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Help me, symbionts, you're my only hope: Approaches to accelerate our understanding of coral holobiont interactions

Authors: Colleen B. Bove[1*], Maria Valadez Ingersoll[1], Sarah W. Davies[1]

[1] Department of Biology; Boston University, Boston, MA 02215 USA
[*] Corresponding author
Email: colleenbove@gmail.com
Phone: (919) 606-4926

ORCID:
Colleen B. Bove: https://orcid.org/0000-0003-0340-1371
Maria Valadez Ingersoll: https://orcid.org/0000-0002-2884-5350
Sarah W. Davies: https://orcid.org/0000-0002-1620-2278

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Abstract:

Tropical corals construct the three-dimensional framework for one of the most diverse ecosystems on the planet, providing habitat to a plethora of species across taxa. However, these ecosystem engineers are facing unprecedented challenges, such as increasing disease prevalence and marine heatwaves associated with anthropogenic global change. As a result, major declines in coral cover and health are being observed across the world's oceans, often due to the breakdown of coral-associated symbioses. Here, we review the interactions between the major symbiotic partners of the coral holobiont – the cnidarian host, algae in the Family Symbiodiniaceae, and the microbiome – that influence trait variation, including the molecular mechanisms underlie symbiosis and the resulting physiological benefits of different microbial partnerships. In doing so, we highlight the current framework for the formation and maintenance of cnidarian-Symbiodiniaceae symbiosis, and the role that immunity pathways play in this relationship. We emphasize that understanding these complex interactions is challenging when you consider the vast genetic variation of the cnidarian host and algal symbiont, as well as their highly diverse microbiome, which is also an important player in coral holobiont health. Given the complex interactions between and among symbiotic partners, we propose several research directions and approaches focused on symbiosis model systems and emerging technologies that will broaden our understanding of how these partner interactions may facilitate the prediction of coral holobiont phenotype, especially under rapid environmental change.

Introduction

Reef-building corals are the framework engineers for coral reef ecosystems, which are one of the most biodiverse habitats in the world (Reaka-Kudla et al., 1996) that are both economically and ecologically critical (Costanza et al., 2014; Moberg & Folke, 1999). However, dramatic losses in coral cover are being documented globally as anthropogenic greenhouse gas emissions increase and other direct human impacts drive environmental change (De'ath et al., 2012; Eddy et al., 2021). When seawater temperatures increase or chemistry changes, these shifts can lead to a loss of the coral's symbiotic relationship with its endosymbiotic algae in a process termed coral bleaching (Glynn, 1991). These coral bleaching episodes are increasing in frequency and severity (Sully et al., 2019). Predicting coral resilience to changing oceans has become a critical goal of coral reef research, however, we are just beginning to understand the key mechanisms underlying symbiosis establishment, maintenance, and loss, which are crucial processes in understanding coral resilience.

Understanding symbiosis outcomes under changing oceans is challenging because corals are holobionts – an assemblage of the coral host and many other living partners, which includes millions of single-celled dinoflagellates (Family Symbiodiniaceae, (LaJeunesse et al., 2018)) living inside coral gastrodermal cells – that together form a discrete ecological unit (Rosenberg et al., 2007; Rosenberg & Zilber-Rosenberg, 2018; Thompson et al., 2014). In tropical, oligotrophic waters, these symbiotic algae provide essential organic byproducts to the host from photosynthesis (Muscatine & Cernichiari, 1969; Muscatine & Porter, 1977). In addition to these algal partners, corals are host to a diverse microbiome, consisting of bacterial and archaeal symbionts, fungi and viruses (Bourne et al., 2016; Pollock et al., 2018; van Oppen & Blackall, 2019). The majority of microbiome research to date has focused on bacteria, which, similar to the algae, provide the host with essential nutrients (Agostini et al., 2012; Robbins et al., 2019), nitrogen cycling (Pogoreutz et al., 2017; Rädecker et al., 2015) and carbon cycling (Brown & Bythell, 2005; Kimes et al., 2010; Rohwer & Kelley, 2004). Therefore, each coral holobiont is a complex metaorganism, and understanding – and ultimately predicting – its response to future climate change depends on an integrated understanding of all partners and their interactions.

One challenge to predicting coral bleaching is that coral genetic diversity both within and between species is vast, and this diversity can lead to varied responses to environmental stressors. As sequencing technologies advance for non-model systems, our ability to resolve this diversity increases and strong genetic divergence between hosts has been detected across multiple spatial scales. For example, strong divergence within conspecific corals across depths in several coral species has been documented (Bongaerts et al., 2011; Prada & Hellberg, 2013; Serrano et al., 2016) and genetic divergence across environmental gradients, such as inshore-offshore gradients, is also common (Aichelman et al., 2021; Fabricius et al., 2004; Kenkel et al., 2013; Kenkel & Matz, 2016). While coral genetic diversity may not always directly predict phenotypic diversity, phenotypic variation has been shown to be inherited by subsequent generations (*i.e.,* narrow-sense heritability, h^2) across a variety of coral traits (reviewed in (Bairos-Novak et al., 2021)), including thermal tolerance (Dixon et al., 2015), settlement responsiveness (Meyer et al., 2011), calcification (Jury et al., 2019), and growth (Kenkel et al., 2015). This genetic variation highlights the need to include multiple genetic backgrounds in mechanistic studies given that each unique genotype may determine a phenotypic response.

There are also emerging examples of strong genetic divergence within previously presumed coral species (*i.e.,* cryptic (or sibling) coral species (Bickford et al., 2007)), where corals are morphologically similar yet form genetically distinct groups. Cryptic speciation is a well-known phenomenon in corals (Knowlton, 1993), however, the prevalence of these cryptic species has been largely overlooked until more recently, facilitated by the onset of genomic sequencing (Fifer et al., 2022; Forsman et al., 2020; Prada et al., 2014; Prada & Hellberg, 2021; Rippe et al., 2021; Rose et al., 2021). This hidden genetic diversity can lead to distinct physiological variation, and these cryptic lineages may exhibit differential responses to global climate change (Gómez-Corrales & Prada, 2020)) and may also host unique algal communities (Rose et al., 2021). Understanding how this genetic variation can interact with a coral's environment, remains a fundamental challenge in coral biology.

As an additional layer of complexity, reef-building corals have a bipartite life cycle that generally involves planula larvae dispersing in the water column, followed by recruitment to a substrate with adults exhibiting a benthic, sedentary existence (Baird et al., 2009). The selective pressures on planula larvae in the water column are likely very different from those experienced by the recruit or adult coral on the reef, which may lead to ontogenetic shifts in selection pressures. For example, trait heritability has been shown to vary across coral life stages, with adult and larval life stages exhibiting higher heritability of coral bleaching and growth when compared to the juvenile (recruit) stage (Bairos-Novak et al., 2021). These differences may constrain evolution through genetic trade-offs between traits and across life stages.

Different life stages also exhibit variation in algal symbiont acquisition, with some larvae being aposymbiotic (without algal cells) until settlement (horizontal symbiont transmission), while others exhibit vertical symbiont transmission (i.e., algal cells from the parent) (Baird et al., 2021; Hartmann et al., 2017; Swain et al., 2018). These differences in transmission strategies can also lead to differences in adaptive potential of the holobiont across life stages (reviewed in (Putnam, 2021)), and may specifically constrain coral dispersal between environments if algae are locally adapted (S. W. Davies et al., 2020; Howells et al., 2011a). In addition to different algal symbiont transmission strategies, corals exhibit different algal symbiont and microbial community structure across life history stages (Ali et al., 2019; Epstein et al., 2019), highlighting the complexity of holobiont fitness across ontogeny. These complex life cycles combined with differences in transmission strategies across hosts and partners makes predicting coral performance under changing environments challenging.

Algal symbiont diversity has also been better appreciated thanks to the implementation of molecular genetics approaches in Symbiodiniaceae (reviewed in (S. Davies et al., 2022; Quigley et al., 2018)), which have resulted in taxonomic revisions resolving at least eleven genera and many species (LaJeunesse et al., 2018, 2021; Nitschke et al., 2020; Pochon & LaJeunesse, 2021). However, how such genetic variation predicts functional variation remains an open question. It has been well-documented that unique algal isolates can be functionally divergent (*e.g.,* (Beltrán et al., 2021; Díaz-Almeyda et al., 2017; Hawkins et al., 2016; Howells et al., 2011b; Mansour et al., 2018; Parkinson et al., 2016; Parkinson & Baums, 2014; Russnak et al., 2021)). This functional diversity is perhaps predictable given that reef habitats are highly variable and that algae are limited in their dispersal (Fitt et al., 1981; Fitt & Trench, 1983), which could lead to adaptive divergence in algal communities(S. W. Davies et al., 2018; Howells et al., 2011b; Kriefall et al., 2022; Marhoefer et al., 2021; Suggett et al., 2017; van Oppen et al., 2018) or long-term acclimatization (Torda et al., 2017).

Although some coral host species exhibit specificity for particular algal symbionts (Baker, 2003; Hume et al., 2020; Thornhill et al., 2014), others can associate with a diverse array of algal symbionts that can potentially shuffle under different environmental conditions (Baker & Romanski, 2007; Silverstein et al., 2012). These different associations are important because not all host-symbiont pairings are equally resilient to stress (Abrego et al., 2008; Berkelmans & Van Oppen, 2006; Hoadley et al., 2019; Howells et al., 2013; Sampayo et al., 2008). For example, whole-genome resequencing of over 250 corals during a bleaching event found that Symbiodiniaceae genera (*Cladocopium* vs. *Durusdinium*) was a stronger predictor of bleaching than host genetic variation (Fuller et al., 2020)). Limited work has also suggested that host symbiont preference could have a host genetic basis (Quigley et al., 2019; Reich et al., 2021) and thus symbiont-driven thermal tolerance may have higher heritability than expected. Thus, changes in symbiont associations (*i.e.* symbiont shuffling (Baker, 2003)) may enhance resilience to future stress events. The genetic and molecular drivers of symbiont selection is an ongoing area of research (as discussed in the following section).

Overall, the complexities of coral symbioses and the life histories of each symbiotic partner make understanding how corals will respond to environmental change challenging. Here, we present what is known about how interactions between corals and their different holobiont partners influence coral physiology and present a research framework leveraging emerging cnidarian model systems that may help disentangle how holobiont partner interactions might influence coral persistence under rapid change.

Known molecular mechanisms underlying cnidarian-algal symbiosis

Understanding how different host-Symbiodiniaceae pairings influence coral resilience to environmental change remains a research priority. To facilitate the study of the biochemical processes that modulate associations and dissociations of a cnidarian host with its microbial partners, certain cnidarian model systems have been developed. The sea anemone *Exaiptasia pallida* (commonly referred to as “Aiptasia”) has provided a platform for many advances in our molecular understanding of these symbioses. Aiptasia is an ideal model for coral symbiosis because it participates in a facultative symbiosis with certain strains of Symbiodiniaceae, is simple to maintain in laboratories of all scales, can produce larvae naive to symbionts under inducible conditions, and is easily biochemically manipulatable ((Grawunder et al. 2015); reviewed in (Weis et al., 2008)). Additionally, the continued development of these molecular tools have allowed for the advancement of our understanding of the molecular basis of symbiosis in cnidarians (Cleves et al., 2018; Levy et al., 2021; Rosental et al., 2017).

Cnidarian hosts can change or shuffle their algal symbiont communities under different pressures ((Baker, 2003; Hume et al., 2020; Thornhill et al., 2014). These different partnerships may confer unique sets of benefits or costs, depending on conditions. Hosts can associate with "homologous" (i.e., symbiont strains with which a cnidarian host associates under ambient conditions) or "heterologous" (i.e., strains with which a cnidarian host associates following a disturbance) (Davy et al., 1997; Weis et al., 2001). A host may associate with multiple strains at any given time, the proportion of which may vary under changing pressures (see Matthews et al., 2018; Reich et al., 2017; Tivey et al., 2022). There remains much to be understood about the mechanisms that drive symbiont strain selection, maintenance, and loss. Here, we synthesize the research performed to date on these mechanisms in an effort to inform future experiments.

Symbiotic species of Cnidaria are faced with the challenge of establishment and maintenance of their commensal, intracellular algal symbionts while surrounded by a soup of microbes (Hernandez-Agreda et al., 2017). While vertebrates have two forms of immunity - adaptive and innate - invertebrates, including cnidarians, only have innate immunity to differentiate between these beneficial and pathogenic microbes (reviewed by (Nyholm and Graf 2012; Schulenburg et al. 2007; Mydlarz et al. 2016)). The primary mechanism for the host to detect friend versus foe is through Pattern Recognition Receptors (PRRs) that are either secreted by or presented on the surface of animal cells. These receptors recognize and bind specific patterns on microbial cells and initiate intracellular signaling cascades that modulate the host's immune system, often through the complement pathway (reviewed in (Mansfield & Gilmore, 2019). One PRR-microbe interaction implicated in the cnidarian-Symbiodiniaceae symbiosis occurs between host lectin receptors and symbiont glycans, which are distinct polysaccharide and peptide patterns presented on the surface of the symbiont cell and which are recognized by specific binding domains in host lectin PRRs (Tortorelli et al., 2022; Wood-Charlson et al., 2006). Different Symbiodiniaceae species have diverse cell-surface glycan assemblages, which may facilitate highly specific recognition and establishment between host-symbiont partnerships (Logan et al., 2010). Phylogenetic analyses have implicated PRRs in symbiosis as demonstrated by an apparent independent expansion of PRRs in symbiotic, but not non-symbiotic Cnidaria (Baumgarten et al., 2015; Emery et al., 2021).

In the original generation of the Aiptasia genome, Baumgarten and colleagues (2015) identified PRRs with immunoglobulin domains (involved in cell-cell recognition) and carbohydrate-binding fibrinogen domains and termed them Cnidarian ficolin-like proteins (CniFLs). CniFLs are unique to symbiotic Cnidaria, are almost always upregulated in aposymbiotic compared to symbiotic (with homologous symbiont) Aiptasia, and are hypothesized to function in highly specific microorganism recognition (Baumgarten et al., 2015). It has been suggested that these CniFLs are important for recognition and uptake of compatible symbiont types and are downregulated following successful symbiont establishment.

Symbiosis establishment with (homologous) algal symbionts induces anti-inflammatory immune pathways and reduces pro-inflammatory immune pathways. Pro-inflammation is often referred to as an immune response and is measured as Reactive Oxidative Species (ROS) production (reviewed by (Weis et al., 2008)). Indeed, symbiosis between Aiptasia and homologous symbionts is associated with lower expression of pro-inflammatory gene pathways relative to aposymbiotic Aiptasia (Lehnert et al., 2014). One gene associated with innate immunity in cnidarians is Transforming Growth Factor β (TGFβ), which codes for a cytokine that can suppress the inflammatory response (reviewed by (Sanjabi et al., 2009; Yoshimura et al., 2010)). TGFβ downregulates certain pathways in the cnidarian immune system (Fuess et al. 2020), including Nuclear Factor κB (NF-κB) in Aiptasia (Mansfield et al., 2019). TGFβ is induced and downstream transcription factors are phosphorylated in Aiptasia harboring homologous symbionts compared to aposymbiotic Aiptasia, and aposymbiotic Aiptasia mount a higher immune response (measured by nitric oxide production) under lipopolysaccharide challenge (Detournay et al., 2012). Additionally, blocking TGFβ hinders establishment of symbiosis with homologous symbionts, while addition of exogenous TGFβ decreases endogenous nitric oxide and symbiont loss in heat-stressed symbiotic Aiptasia (Detournay et al., 2012).

NF-κB is an evolutionarily conserved transcription factor in the innate immune system that has two forms of activation by distinct PRRs in vertebrates (reviewed in (Gilmore, 2006)). One form of NF-κB activation is through ligand detection and signal transduction by Toll-like receptors (TLRs) containing extracellular leucine-rich repeats (LRRs) and cytoplasmic Toll/Interleukin 1 receptor homology (TIR) domains (reviewed in (Akira et al., 2006)). The exact pathway of NF-κB activation in Cnidaria is not fully resolved, but the protein has been gaining attention for its possible role in symbiosis regulation (Baumgarten et al., 2015; Poole & Weis, 2014; Williams et al., 2018). NF-κB mRNA, protein levels, and protein activity are downregulated following establishment of homologous symbionts in adult and larval Aiptasia. In naive Aiptasia larvae and following clearance of homologous symbionts from adult Aiptasia, NF-κB protein levels and activity increase (Mansfield et al., 2017; Wolfowicz et al., 2016). Aposymbiotic branches of the facultative coral *Oculina arbuscula* also exhibit enrichment of gene pathways involved in NF-κB immunity and the inflammatory response compared to symbiotic branches of the same genetic background (Rivera & Davies, 2021). Further, field-collected *Acropora palmata* exhibited upregulation of NF-κB under short-term thermal stress (DeSalvo et al., 2010). This immune response to symbiosis has even been observed in the salamander *Ambystoma maculatum*, where egg cells in symbiosis with the single-celled algae *Oophila amblystomatis* exhibited a reduction in the expression of genes involved in NF-κB signaling (Burns et al., 2017). Taken together, these studies indicate a possible role of NF-κB modulation in the establishment and maintenance of symbiosis across metazoans.

Few studies have examined differences in expression of anti- and pro-inflammatory immune pathways in hosts in symbiosis with heterologous versus homologous species. Interestingly, NF-κB is not downregulated in adult and larval Aiptasia nor adult Pocillopora damicornis harboring heterologous symbionts, indicating that the host is not able to detect heterologous symbionts through the same signal transduction mechanism as homologous symbionts (Mansfield et al., 2019). Additionally, Aiptasia hosts populated by heterologous symbionts induce multiple oxidative responses, including antioxidant defense, gene pathways, and accumulation of ROS and Reactive Nitrogen Species in host tissues compared to Aiptasia hosting homologous symbionts (Matthews et al., 2017). These data suggest that hosts associated with heterologous symbionts are chronically responding to oxidative stress events even under ambient conditions, but it may also suggest conferred tolerance of the holobiont to oxidative stress through antioxidant priming (Matthews et al., 2017). As further evidence, transcriptomic studies have revealed that Aiptasia in symbiosis with homologous symbionts exhibit downregulation of ROSscavenging genes such as catalase, dual oxidase 1, and Superoxide Dismutase compared to aposymbiotic Aiptasia (Lehnert et al., 2014; Oakley et al., 2016; Rodriguez-Lanetty et al., 2006). Upregulation of inflammation, immune, and ROS response pathways in heterologous-hosting Cnidaria compared to homologous-hosting Cnidaria may promote higher survival of those harboring heterologous symbionts under stressful conditions by conferring tolerance to future biotic and abiotic stressors.

The question that remains is: if hosting certain symbiont strains confers maintained immunity and protection against future oxidative stressors, why are these strains not the preferred (homologous) strains for symbiotic cnidarians? The answer most likely lies in nutritional benefits provided to the host by the symbiont under ambient conditions. After establishment of symbiosis, the host exerts control over Symbiodiniaceae cell density and proliferation through regulation of the cell cycle and nitrogen cycling (Gorman et al., 2022; Rivera & Davies, 2021). In Aiptasia, density of heterologous symbionts is lower than that of homologous symbionts (Matthews et al., 2018; Medrano et al., 2019). In some strains of heterologous symbiont, this lower density is maintained through at least the first year of maintenance in Aiptasia, implicating greater host control over symbiont cell proliferation (Tsang Min Ching et al., 2022). Under ambient conditions, a growing body of work has demonstrated that homologous strains confer greater energetic and nutritional benefits to the host compared to homologous strains, as indicated by higher gross symbiont photosynthesis, higher nitrogen assimilation in the host, and more host lipid bodies (Rädecker et al., 2018; Starzak et al., 2014). Additionally, Aiptasia hosting homologous strains assimilate more metabolites from the symbiont central metabolism and rely less on catabolizing their own energy reserves than Aiptasia hosting heterologous symbionts (Matthews et al., 2017, 2018). Further research is required to understand whether greater metabolic benefits are conferred by heterologous strains over homologous strains under stress conditions.

Overall, these model systems have provided evidence to support the following emerging hypotheses of the biochemical and molecular mechanisms that dictate symbiont strain establishment, maintenance (possibly loss), and how these processes might differ between homologous and heterologous algal strains (Figure 1). In general, PRRs on the surface of host cells recognize glycans presented on homologous symbiont cells resulting in a downregulation of inflammatory pathways in the hosts' innate immune system resulting in rapid establishment of homologous symbiont cells (Detournay et al., 2012; Lehnert et al., 2014; Mansfield et al., 2017, 2019; Tortorelli et al., 2022; Wood-Charlson et al., 2006). In heterologous pairings, these same PRRs fail to bind the glycans with high affinity, and, therefore, establishment progresses slower and symbiont density within hosts is lower (at least initially) (Matthews et al., 2018; Medrano et al., 2019; Tsang Min Ching et al., 2022). Once symbiosis with homologous or heterologous symbionts is established, heterologous symbionts tend to confer lower nutrient benefits to the host than homologous symbionts (Matthews et al., 2017, 2018; Rädecker et al., 2018; Starzak et al., 2014). Cnidarians harboring certain heterologous symbionts are frequently classified as more thermotolerant than those harboring homologous symbionts because of two hypotheses, which are not mutually exclusive: 1) hosts are able to maintain constitutively higher innate immunity, which potentially allows for immune priming to better handle and quench increased reactive oxidative species (ROS) under stress and/or 2) lower algal symbiont densities in hosts associated with heterologous symbionts leads to less overall ROS produced.

The work on these symbiosis model systems has resulted in a deeper understanding of the molecular mechanisms underlying symbiosis. However, much remains unknown. Most of the above work has been performed in a limited range of host genetic backgrounds (i.e., CC7 Aiptasia), host species, and symbiont strains (largely limited to one strain of Symbiodiniaceae per genus). Additionally, the microbiome plays a significant role in the regulation of the holobiont immune system and nutrient/metabolite exchange, yet its exploration has only begun. Without a more complete understanding of the way in which a diverse range of hosts, symbionts, and microbial components interact, our ability to predict symbiotic outcomes will remain a challenge.

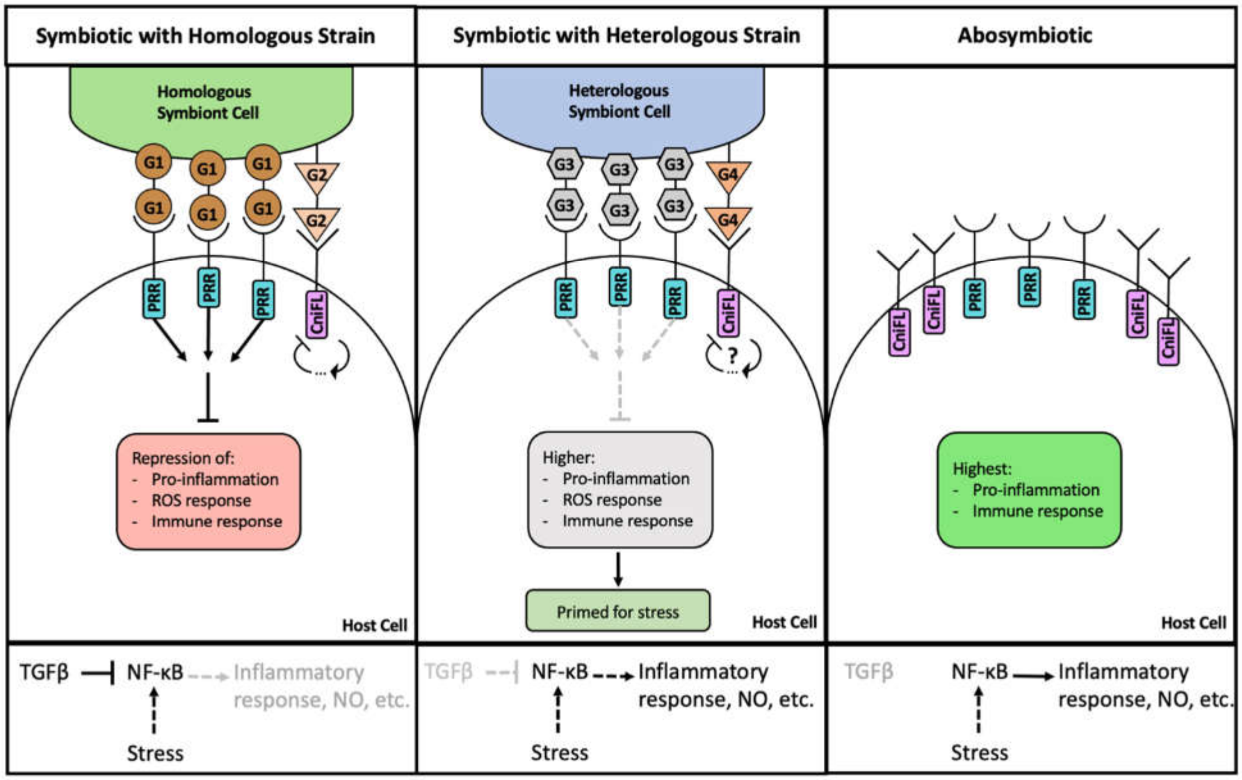

Figure 1. Proposed mechanism by which immunity is regulated in three scenarios of cnidarian-algal symbiosis. When a **homologous** symbiont strain is detected by a host cell, PRRs on the host cell surface recognize and bind glycans (G1) presented on the symbiont cell. This high affinity binding induces an intracellular signaling cascade that downregulates pathways responsible for inflammation, the reactive oxygen species (ROS) response, and the immune response. This repression may be via the inhibition of NF-κB – and therefore the inflammatory response – by TGFβ. Other glycans (G2) bind CniFLs on host cells, which participate in a negative feedback loop. When a **heterologous** symbiont strain is detected by a host cell, PRRs on the host cell surface have lower binding affinity for symbiont glycans (G3). Therefore, the intracellular signaling cascade is either induced to a lower degree or fails to be induced (depending on the binding affinity between G3 and PRRs). The reduced signaling cascade (and lower TGFβ) means that pro-inflammation, the ROS response, and the immune response (characterized by NF-κB

immunity) are not inhibited. This lack of inhibition may prime host cells for future stress. It remains unclear whether CniFLs can recognize and bind heterologous symbiont glycans (G4). In **aposymbiotic** hosts, the intracellular signaling cascade involving TGFβ repression of NF-κB is never induced, so inflammation and the NF-κB immune response remain high. Additionally, there is a high density of CniFLs presented on the host cell to detect symbionts in the environment.

The role of the microbiome in coral holobiont phenotype and resilience

While algal symbionts have gained wide attention in coral symbiosis research, the roles of other diverse members of the microbiome (e.g., bacteria, viruses, fungi, archaea, etc.) in maintaining coral holobiont health have been recently appreciated. These microorganisms are located in microhabitats across the coral holobiont, including the skeleton, gastric cavity, and mucus (Pollock et al., 2018), and can all interact to support a healthy, balanced holobiont through resource allocation (Bourne et al., 2016). However, how these components and spatial arrangement of the microbiome shape the function and fitness of the coral holobiont remain largely unknown (but see (Jiang et al., 2021; Morris et al., 2019; van Oppen et al., 2009; Weis, 2019; Wooldridge, 2010).

Previous work assessing differential coral holobiont phenotypic responses under stress have found varying levels of both inter- and intra-species resilience (Bove et al., 2019; Durante et al., 2019), however, the exact mechanisms behind these differences remain unclear. This variation in phenotypic responses to stress may be partially due to the diversity and community composition of the coral microbiome (Webster & Reusch, 2017). It has been proposed that corals exhibiting higher microbiome flexibility (*i.e.,* restructuring of microbial community) may possess a unique ecological advantage to better adapt under environmental change (Voolstra & Ziegler, 2020). These differences between ‘microbiome regulators’ (corals that maintain stable microbiomes;

e.g., Pocillopora verrucosa (Ziegler et al., 2019) and 'microbiome conformers' (corals that modulate microbiomes in response to environment; *e.g., Acropora hemprichii* (Ziegler et al., 2019)) may predict holobiont phenotypes under stress.

While significant advances in our understanding of Symbiodiniaceae-host (see (Mieog et al., 2009; Morris et al., 2019; Muller-Parker et al., 2015) and bacteria-host (see (Ainsworth et al., 2010; Thompson et al., 2014; Webster & Reusch, 2017) interactions have been made, the relationships between different members of the microbiome (i.e., Symbiodineaceae and bacteria) remain less well understood (but see (Matthews et al., 2020)). Only recently have we begun to characterize bacterial communities associated with cultured Symbiodiniaceae. In doing so, researchers reported highly diverse bacterial communities across algal strains, however, these algae only shared three of the same bacterial operational taxonomic units (OTU) (Lawson et al., 2018). These three OTUs have been proposed to be the core members of Symbiodiniaceae-bacterial assemblages, with Labrenzia being the most abundant (Lawson et al., 2018). Labrenzia is known to produce dimethylsulfoniopropionate (DMSP), which is thought to play a role in coral stress response (Curson et al., 2017; Jones & King, 2015). DMSP production has historically been attributed to Symbiodiniaceae, however, if these bacteria are producing DMSP then they may play a larger role in holobiont health than previously assumed. Further, this group was found to increase in abundance under thermal stress in cultures of *Cladocopium* spp. (Camp et al., 2020), suggesting it may also function in the algal stress response. In addition, algae-associated bacteria have been shown to provide important metabolites, such as carbohydrates (Neave et al., 2017), vitamin B12 (Croft et al. 2005), DMSP (O'Brien et al., 2016), and metals (Amin et al., 2015). These critical interactions between bacteria and Symbiodiniaceae are just beginning to be explored and represent an important avenue for future research to disentangle how these partnerships change under stress.

Corals are composed of several microhabitats (*e.g.,* tissue, mucus, gastrovascular cavity, skeleton) that can host different microorganisms (Bourne et al., 2016; Pollock et al., 2018; Sweet et al., 2011). These compartments each can have unique characteristics that support different microorganism assemblages that perform important biological processes within the holobiont. For example, anoxic conditions within coral gastric cavities at night likely support nitrogen fixation by anaerobic microorganisms (Bourne et al., 2016; Bove et al., 2020). This method of nitrogen fixation (*i.e.,* conversion of elemental nitrogen (N_2) to ammonium (NH_4^+)) can help sustain photosynthesis of primary producers - namely Symbiodiniaceae - within the coral (Rädecker et al., 2015). However, these compartments are often homogenized when sampling coral holobionts (Hughes et al., 2022), potentially concealing more specific microbiome signatures that may impact holobiont interactions (Armitage & Jones, 2019). Indeed, research investigating coral microbiome composition across compartments has found that the mucus microbiome most-closely resembles seawater, while tissue and skeletal communities were more similar to one another (Biagi et al., 2020; Pollock et al., 2018). It is clear that the microbiome plays a significant role in coral holobiont survival and resilience; however, many of the mechanisms underlying these interactions require further elucidation.

The coral microbiome is influenced by many factors, including host developmental stage (Bourne et al., 2016; Damjanovic et al., 2020; Sharp et al., 2012; van Oppen & Blackall, 2019), spatial scales (*e.g.,* across an individual (Rohwer et al., 2002) or geographic regions (Osman et al. 2020; Williams et al. 2022)), and vary in response to environmental change (van Oppen and Blackall 2019; Voolstra and Ziegler 2020) – especially thermal stress (reviewed in Maire et al.

2022). However, the ways that microbes interact with other holobiont partners is particularly important for understanding coral resilience under stress. For example, Symbiodiniaceae strains have been shown to associate with different bacterial assemblages that may promote thermal resilience of certain algal symbionts (Lawson et al. 2018). Indeed, recent work has identified relatively stable bacterial assemblages associated with *Durusdinium trenchii* even under thermal stress that may promote thermal resilience of the coral holobiont (Camp et al. 2020). Similar to warming, the effects of ocean acidification on coral microbiomes is species-specific (Meron et al. 2012; Biagi et al. 2020), with microbiome changes mostly occurring in the mucus layer (Biagi et al. 2020; Glasl et al. 2016). Interestingly, the microbiome at naturally low pH vent sites exhibit declines in putatively symbiotic Endozoicomonas taxa (O'Brien et al. 2016; Morrow et al. 2015) and increases in microorganisms associated with nitrogen fixation and cycling (Biagi et al. 2020), potentially due to concurrent shifts in the nitrogen cycle homeostasis. In fact, as seawater pH continues to decrease, nitrogen cycling in the oceans will also change to favor increased nitrogen fixation and reduced nitrification (O'Brien et al. 2016). This will lead to higher concentrations of biologically available forms of nitrogen (*i.e.,* ammonium and ammonia), which may support higher abundances of nitrogen cycling bacteria in corals (Jessen et al. 2013) and promote invasions of harmful microorganisms. Because alterations to environmental processes will continue to occur as global change persists, a deeper understanding of how multiple stressors interact with these microbial members of the coral holobiont is needed.

Proposed paths forward to disentangle the functional consequences of partner interactions

In order to better understand and predict coral symbiotic partnerships under environmental change, we propose several paths forward that would broaden our understanding of holobiont interactions and resulting phenotypes.

Increasing the number of host-algal pairings in studies

While the work conducted with Aiptasia has significantly shaped our understanding of host-Symbiodiniaceae pairings, the majority of this work has been conducted on a single, or very few, Aiptasia strains. However, we know that there is significant genetic variation in a variety of traits as well as different host-symbiont interactions can drive responses to the environment. Therefore, we encourage researchers to expand their work to include additional strains and to build out strain libraries to pull genotypes from diverse regions that experience unique environmental conditions and make these anemones broadly available to the community (**Figure 2**) (Aiptasia symbiosis resource). *Cassiopea xamachana* also represents a promising model system for some symbiosis related questions (reviewed in (Ohdera et al. 2018), all of the same recommendations exist for this model. We recognize that this work will involve challenges related to feasibility; however, experiments could be conducted sequentially to avoid overburdening researchers. We also encourage careful documentation of genetic background in husbandry and experiments.

This same expansion of cnidarian genetic backgrounds needs to also be considered in Symbiodiniaceae strains (**Figure 2**). Given the diversity of responses of Symbiodiniaceae within and between genera (LaJeunesse et al. 2018), and that each strain can have a unique interaction with a host genotype (Parkinson and Baums 2014), expansion of Symbiodiniaceae strains is critical to build our understanding of symbiosis establishment, maintenance, and loss. Further, it is important to consider how crossing these different host and algal symbiont genetic backgrounds may alter holobiont plasticity under both ambient and stressful conditions.

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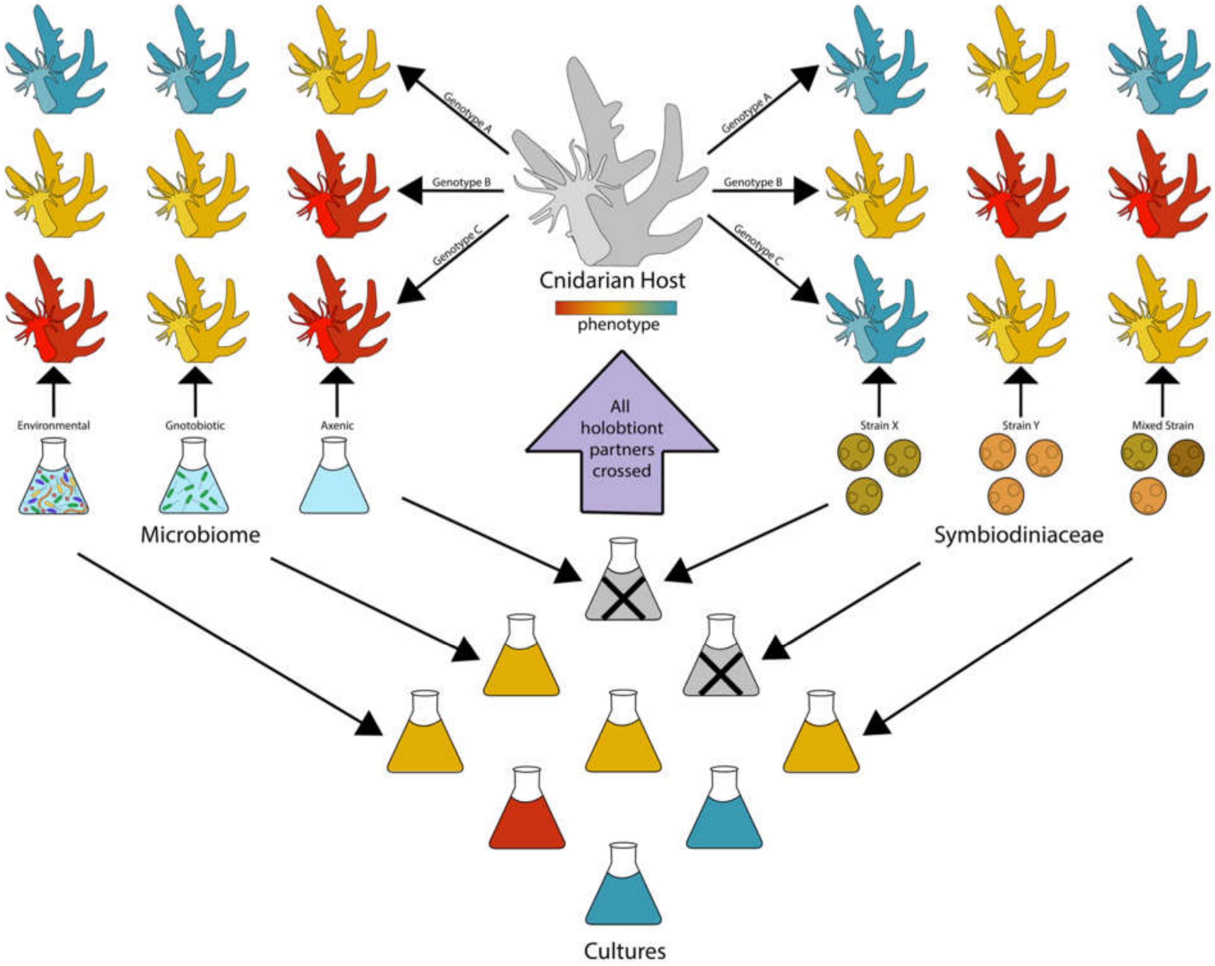

Figure 2. Proposed experimental approaches for future studies on cnidarian holobiont interactions. Focus on increasing host genetic diversity, different monoculture and/or known mixed Symbiodiniaceae strains and microbial communities of varying complexity. We expect that an individual may exhibit multiple phenotypes (depicted by color of host or culture flask; grey flasks represent failed cultures) depending on associated partners that may result in differing levels of resilience or resistance to stress. Crossing these different partnership pairings under both ambient and stressful conditions may elucidate roles of partners in holobiont responses that help better predict holobiont health. The purple arrow depicts future directions once baseline interactions are known across different partner pairings.

Conduct research with and without microbiome members

To study specific interactions between the host, algal symbiont, and microbial partners, we suggest employing the use of axenic and gnotobiotic cnidarian host and symbiont models (Figure 2). Several strains of axenic Symbiodiniaceae have been developed and successfully infected into Aiptasia models, but additional strains from diverse genera must be added and their associations with specific microbial communities should be further investigated (Matthews et al. 2020; Xiang et al. 2013). A protocol for the generation of microbe depleted Aiptasia has already been developed (Costa et al. 2021), showcasing progress towards the generation of completely axenic and eventually gnotobiotic Aiptasia strains. While this same approach applied to corals may be far in the future, researchers can inoculate corals with known beneficial microorganisms for corals (BMC) and evaluate the phenotypic outcomes to better understand the microbiome's role in coral holobiont health (Peixoto et al. 2017; Zhang et al. 2021). Studying the physiological and molecular responses of a host to targeted microorganisms or microorganism communities will uncover the role of these associations and their interactions and how they might be associated with holobiont stress, vulnerability, resistance, and resiliance.

Develop calcifying coral models

While the use of Aiptasia as a model system for corals provides valuable insights into many molecular functions of symbiosis, it remains a solitary polyp that does not calcify like reef building corals. Because of this, other coral models are emerging, including the experimental model species *Astrangia poculata*, a facultative symbiotic coral found across the western Atlantic coastline of the USA (Neff 2020). The use of *A. poculata* as an experimental model may allow for better assessment of the role of each symbiotic partner in coral stress responses (i.e., (Wuitchik et al. 2021; Sharp et al. 2017; Burmester et al. 2017; Burmester et al. 2018; Holcomb et al. 2012), especially because of the importance of symbiosis in coral skeletal growth rate (Chalker and Taylor 1975). In addition to *A. poculata*, several other reef building species are emerging as potential models, including *Oculina arbuscula* (Rivera and Davies 2021) and *Galaxea fascicularis* (Puntin et al. 2022), which can both have their symbiotic states experimentally manipulated.

Developing high throughput screening approaches

Development of additional technologies to rapidly assess phenotypic traits is needed and remains a major bottleneck to the types of experiments needed to understand complex interactions between multiple symbiotic partners. Recent high-throughput approaches for assessing thermal tolerance at the whole coral level (*e.g.,* coral bleaching automated stress systems (CBASS; Voolstra et al., 2020)) and single cell levels (Behrendt et al., 2020) have incorporated short thermal challenges followed by stress characterization through measurement of 1-2 physiological variables such as maximum PSII photochemical efficiency (F_v/F_m) and cell density. However, these approaches are focused on the algal symbiont and further development of additional traits is needed. In addition, while these approaches allow assessment of phenotypic responses of multiple individuals, there is room for growth to improve feasibility of studies crossing multiple genetic backgrounds of holobiont partners as encouraged above.

Leveraging novel technologies

Advances in biomedicine have been facilitated by the development of immortal cell lines for molecular manipulation and microscopy analysis. The generation of cnidarian cell lines has been met with limited success (see Fricano et al. 2020; Nowotny et al. 2021; Rosental et al. 2017;

Kawamura et al. 2021), although achievements have been made with respect to *in vivo* symbiosis (Kawamura et al. 2021). Functional cell lines of cnidarian models will allow researchers to apply exogenous biochemical stimuli – such as TGFβ and nitric oxide –, which would expand our knowledge of gene function. Current studies using mammalian cell lines expressing cnidarian proteins (i.e., Williams et al. 2018)) may be missing key post-translational modifications and/or protein-protein interactions that only occur in native cells.

Single-cell RNA-seq (scRNAseq) is a developing technology that has been successful in several Cnidaria taxa (Hu et al. 2020; Levy et al. 2021; Sebé-Pedrós et al. 2018; Chari et al. 2021). This technique applied to gene expression patterns under different stimuli will unveil the cell types responsible for organismal responses to a stressor or when in symbiosis with different symbiont strains. The addition of spatial scRNAseq (reviewed in Longo et al. 2021) or multiplexed error-robust fluorescence in situ hybridization (Chen et al. 2015) will deepen our understanding of the spatial organization of cell types relative to the organismal body plan. Additionally, proteomic (i.e., (Jaimes-Becerra et al. 2019; Oakley et al. 2016; Sproles et al. 2019; Tortorelli et al. 2022) and metabolomic studies (i.e., (Hillyer et al. 2017; Matthews et al. 2017; Matthews et al. 2018; Williams et al. 2020)) are essential for determining functional patterns of intra- and inter-cellular regulation under different conditions and holobiont partnerships. Further, the development and implementation of the CRISPR-Cas9 system in cnidaria (Cleves et al. 2018) will allow researchers to resolve the functional role of specific genes. Combining these data-rich methods in a multi omic context will improve informed diagnostic approaches to coral work *in situ.*

Conclusions

Coral bleaching is perhaps one of the most intriguing processes in nature because it represents the outcome of cellular dysfunction between symbiotic partners that is easily observed with the naken eye, but its influence spans ecosystem scales. A major goal of coral reef science is to understand and predict these symbiosis outcomes under increasingly changing environments, but this work remains a serious challenge due to the complex interactions between holobiont members, each of which possess immense genetic diversity. While an impressive amount of work has uncovered basic mechanisms involved in cnidarian-algal symbiosis and descriptive studies of microbiome associations are hypothesis generating tools, we propose that mechanistic research would benefit from expanding to use a broader repertoire of genetic diversity and leveraging emerging technologies. We are optimistic that the many ways in which these partners interact will offer hope to the coral reef crisis as we are just scratching the surface of understanding how these partners can mix and match, which may offer fuel to the coral holobiont to adapt and acclimate to their rapidly changing environments.

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Conflict of Interest

The authors declare no conflict of interest.

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References

Abrego, D., Ulstrup, K. E., Willis, B. L., & van Oppen, M. J. H. (2008). Species-specific interactions between algal endosymbionts and coral hosts define their bleaching response to heat and light stress. *Proceedings. Biological Sciences / The Royal Society*, *275*(1648), 2273–2282.

Agostini, S., Suzuki, Y., Higuchi, T., Casareto, B. E., Yoshinaga, K., Nakano, Y., & Fujimura, H. (2012). Biological and chemical characteristics of the coral gastric cavity. *Coral Reefs* , *31*(1), 147–156.

Aichelman, H. E., Bove, C. B., Castillo, K. D., Boulton, J. M., Knowlton, A. C., Nieves, O. C., Ries, J. B., & Davies, S. W. (2021). Exposure duration modulates the response of Caribbean corals to global change stressors. *Limnology and Oceanography*, *66*(8), 3100–3115.

Ainsworth, T. D., Thurber, R. V., & Gates, R. D. (2010). The future of coral reefs: a microbial perspective. *Trends in Ecology & Evolution*, *25*(4), 233–240.

Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell*, *124*(4), 783–801.

Ali, A., Kriefall, N. G., Emery, L. E., Kenkel, C. D., Matz, M. V., & Davies, S. W. (2019). Recruit symbiosis establishment and Symbiodiniaceae composition influenced by adult corals and reef sediment. *Coral Reefs* , *38*(3), 405–415.

Amin, S. A., Hmelo, L. R., van Tol, H. M., Durham, B. P., Carlson, L. T., Heal, K. R., Morales, R. L., Berthiaume, C. T., Parker, M. S., Djunaedi, B., Ingalls, A. E., Parsek, M. R., Moran, M. A., & Armbrust, E. V. (2015). Interaction and signalling between a cosmopolitan phytoplankton and associated bacteria. *Nature*, *522*(7554), 98–101.

Armitage, D. W., & Jones, S. E. (2019). How sample heterogeneity can obscure the signal of microbial interactions. *The ISME Journal*, *13*(11), 2639–2646.

Baird, A. H., Guest, J. R., & Willis, B. L. (2009). Systematic and Biogeographical Patterns in the Reproductive Biology of Scleractinian Corals. *Annual Review of Ecology, Evolution, and Systematics*, *40*(1), 551–571.

Baird, A. H., Yakovleva, I. M., Harii, S., Sinniger, F., & Hidaka, M. (2021). Environmental constraints on the mode of symbiont transmission in corals. *Journal of Experimental Marine Biology and Ecology*, *538*, 151499.

Bairos-Novak, K. R., Hoogenboom, M. O., van Oppen, M. J. H., & Connolly, S. R. (2021). Coral adaptation to climate change: Meta-analysis reveals high heritability across multiple traits. *Global Change Biology*, *27*(22), 5694–5710.

Baker, A. C. (2003). Flexibility and Specificity in Coral-Algal Symbiosis: Diversity, Ecology, and Biogeography of Symbiodinium. *Annual Review of Ecology, Evolution, and Systematics*, *34*(1), 661–689.

Baker, A. C., & Romanski, A. M. (2007). Multiple symbiotic partnerships are common in scleractinian corals, but not in octocorals: Comment on Goulet (2006). *Marine Ecology Progress Series*, *335*, 237–242.

Baumgarten, S., Simakov, O., Esherick, L. Y., Liew, Y. J., Lehnert, E. M., Michell, C. T., Li, Y., Hambleton, E. A., Guse, A., Oates, M. E., Gough, J., Weis, V. M., Aranda, M., Pringle, J. R., & Voolstra, C. R. (2015). The genome of *Aiptasia*, a sea anemone model for coral symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(38), 11893–11898.

Behrendt, L., Salek, M. M., Trampe, E. L., Fernandez, V. I., Lee, K. S., Kühl, M., & Stocker, R. (2020). PhenoChip: A single-cell phenomic platform for high-throughput photophysiological analyses of microalgae. *Science Advances*, *6*(36). https://doi.org/10.1126/sciadv.abb2754

Beltrán, V. H., Puill-Stephan, E., Howells, E., Flores-Moya, A., Doblin, M., Núñez-Lara, E., Escamilla, V., López, T., & van Oppen, M. J. H. (2021). Physiological diversity among sympatric, conspecific endosymbionts of coral (*Cladocopium* C1$^{\text{acro}}$) from the Great Barrier Reef. *Coral Reefs* . https://doi.org/10.1007/s00338-021-02092-z

Berkelmans, R., & Van Oppen, M. J. H. (2006). The role of zooxanthellae in the thermal tolerance of corals: A “nugget of hope”for coral reefs in an era of climate change. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1599), 2305–2312.

Biagi, E., Caroselli, E., Barone, M., Pezzimenti, M., Teixido, N., Soverini, M., Rampelli, S., Turroni, S., Gambi, M. C., Brigidi, P., Goffredo, S., & Candela, M. (2020). Patterns in microbiome composition differ with ocean acidification in anatomic compartments of the Mediterranean coral Astroides calycularis living at CO2 vents. *The Science of the Total Environment*, *724*, 138048.

Bickford, D., Lohman, D. J., Sodhi, N. S., Ng, P. K. L., Meier, R., Winker, K., Ingram, K. K., & Das, I. (2007). Cryptic species as a window on diversity and conservation. *Trends in Ecology & Evolution*, *22*(3), 148–155.

Bongaerts, P., Riginos, C., Hay, K. B., van Oppen, M. J. H., Hoegh-Guldberg, O., & Dove, S. (2011). Adaptive divergence in a scleractinian coral: physiological adaptation of Seriatopora hystrix to shallow and deep reef habitats. *BMC Evolutionary Biology*, *11*, 303.

Bourne, D. G., Morrow, K. M., & Webster, N. S. (2016). Insights into the Coral Microbiome: Underpinning the Health and Resilience of Reef Ecosystems. *Annual Review of Microbiology*, *70*, 317–340.

Bove, C. B., Ries, J. B., Davies, S. W., Westfield, I. T., Umbanhowar, J., & Castillo, K. D. (2019). Common Caribbean corals exhibit highly variable responses to future acidification and warming. *Proceedings. Biological Sciences / The Royal Society*, *286*(1900), 20182840.

Bove, C. B., Whitehead, R. F., & Szmant, A. M. (2020). Responses of coral gastrovascular cavity pH during light and dark incubations to reduced seawater pH suggest species-specific responses to the effects of ocean acidification on calcification. *Coral Reefs* , *39*(6), 1675–1691.

Brown, B. E., & Bythell, J. C. (2005). Perspectives on mucus secretion in reef corals. *Marine Ecology Progress Series*, *296*, 291–309.

Burns, J. A., Zhang, H., Hill, E., Kim, E., & Kerney, R. (2017). Transcriptome analysis illuminates the nature of the intracellular interaction in a vertebrate-algal symbiosis. *eLife*, *6*. https://doi.org/10.7554/eLife.22054

Camp, E. F., Kahlke, T., Nitschke, M. R., Varkey, D., Fisher, N. L., Fujise, L., Goyen, S., Hughes, D. J., Lawson, C. A., Ros, M., Woodcock, S., Xiao, K., Leggat, W., & Suggett, D. J. (2020). Revealing changes in the microbiome of Symbiodiniaceae under thermal stress. *Environmental Microbiology*, *22*(4), 1294–1309.

Cleves, P. A., Strader, M. E., Bay, L. K., Pringle, J. R., & Matz, M. V. (2018). CRISPR/Cas9-mediated genome editing in a reef-building coral. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(20), 5235–5240.

Costanza, R., de Groot, R., Sutton, P., van der Ploeg, S., Anderson, S. J., Kubiszewski, I., Farber, S., & Turner, R. K. (2014). Changes in the global value of ecosystem services. *Global Environmental Change: Human and Policy Dimensions*, *26*, 152–158.

Curson, A. R. J., Liu, J., Bermejo Martínez, A., Green, R. T., Chan, Y., Carrión, O., Williams, B.

T., Zhang, S.-H., Yang, G.-P., Bulman Page, P. C., Zhang, X.-H., & Todd, J. D. (2017). Dimethylsulfoniopropionate biosynthesis in marine bacteria and identification of the key gene in this process. *Nature Microbiology*, *2*, 17009.

Damjanovic, K., Menéndez, P., Blackall, L. L., & van Oppen, M. J. H. (2020). Early Life Stages of a Common Broadcast Spawning Coral Associate with Specific Bacterial Communities Despite Lack of Internalized Bacteria. *Microbial Ecology*, *79*(3), 706–719.

Davies, S., Gamache, M. H., Howe-Kerr, L. I., Kriefall, N. G., Baker, A. C., Banaszak, A. T., Bay, L. K., Bellantuono, A. J., Bhattacharya, D., Chan, C. X., Claar, D. C., Coffroth, M. A., Cunning, R., Davy, S. K., del Campo, J., Diaz-Almeyda, E. M., Frommlet, J. C., Fuess, L. E., Gonzalez-Pech, R. A., … Parkinson, J. E. (2022). Building consensus around the assessment and interpretation of Symbiodiniaceae diversity. In *Preprints*. https://doi.org/10.20944/preprints202206.0284.v1

Davies, S. W., Moreland, K. N., Wham, D. C., Kanke, M. R., & Matz, M. V. (2020). Cladocopium community divergence in two Acropora coral hosts across multiple spatial scales. *Molecular Ecology*, *29*(23), 4559–4572.

Davies, S. W., Ries, J. B., Marchetti, A., & Castillo, K. D. (2018). Symbiodinium functional diversity in the coral Siderastrea siderea is influenced by thermal stress and reef environment, but not ocean acidification. *Frontiers in Marine Science*, *5*. https://doi.org/10.3389/fmars.2018.00150

Davy, S. K., Lucas, I. A. N., & Turner, J. R. (1997). Uptake and Persistence of Homologous and Heterologous Zooxanthellae in the Temperate Sea Anemone Cereus pedunculatus (Pennant). *The Biological Bulletin*, *192*(2), 208–216.

De'ath, G., Fabricius, K. E., Sweatman, H., & Puotinen, M. (2012). The 27-year decline of coral cover on the Great Barrier Reef and its causes. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(44), 17995–17999.

DeSalvo, M. K., Sunagawa, S., Voolstra, C. R., & Medina, M. (2010). Transcriptomic responses to heat stress and bleaching in the elkhorn coral Acropora palmata. *Marine Ecology Progress Series*, *402*, 97–113.

Detournay, O., Schnitzler, C. E., Poole, A., & Weis, V. M. (2012). Regulation of cnidarian-dinoflagellate mutualisms: Evidence that activation of a host TGFβ innate immune pathway promotes tolerance of the symbiont. *Developmental and Comparative Immunology*, *38*(4), 525–537.

Díaz-Almeyda, E. M., Prada, C., Ohdera, A. H., Moran, H., Civitello, D. J., Iglesias-Prieto, R., Carlo, T. A., LaJeunesse, T. C., & Medina, M. (2017). Intraspecific and interspecific variation in thermotolerance and photoacclimation in *Symbiodinium* dinoflagellates. *Proceedings. Biological Sciences / The Royal Society*, *284*(1868). https://doi.org/10.1098/rspb.2017.1767

Dixon, G. B., Davies, S. W., Aglyamova, G. A., Meyer, E., Bay, L. K., & Matz, M. V. (2015). CORAL REEFS. Genomic determinants of coral heat tolerance across latitudes. *Science*, *348*(6242), 1460–1462.

Durante, M. K., Baums, I. B., Williams, D. E., Vohsen, S., & Kemp, D. W. (2019). What drives phenotypic divergence among coral clonemates of Acropora palmata? *Molecular Ecology*, *28*(13), 3208–3224.

Eddy, T. D., Lam, V. W. Y., Reygondeau, G., Cisneros-Montemayor, A. M., Greer, K., Palomares, M. L. D., Bruno, J. F., Ota, Y., & Cheung, W. W. L. (2021). Global decline in capacity of coral reefs to provide ecosystem services. *One Earth*, *4*(9), 1278–1285.

Emery, M. A., Dimos, B. A., & Mydlarz, L. D. (2021). Cnidarian Pattern Recognition Receptor Repertoires Reflect Both Phylogeny and Life History Traits. *Frontiers in Immunology*, *12*, 689463.

Epstein, H. E., Torda, G., Munday, P. L., & van Oppen, M. J. H. (2019). Parental and early life stage environments drive establishment of bacterial and dinoflagellate communities in a common coral. *The ISME Journal*, *13*(6), 1635–1638.

Fabricius, K. E., Mieog, J. C., Colin, P. L., Idip, D., & van Oppen, M. J. H. (2004). Identity and diversity of coral endosymbionts (zooxanthellae) from three Palauan reefs with contrasting bleaching, temperature and shading histories. *Molecular Ecology*, *13*(8), 2445–2458.

Fifer, J. E., Yasuda, N., Yamakita, T., Bove, C. B., & Davies, S. W. (2022). Genetic divergence and range expansion in a western North Pacific coral. *The Science of the Total Environment*, *813*, 152423.

Fitt, W. K., Chang, S. S., & Trench, R. K. (1981). Motility Patterns of Different Strains of the Symbiotic Dinoflagellate Symbiodinium (=Gymnodinium) microadriaticum (Freudenthal) in Culture. *Bulletin of Marine Science*, *31*(2), 436–443.

Fitt, W. K., & Trench, R. K. (1983). The relation of Diel patterns of cell division to Diel patterns of motility in the symbiotic dinoflagellate Symbiodinium microadria ticum Freudenthal in culture. *The New Phytologist*, *94*(3), 421–432.

Forsman, Z. H., Ritson-Williams, R., Tisthammer, K. H., Knapp, I. S. S., & Toonen, R. J. (2020). Host-symbiont coevolution, cryptic structure, and bleaching susceptibility, in a coral species complex (Scleractinia; Poritidae). *Scientific Reports*, *10*(1), 16995.

Fuller, Z. L., Mocellin, V. J. L., Morris, L. A., Cantin, N., Shepherd, J., Sarre, L., Peng, J., Liao, Y., Pickrell, J., Andolfatto, P., Matz, M., Bay, L. K., & Przeworski, M. (2020). Population genetics of the coral Acropora millepora: Toward genomic prediction of bleaching. *Science*, *369*(6501). https://doi.org/10.1126/science.aba4674

Gilmore, T. D. (2006). Introduction to NF-kappaB: players, pathways, perspectives. *Oncogene*, *25*(51), 6680–6684.

Glynn, P. W. (1991). Coral reef bleaching in the 1980s and possible connections with global warming. *Trends in Ecology & Evolution*, *6*(6), 175–179.

Gómez-Corrales, M., & Prada, C. (2020). Cryptic lineages respond differently to coral bleaching [Review of *Cryptic lineages respond differently to coral bleaching*]. *Molecular Ecology*, *29*(22), 4265–4273.

Gorman, L. M., Konciute, M. K., Cui, G., Oakley, C. A., Grossman, A. R., Weis, V. M., Aranda, M., & Davy, S. K. (2022). Symbiosis with Dinoflagellates Alters Cnidarian Cell-Cycle Gene Expression. *Cellular Microbiology*, *2022*. https://doi.org/10.1155/2022/3330160

Hartmann, A. C., Baird, A. H., Knowlton, N., & Huang, D. (2017). The Paradox of Environmental Symbiont Acquisition in Obligate Mutualisms. *Current Biology: CB*, *27*(23), 3711–3716.e3.

Hawkins, T. D., Hagemeyer, J. C. G., & Warner, M. E. (2016). Temperature moderates the infectiousness of two conspecific *Symbiodinium* strains isolated from the same host population. *Environmental Microbiology*, *18*(12), 5204–5217.

Hernandez-Agreda, A., Gates, R. D., & Ainsworth, T. D. (2017). Defining the Core Microbiome in Corals' Microbial Soup. *Trends in Microbiology*, *25*(2), 125–140.

Hoadley, K. D., Lewis, A. M., Wham, D. C., Pettay, D. T., Grasso, C., Smith, R., Kemp, D. W., LaJeunesse, T. C., & Warner, M. E. (2019). Host-symbiont combinations dictate the photo-physiological response of reef-building corals to thermal stress. *Scientific Reports*, *9*(1), 9985.

Howells, E. J., Beltran, V. H., Larsen, N. W., Bay, L. K., Willis, B. L., & van Oppen, M. J. H. (2011a). Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change*, *2*(2), 116–120.

Howells, E. J., Beltran, V. H., Larsen, N. W., Bay, L. K., Willis, B. L., & van Oppen, M. J. H. (2011b). Coral thermal tolerance shaped by local adaptation of photosymbionts. *Nature Climate Change*, *2*(2), 116–120.

Howells, E. J., Berkelmans, R., van Oppen, M. J. H., Willis, B. L., & Bay, L. K. (2013). Historical thermal regimes define limits to coral acclimatization. *Ecology*, *94*(5), 1078–1088.

Hughes, D. J., Raina, J.-B., Nielsen, D. A., Suggett, D. J., & Kühl, M. (2022). Disentangling compartment functions in sessile marine invertebrates. *Trends in Ecology & Evolution*. https://doi.org/10.1016/j.tree.2022.04.008

Hume, B. C. C., Mejia-Restrepo, A., Voolstra, C. R., & Berumen, M. L. (2020). Fine-scale delineation of Symbiodiniaceae genotypes on a previously bleached central Red Sea reef system demonstrates a prevalence of coral host-specific associations. *Coral Reefs* , *39*(3), 583–601.

Jiang, J., Wang, A., Deng, X., Zhou, W., Gan, Q., & Lu, Y. (2021). How Symbiodiniaceae meets the challenges of life during coral bleaching. *Coral Reefs* , *40*(4), 1339–1353.

Jones, G. B., & King, S. (2015). Dimethylsulphoniopropionate (DMSP) as an Indicator of Bleaching Tolerance in Scleractinian Corals. *Journal of Marine Science and Engineering*, *3*(2), 444–465.

Jury, C. P., Delano, M. N., & Toonen, R. J. (2019). High heritability of coral calcification rates and evolutionary potential under ocean acidification. *Scientific Reports*, *9*(1), 20419.

Kenkel, C. D., & Matz, M. V. (2016). Gene expression plasticity as a mechanism of coral adaptation to a variable environment. *Nature Ecology & Evolution*, *1*(1), 14.

Kenkel, C. D., Meyer, E., & Matz, M. V. (2013). Gene expression under chronic heat stress in populations of the mustard hill coral (Porites astreoides) from different thermal environments. *Molecular Ecology*, *22*(16), 4322–4334.

Kenkel, C. D., Setta, S. P., & Matz, M. V. (2015). Heritable differences in fitness-related traits among populations of the mustard hill coral, Porites astreoides. *Heredity*, *115*(6), 509–516.

Kimes, N. E., Van Nostrand, J. D., Weil, E., Zhou, J., & Morris, P. J. (2010). Microbial functional structure of Montastraea faveolata, an important Caribbean reef-building coral, differs between healthy and yellow-band diseased colonies. *Environmental Microbiology*, *12*(2), 541–556.

Knowlton, N. (1993). Sibling Species in the Sea. *Annual Review of Ecology and Systematics*, *24*, 189–216.

Kriefall, N. G., Kanke, M. R., Aglyamova, G. V., & Davies, S. W. (2022). Reef environments shape microbial partners in a highly connected coral population. *Proceedings. Biological Sciences / The Royal Society*, *289*(1967), 20212459.

LaJeunesse, T. C., Parkinson, J. E., Gabrielson, P. W., Jeong, H. J., Reimer, J. D., Voolstra, C. R., & Santos, S. R. (2018). Systematic Revision of Symbiodiniaceae Highlights the Antiquity and Diversity of Coral Endosymbionts. *Current Biology: CB*, *28*(16), 2570–2580.e6.

LaJeunesse, T. C., Wiedenmann, J., Casado-Amezúa, P., D'Ambra, I., Turnham, K. E., Nitschke, M. R., Oakley, C. A., Goffredo, S., Spano, C. A., Cubillos, V. M., Davy, S. K., & Suggett,

D. J. (2021). Revival of *Philozoon* Geddes for host-specialized dinoflagellates, “zooxanthellae”, in animals from coastal temperate zones of northern and southern hemispheres. *European Journal of Phycology*, 1–15.

Lawson, C. A., Raina, J.-B., Kahlke, T., Seymour, J. R., & Suggett, D. J. (2018). Defining the core microbiome of the symbiotic dinoflagellate, Symbiodinium. *Environmental Microbiology Reports*, *10*(1), 7–11.

Lehnert, E. M., Mouchka, M. E., Burriesci, M. S., Gallo, N. D., Schwarz, J. A., & Pringle, J. R. (2014). Extensive differences in gene expression between symbiotic and aposymbiotic cnidarians. *G3* , *4*(2), 277–295.

Levy, S., Elek, A., Grau-Bové, X., Menéndez-Bravo, S., Iglesias, M., Tanay, A., Mass, T., & Sebé-Pedrós, A. (2021). A stony coral cell atlas illuminates the molecular and cellular basis of coral symbiosis, calcification, and immunity. *Cell*, *184*(11), 2973–2987.e18.

Logan, D. D. K., LaFlamme, A. C., Weis, V. M., & Davy, S. K. (2010). Flow-cytometric characterization of the cell-surface glycans of symbiotic dinoflagellates (Symbiodinium spp.)1. *Journal of Phycology*, *46*(3), 525–533.

Mansfield, K. M., Carter, N. M., Nguyen, L., Cleves, P. A., Alshanbayeva, A., Williams, L. M., Crowder, C., Penvose, A. R., Finnerty, J. R., Weis, V. M., Siggers, T. W., & Gilmore, T. D. (2017). Transcription factor NF-κB is modulated by symbiotic status in a sea anemone model of cnidarian bleaching. *Scientific Reports*, *7*(1), 16025.

Mansfield, K. M., Cleves, P. A., Van Vlack, E., Kriefall, N. G., Benson, B. E., Camacho, D. J., Hemond, O., Pedroza, M., Siggers, T., Pringle, J. R., Davies, S. W., & Gilmore, T. D. (2019). Varied effects of algal symbionts on transcription factor NF-κB in a sea anemone and a coral: possible roles in symbiosis and thermotolerance. In *bioRxiv* (p. 640177).

https://doi.org/10.1101/640177

Mansfield, K. M., & Gilmore, T. D. (2019). Innate immunity and cnidarian-Symbiodiniaceae mutualism. *Developmental and Comparative Immunology*, *90*, 199–209.

Mansour, J. S., Pollock, F. J., Díaz-Almeyda, E., Iglesias-Prieto, R., & Medina, M. (2018). Intra- and interspecific variation and phenotypic plasticity in thylakoid membrane properties across two *Symbiodinium* Clades. *Coral Reefs* . https://doi.org/10.1007/s00338-018-1710-1

Marhoefer, S. R., Zenger, K. R., Strugnell, J. M., Logan, M., van Oppen, M. J. H., Kenkel, C. D., & Bay, L. K. (2021). Signatures of adaptation and acclimatization to reef flat and slope habitats in the coral *Pocillopora damicornis*. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.704709

Matthews, J. L., Crowder, C. M., Oakley, C. A., Lutz, A., Roessner, U., Meyer, E., Grossman, A. R., Weis, V. M., & Davy, S. K. (2017). Optimal nutrient exchange and immune responses operate in partner specificity in the cnidarian-dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(50), 13194–13199.

Matthews, J. L., Oakley, C. A., Lutz, A., Hillyer, K. E., Roessner, U., Grossman, A. R., Weis, V. M., & Davy, S. K. (2018). Partner switching and metabolic flux in a model cnidarian-dinoflagellate symbiosis. *Proceedings. Biological Sciences / The Royal Society*, *285*(1892). https://doi.org/10.1098/rspb.2018.2336

Matthews, J. L., Raina, J.-B., Kahlke, T., Seymour, J. R., van Oppen, M. J. H., & Suggett, D. J. (2020). Symbiodiniaceae-bacteria interactions: rethinking metabolite exchange in reef-building corals as multi-partner metabolic networks. *Environmental Microbiology*, *22*(5), 1675–1687.

Medrano, E., Merselis, D. G., Bellantuono, A. J., & Rodriguez-Lanetty, M. (2019). Proteomic

Basis of Symbiosis: A Heterologous Partner Fails to Duplicate Homologous Colonization in a Novel Cnidarian- Symbiodiniaceae Mutualism. *Frontiers in Microbiology*, *10*, 1153.

Meyer, E., Aglyamova, G. V., & Matz, M. V. (2011). Profiling gene expression responses of coral larvae (Acropora millepora) to elevated temperature and settlement inducers using a novel RNA-Seq procedure. *Molecular Ecology*, *20*(17), 3599–3616.

Mieog, J. C., Olsen, J. L., Berkelmans, R., Bleuler-Martinez, S. A., Willis, B. L., & van Oppen, M. J. H. (2009). The roles and interactions of symbiont, host and environment in defining coral fitness. *PloS One*, *4*(7), e6364.

Moberg, F., & Folke, C. (1999). Ecological goods and services of coral reef ecosystems. *Ecological Economics: The Journal of the International Society for Ecological Economics*, *29*(2), 215–233.

Morris, L. A., Voolstra, C. R., Quigley, K. M., Bourne, D. G., & Bay, L. K. (2019). Nutrient Availability and Metabolism Affect the Stability of Coral-Symbiodiniaceae Symbioses. *Trends in Microbiology*, *27*(8), 678–689.

Muller-Parker, G., D'Elia, C. F., & Cook, C. B. (2015). Interactions Between Corals and Their Symbiotic Algae. In C. Birkeland (Ed.), *Coral Reefs in the Anthropocene* (pp. 99–116). Springer Netherlands.

Muscatine, L., & Cernichiari, E. (1969). ASSIMILATION OF PHOTOSYNTHETIC PRODUCTS OF ZOOXANTHELLAE BY A REEF CORAL. *The Biological Bulletin*, *137*(3), 506–523.

Muscatine, L., & Porter, J. W. (1977). Reef Corals: Mutualistic Symbioses Adapted to Nutrient-Poor Environments. *Bioscience*, *27*(7), 454–460.

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

Nitschke, M. R., Craveiro, S. C., Brandão, C., Fidalgo, C., Serôdio, J., Calado, A. J., & Frommlet, J. C. (2020). Description of *Freudenthalidium* gen. nov. and *Halluxium* gen. nov. to formally recognize Clades Fr3 and H as genera in the family Symbiodiniaceae (Dinophyceae). *Journal of Phycology*. https://doi.org/10.1111/jpy.12999

Nyholm, S. V., & Graf, J. (2012). Knowing your friends: invertebrate innate immunity fosters beneficial bacterial symbioses. *Nature Reviews. Microbiology*, *10*(12), 815–827.

Oakley, C. A., Ameismeier, M. F., Peng, L., Weis, V. M., Grossman, A. R., & Davy, S. K. (2016). Symbiosis induces widespread changes in the proteome of the model cnidarian Aiptasia. *Cellular Microbiology*, *18*(7), 1009–1023.

O'Brien, P. A., Morrow, K. M., Willis, B. L., & Bourne, D. G. (2016). Implications of Ocean Acidification for Marine Microorganisms from the Free-Living to the Host-Associated. *Frontiers in Marine Science*, *3*. https://doi.org/10.3389/fmars.2016.00047

Osman, E. O., Suggett, D. J., Voolstra, C. R., Pettay, D. T., Clark, D. R., Pogoreutz, C., Sampayo, E. M., Warner, M. E., & Smith, D. J. (2020). Coral microbiome composition along the northern Red Sea suggests high plasticity of bacterial and specificity of endosymbiotic dinoflagellate communities. *Microbiome*, *8*(1), 8.

Parkinson, J. E., Baumgarten, S., Michell, C. T., Baums, I. B., LaJeunesse, T. C., & Voolstra, C. R. (2016). Gene expression variation resolves species and individual strains among coral-associated dinoflagellates within the genus *Symbiodinium*. *Genome Biology and Evolution*, *8*(3), 665–680.

Parkinson, J. E., & Baums, I. B. (2014). The extended phenotypes of marine symbioses:

ecological and evolutionary consequences of intraspecific genetic diversity in coral–algal associations. *Frontiers in Microbiology*, *5*, 445.

Pochon, X., & LaJeunesse, T. C. (2021). *Miliolidium* n. gen, a new Symbiodiniacean genus whose members associate with soritid foraminifera or are free-living. *The Journal of Eukaryotic Microbiology*, e12856.

Pogoreutz, C., Rädecker, N., Cárdenas, A., Gärdes, A., Voolstra, C. R., & Wild, C. (2017). Sugar enrichment provides evidence for a role of nitrogen fixation in coral bleaching. *Global Change Biology*, *23*(9), 3838–3848.

Pollock, F. J., McMinds, R., Smith, S., Bourne, D. G., Willis, B. L., Medina, M., Thurber, R. V., & Zaneveld, J. R. (2018). Coral-associated bacteria demonstrate phylosymbiosis and cophylogeny. *Nature Communications*, *9*(1), 4921.

Poole, A. Z., & Weis, V. M. (2014). TIR-domain-containing protein repertoire of nine anthozoan species reveals coral-specific expansions and uncharacterized proteins. *Developmental and Comparative Immunology*, *46*(2), 480–488.

Prada, C., DeBiasse, M. B., Neigel, J. E., Yednock, B., Stake, J. L., Forsman, Z. H., Baums, I. B., & Hellberg, M. E. (2014). Genetic species delineation among branching Caribbean Porites corals. *Coral Reefs* , *33*(4), 1019–1030.

Prada, C., & Hellberg, M. E. (2013). Long prereproductive selection and divergence by depth in a Caribbean candelabrum coral. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(10), 3961–3966.

Prada, C., & Hellberg, M. E. (2021). Speciation-by-depth on coral reefs: Sympatric divergence with gene flow or cryptic transient isolation? *Journal of Evolutionary Biology*, *34*(1), 128–137.

Putnam, H. M. (2021). Avenues of reef-building coral acclimatization in response to rapid environmental change. *The Journal of Experimental Biology*, *224*(Pt Suppl 1). https://doi.org/10.1242/jeb.239319

Quigley, K. M., Baker, A. C., Coffroth, M. A., Willis, B. L., & van Oppen, M. J. H. (2018). Bleaching Resistance and the Role of Algal Endosymbionts. In M. J. H. van Oppen & J. M. Lough (Eds.), *Coral Bleaching: Patterns, Processes, Causes and Consequences* (pp. 111–151). Springer International Publishing.

Quigley, K. M., Willis, B. L., & Kenkel, C. D. (2019). Transgenerational inheritance of shuffled symbiont communities in the coral Montipora digitata. *Scientific Reports*, *9*(1), 13328.

Rädecker, N., Pogoreutz, C., Voolstra, C. R., Wiedenmann, J., & Wild, C. (2015). Nitrogen cycling in corals: the key to understanding holobiont functioning? *Trends in Microbiology*, *23*(8), 490–497.

Rädecker, N., Raina, J.-B., Pernice, M., Perna, G., Guagliardo, P., Kilburn, M. R., Aranda, M., & Voolstra, C. R. (2018). Using Aiptasia as a Model to Study Metabolic Interactions in Cnidarian-Symbiodinium Symbioses. *Frontiers in Physiology*, *9*, 214.

Reaka-Kudla, M. L., Wilson, D. E., & Wilson, E. O. (1996). *Biodiversity II: Understanding and Protecting Our Biological Resources*. Joseph Henry Press.

Reich, H. G., Kitchen, S. A., Stankiewicz, K. H., Devlin-Durante, M., Fogarty, N. D., & Baums, I. B. (2021). Genomic variation of an endosymbiotic dinoflagellate (Symbiodinium “fitti”) among closely related coral hosts. *Molecular Ecology*, *30*(14), 3500–3514.

Reich, H. G., Robertson, D. L., & Goodbody-Gringley, G. (2017). Do the shuffle: Changes in Symbiodinium consortia throughout juvenile coral development. *PloS One*, *12*(2), e0171768.

Rippe, J. P., Dixon, G., Fuller, Z. L., Liao, Y., & Matz, M. (2021). Environmental specialization and cryptic genetic divergence in two massive coral species from the Florida Keys Reef Tract. *Molecular Ecology*, *30*(14), 3468–3484.

Rivera, H. E., & Davies, S. W. (2021). Symbiosis maintenance in the facultative coral, Oculina arbuscula, relies on nitrogen cycling, cell cycle modulation, and immunity. *Scientific Reports*, *11*(1), 21226.

Robbins, S. J., Singleton, C. M., Chan, C. X., Messer, L. F., Geers, A. U., Ying, H., Baker, A., Bell, S. C., Morrow, K. M., Ragan, M. A., Miller, D. J., Forêt, S., ReFuGe2020 Consortium, Voolstra, C. R., Tyson, G. W., & Bourne, D. G. (2019). A genomic view of the reef-building coral Porites lutea and its microbial symbionts. *Nature Microbiology*, *4*(12), 2090–2100.

Rodriguez-Lanetty, M., Phillips, W. S., & Weis, V. M. (2006). Transcriptome analysis of a cnidarian-dinoflagellate mutualism reveals complex modulation of host gene expression. *BMC Genomics*, *7*, 23.

Rohwer, F., & Kelley, S. (2004). Culture-Independent Analyses of Coral-Associated Microbes. In E. Rosenberg & Y. Loya (Eds.), *Coral Health and Disease* (pp. 265–277). Springer Berlin Heidelberg.

Rohwer, F., Seguritan, V., Azam, F., & Knowlton, N. (2002). Diversity and distribution of coral-associated bacteria. *Marine Ecology Progress Series*, *243*, 1–10.

Rosenberg, E., Koren, O., Reshef, L., Efrony, R., & Zilber-Rosenberg, I. (2007). The role of microorganisms in coral health, disease and evolution. *Nature Reviews. Microbiology*, *5*(5), 355–362.

Rosenberg, E., & Zilber-Rosenberg, I. (2018). The hologenome concept of evolution after 10 years. *Microbiome*, *6*(1), 78.

Rose, N. H., Bay, R. A., Morikawa, M. K., Thomas, L., Sheets, E. A., & Palumbi, S. R. (2021). Genomic analysis of distinct bleaching tolerances among cryptic coral species. *Proceedings. Biological Sciences / The Royal Society*, *288*(1960), 20210678.

Rosental, B., Kozhekbaeva, Z., Fernhoff, N., Tsai, J. M., & Traylor-Knowles, N. (2017). Coral cell separation and isolation by fluorescence-activated cell sorting (FACS). *BMC Cell Biology*, *18*(1), 30.

Russnak, V., Rodriguez-Lanetty, M., & Karsten, U. (2021). Photophysiological tolerance and thermal plasticity of genetically different Symbiodiniaceae endosymbiont species of Cnidaria. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.657348

Sampayo, E. M., Ridgway, T., Bongaerts, P., & Hoegh-Guldberg, O. (2008). Bleaching susceptibility and mortality of corals are determined by fine-scale differences in symbiont type. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(30), 10444–10449.

Sanjabi, S., Zenewicz, L. A., Kamanaka, M., & Flavell, R. A. (2009). Anti-inflammatory and pro-inflammatory roles of TGF-beta, IL-10, and IL-22 in immunity and autoimmunity. *Current Opinion in Pharmacology*, *9*(4), 447–453.

Schulenburg, H., Boehnisch, C., & Michiels, N. K. (2007). How do invertebrates generate a highly specific innate immune response? *Molecular Immunology*, *44*(13), 3338–3344.

Serrano, X. M., Baums, I. B., Smith, T. B., Jones, R. J., Shearer, T. L., & Baker, A. C. (2016). Long distance dispersal and vertical gene flow in the Caribbean brooding coral Porites astreoides. *Scientific Reports*, *6*, 21619.

Sharp, K. H., Distel, D., & Paul, V. J. (2012). Diversity and dynamics of bacterial communities in early life stages of the Caribbean coral Porites astreoides. *The ISME Journal*, *6*(4), 790–

801.

Silverstein, R. N., Correa, A. M. S., & Baker, A. C. (2012). Specificity is rarely absolute in coral–algal symbiosis: Implications for coral response to climate change. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1738), 2609–2618.

Starzak, D. E., Quinnell, R. G., Nitschke, M. R., & Davy, S. K. (2014). The influence of symbiont type on photosynthetic carbon flux in a model cnidarian–dinoflagellate symbiosis. *Marine Biology*, *161*(3), 711–724.

Suggett, D. J., Warner, M. E., & Leggat, W. (2017). Symbiotic dinoflagellate functional diversity mediates coral survival under ecological crisis. *Trends in Ecology & Evolution*, *32*(10), 735–745.

Sully, S., Burkepile, D. E., Donovan, M. K., Hodgson, G., & van Woesik, R. (2019). A global analysis of coral bleaching over the past two decades. *Nature Communications*, *10*(1), 1264.

Swain, T. D., Westneat, M. W., Backman, V., & Marcelino, L. A. (2018). Phylogenetic analysis of symbiont transmission mechanisms reveal evolutionary patterns in thermotolerance and host specificity that enhance bleaching resistance among vertically transmitted Symbiodinium. *European Journal of Phycology*, *53*(4), 443–459.

Sweet, M. J., Croquer, A., & Bythell, J. C. (2011). Bacterial assemblages differ between compartments within the coral holobiont. *Coral Reefs* , *30*(1), 39–52.

Thompson, J. R., Rivera, H. E., Closek, C. J., & Medina, M. (2014). Microbes in the coral holobiont: partners through evolution, development, and ecological interactions. *Frontiers in Cellular and Infection Microbiology*, *4*, 176.

Thornhill, D. J., Lewis, A. M., Wham, D. C., & LaJeunesse, T. C. (2014). Host-specialist lineages dominate the adaptive radiation of reef coral endosymbionts. *Evolution;*

International Journal of Organic Evolution, *68*(2), 352–367.

Tivey, T. R., Coleman, T. J., & Weis, V. M. (2022). Spatial and temporal patterns of symbiont colonization and loss during bleaching in the model sea anemone Aiptasia. *Frontiers in Marine Science*, *9*. https://doi.org/10.3389/fmars.2022.808696

Torda, G., Donelson, J. M., Aranda, M., Barshis, D. J., Bay, L., Berumen, M. L., Bourne, D. G., Cantin, N., Foret, S., Matz, M., Miller, D. J., Moya, A., Putnam, H. M., Ravasi, T., van Oppen, M. J. H., Thurber, R. V., Vidal-Dupiol, J., Voolstra, C. R., Watson, S.-A., … Munday, P. L. (2017). Rapid adaptive responses to climate change in corals. *Nature Climate Change*, *7*(9), 627–636.

Tortorelli, G., Rautengarten, C., Bacic, A., Segal, G., Ebert, B., Davy, S. K., van Oppen, M. J. H., & McFadden, G. I. (2022). Cell surface carbohydrates of symbiotic dinoflagellates and their role in the establishment of cnidarian-dinoflagellate symbiosis. *The ISME Journal*, *16*(1), 190–199.

Tsang Min Ching, S. J., Chan, W. Y., Perez-Gonzalez, A., Hillyer, K. E., Buerger, P., & van Oppen, M. J. H. (2022). Colonization and metabolite profiles of homologous, heterologous and experimentally evolved algal symbionts in the sea anemone Exaiptasia diaphana. *ISME Communications*, *2*(1), 1–10.

van Oppen, M. J. H., Baker, A. C., Coffroth, M. A., & Willis, B. L. (2009). Bleaching Resistance and the Role of Algal Endosymbionts. In M. J. H. van Oppen & J. M. Lough (Eds.), *Coral Bleaching: Patterns, Processes, Causes and Consequences* (pp. 83–102). Springer Berlin Heidelberg.

van Oppen, M. J. H., & Blackall, L. L. (2019). Coral microbiome dynamics, functions and design in a changing world. *Nature Reviews. Microbiology*, *17*(9), 557–567.

van Oppen, M. J. H., Bongaerts, P., Frade, P., Peplow, L. M., Boyd, S. E., Nim, H. T., & Bay, L. K. (2018). Adaptation to reef habitats through selection on the coral animal and its associated microbiome. *Molecular Ecology*, *27*(14), 2956–2971.

Voolstra, C. R., Buitrago-López, C., Perna, G., Cárdenas, A., Hume, B. C. C., Rädecker, N., & Barshis, D. J. (2020). Standardized short-term acute heat stress assays resolve historical differences in coral thermotolerance across microhabitat reef sites. *Global Change Biology*, *26*(8), 4328–4343.

Voolstra, C. R., & Ziegler, M. (2020). Adapting with Microbial Help: Microbiome Flexibility Facilitates Rapid Responses to Environmental Change. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, *42*(7), e2000004.

Webster, N. S., & Reusch, T. B. H. (2017). Microbial contributions to the persistence of coral reefs. *The ISME Journal*, *11*(10), 2167–2174.

Weis, V. M. (2019). Cell Biology of Coral Symbiosis: Foundational Study Can Inform Solutions to the Coral Reef Crisis. *Integrative and Comparative Biology*, *59*(4), 845–855.

Weis, V. M., Davy, S. K., Hoegh-Guldberg, O., Rodriguez-Lanetty, M., & Pringle, J. R. (2008). Cell biology in model systems as the key to understanding corals. *Trends in Ecology & Evolution*, *23*(7), 369–376.

Weis, V. M., Reynolds, W. S., deBoer, M. D., & Krupp, D. A. (2001). Host-symbiont specificity during onset of symbiosis between the dinoflagellates Symbiodinium spp. and planula larvae of the scleractinian coral Fungia scutaria. *Coral Reefs* , *20*(3), 301–308.

Williams, L. M., Fuess, L. E., Brennan, J. J., Mansfield, K. M., Salas-Rodriguez, E., Welsh, J., Awtry, J., Banic, S., Chacko, C., Chezian, A., Dowers, D., Estrada, F., Hsieh, Y.-H., Kang, J., Li, W., Malchiodi, Z., Malinowski, J., Matuszak, S., McTigue, T., 4th, … Gilmore, T. D.

(2018). A conserved Toll-like receptor-to-NF-κB signaling pathway in the endangered coral Orbicella faveolata. *Developmental and Comparative Immunology*, *79*, 128–136.

Wolfowicz, I., Baumgarten, S., Voss, P. A., Hambleton, E. A., Voolstra, C. R., Hatta, M., & Guse, A. (2016). Aiptasia sp. larvae as a model to reveal mechanisms of symbiont selection in cnidarians. *Scientific Reports*, *6*, 32366.

Wood-Charlson, E. M., Hollingsworth, L. L., Krupp, D. A., & Weis, V. M. (2006). Lectin/glycan interactions play a role in recognition in a coral/dinoflagellate symbiosis. *Cellular Microbiology*, *8*(12), 1985–1993.

Wooldridge, S. A. (2010). Is the coral-algae symbiosis really “mutually beneficial” for the partners? *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology*, *32*(7), 615–625.

Yoshimura, A., Wakabayashi, Y., & Mori, T. (2010). Cellular and molecular basis for the regulation of inflammation by TGF-beta. *Journal of Biochemistry*, *147*(6), 781–792.

Ziegler, M., Grupstra, C. G. B., Barreto, M. M., Eaton, M., BaOmar, J., Zubier, K., Al-Sofyani, A., Turki, A. J., Ormond, R., & Voolstra, C. R. (2019). Coral bacterial community structure responds to environmental change in a host-specific manner. *Nature Communications*, *10*(1), 3092.