023), by revealing the persistence of this transformation and the local extinction of several *Cladocopium* species within *P. ryukyuensis* assemblages. Corals also experienced short-lived increases in *Symbiodinium* symbionts, revealing a transient role of local environmental drivers in post-heatwave symbiotic changes and providing support for *ex situ* experimental evidence of dynamic *Symbiodinium*-*Durusdinium* competition dynamics (McIlroy et al., 2019). Taken together, these results reveal a path of post-heatwave symbiont succession distinct from trajectories observed following milder acute disturbance exposure (Figure 4.5).

4.5.1 Extended heatwave duration disrupts symbiotic reversion: *Durusdinium*

Despite a 7-year period free from additional heat stress exposure following the 2015-16 heatwave, *Durusdinium* remained overwhelmingly dominant in Kiritimati's *P. ryukyuensis* population. This result extends the duration that heat stress-induced *Durusdinium* is found to persist in natural systems by several years, with previous reports ranging between 5 months to 4 years post-heatwave (Thornhill et al., 2006b; LaJeunesse et al., 2009b; Keshavmurthy et al., 2020). Reef-wide replacement of *Cladocopium* by heat-tolerant *Durusdinium* was previously hypothesised to occur by repeated severe heat stress exposure without intervening symbiotic reversion, with step-wise cumulative increase in the abundance of heatwave-transformed assemblages (sensu Baker et al., 2017). This is an increasingly likely trajectory under current projections of marine heatwaves frequency, duration and severity (Laufkötter et al., 2020). Field evidence of such an increase in heatwave-transformed assemblages has been reported for *Durusdinium*-dominated colonies of *Pocillopora* spp. on a Panamanian reef-tract between the 1997-8 and 2015-16 mass bleaching events (Palacio-Castro et al., 2023). The reefs of Kiritimati

had accumulated heat stress above 8 DHW four times in recorded history prior to the 2015-16 event, most recently in 2002 (NOAA Coral Reef Watch, 2019), although these were dwarfed by accumulation of over 24 DHW in 2015-2016. The dominance of genus *Cladocopium* symbionts in 2014-2015 in most *Platygyra ryukyuensis* corals demonstrates that any prior heat-induced shifts towards *Durusdinium* had not persisted on Kiritimati. We therefore suggest that long-term shifts in coral-Symbiodiniaceae assemblages, as theorised following multiple successive heatwaves, can also be brought about by a single extreme heat stress event.

The unprecedented duration and intensity of the 2015-16 marine heatwave may explain the enduring success of *Durusdinium* in Kiritimati's *Platygyra* population. Priority effects are thought to provide a competitive advantage to *Durusdinium* in early bleaching recovery (Buzzoni et al., 2023). However, as symbionts recover, the photic environment within bleached coral tissue is ameliorated and the competition for nitrogen between symbionts intensifies (Enríquez et al., 2005; Krueger et al., 2020). This could facilitate the recovery of stress-sensitive symbionts, such as *Cladocopium*, which can have a competitive advantage over *Durusdinium* in nitrogen assimilation and population growth *in hospite* (McIlroy et al., 2020). Extended heatwaves, such as the 2015-16 heatwave on Kiritimati, may provide opportunity for *Durusdinium* assemblages to become established and remain resistant to competition. Indeed, *Platygyra ryukyuensis* colonies that survived the heatwave recovered *Durusdinium* at densities comparable to pre-heatwave symbiont densities by March 2016 whilst still experiencing heat stress (Claar et al., 2020). The dominance of stress-tolerant generalists, such as *Durusdinium*, is typically restricted to communities of early- or mid- successional taxa exposed to chronic disturbance (Huston & Smith, 1987), as observed on Kiritimati in 2014-15 prior to the heatwave. However, the enduring

persistence of *Durusdinium* has obscured this spatial variation and homogenised symbiont assemblages across the atoll.

The ecological consequences of the long-term entrenchment of thermotolerant symbionts, for coral individuals, populations and the broader reef ecosystem are not clear, but may be detrimental. While these shifts might confer an advantage to corals in a warming ocean, providing increased bleaching resistance during subsequent heatwaves of short duration (Palacio-Castro et al., 2023), this may be outweighed by any putative physiological trade-offs of hosting these symbionts outside of heatwave-periods (Ortiz et al., 2013; Matsuda et al., 2023; Turnham et al., 2023). However, the translation of inferior symbiont photochemical efficiency into a physiological cost for host coral metabolism is disputed, and may only manifest when hosted for periods of several weeks or more (Kemp et al., 2023). Indeed, whilst some *Platygyra ryukyuensis* corals on Kiritimati survived the 2015-16 heatwave by shifting to host *Durusdinium* after bleaching, those already hosting these symbionts on reefs exposed to high human disturbance suffered much higher mortality (Claar et al., 2020b), reflecting either the accumulated costs of hosting *Durusdinium*, the impact of chronic anthropogenic stress or their interaction. Understanding the true cost of symbiont community transformations on the resilience of coral reefs will only be possible when the persistence of these changes is better understood, which our study suggests may be more enduring than previously thought.

4.5.2 Loss of pre-heatwave species disrupts symbiotic reversion: *Cladocopium*

Previously common *Cladocopium* lineages were not detected post-heatwave in the *P. ryukyuensis* population, reflecting a concomitant shift in *Cladocopium* species with the enduring dominance of *Durusdinium*. Symbiont ITS2 profiles comprising mainly C50a and/or C3

sequence variants, found in *P. ryukyuensis* pre-heatwave, are commonly detected in Indo-Pacific corals within the Acroporidae or Merulinidae families (Carballo-Bolaños et al., 2019; Lewis et al., 2022; Marzonie et al., 2024). In contrast, the taxa that replaced pre-heatwave lineages, defined by C15, C15h and/or C116 variants, are most often found in Indo-Pacific *Porites* spp. (Hoadley et al., 2021; Starko et al., 2023; Chen et al., 2024b). The survival of *Porites* sp. on Kiritimati following the 2015-16 heatwave was higher than most Acroporidae and Merulinidae (including *P. ryukyuensis*) species (Baum et al., 2023). Given the capacity for symbiont environmental availability to shape coral symbiont assemblages via horizontal acquisition (Ziegler et al., 2018b; Williamson et al., 2021; Johnston et al., 2025), we postulate that the switch in *P. ryukyuensis*-associated *Cladocopium* species arose as environmental or host reservoirs of C50a and C3 symbionts were severely depleted due to the severity and extended duration of the 2015-16 heatwave. Whilst these recovered *Cladocopium* taxa may have been truly absent within coral colonies pre-heatwave and subsequently acquired through exogenous uptake, it is also possible that they cohabited with corals' dominant *Cladocopium* taxa pre-heatwave below the approximate minimum detection limit of 0.1% for this sequencing method (Quigley et al., 2014). However, prior research supports adult corals' capacity to uptake exogenous symbionts (Boulotte et al., 2016; Scharfenstein et al., 2022; Buzzoni et al., 2023; Johnston et al., 2025). The apparent disappearance of pre-heatwave *Cladocopium* taxa would suggest that extreme heatwaves may reshape the Symbiodiniaceae metacommunity through the differential mortality of Cnidarian hosts, analogous to the population co-extinctions projected for many bacterial symbionts and their insect hosts under climate change (Renoz et al., 2019; Doña & Johnson, 2020).

The *Cladocopium* symbionts hosted post-heatwave, in addition to being taxonomically distinct, were hosted mostly at low relative abundances. Results from experimental inoculations of sea anemones with novel symbionts reach lower symbiont densities compared to normal symbiont densities, attributed to increased rejection by the host immune response (Matthews et al., 2017; Medrano et al., 2019). Hence, the C15-, C15h- or C116-dominated *Cladocopium* species detected in the post-heatwave *P. ryukyuensis* population may lack the coevolved immune-recognition or immune-evasion traits that facilitated host-colonisation of pre-heatwave *Cladocopium* species to higher densities (Voolstra et al., 2009; Johnston et al., 2022). Reduced host-recognition of recovered *Cladocopium* lineages may explain the observed lack of post-heatwave reversion towards *Cladocopium*, as these distinct *Cladocopium* taxa are inferior to *Durisdinium* at colonising recovering host corals. Heatwave-induced local extirpations of *Cladocopium* lineages have been reported in other reef coral populations including Kiritimati's *Porites lobata* corals (Starko et al., 2023). The loss of host-adapted *Cladocopium* symbionts jeopardises the persistence of coral reefs, given the importance of Symbiodiniaceae functional diversity for coral reef ecosystem resilience (Suggett et al., 2017).

4.5.3 Post-heatwave rise of a rare opportunist: *Symbiodinium*

The rapid post-heatwave colonisation success of *Symbiodinium* in Kiritimati may be attributed to its advantage within the harsh photic environment that is bleached coral tissue (Enríquez et al., 2005). The genus *Symbiodinium* has been characterised as competitively dominant under higher light intensities, occupying spatial niches in shallower reefs or in the upper surfaces of colonies (Rowan & Knowlton, 1995; LaJeunesse, 2002). The influx of *Symbiodinium* contributed to three years of highly dynamic symbiont assemblages within coral colonies, with the codominance of

Durusdinium and *Symbiodinium* oscillating year-to-year. This is consistent with prior studies reporting transient detections of *Symbiodinium* as it increases in relative abundance during or shortly after a bleaching event (Toller et al., 2001; Kemp et al., 2014; Leinbach et al., 2022). The effects of symbiont post-heatwave dynamism on coral host fitness are uncertain but likely significant, considering the central role symbionts play in host metabolism and the host stress response (Jones & Berkelmans, 2011; Silverstein et al., 2015), and any resource partitioning towards competition by cohabiting symbiont taxa may reduce energetic translocation to the coral host (Kenkel & Bay, 2018). Moreover, energetic trade-offs for corals hosting *Symbiodinium* at ambient temperatures have been reported. (Stat et al., 2008b), resembling those incurred by hosting *Durusdinium*. Furthermore, whilst the *Symbiodinium* taxa detected (in the ‘A5’ lineage, Figure B3) are also found in apparently healthy corals on Kiritimati (Claar et al., 2020b; Starko et al., 2023), another species within this genus (*Symbiodinium necroappetens* ‘A13’) has been characterised as an opportunist that colonises necrotic host tissue (LaJeunesse et al., 2009a, 2015).

In addition to its increase from pre- to post-heatwave, *Symbiodinium* relative read abundance varied spatially in corals across Kiritimati. The post-heatwave increase in *Symbiodinium* was greatest at sites experiencing lower local human disturbance and with higher water clarity, exposed to higher benthic solar radiation compared to more turbid sites. The eventual competitive exclusion of *Symbiodinium* by *Durusdinium* as a function of water clarity (in-water visibility) is consistent with previous experimental evidence of *Symbiodinium* persistence under high light and competitive exclusion by *Durusdinium* under low light conditions (McIlroy et al., 2019). This supports a role of local anthropogenic impacts on the recovery trajectories of coral symbioses, as heat-tolerant symbionts are recovered following an

intermediate symbiotic state under minimal local disturbance. However, the nuances of the symbiotic dynamism reported here were only revealed by time-series sampling within tracked coral colonies, which would otherwise have been obscured without these longitudinal data.

As the frequency and intensity of marine heatwaves increases, the persistence of heatwave-transformed symbioses and homogenisation across gradients of human disturbance reported here may come to characterise Symbiodiniaceae associations in many symbiotically flexible coral species.

4.6 Figures

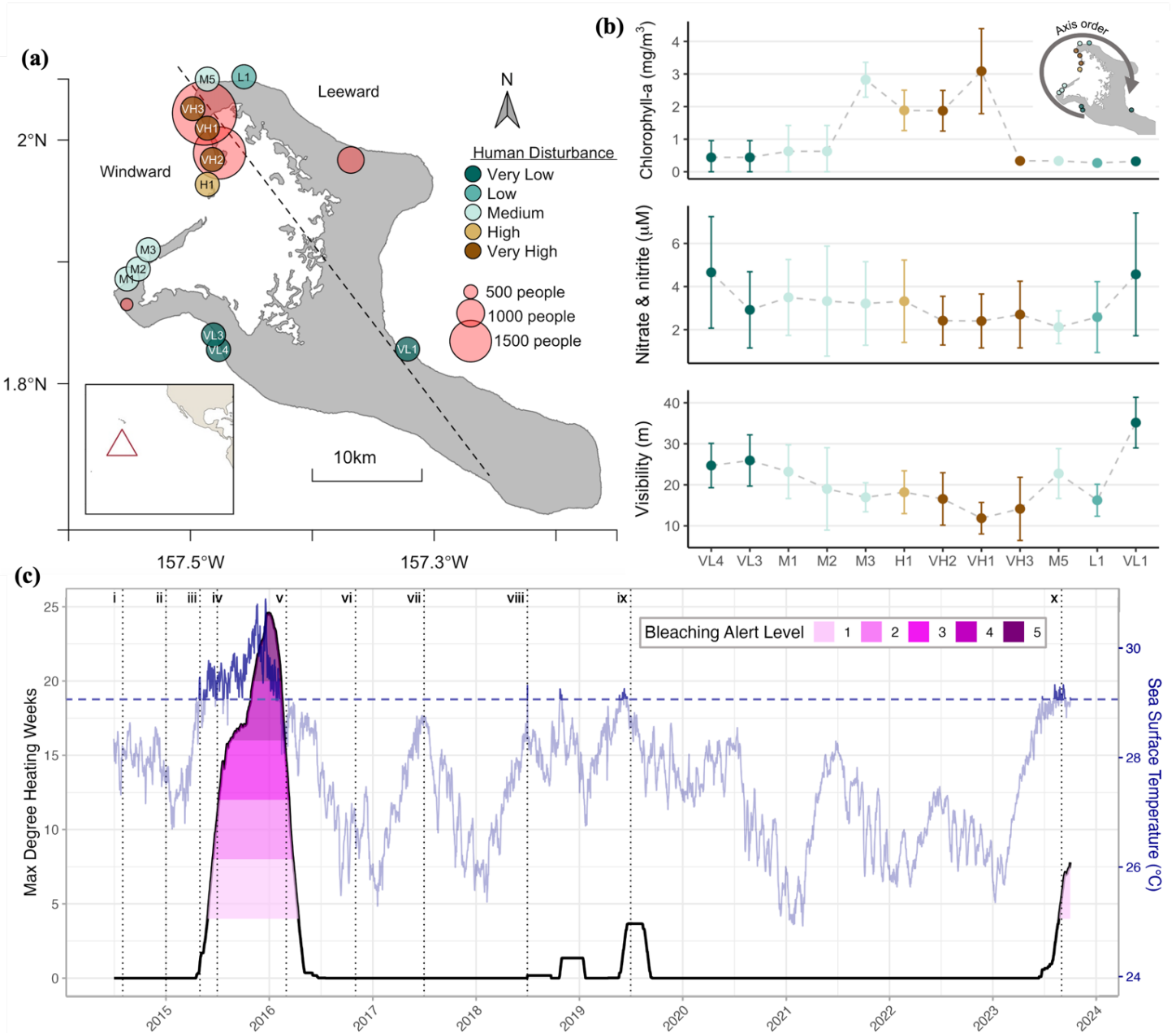


Figure 4.1: Spatiotemporal patterns of chronic and acute stressors on Kiritimati reefs. **a)** Labelled points indicate locations of the 12 reef sites sampled on a map of Kiritimati. Sites are categorised as very low, low, medium, high or very high disturbance based human disturbance. Permanent settlements are depicted by red circles scaled according to population size. A dashed line dissects the island to indicate shores designated as windward or leeward. **b)** Mean ($\pm$ standard error) measurements of environmental metrics at each site from 2014-2023. Satellite-derived sea-surface chlorophyll-a concentration, surface water nitrate and nitrite concentration, and in-water visibility are shown. Study sites are ordered along the x-axis by location, moving clockwise windward to leeward. **c)** Satellite-derived sea-surface temperature ($^{\circ}\text{C}$) and accumulated heat stress (maximum degree-heating-weeks, DHW) are shown by blue and black line graphs, respectively. Colour fills under the DHW line represent periods over which thresholds of 4, 8, 12, 16 and 20 DHW were breached, corresponding to respective Coral Reef Watch bleaching alert levels 1-5. The dashed blue horizontal line indicates the average threshold for SST anomalies (1°C above Maximum Monthly Mean for all sites), above which DHW begin to accumulate. Dotted vertical lines mark ten sampling expeditions carried out between August 2014 and September 2023.

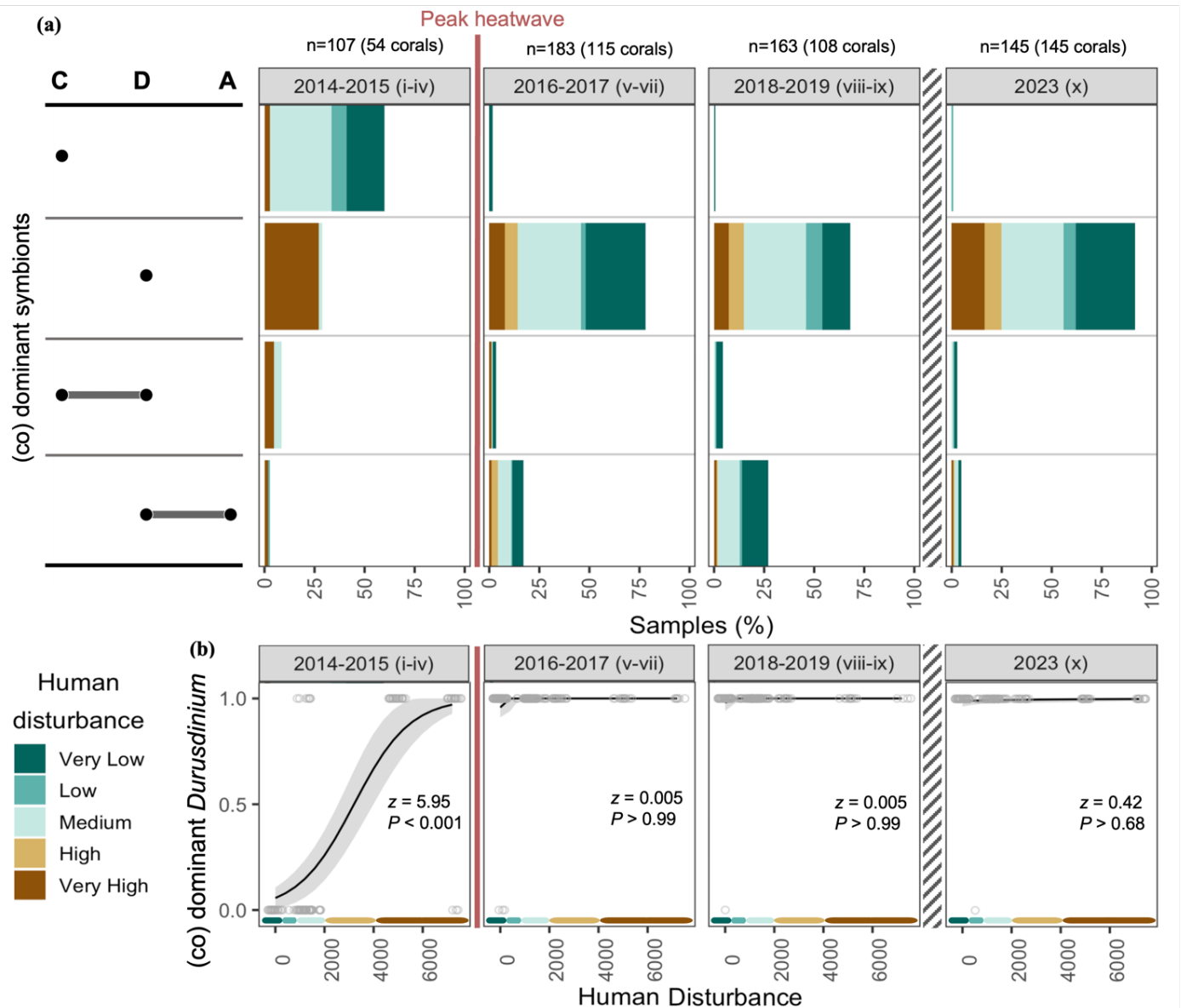


Figure 4.2: Symbiont assemblages hosted by *Platygyra ryukyuensis* colonies are characterised by their dominant or codominant genera. 598 samples collected across expeditions i-x are grouped into four time-periods. **a)** Symbiont assemblages are grouped by the incidence of genera with relative read abundance (RRA) > 10%. C = *Cladocopium*; D = *Durusdinium*; and A = *Symbiodinium*. One sample co-dominated by all three genera (C+D+A), and two samples hosting codominant C+A not shown. Bars represent proportion of samples within each period and are filled by human disturbance category. **b)** Logistic regressions for each time-period showing colonies with dominant or co-dominant *Durusdinium* against site-level human disturbance exposure (categorical designations shown by coloured bars above x-axis). Points represent individual samples, with some random horizontal variation added to reduce point overlap. Peak heat stress accumulation is denoted with a red line between expeditions iv and v and the hashed vertical column marks a hiatus in sampling between 2019 and 2023.

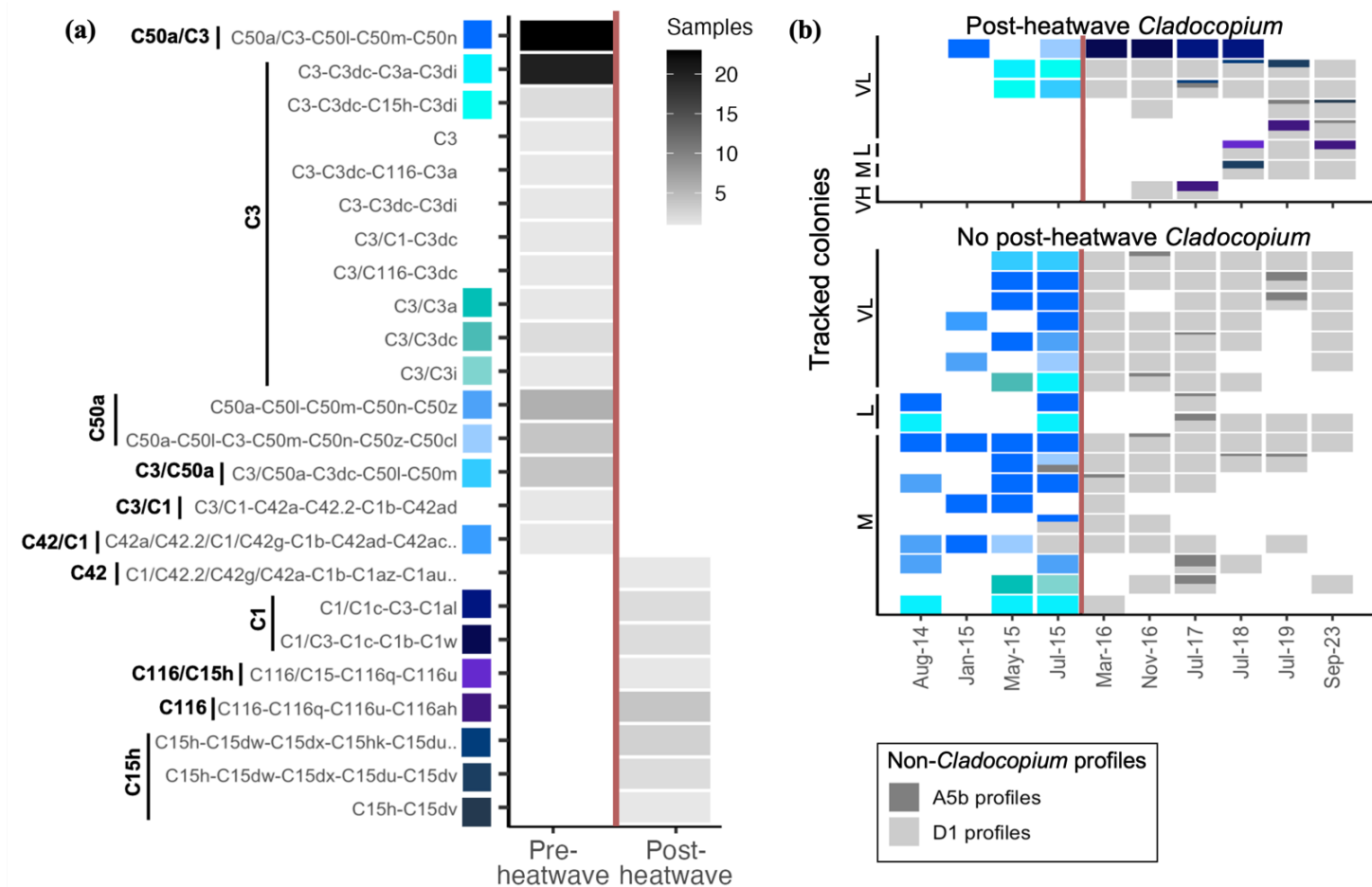


Figure 4.3: Pre- to post-heatwave shift in *Cladocopium* lineages hosted. **a)** Samples in which *Cladocopium* was dominant or codominant are shown (n=88). ITS2 profiles are ordered by their majority sequence variant(s), indicated by bold black labels. **b)** Each row represents a distinct tracked colony. *Cladocopium* ITS2 profiles present are indicated by the blue colour-scale in a), whilst *Symbiodinium* and *Durisdinium* profiles are shown in grey. Colonies are grouped by those with at least one post-heatwave sample containing *Cladocopium*, and those without ('no post-heatwave *Cladocopium*'). Human disturbance exposure is denoted by 'VL', 'L', 'M' and 'VH', representing very low, low, medium and very high disturbance categories. Vertical red lines indicate peak heatwave exposure.

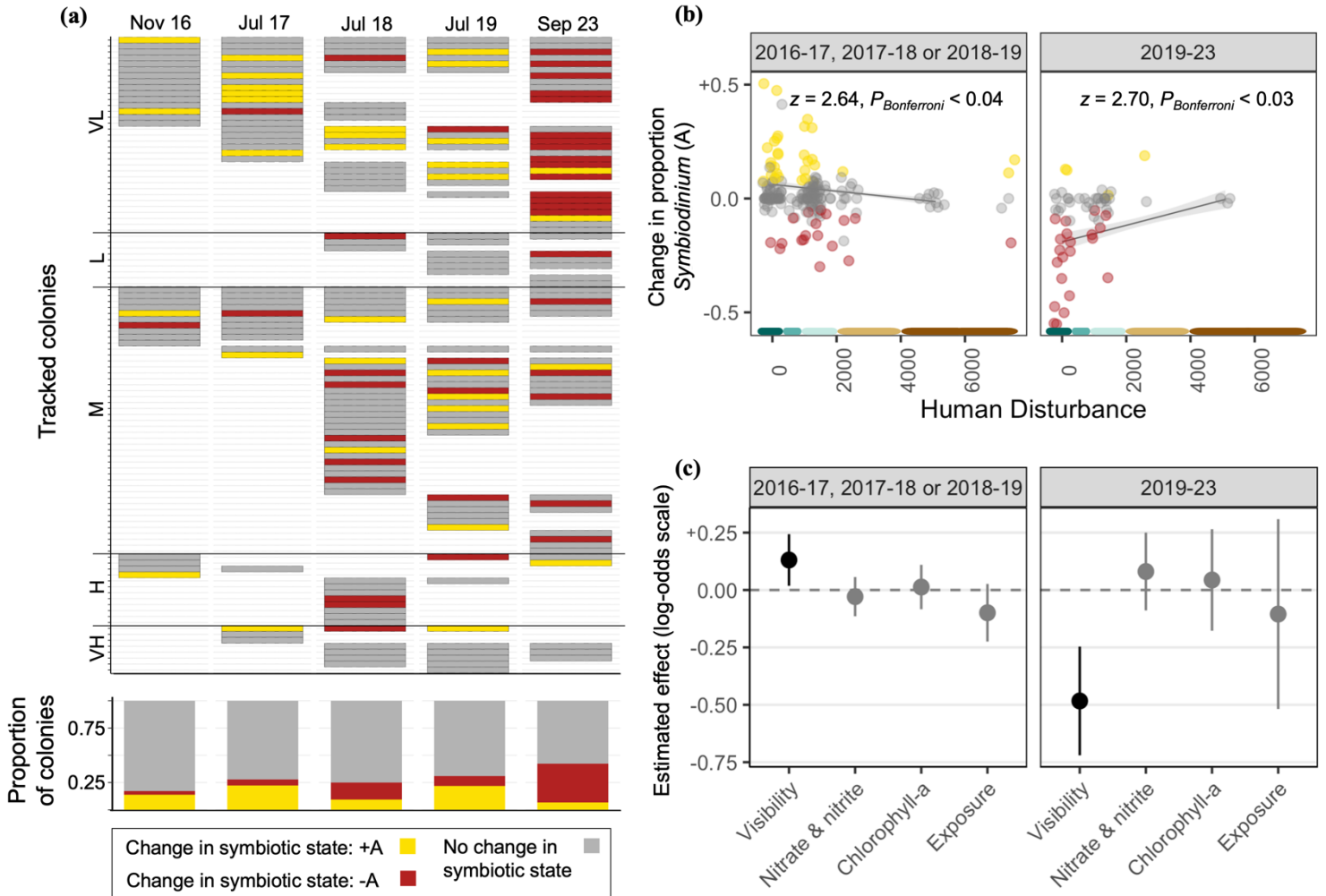


Figure 4.4: Post-heatwave dynamism of *Symbiodinium* in *Platygyra ryukyuensis* colonies that were sampled at least twice post-heatwave over successive expeditions. For list of the 107 re-sampled colonies included in this figure see Figure S2. **a)** Symbiotic state changes are characterised by dominant/codominant Symbiodiniaceae genera in a coral colony compared to its previous assemblage: only state changes between *Durusdinium* ('D') and *Durusdinium-Symbiodinium* ('D+A') assemblages are coloured. Each row represents a colony, ordered by human disturbance exposure denoted by 'VL', 'L', 'M' and 'VH', with bar colour indicating whether the colony transitioned from *Durusdinium* to *Durusdinium-Symbiodinium* ('+A') or from *Durusdinium-Symbiodinium* to *Durusdinium* ('-A') with respect to the previous expedition. The proportions of tracked colonies that underwent these state changes is summarised beneath. **b)** Beta regressions of the change in *Symbiodinium* RRA in re-sampled corals against human disturbance exposure. Points represent colonies with some random horizontal variation added to reduce point overlap, coloured by colony transition categories used in a). Points from site VH1 (the highest continuous disturbance exposure) were not included in the regression in the left-hand panel, since this site was not represented in 2019-2023 resamples. **c)** Estimated effects on the change in *Symbiodinium* RRA in re-samples corals attributed to variation in site environmental variables. Lines represent 95% confidence estimates of effect sizes (points) based on beta mixed-effects model coefficients, shown on the model's log-odds scale. Effects significantly different from zero are shown in black, those not significantly different from zero are grey.

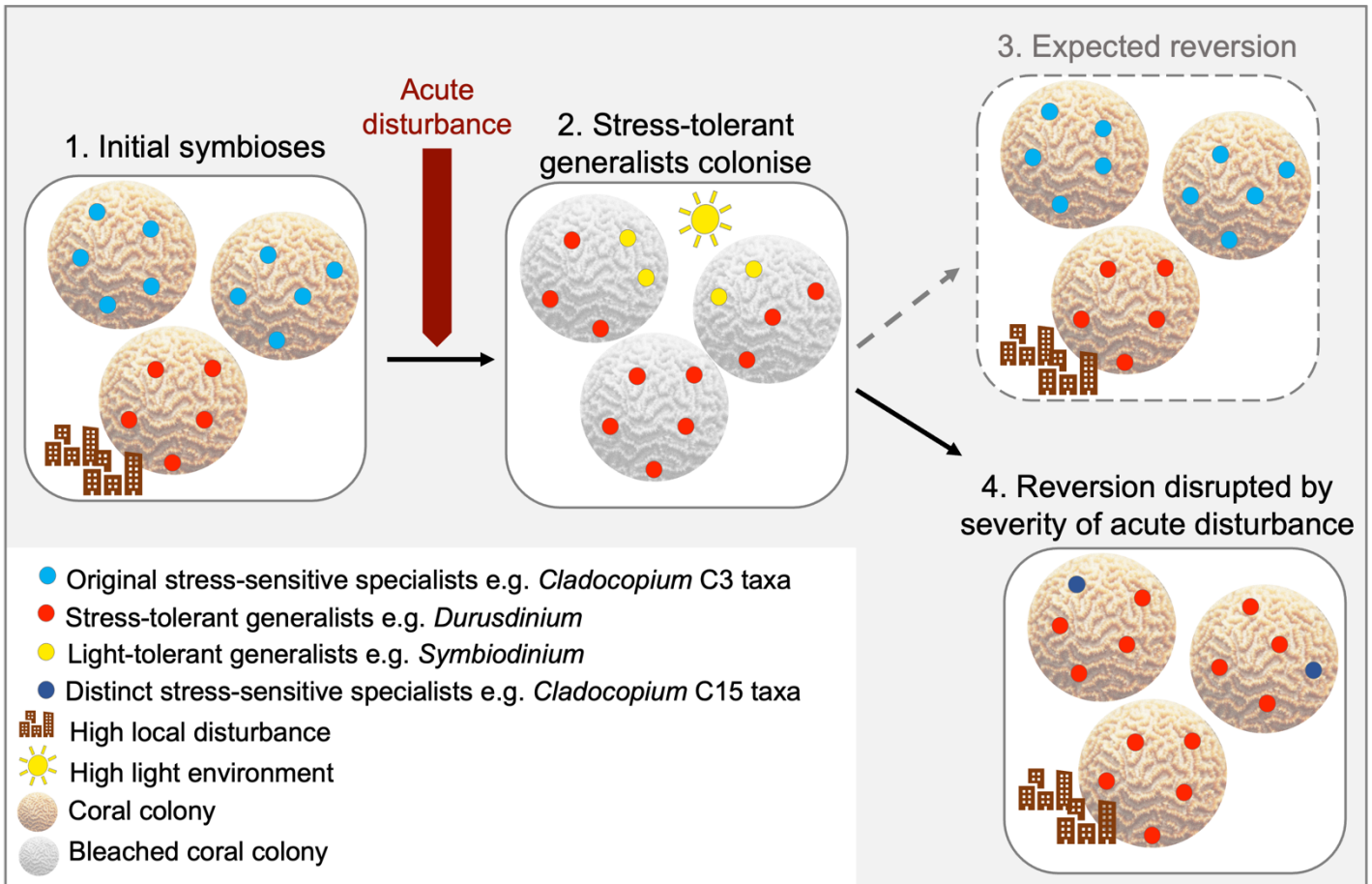


Figure 4.5: Proposed pathway for Symbiodiniaceae succession *in hospite* following an extreme heatwave. **1.** Initial symbioses: Symbiont genera are spatially distributed according to human disturbance. **2.** Stress-tolerant generalists colonise: Following coral bleaching, stress-tolerant symbionts re-colonise bleached hosts. Light-tolerant symbionts may also colonise in high light environments. **3.** Expected reversion: Pre-heatwave symbionts with faster growth rates and competitively superior nitrogen acquisition competitively dominate or exclude stress-tolerant generalists. **4.** Reversion disrupted by severity of acute disturbance: Extended duration or extreme severity of the acute disturbance instead favours the persistence of heatwave-transformed symbioses. Priority effects facilitate colonisation of stress-tolerant symbionts over extended stress exposure. High host mortality following extreme events reduces the metacommunity availability of original symbionts, whilst those specialist symbiont species with greater availability may have reduced capacity for host-colonisation.

Chapter 5 – Symbiotic indicators of heatwave vulnerability in corals from thermal hotspots

Adapted from “Algal symbionts indicate heatwave vulnerability in corals from hotspots but not from thermal refugia.”, manuscript in revision.

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5.1 Abstract

Reef-building corals face continued declines due to climate change-amplified marine heatwaves. In addition to affecting coral heat tolerance, corals' algal endosymbionts (family Symbiodiniaceae) can reflect their prior heatwave exposure, although understanding is often limited to heatwave-induced shifts between symbiont genera. Here, we used ITS2 metabarcoding to characterise Symbiodiniaceae assemblages in 293 individuals of the common Indo-Pacific coral *Acropora aff. digitifera* in Palau (Western Pacific), between two outer-reef regions with contrasting heatwave histories. During the strongest recorded heatwaves, southwestern 'hotspot' reefs have typically accrued an additional 2 °C-weeks of heat stress compared to thermal 'refugia' located 60 km north. In contrast to previous studies that observed declines in symbiont richness following heat stress, we found a greater diversity of symbiont taxa and low-abundance sequence variants in 'hotspot' corals, in genus *Cladocopium* predominantly within the C40 lineage (*Cladocopium madreporum*). Combining these data with experimental heatwave performance from 168 of these corals revealed that approximately 10% of heat tolerance variability at hotspot reefs was associated with hosting different symbiont taxa. Compared to other hotspot corals, those hosting symbionts with the C15h sequence variant suffered bleaching mortality at 0.8 °C-weeks lower heat stress. Despite higher variability in heat tolerance among corals from thermal refugia compared to hotspot reefs, we found no association between heat tolerance and the symbionts hosted by refugium corals. As the world's coral reefs are exposed to intensifying marine heatwaves under accelerating climate change, the low-abundance variants that characterise symbionts within genera or lineages may become increasingly important indicators of poor heatwave tolerance.

5.2 Introduction

Marine heatwaves are restructuring populations, imposing directional selection through mass-mortality and favouring specific genotypes whilst simultaneously eroding standing genetic and phenotypic variation (Coleman & Wernberg, 2020; Gurgel et al., 2020; Burgess et al., 2021; Starko et al., 2023; Lachs et al., 2024a). Reef-building corals (order Scleractinia) are especially vulnerable to the effects of marine heatwaves, since they exist in obligate symbioses that are highly sensitive to environmental disturbance (Glynn, 1996) and are often distributed close to their thermal tolerance limit (Smith et al., 2023). Some of the variation in coral heat tolerance over scales of 300-1,300 km (across latitudinal gradients of the Great Barrier Reef for example) has been attributed to coral adaptation or acclimatisation to differential historical heat stress (Guest et al., 2012; Denis et al., 2024; Naugle et al., 2024). However, the effects of marine heatwaves on coral heat tolerance are also influenced by the timing and the spatial extent of prior exposure (Hughes et al., 2019; Marzonie et al., 2022). In comparison to broad-scale patterns, our understanding of variation at the smaller spatial scales (<100 km) relevant for most reef-management, remains limited (van Woesik et al., 2012; Thomas et al., 2018; Voolstra et al., 2020; Lachs et al., 2024b). Heatwave tolerance may increase in coral populations through adaptation to frequent heat stress (Lachs et al., 2024a), although reefs that experience consistently less heatwave stress, which we refer to here as thermal ‘refugia’, may represent crucial reservoirs of genetic variation and adaptive potential as heatwaves force population bottlenecks in surrounding habitat (Keppel et al., 2012; Leiva et al., 2025). As marine heatwaves continue to increase in severity, duration and frequency under accelerating climate change

(Oliver et al., 2021), few reefs are expected to escape regular heat stress in the coming decades (McWhorter et al., 2022).

Environmental variation can drive local adaptation even in populations with high gene flow and as such population structure can persist despite long-range dispersal of coral larvae (Richardson et al., 2014). The reef-building coral *Acropora digitifera* has shown high population connectivity between islands of the tropical Pacific over 1,000 km apart and has the capacity for extensive dispersal (Davies et al., 2015). Despite high connectivity, considerable variation in *A. aff. digitifera* heatwave tolerance, with a substantial heritable coral genetic basis, is evident between nearby reefs (<100 km) and even within reefs (<100 m) (Humanes et al., 2022; Lachs et al., 2024b). The north of Palau's barrier reef system has consistently been exposed to milder heat stress conditions during the most intense marine heatwaves (van Woesik et al., 2012; Lachs et al., 2024b), potentially owing to frequent flushing of northern lagoons through reef channels compared to higher tidal retention surrounding southwestern reefs (Golbuu et al., 2012, 2016). This marked local variation in historical heat stress between Palau's northern thermal 'refugia' and southwestern 'hotspots', delineated recently from satellite data (Lachs et al., 2024b), is associated with corresponding genetic divergence in Palauan populations of several reef-building corals (Cros et al., 2016; Rivera et al., 2022).

Phenotypic variation within coral populations can reflect differences in microbial holobiont composition, with corals' associated microalgal endosymbionts (Family Symbiodiniaceae) capable of generating substantial variation in thermal tolerance (Berkelmans & van Oppen, 2006; Silverstein et al., 2015). Many species in the genus *Acropora* exhibit flexibility in their associations with different symbiont genera, which is characteristic of the flexibility attributed to corals that acquire their symbionts from the environment rather than

inheriting them from a parent (Fabina et al., 2012; Turnham et al., 2021). However, *Acropora* spp. have shown low flexibility in their symbiont associations within *Cladocopium*, the most phylogenetically and phenotypically diverse genus within Symbiodiniaceae (LaJeunesse et al., 2018), commonly hosting regionally-specific *Cladocopium* lineages (Thomas et al., 2014; Zarate et al., 2024). Palau's *A. aff. digitifera* population has been found to host symbionts in genera *Durusdinium* and *Symbiodinium* (Humanes et al., 2024), but its associations with *Cladocopium* are almost exclusively with the 'C40' lineage, formally described as *Cladocopium madreporum* (Davies et al., 2020; Humanes et al., 2022; Butler et al., 2023; Lachs et al., 2023; Lewis et al., 2024). Despite this C40-specificity, spatial structuring of symbionts within a single *Cladocopium* lineage has been detected within other highly connected *Acropora* spp. host populations (Davies et al., 2020; Kriefall et al., 2022; Armstrong et al., 2024; Naugle et al., 2024), and therefore may also exist between Palau's *A. aff. digitifera* population and different C40 symbiont taxa.

Exposure to heat stress has been linked to reductions in the diversity of Symbiodiniaceae in reef-building coral hosts. The erosion of symbiont diversity with heat stress can reflect the replacement of heat-sensitive *Cladocopium* symbionts by less taxonomically diverse, stress-tolerant genera *Durusdinium* or *Symbiodinium* (Pettay et al., 2015; Claar et al., 2020b; Jain et al., 2020; Quigley et al., 2022). The loss of symbiont richness from within a single *Cladocopium* lineage has also been documented in some coral populations following heat stress (Kriefall et al., 2022; Starko et al., 2023; Leiva et al., 2025). Therefore, local thermal refugia that have avoided such losses may prove important reservoirs for symbiotic diversity at this fine taxonomic scale.

Contrary to expectations of increased heat tolerance at hotspot reefs in Palau, owing to coral adaptation or acclimatisation with higher exposure to historical heat stress, Lachs et al (2024b) observed reduced heat tolerance of *Acropora aff. digitifera* from hotspots, compared to

their northern refugium-sourced conspecifics which also exhibited greater variation in heat tolerance. Here, we investigate whether differences in historical heat stress in this reef system are associated with the Symbiodiniaceae taxa hosted by *Acropora aff. digitifera*, with corresponding links to coral heat tolerance. We hypothesise that i) corals from hotspot reefs host less diverse Symbiodiniaceae assemblages due to the loss of symbiont richness with historical heat stress, and ii) variation in heat tolerance between corals from reefs that have experienced comparatively less historical heat stress corresponds to the Symbiodiniaceae taxa they host. This study provides insights into associations between symbionts and historical heat stress regimes beyond shifts among Symbiodiniaceae genera. Our results also contribute to a burgeoning appreciation of symbiont variation at fine taxonomic scales within *Cladocopium* and corresponding links to heat tolerance within coral populations.

5.3 Materials & methods

5.3.1 Historical thermal regimes

The coral individuals investigated in this study originated from five replicate sites from thermal refugia and hotspot reefs. Three replicate sites in each region correspond to the sites (and corals) used in Lachs et al. (2024b). The characterisation of historic thermal stress regimes, given in full detail in Lachs et al. (2024b), was based on 0.05° by 0.05° latitude-longitude resolution maps of annual peak cumulative heat stress for the period 1985-2020. Heat stress was calculated in Degree Heating Weeks (DHW, in units of °C-weeks)– a metric that incorporates both the duration and intensity of heat stress, such that > 8 °C-weeks is strongly associated with reef-wide coral bleaching (Heron et al., 2016; Lachs et al., 2021). By mapping the annual heat stress reefs

received relative to one another (DHW anomaly), and the inter-annual consistency with which reefs were ranked (in DHW percentile), Lachs et al. (2024b) delineated persistent hotspots and thermal refugia, as reefs that consistently experience higher-than-average heat stress and lower-than-average heat stress, respectively (Figure 5.1).

5.3.2 Coral collection and experimental heatwave exposure

Acropora aff. digitifera corals have a corymbose morphology and are widely distributed across Indo-Pacific high wave-energy reefs. Fore reefs between 3-8 metres below sea level were chosen as collection sites within target thermal hotspot or refugium pixels. At each site, transects were laid along a 5-6 m isobath and *A. aff. digitifera* corals with at least 20 basal branches (approximately 20 cm in diameter) and at least 5 m apart were selected, to reduce the probability of selecting clones (Baums et al., 2019). Recent molecular phylogenetic work continues to reveal changes in the systematics of the Acroporidae family that had previously been obscured by morphological similarities (Bridge et al., 2024; Rassmussen et al., 2025). Our approach to identifying and collecting corals in Palau with a morphological affinity to *Acropora digitifera* was the same as that used by (Lachs et al., 2024b), but coral taxonomists have recently noted that these corals have not been verified as *Acropora digitifera* using molecular phylogenetics, hence we refer to these corals conservatively as *Acropora aff digitifera*. Corals were selected based on their morphological affinity to the nominal species *Acropora digitifera*, hence the ‘*aff.*’ qualifier is used here. Each coral was photographed for subsequent morphological species identification, and branches (6 for heat stress evaluation and 1 for DNA sequencing) were broken off at their bases and stored in seawater coolers until they were transferred to experimental tanks at the Palau International Coral Reef Center (Koror, Palau) later the same day.

Coral branches were allowed to acclimate to conditions in a series of 40 litre flow-through race-way tanks for 7-10 days following collection in April 2022. Five branches per coral were transferred to randomised positions across 10 experimental tanks and the sixth to one of two control tanks. After acclimation, experimental tank temperatures were increased by 0.4 °C on day 1, and by 0.5 °C on days 3, 5, 7, and 13 to reach a final temperature of approximately 32.5 °C. This temperature was maintained for over 5 weeks until all branches had died, accumulating up to 16 °C-weeks. One branch in the control treatment suffered mortality and as such all experimental branches originating from this coral were removed from subsequent analyses, resulting in 168 corals (816 branches) retained for analysis (Table S1). Further details of this experimental heatwave exposure are given in Lachs et al. (2024b).

Coral bleaching does not reliably predict subsequent mortality (Hughes et al., 2019; Matsuda et al., 2020), yet both responses provide insight into coral and Symbiodiniaceae performance under heat stress. To capture both, Lachs et al. (2024b) recorded the visual progression of bleaching and mortality in experimental fragments and calculated a bleaching and mortality index (from 0 to 1) for each coral as heat stress advanced. This facilitated the calculation of the 50 % effective dose (ED₅₀) for heat stress, DHW₅₀, the accumulated heat stress at which a coral's bleaching and mortality index reaches 0.5 (equivalent to all replicate branches being bleached), used here as an ecologically meaningful measure of tolerance to simulated long-term marine heatwave stress.

5.3.3 Symbiodiniaceae DNA extraction and sequencing

Coral branches designated for sequencing were wrapped in aluminium foil and stored at -80 °C by the end of the collection day upon removal from a seawater cooler. Each wrapped branch was

flash-frozen in liquid nitrogen and crushed into a fine powder using a sterilised pollen press and hammer. The powdered branch was briefly mixed with a spatula and ~2 mm³ of sample was retained for DNA extraction using a DNeasy Blood and Tissue kit (Qiagen). The manufacturer's protocol was modified to include an overnight lysis, and samples were carefully inverted rather than vortexed prior to being passed through spin columns to avoid shearing of DNA by skeletal fragments (Baums & Kitchen, 2020). In addition, the supernatant of lysed samples was transferred into new microcentrifuge tubes to remove large skeletal fragments, and then again following 1-minute at 6,000 rpm in a centrifuge to remove smaller skeletal fragments, and two 200 µl elutions were performed and combined to maximise final DNA yield. The internal transcribed spacer 2 (ITS2) ribosomal region was amplified using SYM_VAR primer pairs (5'CAGCTTCTGGACGTTGYGTTGG3'; 5'CGGGTTCWCTTGTYTGACTTCATGC3') (Hume et al., 2013, 2018) and combined to form unique paired indexes for sample demultiplexing. A no-template control from each 96-well amplification and a total of three negative extraction controls were used to check for contamination using gel electrophoresis. Amplicon purification and normalisation was performed using Just-a-plate 96 PCR Normalization and Purification kits (Charm Biotech). The resulting PCR products were pooled and quantified with Qubit 3.0 fluorometry (Thermo Fisher Scientific). Amplicon sequence data were generated using Illumina MiSeq v2 (Illumina Inc.) performed at the John G. Shedd Aquarium (Chicago) Molecular Ecology Laboratory, generating paired-end 250 base-pairs and yielding on average 25,154 raw contigs per sample.

5.3.4 Bioinformatics & classifying Symbiodiniaceae sequence data

ITS2 sequence data were analysed using two workflows to classify distinct sequence variants: i) SymPortal, which uses minimum entropy decomposition to identify defining intragenomic variants (DIVs) and uses co-occurrence patterns to further cluster these into ITS2 profiles, representing putative species (Hume et al., 2019) and ii) DADA2, which identifies amplicon sequence variants (ASVs) using a denoising algorithm that models sequencing errors (Callahan et al., 2016). These two bioinformatics pipelines represent different approaches to resolving intra- and inter-genomic sequence variants from the multi-copy ITS2 region in Symbiodiniaceae, hence combining these methods allows for more robust interpretation of sequencing results (Quigley et al., 2022; Davies et al., 2023). The DADA2 approach offers greater sensitivity in differentiating sequence variants, whilst the SymPortal approach aims to distinguish most intra- and inter-genomic variation, although ASVs and DIVs cannot confidently be confirmed as intra- or inter-genomic from these data and are therefore referred to as sequence variants. Sequence data were submitted to the remotely hosted SymPortal database. For the DADA2 pipeline, primer sequences were removed using the ‘cutadapt’ tool (Martin, 2011), then forward and reverse reads were denoised and merged using default parameters (truncating reads at the first instance the quality score falls below 2 and permitting a maximum of 2 expected errors in each forward or reverse read). To minimise the effect of uneven sample sequencing depths on the detection of low abundance variants, sample coverage of ASV and DIV richness was modelled against sequencing depth using the ‘iNEXT’ package (Hsieh et al., 2024), revealing estimated sample coverage greater than 0.999 at 1,000 reads (Figure C1). Hence, samples with <1,000 total post-processing Symbiodiniaceae reads (6 of 299) were removed from analyses (Table C1).

SymPortal sequence data yielded a mean of 10,587 post-processing Symbiodiniaceae reads per sample from a total of 61 distinct DIVs which were further collapsed into 23 distinct

ITS2 profiles. A mean of 9,996 Symbiodiniaceae reads per sample were retained from the DADA2 output for a total of 92 distinct ASVs. The most numerically abundant (the highest relative read abundance) ITS2 profile in each sample comprised on average 99.7% (over 75% in all cases) of Symbiodiniaceae reads, the dominant profile. Out of 293 samples, 13 contained an additional background ITS2 profile, comprising on average 7.9% of Symbiodiniaceae reads. Where ITS2 profiles are displayed, a colour scale has been assigned based on profiles' average defining proportions of sequence variants (Table C2, Figure C2). All ITS2 profiles, DIVs and ASVs detected were within genus *Cladocopium*, with DIVs and ITS2 profile taxonomies assigned by using reference sequences within the SymPortal community database. Unique ASVs were numbered and assigned to the genus level within DADA2 using a naïve Bayesian classifier algorithm (Wang et al., 2007) with a minimum bootstrap confidence of 80 using a comprehensive Symbiodiniaceae reference database available within the online data repository (Franklin et al., 2012; Bongaerts et al., 2013; Nitschke, 2020). Subsequently, ASV ancestral *Cladocopium* lineages were assigned based their closest match within the same reference database. ASV and DIV reference sequences were aligned using the 'MUSCLE' algorithm implemented in the 'msa' R-package (Edgar, 2004; Bonatesta et al., 2024). Pairwise distance calculations were performed on aligned sequences in 'seqinr' (Charif, 2023) and these were used to construct neighbour-joining trees of ASVs and DIVs using the 'ape' R package (Paradis, 2024). Pairwise distances between ITS2 profiles for the construction of a neighbour-joining tree were provided by SymPortal (Figure C3).

5.3.5 Statistical analyses

Indicator species analyses were performed using multi-level pattern analyses on symbiont assemblages followed by permutation testing (De Cáceres & Legendre, 2009; De Cáceres et al., 2010): The ‘Indicator Value’ method was used to detect symbiont ASVs or DIVs that, when present, were significant predictors of a coral’s hotspot or refugium origin, based on both the regional specificity and regional occurrences of sequence variants. To compare the heterogeneity of symbionts hosted by corals collected from hotspot reefs and from refugia, both alpha diversity (diversity within coral samples) and beta dispersion (symbiont assemblage dissimilarity between samples) were assessed. Alpha diversity of ITS2 profiles within each coral was estimated using the phylogenetic-Hill richness metric, which incorporates profile richness and phylogenetic spread by integrating the sum of neighbour-joining tree branch lengths (Figure C3) connecting the profiles in each sample (Li, 2023). Dissimilarities between symbiont assemblages were calculated as weighted unifracs distances (sequence dissimilarity between composite DIV or ASV variants) and ordinated. The beta dispersion between hotspot samples and between refugium samples was compared based on sample distances to centroids from weighted unifracs ordinations.

The Bartlett test for homogeneity of variances (around group means) was used to compare the variances in coral heat tolerance (DHW_{50}) between regions. Indicator species analyses of heat tolerance were performed separately on ASVs, DIVs and ITS2 profiles to detect symbiont indicator candidates: a point-biserial correlation method was used to test symbiont associations with corals in a variety of heat tolerance quantiles (e.g. tertiles, quartiles and pentiles). The strength of the relationships between coral DHW_{50} and symbiont heat tolerance indicators relative read abundance (RRA) within samples was formally assessed using generalised linear mixed-effects models with Gamma error distributions (to relax the assumption

of homoscedasticity). Collection site was included as a random effect in linear mixed-effects models and four putative outlier points (2 hotspot corals and 2 refugium corals) were excluded due to their studentised residuals exceeding ± 3 (Fox & Weisberg, 2018). Differences in the relative read abundances of sequence variants between samples comprising the same ITS2 profile may reflect sequencing biases rather than real differences in variant relative abundances (Hume et al., 2019). Hence, linear models of heat tolerance were constructed using the RRA of a variant in each sample ('sample RRA' models) in case of erroneous clustering of distinct taxa into ITS2 profiles and analogous 'mean profile RRA' models were created using the mean RRA of the variant across samples hosting the same ITS2 profile(s). Figure S4 shows the variability in sample RRA that was collapsed into mean profile RRA for four variants of interest. The variance in DHW₅₀ explained by symbiont fixed effects was estimated as the marginal R² of linear mixed-effects models (Nakagawa & Schielzeth, 2013). Post-hoc Tukey's pairwise tests were used to test for significant between-group differences in mean DHW₅₀ according to the dominant ITS2 profile hosted.

5.4 Results

5.4.1 Hotspot colonies host a greater diversity of symbiont species

Cladocopium C40-C3-C115-C40h-C40e was the most common profile detected in *A. aff. digitifera* corals sampled across all 10 sites, yet this was the dominant profile in only 51% of hotspot corals compared to 67% of refugium corals (Figure 5.2a). The six profiles containing the C15h sequence variant were more common in hotspot corals, detected in 21% of individuals compared to only 11% of refugium corals, largely driven by profiles C40-C3-C15h-C115-C40h

and C40/C15h-C3-C15do-C115 (in which C15h is present at defining proportions of 0.05 and 0.38 respectively, Table C2). Other region-specific symbiont distributions include the detection of C116 variants in the C40-C116a-C3-C115-C116v profile in seven corals exclusively at hotspot reef sites, and profiles containing C50 variants in two refugium corals. Indicator analyses revealed sequence variants deemed to indicate a coral's hotspot or refugium origin, when present: six ASVs and one DIV were significant refugium indicators, compared to six ASV and seven DIV indicators of hotspot corals (Figure 5.2b, indicator value test statistics given in Table C3). Across both ASV and DIV bioinformatics methods, the presence of several C15 and C116 variants were significantly associated with corals originating from hotspot reefs, in accordance with increased occurrences of C15- and C116-containing ITS2 profiles across hotspot corals (Figure 5.2a). All C15 and C116 variants were found either in background profiles (as C15h-C15vr-C15do or C15/C93a hosted alongside a dominant profile) or at lower defining proportions than the C40 variant, except in two cases where the C15h/C40-C15-C3-C115 profile was detected (Table C2). Despite their low defining proportions in *Cladocopium* symbiont profiles, these indicator variants served to differentiate regional symbiont assemblages.

Symbiodiniaceae assemblages hosted by corals from hotspot reefs were more heterogenous than those from thermal refugia, with higher symbiont diversity within and between hotspot corals, quantified as alpha diversity and beta dispersion respectively. A total of 18 distinct *Cladocopium* ITS2 profiles (putative species) were detected in corals sampled from thermal refugia (14 dominant and 4 background profiles, composed of 42 distinct DIVs or 53 ASVs), but similarly, 16 distinct profiles (13 dominant and 3 background, composed of 50 distinct DIVs or 63 ASVs) were detected in hotspot corals. However, the beta-dispersion between Symbiodiniaceae assemblages was significantly higher between hotspot corals than

between refugium corals (ASVs; $F = 19.08$, $P < 0.001$, DIVs; $F = 21.38$, $P < 0.001$), based on sequence variant unifrac-distance ordinations (Figure 5.3a). In addition, hotspot corals hosted a higher alpha diversity of symbiont ITS2 profiles than refugium corals based on the richness and phylogenetic spread of profiles (phylogenetic divergence between profiles hosted, or divergence relative to profiles in this dataset if only one profile was present) (Figure 5.3b, $t = 2.34$, $P = 0.020$, Figure C3). The greater symbiont beta dispersion between hotspot corals along with their higher alpha diversity of ITS2 profiles remain evident when restricting analyses to the three hotspot reefs and three refugia sampled for experimental heat stress (ASV dispersion; $F = 11.54$, $P = 0.001$, DIV dispersion; $F = 12.05$, $P = 0.001$, phylogenetic-Hill richness; $t = 2.30$, $P = 0.023$).

5.4.2 Higher heat tolerance mean and variance in thermal refugia

The mean effective dosage of heat stress required to elicit a 50% bleaching mortality response (DHW_{50}) was significantly higher for corals from refugia than from hotspot reefs under experimental heat stress (Figure 5.4; Welch $t = 2.48$, $P = 0.014$; Wilcoxon rank $W = 2304$, $P < 0.001$). This difference represents a mean increased DHW_{50} of 0.54 in refugium corals compared to hotspot corals (refugia mean; 10.09 °C-weeks, hotspots mean; 9.55 °C-weeks). Furthermore, the worst- and the best- performing corals under experimental heat stress all originated from thermal refugia (DHW_{50} ranged from 3.8 to 12.5 °C-weeks), reflecting the higher variance in DHW_{50} between refugium corals compared to hotspot corals (Bartlett's $K^2 = 9.35$, $P = 0.002$).

5.4.3 Symbionts with indicator variants linked to low heat tolerance in hotspot corals

Relative read abundances (RRA) of the C40 variant (DIV C40/ASV1 C40), the numerically dominant sequence variant in almost all heat-stressed corals (mean RRA > 0.8), correlated positively with heat tolerance under experimental heat stress in all four quantile sensitivity analyses (Figure 5.5, indicator correlation test statistics given in Table C4). Six variants were found to correlate negatively with heat stress performance. Most notable among these are DIV C15h and its close counterpart ASV4 C15 (both with a mean RRA of 0.14 when present), which indicated poor heat stress performance in all four quantile analyses. In addition, ITS2 profile C40-C3-C15h-C115-C40h, the most common profile containing the C15h variant and one with higher occurrence in hotspot corals (Figure 5.2a), correlated negatively with heat tolerance in two of the four quantile analyses.

The strength of correlations between indicator symbiont candidates and coral heat stress performance was assessed, revealing significant symbiont-heat tolerance associations for hotspot corals only. The DHW₅₀ of hotspot corals decreased significantly with increasing C15h DIV RRA (Figure 5.6a, sample RRA; $z = 2.26$, $P = 0.024$, mean profile RRA; $z = 2.16$, $P = 0.031$), but no analogous effect was detected in corals from thermal refugia (sample RRA; $z = 0.40$, $P = 0.687$, mean profile RRA; $z = 0.26$, $P = 0.795$), based on corals with non-zero C15h DIV RRA only. Additionally, DHW₅₀ increased significantly with C40 DIV RRA in hotspot corals (Figure 5.6b, sample RRA; $z = 3.18$, $P = 0.001$, mean profile RRA; $z = 2.74$, $P = 0.006$), but not in refugium corals (sample RRA; $z = 0.39$, $P = 0.697$, mean profile RRA; $z = 0.27$, $P = 0.789$). Similarly, hotspot coral DHW₅₀ significantly decreased with ASV4 C15 RRA (Figure C5a, sample RRA; $z = 2.80$, $P = 0.005$, mean profile RRA; $z = 2.34$, $P = 0.019$) and increased with ASV1 C40 RRA (Figure C5b, sample RRA; $z = 2.93$, $P = 0.003$, mean profile RRA; $z = 2.67$, $P = 0.008$). In a combined model with both C15h DIV RRA and C40 DIV RRA included as

predictors, these symbiotic variables were estimated to explain 10.0-10.8% of DHW₅₀ variation between hotspot corals (sample RRA marginal R²; 0.108, mean profile RRA marginal R²; 0.100) compared to 0.2-0.4% of variation between refugium corals (sample RRA marginal R²; 0.004, mean profile RRA marginal R²; 0.002). The symbiont-heat tolerance correlations in hotspot corals seem to be largely driven by those hosting ITS2 profiles C40-C3-C15h-C115-C40h or C40/C15h-C3-C15do-C115 (Figure 5.6a, 5.6b). The mean DHW₅₀ for hotspot corals hosting any profile with the C15h variant was only 8.9 compared to the mean DHW₅₀ of 9.7 across remaining hotspot corals, equating to these C15h-containing corals having a lower heat tolerance of 0.8 °C-weeks (Figure 5.6c).

5.5 Discussion

Using field-sampling, experimental heatwave exposure, and indicator symbiont analyses, we characterised Symbiodiniaceae assemblages in *Acropora aff. digitifera* across Palauan reefs with different thermal histories and assessed how symbiotic variation relates to coral tolerance to a simulated marine heatwave. We observed a higher diversity of *Cladocopium* symbiont species and their low-abundance sequence variants in corals from hotspot reefs that had consistently experienced higher levels of heat stress, rather than the symbiont homogenisation or decline in richness reported by previous studies (Kriefall et al., 2022; Starko et al., 2023; Leiva et al., 2025). Although symbiotic differences could not explain the variation in heat tolerance amongst corals from thermal refugia reported by Lachs et al. (2024b), yet symbiont associations could explain some hotspot heat tolerance variability. Together, these results represent a region-dependent distribution of indicator symbionts coupled with their region-dependent association

with low coral heat tolerance (Figure 5.7): *Acropora aff. digitifera* from hotspot reefs were more likely to host diverse symbiont species (characterised by their low-abundance sequence variants) and those hosting such assemblages were more likely to exhibit lower heat stress tolerance compared to other hotspot corals.

5.5.1 Symbiont diversity under chronic environmental stress

Symbiont assemblages hosted by *Acropora aff. digitifera* corals from southwestern hotspot forereefs were more diverse and more distinct from each-other compared to those hosted by their northeastern conspecifics from thermal refugium forereefs. This difference was largely driven by hotspot corals that hosted *Cladocopium* ITS2 profiles containing small relative abundances of C15 or C116 variants, which were more phylogenetically distinct from the typical C40-dominated profiles detected in corals in this study. These symbiont differences may reflect differential historical heat stress between these two regions, with thermal hotspots receiving on average an additional 2 °C-weeks during Palau’s marine heatwaves in 1998, 2010 and 2017. From 1985 to 2020, hotspots had only 3-4 years without heat stress accumulation, compared to 8-12 heat-stress free years at thermal refugia (Lachs et al., 2024b). However, due to spatial autocorrelation of hotspots and refugia on Palau’s southwestern and northeastern forereefs respectively, the effects of other regional environmental variables on *Acropora aff. digitifera*’s symbiont assemblages cannot be overlooked. Specifically, corals in the southern lagoon enclosed by Palau’s barrier reef system host Symbiodiniaceae distinct from their outer barrier reef conspecifics (Turnham et al., 2025), linked to the lagoon’s comparatively warm, acidic and turbid waters which wash over southern forereefs (including hotspot sites) during receding tides (van Woesik et al., 2012; Golbuu et al., 2016). Although symbiotic differences between hotspot

and refugium reefs cannot confidently be attributed to historical heat stress, hotspot reefs do characterise increased exposure to chronic environmental stressors compared to their northern refugium counterparts.

Contrary to expectations of environmental filtering of stress-sensitive symbiont taxa from hotspot reefs, no notable difference in the number of distinct ITS2 profiles was detected between hotspot and refugium reefs. Prior evidence of decreased richness or beta dispersion of Symbiodiniaceae taxa in corals, populations or communities comes from a variety of locations in response to recent heatwave exposure (Claar et al., 2020b; Jain et al., 2020; Quigley et al., 2022; Starko et al., 2023) or chronic exposure to unfavourable environmental conditions such as high turbidity, salinity, nitrogen, acidity or temperature variability (Fabricius et al., 2004; Ziegler et al., 2017; Hoadley et al., 2019; Smith et al., 2020; Kriefall et al., 2022; Davies et al., 2023; Gantt et al., 2024). In contrast, evidence of increases in Symbiodiniaceae diversity with environmental stress is seemingly limited to increased dispersion between assemblages with the incursion of *Durusdinium* under local anthropogenic stress (Claar et al., 2020a) and increased *Cladocopium* richness in *Pocillopora damicornis* on more thermally variable reef flats compared to neighbouring reef slopes (van Oppen et al., 2018). Indeed, increases in richness and heterogeneity with stress exposure are more characteristic of corals' bacterial microbiomes (McDevitt-Irwin et al., 2017), often attributed to opportunistic microbial colonisation in compromised hosts (Zaneveld et al., 2017).

5.5.2 Low-abundance symbiont variants associated with poor coral heat tolerance

A. aff. digitifera corals sourced from hotspot reefs that hosted different Symbiodiniaceae taxa displayed significantly different experimental heat tolerances. Two non-mutually exclusive

hypotheses may explain this result; functional differences between symbionts drove differences in holobiont heat tolerance, or certain symbiont taxa were hosted by corals with different heat tolerances without a direct effect of symbiont composition. Considering the increased symbiotic diversity detected in these hotspot corals, a potential mechanism for the latter hypothesis is a reduced host control over symbiont associations in corals weakened by prior heat stress exposure or other environmental stress, which then exhibited low heat tolerance (Carilli et al., 2010; Prada et al., 2017; Baum et al., 2023). Alternatively, the lack of correlation between heat tolerance and symbiont composition in refugium corals may reflect an interaction between chronic environmental stress and symbiont phenotype. Experimental evidence has revealed the degree of energetic mutualism or parasitism exhibited by Symbiodiniaceae *in hospite* to be dynamic in response to environmental change, with direct effects on host physiology (Baker et al., 2018; McIlroy et al., 2020), hence the same Symbiodiniaceae taxon may theoretically confer reduced host benefits to hotspot corals compared to their refugium conspecifics.

The use of the C15h sequence variant as an indicator for low heat tolerance allowed us to distinguish a group of related symbiont taxa (profiles) containing C15h (Figure S3), which were significantly associated with reduced coral heat tolerance. Notably, the difference of 0.8 °C-weeks in mean heat tolerance between hotspot corals hosting these different symbiont profiles is greater than the difference in mean heat tolerance between hotspot and refugium-sourced *A. aff. digitifera* corals (Lachs et al., 2024b). These C15h-containing symbiont profiles, whilst mostly within the C40 *Cladocopium* lineage, may have exhibited functional differences, based on prior evidence of variation in thermotolerance at these fine taxonomic scales, namely between taxa within *Cladocopium* C15 (Hoadley et al., 2021). The C40 variant was also identified as a potential indicator of heat tolerance, although its relative read abundance was often inversely

proportional to both that of C15h and to the presence of background ITS2 profiles. This raises the possibility of an emergent property of symbiont assemblages, such as ITS2 profile richness, being associated with holobiont heat tolerance (Kenkel & Bay, 2018; Howe-Kerr et al., 2020). Indeed, despite very low relative read abundances, symbionts hosted at background levels can have disproportionately large effects on holobiont phenotype (Ziegler et al., 2018a; Buzzoni et al., 2023).

5.5.3 Non-symbiotic correlates of coral heat tolerance in thermal refugia

Experimental heat tolerance of thermal refugium-sourced corals ranged from 3.8 to 12.5 °C-weeks, yet differences in the *Cladocopium* symbionts hosted were not linked to heat tolerance differences as they were for hotspot corals. A range of phenotypic differences between coral hosts can influence heat tolerance variability in holobionts (Kenkel et al., 2013; Drury, 2020), such as coral tissue biomass, which has been linked to increased heat stress survival in several coral species (Anthony et al., 2009; Thornhill et al., 2011; Huffmyer et al., 2021). The coral tissue biomass of replicate branches from the corals heat-stressed in this study were reported by Lachs et al. (2024b), revealing a correlation between increased tissue biomass and heat tolerance in refugium corals, with no discernible relationship in hotspot corals. Variation in tissue biomass could explain 5.3% of heat tolerance variation between refugium corals (Figure C6), which when considered alongside the 10% of variation explained by Symbiodiniaceae in hotspot corals reported here, leaves a large amount of unexplained heat tolerance variation in *Acropora aff. digitifera* corals from both regions. Some of this remaining variability may be linked to the additive genetic variance between hosts: the narrow-sense heritability of heat tolerance in *A. aff. digitifera* corals from the southwest of Palau's reef system has previously been estimated as 0.23

(23% of variation explained), following the same experimental heat stress procedure reported here (Humanes et al., 2024). The region-specific associations of symbiont assemblage and tissue biomass with heat tolerance in these *A. aff. digitifera* corals highlights the potentially transformative effect of local environmental history on the relationship between host, Symbiodiniaceae and heatwave performance (Howells et al., 2012; Drury & Lirman, 2021; Naugle et al., 2021), even within a single coral population.

As the world's coral reefs face intensifying marine heatwave exposure under accelerating climate change (Oliver et al., 2021; Cooley et al., 2022), we reveal aspects of coral-Symbiodiniaceae ecology likely to become increasingly relevant for the persistence of these ecosystems. Namely, coral populations that consistently host symbionts within one ancestral lineage of genus *Cladocopium* may still show a degree of symbiotic flexibility in response to environment change. Furthermore, symbiont differences at these fine taxonomic scales can be associated, whether causal or correlational, with definitive differences in tolerance and survival through marine heatwaves.

5.6 Figures

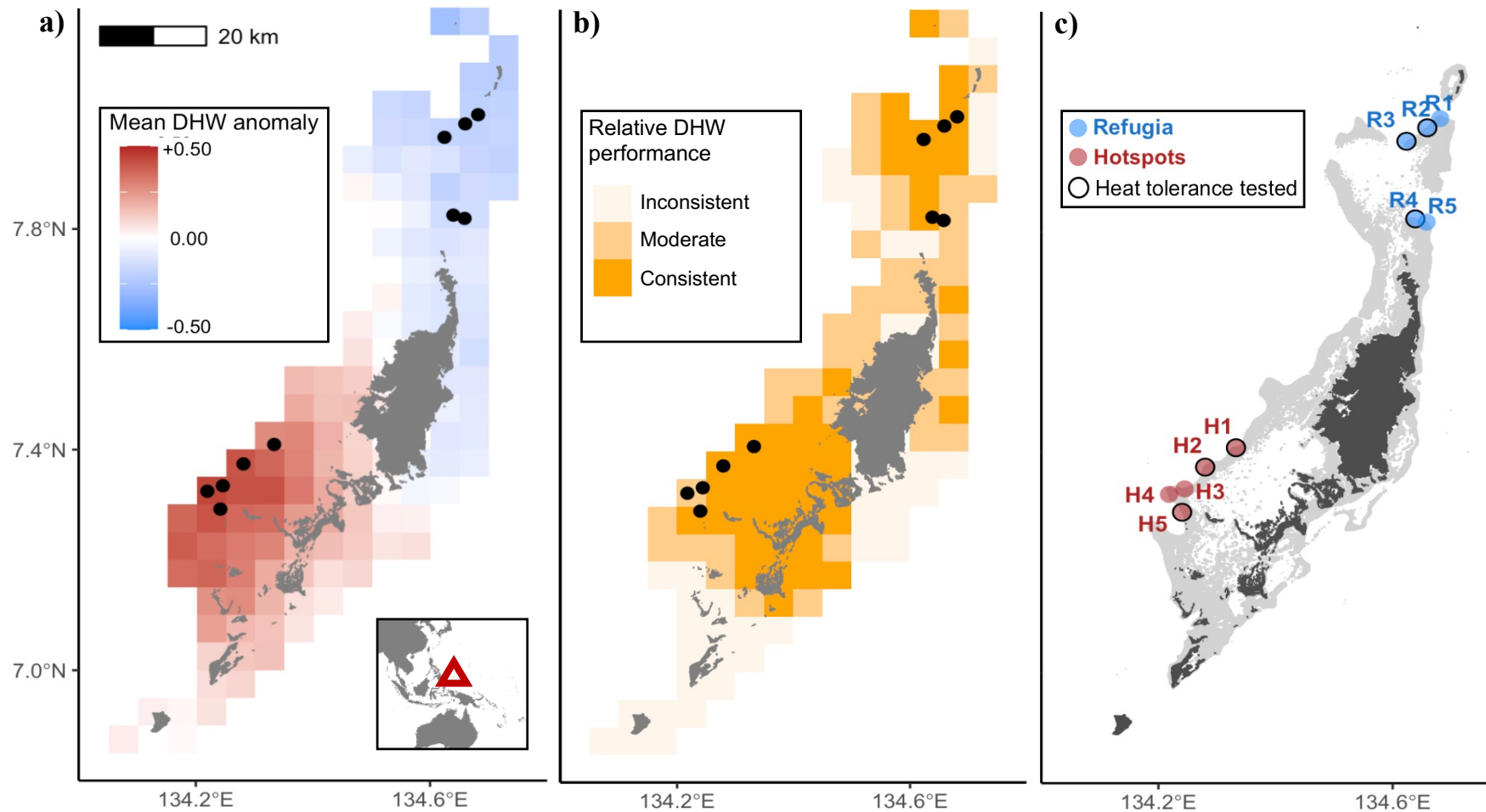


Figure 5.1: Sampling across Palau's gradient of historical heat stress, modified from Lachs et al (2024b). **a)** Coral collection sites are shown in black, against a map of reef pixels. Pixels are coloured according to mean annual Degree Heating Week anomaly between 1985 and 2020 above or below the average across Palau's reefs. **b)** Pixel shading indicates standard deviation of annual percentile rankings of reef pixels, such that lower values (darker shading) correspond to higher consistency in DHW ranking. **c)** Locations of the five thermal 'Refugia' reefs (R1-R5) and the five thermal 'Hotspots' (H1-H5) where *Acropora aff. digitifera* branches were collected. Replicate branches from three of the five sites in each region were subjected to an experimental heatwave in addition to symbiont characterisation (sites outlined in black). Dark grey areas represent land and light grey indicates reef extent (extracted from; Anderson, 2007).

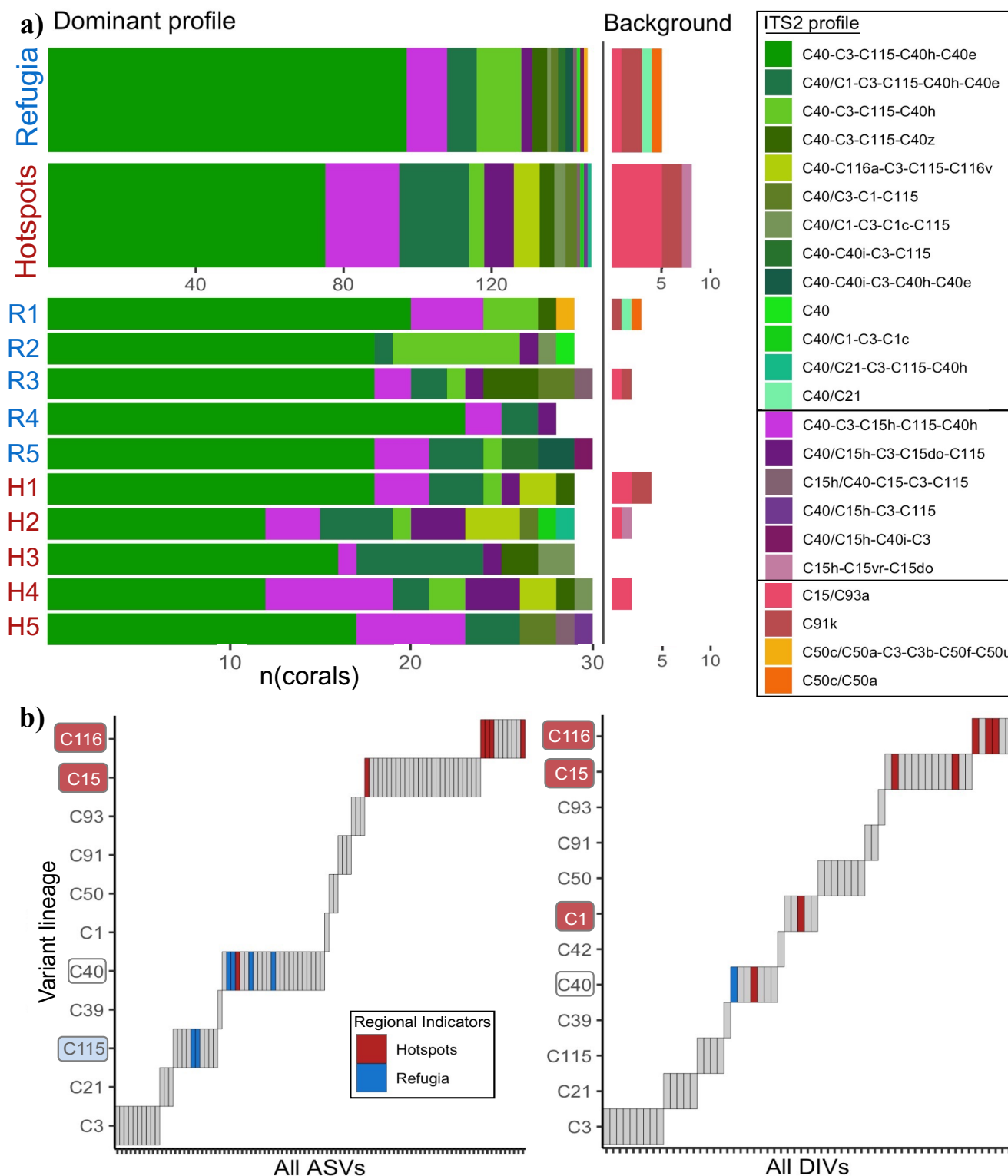


Figure 5.2: Hotspot and refugium symbionts characterised by their low-abundance variants. **a)** Number of corals hosting each *Cladocopium* ITS2 profile from hotspot reefs (n = 147) and thermal refugia (n = 146). The dominant profile and additional background profile (if present, comprising < 10 % of sample symbiont reads) are shown. Unique ITS2 profiles are represented by a colour scale, grouped in the figure

legend by those comprising mainly the C40 variant (top, green hues, C40 defining sequence proportions ≥ 0.5) and those containing the C15h variant (middle, purple hues), with additional profiles grouped below. Profiles are ordered within each group by decreasing occurrence in the dataset in the figure legend. **b)** Results of presence/absence indicator analysis, with symbiont sequence variants (ASVs or DIVs) represented by x-axis ticks (ordered by lineage). Colours represent sequence variants that are significant indicators of refugia (blue) or hotspot (red) reefs, otherwise variants are shown in grey. Lineages containing indicator variants are surrounded by borders and those containing only hotspot or only refugium indicators are shaded red or blue respectively.

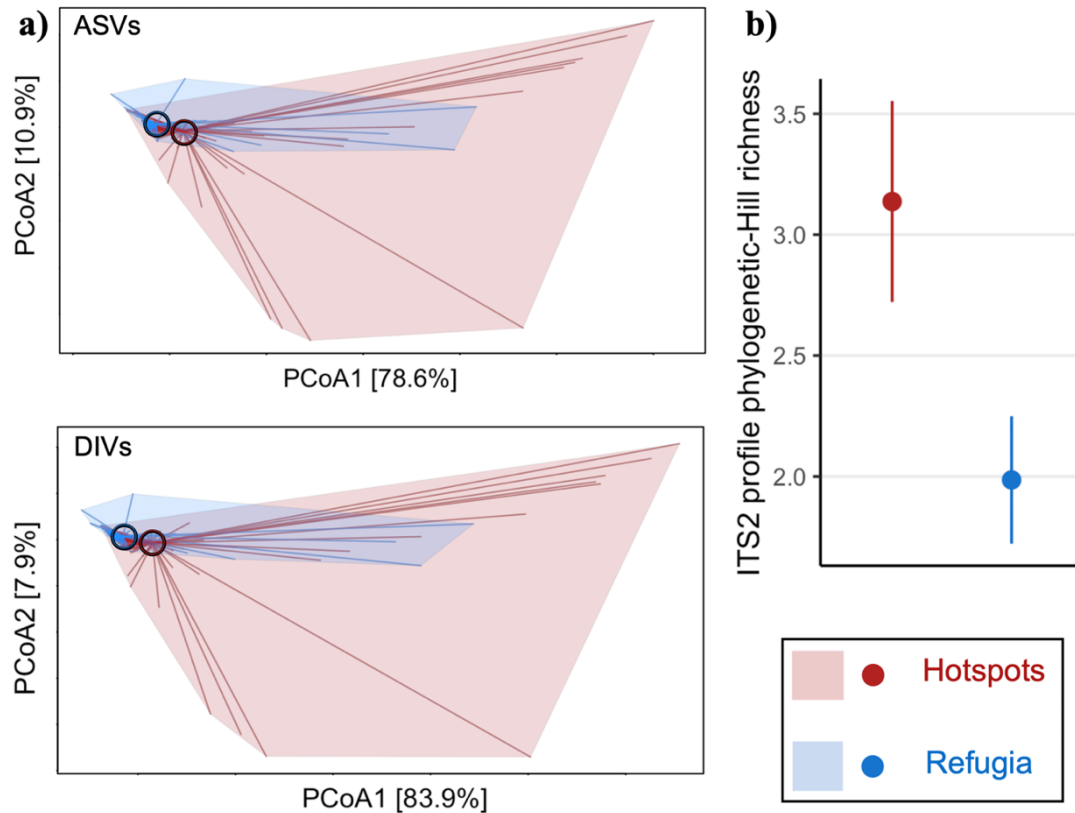


Figure 5.3: Increased symbiont diversity between and within hotspot corals. **a)** Principal coordinate analysis of weighted unifrac distances between symbiont sequence variants. Lines connect the multivariate locations of individual samples to their regional centroids (circled). Shaded red and blue polygons indicate the full multivariate expanse of samples in hotspot and refugium regions respectively. **b)** Within-sample alpha diversity of ITS2 profiles is summarised for each region (hotspot reefs in red, refugium reefs in blue) using phylogenetic-Hill richness (incorporating richness and summed phylogenetic tree branch lengths, then multiplied by 10^3 branch units for y-axis interpretability). Points and error bars indicate regional means $\pm$ 1 standard error.

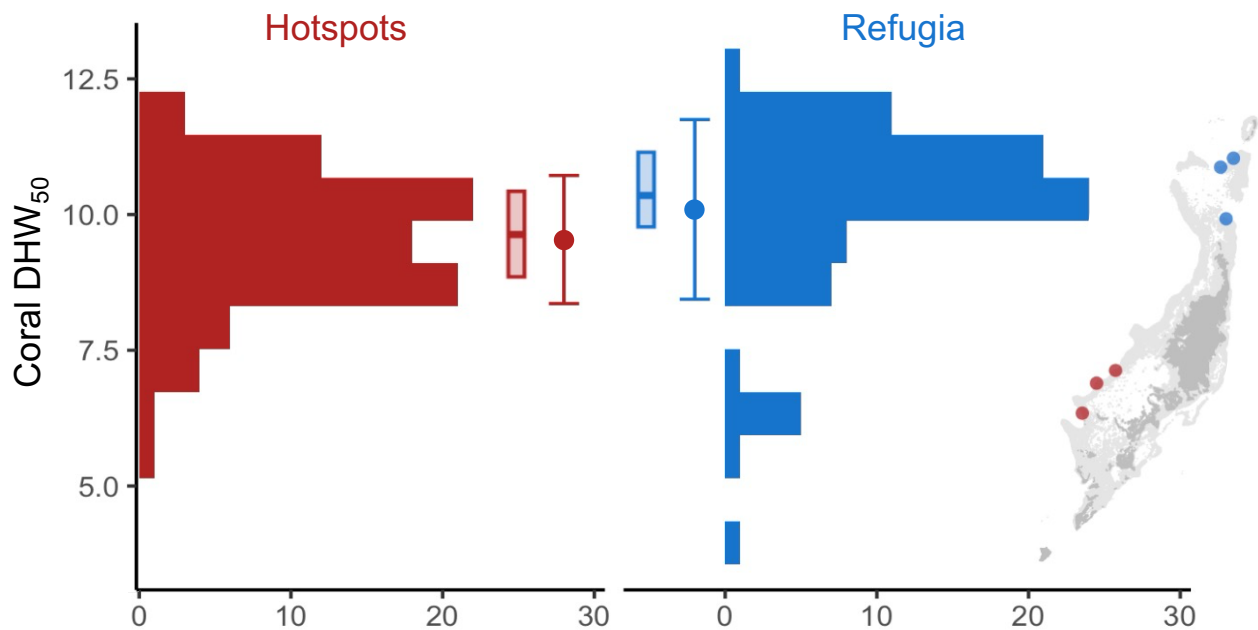


Figure 5.4: Higher mean, median and variance in heat tolerance among refugium corals. Modified from Lachs et al (2024). Histograms of DHW₅₀ for corals collected from three hotspot sites (n = 88) and three thermal refugia (n = 80), marked on map inset. Regional means $\pm$ 1 standard deviation are marked by circular points and error bars, whilst boxplots indicate 1st quartile, median and 3rd quartile for each region.

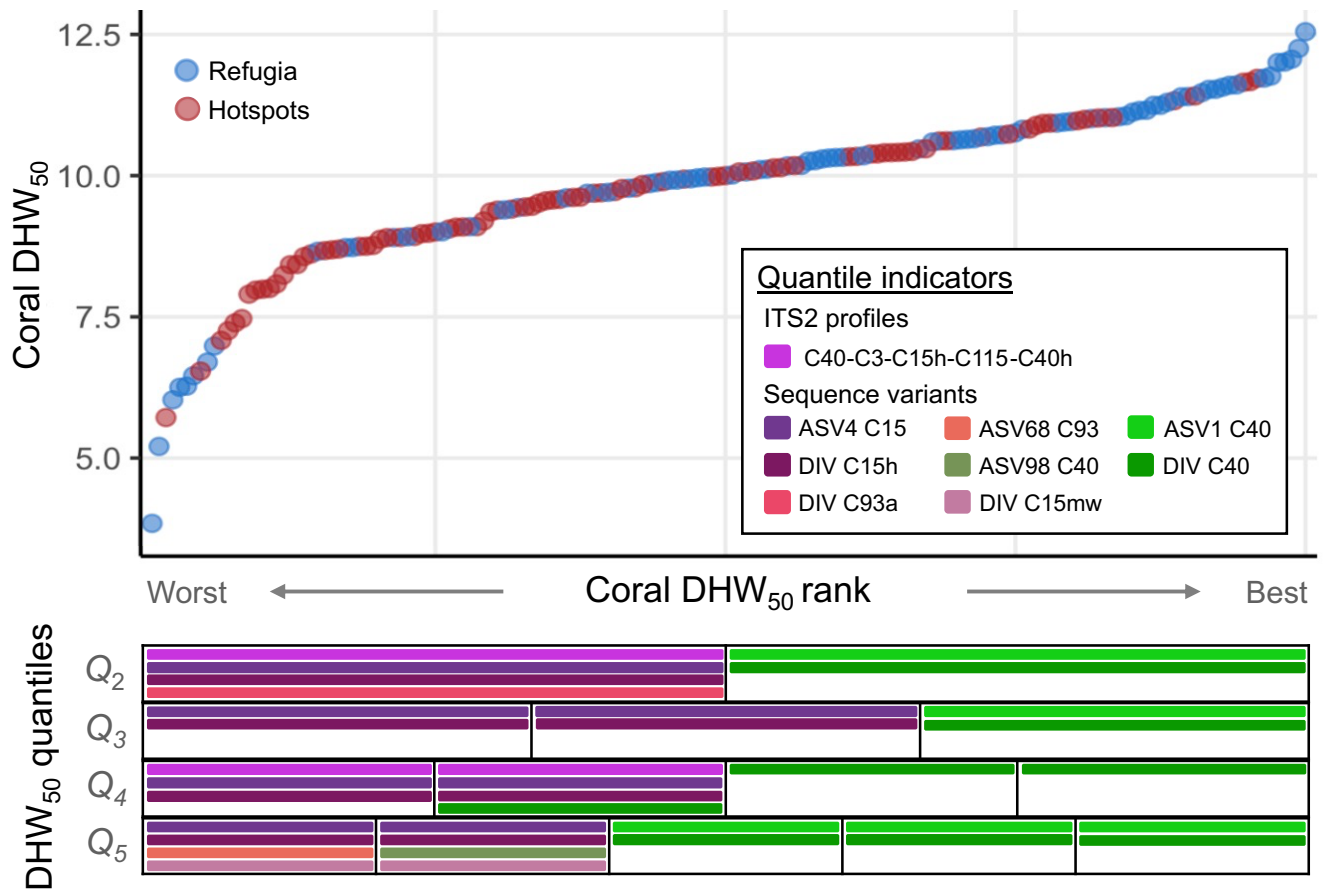


Figure 5.5: Low-abundance symbiont sequence variants as low heat tolerance indicators. Corals from the experimental heat stress ($n = 168$) are ranked by increasing DHW_{50} along the x-axis, with blue and red points representing refugium and hotspot corals respectively (top panel). Indicator analyses were performed separately for ASVs, DIVs and ITS2 profiles and were repeated with ranked corals divided into 2, 3, 4 or 5 quantiles (bottom panel). Significant indicators of quantiles are represented by coloured horizontal bars.

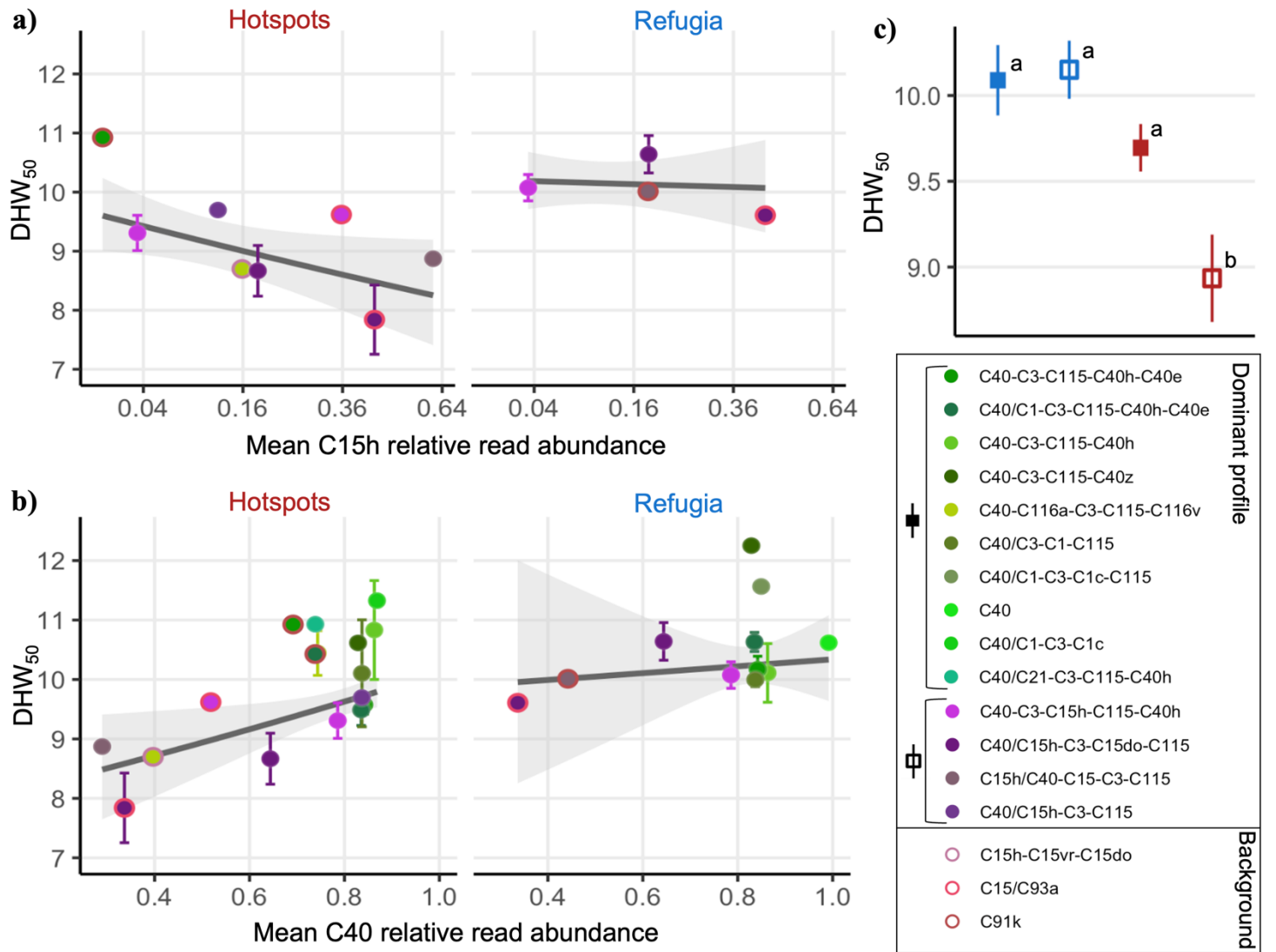


Figure 5.6: Symbiont indicators only predict heat tolerance at hotspot reefs. **a,b)** Points and error bars represent mean DHW₅₀ $\pm$ one standard error for each unique combination of dominant and background (if present) ITS2 profiles. Generalised linear models with Gamma error distributions are indicated by grey lines, surrounding shading shows $\pm$ 1 standard error around model predictions. **a)** Mean relative read abundance of DIV C15h in profile combinations (x-axis scale is square root transformed). Only profile combinations with non-zero C15h relative read abundances are shown (hotspots $n = 19$, refugia $n = 8$). **b)** Mean relative read abundance of DIV C40 in profile combinations (hotspots $n = 86$, refugia $n = 78$). **c)** Mean ($\pm$ 1 standard error) DHW₅₀ of refugia (blue) and hotspot (red) corals hosting dominant ITS2 profiles with the C15h variant (empty squares) or without the C15h variant (filled squares). Letter designations represent group differences gleaned from post-hoc Tukey's pairwise tests.

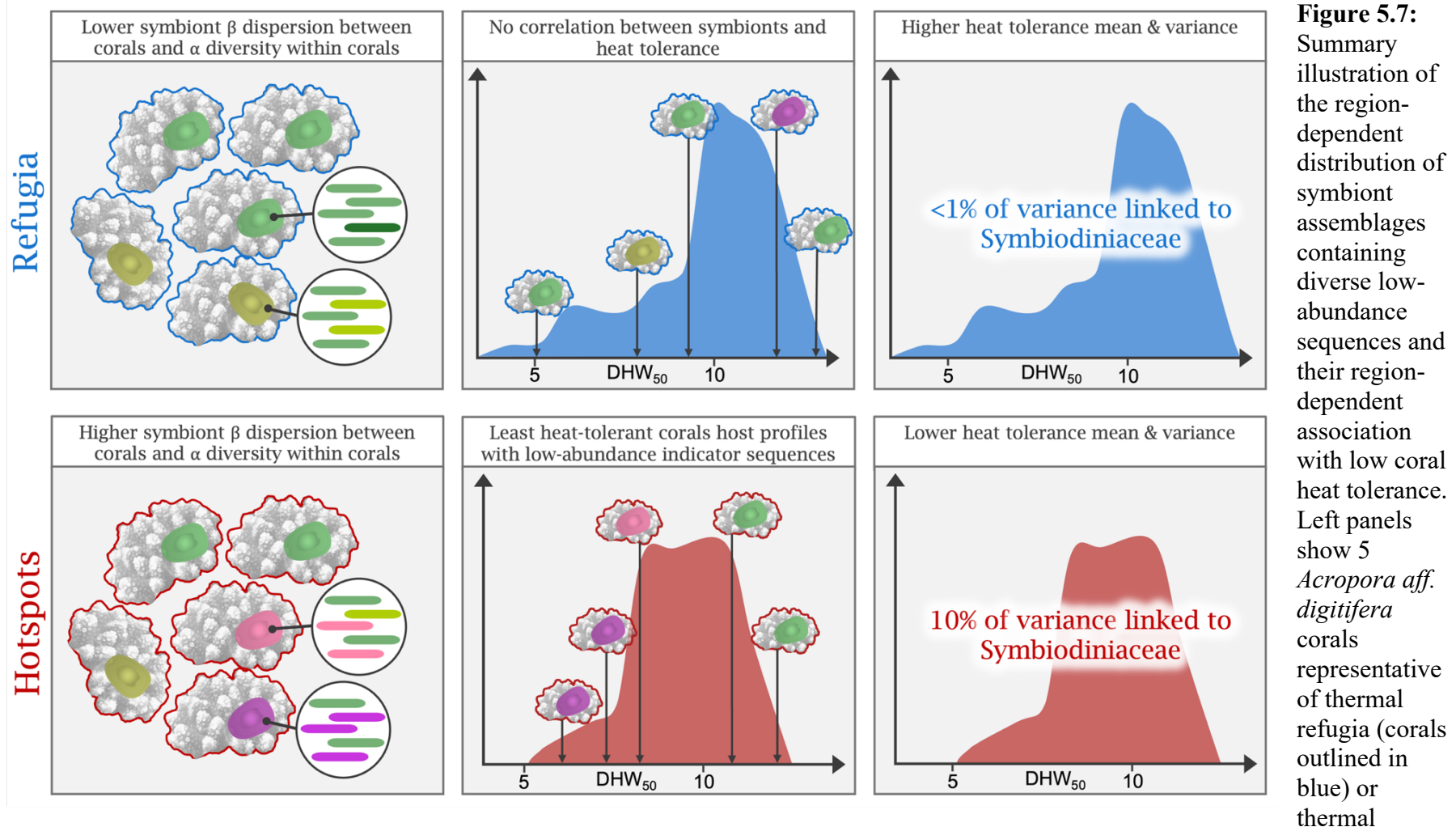


Figure 5.7: Summary illustration of the region-dependent distribution of symbiont assemblages containing diverse low-abundance sequences and their region-dependent association with low coral heat tolerance. Left panels show 5 *Acropora aff. digitifera* corals representative of thermal refugia (corals outlined in blue) or thermal

hotspot reefs (corals outlined in red) identified around Palau. Coloured ovals in corals represent Symbiodiniaceae, with those in refugium corals more similar to each other than those from hotspot corals. Magnified views of symbionts represent the sequence variants detected in symbiont taxa using DNA metabarcoding. Symbionts in hotspot-corals contained a greater diversity of low-abundance sequence variants (represented by pink/purple bars) in addition to the C40 variants (shades of green) that dominated this dataset. Middle and right panels show the approximate distributions of coral heat tolerance (DHW_{50}) through an experimental heatwave, with a higher mean and variance in heat tolerance for refugium corals. Middle panels are annotated to illustrate the relationship between indicator symbionts (pink/purple) and low heat tolerance among hotspot corals and the lack of association between heat tolerance and symbiont identity among refugium corals.

Chapter 6 – Conclusion

The intensification of marine heatwaves in the global tropics, together with a barrage of local anthropogenic impacts threaten the functioning and persistence of coral reef ecosystems this century (Gove et al., 2023; Quigley & Baird, 2024; Halpern et al., 2025). The obligate microalgal symbionts (Symbiodiniaceae) hosted by corals are major drivers of variation in the sensitivity of coral colonies and taxa to these global or local stressors, with corals' symbiont assemblages often undergoing compositional changes in response to disturbance (Silverstein et al., 2015; Palacio-Castro et al., 2023). Increasing understanding of the ongoing shifts in coral-algal symbioses is a crucial facet of predicting coral environmental sensitivity with a view to informing reef management and restoration (Logan et al., 2021; Shaver et al., 2022). To this end, this dissertation aimed to clarify the flexibility and persistence of coral-algal symbioses under chronic human disturbance, chronic heat stress and acute heatwave exposure, with wider relevance to other microbial symbiotic systems under climate change and multiple stressors.

Reviewing evidence of climate change-induced shifts in host-symbiont ecology across terrestrial and aquatic ecosystems (Chapter 2) revealed a pervasive paucity of long-term studies documenting the transformation, persistence, or reversion of microbial symbioses under the effects of climate change. However, it did reveal commonalities across ecosystems in their ongoing and predicted climate change-induced symbiotic shifts, including an increased dominance of generalist symbiont taxa, a vulnerability of hosts to symbiotic parasitism and invasions by novel or opportunistic symbionts following disturbance-induced dysbiosis. Population-level comparisons of symbiont-host associations spanning more than 5 years in natural populations remain rare (Baker et al., 2004, 2013; Riegler et al., 2005; Palacio-Castro et

al., 2023) and time series of at least 5 years that have tracked symbiont community composition within individual hosts have seemingly been limited to sample sizes under 60 (Edmunds et al., 2014; Lewis et al., 2024). Insights from the 5-year population-level tracking of symbiont assemblages in 973 corals and 2-5 year time series within 121 colonies from Chapter 3, together with population-level sampling of 598 corals spanning nearly a decade and 2-7 year time series within 107 colonies from Chapter 4, are therefore of particular value. Incursions of generalist and putatively opportunistic symbiont taxa with climate change-driven warming have been observed or predicted in systems from the bacteriomes inside mangrove leaves to the ectomycorrhizae of temperate forest root-systems (Markkola et al., 2016; Nethmini et al., 2025), foreshadowing analogous conclusions from subsequent chapters in this dissertation.

Results from Chapter 3 were consistent with this expectation of a climate change-induced shifts towards host-generalist symbiont taxa. I investigated whether corals with horizontal and vertical symbiont inheritance differ in their temporal and spatial symbiont flexibility upon exposure to both acute heat stress and/or chronic human disturbance. Previous studies have revealed a higher diversity of Symbiodiniaceae associations in coral taxa that employ horizontal symbiont acquisition, more strongly associated with historical environmental conditions compared to taxa with vertical symbiont inheritance (Marzoni et al., 2024; Zarate et al., 2024). However, understanding of changes in coral-Symbiodiniaceae associations under exposure to multiple stressors was seemingly limited to 3 coral taxa on Kiritimati Island (Claar et al., 2020b; Starko et al., 2023), with no prior studies addressing multiple stressor effects on coral-Symbiodiniaceae associations across corals with different reproductive life histories. I uncovered greater flexibility in the Symbiodiniaceae genera and lineages hosted by coral species with horizontal symbiont-inheritance compared to those with vertical inheritance, over 5 years under

exposure to multiple stressors. Unexpectedly, I found the likelihood of a heatwave-induced change in symbionts increased with human disturbance in vertical-inheritance corals but decreased with human disturbance in horizontal-inheritance corals, reflecting a saturating response towards the dominance of generalist genus *Durusdinium*.

Then, using time series data spanning a decade from one coral species (Chapter 4), I tested whether exposure to chronic human disturbance affected the post-heatwave rate and trajectory of reversion from heatwave-transformed symbiont assemblages. I uncovered an association between differential local human disturbance exposure and the succession of recovered symbiont assemblages through intermediary states, providing field evidence of the environmentally-mediated competition dynamics between Symbiodiniaceae genera observed *ex situ* (McIlroy et al., 2019). *Durusdinium* persisted for more than 7 years post-heatwave in contrast to previous studies that have observed reversion to pre-disturbance symbiont assemblages within a few years (Thornhill et al., 2006b; LaJeunesse et al., 2009b; Keshavmurthy et al., 2020), revealing a path of post-heatwave symbiont succession distinct from trajectories observed following milder acute disturbance exposure. This chapter demonstrated that a single extreme acute heat stress exposure can disrupt long-term reversion from stress-tolerant generalist symbiont assemblages following bleaching recovery, analogous to the proposed persistent transformations of coral-symbiont associations following multiple successive heatwaves (Baker et al., 2017).

Finally, in a coral species that forms more specific associations with one lineage within genus *Cladocopium* (Chapter 5), I tested for fine-scale differences in Symbiodiniaceae taxa hosted by corals from reefs exposed to different amounts of historical heat stress over the 20 years prior. I found an increased diversity of symbionts within and between corals at reefs

exposed to greater historical heat stress compared to nearby reefs, in contrast to prior reports of decreased diversity in associations within genus *Cladocopium* following heat stress (Kriefall et al., 2022; Starko et al., 2023; Leiva et al., 2025). By employing indicator species analysis, a method only recently first utilised in Symbiodiniaceae research (Chen et al., 2024a; Klein et al., 2024; Villela et al., 2024), I deduced that the symbionts responsible for this increased diversity were associated with reduced bleaching resilience, but only in corals from those reefs exposed to more chronic heat stress. This result supports previous work showing decreased bleaching resilience in corals exposed to chronic stress (Carilli et al., 2010; Prada et al., 2017; Baum et al., 2023) and may also reflect differences in Symbiodiniaceae performance within coral hosts in response following environmental changes (Baker et al., 2018; McIlroy et al., 2020).

Taken together, trends emerge in the findings from chapters in this dissertation. Firstly, Chapters 3, 4 and 5 all revealed significant effects of local environmental conditions (at the reefal, rather than regional or global scale) on the stability of coral-Symbiodiniaceae associations: From the flexibility and post-heatwave dynamism of symbioses under local human disturbance in Kiritimati, to the incursion of diverse cogeneric symbionts on more chronically-heat stressed reefs in Palau and the local context of their links to bleaching resilience. Similarly, in the 6 coral species investigated on Kiritimati, heatwave exposure and human disturbance both drove shifts in corals' assemblages from genus *Cladocopium* towards generalist and putatively opportunistic genera *Symbiodinium* and *Durusdinium* (Stat et al., 2008b; Jones & Berkelmans, 2010), with *Durusdinium* still dominating the assemblages of one of these species almost 8 years post-heatwave. The *Cladocopium* C40-lineage symbionts containing the C15h sequence variant, detected in Palauan *Acropora digitifera* from thermal hotspots, whilst not necessarily generalists in their associations with coral hosts, could be characterised as opportunists based on their

incursion in chronically stressed corals and their links to poor host heatwave tolerance. One notable contrast between chapter results lies in the heatwave-induced loss of *Cladocopium* diversity at the lineage and profile scale accompanied by a shift towards C15 and C116 symbionts in Kiritimati's symbiotically flexible corals, compared to the increased *Cladocopium* C40 diversity at the profile and sequence variant scale in chronically stressed *Acropora digitifera*, reflecting differences in responses to disturbance between symbiont taxonomic scales.

Limitations

Despite these advances, several limitations in the design and methodology of this research constrained the conclusions drawn. Firstly, spatial autocorrelation between human disturbance and other environmental variables on Kiritimati's reefs, and between reef location and historical heat stress in Palau, limited my capacity to estimate individual variable effects on coral-symbiont associations. Additionally, the minimal heatwave survival of *Pocillopora grandis* on Kiritimati reduced the applicability of chapter 3 results to other vertical-inheritance corals, as vertical-inheritance coral results were based mainly on *Porites lobata*. However, results from *Pocillopora* spp. across the Tropical Pacific have revealed inflexible associations of host species and phylotypes to specific *Cladocopium* lineages, with increased spatial flexibility in populations exposed to higher historical temperatures (Armstrong et al., 2023), analogous to results from *Acropora digitifera* reported in Chapter 5. Furthermore, classification of coral species with predominantly vertical or horizontal symbiont inheritance in Chapter 3 was based on the respective presence or absence of symbionts in larvae (Baird et al., 2009; Hartmann et al., 2017). However, a variety of coral taxa may in fact employ mixed (horizontal and vertical) modes of

symbiont transmission (Quigley et al., 2018a, 2018b), prompting the need for further clarification of corals' symbiont inheritance modes to better understand associated symbiotic flexibility.

A major methodological limitation of results presented in this dissertation is rooted in the choice of the ITS2 region for sequencing and characterising Symbiodiniaceae taxa. This multi-copy target is widely used to characterise Symbiodiniaceae, enhancing comparability between studies, but introduces uncertainty in differentiating intra- and inter-genomic variation in symbiont assemblages. The use of the ITS2 marker in tandem with an additional marker such as the hyper-variable non-coding region of the *psbA* minicircle, can help to differentiate Symbiodiniaceae taxa whilst providing increased resolution of symbiont genotypes (LaJeunesse and Thornhill 2011). In addition to metagenomic sequencing of symbiont assemblages, the use of quantitative PCR with genus-specific targets can add quantitative information to the composition of corals Symbiodiniaceae assemblages and has previously provided valuable insights into disturbance-induced fluctuations in Symbiodiniaceae relative abundances over time (McIlroy et al., 2019).

Future directions

Ex situ time series experiments have previously revealed the rate and trajectories of competitive dominance or competitive exclusion between Symbiodiniaceae in genera *Symbiodinium*, *Breviolum* and *Durusdinium* in a Cnidarian model system (McIlroy et al., 2019): Specifically, this revealed the reversal of symbiont competitive hierarchies in response to environmental light availability. Given the challenges associated with obtaining long-term time series of coral-

Symbiodiniaceae symbioses from natural reef systems under intensifying climate change, additional *ex situ* time series could prove valuable in elucidating the rates and trajectories of symbiont assemblage compositional changes in response to combinations of chronic human disturbance, chronic heat stress and acute heatwave exposure. Further research into the persistence of disturbance-transformed coral-algal symbioses may also benefit from ongoing insights into other symbiotic systems. In particular, the associations between vascular plants and mycorrhizal fungi resembles the coral-Symbiodiniaceae system as an interaction between two eukaryotic partners, with low-diversity symbiont assemblages compared to those of bacterial microbiomes. Whilst arbuscular mycorrhizal (AM) hosts are largely distributed across the tropics and subtropics, and ectomycorrhizal (EM) hosts across temperate regions, transition zones exist where hosts associate with both host-generalist AM symbionts and host-specialist EM symbionts (Rog et al., 2025). The dynamics between these symbiotic partners in response to disturbance may inspire parallels with dynamics between specialist *Cladocopium* taxa and generalist *Symbiodinium* and *Durusdinium* on coral reefs.

Reef managers and coral conservationists may benefit from results reported here of post-disturbance incursions of putatively opportunist taxa in genus *Symbiodinium*, warning of the vulnerability of disturbed Symbiodiniaceae assemblages to invasion. Such invasions may threaten the post-heatwave recovery of coral colonies and reefs, exemplified by recent novel detections of a Caribbean putative necrophile of dead coral tissue, *Symbiodinium necroappetens* (A13, (LaJeunesse et al., 2015)), on Brazilian coral reefs following extreme heat stress, (Villela et al., 2025). Predictive models of coral reef persistence this century have previously been improved by accounting for increased bleaching resilience afforded to corals through bleaching-induced shifts towards hosting *Durusdinium* (Logan et al., 2021). Results from this dissertation

demonstrate the increasing persistence of heatwave-transformed symbioses and homogenisation of Symbiodiniaceae assemblages in symbiotically flexible coral species under acute and chronic disturbance, showing limited additional capacity for increased resilience via symbiont assemblage changes in these corals. On the other hand, predictive models may be improved by considering shifts between symbionts at fine taxonomic scales within genus *Cladocopium* in those coral taxa with greater fidelity to *Cladocopium*, given the associated differences in heatwave tolerance reported here in *Acropora digitifera* corals. These findings are applicable to symbiont-informed predictions of heatwave vulnerability and in mapping the distribution of ‘at-risk’ reefs (Fuller et al., 2020), a useful tool in the prioritisation of reef management efforts (McLeod et al., 2021). Furthermore, our results demonstrate the potential for using symbiont metabarcoding to detect holobionts with low heat tolerance within a population, even in coral taxa associated with low Symbiodiniaceae diversity. Similar approaches could be incorporated into reef restoration to screen for potentially maladaptive indicator symbionts, thereby avoiding propagating them through coral nurseries and onto restored reefs (Baums et al., 2019).

In chapters 3, 4 and 5, I have demonstrated that legacy effects of acute or chronic disturbance can be detected in corals’ Symbiodiniaceae assemblages across timespans of 5, 10 and 20 years, respectively. Transformations of corals’ Symbiodiniaceae assemblages, driven by both chronic and acute stressors, mirror rapid global increases in species turnover as these interacting stressors increase in frequency and intensity (Pinsky et al., 2025). Results from coral reefs may also foreshadow the extended persistence of disturbance-transformed states in other microbial symbiotic systems (Hernandez et al., 2021; Radujković et al., 2025), threatening ecosystem function (Cavicchioli et al., 2019). This dissertation offers unique insights into the endurance of

disturbance-transformed microbial communities and underscores the value of these critically vulnerable coral reefs as a globally relevant model for understanding how climate extremes impact the resilience of symbiotic species.

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Appendices

Appendix A: Chapter 3 Supplemental Materials

Sites: clockwise from south-facing shore				N(samples)					
Disturbance category	Human population 2km	Fishing pressure 10km	Total disturbance	Dipsastraea	Favites	Hydnophora	Platygyra	Pocillopora	Porites
Low	0	0	0	6	3		15	1	19
Low	0	0	0	8	3	6	27	13	38
Medium	351	809	1160	2	4	4	6	12	10
Medium	0	1213	1213	9	8	13	19	20	30
Medium	0	1213	1213	23	4	8	19	19	38
Medium	0	1213	1213	13	10	11	21	18	27
Low	0	809	809						8
Medium	0	1213	1213						8
High	0	2425	2425	3	8	3	13	1	14
High	1223	3638	4861	6	20	5	11	19	10
High	1691	3638	5329	2	2	2	2	6	
High	4042	3234	7276	9	13	8	14	15	19
High	3065	2021	5086	17	8	7	8	18	27
Medium	0	1617	1617	8	7	5	17	11	17
Low	0	809	809	8	5	6	17	14	21
Low	0	809	809	6	5	5	1	13	15
Low	0	0	0	2	2		1		4
Low	0	0	0	2	19	12	7	14	26
Low	0	0	0		2	5		12	12

Table A1: The total number of coral samples collected and successfully sequenced from each of 19 sampling sites around Kiritimati, partitioned by coral genus. Sites are listed in clockwise order starting from Kiritimati's south-facing shore, as per Figure 3.1. Total disturbance represents estimated site-level human disturbance exposure (collapsed into categories 'low', 'medium' and 'high'), calculated as the sum of the human population within 2 km and a kernel density function representing estimated fishing pressure within 10 km or each site.

Expedition →	Aug 2014 (n=187)			Jan 2015 (n=25)			May 2015 (n=35)			Jul 2015 (n=358)		
Disturbance →	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High
Dipsastraea	1	13	5		5			2	4	15	21	14
Favites		6	4					1	1	15	10	16
Hydnophora	2	17	5							24	14	7
Platygyra	2	10	7					2	1	14	11	11
Pocillopora	12	36	12	2	3	5		4	5	49	33	19
Porites	10	35	10	2	3	5	4	7	4	38	29	18

Expedition →	Mar 2016 (n=184)			Nov 2016 (n=53)			Jul 2017 (n=149)			Jul 2018 (n=50)			Jul 2019 (n=53)		
Disturbance →	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High	Low	Med	High
Dipsastraea	4	9	8	3			7	3	3	1			1	2	3
Favites	6	5	13	3		2	10	7	2	4	3	5	1	1	8
Hydnophora	5	7	6	1	1	2	1	1	1		1	2	1		2
Platygyra	17	14	9	9	3	3	13	31	13	7	8	2	6	3	2
Pocillopora			5				1	1	3	2	1	3	1	2	7
Porites	39	20	17	15	6	5	24	21	7	6	4	1	5	5	3

Table A2: The total number of coral samples collected and successfully sequenced from each expedition before peak heatwave exposure (August 2014 - July 2015) and after peak heatwave exposure (March 2016 – July 2019), partitioned by site-level human disturbance category and coral genus.



Figure A1: Neighbour-joining tree showing estimated Unifrac distances between *Cladocopium* ITS2 profiles obtained from Symportal (based on sequence dissimilarities between profiles' composite defining intragenomic variants). ITS2 profiles were assigned to *Cladocopium* lineages (represented by bold labels and coloured bars) based on the clustering of profiles in this tree, supported by the dominant sequence comprising profiles (indicated by profile names).

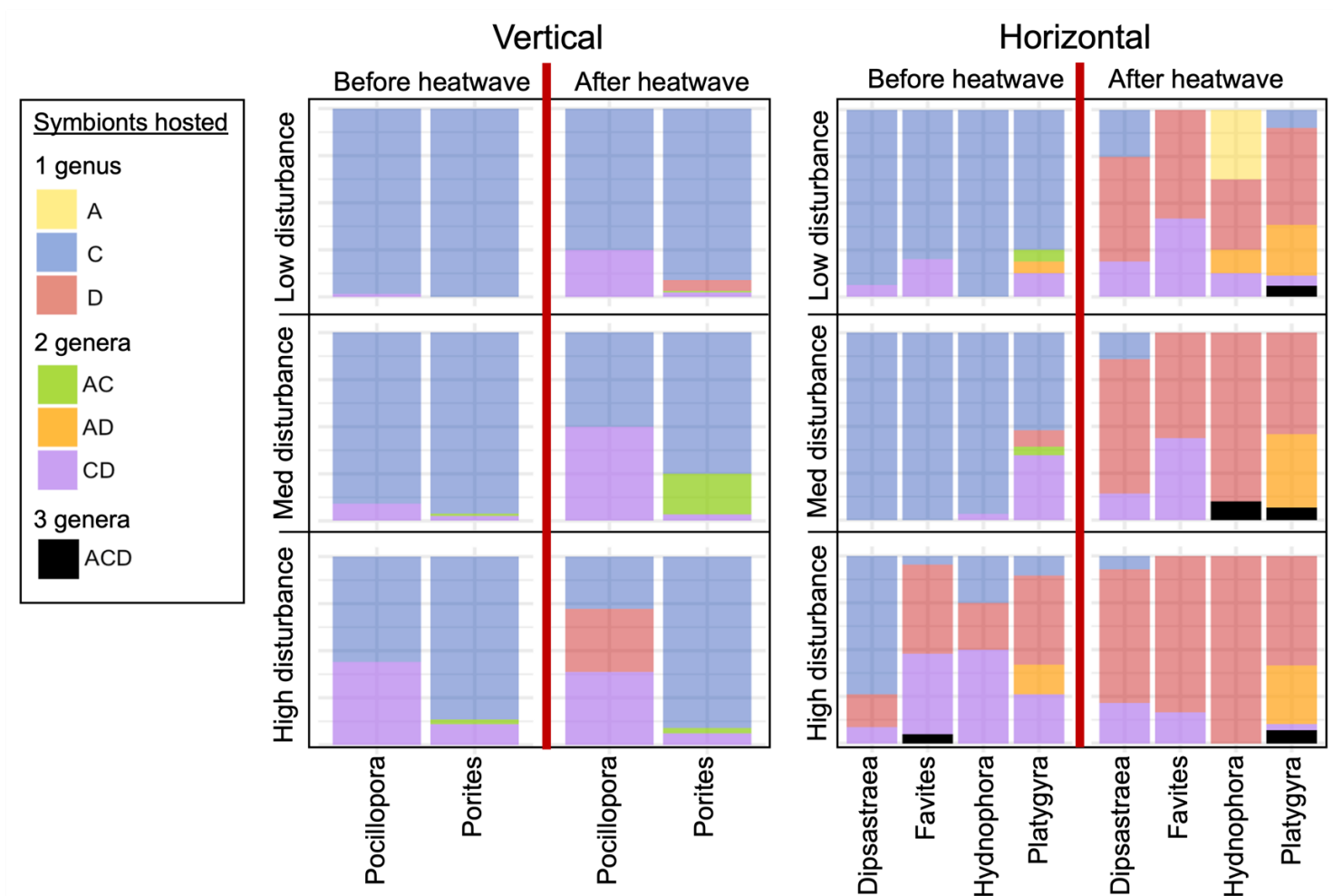


Figure A2: Symbiodiniaceae genera detected in coral genera with vertical (left) or horizontal (right) symbiont inheritance, before or after peak heatwave exposure and from sites exposed to low, medium or high human disturbance (total n = 1,094 samples). Coloured areas represent the proportion of corals in each group found to host each combination of symbiont genera *Symbiodinium* ('A'), *Cladocopium* ('C') and *Durisdinium* ('D').

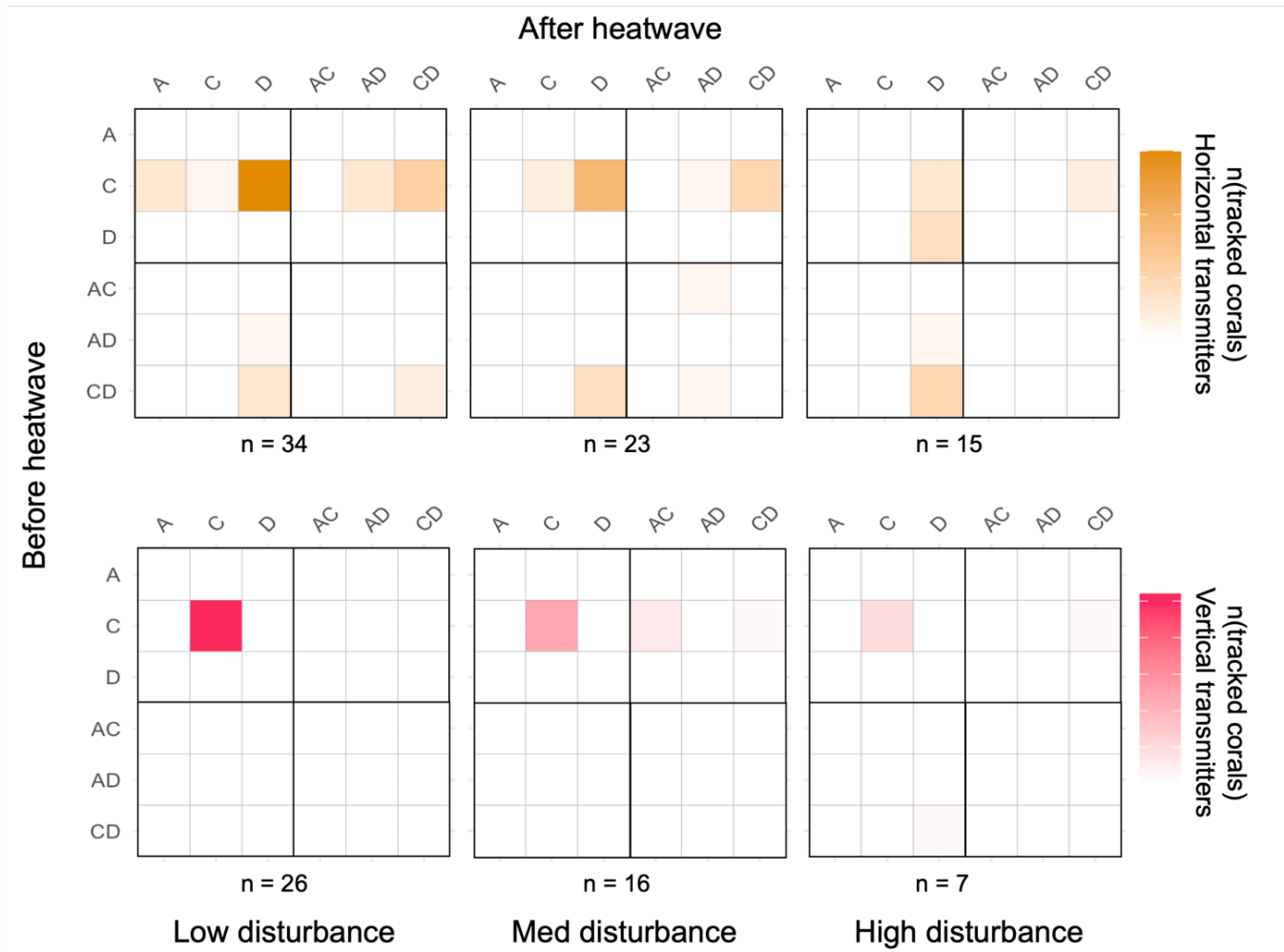


Figure A3: Symbiodiniaceae genera hosted by each tracked coral with horizontal (n = 72) or vertical (n = 49) symbiont inheritance before and after peak heatwave exposure. Grid squares rows and columns represent each distinct combination of symbiont genera hosted by tracked corals.

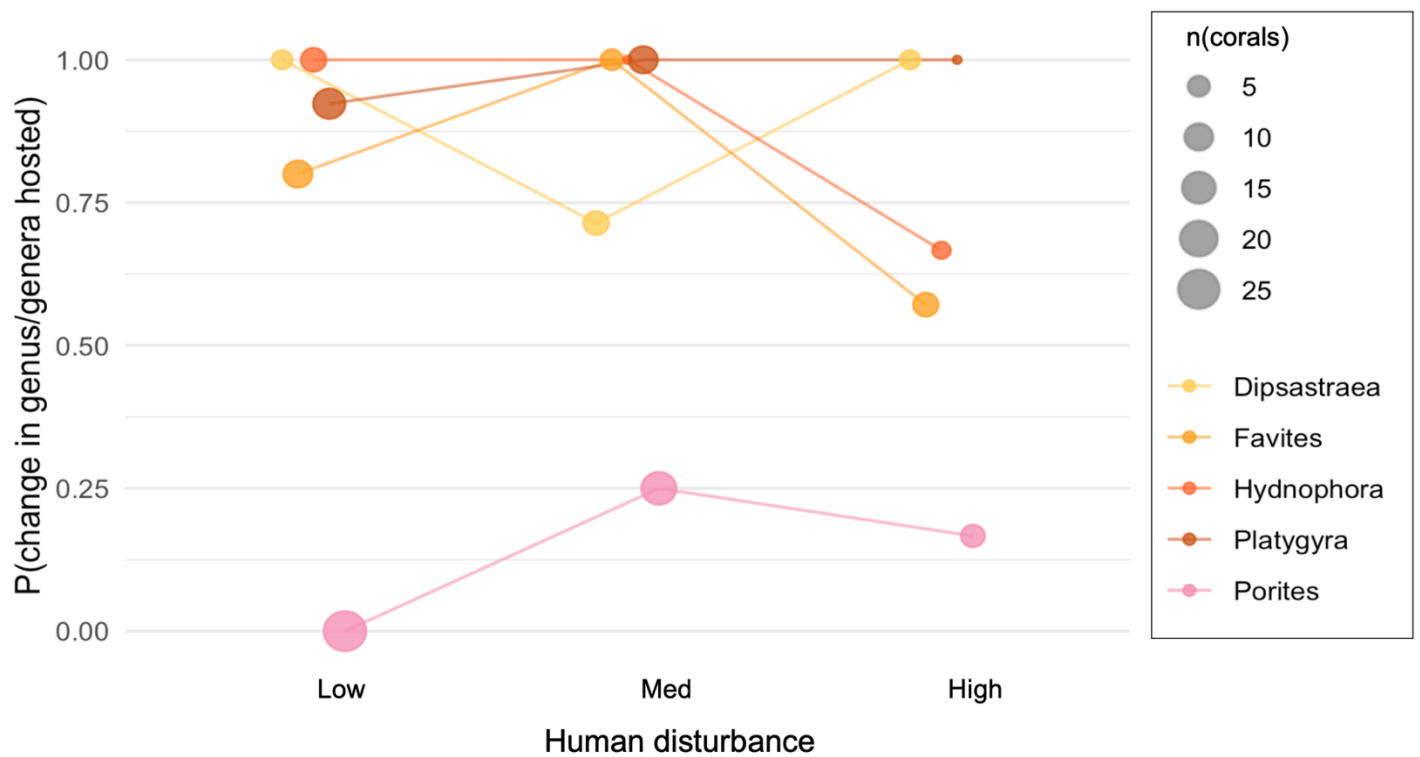
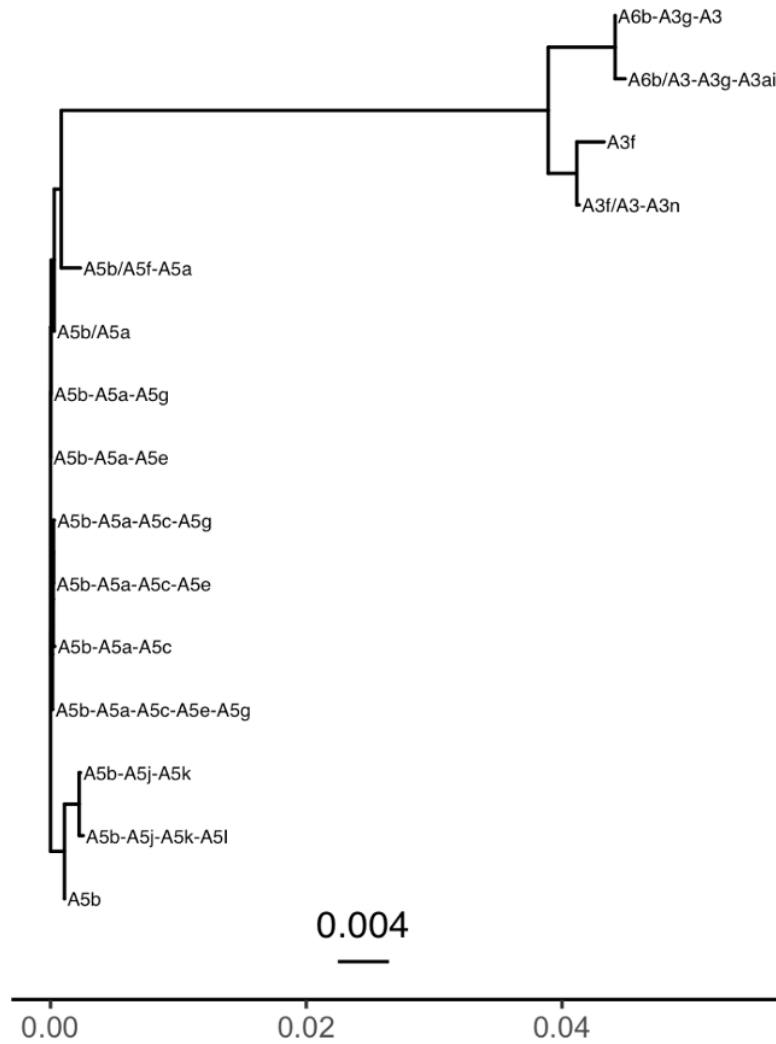


Figure A4: Probability of a change in the combination of Symbiodiniaceae genera hosted by a tracked coral from before peak heatwave exposure to after peak heatwave exposure. Points represent means of a binary variable of change per human disturbance category for each coral genus, coloured by coral genus. Lines connect points for each coral genus and point sizes reflect the number of tracked corals from which each mean was calculated. In total, 72 tracked corals with horizontal symbiont inheritance and 48 with vertical symbiont inheritance are represented here (one tracked *Pocillopora* coral is not shown here).

a)

Before heatwave

After heatwave

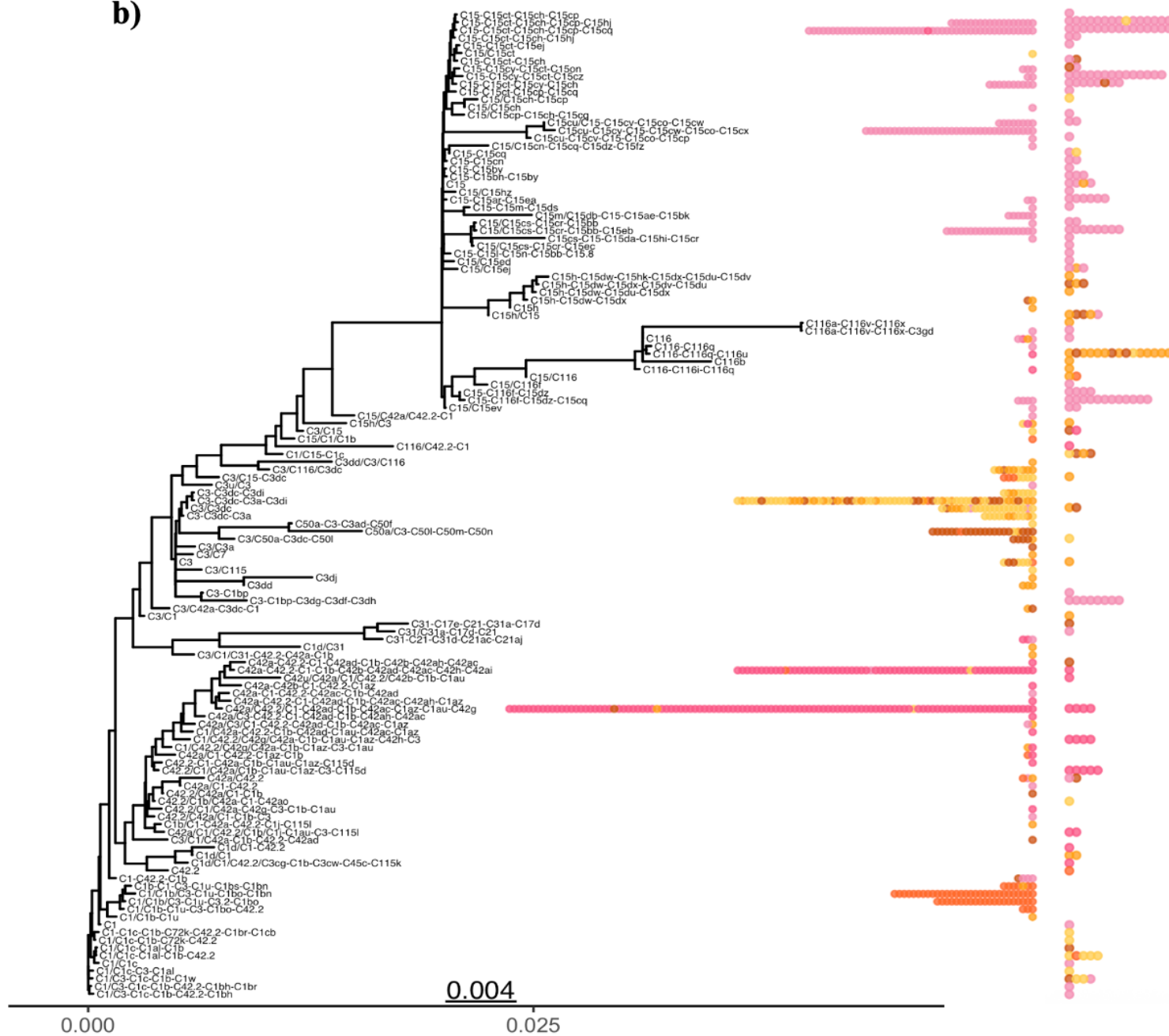


- Dipsastraea
- Favites
- Hydnothra
- Platygryra
- Pocillopora
- Porites

b)

Before heatwave After heatwave

- Dipsastraea
- Favites
- Hydnoophora
- Platygyra
- Pocillopora
- Porites



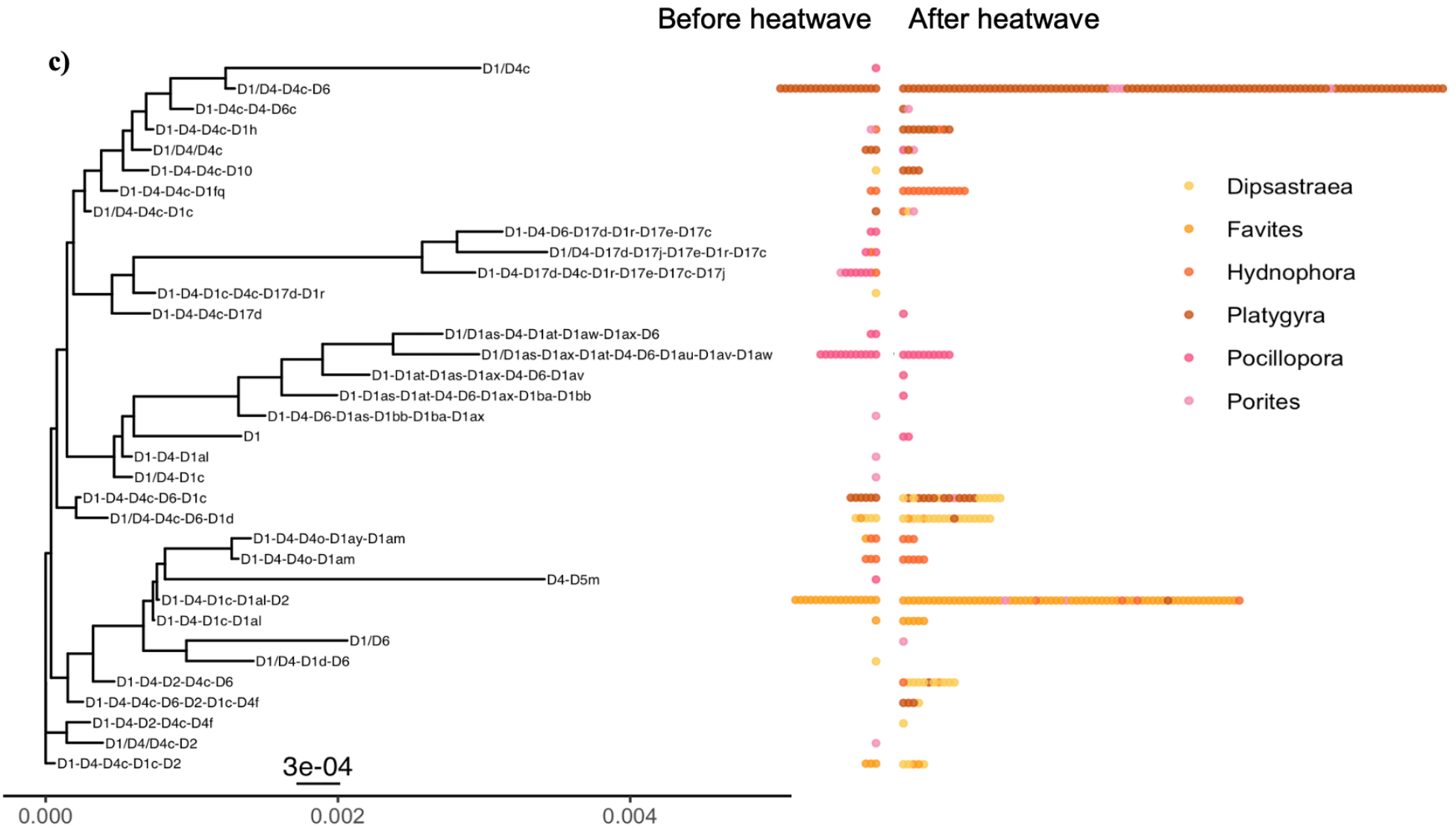


Figure A5: Neighbour-joining trees showing Unifrac distances between symbiont ITS2 profiles within **a)** genus *Symbiodinium*, **b)** genus *Cladocopium* and **c)** genus *Durusdinium*. Between-profile Unifrac distances are calculated within the Symportal algorithm based on sequence dissimilarities between profiles' composite DIVs (defining intragenomic variants). Branch lengths are scaled to Unifrac distances (shown by scale below trees). Points shown to the right of profile trees represent samples (coloured by coral genus) containing the profile from before and after peak heatwave exposure.

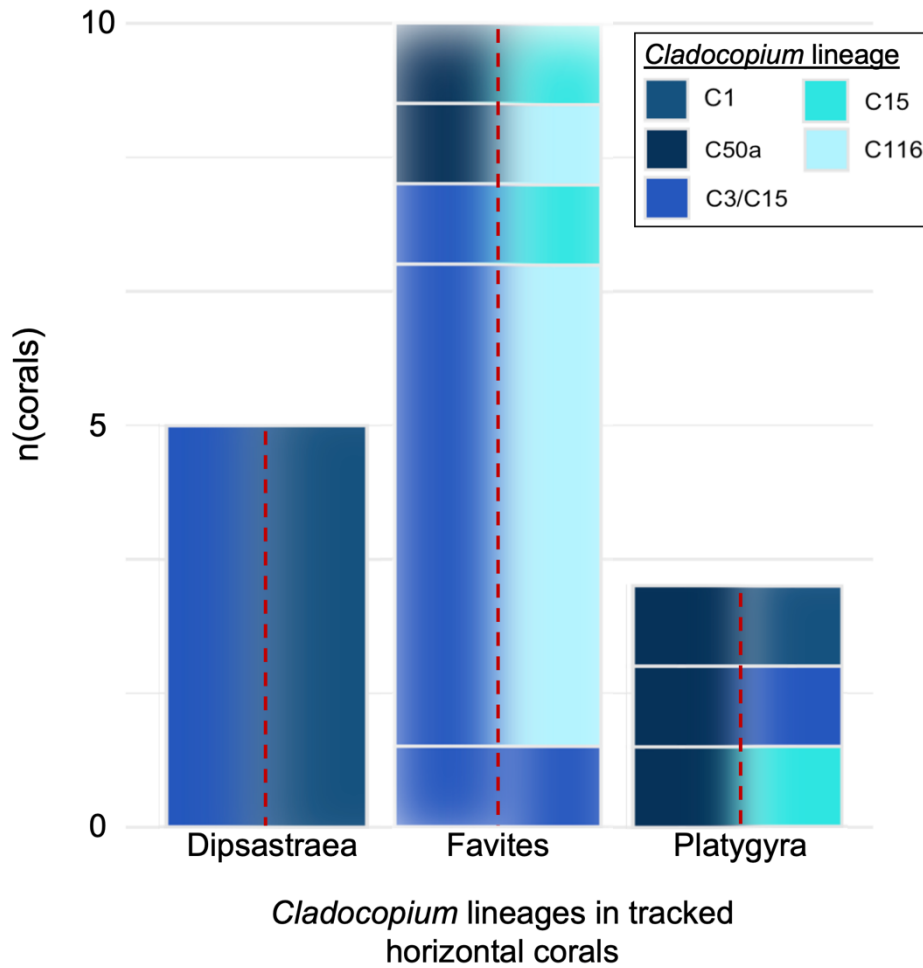


Figure A6: The lineages of *Cladocopium* profiles hosted by the subset of tracked horizontal corals hosting *Cladocopium* both before and after peak heatwave exposure ($n = 18$). Bar colours represent the *Cladocopium* lineage of the profile hosted by a coral before peak heatwave (left of red-dashed line) to after peak heatwave (right of red-dashed line).

Appendix B: Chapter 4 Supplemental Materials

Site	Human population 2km	Fishing pressure 10km	Combined metric
Very Low 1	0	0	0
Very Low 3	0	0	0
Very Low 4	0	0	0
Low 1	0	809	809
Medium 1	0	1213	1213
Medium 2	0	1213	1213
Medium 3	0	1213	1213
Medium 5	0	1617	1617
High 1	0	2425	2425
Very High 1	4042	3234	7276
Very High 2	1223	3638	4861
Very High 3	3065	2021	5086

Table B1: Site-level metrics of local human disturbance. Metrics from a geographic buffer of human population size within 2 km for point-source waste-water exposure, and from a 10 km kernel density spatial layer of subsistence-fishing pressure. The two metrics are summed to give a combined metric of site-level local human disturbance exposure.

Expedition →	Aug-14 (i)		Jan-15 (ii)		May-15 (iii)		Jul-15 (iv)		Mar-16 (v)		Nov-16 (vi)		Jul-17 (vii)		Jul-18 (viii)		Jul-19 (ix)		Sept-23 (x)	
Site ↓	Vis/m	Nit/mM	Vis/m	Nit/mM	Vis/m	Nit/mM	Vis/m	Nit/mM	Vis/m	Nit/mM	Vis/m	Nit/mM	Vis/m	Nit/mM	Vis/m	Nit/mM	Vis/m	Nit/mM	Vis/m	Nit/mM
Very low 1	NA	NA	NA	NA	30.48	2.77	30	2.66	NA	9.72	NA	NA	30	2.66	NA	9.72	NA	NA	45	NA
Very low 3	NA	NA	NA	NA	18.29	3.38	30	2.49	NA	2.11	25	NA	30	2.49	NA	2.11	25	NA	25	NA
Very low 4	NA	NA	NA	NA	NA	NA	25	2.61	NA	3.78	20	NA	25	2.61	NA	3.78	20	NA	18.5	NA
Low 1	14.5	0.52	NA	NA	NA	NA	22.5	NA	NA	4.51	17	2.91	22.5	NA	NA	4.51	17	2.91	15	NA
Med 1	16.8	2.62	15.42	NA	24.38	2.56	20	3.82	NA	3.28	25	2.87	20	3.82	NA	3.28	25	2.87	29	NA
Med 2	16.8	1.53	15.42	NA	9.14	2.08	25	3.42	NA	8.61	40	NA	25	3.42	NA	8.61	40	NA	27.5	NA
Med 3	12.2	1.86	NA	NA	21.34	3.41	20	2.64	NA	4.19	15	3.67	20	2.64	NA	4.19	15	3.67	17.5	NA
Med 5	16	1.52	NA	NA	NA	NA	25	NA	NA	3.02	20	2.47	25	NA	NA	3.02	20	2.47	30	NA
High 1	NA	NA	NA	NA	NA	NA	20	2.38	NA	1.98	12	3.10	20	2.38	NA	1.98	12	3.10	25	NA
Very high 1	13.7	1.63	10.80	NA	13.72	1.58	20	2.36	NA	3.29	10	NA	20	2.36	NA	3.29	10	NA	10	NA
Very high 2	12.2	3.45	NA	NA	9.14	2.15	20	1.98	NA	2.08	17	2.57	20	1.98	NA	2.08	17	2.57	15	NA
Very high 3	14.2	1.40	10.80	NA	10.67	1.58	30	2.62	NA	5.38	12	3.29	30	2.62	NA	5.38	12	3.29	20	NA

Mean ±SE 2014-2023			
Site ↓	Vis/m	Nit/mM	Chl/mgm ⁻³
Very low 1	35.16±2.52	4.57±1.16	0.32±0.02
Very low 3	25.94±2.36	2.92±0.72	0.44±0.17
Very low 4	24.70±2.42	4.66±1.29	0.44±0.17
Low 1	16.24±1.74	2.58±0.82	0.27±0.01
Med 1	23.20±2.31	3.49±0.62	0.63±0.25
Med 2	19.01±3.35	3.33±0.96	0.63±0.25
Med 3	16.97±1.25	3.21±0.68	2.82±0.17
Med 5	22.75±3.04	2.12±0.38	0.34±0.01
High 1	18.20±2.33	3.32±0.85	1.88±0.20
Very high 1	11.87±1.28	2.40±0.47	3.09±0.41
Very high 2	16.56±2.26	2.42±0.40	1.87±0.20
Very high 3	14.15±2.57	2.70±0.55	0.34±0.01

Table B2: Site-level seawater metrics by expedition. Measurements for in-water visibility in metres (Vis/m) and surface water nitrate & nitrite concentration (Nit/μM) shown are all calculated as the mean from three measurements collected per expedition. Cells filled with ‘NA’ represent sites/expeditions where no visibility or inorganic nitrogen concentration data were collected. The mean (± standard error of the mean) of values across expeditions i-x are given for in-water visibility and nitrate & nitrite concentration, whilst mean (± standard error of the mean) surface chlorophyll-a concentration (Chl/mgm⁻³) at each site was calculated from annual means of daily satellite-derived data from 2014 to 2023.

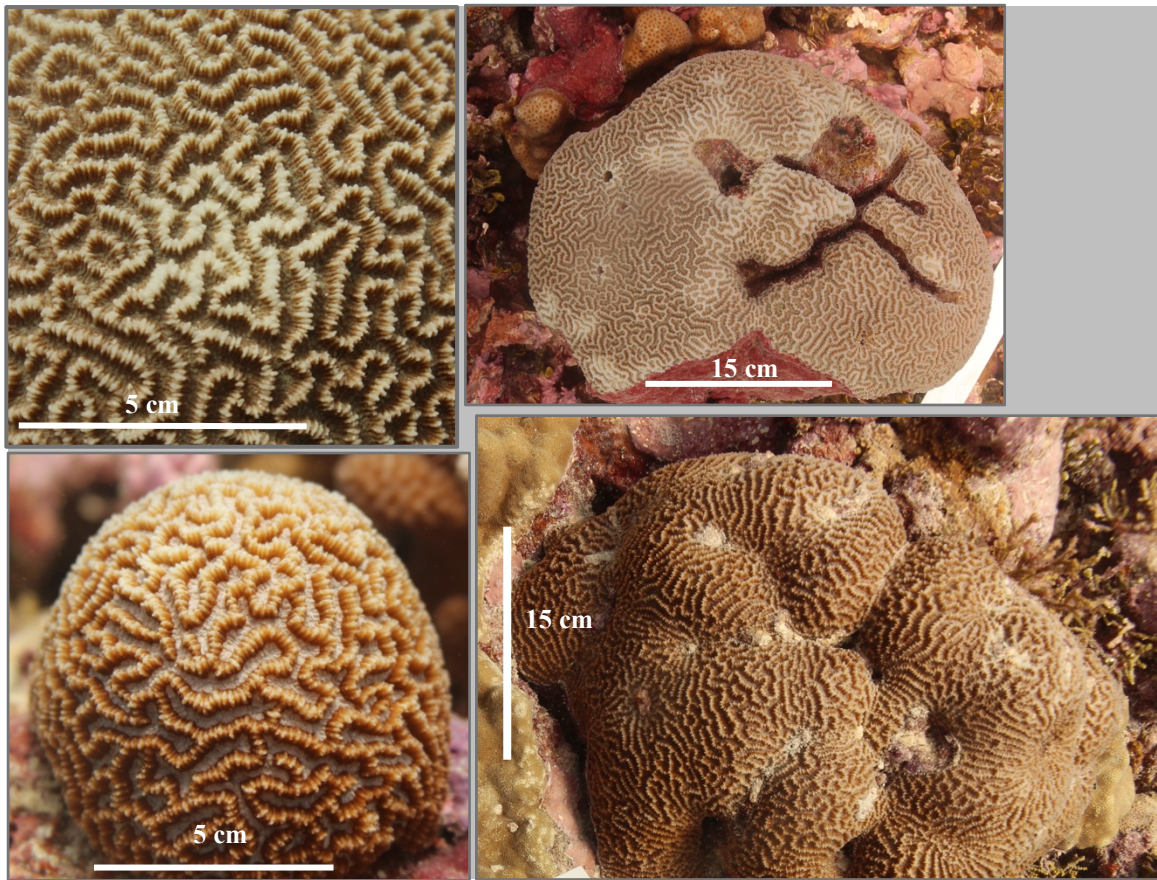


Figure B1: Morphological basis of taxonomic classification. Examples of the macro- and micro-structure of the *Platygyra ryukyuensis* colonies sampled are shown (photographs: D. Buzzoni).

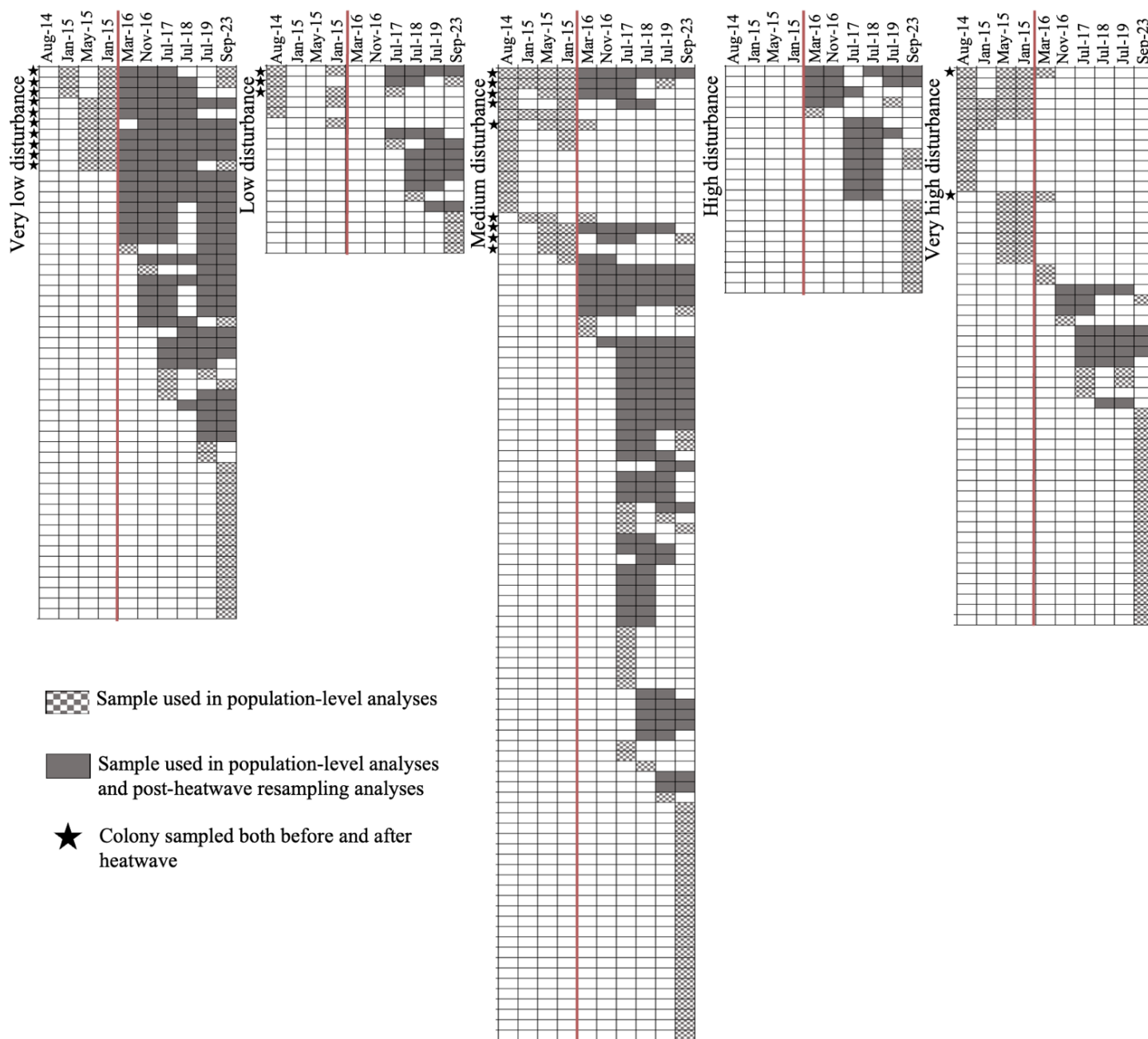
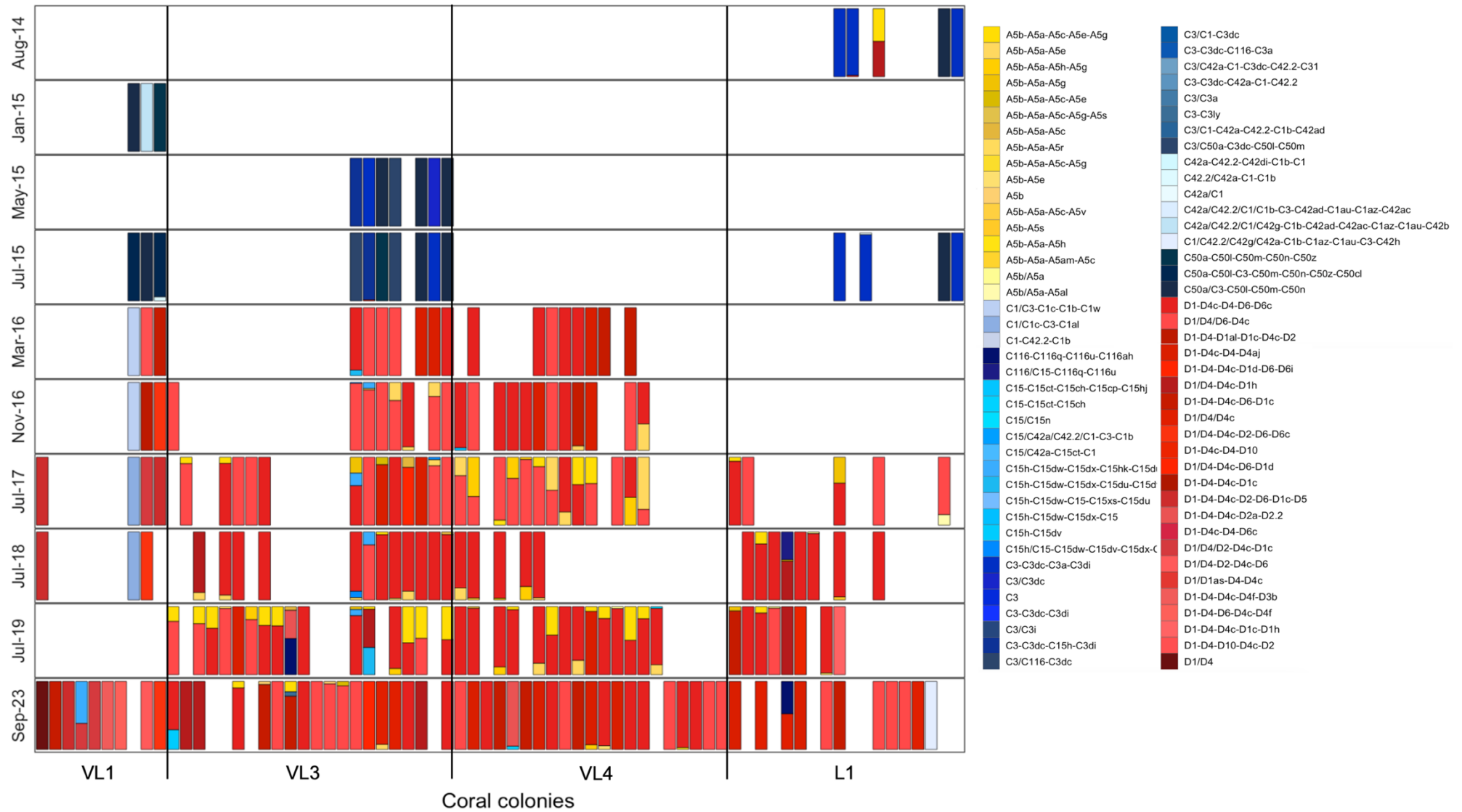
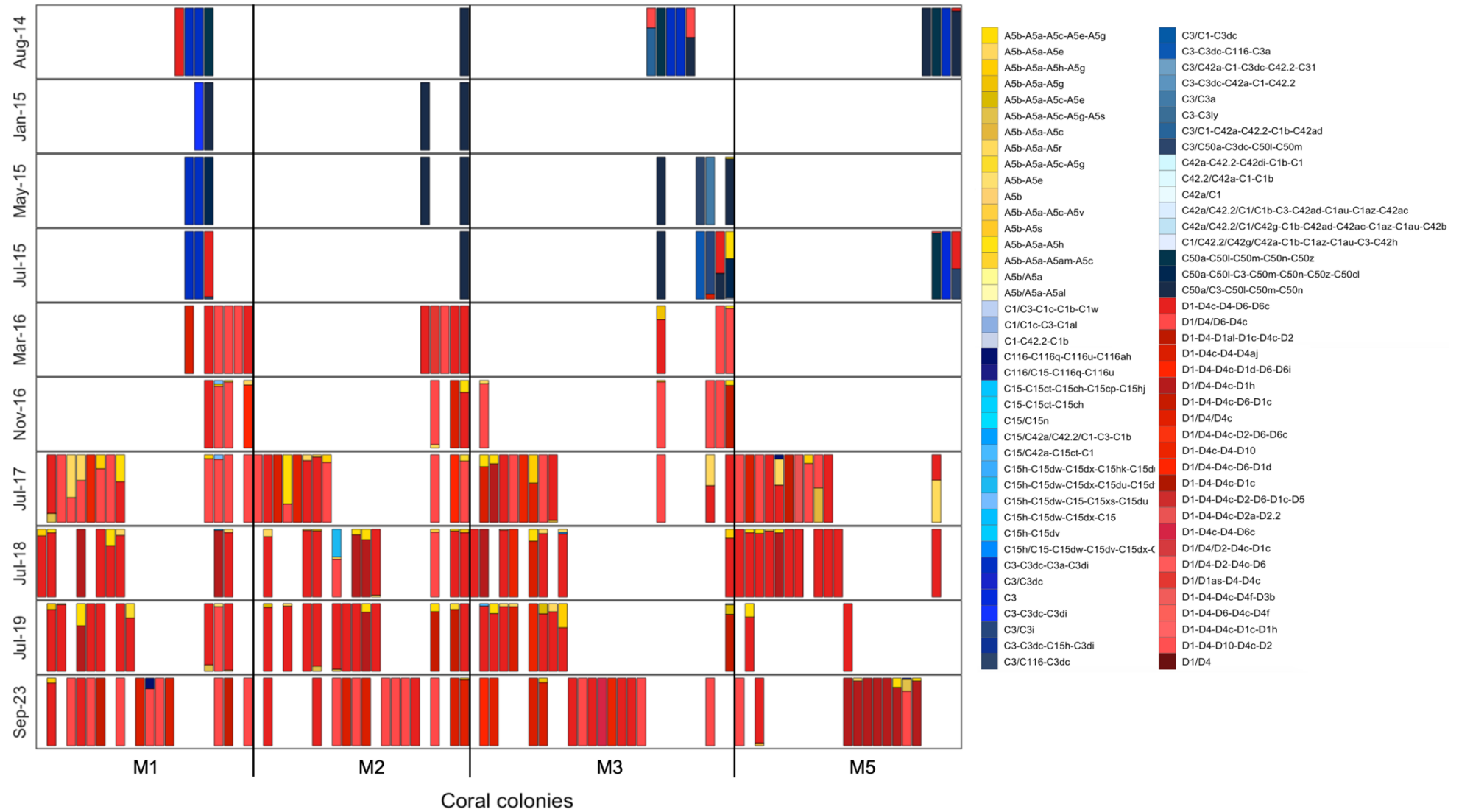


Figure B2: Samples used in population-level and tracked colony analyses. A representation of sampling across ten expeditions, with individual colonies represented by grid rows, separated by human disturbance category. Each filled grid cell represents one of the 598 samples from 237 distinct colonies that passed post-sequencing quality control. Red vertical lines represent exposure to peak heat stress. For population-level analyses, 188, 41, 229, 44 and 96 individual samples were collected from very low, low, medium, high and very high human disturbance sites respectively. For tracked colony analyses, 363 samples from 108 distinct coral colonies were used, ranging from 2 to 6 sequential samples per colony. The 24 colonies sampled both before and after the heatwave are indicated by star icons.

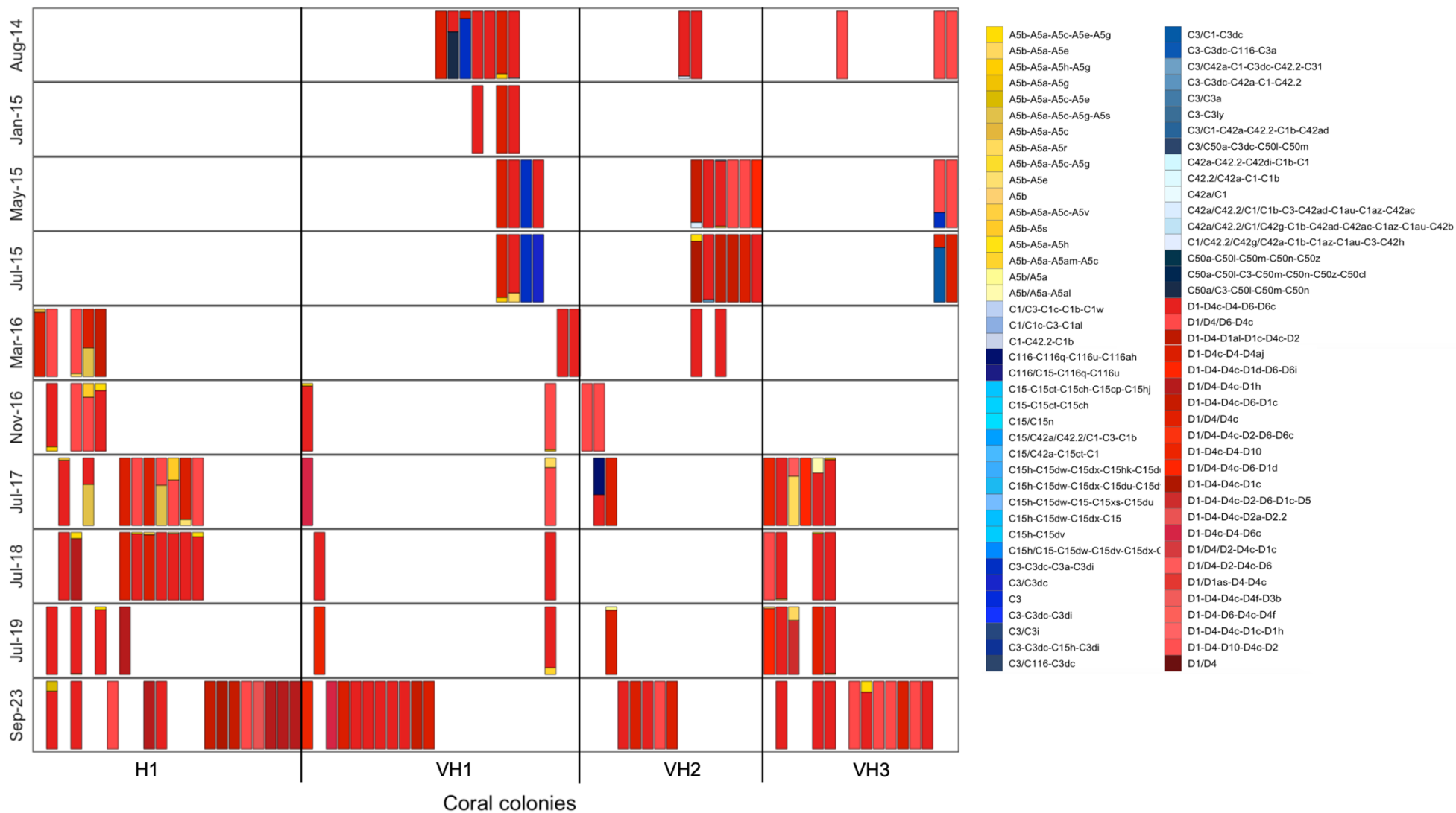
a) ITS2 Profiles



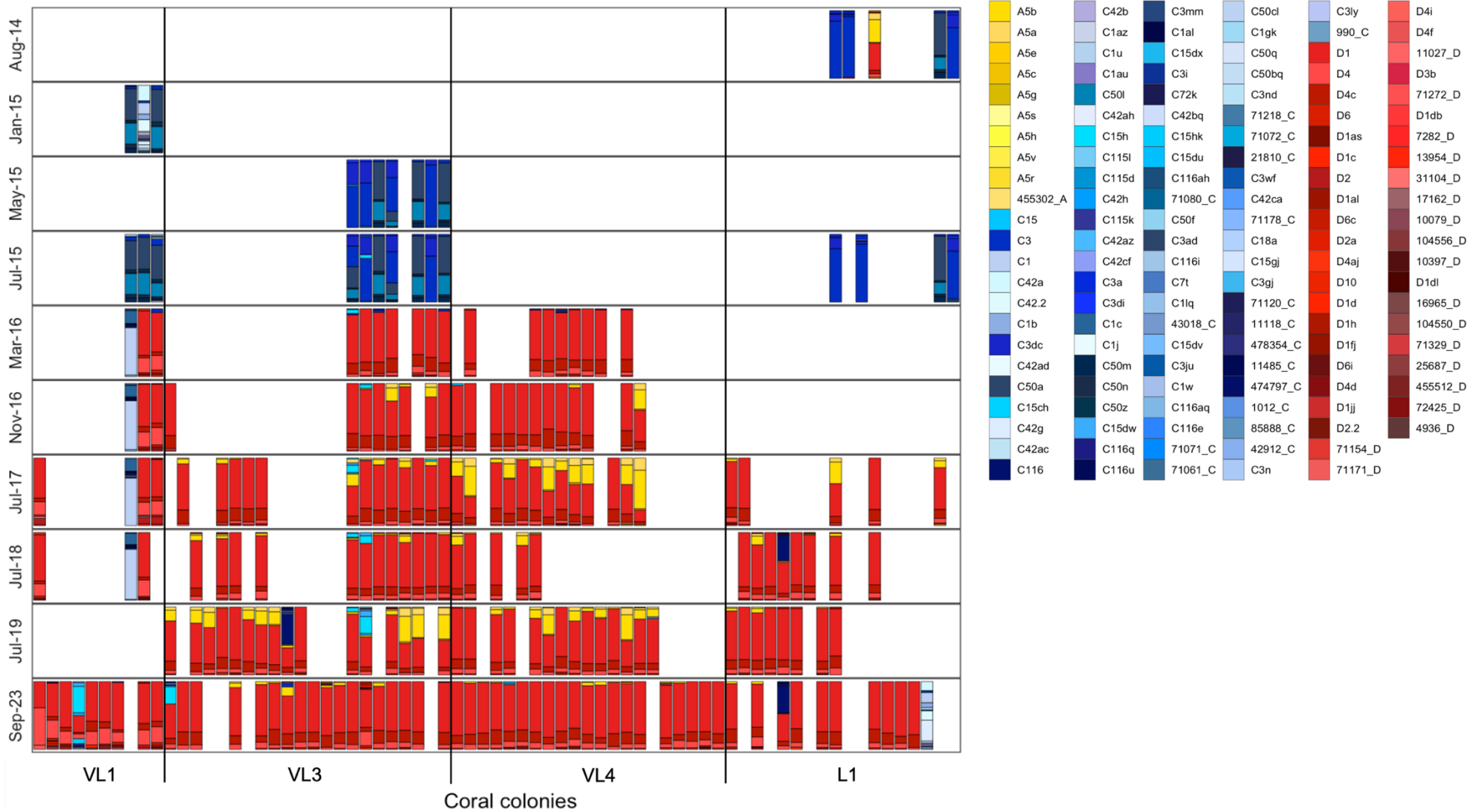
b) ITS2 Profiles



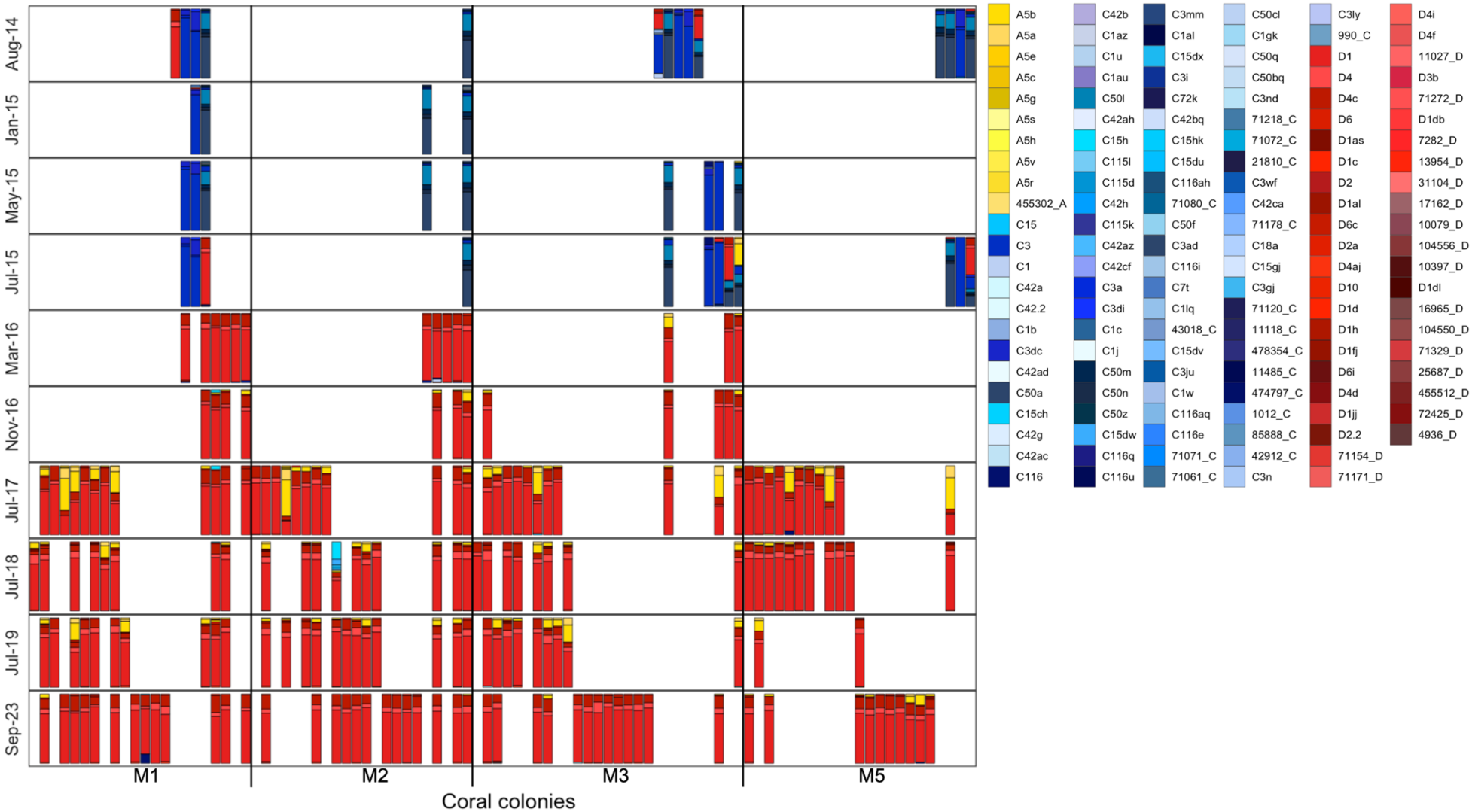
c) ITS2 Profiles



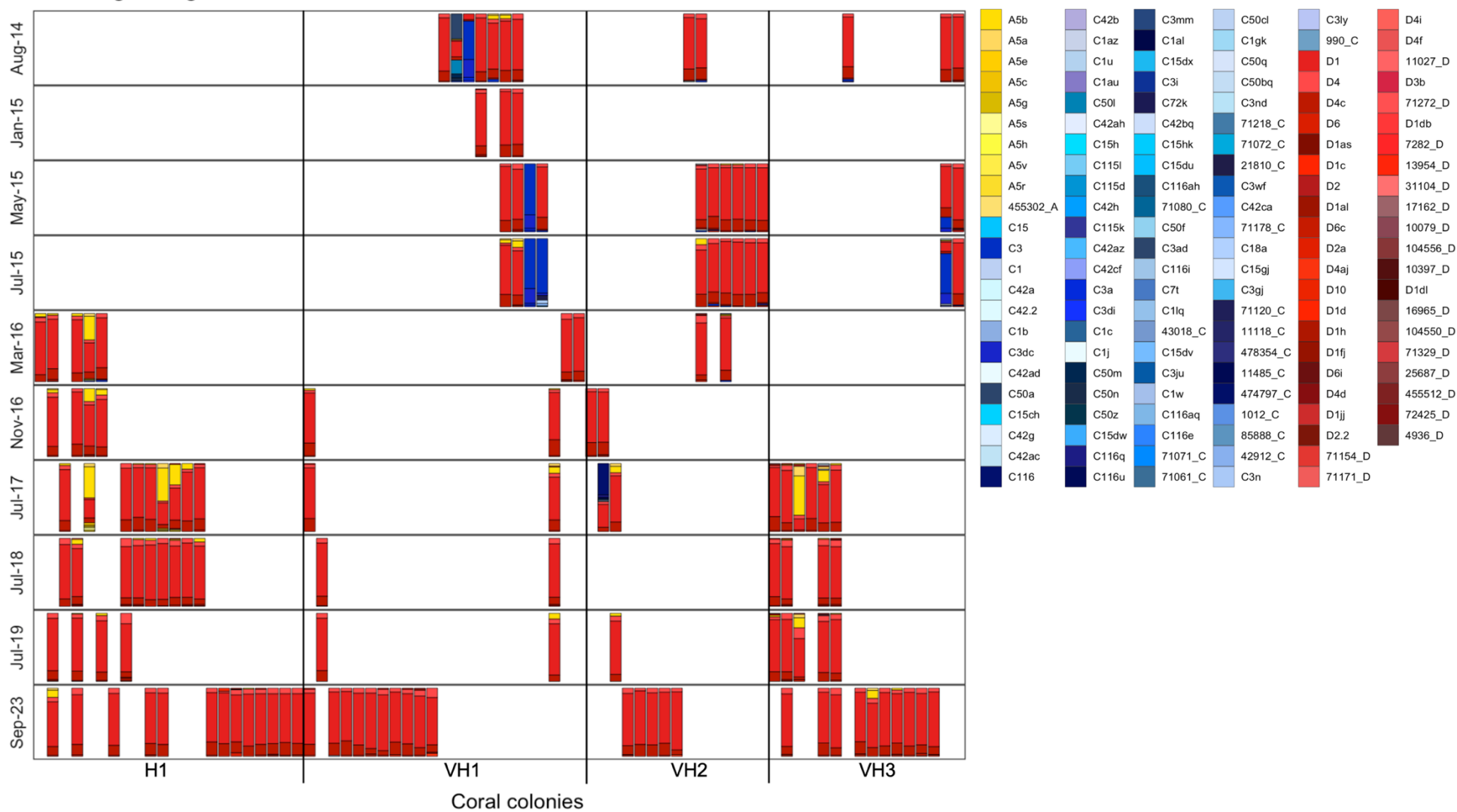
d)Defining Intragenomic Variants



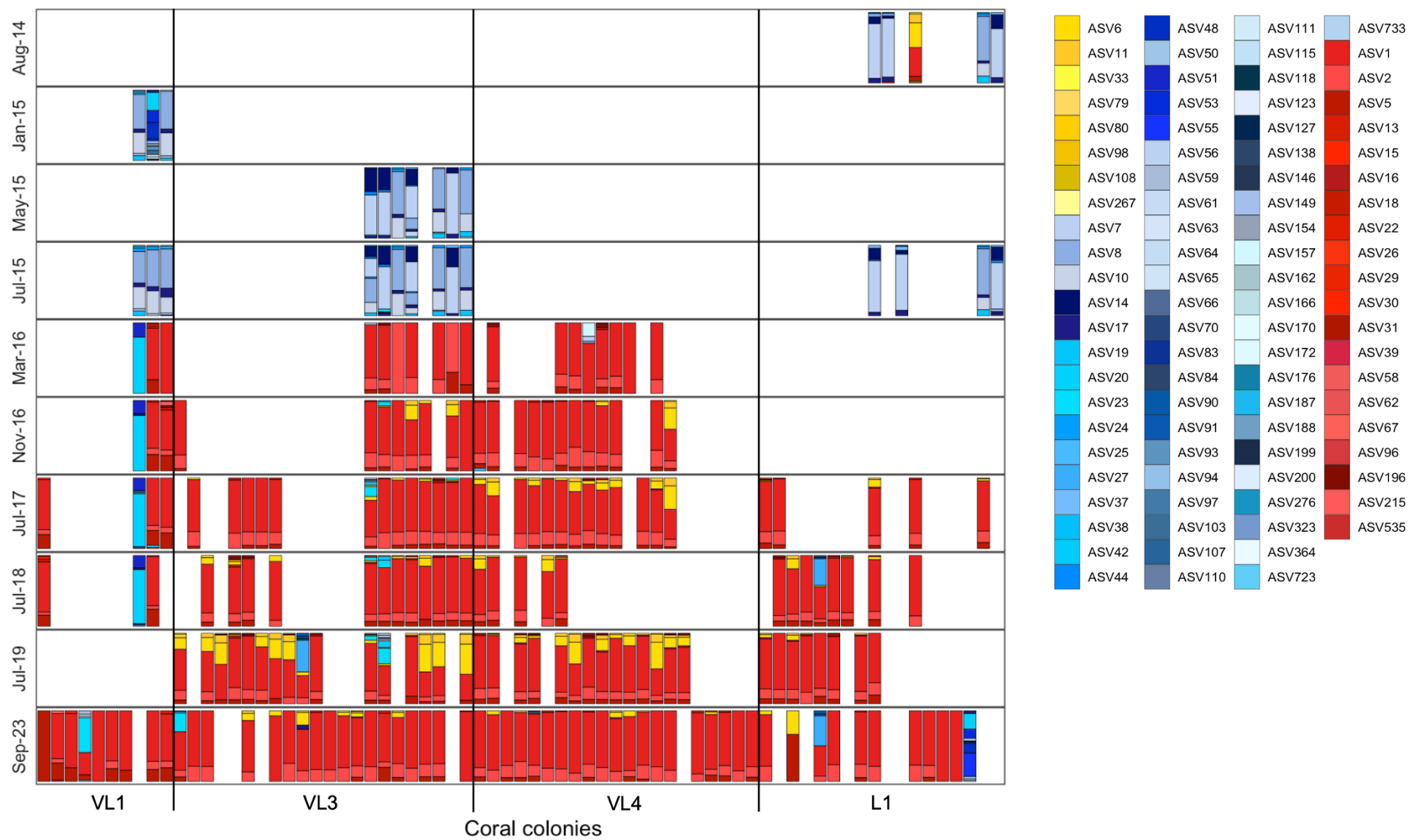
e) Defining Intragenomic Variants



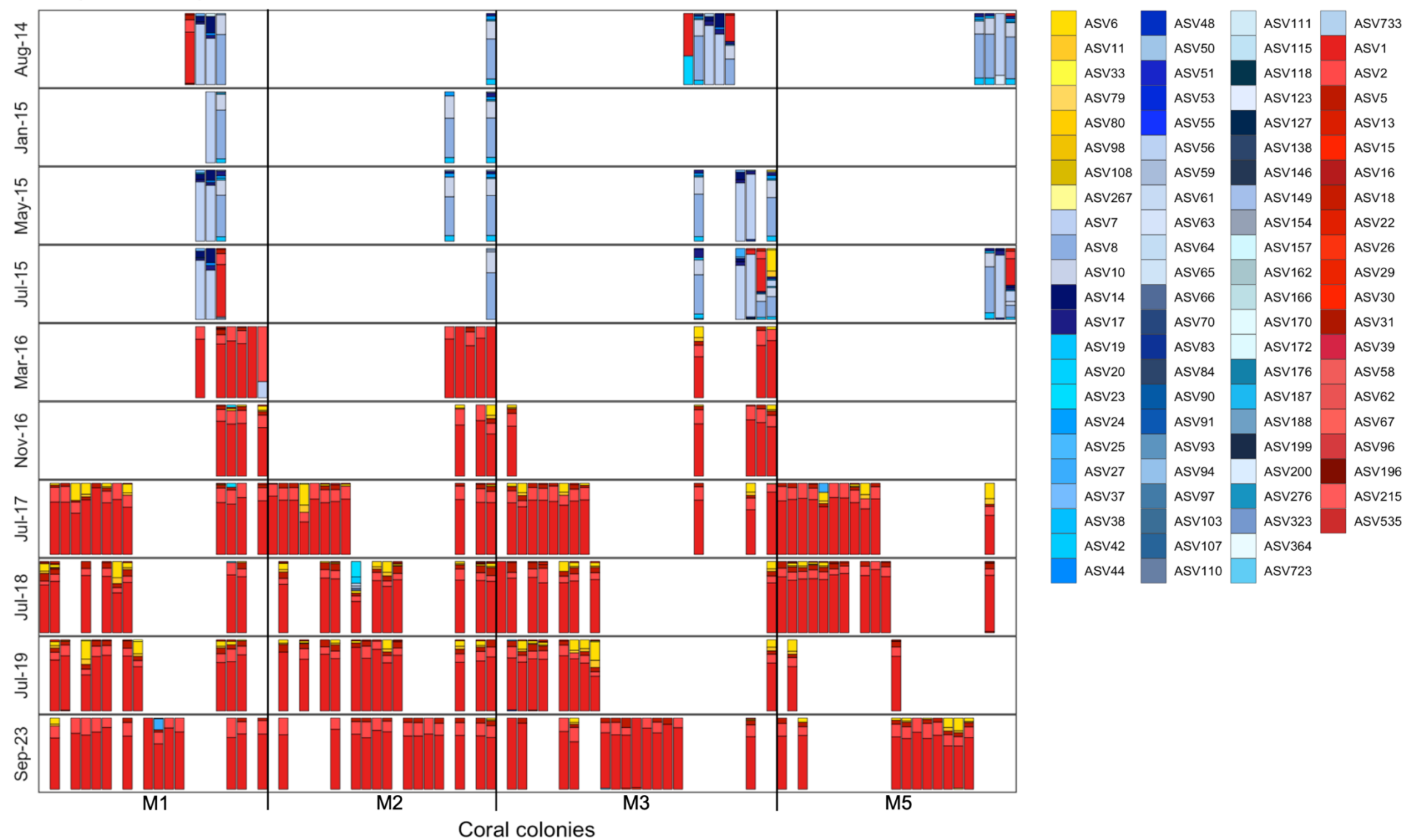
d) Defining Intragenomic Variants



g) Amplicon Sequence Variants



b) Amplicon Sequence Variants



i) Amplicon Sequence Variants

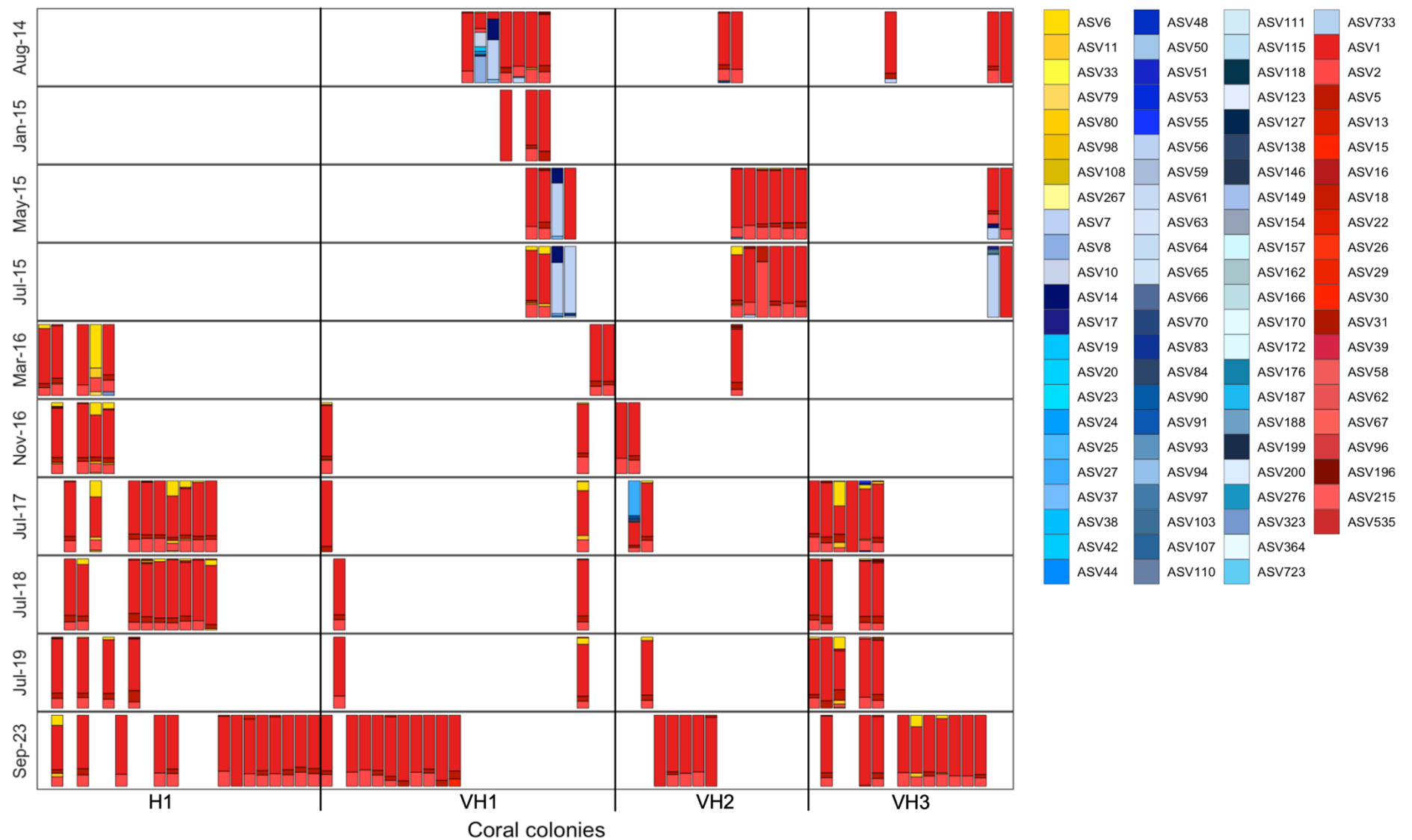


Figure B3: Symbiont composition of all samples according to different read-clustering methods used. Plots show the symbiont composition of each of the 598 samples that passed post-sequencing quality control. Each stacked column represents a single sample sequenced from a single colony, with the height of coloured stacks representing the relative abundance (proportion of Symbiodiniaceae reads) of the taxon, ordered from highest (bottom) to lowest (top). Taxa comprising <1% of a sample's symbiont assemblage are not shown. Blue colours represent taxa in genus *Cladocopium*, red represents genus *Durusdinium*, and yellow represents genus *Symbiodinium*. Samples are ordered along the x-axis by site from low to high human disturbance. Samples are ordered within each site by coral colony, such that samples collected from the same colony during different expeditions are all shown at the same x-axis position. **(a-c)** ITS2 profiles detected by SymPortal's intragenomic clustering algorithm, following minimum entropy decomposition (MED) clustering. **(d-f)** Defining Intragenomic Variants (DIVs) detected by MED-clustering. **(g-i)** Amplicon Sequence Variants (ASVs) detected by DADA2's minimum sequence alignment clustering.

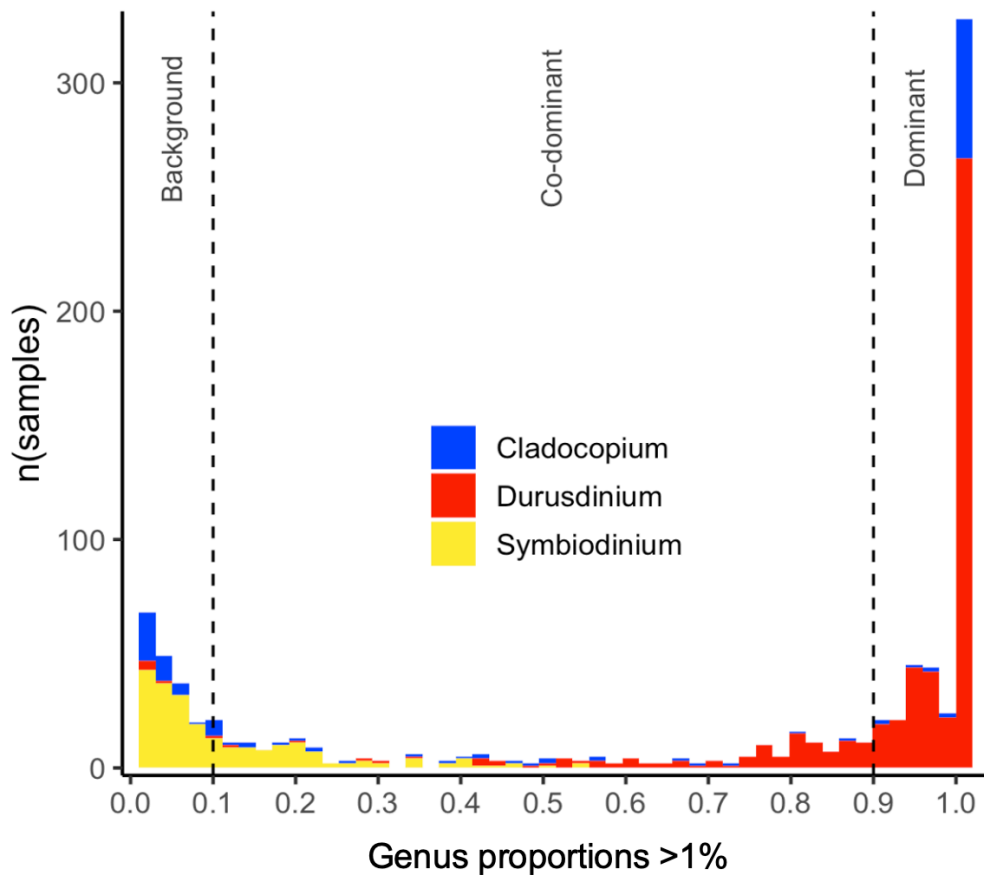


Figure B4: Symbiont per-sample relative abundances inform ‘background’ and ‘dominant’ designations. Histograms of the per-sample relative abundances of Symbiodiniaceae genera *Cladocopium*, *Durusdinium* and *Symbiodinium* after sequence variants comprising <1% of samples have been removed, binned by increments of 0.02, calculated from minimum sequence alignment results (amplicon sequence variant proportions). Dashed vertical lines indicate symmetrical threshold values of 0.1 and 0.9 used to designate the dominant symbiont genus in a sample (≥ 0.9), co-dominant genera (≥ 0.1 , < 0.9) and background genera (≥ 0.01 , < 0.1). Thresholds were selected to best capture the bimodality of proportions of all three genera of Symbiodiniaceae.

Appendix C: Chapter 5 Supplemental Materials

Site	Latitude	Longitude	Collection date	Branches in heat stress	Colonies in heat stress	Colonies sequenced
Hotspot 1 (H1)	7.409	134.332	05-Apr	143	29	29
Hotspot 2 (H2)	7.374	134.280	04-Apr	145	29	29
Hotspot 3 (H3)	7.334	134.245	15-May	0	0	29
Hotspot 4 (H4)	7.324	134.218	16-May	0	0	30
Hotspot 5 (H5)	7.292	134.240	05-Apr	148	30	30
Refugium 1 (R1)	8.006	134.681	20-May	0	0	29
Refugium 2 (R2)	7.989	134.659	03-Apr	138	28	29
Refugium 3 (R3)	7.965	134.624	03-Apr	102	24	30
Refugium 4 (R4)	7.824	134.639	03-Apr	140	28	28
Refugium 5 (R5)	7.818	134.6580	19-May	0	0	30
Total				816	168	293

Table C1: Coral collection sample sizes and site metadata. Sample sizes represent those used in analyses, following sample sequence quality control and the removal of colonies with unexplained control mortality in experimental heatwave.

ITS2 profile (dataset detections)	Average defining sequence proportions and [stdev]
C40 (1)	1.000[NA]
C40/C1-C3-C1c (1)	0.911[NA]-0.054[NA]-0.026[NA]-0.009[NA]
C40-C3-C115-C40h (16)	0.895[0.030]-0.058[0.018]-0.037[0.019]-0.010[0.002]
C40-C3-C115-C40h-C40e (173)	0.871[0.029]-0.061[0.015]-0.040[0.010]-0.019[0.006]-0.009[0.004]
C40/C1-C3-C1c-C115 (4)	0.872[0.029]-0.055[0.019]-0.036[0.012]-0.029[0.016]-0.008[0.005]
C40/C3-C1-C115 (6)	0.870[0.023]-0.071[0.022]-0.035[0.010]-0.024[0.015]
C40-C3-C115-C40z (8)	0.863[0.040]-0.073[0.019]-0.048[0.022]-0.017[0.004]
C40-C40i-C3-C115 (2)	0.862[0.026]-0.062[0.007]-0.039[0.016]-0.037[0.003]
C40/C1-C3-C115-C40h-C40e (27)	0.858[0.031]-0.068[0.018]-0.042[0.013]-0.015[0.005]-0.011[0.013]-0.007[0.002]
C40-C40i-C3-C40h-C40e (2)	0.775[0.008]-0.144[0.006]-0.053[0.005]-0.017[0.002]-0.011[0.001]
C40/C21-C3-C115-C40h (1)	0.777[NA]-0.116[NA]-0.061[NA]-0.031[NA]-0.015[NA]
C40/C21 (1)	0.500[NA]-0.500[NA]
C40-C116a-C3-C115-C116v (7)	0.729[0.116]-0.144[0.099]-0.057[0.014]-0.036[0.007]-0.035[0.026]
C40-C3-C15h-C115-C40h (32)	0.830[0.067]-0.060[0.018]-0.053[0.066]-0.040[0.013]-0.017[0.006]
C40/C15h-C3-C115 (1)	0.856[NA]-0.125[NA]-0.011[NA]-0.009[NA]
C40/C15h-C3-C15do-C115 (11)	0.532[0.199]/0.375[0.199]-0.041[0.013]-0.029[0.012]-0.023[0.008]
C40/C15h-C40i-C3 (1)	0.468[NA]-0.462[NA]-0.044[NA]-0.027[NA]
C15h/C40-C15-C3-C115 (2)	0.467[0.238]/0.447[0.259]-0.043[0.010]-0.029[0.009]-0.015[0.002]
C15h-C15vr-C15do (1)	0.843[NA]-0.104[NA]-0.054[NA]
C15/C93a (6)	0.633[0.096]/0.367[0.096]
C91k (4)	1.000[0.000]
C50c/C50a-C3-C3b-C50f-C50u (1)	0.500[NA]-0.380[NA]-0.065[NA]-0.035[NA]-0.016[NA]-0.004[NA]
C50c/C50a (1)	0.541[NA]-0.459[NA]

Table C2: ITS2 profile within-dataset mean defining sequence proportions and their standard deviations.

Sequence variant	Region indicated	Test statistic (IndVal)	$P(\text{IndVal} \text{H}_0)$
ASV19 C40	Hotspots	0.44	<0.001
ASV4 C15	Hotspots	0.42	0.010
ASV14 C116	Hotspots	0.22	0.014
ASV75 C116	Hotspots	0.22	0.014
ASV51 C116	Hotspots	0.20	0.027
ASV40 C116	Hotspots	0.20	0.027
ASV13 C40	Refugia	0.49	<0.001
ASV87 C115	Refugia	0.22	0.007
ASV86 C115	Refugia	0.20	0.015
ASV7 C40	Refugia	0.19	0.030
ASV95 C40	Refugia	0.19	0.030
ASV60 C30	Refugia	0.19	0.031
DIV C40z	Hotspots	0.73	<0.001
DIV C1	Hotspots	0.41	0.002
DIV C15h	Hotspots	0.41	0.003
DIV C116v	Hotspots	0.22	0.015
DIV C116a	Hotspots	0.22	0.015
DIV C116x	Hotspots	0.20	0.028
DIV C15hg	Hotspots	0.20	0.031
DIV C40i	Refugia	0.19	0.030

Table C3: Statistical test results for regional indicator analyses.

Profile/variant	Test iteration (Q_n)	Quantile(s) indicated	Test statistic (r)	$P(r H_0)$
C40-C3-C15h-C115-C40h	Q_2	Q_2	0.20	0.018
C40-C3-C15h-C115-C40h	Q_4	$Q_4 Q_3$	0.20	0.042
ASV4 C15	Q_2	Q_2	0.21	0.006
ASV4 C15	Q_3	$Q_3 Q_2$	0.20	0.022
ASV4 C15	Q_4	$Q_4 Q_3$	0.21	0.032
ASV4 C15	Q_5	$Q_5 Q_4$	0.24	0.020
DIV C15h	Q_2	Q_2	0.21	0.004
DIV C15h	Q_3	$Q_3 Q_2$	0.21	0.023
DIV C15h	Q_4	$Q_4 Q_3$	0.21	0.029
DIV C15h	Q_5	$Q_5 Q_4$	0.25	0.013
DIV C93a	Q_2	Q_2	0.15	0.050
DIV C15mw	Q_5	$Q_5 Q_4$	0.21	0.035
ASV68 C93	Q_5	Q_5	0.27	0.015
ASV98 C40	Q_5	Q_4	0.22	0.030
ASV1 C40	Q_2	Q_1	0.18	0.018
ASV1 C40	Q_3	Q_1	0.20	0.017
ASV1 C40	Q_5	$Q_1 Q_2 Q_3$	0.23	0.031
DIV C40	Q_2	Q_1	0.21	0.004
DIV C40	Q_3	Q_1	0.20	0.017
DIV C40	Q_4	$Q_1 Q_2 Q_3$	0.23	0.012
DIV C40	Q_5	$Q_1 Q_2 Q_3$	0.25	0.016

Table C4: Statistical test results for DHW₅₀ quantile indicator analyses.

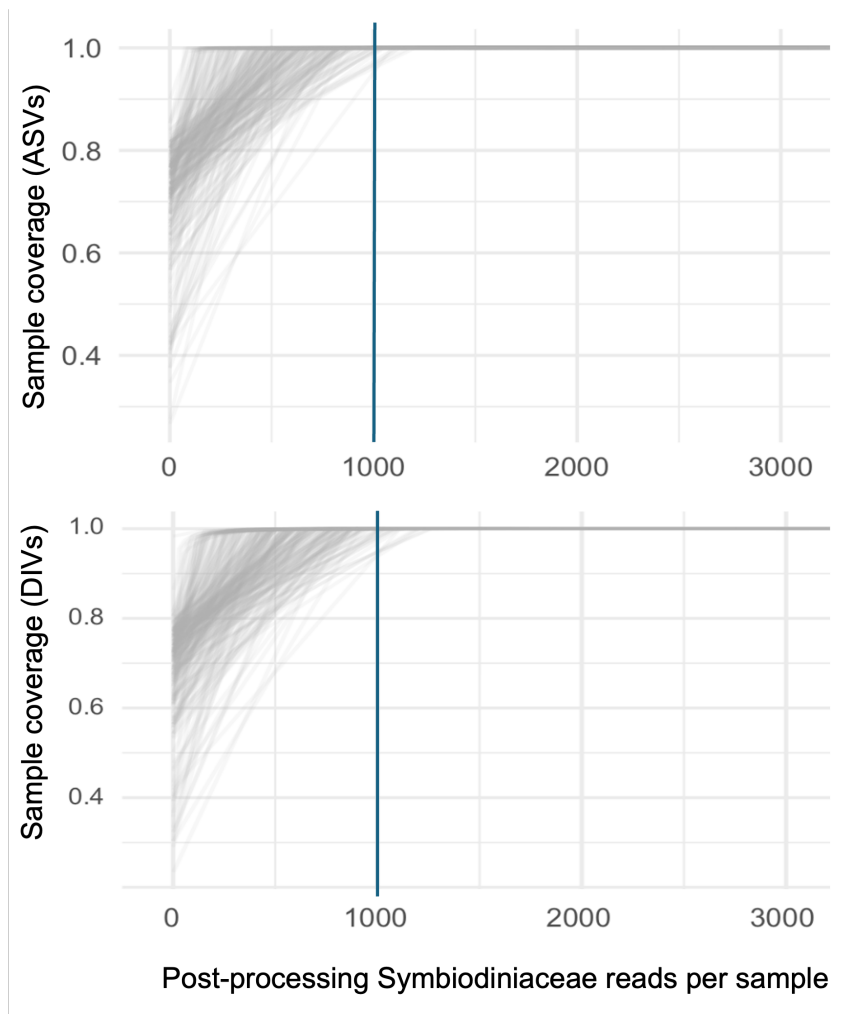


Figure C1: Estimated sequence variant coverage informs sample minimum read threshold. Per-sample estimated coverage of true symbiont sequence richness of ASVs (top) and DIVs (bottom). Each grey line represents a single sample.

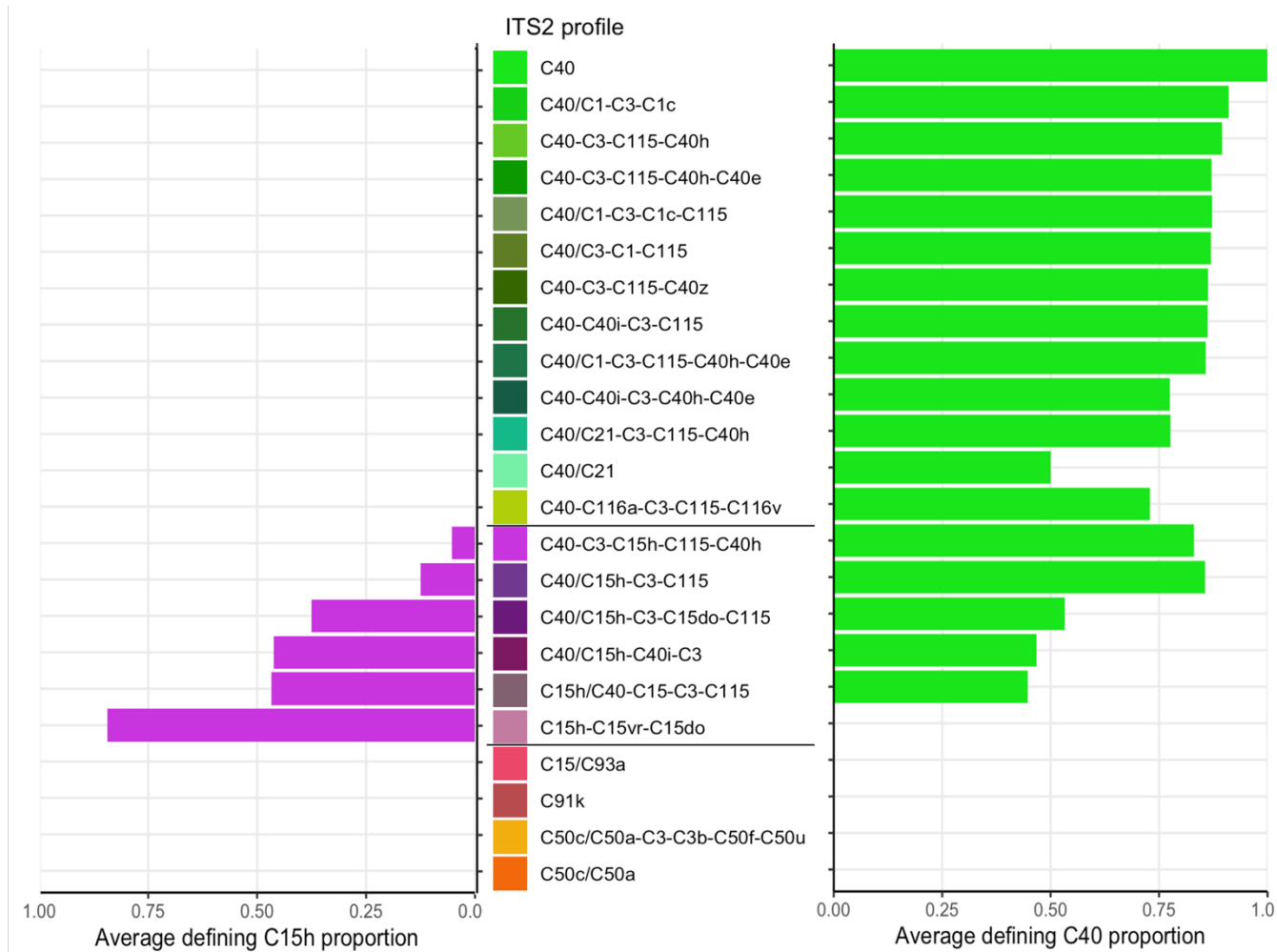


Figure C2: Variant average defining proportions inform ITS2 profile colour scale. ITS2 profiles can most simply be delineated by the average defining proportions (mean relative read abundances) of sequence variants comprising profile detections within this dataset. Specifically, defining proportions of the C40 (left) and C15h (right) sequence variants have been used to inform the colour-scheme used for profiles due to their high relative read abundances and commonness across samples. Profiles comprising mainly the C40 variant are shown along a green colour-scale (top group) unless the C15h variant is present, in which cases profiles are indicated by purple hues (middle group), followed by profiles comprising neither variant (bottom group).

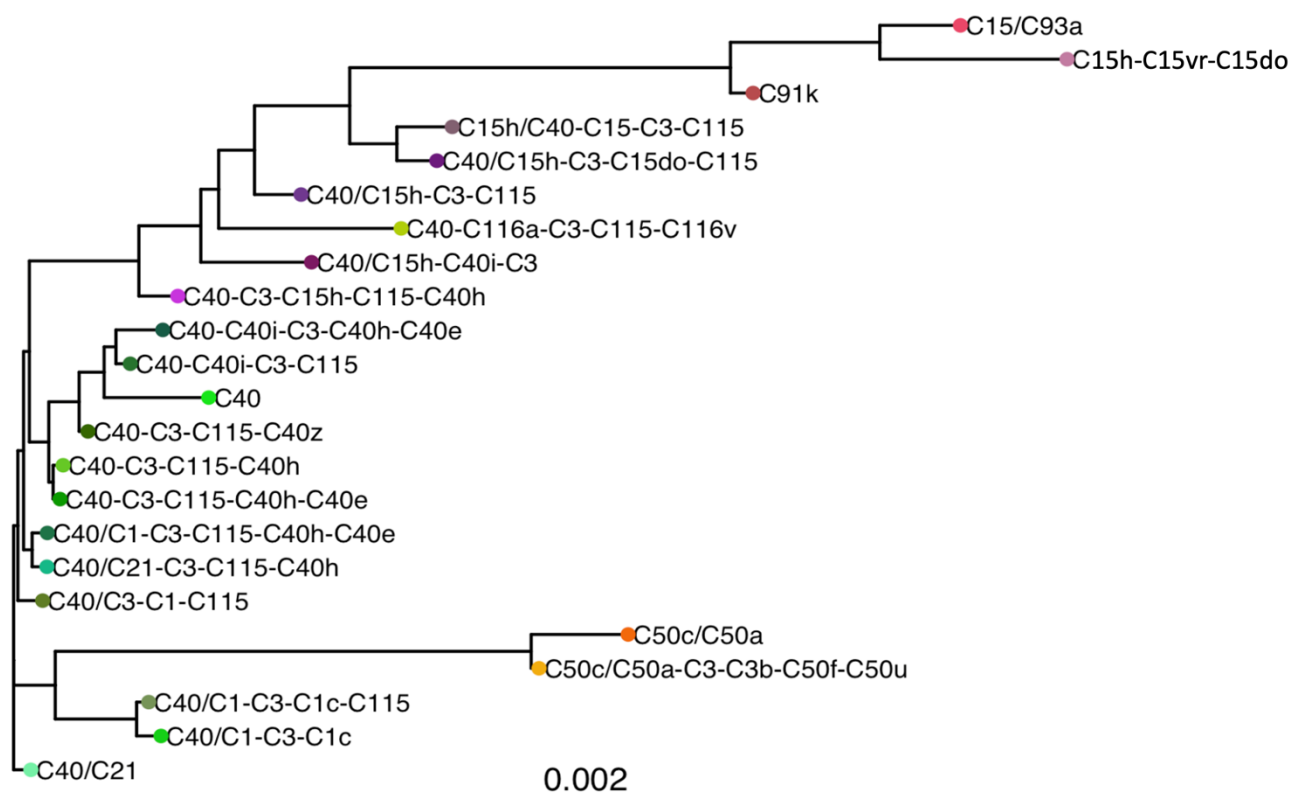


Figure C3: Neighbour-joining tree of between-ITS2 profile distances. Tree built using square root unifracs distances between ITS profiles, provided by SymPortal. Branch lengths are scaled to represent distances, with scale provided below tree.

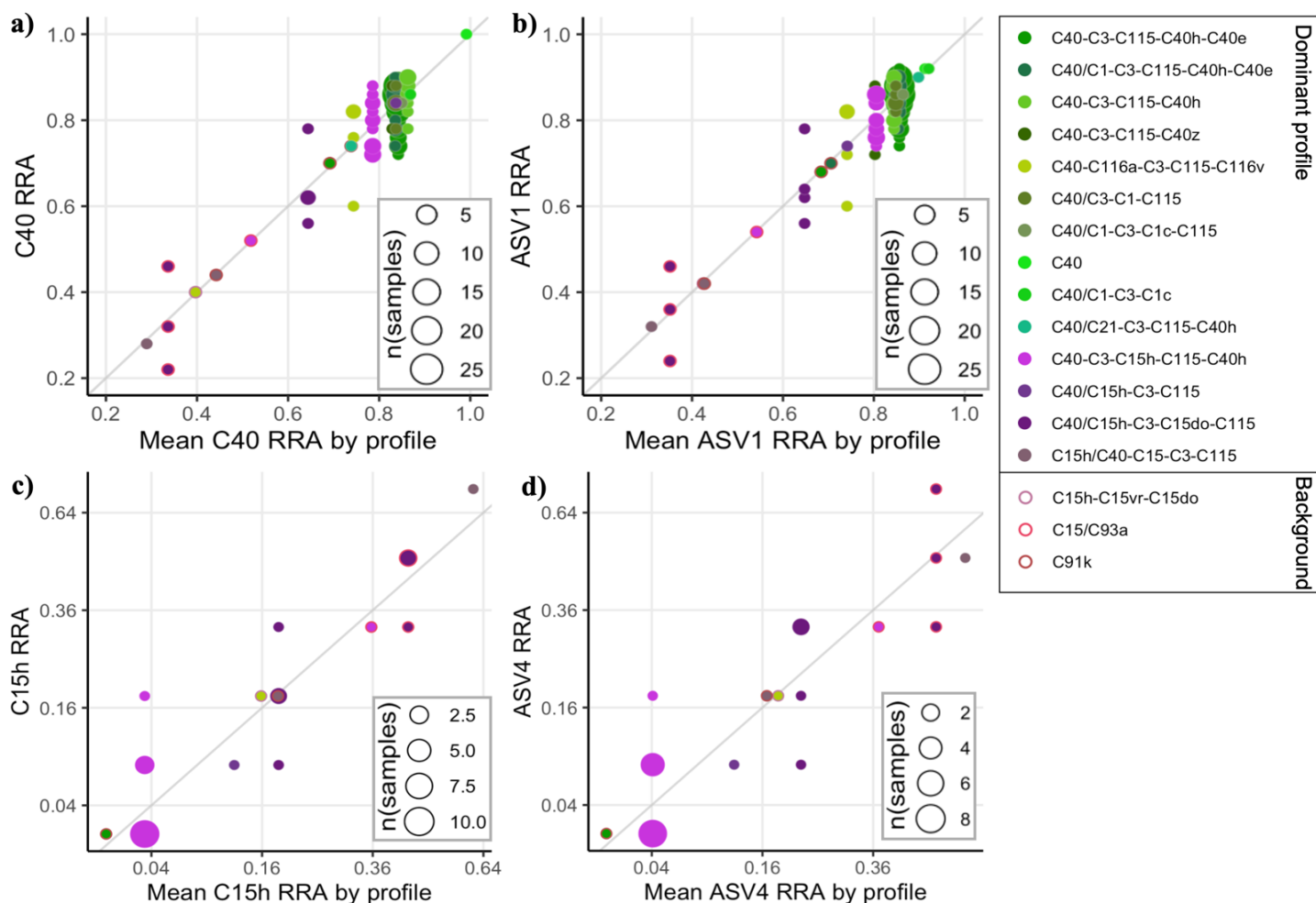


Figure C4: Variability in sample RRA within mean profile RRA for four variants of interest. Within-sample relative read abundance (RRA) of variants is shown on y-axes, binned into increments of 0.02 RRA with point size representing sample counts. Samples are also grouped by the ITS2 profiles hosted, indicated by point fill (dominant profile) and border colour (background profile, if present). X-axes show the mean RRA for samples within each profile group. **a)** RRA of DIV C40 (n = 164). **b)** RRA of ASV1 C40 (n = 164). **c)** RRA of DIV C15h, only including profile groups containing C15h (axes scales are square root transformed, n = 27). **d)** RRA of ASV4 C15, only including profile groups containing C15h (axes scales are square root transformed, n = 27).

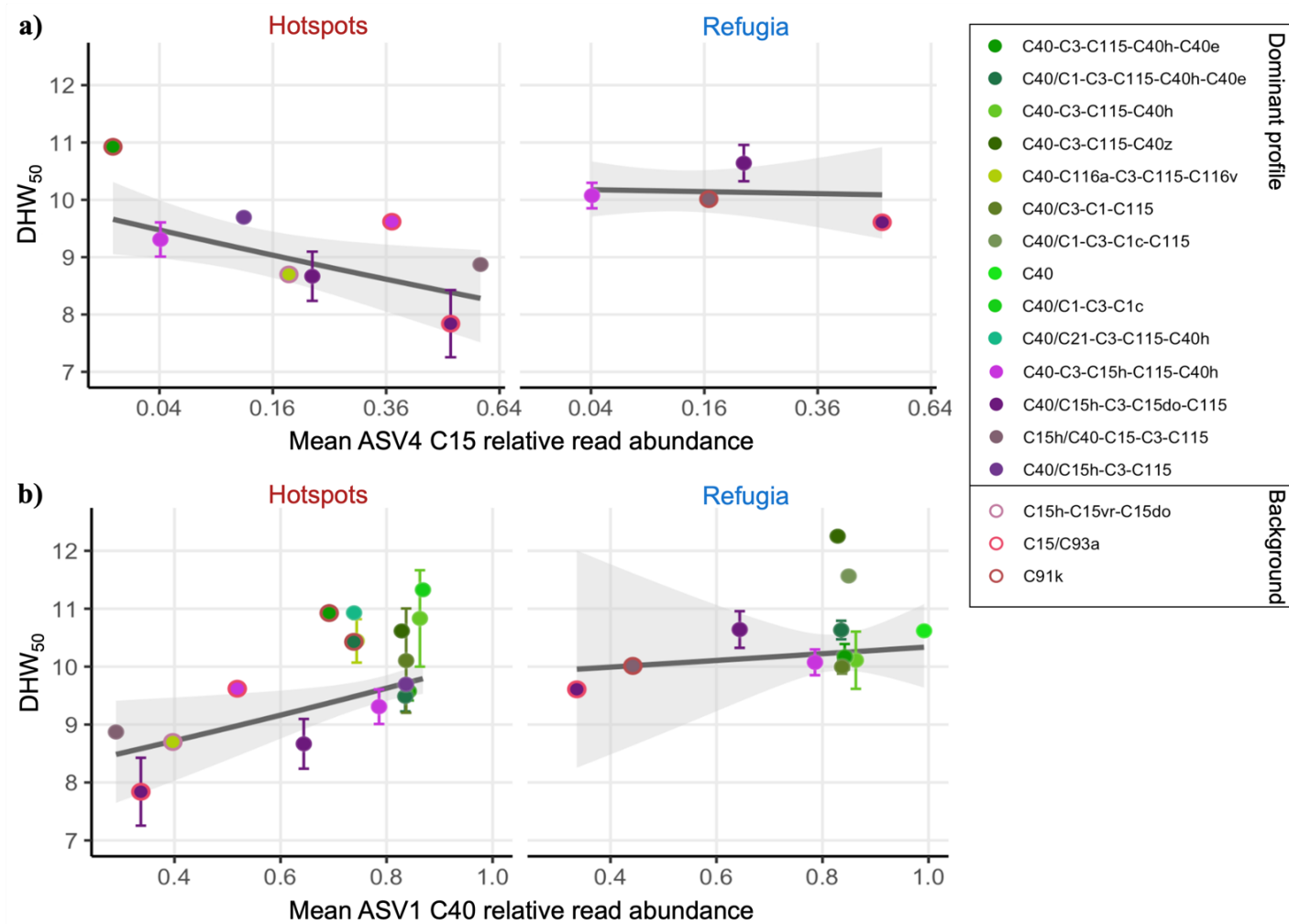


Figure C5: Symbiont indicators only predict heat tolerance at hotspot reefs. Points and error bars represent mean DHW₅₀ $\pm$ one standard error for each unique combination of dominant and background (if present) ITS2 profiles. Generalised linear models with Gamma error distributions are indicated by grey lines, surrounding shading shows $\pm$ 1 standard error around model predictions. **a)** Mean relative read abundance of ASV4 C15 in profile combinations (X-axis scale is square root transformed). Only profile combinations with non-zero ASV4 relative read abundances are shown (hotspots $n = 19$, refugia $n = 8$). **b)** Mean relative read abundance of ASV1 in profile combinations (hotspots $n = 86$, refugia $n = 78$).

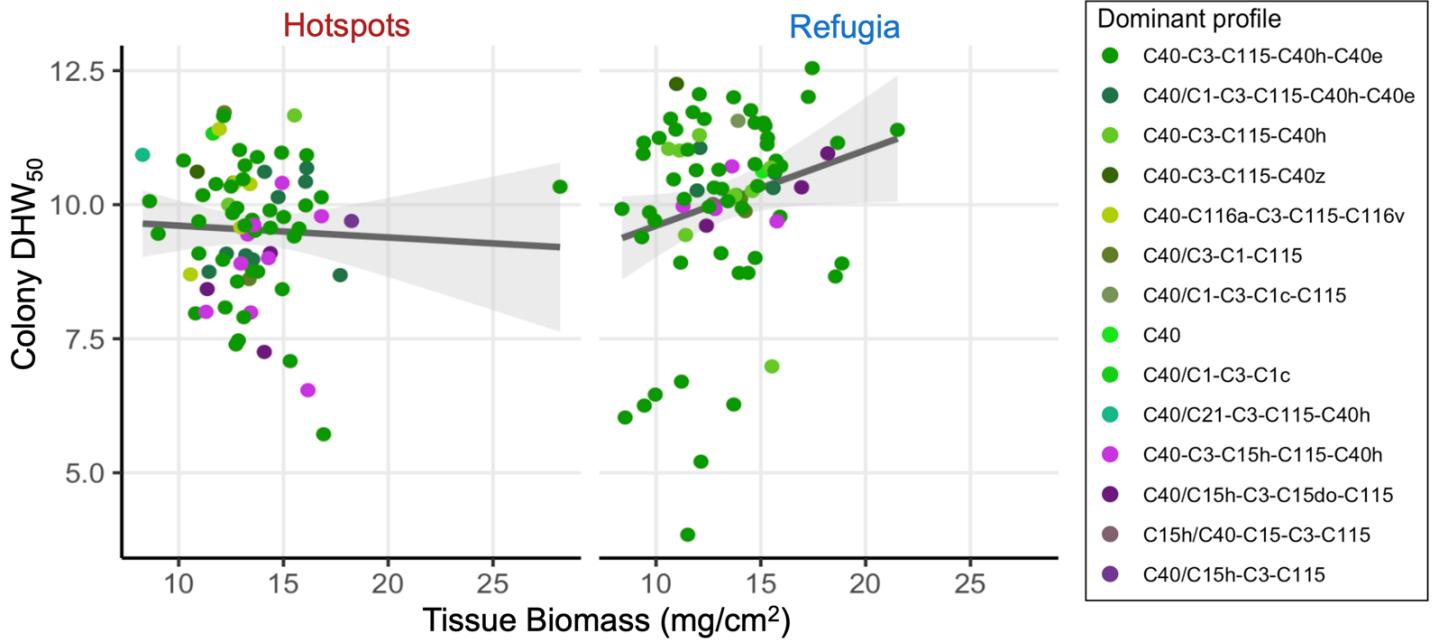


Figure C6: Non-symbiotic correlates of heat tolerance at refugium reefs. Modified from Lachs et al. (2024). Each point represents a coral colony, coloured by Symbiodiniaceae dominant ITS2 profile. Generalised linear models are indicated by grey lines, surrounding shading shows ± 1 standard error around model predictions. The mean tissue biomass (from 3 replicate branches) of colonies is shown against colony DHW₅₀. A generalised linear mixed effects models (with site included as a random effect) revealed a significant positive effect of tissue biomass on DHW₅₀ in refugia ($z = 2.49$, $P = 0.013$), with no significant effect in hotspot corals ($z = 0.59$, $P = 0.555$). Tissue biomass explained an additional 5.3% of heat tolerance variation between refugium corals (marginal R^2 ; 0.053) when included in symbiont generalised mixed effects models.