THE ROLE OF VARIABILITY IN THE ECOLOGY AND EVOLUTION OF CORALS

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ABSTRACT

Climate change is impacting multiple habitats and one of the most susceptible ecosystems is coral reefs. There are some examples of corals that can acclimatize and adapt to stress events. Measuring the variability in different processes will improve our knowledge of the mechanisms of coral persistence.

The variability in competence time among larvae of different species of coral was tested. There was extensive variation in settlement even in larvae from the same brood. Coral larvae from multiple species had flexible settlement ecology, potentially influencing connectivity among populations.

To better understand the impact of stress events on different coral phenotypes, corals were monitored in Kāne'ohe Bay on O'ahu during two successive bleaching events in 2014 and 2015. Different species showed variation in susceptibility to thermal stress. One hundred and fifty individual colonies were tagged and visually monitored for their bleaching status and recovery starting in October 2014. The tagged colonies had low rates of total mortality with 19% of *P. damicornis*, 10% of *M. capitata* and no *P. compressa* that died after 19 months of monitoring. There were different rates of recovery, with *P. compressa* recovering more rapidly than *M. capitata*.

Individual *P. compressa* and *M. capitata* in Kāne'ohe Bay were tagged as pairs, one colony severely bleached adjacent to a healthy colony. Reduced representation sequencing was conducted on 16 pairs of *P. compressa* to elucidate the role of genetics in the bleaching susceptibility of these corals. One hundred and two genes were found that

segregated two clades of *P. compressa* in Kāneʻohe Bay, but these clades did not correspond to bleaching status or location. Of these one hundred and two genes, thirty-four were annotated of which three were from the mitochondrial genome and thirty-one were from nuclear regions. The difference in these one hundred and two loci suggested that there are two cryptic species of *P. compressa*. This research describes extensive standing variability in the processes of coral recruitment, coral resistance to stress, and coral genetic diversity. In marine ecology and evolution we need to understand the role of variability to evaluate the persistence of corals into the future.

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CHAPTER 1

Potential Mechanisms of Coral Acclimation and Adaptation

Abstract

Human induced habitat degradation and climate change are already impacting many different ecosystems on the planet. Coral reefs are extremely susceptible to changing environments, partially due to the physiology of the corals themselves. Critical to predicting and managing corals for persistence in the future is to measure the extent to which they can acclimatize and adapt, since the current rate of change is pushing corals to their physiological limits. This review summarizes our current understanding of coral acclimatization and adaptation. The review uses experiments and reviews from the plant and animal literature to highlight modern methods including next generation sequencing. Multiple mechanisms can drive coral acclimatization including phenotypic plasticity, symbiosis, gene regulation and transgenerational acclimatization. All of these mechanisms have the potential to enable the persistence of coral individuals through multiple types of stress events. There is promising evidence that corals are capable of rapid acclimatization through physiological plasticity and symbiotic associations. However, acclimatization is a short-term response to local conditions, and the ability of corals to adapt is critical for the survival of species within and among populations during stress events. There are multiple genetic mechanisms that might drive coral survival in stressful habitats including selection for stress resistance, local adaptation to extreme habitats, and the heritability of stress resistant traits. There are multiple examples of coral local adaptation to challenging habitats that mimic future conditions under climate change, suggesting their effective population size is large enough to allow corals to adapt to new and changing habitats. However, there is no clear estimation of the rate of adaptation in coral populations, and we know little about how increased frequency of stress events will impact the long-term demographic processes for multiple coral species. These multiple aspects of coral acclimatization and adaptation are critical to understand for the conservation of coral reefs.

Introduction

Most ecosystems are being impacted by human activities and land development. Increasingly, near-shore marine habitats are being degraded by local land use threatening coral reefs around the world. Concurrently, a rapid rise in atmospheric CO_2 is changing ocean temperatures and alkalinity, additionally threatening coral reefs (Hughes et al., 2003, Hoegh-Guldberg et al. 2007). This era has been termed the Anthropocene due to the variety of changes that humans are imposing on natural systems (Steffen et al. 2007). If atmospheric carbon concentrations continue to rise it is predicted that ocean habitats will be seriously compromised by 2100 (Gattuso et al. 2015). Even though reefs fulfill many ecosystem services there is inadequate data to determine if corals can survive an increasing number and intensity of stressors. In this Anthropocene era, a critical goal for humanity should be to preserve the ecosystem services that many different habitats provide.

The potential for organisms to respond to stressful events including climate change has been reduced to three major outcomes; organisms adjust their phenotype through acclimatization and adaptation to the changing habitats, organisms move to habitats that are more suitable for success, or a species dies. For those organisms with a sessile life history stage, including plants and many marine invertebrates, acclimatization and adaptation hold the greatest promise for persistence in the future. As climate change impacts habitats it will create both acute and chronic stress for many organisms, with stress defined as a state of "threatened" homeostasis (Johnson et al. 1992). Johnson et al. (1992) provide a thorough review of our understanding of stress going as far back as the ancient Greeks. Many animals adjust their physiology and hormones to respond to stress (Sapolsky et al. 2000, McEwen 2007). The genomic components of stress response have also been studied for multiple decades (Gasch et al. 2000). In plants the physiological mechanisms of stress response have been studied in response to a variety of abiotic and biotic interactions (Hsiao 1973, Cushman and Bohnert 2000, Wang et al. 2003). For mobile organisms there are often behavioral components of stress responses, but for sessile marine invertebrates cellular and genetic processes have the potential to drive the majority of the stress response.

Individual phenotypes exhibit a range of stress responses that probably help to dictate niche breadth within and among species especially in habitats that have variable environmental factors. For the purposes of this review we have defined genomes, traits and phenotypes in the glossary (Box 1). Importantly, the traits and phenotypes of an organism can be on a continuum from plastic to fixed. The stress response can be multifaceted, for instance when corals bleach they are stressed, which can result in recovery or mortality. But some individual coral colonies are capable of reducing the stress on their physiology so that they don't bleach, which is considered a bleaching resistant phenotype. An individual gene might code for different trait (for example a structurally different heat shock protein) that reduces stress. A coral that has a better heat shock protein and different antioxidant enzymes (another trait) might not bleach in elevated seawater temperatures, thus multiple traits contribute to the bleaching resistant phenotype. Understanding the genetic basis of stress phenotypes will be critical to

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predicting corals' ability to acclimatize and adapt (Madin et al. 2016), and new genomic tools have the potential to discover the genetic architecture underlying many stress response traits.

Much like in corals, juvenile and adult plants can not move from their local environment and the individuals with appropriate phenotypes persist, often through acclimatization. Stress in every habitat can vary from extreme acute events to constant chronic stressors (Miller 2016). The stress response can vary among species, among populations of the same species, as well as among individuals in one population. Different physiological responses among individuals often drive fitness variation among individuals within an organisms lifetime, but evolutionary processes might be critical to understanding a population, or group of populations susceptibility to stress and potential for extinction. Adaptation acts on allele frequencies, which can over generations to drive a local match between mean phenotypes and habitat. As disturbance and multiple stressors increase their pressure on natural systems some populations can resist and recover from stress events by physiological processes, while others appear to be changing in their genetic composition. Thus, there is evidence that both acclimatization and adaptation contribute to resilience.

In this era of environmental change there is a critical need to understand the mechanisms driving the processes of acclimatization and adaptation in both terrestrial and marine ecosystems. Coral reefs are habitats that are especially susceptible to climate change but their ability to resist stress has only been studied using a few species in a few locations. Corals themselves are critical to conserve since they build the structure of reefs that sustain a huge diversity of organisms. Recent research has focused on describing coral mechanisms of acclimatization and adaptation, but compared to plants we still know very little about the mechanisms that corals use to survive stress events.

> **Box 1. Glossary**
> **Acclimatization**: An individual organism's ability to maintain homeostasis, especially in response to changing abiotic and biotic features of the local habitat. Typically quantified using physiological techniques.
> **Adaptation**: Change in mean fitness of a population. Requires heritable trait variation within populations.
> **Quantitative Trait Locus** (QTL): A region of DNA that contributes to phenotypic variation in a particular trait. Most traits are polygenic, anywhere from 2-1000s of QTL contribute to their variation.
> **Expression Quantitative Trait Locus** (eQTL): A QTL that contributes to variability in mRNA expression. A mechanism for heritable regulation of gene expression.
> **Transcriptome**: All of the mRNA in the cells. Measuring the transcriptome gives a snap shot of the genes that are actively being expressed.
> **Epigenetics**: Heritable variation that is not associated with changes in DNA sequences, but may be caused by processes that modify DNA, like methylation.
> **Genome**: All of the genetic material in an organism including coding and non-coding gene regions, including the nucleus and organelles.
> **Heritability**: The proportion of phenotypic variation with a genetic basis. Can be further partitioned into narrow sense heritability (h^2), which is the additive genetic variation upon which natural selection operates.
> **Traits**: A measurable character of an organism that may be the target of natural selection or is of interest to plant/animal breeders. Most traits are quantitative (see QTL above) and are determined by genetic and environmental components.
> **Phenotype**: The measurable expression of genes and environment at the trait or multiple trait level.

Critical to conserving coral reefs is managing them for improved acclimatization and adaptation in the face of a changing climate. In this review acclimatization has been separated from adaptation, but it is important to realize that these processes act in concert and some mechanisms such as epigenetics could influence both processes. There have been several previous reviews on coral acclimation to thermal stress (Gates and Edmunds 1999, Edmunds and Gates 2008) and genetic adaptation and acclimatization (Barshis 2016, Putnam et al. 2017). This review is not meant to repeat those reviews but instead is designed to showcase a large body of literature for plants to highlight processes that might influence coral persistence. In the last 20 years extensive research on physiological and adaptive responses of plants to climate change provides many examples of methods and techniques that could be applied to coral research. Plants share many features and traits that are comparable to corals, including but not limited to: a sessile adult phase, the ability to produce clones, the ability to conduct selective breeding, interaction with multiple types of symbionts and a reliance on photosynthesis for some of their energy supply. Our understanding of plants can foster novel questions that could increase our understanding of the processes of acclimatization and adaptation in corals. Since this field has been revolutionized by multiple tools using next generation sequencing (NGS) and the age of "-omics" (Mardis 2008), this review highlights the diverse applications of these tools to advance our understanding of the mechanisms of coral persistence.

Acclimatization
Plastic Physiology, Traits and Phenotypes

Phenotypic plasticity is a key trait that has been implicated in plant success (Sultan 1995, Bohnert and Sheveleva 1998). The ability of certain traits to be flexible in response to different abiotic conditions can significantly contribute to the breadth of an organism's realized niche. Phenotype arises from individual or multiple traits that are a product of the interaction of genotype and the environment (Marais et al. 2013). Phenotypic plasticity can produce different morphologies that maximize the efficiency of a plant's use of light (Sultan 2000), different physiological mechanisms to deal with local stressors such as desiccation and salinity (Hasegawa et al. 2000), and different biochemical pathways to use variable concentrations of local nutrients (Hodge 2004). The impact of heat stress on plant physiology has been reviewed (Kotak et al. 2007, Wahid et al. 2007), and many plants have enough physiological plasticity to deal with small increases in temperature. Plasticity might be broader than predicted by our experimental methods; for example, some trees have modified their rates of respiration when exposed to higher temperatures to release less CO_2 than predicted under future temperature increases (Reich et al. 2016). But plasticity has its limits, some proteins and enzymes are constrained by their structure, many proteins denature above threshold temperatures (Somero 2012). This upper physiological tolerance establishes a limit past which no level of plasticity can help an organism survive. In intertidal crabs the individuals with the most physiological plasticity are actually the most susceptible to a changing climate, probably since these individuals are already close to their thermal tolerance limits (Stillman 2003).

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There has been extensive research on the plasticity of traits in plants (Sultan 2000) and corals (Gates and Edmunds 1999). Some species of corals have plastic morphology determined by abiotic features of their habitat including light and wave energy (Todd 2008). It is relatively easy to document morphological plasticity as a range of phenotypes in response to environmental gradients, but documenting physiological plasticity can be challenging. Coral physiology was recently reviewed (Sweet and Brown 2016), and much of that work is showing plenty of scope for physiological plasticity in response to anthropogenic stress.

Quantifying a phenotype can be challenging. Typical experiments measure a trait in both a treatment and a control. However, this doesn't adequately represent the breadth of that trait. A much better approach is to measure a response over a continuum of the treatment variable, for instance respiration can be measured across multiple temperatures using a response curve (Angilletta Jr. 2009). This curve gives us a much more accurate measure of the range of temperatures that an individual can tolerate. In most animals measuring an individual's response to a range in temperature could be biased based on testing different genotypes, but the clonality of plants and corals provide an opportunity to test the same genotype with a range of temperatures. This experimental method allows researchers to remove genotypic variation as a confounding factor to understand the breadth of traits associated with a genotype. There is a real paucity of stress response curves for multiple coral stress traits, especially comparisons among populations, across the diversity of species and among ontogenetic stages, but a database of coral traits should help to build these resources (Madin et al. 2016).

While it is poorly studied in corals there may be an important ontogenetic shift in phenotype flexibility, referred to as developmental plasticity. Adaptive developmental plasticity can be categorized as either somatic state based (a phenotype is switched from one type to another, i.e. small versus large body size) or informational, which is when the developing embryo/juvenile is exposed to environment conditions that modify its phenotype to the future environment (Nettle and Bateson 2015). Currently it is not known if juvenile corals have fixed phenotypes or if new recruits can "tune" their phenotype to their recruitment habitat, let alone what is driving any phenotypic difference. While it is difficult to disentangle maternal effects from increased flexibility in new recruits it should be possible to see if the breadth of a phenotype is wider during a coral's early life history stages. With experiments during the early ontogeny of corals it could be determined whether there is a window of developmental plasticity that closes, as the juveniles get older. One study measured growth and survival in new recruits of *Porites astreoides* that originated from two different populations with different thermal histories (Kenkel et al. 2015b). In a common garden the juveniles relied on energy stores for survival in the first five weeks, and after that there was less of a difference between the juveniles, although there remained a difference in their ability to grow when exposed to temperature stress (Kenkel et al. 2015b). One aspect of a window of phenotypic flexibility that has been studied in corals is the uptake of different symbiont types, which is discussed in the symbiosis section below. Whether juvenile phenotypes are more flexible than adult phenotypes has important conservation implications, as there might be

an increased chance of success of restoration efforts that transplant juvenile corals into a novel habitat compared to transplanting fragments of adult corals.

Variable physiology allows some corals to survive in extreme conditions such as elevated temperatures and salinity in the Persian Gulf (D'Angelo et al. 2015), decreasing pH (Fabricius et al. 2011, Kroeker et al. 2011), and reefs in "poor" habitats (Schoepf et al. 2015, Guest et al. 2016, Morgan et al. 2016). The fact that coral communities survive in these suboptimal habitats suggests that phenotypes already exist that are capable of persistence even in the stressful conditions predicted for the near future (next 20-50 years) of climate change. However, it is difficult to know the long-term demographic consequences of sub-lethal stress on these coral species.

Physiologists have been measuring proteins in plants and animals to better understand sub-lethal stress (Kotak et al. 2007). A few coral studies have measured important biomarkers, especially heat shock proteins and enzymes that compose the oxidative stress pathway (Lesser 2006). Sub-lethal stress can be insidious and corals that look healthy might actually be compromised. This is especially important to consider with the interaction of multiple stressors (Ban et al. 2014), as each stressor might act independently, or there might be synergetic interactions. For instance in a study of coral larvae temperature stress of +3 °C did not cause larval mortality, but it did cause oxidative stress (Ritson-Williams et al. 2016). When temperature stress was added to a competitive interaction with a benthic cyanobacterium more larvae died than when exposed to either stressor alone. Unfortunately the methods for identifying sub-lethal stress are varied and rarely standardized, but proteomic techniques hold great promise to quantify multiple proteins involved in stress response as has been shown in many plants (Bantscheff et al. 2007, Yates et al. 2009, Bantscheff et al. 2012). To better understand the impact of sublethal stress on physiology it is critical to evaluate the impact of individual and multiple stressors on coral cellular processes.

There is growing evidence that corals exposed to a short duration of a low level of thermal stress are more resistant to future thermal stress events (Bellantuono et al. 2012, Howells et al. 2013, Ainsworth et al. 2016). However, the mechanisms of this acquired resistance are unclear. It may be that the corals produce more anti-oxidant enzymes during mild stress events, providing higher constitutive concentrations in preparation for a more significant stress event. Or it may be that gene regulation is already activated allowing a coral to rapidly fine tune the magnitude of gene expression to any subsequent stress. The idea of "hardening" was described for plants' resistance to cold temperatures (Beck et al. 2004), and may provide an important mechanism to pre-condition corals to a future stress event. The mechanisms of acclimatization are rarely measured in a holistic context but potentially include a combination of upregulated stress response enzymes, shifting symbiont communities, differential gene expression, and epigenetics.

Symbiosis

Plants and animals form symbioses with a variety of organisms (van der Heijden et al. 2008, McFall-Ngai et al. 2013, McFall-Ngai 2014). Symbionts are critical for nutrient acquisition (Bonfante and Anca 2009, Lugtenberg and Kamilova 2009), and have

even been implicated in increased thermal tolerance in the plant *Dichanthelium lanuginosum* (Redman et al. 2002).

The dinoflagellate symbionts in the genus *Symbiodinium* are the best studied component of the coral holobiont. Much like plants, *Symbiodinium* can photoacclimatize, which includes increasing photosynthetic pigments within the same number of cells. Additionally, the coral can host different abundances of *Symbiodinium* cells to respond to small differences in light environment (Cunning and Baker 2013). There are examples of hosting different clades of *Symbiodinium* in different habitats (Abrego et al. 2008), as well as in different light environments within the same coral colony (Rowan et al. 1997). There are costs to hosting some clades (Lesser et al. 2013), with reduced growth in both *Acropora millepora* and *Acropora tenuis* juveniles that hosted clade D compared to clade C (Little et al., 2004). If not regulated by the host high densities of *Symbiodinium* can make corals more susceptible to thermal stress (Cunning and Baker 2014, Cunning et al. 2015b). The trade-offs are complex and poorly studied in natural settings, but recent work with dynamic energy budget modeling should help us to construct a theoretical framework to better understand these host-symbiont trade-offs (Cunning et al. 2017).

Symbiodinium diversity has been measured using various molecular loci since there are few useful morphological characters. The current literature typically refers to diversity with clades, which are a rough grouping of genotypes showing relationships from the family to genera level. Clade level diversity has recently been measured using the ITS2 loci with amplicon sequencing methods. In addition, here the term phylotype refers to genetic diversity at the "strain" level (comparable to species), which has been measured using a variety of loci, including the chloroplast gene psbA (D'Angelo et al. 2015). Symbiosis with only one clade of *Symbiodinium* has been shown to be critical for *Pocillopora* in Moʻorea to respond to stress events (Putnam et al. 2012). Multiple clades and even phylotypes within a clade have different physiologies (Rowan 2004) and are capable of rapid adaptation to light and stress conditions, conferring traits to their coral host (Howells et al. 2013, D'Angelo et al. 2015). But it is not clear if corals that associate with one specialized symbiont or those that host a variety of symbionts are more able to cope with climate change. Flexibility in some corals for multiple symbiont partners can increase survival during a bleaching event (Rowan et al. 1997, Cunning et al. 2015a). On the Great Barrier Reef in Australia *Pocillopora damicornis* and *Stylophora pistillata* symbiont communities were dominated by clade C *Symbiodinium* (Boulotte et al. 2016). Their abundance was greatly reduced after two bleaching events and novel clade D symbionts were detected after the bleaching stress. For some species that host multiple clades such as *Montipora capitata* there was no evidence for shuffling during a stress event (Cunning et al. 2016). The dynamics of symbiont shuffling in natural populations needs to be better quantified among coral species. Low background concentrations of clade D were also thought to mediate bleaching recovery and survival in *Acropora millepora* (Bay et al. 2016). However, the consequences of hosting specific *Symbiodinium* phylotypes in low concentrations are not well understood.

Early life history stages of corals might be more flexible in their symbiosis than adults, but how stress events during the juvenile stages shape future patterns of symbiosis are poorly understood. Larvae of *Acropora millepora* and *A. tenuis* took up a wide variety

of symbiont types as larvae and juveniles (Cumbo et al. 2013), suggesting flexibility in symbiont diversity due to environmentally available *Symbiodinium* phylotypes. It appears that there is increased flexibility in symbiont partners in juvenile corals compared to adults on the same reefs. Over 3.5 years the *Symbiodinium* community in juvenile *Acropora tenuis* eventually matched the adult symbiont community, but in *Acropora millepora* the symbiont community in juvenile corals did not change during 3.5 years and it was not the same as the symbiont community in adult corals (Abrego et al. 2009). A similar trend was found in a Caribbean gorgonian, *Briarium asbestinum*, which hosted a greater range of symbionts as a juvenile, and only had the symbionts found in adults after 4 years (Poland and Coffroth 2017). A novel experiment described the consistency of the symbiont community from parent corals to the next generation as heritability. Although not an evolutionary measure of heritability, the mean heritability of *Symbiodinium* type was greatly increased in *Montipora digitata* that vertically acquired their symbionts (h^2=0.57) compared to *Acropora tenuis* that horizontally acquired their symbionts (h^2=0.36). But it is important to note that neither of these estimates were as extreme as what would be predicted by these corals' symbiont acquisition mode (Quigley et al. 2017). Theoretically increased phenotypic flexibility in coral juveniles would allow for rapid acclimatization to local conditions, giving coral larvae a wider range of settlement habitat that would allow growth and survival after settlement. For hosting different symbionts there very well may be a window of opportunity that would allow acclimatization, but this is less likely (but not impossible) for the coral species that acquire their symbionts horizontally from their parents. There may be phylogenetic constraints on flexibility but so few corals species have been studied it is difficult to know the breadth of symbiotic associations during ontogeny.

Modern techniques using multiple molecular markers are showing extensive standing diversity in *Symbiodinium* even within clades (LaJeunesse et al. 2004). Low concentrations of rare *Symbiodinium* phylotypes have been found in some corals (Boulotte et al. 2016), and we expect the documentation of the diversity of *Symbiodinium* communities to increase with the application of NGS methods, especially using amplicon sequencing which could be modified to measure multiple loci to better characterize phylotypes. This diversity has a consequence for the coral host with some types of *Symbiodinium* providing more carbon to their host than others (Little et al. 2004). The origin of *Symbiodinium* diversity remains poorly understood. *Symbiodinium* in the coral host reproduce clonally, reducing the opportunity for recombination, but there is genetic evidence for sexual reproduction (Baker 2003, Chi et al. 2014). The *Symbiodinium* genome is 1.5 GBPs, which is much larger than most animals but smaller than other dinoflagellates (Shoguchi et al. 2013). Orthologous genes are common (Shoguchi et al. 2013), which might be an important mechanism for *Symbiodinium* to generate novel genotypes. The importance of genetic architecture in *Symbiodinium* acclimation and adaptation is unclear and will remain difficult to interpret until *Symbiodinium* genomes for multiple clades have been sequenced and assembled (Aranda et al. 2016). *Symbiodinium* transcriptomes are beginning to be studied (Bayer et al. 2012, Xiang et al. 2015), but it is unclear whether the physiological differences among clades and

phylotypes represents differences in standing genetic architecture or in gene expression (Ladner et al. 2012, Barshis et al. 2014).

Research on corals is also measuring the importance of bacteria and viruses as symbionts in the holobiont (Knowlton and Rohwer 2003, Ainsworth et al. 2010, Bourne et al. 2016). The microbial community is typically measured using amplicon sequencing of the 16S gene. The community diversity of the microbiome and its potential to shape the stress response of marine invertebrates is just beginning to be appreciated (McFall-Ngai et al. 2013). Many corals host a unique microbiome with a few taxa detected consistently among coral species that make up a "core" portion of the microbiome (Ainsworth et al. 2015). Interestingly, the diversity of microbes can be regulated by antibiotics produced by bacteria within the coral microbiome (Kvennefors et al. 2012). The ability of corals to influence the relative dominance of individual taxa within their microbial community is poorly understood. The components of the microbiome are thought to influence the susceptibility of corals to thermal stress (Ziegler et al. 2017). The function of different players in the microbial community and their role in coral health could be a key feature of coral acclimatization to their environment.

Microbes have the potential to shape coral metabolism in many ways, including horizontal gene transfer (Schonknecht et al. 2013, Bhattacharya et al. 2016). Microbial symbionts are known to fulfill important functions for coral survival, such as the fixation of nitrogen by symbiotic cyanobacteria (Lesser et al. 2004, Lesser et al. 2007). Since corals live in oligotrophic environments this symbiotic source of critical amino acid building blocks could be critical to their success. Microbes may be assisting with many physiological functions for their coral hosts that are not yet described. The effectiveness of microbial transplants (Scheuring and Yu 2012) to increase coral stress resistance has not been tested and any intervention techniques require a much greater knowledge of the natural fluctuations in the microbial community found in corals (Ainsworth et al. 2015), as well as the proteins they produce and their role in coral metabolism (Lee and Hase 2014, Sogin et al. 2016). There is a paucity of information about the ecological or physiological function of particular microbes and even less information about how communities of microbes interact to increase coral stress resistance.

For plants, fungal symbionts are also known to modify thermal tolerance (Redman et al. 2002). This research showed that aposymbiotic plants died when exposed to a regime of temperatures at 50 °C for 8 hours/day for 10 days, but those plants infected with *Curvularia* sp. symbionts had 100% survival. These experiments showed that fungal symbionts can be critical for thermal tolerance, which is similar to the work showing different thermal resistance associated with different clades of *Symbiodinium*. However, the role of fungal symbionts in marine systems is very poorly known. Recent research using molecular tools is showing that marine fungi are very diverse (Amend 2014) and some species of fungi may carry out critical physiological functions for their coral hosts (Amend et al. 2012). The role of fungal symbionts in any coral stress response is not clear, but researchers must be careful to not limit the taxonomic scope of their sampling when considering the dynamic role of symbionts in corals' response to stress. Our current methods of amplicon sequencing one gene region have the potential to bias our understanding of symbiont communities by identifying only a subset of the microbes. A

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better method would be to multiplex loci to detect a greater diversity of the potential symbionts within the same coral samples.

Gene regulation

Physiological plasticity has been measured using traditional physiological methods in a range of plants and animals. Recently NGS techniques, such as transcriptomics (Box 1), have been developed to measure gene expression in a range of genes simultaneously. Gene expression is a rapid response (from seconds to hours) that can "tune" a trait to recent changes in the local environment (Lasky et al. 2014), and gene expression has been implicated in acclimatization in plants (DeBiasse and Kelly 2016) and in corals (Barshis et al. 2013). Transcriptomes have great power to detect not only what genes are up-regulated to respond to a stressor but also which genes are down-regulated to quantify physiological trade-offs during stress. RNAseq is a powerful tool that measures the mRNA being expressed at a snap shot in time. Pipelines to assemble and annotate RNAseq data such as Trinity (Haas et al. 2013) can be incorporated with additional analyses to identify the genes that are differentially expressed, as well as quantify the extent of increased or decreased expression. While transcriptomes can measure a wide variety of genes and their patterns of expression, there are some important caveats to this method. A recent study with the coral *Seriatopora hystrix* paired gene expression with proteomics and found a mismatch between the genes that were expressed and the concentrations of proteins encoded by those genes (Mayfield et al. 2016). A recent review highlighted the difference between gene expression and the production of proteins, suggesting we may be over emphasizing the impact of gene expression (Evans 2015). If an enzyme is already present in adequate concentrations in an organism exposed to stress, is there a need to increase the production of this enzyme through gene regulation? These proteins are the primary driver of a rapid change in phenotype. This potential mismatch between gene expression and protein concentration could potentially be addressed by integrating transcriptomics with proteomics and metabolomics to determine the actual contribution of gene regulation to the standing concentrations of stress response proteins.

Most of the recent coral transcriptome experiments track gene expression at one or two time points. After three days of exposure to temperature stress, sixteen *Acropora hyacinthus* genotypes were shown to up-regulate hundreds of genes as they already showed visual signs of bleaching (Barshis et al. 2013). Another study showed that even after five hours of exposure to elevated temperatures gene regulation was already responding to temperature stress (Seneca and Palumbi 2015). A few studies have shown that there is large variation in gene expression even across a diel cycle (Levy et al. 2011), with some genes being expressed during the day and others expressed at night (Ruiz-Jones and Palumbi 2015). In *Acropora millepora* the genes that were expressed after three days of thermal stress were different than those expressed after nine days of exposure (Moya et al. 2015). These studies illustrate the bias that time of sampling might introduce into a study of gene expression. If a gene is only expressed at night and a coral was sampled during the day, this experimental design would miss those patterns of

differential expression. Additionally, the symbiont type hosted by a coral can influence corals' gene expression (DeSalvo et al. 2010, Yuyama et al. 2012). Quantifying gene expression is complex and coral researchers must be aware of these confounding factors when designing experiments that measure gene expression.

One of the greatest challenges in understanding gene expression in both plants and animals is identifying the individual genes, referred to as annotation (Alvarez et al. 2015). Currently researchers rely on public databases to annotate genes, and even though these databases contain a lot of information, there are only a few cnidarian genomes with very limited quality and taxonomic scope (Shinzato et al. 2011, Steele et al. 2011, Baumgarten et al. 2015). Recently a paper compared Cnidarians using a much broader taxonomic scope to better understand the genes underlying calcification (Bhattacharya et al. 2016). Even with relatively few resources genomic studies hold great promise to better understand corals and their symbionts (Shinzato et al. 2014). New methods have been developed to localize gene expression among different tissues, which was used to show that gene regulation associated with bleaching was expressed in the oral gastrodermis of corals (Traylor-Knowles et al. 2017). But our current databases are limited in the number of identified genes and most coral studies can annotate less than half of the genes that might be differentially expressed. Advances in crispr CAS9 (Doudna and Charpentier 2014), small RNAs (Banerjee et al. 2016) and morpholinos (Heasman 2002) hold great promise as experimental techniques to determine the importance and function of many previously unidentified genes.

Perhaps just as important as the identity of the genes that are being expressed is the duration and magnitude of gene expression. The extent of gene expression within individual corals can be critical for thermal tolerance (Barshis et al. 2013). While we assume that gene regulation is a relatively plastic response to short term environmental changes (Ruiz-Jones and Palumbi 2017), there is evidence that *Acropora hyacinthus* exposed to higher extreme temperatures had 60 genes that were "frontloaded" to constantly be expressed at higher levels than corals from a pool with lower variability in temperatures (Barshis et al. 2013). The authors suggest that this frontloading is an adaptation to higher temperatures. Gene expression was measured in colonies of *Porites astreoides* from Florida in response to a six week treatment of thermal stress (Kenkel et al. 2013b). Those corals from the thermal stressed population also constitutively upregulated stress response genes. However, in both of these experiments it is unclear if this level of expression is heritable or a consequence of different gene regulation during a developmental window of plasticity. Also there is very little information on the significance of genes that are up/down regulated for an hour versus those genes that are expressed for days to weeks. Again we have a very poor understanding of how many proteins are produced during the duration of gene expression. While challenging on many levels, a controlled experiment with a combination of gene expression, proteomics and physiology would greatly advance our understanding of the role of gene expression in stress response to short and long term stressors.

For corals there needs to be better understanding of the suite of genes undergoing differential expression, which will help us to understand how gene networks interact to control gene expression (Feltus 2014). Gene expression is controlled by transcription

factors. An excellent review of plant transcription factors showed that these expression networks were critical in heat stress response (Guo et al. 2016). In plants, transcription factors interact to form regulatory networks that are critical in gene expression in response to stressors (Bemer et al. 2017, Ohama et al. 2017), and novel methods are being developed to map these networks (Yazaki et al. 2016). Expression networks have been studied in *Acropora hyacinthus* (Rose et al. 2016), and this systems genetics approach has a great potential to identify expression networks that might influence stress resistance. Transcription factors can control gene regulation and so can epigenetic mechanisms as described below. Understanding the regulation of gene expression in plants and animals and the functions of novel unidentified genes is a huge gap in our understanding of coral acclimatization.

Epigenetics and Transgenerational Acclimatization

Stress in the maternal environment might allow an organism to "tune" its gametes for increased survival in a stressful habitat, which has been termed "informational developmental plasticity". For instance plant broods were split from parents grown in wet and dry conditions, and then evaluated for their drought resistance (Sultan et al. 2009). For *Polygonum persicaria*, a weedy generalist, the next generation of plants exhibited drought resistant phenotypes if they were from parents exposed to drought conditions. But this was not true for the congeneric species *Polygonum hydropiper*, a non-weedy species that requires moist habitats. *P. hydropiper* adults were thought to be stressed in the drought treatment thus reducing its maternal contributions to the seeds it produced (Sultan et al. 2009). The power of these techniques to measure acclimatization and adaptation is illustrated in a study of plants that exposed multiple generations to high CO_2, effectively testing a strong selection event of climate change on multiple generations (Watson-Lazowski et al. 2016). While species specific, transgenerational acclimatization has important implications for the persistence of a variety of marine organisms in a changing climate, and is an exciting approach of several recent publications (Putnam and Gates 2015, Munday et al. 2017).

The mechanisms of transgenerational acclimatization can take multiple forms, from increasing larval energy reserves to epigenetic control of gene expression. Transgenerational acclimatization has been found in the damsel fish *Acanthochromis polyacanthus* (Munday et al. 2017), and the potential mechanisms of this acclimatization included high heritability of metabolic traits and the upregulation of 53 metabolic genes (Veilleux et al. 2015). So far most of the work with corals has looked at the differential lipid content among coral larvae (Graham et al. 2013, Hartmann et al. 2013). These studies documented differential lipid reserves in offspring, but did not treat the parental colonies to assess transgenerational acclimatization. Brooding corals of *Pocillopora damicornis* were exposed to +2.4 °C temperatures and low pH, and those parental colonies then produced larvae that had higher size normalized respiration in the dark when exposed to the same treatment (Putnam and Gates 2015). Brooding species make excellent candidates for these experiments since many brooded larvae are a product of self-fertilization (Brazeau et al. 1998, Carlon and Lippe 2011), reducing the genetic

diversity among offspring produced by outcrossing. This leverages coral biology to better understand the nature of phenotypic plasticity in offspring to test for transgenerational acclimatization, but we still have little information about how the mechanisms of transgenerational acclimatization might vary among coral species with different reproductive modes.

A variety of offspring phenotypes might be advantageous, especially in variable habitats. In insects and plants there is evidence for this type of "bet hedging" (Philippi and Seger 1989), especially in seed ecology (Venable and Brown 1988), which is the dispersal phase of many plants. Work on desert plants shows they produce seeds with different dormancy times, which allows the seeds to sprout when the rainfall is appropriate for plant growth (Gremer and Venable 2014, Gremer et al. 2016). This bet hedging for habitats that vary temporally is thought to be a strategy that increases phenotypic variance, so even though it might decrease the maximum fitness of a species in a good year, it has the potential to increase fitness in the bad years, allowing overall greater fitness in habitats that have both good and bad years (Philippi and Seger 1989). Similar to plants the early life history stages of corals is a dispersal phase, but there are only a few studies that have considered bet hedging in dispersal duration (Figueiredo et al. 2013). Variable time until settlement has been found in the larvae of a variety of coral species (Chapter 2). This variation could be important for marine larval connectivity, which was studied extensively in fish and some invertebrates to better understand the ability of marine reserves to be a source of larvae to other sites (Cowen and Sponaugle 2009). The integration of variable pelagic durations has helped to match patterns of genetic connectivity to larval dispersal modeled with oceanographic currents (Baums et al. 2006, Figueiredo et al. 2013). Understanding connectivity should help us to predict which populations might contribute resistant genotypes to populations recovering from a mortality event. There is a critical need to understand connectivity on relevant spatial and temporal scales. While there is evidence that the time until competence is variable in coral larvae (Chapter 2), it is unclear what mechanisms determine coral planktonic duration, but in plants variability in seed dormancy is hypothesized to be controlled by epigenetic processes (Herman and Sultan 2016).

An important mechanism of transgenerational acclimatization is epigenetics. The term epigenetics includes a variety of mechanisms that all influence gene regulation without changing the sequence of the DNA (Feil and Fraga 2012). Epigenetics is relatively well studied in plants because it is possible to raise clones or inbred lines in different environments, producing an experimental framework for understanding the influence of the environment on the expressed phenotypes. Again this is an advantage for coral researchers that can make many clones from one coral colony. Epigenetics plays an important role in plant stress response (Boyko and Kovalchuk 2008), including heat stress (Liu et al. 2015). Epigenetics can include the methylation of gene regions that inhibits transcription (Boyko and Kovalchuk 2008) and the use of small RNAs (both small interfering RNAs and micro RNAs) to inhibit translation (Sunkar et al. 2012, Banerjee et al. 2016). These small RNAs are known to control the expression of genes critical in plant development, stress response and nutrient homeostasis (de Lima et al. 2012). In *Stylophora pistillata* eight small RNAs were implicated in symbiosis and

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calcification (Liew et al. 2014). A study with *Acropora digitifera* surveyed small RNAs and suggest a role for them in coral thermal tolerance (Gajigan and Conaco 2017). A recent study also found small RNAs that might control stress response in *Symbiodinium* (Baumgarten et al. 2013). Additionally, post translational RNA editing has been detected in *Symbiodinium* probably leading to differences in gene regulation (Liew et al. 2017). Given the potential of these small RNAs to control gene expression, more studies are needed to characterize the diversity and function of small RNAs in corals.

Epigenetics is receiving increased attention as it may explain phenotypic plasticity in response to stress events and epigenetics can be heritable providing a mechanism of transgenerational acclimatization (Angers et al. 2010, Herman and Sultan 2016). An elegant study grew clonal lines of *Populus* trees in a common garden in drought conditions, but each clone started in nurseries with different environments (Raj et al. 2011). This study found that clonal plants with different phenotypes of drought resistance differed in their gene expression patterns, and further work identified different levels of whole genome methylation among the clonal plants started in different habitats (Raj et al. 2011). Epigenetics has the potential to broaden phenotypic plasticity thus allowing similar plant genotypes to survive in different habitats (Herman et al. 2014). A similar type of experiment could be conducted with coral larvae, especially closely related brooded larvae.

Epigenetics in marine organisms has recently been reviewed (Hofmann 2017) so for brevity only the coral literature is highlighted here. In a genome wide scan there were different levels of total methylation found between *Pocillopora damicornis* and *Montipora capitata* exposed to elevated concentrations of CO_2 (Putnam et al. 2016). This experiment showed that bulk methylation can be quantified and might be important for phenotypic plasticity in corals. Further work using bisulfide sequencing, has shown the specific genes that are being methylated (Dixon et al. 2015, Dixon et al. 2016). The tools to measure broad scale patterns of methylation and gene specific methylation are available and could be applied to a variety of experimental designs to measure epigenetic mechanisms of coral acclimatization.

Adaptation

Adaptation is an evolutionary process that can structure natural populations and communities even on relatively short time scales (decades to centuries). NGS technology has revolutionized the techniques available to sequence genes and genomes, providing more data to measure and quantify selection (Mardis 2008, Stapley et al. 2010). Much of this work has focused on humans, where genomic variation has been described among multiple individuals and populations (Altshuler et al. 2010). In humans, just as for most organisms, both demographic processes and natural selection act on genomes (Lachance and Tishkoff 2013). Using NGS techniques a variety of aspects of plant genomic evolution have been studied including, selective sweeps and genetic draft (Neher 2013), local adaptation to stressful environments (Siol et al. 2010), and heritability of stress resistant traits (Visscher et al. 2008). Critical to measuring the impact of selection on plant genomes is to survey the genotypic diversity within and among populations and use

that as a baseline to understand how genotypic diversity changes in response to stress events (Pauls et al. 2013). Coral experiments testing the role of adaptation in coral persistence should span multiple scales including, internal microbiome, individual variability, populations' resistance to stress and changes in genotypic and species diversity within communities.

Climate change continues to destabilize most habitats, even as our ability to measure selection on genomes rapidly expands. There is a pressing need to understand mechanisms, rates and patterns of adaptation and to use that data to manage natural habitats (Christmas et al. 2016b). Climate change can reduce genetic diversity within populations and species (Pauls et al. 2013). Climate change is a strong selection pressure (although it might be more diffuse in some marine habitats already exposed to variable temperature and alkalinity), reducing genetic diversity within a population leading to reduced resistance and resilience to future stress events (Jump and Penuelas 2005). Plants are known to adapt to different soil habitats (Brady et al. 2005), heavy metal pollution (Watanabe and Osaki 2002), different thermal environments (Berry and Bjorkman 1980) and climate change (Jump and Penuelas 2005). The limits of adaption are driven by a combination of genetic and environmental factors. With model systems that can be cloned and bred such as *Arabidopsis thaliana,* phenotypic variation has been leveraged to map the genetic sites that contribute to a phenotype (Trontin et al. 2011). In plants the genomic methods used to determine local adaptation and potential pitfalls were reviewed (Franks and Hoffmann 2012).

NGS tools are providing a greater understanding of the impact of selection on plant genomes. Selection can act on a wide variety of plant phenotypes including ploidy level, mating systems and demographic history (Hough et al. 2013). As our ability to identify sites of selection has greatly improved we can start to determine the genes responsible for a trait, even a complex trait like flowering time (Zuellig et al. 2014). Quantitative trait locus (QTL) has been developed to measure how traits vary among individuals and has been especially important for the selective breeding of domesticated plants (Marais et al. 2013). However, understanding the genetic architecture of traits and which sites are under selection especially for additive traits continues to be a challenge even in controlled environments (Ehrenreich and Purugganan 2006). Critical to understanding adaptation is testing genotypes both in the laboratory and in field settings (Anderson et al. 2011). Landscape genomics is emerging as a useful discipline to understand plant adaptive response to climate change (Sork et al. 2013).

For corals the critical question remains, can corals adapt as rapidly as the climate changes? On a geologic time scale we know that corals can adapt (Pandolfi 2015) but the pace of evolution is critical for survival in the Anthropocene (Steffen et al. 2007). NGS data can detect signatures of adaptation among species, especially in relation to corals' ability to calcify (Bhattacharya et al. 2016). Much of our understanding of the future of coral reefs comes from modeling their performance in response to climate change and the associated change in seawater temperatures and ocean acidification (Hughes et al. 2003, Hoegh-Guldberg et al. 2007). However, for the most part these models do not incorporate the role of selection in driving adaptation to climate change. It may be that adaptation in corals cannot act fast enough to keep pace with climate change. Corals exhibit a large

range in generation time, which is central to understanding their ability to rapidly adapt. Some of the brooders and weedy species have 1-3 year generation times (Baria et al. 2012), while other species such as some of the massive growth forms have generation times on the scale of decades. This disparity in generation times highlights the fact that the rate of adaptation will be dependent on the species studied.

Local selection has been detected in some coral genomes, *Porites astreoides* colonies from near shore and off shore environments had different thermal tolerances even though they hosted the same clade of *Symbiodinium* (Kenkel et al. 2013a). Perhaps selection is acting on the hosts' ability to be flexible in the range of symbiont associations. Since coral symbiotic dinoflagellates have relatively short generation time it might be expected that selection can act on the coral holobiont, but a recent review discusses how rapid shuffling of symbionts effectively ensures that adaptation due to symbionts is not heritable (Skillings 2016). There is evidence that adaptation in the symbionts might increase coral thermal tolerance. A thermally tolerant *Symbiodinium* phylotype has been found in the Persian Gulf (Hume et al. 2016). This *Symbiodinium thermophilum* phylotype is closely related to other types in adjacent habitats such as the Gulf of Oman and the Red Sea coastline, and has probably arisen relatively rapidly in the last 6,000 years (Hume et al. 2016). Laboratory experiments have also documented rapid (3 years) increased thermal tolerance in *Symbiodinium* phylotypes that are cultured under higher temperature conditions (Chakravarti et al. 2017). While these studies offer hope for a mechanism of rapid adaptation to thermal stress it is unclear if these processes can keep pace with rapid climate change.

Critical to understanding the potential rate of adaptation is measuring the effective population size of coral species in populations most threatened by higher seawater temperatures. There is a paucity of data for coral population size among species, but large effective population sizes are critical for rapid adaptation (Charlesworth 2009). Charlesworth (2009) reviews the many methods of calculating effective population size, the caveats of this metric, and the importance of effective population size to determine the relative importance of selection or drift. Matz et al. (2017) showed that for *Acropora millipora* at five populations the effective population size was between 10,000 and 50,000. The authors argue that this population size is adequate for rapid adaptation in response to climate change, at least for the next 100 years (Matz et al. 2017). A large effective population size and standing genetic variation was critical for killifish adaptation to habitats that contained harmful pollutants (Reid et al. 2016). For these killifish the selection pressure of pollution was found at multiple sites along the East Coast of the USA and while there was a strong signal for local adaptation at each site, there was also a consistent mutation in the aryl hydrocarbon receptor signaling pathway in every population exposed to pollution (Reid et al. 2016). Since this adaptation was in response to human pollution, it shows that killifish can rapidly adapt to stressors, on the scale of decades.

Theoretically, the fact that coral populations are all within 1-2 °C of their thermal maximum suggests that the local populations have already adapted to their thermal environment and thus most species must be capable of adaptation. However, there is little baseline data to understand if the abundance of genotypes are changing in response to

local and global stressors and whether populations sizes are being reduced to eliminate adaptive potential (Hansen et al. 2012). This is also an important caveat of restoring a coral reef, maintain genotypic diversity within restored habitats is necessary to ensure those populations can adapt to future stressors (Baums 2008). Again, the effective population size is not known for most coral populations, even though this is a critical metric to assess the potential pace of coral adaptation.

There is a pressing need to document the standing genetic diversity in natural populations (Hansen et al. 2012). How can we detect a change in genetic diversity within a population if there is no baseline of current diversity? Some NGS methods have been developed to directly address this question including genotyping by sequencing (Elshire et al. 2011), which is similar to reduced representation sequencing (RADseq) methods (Davey et al. 2011). A genotype by sequencing study was conducted on *Pocillopora damicornis* from multiple populations in Australia, and multiple sites of selection were identified, especially in genes related to stress tolerance in the most northerly populations (Thomas et al. 2017). These tools also allowed Thomas et al., (2017) to identify populations with less genotypic richness, suggesting that local adaptation was reducing genetic diversity. Using genotyping by sequencing on populations of *Acropora cervicornis* in Florida, there was relatively high diversity among populations (Drury et al. 2016). Using reduced representation sequencing with targeted gene regions, termed RAD capture, a plant study compared 970 genes among 17 populations of the narrow-leaf hopbush used in restoration in Australia (Christmas et al. 2016a). This study tracked multiple genes that were responsible for local adaptation to temperature, water availability, and elevation within this plant species. Methods designed to target multiple select loci like RAD capture and RADtags (Jones and Good 2016), hold great promise for identifying genotypic diversity within and among populations of 100's to 1000's of individuals. While these include an ascertainment bias, RADseq on a small number of populations, followed by a targeted RAD approach for those genes thought to be important among many individuals of a species is a powerful experimental design to determine drivers and patterns of adaptation among populations.

There is also increasing evidence that there is great standing variability within populations for phenotypes and genotypes that are resistant to stress. A few studies have documented this variability within populations, but very few have documented the long-term fate of variable phenotypes. A simple method to determine how different phenotypes perform in a changing climate is to monitor individual corals within and among populations (Jones et al. 2008, Neal et al. 2017) (Chapter 3). Studies that link RADseq and individual coral performance in the field will greatly improve our ability to detect the sites in the genome that confer resilience to individual corals (Chapter 4).

Local Adaptation

Local adaptation is a critical concept in population genetics, and the methods and caveats of looking for the signatures of selection across a variety of habitat has been reviewed (Kawecki and Ebert 2004, Savolainen et al. 2013, Tiffin and Ross-Ibarra 2014). Much of our understanding of local adaptation comes from genomic studies on animals

and plants (Wright and Gaut 2005, Neale and Kremer 2011). Genetic change can now be measured in thousands of SNP's across the genome using whole genome and RADseq methods, which have been compared and reviewed recently (Puritz et al. 2014, Andrews et al. 2016). Local adaptation is known to enable plants to survive in different habitats. In a large transplant experiment with 3 plant species, genetic clones of these plants always performed the best in their natal habitat (Joshi et al. 2001). The genomes and patterns of methylation for three different populations of oak trees were compared across a gradient of climates within California and found to have CpG methyl polymorphisms that drove local adaptation (Platt et al. 2015). There is evidence that signatures of local selective pressures can persist even in populations with gene flow (Tigano and Friesen 2016). Some trees have gene flow across long distances suggesting standing diversity can interact with gene flow to help drive plants' adaptive response to climate change (Kremer et al. 2012). Trees create essential habitats and current genomic resources can be leveraged to better understand their diversity within populations and to set conservation goals in the face of shrinking habitats and climate change (Holliday et al. 2017).

Local adaptation might limit future rates of adaptation, and a synthesis of experimental results showed little evidence that the rate of plant evolution could keep pace with climate change (Jump and Penuelas 2005). A meta-analysis of twelve studies on evolutionary or plasticity mechanisms of adaptation found that in eight of the plants studied they were unable to adapt at a rate rapid enough to keep pace with climate change (Franks et al. 2014). These techniques are becoming more refined, in balsam poplar researchers used spatial modeling of multiple loci to determine nonlinear genomic patterns in response to environmental gradients in temperature (Fitzpatrick and Keller 2015). Much of the genomic methods to test local adaptation have focused on RADseq to find loci that might be under selection among multiple populations. Recent reviews warn of the frequency of false positives in the analysis of sites of selection (Francois et al. 2016), and the potential for selective sweeps to over represent the detected loci under selection (Lowry et al. 2017). While there is potential for false positives using RADseq, it is still a powerful tool to identify some sites of selection. It will be impossible to detect all of the sites of selection until high quality genomes from multiple individuals within multiple populations are sequenced.

Coral researchers are leveraging field observations to look for local adaptation in habitats that are refuges from stressors (Thompson and van Woesik 2009, van Woesik et al. 2012). These refuges scale from ocean basins to specific reefs within an island group. Refuges contain species that are locally adapted to their habitats and in some cases include corals that exhibit resistance to climate change. For instance the coral reefs in Kāne'ohe Bay, O'ahu, Hawai'i, showed resistance to bleaching in two consecutive years of thermal stress, both 2014 and 2015 (Chapter 3). Kāne'ohe Bay has been exposed to extensive historical stress (Bahr et al. 2015), and as a function of being at 21 °N latitude is exposed to a 6-10 °C range in seawater temperatures on an annual basis. Multiple factors probably contribute to this reef showing resilience. The reefs in Kāne'ohe Bay are dominated by two coral species that make up more than 95% of the coral cover. This low species diversity is extreme even for Hawaiian reefs, and may reflect the consequences of local adaptation to a suboptimal habitat.

Coral populations in refuge habitats may be important sites for conservation as they might supply adjacent habitats with individuals that are more resistant to modern threats. However, local adaptation can be a consequence of disrupted gene flow among populations. Research on the coral *Platygyra daedalea* showed that there was local adaptation of the coral genome as well as symbiosis with different clades of *Symbiodinium* between the Persian Gulf and the Sea of Oman (Howells et al. 2016). Higher thermal tolerance was found in the Persian Gulf population and this was maintained even after a six-month common garden acclimatization experiment. Importantly, local adaptation may create a phenotype to habitat mismatch when corals are transplanted from one site to another. Many coral nurseries are growing 10's to 100's of fragments sourced from a few coral genotypes, effectively transplanting many clones to a restoration site. This restoration work can increase coral cover, but it does not guarantee that the transplanted genotypes will survive in their new habitat. Howells et al. (2013), showed that two populations of *Acropora millepora* were adapted to their local conditions and had 40-50% mortality when the corals were transplanted between two sites on the Great Barrier Reef. Adult colonies of *Porites astreoides* from two different populations with different thermal histories did not survive in reciprocal transplants, which the authors argue is local adaptation to their natal habitats (Kenkel et al. 2015a). In the Persian Gulf local adaptation appears to have driven a genetic resistance to both high seawater temperatures and to high salinity in *Porites* spp. (D'Angelo et al. 2015). This local adaptation meant that corals transplanted out of the Persian/Arabian Gulf died because of a different salinity regime. An alternative to using locally adapted corals as nursery stock would be to identify those populations that contain genotypes that augment the *potential* for local adaptation. Another restoration strategy has been to use sexually produced larvae for restoration, potentially leveraging larval phenotypic plasticity (see acclimatization section).

Recently multiple reviews call for more human intervention to promote adaptation, termed prescriptive or assisted evolution (Smith et al. 2014, van Oppen et al. 2015). Human assisted evolution is a logical response to rapid climate change, since humans could facilitate the rate of evolution for some organisms, helping them to persist in rapidly changing environments. This could take the form of assisted gene flow where resistant genotypes are transplanted to impacted habitats that closely match the original habitat (Aitken and Whitlock 2013). Assisted gene flow could be modeled as migration in population genetics (Matz et al. 2017), and has the potential to augment standing genetic diversity in an impacted habitat where mortality caused a loss in diversity.

Heritability

The heritability of traits and phenotypes is central to evolution in response to selective pressures. Quantitative genetics in plants has a rich history as a method to map and quantify the genetic loci responsible for traits and phenotypes. Quantitative genetics has been used extensively in plant breeding experiments, which is instrumental in the

domestication of many plant species. A major advance in our understanding of plant's responses to climate change is describing the genomic architecture underlying traits that confer resilience to climate change (Jump and Penuelas 2005, Nordborg and Weigel 2008), which often results from studies of local adaptation (see above). Plant breeding and selective breeding is a proven method to better understand the narrow sense heritability of important traits such as rapid growth or larger fruit (Marais et al. 2013). Selective breeding of plant clonal lines and inbred strains have proven invaluable as a tool to map QTL's (Kover and Mott 2012). These tools are just beginning to be applied to corals, especially with the concept of assisted evolution, where coral selective breeding could create resistant phenotypes (van Oppen et al. 2015, van Oppen et al. 2017).

Heritability of stress resistance is rarely studied in corals, and relatively few studies have conducted controlled crosses to determine heritability among siblings and cousins (but see Dixson et al. 2015). Heritability has been estimated for coral morphology (Carlon et al. 2011). A simple method to measure broad sense heritability is to compare the phenotypic variability within and among populations, which has been done for calcification in multiple coral species in Hawai'i (Jury et al., unpublished data). Further research with known crosses measuring a greater variety of traits would greatly inform future selective breading efforts in corals. Ecologically, heritability of stress resistant traits is promising since there can be connectivity among habitats with different thermal regimes (Kleypas et al. 2016), suggesting that if thermal tolerance is heritable there could be evolutionary rescue of connected reefs supplied with thermally adapted larvae.

Selective breeding is very challenging with corals that brood their larvae, but should be achievable with corals that have external fertilization (Fogarty et al. 2012), especially in some of the weedy species that have short generations times. These spawning corals provide an experimental technique for crossing individuals exhibiting a continuum of stress resistance. In this way selective breeding could be used to identify QTLs critical for coral thermal tolerance. Some larvae have different gene expression in response to thermal stress (Meyer et al. 2009, Meyer et al. 2011, Baums et al. 2013, Polato et al. 2013), but this is rarely linked to parental phenotypes, making it difficult to disentangle the mechanisms of this variance. A study by Dixson et al. (2015) included a few individual crosses between two populations of *Acropora millepora*, and showed that thermal tolerance was greatest in larvae derived from crosses of parents from the higher temperature site. While these studies of heritability are promising, we are only beginning to understand the heritability of coral phenotypes and whether this heritability is consistent among species and among populations. No studies have quantified phenotype among sibling crosses and across F_2 generations, which would be a better method to understand narrow sense heritability (h^2). To understand the heritability of traits researchers could track the parental genotypes and phenotypes in the field combined with research on specific crosses of gametes in the laboratory. Long-term studies of parents to document their phenotypes in response to multiple stressors and specific crosses of their offspring will reveal the heritability of specific traits that enable coral survival during stress events. Corals that broadcast spawn their gametes are excellent models for

selective breading experiments, and these methods have great potential to advance our understanding of the heritability of coral traits.

Quantitative Trait Loci (QTLs) have been mapped in plants for many traits (Nordborg and Weigel 2008). This is a process by which the genes that code for a trait are measured for the relative amount they influence that trait. Typically this is done by breeding individuals from each extreme of a trait (for instance corals susceptible to bleaching and corals resistant to bleaching), and then measuring the F1 and F2 generations to determine where they fall on this continuum. Thus the genes that are responsible for the trait of interest can be evaluated for their impact on the strength of expression of that trait. Importantly it is rare for traits to be determined by single locus, and our ability to detect QTL's is limited to those gene regions that show the strongest effect on a trait, likely leading to an under representation of the genes that determine a trait (Marais et al. 2013). Most plant traits are associated with multiple QTL's and mapping these loci in plants has revealed that most QTL's have small effects but are additive. QTL research has focused on breeding experiments (Jimenez-Gomez 2011), and have greatly benefited from breeding inbred strains such as those in *Arabidopsis* (Kover and Mott 2012).

We have almost no knowledge of QTL's for corals. Coral generation times are typically long and the methods to breed corals are still poorly developed. With controlled crosses using rapidly growing corals such as some of the Pacific *Acropora* species it should be possible to generate F_2's in 3-5 years (Baria et al. 2012). By integrating a selective breeding experiment with a system of monitoring individual genotypes coral biologists could begin to generate the right type of genetic samples to identify and map QTLs for important coral traits.

Genome wide association mapping (GWA) is a relatively recent technique to identify QTLs without breeding experiments (Nordborg and Weigel 2008). GWA mapping is especially useful in self-fertilizing plants using clonal material, an advantage of studying colonial organisms such as corals. However, the results of GWA are very context dependent and can be confounded by different populations. The model organism *Arabidopsis thaliana* has been extensively studied using GWA (El-Soda et al. 2014) and genes that are important for stress tolerance have been identified (Olivas et al. 2017). This approach has been applied to other plants in response to thermal stress (Lafarge et al. 2017), and GWA should be especially important for identifying QTLs in non-model organisms including corals. A structural equation modeling framework can integrate GWA and gene regulatory networks to identify QTL's (Nuzhdin et al. 2012). The coral *Acropora millepora* was surveyed with a targeted genome association approach to look for genes associated with stress tolerance (Jin et al. 2016). Jin et al., (2016) found that at 19 genomic loci there was strong correlation with environmental conditions, and they track changes in the antioxidant stress response pathway across multiple populations. A GWA approach combined with controlled crosses will likely provide the highest resolution data for understanding the combination of genes that contribute to a trait.

In human genomes, research has leveraged our understanding of the genome to determine loci where patterns of gene expression is heritable (Majewski and Pastinen 2011, Nica and Dermitzakis 2013), and methods to find these eQTLs were recently

reviewed (Battle and Montgomery 2014). In plants and some animals there are examples of heritable gene expression (Gilad et al. 2008, Bonduriansky and Day 2009, Skelly et al. 2009). These eQTLs are critical sites that create variation in traits that might confer a selective advantage (Cubillos et al. 2012). There are no published studies on eQTLs in corals, but they probably improve the ability of corals to adapt to their environment. Importantly, gene expression could be a mechanism of acclimatization and could also be an adaptive response.

Conclusions

This review has stressed the importance of measuring genetic variability. This variability results in phenotypes that are the raw material for natural selection. Maintaining this variability in traits, phenotypes, and genotypes is critical to ensure that populations can adapt to natural and anthropogenic stressors. Surprisingly there is relatively little knowledge of the long-term fate of different phenotypes in natural settings. While research on trees has highlighted the successful approach of measuring every individual tree in a plot and tracking the demographics of genotypes over time (Condit 1995), these techniques are rarely applied to coral reef habitats (but see Neal et al. 2017). There are only a few sites where individual corals have been tagged and monitored for 2-10 years including the Florida Keys, USA (Baker, unpublished data), St. Johns in the US Virgin Islands (Edmunds 2000, 2002), Bocas del Toro, Panama (Neal et al. 2017), the Great Barrier Reef, Australia (Jones et al. 2008), and Kāneʻohe Bay, Hawaiʻi (Chapter 3). These sites will be invaluable to measure change over time, especially as thermal stress increases in extent and frequency. These *in situ* phenotypes could be leveraged with controlled breeding experiments to better understand the genomic components of coral resilience. There is a pressing need to understand patterns in natural populations to evaluate the potential for acclimatization and adaptation to climate change (Hansen et al. 2012).

Box 2. The role of variability in coral acclimatization and adaptation

Variability is a key trait in biology, whether it be genotypic, environmental, or phenotypic variability. While many studies focus on the mean response of multiple individuals to a stress, it could be argued that the variance is more critical, especially for the process of adaptation. Measuring variance highlights the range of responses and variance is a critical aspect of the breadth of a phenotype. Surprisingly few studies have considered phenotypic variance within and among populations of corals. There remains a paucity of information about the standing genotypic diversity on reefs (Chapter 4). To understand the role of adaptation in coral persistence we must understand the inherent variation in genotypes within and among populations to be able to measure future changes in genotypic diversity in response to stress events.

Variation in phenotypes can arise from genotypic variation derived from sexual recombination, variation in gene expression, and variation in the interaction of genotype with the environment. While it is quite easy to identify different phenotypes especially in laboratory manipulations, there is a great need for long-term monitoring of different genotypes in the field to predict what traits are necessary for coral persistence through repeated and varied stress events (Chapter 3).

Variance in traits has consistently been understudied for early life history stages in corals. Do coral larvae have certain traits that are more variable than adults? For instance a variable pelagic duration or different settlement requirements has important implications for the connectivity among habitats and thus migration of genotypes among populations (Chapter 2). Since larval settlement determines the adult habitat for sessile organisms, are physiology traits, symbiotic associations and gene expression more variable during the early life history stages? Is there a window of flexibility with a certain amount of time in which the young coral can fine-tune their physiology to their settlement habitat? Or are variable traits determined by the parents, with transgenerational acclimatization influencing larval/juvenile phenotypes? More research specifically designed to address the role of variability is needed to better understand the

Critical to understanding the variability in a trait, individual or population is measuring a response curve for individual traits. This response curve (i.e., Reaction Norms) helps to define the breadth of a phenotype and is critical to understand the range of conditions that genotypes can tolerate. A response curve is also critical for modeling studies to evaluate the impact of a continuum of stress across a continuum of phenotypes. To increase our understanding of the phenotype, modern experimental physiology needs to include response curves to better document the variability inherent in coral phenotypes.

Since we are just beginning to measure coral genomes and gene expression the overwhelming majority of studies have focused on adult corals, but there is great potential for phenotypic plasticity during the larval and juvenile stages of corals. Through multiple mechanisms of maternal effects, transgenerational acclimatization, epigenetics and bet hedging it may be that future generations of corals will be more capable of dealing with climate change than the adult corals more typically used in experiments. Much more research is needed to understand the phenotypic plasticity in coral larvae and juveniles.

This review has artificially separated acclimatization from adaptation since both of these processes interact and are critical for coral persistence. This approach was chosen to provide a framework for the discussion of how corals can survive and persist in changing environments. In any trait there will be a combination of short-term local pressures as well as long-term selective pressures on different genotypes within populations. Fundamentally both acclimatization and adaptation are working together to

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influence coral persistence. However, there are multiple lines of evidence that some species will be losers and it is very likely that the coral reefs of the future will look very different than modern reefs (Loya et al. 2001, Fabricius et al. 2011, Edmunds et al. 2014). Fundamental to understanding the future of corals reefs is establishing a modern baseline against which the rates and patterns of change can be tested. This will require collaborations among field and experimentally ecologists, physiologists, and quantitative geneticists. With the advent of NGS there are a variety of tools available to measure corals from genomes to communities, but due to the rapid rate of climate change there is a pressing need to better document the standing diversity on reefs and the future trajectory of that diversity in response to multiple acute and chronic stress events.

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CHAPTER 2

Variability in Larval Settlement of Multiple Coral Species

Abstract

Many marine invertebrates have variable patterns of larval settlement. A novel method was developed to measure the settlement variability among larvae of different species of coral. Larvae from multiple parents of *Favia fragum, Porites astreoides, Pocillopora damicornis, Leptastrea purpurea, Orbicella faveolata, Acropora cervicornis* and *Montipora capitata* had just as much variability in settlement in response to the same individual coralline alga as among different individuals of *Hydrolithon boergesenii* (Belize) and *Hydrolithon reinboldii* (Hawai'i). There were significantly different amounts of settlement variation between the species that brood their larvae and the coral species with external fertilization. When broods from the same coral colony were tested there was just as much settlement variation within a brood as among colonies for *Favia fragum* and *Porites astreoides*. However, *Pocillopora damicornis* had more variability in settlement among colonies with some colonies showing high variability and other colonies showing low variability. Multiple broods of larvae were tracked for their time until settlement and individual broods had constant rates of settlement until day 8-12 when 80 % of the larvae of *Favia fragum* and *Pocillopora damicornis* had settled. These experiments show that multiple species of coral larvae have extensive variability in their time until settlement.

Introduction

 Supply side ecology is critical for building populations in both terrestrial and marine habitats (Gaines and Roughgarden 1985, Lewin 1986, Nathan and Muller-Landau 2000, Nathan et al. 2008). Larval dispersal is a critical demographic processes that contributes to population structure, community dynamics, and recovery after a disturbance event. Dispersal and connectivity are features of habitats that control recovery as well as gene flow among populations (Ronce 2007, Cowen and Sponaugle 2009). In marine systems successful recruitment can determine connectivity, which is a critical aspect of marine conservation (Grantham et al. 2003, Almany et al. 2007, Jones et al. 2009).

 Settlement is a well studied aspect of larval ecology since it determines the pelagic duration of larvae, which integrates with physical currents to determine the connectivity of species among populations (Hellberg et al. 2002, Byrne 2012). For sessile organisms settlement is a major transition from a dispersive to sedentary life history stage. Marine larvae exhibit a wide range of dispersal strategies (Strathmann 1993, Pechenik 1999, Strathmann 2007, Toonen and Tyre 2007) and settle in response to a range of abiotic and biotic cues (Pawlik 1992, Rodriguez et al. 1993, Hadfield and Paul 2001, Hadfield 2011).

 The time until settlement is one of the most commonly measured traits of marine larvae. Among marine invertebrates there is variability in the time until settlement (Hadfield and Strathmann 1996). Variation in larval duration until settlement has been shown in echinoderms (Birkeland et al. 1971), opisthobranchs (Gibson and Chia 1995, Krug 2001, 2009) and polychaete larvae (Toonen and Pawlik 2001a, b). Some larvae in the same brood exhibit multiple strategies, a larval phenotype that crawls away from the egg mass together with larvae that swim for days to months (Gibson and Chia 1995, Krug 2001, 2009). In polychetes some larvae settled gregariously and some larvae had a longer dispersal time and settled without conspecific cues (Toonen and Pawlik 2001a, b). Variability in settlement timing might be an important mechanism for larvae to settle in habitats that vary in the quality of settlement habitat both spatially and temporally (Toonen and Pawlik 1994, Strathmann et al. 2002). The importance of variability in multiple larval traits and methods to quantify that variability was reviewed for marine invertebrates (Jacobs and Podolsky 2010).

 Corals also have extensive variability in their larval traits. *Pocillopora damicornis* larvae have variation in physiologic traits including; larval size, symbiont density, protein content, total lipids, respiration, and citrate synthase (Putnam et al. 2010, Cumbo et al. 2012, Rivest and Hofmann 2014, 2015). Larvae of *Favia fragum* in Bermuda had variable settlement depending on the time of release within a brood even on the same day (Goodbody-Gringley 2010). *Porites astreoides* had variability in larval physiologic traits including respiration, *Symbiodinium* density, and mortality after 24 hours (Edmunds et al. 2001) and there was variation in larval size and total lipid content of *P. astreoides* among sites in Bermuda (de Putron et al. 2017). Larval planktonic survival was found to vary with larval size for *P. damicornis*, *Stylophora pistillata* and *Seriatopora histrix* (Isomura

and Nishihira 2001). Variation in settlement competence among larvae was also found for the spawning corals *Favites chinensis* and *Goniastrea aspera* in Okinawa, Japan (Nozawa and Harrison 2005). *Goniastrea aspera* exhibited both internal and external fertilization, which appears to be a unique adaptation to increase the range of time until competence for this species, although these methods have only been applied to *G. aspera* (Nozawa and Harrison 2005). All these experiments suggest that coral colonies are producing a variety of larval phenotypes among broods across space and time. However, all these studies use different methods or measure different response variables making among species comparisons difficult.

A few species of coral larvae have been shown to have variation in their settlement (Figueiredo et al. 2013, Chamberland et al. 2017). However, variability in settlement could be driven by multiple factors including competence time, larval condition and variable settlement cues. The benthic substrata that coral larvae use to indicate appropriate settlement habitat has been described as crustose coralline algae (CCA) for some coral species (Raimondi and Morse 2000, Harrington et al. 2004, Ritson-Williams et al. 2010), and biofilms for other species (Negri et al. 2001, Webster et al. 2004, Tran and Hadfield 2011). Further work showed settlement cues can be attributed to unidentified cellular components of the CCA itself (Morse and Morse 1991, Kitamura et al. 2007, Tebben et al. 2015). However, microbial strains isolated from CCA have also been shown to induce coral larval settlement and metamorphosis (Negri et al. 2001, Tran and Hadfield 2011, Sneed et al. 2014). Different species of CCA are known to host different microbial communities (Johnson et al. 1991, Sneed et al. 2015), and it may be that the microbes associated with specific species of CCA produce settlement cues for coral larvae.

Understanding patterns of settlement variability among coral species has been confounded by experiments that use different settlement substrata for different coral species. Different coral species have different habitat requirements and might cue into different characteristics of their preferred habitat. For instance, coral larvae that live at different depths had the greatest settlement in response to ruble or tiles conditioned at their natal depths (Carlon 2002, Baird et al. 2003). Many of the shallow water corals had high rates of settlement in response to the same CCA species, *Hydrolithon boergesenii* (Ritson-Williams et al. 2016). But throughout these coral larval experiments there were always some larvae that did not settle. Since each replicate in these experiments was a different individual of CCA it is impossible to know if the observed variance was due to characters of individual algae or due to variation in the larvae tested. Additionally, the fate of those larvae that don't settle is unclear, as they are rarely monitored after the experiments.

The experiments presented here were designed to better understand the variability in settlement of multiple species of coral larvae exposed to similar settlement substrata. Since many coral species will settle in response to the CCA species *Hydrolithon boergesenii* in the Caribbean and the sister species *H. romboldii* in the Pacific, these species were used to ensure that any variance in settlement was not due to differences among species of CCA. Larval settlement variability was measured in response to multiple fragments of the same algae and in response to multiple individual algae (Figure

2.1). This provided a method to test if settlement variability is driven by differences among individual algae of the same species. These experiments used larvae pooled from multiple parents, which could be another source of the observed variation in settlement. Further experiments with individual broods of larvae were also compared for their settlement variability. To determine the fate of larvae that did not immediately settle, individual broods of larvae were maintained in the presence of settlement substrata for up to 30 days and their rates of settlement were assessed. For most of these experiments the coefficient of variation (CV) is shown to highlight differences in variability instead of differences in mean settlement (Jacobs and Podolsky 2010). CV is not a statistical test, but instead was used to display variance comparing within and among CCA individuals on the same figure.

Materials and Methods
Species Studied

Coral species with internal fertilization of their gametes (brooders) included in this study were *Leptastrea purpurea* (Hawai'i), *Favia fragum* (Belize), *Pocillopora damicornis* (Hawai'i), and *Porites astreoides* (Belize). The species that have external fertilization (spawners) included *Acropora cervicornis* (Belize), *Montipora capitata* (Hawai'i), and *Orbicella faveolata* (Belize). The dates of larval collection for each species are listed in Table 2.1. For the spawning species the dates listed in the table are the dates the gametes were collected and fertilized as described below in the larval rearing section.

To reduce variation in settlement substrata only one species of crustose coralline algae (CCA) was used in each ocean basin. In Belize *Hydrolithon boergesenii* was selected because it is relatively common on reefs and can facilitate coral larval settlement (Ritson-Williams et al. 2016). In Hawai'i *Hydrolithon reinboldii* was selected because it is the most similar to *H. boergesenii* in habitat and morphology and can facilitate the larval settlement of Pacific *Acropora* species (Harrington et al. 2004). The algae were identified using morphological characters (Adey et al. 1982, Ritson-Williams et al. 2014).

Larval rearing

For coral species that brood their larvae, coral colonies were brought into the laboratory and held in containers with flow-through seawater. Adult corals and larvae were held in semi-enclosed or shaded (approximately 70% attenuation of sunlight) outdoor facilities that ensured natural light cycles. The corals were constantly held in flow-through seawater baths to ensure natural seawater temperatures. The brooders release their larvae at night, and every evening individual coral containers were fitted with a larval collector. The larval collector was a 800 ml tripour beaker with its bottom replaced with 180 μm nitex mesh. As fresh seawater flowed into each adult coral container the outflow was directed into the larval collector. In this way larvae would spill over the adult container but would be trapped in the larval collector until the morning. Every morning larvae were collected and immediately used in the experiments described

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below. Brooding coral species are known to have larvae that are competent to settle immediately after release (Szmant 1986, Richmond 1988, Richmond and Hunter 1990).

For the spawning corals, gametes were collected from the field and fertilized in the laboratory. For both *A. cervicornis* and *O. faveolata* individual adult corals were fitted with a custom designed net that trapped gametes in a cup that floated above the net as described for *Acropora* spp. (Ritson-Williams et al. 2010). For *M. capitata* gametes were collected from the ocean surface by scooping them into a 2 L bucket during spawning. All gametes were returned to the laboratory and fertilized within an hour of release. Eggs were held with sperm of another individual colony of the same species, or as a random mix for *M. capitata* collected from the surface slick. After one hour excess sperm was rinsed from the eggs and the eggs were placed in a rearing container. For *A. cervicornis* the fertilized eggs were held in six 4 L buckets with their bottom replaced with 100 μm mesh, which was held in a larger bucket that contained seawater. In this way, the seawater could overflow from the outside container but the embryos were held in the inner container without spilling over the top. There was a slow addition of water to each bucket that gently moved the fertilized eggs and ensured fresh seawater was in each container. For *O. faveolata* the fertilized eggs were held in the same type of containers but without the addition of flowing seawater. The fertilized eggs of *M. capitata* were held in six 1 or 2 L plastic containers that were floated on the surface of a large outdoor seawater tank. For both *O. faveolata* and *M. capitata* half of the seawater in their containers was changed twice daily. For all species the larval containers were checked frequently and any dead embryos were removed with a transfer pipette to ensure the survival of the larval culture. Larvae of *A. cervicornis* were used in experiments seven days after fertilization, larvae of *O. faveolata* were used eight days after fertilization and larvae of *M. capitata* were used both seven and nine days after fertilization. For all of these species competence was assessed by confirming the larvae were elongated and actively probed the bottom of their containers.

Settlement variability in response to individual alga

To assess settlement variability in response to the same individual alga and among multiple algae, larvae from multiple parents were pooled. Larvae from the brooders (5-20 individual parent colonies) were immediately pooled the morning of release. Larvae from the spawners were raised as a pooled cohort with 5-10 adult colonies supplying gametes, except for gametes of *M. capitata,* which were collected from the field where greater than 10 colonies were observed spawning.

For all of the brooding coral species 9-10 larvae from the pooled culture were placed into a 60 mm petri dish with 0.2 or 0.45 um filtered seawater (FSW). Ten larvae were used in *O. faveolata* experiments but twenty larvae were used for experiments with *A. cervicornis* and *M. capitata*.

For every experiment individual algae of *H. boergesenii* (Belize) or *H. reinboldii* (Hawai'i) were collected from shallow (<5 m) reef habitats. Individual algae were cut into 1 x 1 cm fragments to be added to the petri dishes with the coral larvae. Each fragment of one alga (4-6 fragments/alga) was randomly assigned a petri dish and placed

so that the CCA surface was face up. Five or six individual coralline algae were used for each coral species to test for a different settlement response within and among multiple algae (Figure 2.1).

Settlement was calculated by adding the number of larvae that settled and metamorphosed onto the CCA surface, the rock under the CCA, and the dish surface. The proportion settlement was calculated by dividing the total number of settlers (on all potential substrata) by the initial number of larvae placed in each dish. Since proportion data are constrained between 0-1, data were arcsine square root transformed to ensure normality. The coefficient of variation (CV, calculated as the SD/mean) was calculated for settlement within each individual CCA (n=4-6). Among coralline algae CV was also calculated from the total proportion settlement (totals were calculated by combining individual dishes for each CCA alga, for a denominator of 40-60 larvae per alga) and calculating the CV using each CCA as a replicate (n=5-6). A Levene's test was used to determine if different individual alga caused different variability in time until settlement within a coral species. A Levene's test was also used on all the replicate algae for a coral species to determine if there was a significant difference in settlement variability among species. The total settlement on an individual CCA data for all species were coded categorizing them as either a brooder or a spawner, and a Levene's test was used to determine if reproductive mode influenced the observed variation in settlement. Data from 9 day old *M. capitata* was excluded for the brooder and spawner comparison.

Within brood settlement variability

Three brooding species were studied further to determine if individual coral colonies produced larvae that had variable settlement or if among colony variation was responsible for the patterns observed in the experiment that used larvae pooled from multiple colonies. *F. fragum*, *P. astreoides* and *P. damicornis* were used to track individual broods of larvae as these were the only species that had individual colonies that released enough larvae for replicated experiments. In all experiments ten larvae were added to each 60 mm petri dish. A Levene's test was used to determine if the settlement variation was significantly different among different algae for the larvae from one parent colony. A Levene's test was used to compare variation in settlement among colonies within a species, and among species using total settlement on each CCA individual as replicates (n=2-5 per colony).

Larval Duration Until Settlement

Time until settlement was assessed with larvae of *F. fragum* and *P. damicornis*. Larvae of *F. fragum* and *P. damicornis* were maintained in 800 ml tripour beakers with 153 μm nitex mesh on their bottom that were held in larger containers of flow-through seawater. Each brood (ranging from 34 to 142 larvae) from an individual colony was maintained in a different larval container independently. A 1 x 1 cm fragment of *Hydrolithon* was added to each larval container and was removed and replaced after 24 hours and then after every two day. All swimming larvae were counted initially, after 24

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hours and every two or three days after that. New recruits on the CCA fragments were counted and at every time point the CCA fragment was removed from the larval container and replaced with a different CCA fragment of the same species but not the same alga. After 3-7 days the larvae were moved to containers that had been cleaned with freshwater and dried. The proportion of swimming larvae for every time point was calculated using the initial number of larvae placed in the containers as the denominator. Very few larvae died in each brood (0-5) so the percent swimming larvae reported reflects the cumulative number of larvae that had settled.

Results
Settlement variability in response to individual alga

Figure 2.2 shows the CV for replicates within individual CCA and among the different CCA individuals for each coral species. A Levene's test determined that there was no difference in the larval settlement variation among any of the individual CCAs for each coral species (Table 2.2). A Levene's test found no significant difference of settlement variability among the different coral species (F=0.893, p=0.523). There was significantly more settlement variance in brooders when compared to spawners (F=9.309, p=0.004), even when 7 day old *M. capitata* (the only spawner tested that contains *Symbiodinium* in the larvae) was excluded (F=8.942, p=0.006), or if the data for 9 day old *M. capitata* was used instead of the 7 day old data (F=8.195, p=0.007).

Within brood settlement variability

The CV for settlement variability within and among CCA individuals is shown in Figure 2.3. A Levene's test determined that there was no difference in the variance in settlement among CCA individuals from larvae produced within a single colony for *F. fragum* (F=0.787, p=0.573), *Porites astreoides* (F=0.492, p=0.742) and *Pocillopora damicornis* (F=0.291, p=0.831). There was significantly more variance in settlement among individual colonies in *Pocillopora damicornis* when compared to among colony variation in both *F. fragum* and *P. astreoides* (F=4.365, p=0.019).

Planktonic Duration Until Settlement

Over 50% of the *F. fragum* larvae were still swimming four days after release (Figure 2.4a). For all but one colony, only 20% of the larvae continued swimming until the twelfth day. After fourteen days most of the larvae had settled but there were a few larvae from each colony that continued to swim until the end of monitoring on the nineteenth day.

For all *P. damicornis* colonies, 50% of the larvae had settled by day seven (Figure 2.4b). By the twelfth day, less than 20% of the larvae remained swimming for the majority of the colonies. All of the larvae from one colony settled by day eight and another colony had 100% settlement by the nineteenth day. However, the other three

colonies had a few larvae that continued to swim until the thirtieth day, which was the end of monitoring.

Discussion

Throughout these experiments there was extensive variability in the number of larvae that settled in response to an individual alga. The mean rates of settlement in response to *Hydrolithon* spp. were consistent with previous reports (Figure 2.2a), but analyzing the variance showed as much variability in settlement in response to the same individual alga as there was among different individuals of the CCA (Figure 2.2b). This settlement variability was similar for all of the coral species tested. Additionally, for three brooding coral species variance in settlement response to an individual alga was found within a single brood of larvae.

For the corals studied here there was variation in settlement in all of the species, but there was a significant difference in variance between brooders and spawners. A recent synthesis suggested that reproductive mode could influence the dispersal strategies of organisms with outcrossing favoring dispersal and self-fertilization favoring little or no dispersal (Auld and de Casas 2013). While reproductive mode is somewhat flexible in corals there is a trend for outcrossing in spawners (Carlon 1999) and self-fertilization in brooders (Carlon and Lippe 2011). Higher variability in brooded larvae could also be due to larger larval size and the presence of *Symbiodinium* that might contribute energy during dispersal (Isomura and Nishihira 2001, Harii et al. 2010). Since the brooders can get energy from symbionts while in the plankton it could be assumed that only coral species that brood their larvae are capable of having variable settlement (Cumbo et al. 2012). However, variability in the time until settlement should not be considered a dichotomy between brooders and spawners, instead the data presented here show that corals with both life history strategies exhibit some degree of settlement variability.

Broods from each colony had some larvae that settled immediately and some that had greater dispersal potential, more than 30 days for some *P. damicornis* larvae (Figure 2.4). For *P. damicornis* there was significant variation among colonies, with some colonies producing larvae that settled at high rates immediately (with a low CV) and some colonies produced larvae with low rates of settlement in 24 hours (Figure 2.3c). Most of the *F. fragum* colonies produced larvae with low rates of settlement after 24 hours (with high CV) even though there was no significant difference in their variance (Figure 2.3a). Measuring variance illustrates three patterns in larval settlement; 1. Low variance with high rates of settlement immediately, 2. High variance with larvae that settle over a broad range of times, 3. Low variance with delayed settlement. Larvae that exhibit each of these patterns were found among colonies of *P. damicornis*.

What controls the variance in time until settlement in larvae from different colonies? Some studies are showing that maternal effects in marine invertebrates can affect their larval size, which influences much of their larval ecology (Marshall et al. 2008a, Marshall et al. 2008b, Monro et al. 2010). While maternal effects and larval size did not significantly influence post-settlement survival in *Agaricia agarcites* (Hartmann

et al. 2013), maternal affects have not been tested with most coral species. Alternatively, variation in settlement competence could be a consequence of different mating systems as some brooded coral larvae can be produced sexually (Combosch and Vollmer 2013), from self-fertilization (Brazeau et al. 1998, Gleason et al. 2001, Carlon and Lippe 2011), and asexually through parthenogenesis (Ayre and Miller 2004). It may be that each of these mating systems produce larvae with different phenoytypes, but this remains untested. Additionally, it has been proposed that epigenetics has the potential to control the frequency of seed germination time (Herman et al. 2014), but epigenetic control of time until settlement has not been tested in coral larvae. The mechanisms driving variability in larval planktonic duration could be studied to determine the extent that parent colonies can "tune" their larval phenotypes for dispersal.

Testing larval behavior is challenging due to the small scale and complex physical factors that contribute to larval settlement. While settlement variance might be due to different larval traits it could also be due to micro spatial variance within the settlement substrata itself. A few studies have identified bacteria on the surface of CCA that induce coral larval settlement (Johnson et al. 1991, Negri et al. 2001, Sneed et al. 2015), and it is likely that the biofilm communities vary on small spatial scales. Even though the experimental design in this study used replicate fragments of the same individual alga to test variability, each fragment of these algae could have had different biofilm communities or variable surface textures at the scale a larva might detect.

While these studies show extensive variability much more work is necessary to determine if the variability measured in laboratory experiments apply to larval behavior in natural settings. These experiments were not conducted with natural water flow. Many larvae are known to respond to flow and it may be that larvae trapped in still water behave differently than those found on the reef. Laboratory measured pelagic duration is not a measure of dispersal distance, if larvae immediately swim to the benthos and rapidly attach it is unlikely they will disperse among reefs (Carlon and Olson 1993). If larvae are blown off a reef by strong currents they may disperse to the open ocean where there is no settlement substrata. Many larvae are eaten or die in the plankton (Fabricius and Metzner 2004), making larval connectivity among reefs a relatively rare occurrence (Strathmann et al. 2002). All of these caveats of laboratory experiments provide a challenge for understanding coral larval behavior, even as modeling coral larval dispersal and connectivity is increasingly important to understand coral resilience.

Variability in multiple larval traits have been documented and variance in settlement competence is being incorporated into models of marine connectivity (Metaxas and Saunders 2009). One study incorporated a settlement window to model the dispersal of *Acropora palmata* within two ocean basins in the Caribbean (Baums et al. 2006). There is extensive research to understand the pelagic larval duration of corals (Richmond 1987, Wilson and Harrison 1998, Harii et al. 2002, Nishikawa and Sakai 2005, Graham et al. 2008, Heyward and Negri 2010, Nozawa and Okubo 2011). However, many of these experiments did not provide continuous settlement substrata. Two recent studies measured the time until settlement in coral larvae constantly exposed to a mixed benthic assemblage pre-conditioned on settlement tiles (Figueiredo et al. 2013, Chamberland et al. 2017). The experiments by Figueiredo et al. (2013) showed variable

settlement over time for a different suite of spawning and brooding species, including; *Acropora humilis, A. gemmifera, A. millepora, A. valida, Goniastrea retiformis,* and *Platygyra daedalea* (spawners), and the brooders *Seriatopora hystrix* and *Stylophora pistillata*. When this variability was incorporated into an oceanographic model these species had mostly local recruitment consistent with observed patterns of recruitment in the field (Figueiredo et al. 2013). The majority of settlement experiments do not consider the fate of larvae after the first day of competence, but some settlement during the first two weeks (Figure 2.4) suggests that larvae that don't settle immediately could still contribute to patterns of recruitment.

For other marine invertebrate larvae it is known that delayed metamorphosis can have a latent effect on later life history stages (Pechenik et al. 2001, Marshall et al. 2003, Pechenik 2006). Coral larvae can settle in response to biofilms and a few CCA species but cues from CCA were not required for settlement and metamorphosis (Ritson-Williams et al. 2016). Additionally, coral larvae can slow their metabolism during dispersal probably allowing greater flexibility in planktonic duration (Graham et al. 2013a). For *Acropora tenuis*, a species that does not contain symbionts in the larvae, there were no latent affects of delayed settlement among larvae settled 2, 4 and 6 weeks after spawning (Graham et al. 2013b). It may be that multiple adaptations allow coral larvae to survive in the plankton for long durations, increasing the potential for successful recruitment even if they disperse for days to weeks.

Why do some larvae settle immediately and others swim for weeks? It may be microscale variation in the settlement cues but it also might be differences in larval competence. How competence is "programed" into larvae remains unknown, but experiments with neurotransmitters and signaling compounds (Bishop et al. 2006) and experimental techniques to quantify gene regulation (Meyer et al. 2009) and epigenetics (Herman et al. 2014) hold great promise to elucidate the underlying cellular and genetic regulation of variable larval competence. It is likely that the maternal provisioning and the maternal environment all contribute to variable traits in larvae (Crean and Marshall 2009), but this remains to be tested in coral larvae. However, it is clear from the experiments presented here that considerable variability in time until settlement exists for many coral species and this could have important ecological and evolutionary implications for coral recruitment and persistence.

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Table 2.1. The corals species used and the date of their larval release. Reproductive Mode is either brooder (B) or spawner (S). Population corresponds to the variability experiments using larvae from multiple parents. Individual broods corresponds to the experiments comparing individual broods of larvae, and Duration corresponds to the time until settlement experiments. Any cells left empty represents an experiment not conducted with that coral species.

| Species | Repro. Mode | Date of Larval Release | | |
		Population	Individual Broods	Duration
Leptastrea purpurea	B	Aug. 28, 2015		
Favia fragum	B	July 11, 2011	June 21, 2013	June 21,2013
Pocillopora damicornis	B	Aug. 21, 2016	Sept. 28, 2015 Aug. 21, 2016	Oct. 17, 2016
Porites astreoides	B	April 20, 2012	April 20 & 21, 2012	
Acropora cervicornis	S	Aug. 18, 2011		
Montipora capitata	S	July 17, 2015		
Orbicella faveolata	S	Sep. 19, 2011		

Table 2.2. The results of a Levene's test that compared the variance among CCA individuals for each coral species. Larvae of *M. capitata* were tested at both 7 and 9 days old (do).

Species	F value	P value
F. fragum	0.815	0.551
L. purpurea	0.135	0.938
P. astreoides	1.161	0.358
P. damicornis	0.408	0.801
A. cervicornis	0.908	0.493
O. faveolata	1.041	0.412
M. capitata 7do	0.294	0.912
M. capitata 9 do	0.536	0.711

Individuals of *Hydrolithon* sp. (5 large squares) were cut into 5 equally sized fragments and placed in independent petri dishes (circles).

CCA #1   CCA #2   CCA #3   CCA #4   CCA #5

Within CCA individual comparison. Each petri dish in a column has a fragment of the same CCA individual. Settlement = # of settlers/10 per dish, n=5

Among individual CCA comparison. Total settlement is the sum of all petri dishes for a CCA individual (a column). Settlement = total # of settlers/50, n=5.
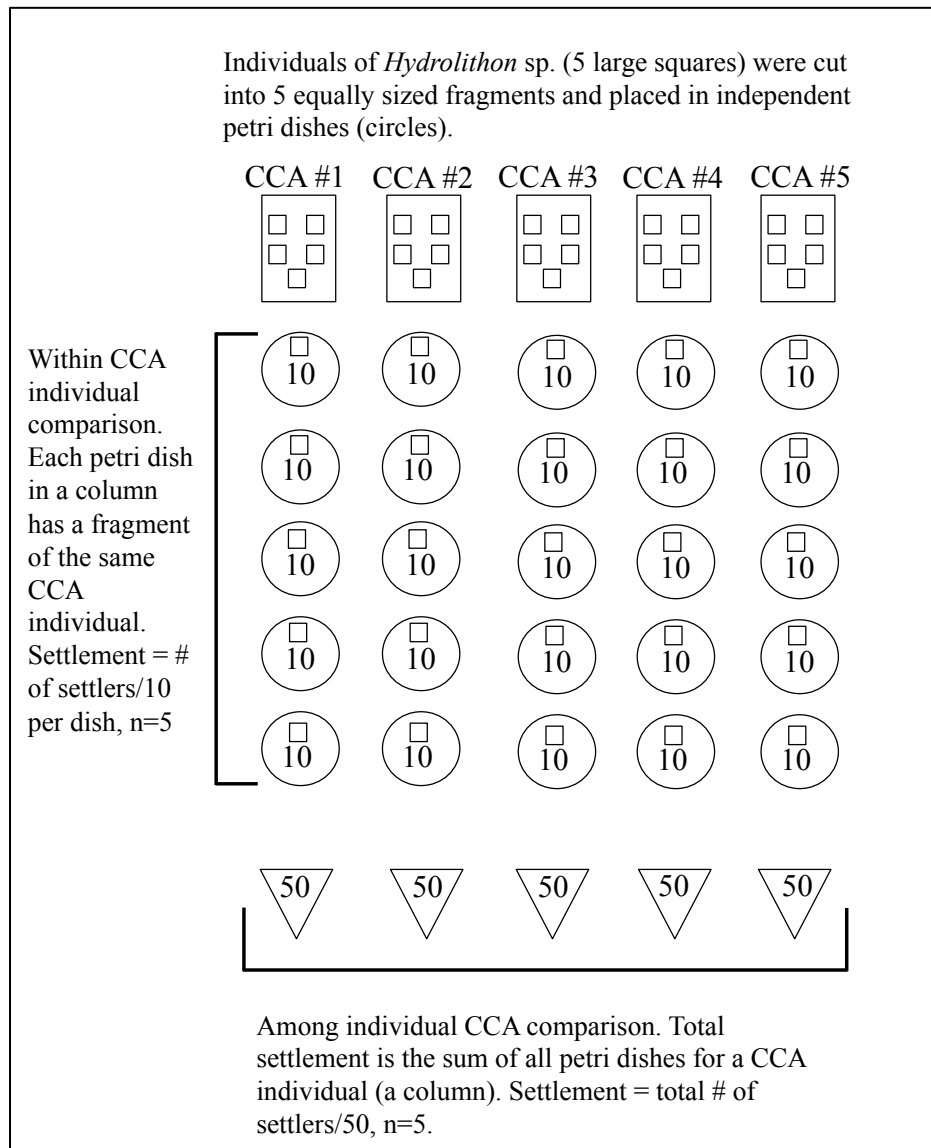
Figure 2.1. A diagram illustrating the experimental design to compare settlement within and among individual CCA. Numbers inside the circles and triangles represent the typical number of larvae used to calculate proportion settlement (see the methods for number of larvae used for each coral species).
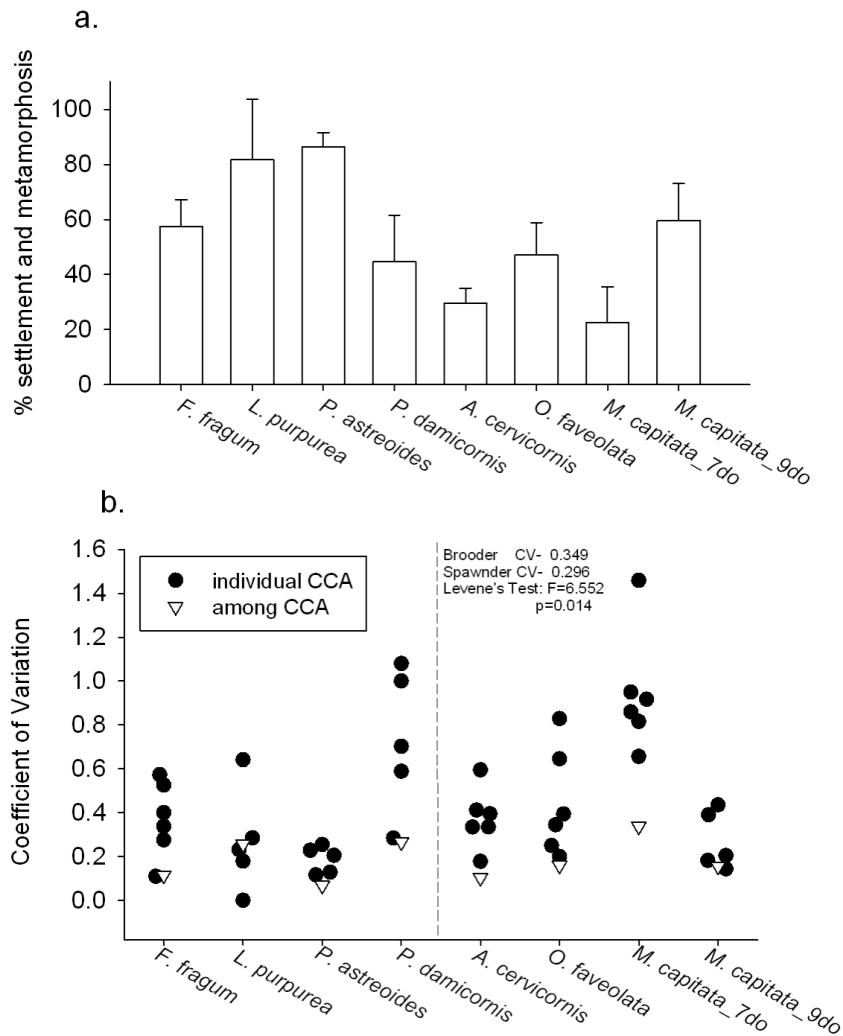
Figure 2.2. Settlement in response to individual and multiple coralline algae for multiple coral species. a. Mean settlement among CCA individuals, error bars are + 1 SD. b. The variation in settlement among larvae of different coral species. Filled circles represent the coefficient of variation (CV) of settlement in response to different fragments of the same individual crustose coralline algae (CCA). The open triangles represent the CV of settlement among the different CCA. Dotted line separates brooding species (left) from the spawning species (right). Data for larvae of *Montipora capitata* were tested at 7 or 9 days after fertilization (do).
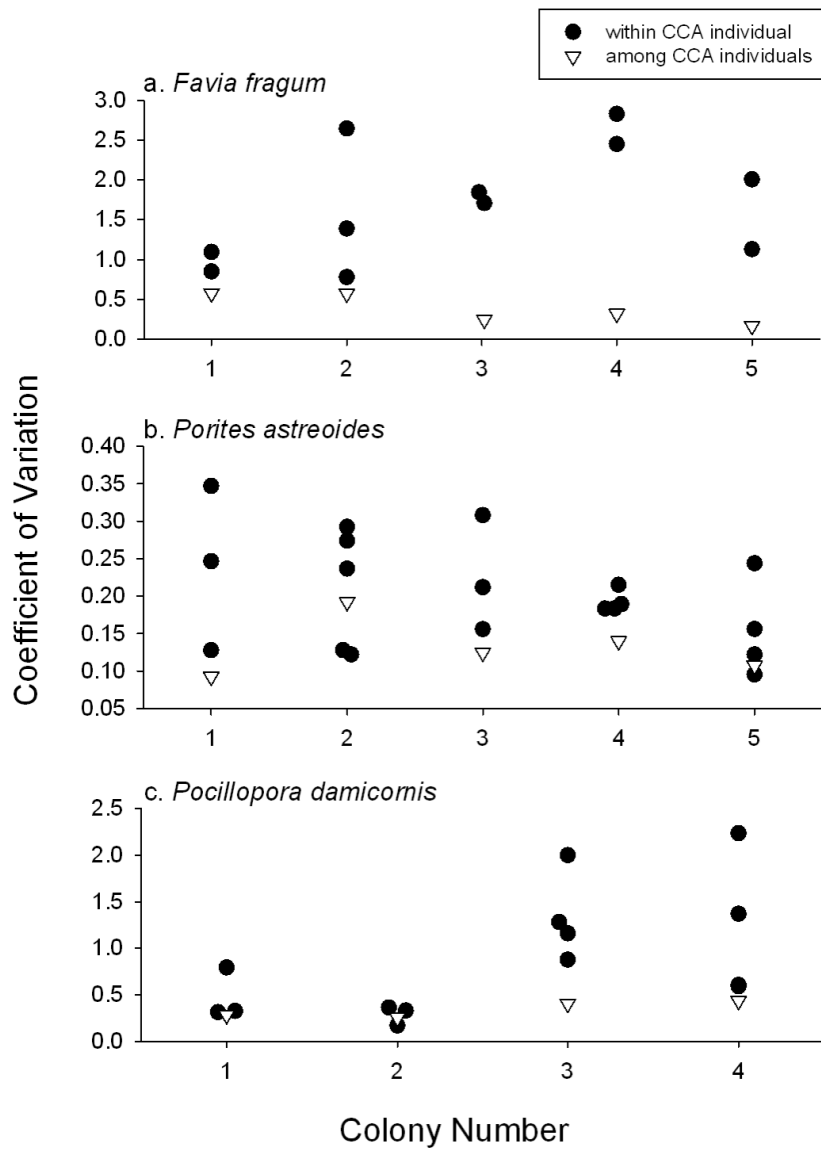
Figure 2.3. The variation in settlement using individual broods of larvae from; a. *Favia fragum*, b. *Porites astreoides*, c. *Pocillopora damicornis*. Filled circles represent the coefficient of variation (CV) of settlement in response to different fragments of the same individual crustose coralline algae (CCA). The open triangle represents the CV of settlement among the different CCA individuals.

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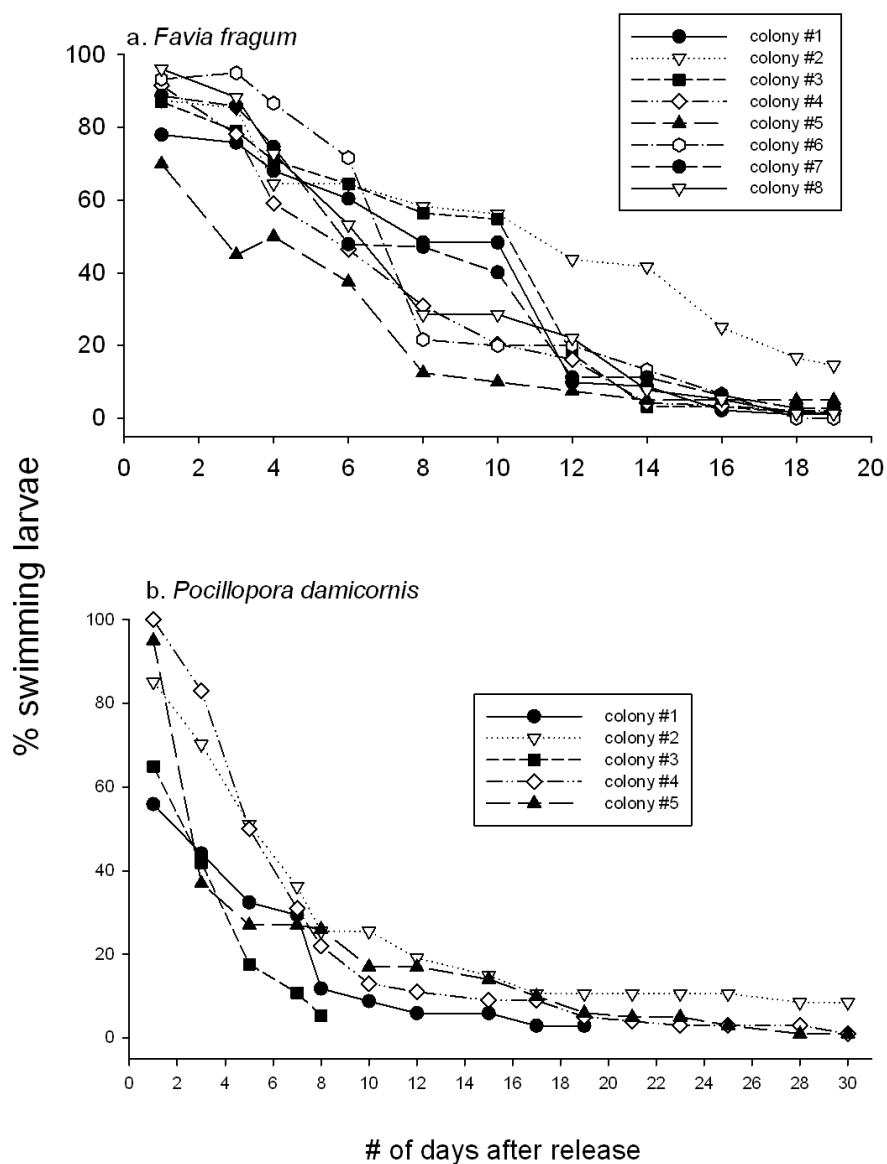Figure 2.4. The duration until settlement for larvae of a. *Favia fragum* and b. *Pocillopora damicornis*. Each line is a brood released from a different colony. Larvae were constantly exposed to settlement substrata for the duration of the experiments.

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CHAPTER 3

Multiple Scales of Coral Resilience to Successive Bleaching Events

Abstract

Coral bleaching is increasing in extent and frequency across the globe. Historically, coral bleaching in the main Hawaiian Islands was extremely rare and only occurred in 1996. However, in the summers of both 2014 and 2015, successive bleaching events occurred in Kāneʻohe Bay, Oʻahu. Seawater temperatures were above 28 °C for approximately one month in 2014 and 3 months in 2015, and peaked above 30 °C in both years. Severe bleaching and paling covered 77 and 55 % of reefs in 2014 and 2015, respectively. Different species showed a range of susceptibility with 80-100% of *Pocillopora spp.* bleaching in both years, but less than 50 % bleaching of *Porites compressa* and *Montipora capitata,* the dominant corals in Kāneʻohe Bay. The encrusting coral *Leptastrea purpurea* had less than 1 % of the colonies bleached in both years. Sixty individual colonies of *P. compressa* and *M. capitata* and 28 colonies of *Pocillopora damicornis* were tagged and visually monitored throughout the two-year period. Cumulatively, 19 % of *P. damicornis*, 10 % of *M. capitata* and no *P. compressa* died by May 2016. Partial mortality did not occur in 2014, but impacted 13 % of the colonies in 2015, with *P. damicornis* and *M. capitata* having higher rates of partial mortality than *P. compressa*. Most of the colonies that bleached recovered their symbionts within 3-4 months in both years, though *P. compressa* visual recovered more rapidly than *M. capitata* and *P. damicornis*. Intermediate bleaching of coral cover, relatively low susceptibility in the dominant species, and low mortality combined with rapid rates of recovery of individual coral colonies in Kāneʻohe Bay suggest that these reefs are resilient to the anomalously high temperatures experienced during 2014 and 2015.

Introduction

Climate change is impacting many types of ecosystems at rapid rates. These changing environmental conditions can cause sublethal stress and mortality for many different organisms (Parmesan 2006). Reef building corals, the ecosystem engineers of reefs, are some of the most susceptible organisms to climate change (Pandolfi et al. 2003, Hoegh-Guldberg et al. 2007). Corals live close to their temperature limits and as seawater temperatures increase some corals bleach (Jokiel and Coles 1973, Glynn 1983, 1984). Coral bleaching is the expulsion of single-celled dinoflagellates from a coral's tissue (Brown 1997, Douglas 2003). This symbiosis is critical to the success and survival of corals (Muscatine and Porter 1977, Goreau et al. 1979), and the breakdown of symbiosis is a sign of sublethal stress that, if not reversed in weeks to months, can result in coral mass mortality (Glynn 1996, Baker et al. 2008). The condition of individual corals (Brown et al. 2002, Thompson and van Woesik 2009, Carilli et al. 2012, Howells et al. 2013) and multiple local abiotic conditions (Fitt et al. 2001) all contribute to the susceptibility of corals to bleaching events.

Coral bleaching is increasing in frequency as seawater temperatures continue to warm due to climate change (Baker 2003, Hughes et al. 2003, Baker et al. 2008). As technology has advanced we are better able to predict bleaching events and predict bleaching across wide geographic scales using satellite data (Donner et al. 2005, Liu et al. 2014, Logan et al. 2014). These tools are a useful method to quantify regional bleaching impacts but little is known about the variation in bleaching among coral populations in local habitats. The overwhelming trend on reefs after a bleaching event is an extensive reduction in the live percent cover of corals (Edwards et al. 2001, Loya et al. 2001), and recovery can take 5-10 years after a bleaching event (Golbuu et al. 2007, Diaz-Pulido et al. 2009, Gilmour et al. 2013). Some corals bleach but recover their symbionts within months without dying (Levitan et al. 2014, Guest et al. 2016). As seawater temperatures increase around the world we must understand the features of a habitat that enable corals to resist and or rapidly recover from bleaching if we hope to maintain reef ecosystems.

Even though there have been extensive laboratory studies to understand the cellular and organismal impacts of bleaching (reviewed in Baker 2003) there has been relatively little documentation on individual populations that resist and recover from changing conditions (Coles and Brown 2003, Edmunds and Gates 2008, Palumbi et al. 2014, Cacciapaglia and van Woesik 2015). Some coral populations were hypothesized to have acquired resistance to bleaching in consecutive events separated by 3-10 years (Maynard et al. 2008, Guest et al. 2012, Pratchett et al. 2013). However, there is limited information on the mechanisms responsible for increased resistance. Both resistance to stressful events and the patterns and rates of recovery from stress is critical to understanding resilience (Hodgson et al. 2015). However, resistance and recovery are difficult to quantify with typical reef surveys and should be measured at multiple scales. Assessing variability among individuals, among species, and among reef areas is necessary to accurately document the fate of bleached reefs (Chapter 1). Integrating multiple scales of bleaching provides data about the extent of bleaching as well as the

capacity for recovery and the impact of bleaching on coral community diversity. Individual coral colonies can be monitored for the rates of bleaching and recovery, but are rarely monitored in a natural setting (Baird and Marshall 2002, Jones et al. 2008). As the frequency and extent of bleaching intensifies we need to understand patterns of individual and population scale performance to better manage for increased resilience.

To better understand the patterns of coral resilience *in situ* three patch reefs were studied in Kāneʻohe Bay on the island of Oʻahu. Coral reefs in Hawaiʻi provide an important case study because they bleach very rarely. Before 2014 the only bleaching event recorded in the main Hawaiian Islands was in 1996 (Jokiel and Brown 2004). However, more recently the reefs in Hawaiʻi experienced elevated seawater temperatures and subsequent coral bleaching in both summers of 2014 and 2015. This study uses multiple ecological survey methods to assess coral resilience in response to repeated stressors. Natural patterns of bleaching resistance and recovery are documented by monitoring coral populations and individuals in the field. By studying populations in the wild that survive bleaching we begin to understand the key traits of resilient reefs. With increased knowledge of resistance and recovery reefs can be better managed for long-term resistance to climate change.

Materials and Methods
Sites Studied and Physical Environmental Parameters

In September 2014 bleaching was observed throughout Kāneʻohe Bay on the east coast of Oʻahu. Field surveys were conducted at three reefs in the bay to characterize the extent of coral bleaching and to document patterns of recovery. Reef 44 is located at the northern end of Kāneʻohe Bay, reef 25 is in the middle bay and the reef at the northwestern side of the Hawaiʻi Institute of Marine Biology (HIMB) is located in the southern portion of the bay (Figure 3.1). These reefs were selected to encompass a gradient of exposure to different abiotic conditions with reefs in the north of the bay (44) characterized by greater amounts of freshwater input as well as greater oceanic influence, and reefs in the south (HIMB) with less mixing and a longer retention time of seawater (Lowe et al. 2009).

At each of these reefs abiotic parameters were monitored at 2 m depth starting October 2014. Temperature was recorded every 15 minutes using a HOBO pendant logger placed inside the cement block so that it was shaded from direct light. The temperature loggers were calibrated together and a linear regression conversion factor was applied to ensure the data from different loggers were comparable. Photosynthetic Active Radiation (PAR) was recorded every 15 minutes using an Odyssey PAR logger. PAR loggers were calibrated in laboratory flow-through seawater tanks by comparison to a Li-Cor model LI-1400 to create a standard curve of $\mu mol \ s^{-1} \ m^{-2}$ using a linear regression equation. Sediment was measured in 5 cm diameter PVC tubes that were capped at the bottom. The tubes were 42 cm long giving a 7:1 ratio of trap height to mouth width. Each sediment tube was held upright by the cement block. Each tube was collected monthly and the sediment was filtered away from the seawater, rinsed with fresh water (200 ml), dried at 60 °C for 3-7 days and weighed. Sedimentation rates

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(grams of sediment per day) were calculated by dividing the dry weight of the sediment by the number of days the trap had been in the water.

Twenty years of temperature data was downloaded from the NOAA buoy 1612480 Mokuoloe in Hawaiʻi and from the NOAA buoy 1630000 Apra Harbor in Guam. Guam was chosen as a typical tropical location (13 °N) because it has seawater temperature data dating back to 2004 (with a few gaps). This allows a comparison of the extent of temperature fluctuation in Hawaiʻi (subtropical) with a more typical tropical location. Monthly maximum and minimum temperatures were calculated from the data to better characterize the range of temperature fluctuations that occur in Kāneʻohe Bay and Guam over 20 years, from 1994-2014. The range of temperatures in a month and a year were calculated and the means were compared using a t-test between Hawaiʻi and Guam to determine if there was a difference in the range of temperatures that corals experienced at these two sites.

Community scale bleaching

Percent bleaching was assessed with 5 replicate 10 m long video transects on reef 44, reef 25 and the north side of HIMB. The video was taken on October 23, 2014 at 2 m and October 30, 2014 at 0.5 m depth and for 2015 all transects were recorded on October 22. The video recorder was held forty cm above the benthos and ten still frames of approximately 0.6 m^2 were extracted from each replicate video. Five of these still frames were selected at random (random numbers generated in excel) for analysis using Coral Point Count with Excel (CPCE v4.1). Fifty random points were overlaid and categorized on each still image. Each point that fell on a coral was visual assessed and marked as bleached, partially bleached or dark. The mean percent of coral cover, percent severely bleached and percent pale were calculated as the mean from each of the five replicate transects for each depth at each reef. Differences in proportion bleached between depth, between 2014 and 2015 and among reefs were tested with a three-way ANOVA of proportion severely bleached data that were normally distributed and had equal variances after an arcsine square-root transformation.

Among species bleaching susceptibility

Thirty minute timed swims were used to assess the susceptibility of different coral species in Kāneʻohe Bay. Nine reefs (44, 43, 42, 25, 22, 20, 5, 3, North side of HIMB) were surveyed on October 29 to November 6, 2014 and October 19-22, 2015 at depths between 0-7 m and every individual coral colony encountered was counted and categorized as bleached (>90 % bleached or very pale) or healthy. The proportion bleached was calculated as the number of individual colonies bleached divided by the total number of colonies counted. The percent bleached was calculated for each species on each reef and the data shown are the means calculated from replicate reefs, with n-reefs the number of reefs on which that species was found. If a coral species was found on less than three reefs it was not included in the data analysis. For bleaching susceptibility in *Porites compressa* and *Montipora capitata* the number of bleached or

healthy colonies were counted from the 5 replicate video transects at 2 m and 0.5 m described above. Due to the high coral cover of *P. compressa* and *M. capitata* only the data from three reefs (44, 25, North side of HIMB) were used to calculate susceptibility for these two species. The percent of colonies severely bleached was rank transformed because it didn't meet the assumptions of normality or equal variances. A two-way ANOVA was used to compare among species and between 2014 and 2015. A post hoc Tukeys Test was run to determine groups with significantly different means.

Individual colony scale bleaching, recovery, and mortality

One hundred and forty-eight individual coral colonies were tagged and assessed for bleaching recovery and partial to full mortality from October 2014 to March 2016. At each reef twenty individual *P. compressa* and *M. capitata* were tagged 24 October 2014. The corals were tagged as adjacent pairs, where one colony was bleached and the other was dark brown (Figure 3.2). In addition, 9-10 colonies of bleached *Pocillopora damicornis* were tagged at each reef (since there was such a high rate of bleaching in *P. damicornis* adjacent pairs were not available). Tagged colonies were photographed every 3-6 weeks and for each time point the colonies were ranked with a visual bleaching score, similar to the scoring scheme used in Guest et al. (2016). A score of 0 was used to indicate a dead colony. A score of 1 was assigned to any coral that was severely bleached (greater than 90 percent of the colony area bleached or extremely pale). A score of 2 was assigned to a coral that was partially bleached or appeared pale compared to a normal color for that colony ("normal" colony color was determined from photographs of that individual during the March or April time point in 2015). A score of 3 was assigned to any coral that had a dark brown color that was "normal". Using only the corals that bleached, the mean bleaching scores were calculated for each species at each reef at each time point. To assess for a difference in recovery rates a three way repeated measures ANOVA was performed with species, reef and time as fixed factors and the bleaching score as the dependent variable. In January and February 2015 a windstorm toppled some colonies, changing the number of bleached corals used to calculate mean bleaching scores; Reef 44 *M. capitata* n=9, *P. compressa* n=10 and *P. damicornis* n=9. Reef 25 *M. capitata* n=9, *P. compressa* n=10 and *P. damicornis* n=10. HIMB, *M. capitata* n=7, *P. compressa* n=8 and *P. damicornis* n=8.

The photographs of individual colonies from the March 31, 2016 were assessed for mortality analysis and the frequency of the number of colonies experiencing full, partial and no mortality was compared among species and among reefs using a chi-squared test.

Results
Environmental data

The 2014 and 2015 data for temperature (Ritson-Williams and Gates 2016c), light (Ritson-Williams and Gates 2016a), and sediment (Ritson-Williams and Gates 2016b) at each reef are downloadable data sets archived at Zenodo. The temperatures and PAR

from October 2014 to December 2015 at the three reefs were similar (Figure 3.3). Sediment load at each reef was variable during the monitoring period (Figure 3.3c). From 1994 to 2014 temperatures in Kāneʻohe Bay fluctuated from a maximum of 31.4 °C to a minimum of 19 °C, with temperatures above 30 °C only in 1996 and 2014 (Figure 3.4a). These data were compared to Guam, which has temperature fluctuations more typical of a tropical reef. At Kāneʻohe Bay the difference in seawater temperature maximum to minimum was a monthly mean of 2.7 °C (n=231) and of 7.7 °C per year (n=20). In Apra Harbor on Guam there was a mean range of 1.9 °C (n=154) per month and of 5 °C (n=16) per year. There were significant differences in the monthly (Mann-Whitney, $p<0.001$, U=8248) and yearly (t-test, $p<0.001$, T=7.57, df=34) range of seawater temperatures between Hawaiʻi and Guam. A comparison of seawater temperatures in Kāneʻohe Bay during June-December in 2014 and 2015 shows that the corals were exposed to different thermal regimes that both resulted in extensive bleaching (Figure 3.4b).

Community scale bleaching

The area surveyed for bleaching in video transects for Kāneʻohe Bay was assessed in both 2014 and 2015 at two different depths (0.5m and 2m; Table 3.1). The data for proportion of coral cover severely bleached showed no difference among the reefs (3-way ANOVA, $p=0.667$, F=0.408), but at every reef there was significantly less bleaching at 2 m depth ($p<0.001$, F=29.379). There was significantly less bleaching in 2015 compared to 2014 ($p=0.013$, F=6.608). There was only one significant interaction between year and reef ($p=0.045$, F=3.307) that showed reef 25 in 2015 had less bleached coral cover than in 2014.

Among species bleaching susceptibility

There were different bleaching susceptibilities among coral species in Kāneʻohe Bay (Table 3.2). There was a significant difference in the proportion of severely bleached colonies among coral species (2-way ANOVA, $p<0.001$, F=27.40). A Tukeys HSD post hoc test showed that *Pocillopora* spp. were the most susceptible and *Leptastrea purpurea* was the least susceptible. There were significantly fewer colonies bleached in 2015 compared to 2014 ($p<0.001$, F=12.79). There was no interaction between coral species and year ($p=0.378$, F=1.09).

Individual colony scale bleaching, recovery, and mortality

The individually tagged corals showed different rates of visual recovery from bleaching among species, with *P. compressa* recovering faster than *M. capitata* and *P. damicornis* (Figure 3.5, 3-way RM ANOVA, $p=<0.001$, F=5.98). There was no difference in the rate of recovery among the three reefs (3-way RM ANOVA, $p=0.321$, F=1.156). There was an interaction between reef and time ($p=0.01$, F=1.62), with *M. capitata* showing reduced recovery at HIMB for one time point, December 17, 2015. There was also a significant interaction between species and time ($p<0.001$, F=3.97).

Overall there was 7.5 % full mortality of tagged colonies, with five (10 %) *M. capitata* (three bleached and two healthy colonies) and five (19 %) *P. damicornis*, but no colonies of *P. compressa* that died during the monitoring (Figure 3.6). The frequency of colonies experiencing full and partial mortality varied among species with similar rates in *P. damicornis* and *M. capitata* but lower rates of mortality in *P. compressa* (Figure 3.6, chi squared=14.416, p=0.006). The mortality rate was similar at HIMB (7.9 %), at reef 25 (6.3 %) and at reef 44 (8.5 %). Mortality was not significantly different among reefs (chi squared=9.242, p=0.055), although the power was low on this analysis.

Discussion

The corals in Hawai'i experienced two successive summers of high seawater temperatures that resulted in extensive coral bleaching. Bleaching transects in Kāne'ohe Bay showed that both years had similarly severe bleaching, impacting 60-80 % of the coral cover. However, most of the corals in Kāne'ohe Bay recovered from both bleaching events and cumulative mortality was less than 10 %. The extent of a temperature anomaly and its duration are critical variables for the severity of coral bleaching (Glynn et al. 2001, McClanahan et al. 2007). In both 2014 and 2015 there was a peak in seawater temperature during September to above 30 °C. In October and November the seawater temperatures dropped rapidly, probably contributing to the high rates of survival and rapid recovery of the corals in Kāne'ohe Bay (Figure 3.5). Coral bleaching has only previously happened in the main Hawaiian Islands in 1996, which is the only other year that seawater temperatures peaked above 30 °C. Bleaching in 2014 and 2015 triples the observed occurrence of coral bleaching in Hawai'i. While frequency, severity and extent of coral bleaching is increasing across the planet, there is a pressing need for monitoring long-term trajectories of coral populations to better understand which species will persist in these habitats.

There were different rates of susceptibility to bleaching among different coral species. These rates of susceptibility for Hawaiian coral species match published species susceptibilities from other locations (Marshall and Baird 2000). However, our data show that *Pocillopora spp.* were especially vulnerable to high seawater temperatures in both summers. Of the three coral species in which individual colonies were monitored, *P. damicornis* had the highest mortality. Very low rates of bleaching were observed in the encrusting species *Leptastrea purpurea*. This small encrusting species had a bleaching susceptibility of less than 1% both years of study. The fact that some species will be winners and others will be losers in the future (Loya et al. 2001, Edmunds et al. 2014) is important as this data can help predict the species composition of future reef communities.

The among species susceptibility data suggest that the reefs in Kāne'ohe Bay are becoming increasingly resistant to thermal stress events since there were significantly less colonies affected by bleaching in 2015 than in 2014. However, this data could be biased by different duration of thermal stress since seawater temperatures were above 28 °C in June 2015 but not until August in 2014. Some research shows that corals are more likely to resist bleaching if they are exposed to a longer duration of warm temperatures

prior to thermal stress (Ainsworth et al. 2016), which corresponds to the seawater temperature pattern in 2015. However, all of the tagged colonies that bleached in 2014 bleached again in 2015, suggesting that individual corals did not acclimatize to thermal stress. Additionally, individual *M. capitata* colonies showed no shuffling of their dominant *Symbiondium* clade in 2015 (Cunning et al., 2016). Monitoring individual coral colonies is necessary to determine if reefs are becoming increasingly resistant over time. Monitoring at the individual scale gives us a powerful tool to tease apart how reefs are adapting to thermal stress; are individuals dying leaving only the resistant individuals alive, or are individuals acclimatizing allowing the preservation of genotypic diversity through a bleaching event?

Overall the 148 individual coral colonies that we monitored showed high variability in bleaching susceptibility, but those that did bleach showed consistent recovery of their symbionts. The paired corals monitored in this study are not a random sampling of *P. compressa* or *M. capitata*. These colonies were intentionally selected as pairs to minimize the potential confounding effects of microhabitat heterogeneity. But these pairs were not an anomaly, there were bleached and unbleached corals adjacent to each other at every reef visited, indicating high phenotypic diversity within this population.

In situ recovery rates were relatively rapid with most corals becoming darkly pigmented three months after experiencing maximum seawater temperatures (Figure 3.4). There was no difference in the recovery rate of individuals among reefs in this analysis. In 2014 and 2015 *Montipora capitata* colonies were tracked for their *Symbiodinium* abundance and the health scores reported here corresponded very well to quantification of the abundance of *Symbiodium* cells normalized to host coral cells (Cunning et al. 2016). However, while Cunning et al. (2016) report a slower recovery of corals at HIMB, there was no difference in the recovery rate among reefs detected in the current study. This is probably due to reduced resolution using the visual scores, but these visual assessments are non-invasive and rapid allowing for monitoring at a higher frequency than is found in most studies.

In fifteen months only five *Montipora capitata* and five *Pocillopora damicornis* individual colonies died. There was no partial mortality within a coral colony after the 2014 bleaching but there was some after the bleaching in 2015, probably due to the cumulative effect of two consecutive stress events. Since partial mortality does not eliminate a genotype from the population this impacts coral cover but not genotypic diversity. These low rates of mortality are probably due to relatively rapid rates of seawater cooling in November of both 2014 and 2015 (Figure 3.4). Kāneʻohe Bay also has relatively high rates of sedimentation and has abundant plankton for heterotrophy. Reefs that have low light stress and high potential for hetertrophic nutrition are probably more capable of recovery after a bleaching event (Guest et al. 2016).

There was variation in bleaching susceptibility among species and also within species. Some studies found that hosting different *Symbiodinium* types contributed to variation in bleaching susceptibility (Rowan et al. 1997, Glynn et al. 2001). However, analysis of the ITS2 gene region of the *Symbiodinium* in *M. capitata* tagged in this study showed that while colonies hosting type D1a did not bleach, only some colonies hosting

C31 bleached, while other colonies did not (Cunning et al. 2016). Furthermore, *Porites compressa* in Hawaiʻi is only known to host ITS2 type C15 (LaJeunesse et al. 2004, Stat et al. 2013), suggesting that intraspecific bleaching resistance is not driven by *Symbiodinium* type. There are many other potential factors that contribute to variation in intraspecific phenotypes and further work on coral genetic adaptation (Palumbi et al. 2014), gene expression and physiology (Csaszar et al. 2009, Barshis et al. 2013) and microbiome (Ainsworth et al. 2010, Littman et al. 2011) is needed. Regardless of the cause these field surveys document the presence of within species variation in populations that could be important raw material for adaptation to climate change.

Local adaptation is one mechanism that has been studied in terrestrial environments that allows plants to survive in degraded habitats (Joshi et al. 2001, Siol et al. 2010, Anderson et al. 2011). Local adaptation to stressful conditions is well documented, but has only recently been studied for corals (Palumbi et al. 2014) and *Symbiodinium* (D'Angelo et al. 2015). Local adaptation may be driving the resilience of Kāneʻohe Bay corals because these corals have been exposed to annual temperature variations greater than 10 °C (Figure 3.4) and Kāneʻohe Bay has a rich history of human induced disturbance (Bahr et al. 2015). Currently, in Kāneʻohe Bay *P. compressa* and *M. capitata* make up greater than 95% of the coral cover on these reefs, and reduced diversity can be found in other disturbed habitats, which may be an important consequence of local adaptation. Climate change is known to lower genotypic diversity through multiple mechanisms (Pauls et al. 2013), and thermal stress can reduce genetic diversity on reefs (Selkoe et al. 2016). Resilience is a double edged sword, increased resistance to and recovery from stressors in Kāneʻohe Bay appear to correspond with a reduction in species diversity and potentially a reduction in genotypic diversity.

Resilience has been attributed to a few reefs, but the type of response to bleaching is quite variable. Corals in French Polynesia were assessed for bleaching susceptibility during four bleaching episodes from 1991-2007 (Pratchett et al. 2013). While the trends show increased resistance, since these authors did not monitor the same colonies it is impossible to tell if this is a result of acclimatization, differential mortality, or an artifact of different environmental stressors during different bleaching events. Macroscale refugia have been predicted for multiple locations in the Pacific and Indian Oceans, but there is relatively little known about refugia on a local scale (Cacciapaglia and van Woesik 2015). In 2010 in Singapore the reefs were characterized by relatively low rates of bleaching and rapid recovery within a few months (Guest et al. 2016). Corals in nearshore bays of Palau were more resistant to bleaching than offshore reefs even though they had higher seawater temperatures (van Woesik et al. 2012). Sites in Africa showed less bleaching associated mortality if they experience the largest temperature variation (McClanahan et al. 2007). The reefs at Singapore and Palau are characterized by highly variable temperature regimes and high sedimentation, and so are the reefs in Kāneʻohe Bay. Additionally, these reefs have a reduced coral fauna compared to other nearby reefs. Using these case studies there is a trend for corals living in fluctuating temperatures and high turbidity to be more resistant to coral bleaching even though they live in "degraded" conditions. This suggests that corals adapted to local stress may be more resilient in the

face of climate change, which is critical information to detect other sites that could be local refugia from bleaching.

Two successive bleaching events are unprecedented in Hawai'i and the corals monitored here offer an important example of some colonies and reefs that can recover from two thermal stress events. Rarely are multiple scales of coral reefs monitored for their resistance to and recovery from bleaching, but it is critical that we study multiple scales to fully measure coral resilience. No one monitoring protocol is perfect but integrating methods can better assess coral resilience to fully understand which coral populations might persist in a future of climate change. Multiple studies show that corals that are locally adapted to variable habitats can resist stress and recover from exposure to a more extreme climate.

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Table 3.1. The extent of bleaching on three reefs in Kāneʻohe Bay, Oʻahu, Hawaiʻi. Bleaching is defined as points that were pure white, paling is defined as brown but paler than a normal brown. % Bleached and % pale were calculated as a proportion of total coral cover. n=5 for each reef at each depth in both years.

Reef	Year	Depth (m)	% coral cover (SE)	% bleached (SE)	% pale (SE)
HIMB	2014	0.5	60.1 (3.4)	22.9 (5.1)	62.1 (4.6)
		2	56.2 (5.9)	12.3 (3.3)	45.6 (2.8)
	2015	0.5	61.6 (2.9)	27.5 (8.5)	45.2 (5.8)
		2	51.3 (4.3)	13.0 (3.9)	31.7 (5.1)
25	2014	0.5	72.0 (4.2)	36.3 (4.2)	56.1 (5.1)
		2	85.0 (3.0)	17.1 (4.6)	55.7 (7.6)
	2015	0.5	72.4 (7.1)	17.5 (4.7)	49.8 (3.1)
		2	85.4 (2.5)	6.9 (0.5)	35.7 (5.3)
44	2014	0.5	54.7 (4.6)	25.0 (4.9)	57.7 (5.0)
		2	78.1 (3.0)	14.8 (3.1)	58.5 (4.0)
	2015	0.5	47.7 (3.2)	22.5 (5.8)	45.7 (6.4)
		2	76.8 (3.8)	4.9 (1.2)	39.5 (4.1)

Table 3.2. The bleaching susceptibility of different coral species in Kāneʻohe Bay, Oʻahu, Hawaiʻi. Susceptibility was calculated based on proportion of individual bleached colonies per patch reef. Mean was calculated from replicate reefs. There was a significant difference in the amount of bleaching between 2014 and 2015 and letters next to species names indicate significant groupings as determined by Tukey's post hoc test.

Coral species	Year	% bleached (SE)	n reefs	# of colonies
Pocillopora meandrina[a]	2014	100 (0)	4	7
	2015	100 (0)	4	8
Pocillopora damicornis[a]	2014	90.6 (2.4)	9	1518
	2015	81.4 (7.6)	9	1177
Montipora spp.[b]	2014	65.3 (7.1)	8	161
	2015	44.3 (10.8)	7	146
Pavona varians[b]	2014	62.9 (13.8)	4	36
	2015	24.9 (9.2)	7	41
Porites compressa[b]	2014	43.7 (2.6)	3	903
	2015	19.7 (0.7)	3	980
Montipora capitata[bc]	2014	36.7 (1.9)	3	338
	2015	16.6 (4.7)	3	388
Fungia scutaria[b]	2014	35.4 (4.0)	9	662
	2015	26.5 (10.7)	9	584
Leptastrea purpurea[c]	2014	0.75 (0.8)	7	563
	2015	0 (0)	6	823
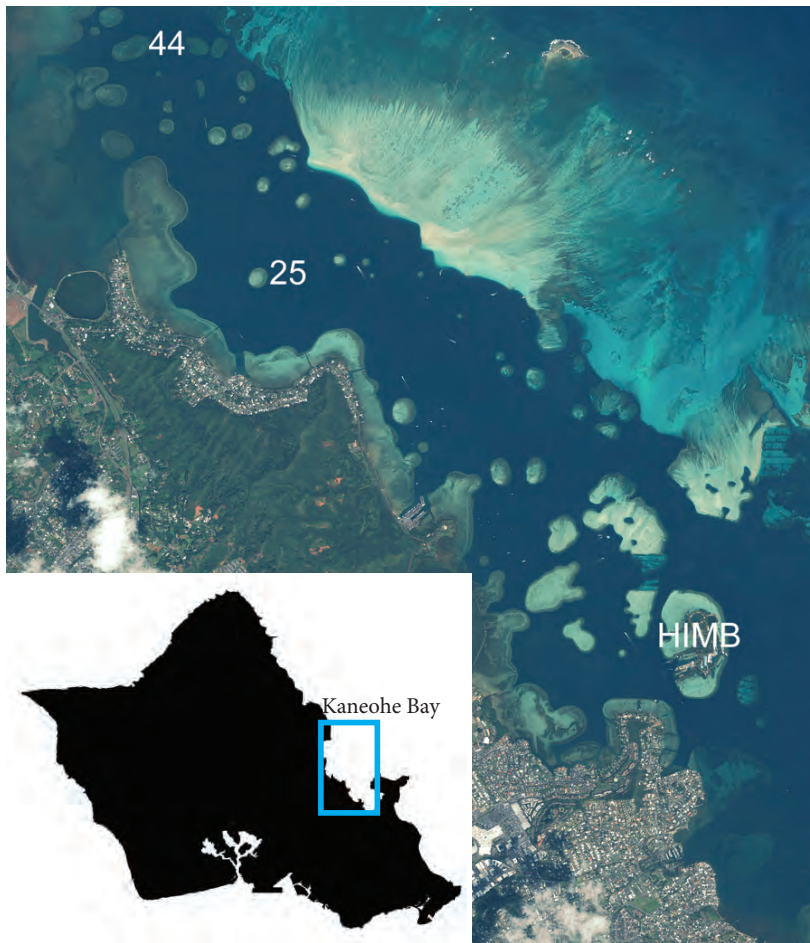
Figure 3.1. A map of Kāneʻohe Bay showing the locations of the patch reefs studied. The inset shows the location of Kāneʻohe Bay on Oʻahu Hawaiʻi, USA.
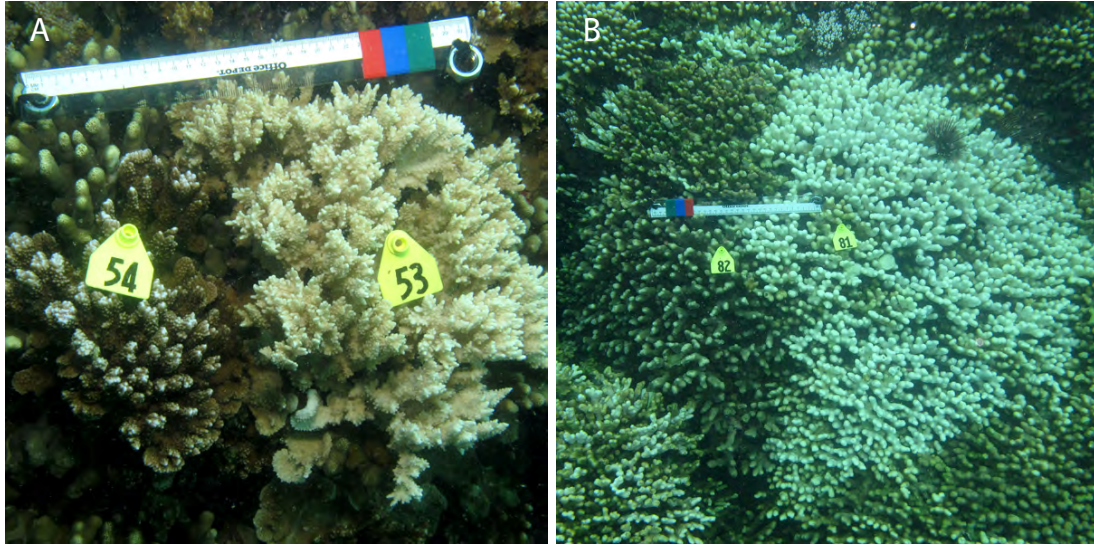
Figure 3.2. A photograph of the paired coral colonies from October 2014. A. a tagged pair of *Montipora capitata* colonies, B. a tagged pair of *Porites compressa* colonies.
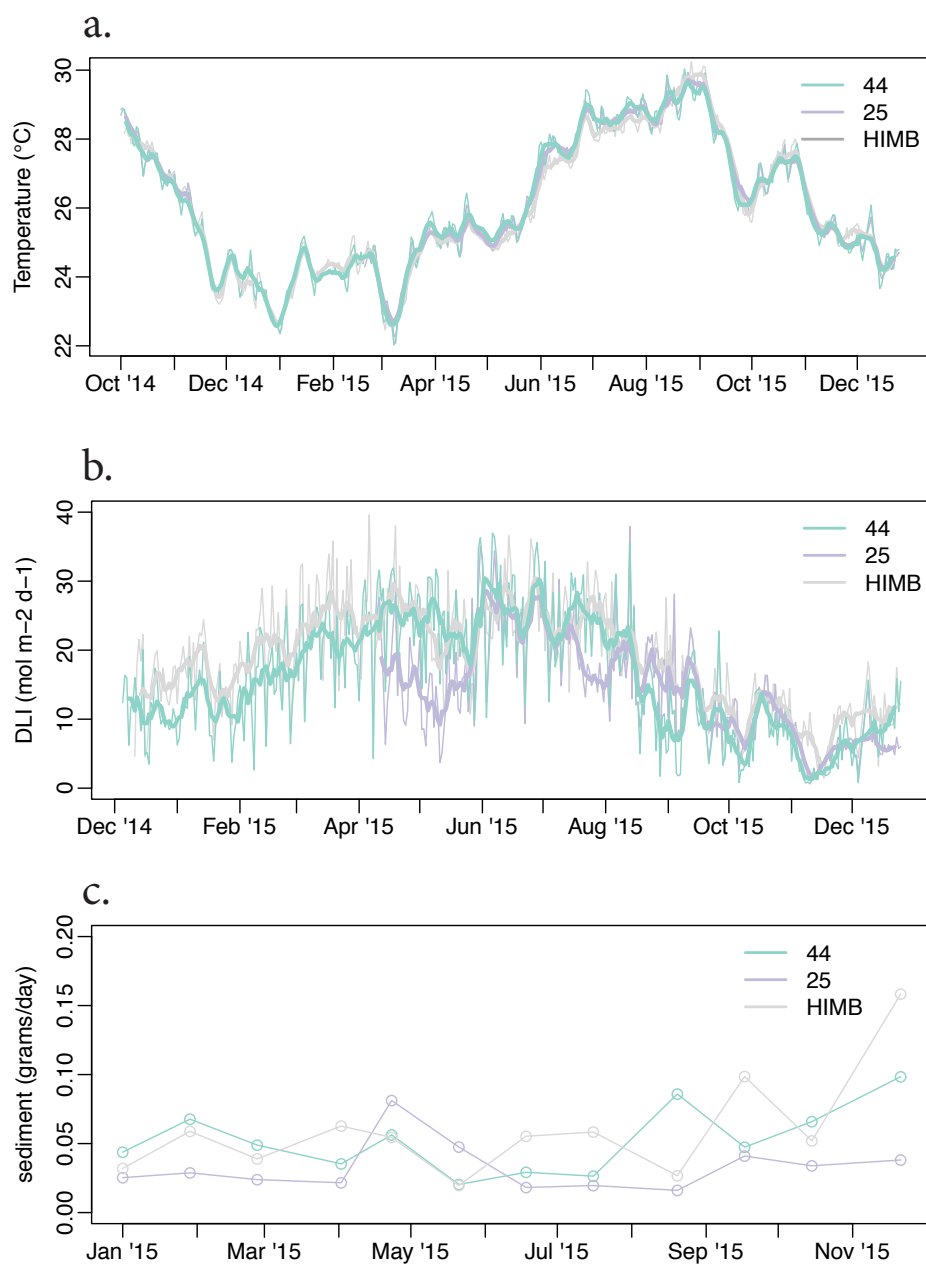
Figure 3.3. The physical parameters in Kāneʻohe Bay at each of the three reefs from October 2014 to December 2015. a. The temperature at 2 meters depth at each reef. b. The light regime at 2 meters depth at each reef. c. The sediment load at 2 meters depth at each reef.
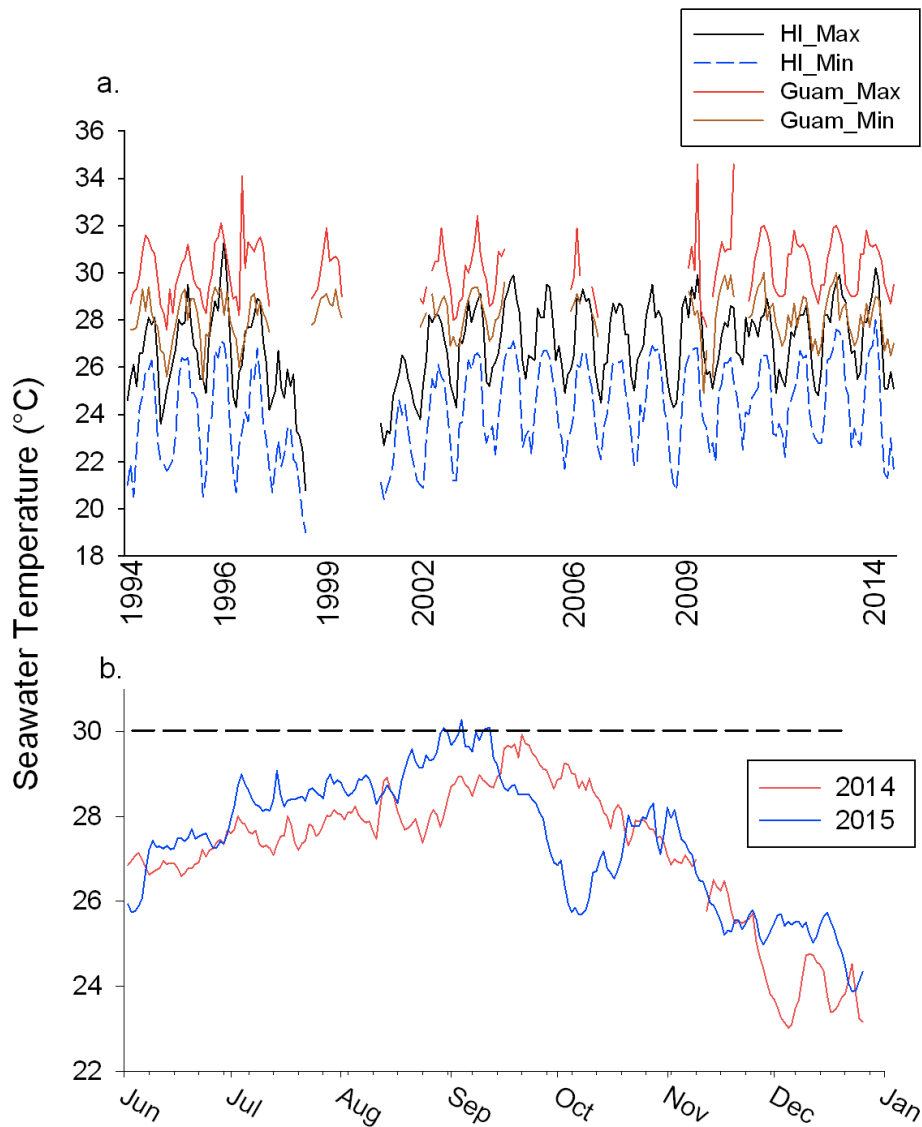
Figure 3.4. Seawater temperature profiles. Temperature data was downloaded from NOAA buoys as described in the methods. A. a comparison of the temperature variation at Kāneʻohe Bay and Guam during 20 years. Black and red colors correspond to the temperature maximum and blue and brown colors are temperature minimum for Hawaiʻi and Guam, respectively. B. Kāneʻohe Bay seawater temperature comparison of 2014 and 2015 from June to January.
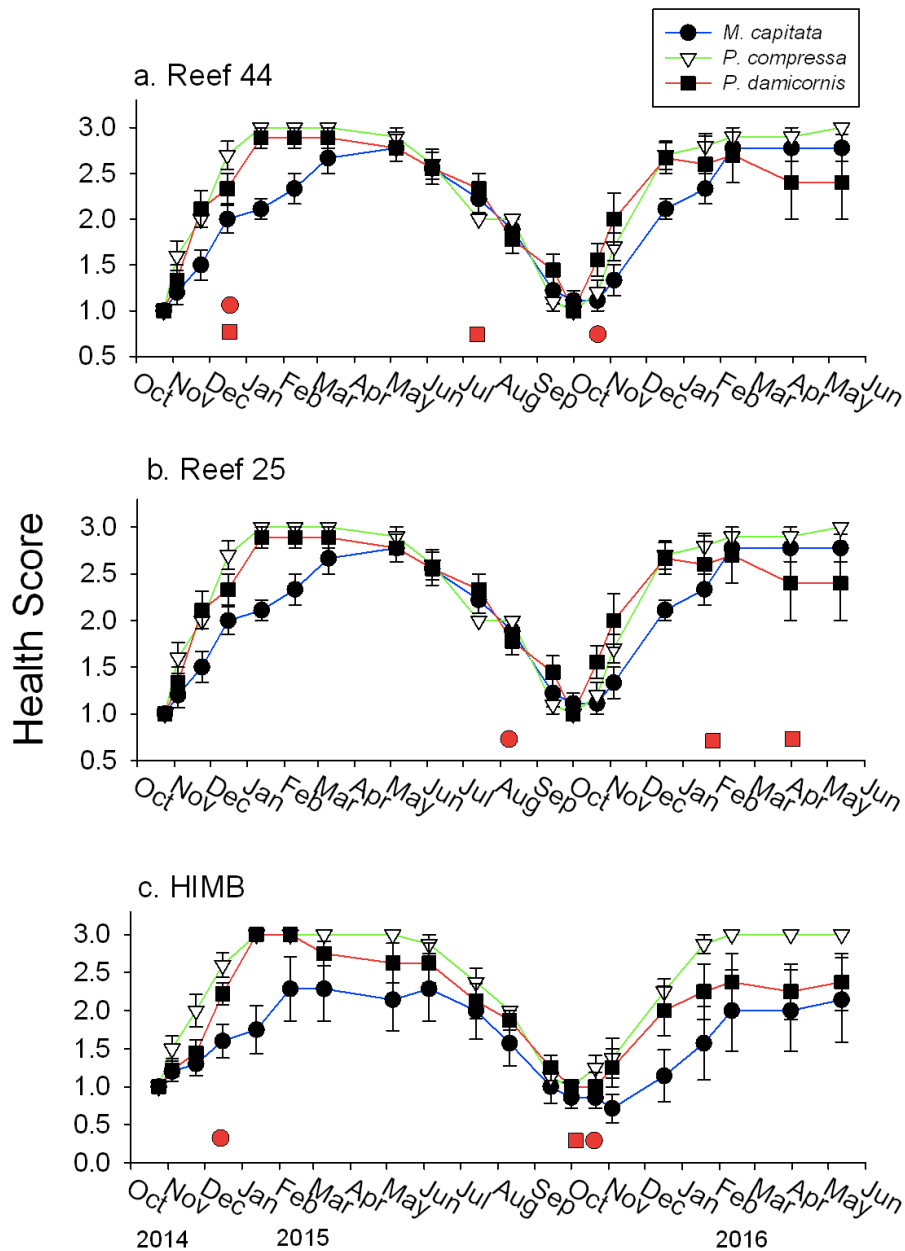
Figure 3.5. Coral condition in three species of corals. Only bleached colonies from the pairs are used to calculate the means. Bleaching was assessed visually with a score of 0 (dead), 1 (severely white), 2 (partially bleached or pale), 3 (darkly pigmented). A. Reef 44 B. Reef 25 C. North side of Hawai'i Institute of Marine Biology (HIMB).
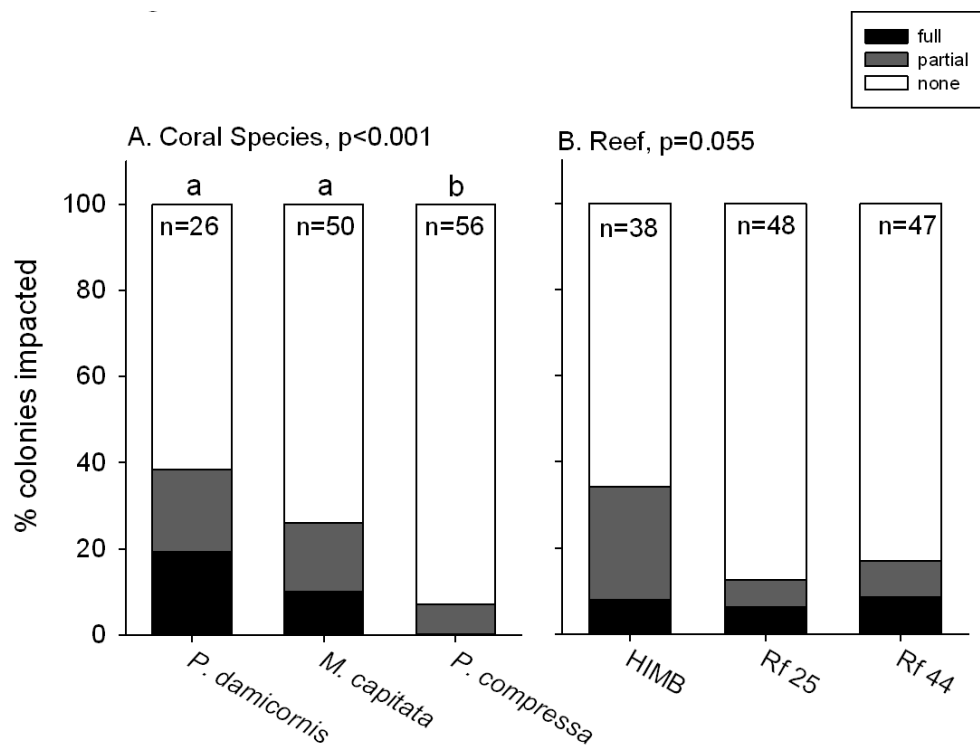
Figure 3.6. The frequency of mortality in coral colonies in March 2016 following 2 successive bleaching events. Different letters above the bars indicate significantly different groups. A. sorted by coral species, B. sorted by patch reef.

CHAPTER 4

Patterns of Genomic Variation Among Individuals of *Porites compressa*

Abstract

Coral reefs are threatened by multiple stressors including climate change. In 2015 there was a coral bleaching event that impacted reefs all around the globe. While surveying coral bleaching in Kāneʻohe Bay, some corals remained darkly pigmented even though they were adjacent to corals that were pale white and bleached. To better understand the genetic components of thermal tolerance reduced representation genomic sequencing (ezRAD) was applied to 16 pairs of bleached and unbleached *Porites compressa* colonies. This sequencing produced extensive genetic data that was processed through the dDocent pipeline. Analysis with pcadapt found that there were no consistent genetic differences between coral colonies that had bleached or not. However, this approach did identify two separate clades of *P. compressa* corals. 166 biallelic SNP's were found as outliers driving genetic structure that separated these two clades. These SNP's were found in 102 genes, 34 of which could be annotated against the NCBI database. Of these 34 genes, 3 were from the mitochondrial genome and 31 of them were from other nuclear regions. The genes that separated these two groups of *P. compressa* were consistent with gene regions that can be used to barcode different species, including the mitochondrial region COI. These data suggest that there are two cryptic species of *P. compressa* in Kāneʻohe Bay, however these two groups do not correlate with any obvious phenotypic difference among the corals. Cryptic species are known in some groups of corals, especially those with relatively few morphological characters. These data show the multiple sites of diversity among genotypes of the Hawaiian *P. compressa*.

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Introduction

        Many habitats are threatened by climate change (Parmesan 2006), and coral reefs are considered especially vulnerable (Hughes et al. 2003). Coral diversity is threatened by climate change (Hoegh-Guldberg et al. 2007) and already many reefs have lost coral cover (Gardner et al. 2003, Bruno and Selig 2007). The long-term impact of stress events on community shifts on reefs shows degradation (De'ath et al. 2012), yet there remains little knowledge of the impact of these events on genetic diversity. Measuring the current genotypic diversity on natural reefs is critical to set a baseline to understand the impact of future stress events on coral ecology and evolution.

        Both acclimatization (Gates and Edmunds 1999, Edmunds and Gates 2008, Barshis et al. 2013) and adaptation (Bay and Palumbi 2014, D'Angelo et al. 2015) are important processes that could help corals persist in a changing climate. Local adaptation has been described between regions with different temperature regimes (D'Angelo et al. 2015). Local adaptation to the Persian Gulf included adaptation to higher salinity levels, thus when these corals were transplanted to adjacent cooler habitats the corals did not survive presumably due to lower salinities. Local adaptation was also implicated in *Acropora millepora* colonies that were reciprocally transplanted from different regions of the Great Barrier Reef (Howells et al. 2013). Corals from their natal habitats performed well, but as temperatures increased or decreased the transplanted corals bleached and died. This work suggests that corals locally adapted to warmer temperatures could not tolerate different thermal exposures. *Acropora hyacinthus* that live in highly variable pools in American Samoa are thought to use a combination of gene regulation and genetic "frontloading" to persist in habitats with more extreme temperatures (Palumbi et al. 2014). Critical to understanding the ability of corals to adapt to their local conditions is an understanding of the standing genetic diversity on reefs.

        Modern next generation sequencing (NGS) has been used to show genomic sites of selection for many different taxa (Stapley et al. 2010). NGS can measure millions of bases and has provided data on coral genomes (Shinzato et al. 2011), transcriptomes (Barshis et al. 2013, Ruiz-Jones and Palumbi 2017), and selected regions within the genome (Forsman et al. 2017) to reveal patterns of genetic diversity within and among populations. Restriction Assisted Digestions sequencing (RADseq) can be used to quantify the standing genetic diversity of multiple individuals within and among populations, especially of non-model organism (Davey et al. 2011). RADseq techniques use restriction enzymes to digest genomic DNA and then sequences some of the same regions of the genome among individual samples and populations allowing the detection of single nucleotide polymorphisms (SNP's) at many loci (Davey et al. 2011). Multiple RADseq techniques have been developed and mostly vary according to the restriction enzyme used and the length of sequenced reads produced (Puritz et al. 2014b). ezRAD is one method that provides longer reads (200-300bp) enabling better annotation of the genes that contain SNP's (Toonen et al. 2013). While these methods are relatively new they hold great promise to identify the underlying genetic variation that might enable corals to adapt to climate change.

In 2014 and 2015 Hawaiian reefs experienced extensive bleaching events (Chapter 3). *Porites compressa* can be dominant in some habitats and this species showed extensive phenotypic plasticity in the bleaching events with some corals that bleached and others that maintained their symbionts. *Porites compressa* is thought to consistently host *Symbiodinium* C15 (LaJeunesse et al. 2004), so it is unlikely that symbiont diversity is contributing to a difference in coral susceptibility to thermal stress. Additionally colonies adjacent to each other showed variability in their bleaching susceptibility (Figure 4.1) greatly reducing the potential for habitat heterogeneity to drive differential bleaching. Genomic differences among these corals might explain the differential susceptibility of *P. compressa* to thermal stress but little is known about the genotypic diversity of this species.

To better understand the standing genotypic diversity and the possible genomic component of thermal tolerance thirty-two individual colonies of *P. compressa* were sequenced using the ezRAD protocol. Bleached and unbleached paired colonies were selected from the previously described tagged colonies in Kāneʻohe Bay (Chapter 3). Sixteen colonies (eight pairs) were selected from each reef (25 and 44) to better understand the potential standing diversity among reefs in Kāneʻohe Bay. In this way two hypotheses were tested: 1. What is the standing genotypic diversity on two reefs in Kāneʻohe Bay, and 2. Are there SNP's in the genome that correlate with thermal tolerance among colonies in the same habitat.

Materials and Methods

Individual *Porites compressa* colonies were tagged in October 2014 as described in Chapter 3. Each individual colony was photographed and a small fragment of approximately 3 cm length was removed from the colony and frozen in liquid nitrogen within 3 minutes. At regular time points, coral biopsies (less than 1 cm^2) were removed from each colony and immediately placed in 500 μl of DNA buffer (40ml of 5M NaCl, 50ml of 0.5M EDTA, and 490ml of HyClone water) with 1% SDS. If corals were preserved in liquid nitrogen, a small biopsy of the fragment was removed in the laboratory and placed in DNA buffer. As soon as possible (no more than 4 hours), the coral biopsies in DNA buffer were heated to 65 °C for 60-90 minutes. After this the tubes containing the coral biopsies in DNA buffer were stored at 4 °C.

DNA extraction followed the phenol chloroform protocol as described (dx.doi.org/ 10.17504/protocols.io.dyq7vv). 25 μl of Proteinase K (at 10mg/ml) was added to 500 μl of DNA buffer in each sample. The tubes were vortexed and then incubated at 55 °C for 2-3 hours. 100 μl of buffer was removed from each tube and used for subsequent DNA extraction. 200 μl of CTAB were added to each tube of buffer and incubated at 65 °C for 30 minutes. 300 μl of Chloroform was added to each tube, and then the tubes were rotated for 2-3 hours. Tubes were centrifuged at 10,000g for 10 minutes and 250 μl of the top layer was removed and placed in a new tube. 500 μl of 100% ethanol was added to each tube, vortexed and then placed in the freezer for 2 hours or longer. Tubes were centrifuged at 10,000g for 10 minutes and the ethanol was removed. Tubes were dried in a speedvac at 45 °C for 45 minutes. 100 μl of 0.3M

NaOAc was added to each tube and then vortexed. 200 µl of 100% ethanol was added to each tube and placed in the freezer for 2 hours or longer. The tubes were centrifuged at 10,000g for 10 minutes the supernatant was removed. 100 µl of 70% ethanol was added to each tube and then the samples were vortexed and centrifuged at 10,000g for 10 minutes and then the ethanol was removed. The tubes were dried in the speedvac for 45-60 minutes at 45 °C. The DNA was then re-suspended in 30 µl of TE buffer (10mM Tris, 0.1 mM EDTA) and stored at -20 °C.

The libraries of genomic DNA were prepared for ezRAD following the protocol described in Toonen et al., (2013). Briefly, DNA was run on a 0.7 % agarose gel to ensure that each sample had large fragments of DNA. DNA was quantified in each extract using the Accuclear dsDNA quantification kit following the manufacturer's instructions. The volume was adjusted for each sample to ensure that library preparation started with 1.3 µg of DNA in 25 µl of TE buffer. Each DNA sample was digested with 25 µl of DPNII master mix (1 µl DPNII, 5 µl buffer, 19 µl of HyClone water) at 37 °C for 3 hours, 20 minutes at 65 °C and then held at 15 °C. After digestion samples were cleaned using Agencourt AMPure XP beads at a ratio of 50 µl of DNA to 90 µl of beads. After the bead cleaning, the DNA was dissolved in 28 µl of water. The samples were checked to ensure digestion on a 1.4 % agarose gel run at 100 volts for 45 minutes.

Individual libraries were prepared using the KAPA Hyper Prep Kit following the manufacturer's protocol, except each reaction volume was halved. Each individual library was barcoded with 2 Illumina adapters in a forked design. The DNA for 6 individual corals (colony #: 63, 64, 95, 104, 107, 116) were split in half and barcoded with different adapters. The libraries were size selected for a target of 400 bases using SPRI beads. Every sample was run on PCR for 12 cycles (as is recommended in the KAPA kit) to ensure adequate concentration of DNA for sequencing. After PCR the DNA was cleaned using a 1:1 ratio of DNA to AMPure XP beads. The DNA was submitted to the core genetics lab at HIMB and all 32 libraries were sequenced on an Illumina MiSeq (RUO) with 300 bp paired end reads.

Resulting sequences were trimmed of their adapters and analyzed with the dDocent pipeline (Puritz et al. 2014a, Puritz et al. 2014b). Default values were used in the dDocent pipeline and minimum coverage for sequences was set at 3. The minimum number of individuals with a unique sequence was set at 4. The reference assembly was built *de novo* from the *P. compressa* sequences. The output of dDocent uses the program VCFtools (Danecek et al. 2011) to produce a variant call file (VCF) that was used for further analysis.

The VCF file was analyzed using the pcadapt package in R (Luu et al. 2017). In pcadapt a k of 2 explained the majority of the variance, so further analyses were constrained to k=2. A false discovery rate was set at an alpha value of 0.1, and subsequently more rigorous alpha values to identify the number of SNP's that consistently segregated these two groups. A manhattan plot was created to visualize the outlier loci. Reads were identified and contigs were extracted from the vcf file, and they were annotated by comparing them to the NCBI database using blastn. Genes were categorized as either "no hit", similar to a known gene at a Q-score of $> e^{-10}$, or identified at a Q-score of $< e^{-10}$.

Results

Sequencing was performed on all 32 coral colonies. Colony number 86 had relatively poor-quality sequences, but was not excluded from analysis (Table 4.1). Most colony libraries produced between one and three million sequences (Table 4.1). The six colonies that were split in half and sequenced with two different barcodes were consistently more similar with their pair than with any other coral library. This technical control showed relatively low error rates inherent in the methods. The dDocent pipeline provided 47,007 sites that were variable for further comparison, of these 37,030 sites were biallelic sites.

Analysis of the VCF file with pcadapt identified two clades within *P. compressa*. From the PCA plot most of the difference that separated the two groups was in PC1. With an alpha value (false discovery rate) of 0.1 in pcadapt 166 SNP's in PC1 were identified that segregated the *P. compressa* individuals into two groups. The two groups did not segregate according to visually differences (Figure 4.1), reef site (Figure 4.2), bleaching history (Figure 4.3), colony depth or sex of the colony (Table 4.2). At an alpha value of 0.1 there were 166 SNP's that segregated these two clades on PC1, and 220 SNP's that contributed to PC2. The manhattan plot illustrates the number of outlier loci that structure these two groups (Figure 4.4). At an alpha value of 1^{-05} only SNP's in PC1 were significant outliers, and there were thirty-four loci (indicated with an asterisk in Table 4.3). At an alpha value of 1^{-20} there were still thirteen SNP's that were outliers in PC1. At an alpha value of 1^{-30} there were still two SNP's that were outliers and structured PC1.

At an alpha value of 0.1 the 166 SNP's that were outliers on PC1 could be placed in 102 genes that separate these two clades of *P. compressa*. When these genes were annotated; thirty-two reads had no hit (31%), thirty-six reads had greater than 1^{-10} e-scores (35%), and thirty-four reads were identified at an e-scores of less than 1^{-10} (31%) (Table 4.3).

Discussion

The genomic analysis of thirty-two individual colonies of *P. compressa* showed two different groups within this species. These two groups do not correspond to any visual phenotype associated with habitat or bleaching history (Table 4.2). However, the same dichotomy between these two groups were consistently resolved regardless of whether the false discovery rate was set at 0.1 or 1^{-20}. The one hundred and two genes that were outliers had annotations that showed that there were three mitochondrial gene regions, thirty-one protein coding regions (including histones and transposons) (Table 4.3), and sixty-eight gene regions that could not be reliably annotated.

This dataset did not identify any sites within the genome that were associated with bleaching history. This is possibly due to reduced representation of the genome using a RADseq approach (Davey et al. 2011). RADseq is a random sampling of the genome and it is very possible that the regions responsible for thermal tolerance were not sequenced (Lowry et al. 2017), or that significant SNP's were not sequenced at a high enough

coverage to identify those genes as outliers. While eliminating low coverage reads is important to avoid potential sequencing errors, it also limits our ability to detect biologically important regions that do not have high copy number in the cells. Thermal tolerance is a complex trait and may be driven by multiple loci within the genome. If thermal tolerance loci are additive as has been found in *Arabidopsis* (Kover and Mott 2012) and rice (Lafarge et al. 2017), it may be that the phenotype of thermal tolerance is diluted across many loci. This type of trait is difficult to detect using RADseq methods and would require extensive sampling of multiple populations that were sequenced for their full genome.

Local adaptation has been proposed as a mechanism for corals to survive in habitats that are exposed to higher temperatures. In Florida *Porites astreoides* collected from inshore reefs were genetically distinct from colonies collected from more oceanic environments (Kenkel et al. 2013). When larvae from each of these populations were grown in a common garden they were found to have similar thermal tolerance as their parents, suggesting a heritable component of thermal tolerance (Kenkel et al. 2015). *Acropora millepora* in Australia were shown to host different clades of *Symbiodinium* which resulted in different rates of mortality in novel habitats (Howells et al. 2013). *Acropora millepora* from two different populations (South and Central Great Barrier Reef in Australia) were exposed to different historical thermal regimes (Dixon et al. 2015). When these two populations were crossbred, larvae from the northern population could tolerate higher temperatures than offspring from the southern population, showing that at least some components of thermal tolerance were heritable (Dixon et al. 2015). It is most likely corals use a combination of acclimatization and adaptation to survive thermal stress (Palumbi et al. 2014). For *P. compressa* in Kāneʻohe Bay there was no signature of adaptation to obvious habitat characteristics such as depth or site of collection. However, the majority of coral species studied have focused on *Acropora* spp., and the mechanisms of adaptation remain poorly tested for most coral species.

There is a genetic signature of two distinct clades of *P. compressa* within Kāneʻohe Bay. These clades are supported by differences in one hundred and two genes, which include three mitochondrial and thirty-one nuclear genes (Table 4.3). One of the mitochondrial regions was cytochrome oxidase (COI), and this region has often been used as a species level molecular marker in barcoding studies (Waugh 2007, Bucklin et al. 2011). The mitochondrial genome is maternally inherited and the two clades of *P. compressa* are consistently resolved using the whole mitochondrial genome (Forsman et al., personal communication). Finding two clades using mitochondrial genes suggests that these two groups of *P. compressa* are distinct maternal lineages and finding additional nuclear loci that segregate these groups suggests they are not interbreeding. The two clades were further supported by thirty-three nuclear genes that could be annotated. Additionally, there were sixty-six genes that could not be annotated at a high enough e-score to trust the annotation. These un-annotated genes may carry out important functions for the coral but more work is necessary to determine their function.

It is unclear what mechanisms might drive the separation of these two clades of *P. compressa*. The one hundred and two loci that are outliers represent a relatively small part of the genome, and this may or may not be enough differences to call these two

groups different species. More *Porites* species need to be sequenced with similar methods to determine the typical genomic distance that distinguishes among species in this genus. Since the mitochondrial genomes are different it suggests that these two distinct clades in Kāneʻohe Bay are not interbreeding. However, this pattern of two lineages could arise from multiple mechanisms of both divergence and coalescence (Marko and Hart 2011). These two clades could be the consequence of two different events of *P. compressa* recruiting into Kāneʻohe Bay. A recent migration of *P. compressa* from another source population could have some mutations derived from drift, and even though these two clades are living next to each other it may be that there has not been enough time for cross fertilization allowing the two populations to coalesce. This seems unlikely due to the abundance of both clades in the sampled populations. In thirty-two individual colonies there were seventeen in one clade and fourteen in the other, with one colony that had poor quality sequences. Alternatively, these clades could have diverged due to an isolation mechanism other than allopatry, which is rare but possible (Bowen et al. 2013). It may be that the observed genetic diversity in Kāneʻohe Bay is a result of reproductive isolation suggesting that these corals are incipient species.

Overall speciation is thought to be driven by allopatry, but there are some examples of speciation in sympatry (Bowen et al. 2013). While this is still debated in the literature, at least for corals there are multiple mechanisms that have been described for incipient speciation, even in the absence of physical reproductive barriers. Broadcast spawning organisms synchronously release their gametes into the seawater, eliminating selective mating that might be found in mobile organisms. But in some species of *Acropora* there are weak prezygotic barriers to prevent interbreeding among species (Fogarty et al. 2012), and there are records of hybridization among species (Carlon 1999). In *Favia fragum* two morphotypes were described in adjacent habitats in Panama (Carlon and Budd 2002). Genetically these two morphotypes were distinct using eleven microsatellite markers (Carlon et al. 2011). The reproductive isolation of these two morphotypes was attributed to high rates of self-fertilization in this brooding species (Carlon and Lippe 2011). In the *Orbicella* species complex reproductive isolation was found to occur by both gamete incompatibility and temporal isolation (Levitan et al. 2004). Two of the species, *O. annularis* and *O. faveolata* had incompatible gametes, but the other species, *O. franksi* was observed to spawn 1.5 hours earlier than the other two species. Temporal reproductive isolation or differential sperm binding proteins are both potential mechanism that could create reproductive isolation in a broadcast spawning species with no physical barrier such as *P. compressa*.

Importantly this work describes previously unknown genetic diversity in *P. compressa* from Kāneʻohe Bay. While recent work on *Porites* spp. in Hawaiʻi has described *P. compressa* and *P. lobata* as a hybrid group with extensive interbreeding (Forsman et al. 2017). Preliminary analysis suggests that the *P. compressa* samples sequenced here continue to segregate when added to the larger database of Hawaiian *Porites* (Forsman, personal communication). With additional samples it seems that both *P. lobata* and *P. compressa* continue to be "species", but additionally there is a complex clade that contains some colonies of both morphologies, suggesting there is a hybrid zone between these two species. Species in the genus *Porites* are a challenge to identify due to

relatively few morphological characteristics that distinguish among species. It is conceivable that this genus has extensive sibling species with little to no morphological divergence. For instance a recent paper found a type of *Porites cylindrica* that is brooding its larvae (Abecia et al. 2016). This paper suggests that either there is more reproductive flexibility within *P. cylindrica* or their species is actually a cryptic species with the same colony morphology as *P. cylindrica*. NGS is changing our ability to identify species, and as more "species" are discovered that don't fit our expectations sequence data will be invaluable to discover and identify novel species.

　　While this study documents two different lineages in the genomes of *P. compressa*, it is not clear what evolutionary process might be segregating these sympatric populations. The gene annotations show that the genes typically used to identify species are divergent and some of these genes are maternally inherited, suggesting that there is incipient speciation in *P. compressa* in Kāneʻohe Bay, Oahu. Future work with *P. compressa* should monitor spawning time to determine if there is reproductive isolation between these two clades. Additionally, breading trials for gamete compatibility could be conducted to determine if the gametes are compatible among these two groups of *P. compressa*, and among *P. compressa* and *P. lobata*. And future genetic work could be done to test for the genomic diversity among multiple species of *Porites*.

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Table 4.1. Statistics on the sequencing of *P. compressa* ezRAD libraries. Genomic group refers to the genetic groupings derived from further analysis (see results).

colony #	genetic clade	# of sequences /library	% GC /library	mean sequence depth
61	B	2556098	39	53.9
62	A	2522160	39	61.7
63	B	1840585	39	36.2
64	B	1993672	39	38.8
67	A	2901320	40	74.4
68	A	2731523	40	78.6
73	A	1830271	39	40.4
74	B	2316279	39	58.1
81	A	1859639	39	34.7
82	A	1250297	39	28.3
85	B	2197737	39	49.5
86	neither	2291333*	**62**	**1.9**
93	A	1510556	39	33.0
94	A	2483267	40	26.3
95	B	2064439	40	44.0
96	A	2298917	40	47.5
103	B	2333932	39	36.5
104	A	2300237	39	43.7
105	A	2151759	39	36.3
106	B	2031091	40	57.2
107	B	2313999	41	43.0
108	A	2789971	41	44.6
113	A	2691503	39	48.4
114	A	2369099	40	33.3
115	A	2848825	39	69.1
116	B	2638116	40	54.5
117	A	2452598	40	44.1
118	B	2674820	40	49.6
133	B	2330206	40	42.6
134	A	2744259	40	43.1
139	B	2478739	40	53.9
140	B	2174117	41	44.2

* indicates high sequence duplication in this library. % of sequences remaining after de-duplication was only 9.05.

Table 4.2. Characteristics of individual coral colonies of *Porites compressa* used in this study. Genomic group is based on the 102 genes identified as outliers that consistently separate these two groups. Reef refers to the patch reef where these corals were tagged in Kānoʻehe Bay. Sex is either male (M) or female (F) as these corals are gonochoric, na indicates colonies that have not been analyzed for sex. Bleaching status is based on surveys from 2014 and the corals were characterized as either bleached (Bl) or not bleached (NB).

Colony #	Genomic group	Reef	Depth (m)	Sex	Bleaching Status
61	B	25	1.33	na	Bl
62	A	25	1.33	na	NB
63	B	25	1.66	na	Bl
64	B	25	1.66	na	NB
67	A	25	1	na	Bl
68	A	25	1	na	NB
73	A	25	0.33	na	Bl
74	B	25	0.33	na	NB
81	A	25	1.66	na	Bl
82	A	25	1.66	na	NB
85	B	25	1	na	Bl
86	neither	25	1	na	NB
93	A	25	1.33	na	Bl
94	A	25	1.33	na	NB
95	B	25	0.33	na	Bl
96	A	25	0.33	na	NB
103	B	44	2.33	F	Bl
104	A	44	2.33	F	NB
105	A	44	0.66	M	Bl
106	B	44	0.66	F	NB
107	B	44	1.66	F	Bl
108	A	44	1.66	F	NB
113	A	44	2.66	F	Bl
114	A	44	2.66	F	NB
115	A	44	1.66	F	Bl
116	B	44	1.66	F	NB
117	A	44	2.66	F	Bl
118	B	44	2.66	F	NB
133	B	44	2	F	Bl
134	A	44	2	F	NB
139	B	44	0.33	F	Bl
140	B	44	0.33	M	NB

Table 4.3. A list of the significant genes that determined genomic clades. These genes were significant outliers at an alpha value of 0.1 (an * indicates outliers that were still significant at an alpha value of 0.00001). Only the genes with significant hits to the NCBI database were listed (32 genes that were significant outliers had no hit and are not shown). The annotations were "identified" if the e-value was less than 1^{-10} and genes were categorized as similar if the e-value was greater than 1^{-10} (highlighted in red). Genes highlighted in blue were from the mitochondrial genome and genes highlighted in brown were from nuclear gene regions. Cnidarian taxonomic names are in bold. When multiple loci were present in a gene the sum coverage shown is for the site with the lowest coverage.

dDocent Gene #	Top Taxonomic Hit	Annotated Function in NCBI	e-score	sum coverage
*1184	*Dendrophyllia cribrosa*	cytochrome B	3.00E-16	3527
*2740	*Porites sp.*	cytochrome oxidase subunit I	1.00E-71	2499
3163	*Saccoglossus kowalevskii*	hypothetical protein LOC	1.00E-35	217
7168	*Exaiptasia pallida*	hypothetical protein AC249	5.00E-18	494
*7315	*Porites porites*	NADH dehydrogenase subunit 3	2.00E-32	2109
1754	*Acropora digitifera*	predicted histone h2a	3.00E-36	20486
2532	*Nematostella vectensis*	predicted protein	1.00E-17	20253
3465	*A. digitifera*	predicted uncharacterized protein LOC	4.00E-68	605
12843	*A. digitifera*	predicted uncharacterized protein LOC	1.00E-19	467
13586	*A. digitifera*	predicted uncharacterized protein LOC	5.00E-22	882
11511	*Strongylocentrotus purpuratus*	predicted: RNA-directed DNA polymerase	5.00E-19	271
4616	*A. digitifera*	Predicted: uncharacterized protein	1.00E-24	685
492	*Orbicella faveolata*	uncharacterized protein LOC	4.00E-23	417
4429	*A. digitifera*	Predicted: uncharacterized protein	4.00E-74	662
14893	*E. pallida*	hypothetical protein AC249	9.00E-43	246
12786	*A. digitifera*	predicted uncharacterized protein	2.00E-17	597
2975	*A. digitifera*	predicted uncharacterized protein LOC	8.00E-52	590

Table 4.3. (Continued) A list of the significant genes that determined genomic clades.

6983	*A. digitifera*	predicted uncharacterized protein LOC	4.00E-45	2222
838	*O. faveolata*	uncharacterized protein LOC	2.00E-39	738
5636	*A. digitifera*	predicted uncharacterized protein LOC	2.00E-39	1148
5761	*A. digitifera*	predicted uncharacterized protein LOC	4.00E-22	433
*4535	*A. digitifera*	predicted: RNA-directed DNA polymerase	3.00E-27	505
*8511	*A. digitifera*	predicted uncharacterized protein LOC	4.00E-18	1001
8885	*S. kowalevskii*	predicted uncharacterized protein	2.00E-13	1528
3700	*E. pallida*	hypothetical protein	2.00E-11	1002
*3472	*A. digitifera*	predicted uncharacterized protein	2.00E-11	342
10503	*A. digitifera*	predicted: repressor of the inhibitor of the protein kinase-like	8.00E-20	738
1450	*N. vectensis*	predicted protein	1.00E-12	319
2834	*A. digitifera*	predicted uncharacterized protein	3.00E-22	189
7365	*Biomphalaria glabrata*	predicted uncharacterized protein	5.00E-10	9368
3902	*A. digitifera*	predicted:trihelix transcription factor GTL1-like	4.00E-10	494
10569	*E. pallida*	transposon TX1 uncharacterized 149 kDa protein	2.00E-11	233
523	*A. digitifera*	uncharacterized protein	5.00E-22	438
8068	*O. faveolata*	uncharacterized protein	6.00E-29	326

Table 4.3. (Continued) A list of the significant genes that determined genomic clades.

8110	*Zootermopsis nevadensis*	dipeptidyl peptidase 9	2.30E+00	6722
561	*Flavobacterium fontis*	DNA mismatch repair protein MutS	1	1601
*2668	*Pedobacter rhizosphaerae*	glycosyltransferase	3.60E+00	182
7758	*Agaricus bisporus var. bisporus*	hypothetical protein	8.50E+00	558
10246	*C. briggsae*	hypothetical protein	5.00E-03	954
3686	*C. briggsae*	hypothetical protein	0.12	1196
*1091	*Congregibacter litoralis*	hypothetical protein	5.7	812
4607	*E. pallida*	hypothetical protein	6	948
4742	*Helobdella robusta*	hypothetical protein	4.7	526
*260	*Laccaria amethystina*	hypothetical protein	3.2	811
10879	*Prevotella histocola*	hypothetical protein	1.20E-01	801
9176	*Trichomonas vaginalis*	hypothetical protein	3.20E+00	776
11536	**E. pallida**	hypothetical protein AC249	8.00E-09	568
6092	*Chondromyces apiculatus*	hypothetical protein CAP 1616	7.5	833
12375	*C. briggsae*	hypothetical protein Cbg 10223	2.00E-09	2374
6925	*C. briggsae*	hypothetical protein CBG10223	0.003	4217
*6462	*Stylonychia lemnae*	polyadenylate-binding cytoplasmic	6.9	446
1084	*Mycobacterium tuberculosis*	PPE family protein	5.7	342
1703	**A. digitifera**	predicted uncharacterized protein	3.00E-06	406
3231	**A. digitifera**	predicted uncharacterized protein	4E--05	6439

Table 4.3. (Continued) A list of the significant genes that determined genomic clades.

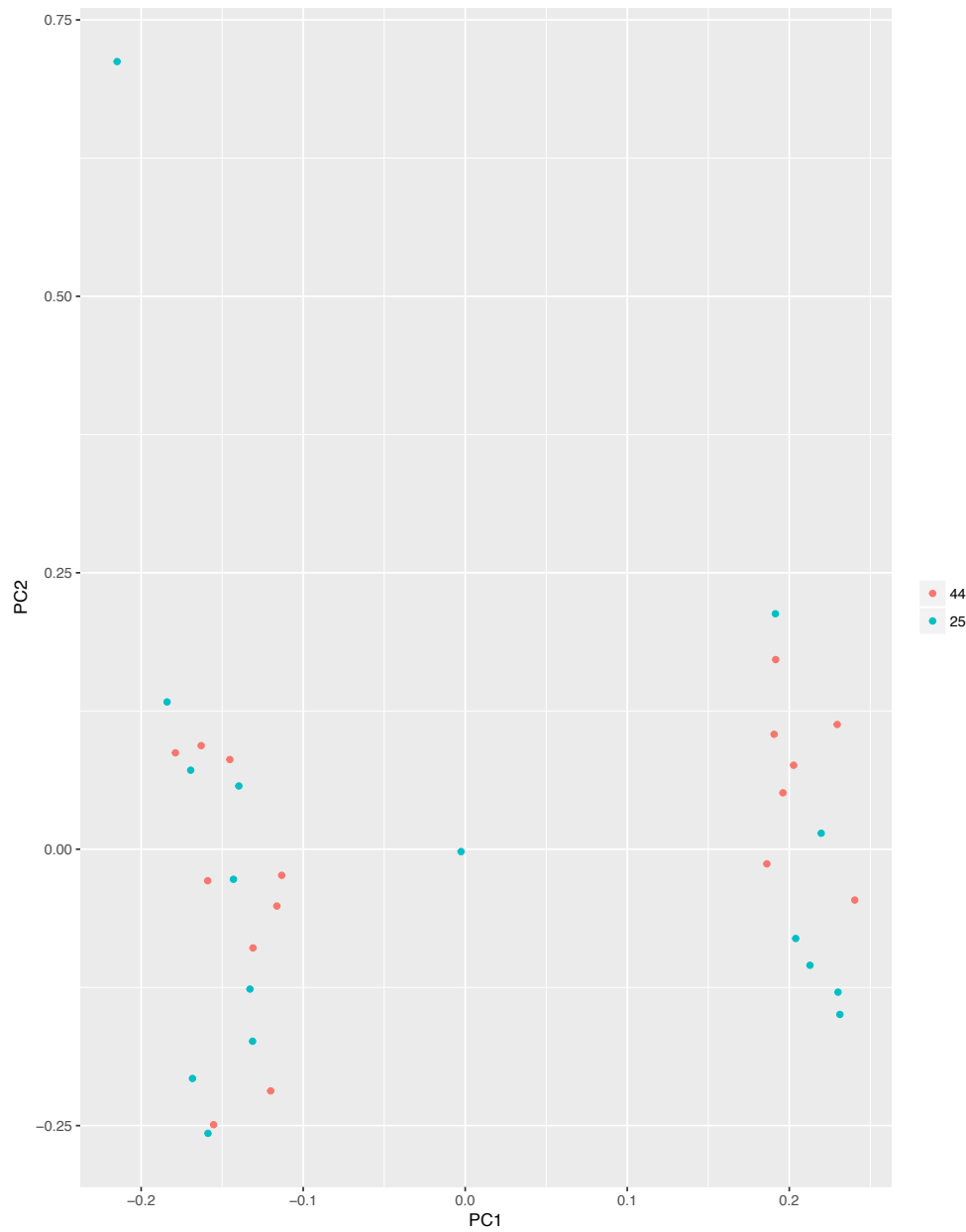| 6575	*A. digitifera*	predicted uncharacterized protein	0.011	376
10273	*A. digitifera*	predicted uncharacterized protein	5.00E-08	1127
10318	*A. digitifera*	predicted uncharacterized protein	9.00E-02	593
1735	*Biomphalaria glabrata*	predicted uncharacterized protein	5.00E-03	3109
*1826	*Cupriavidus necator*	predicted uncharacterized protein	2.50E-01	1574
9912	*Pygocentrus nattereri*	predicted uncharacterized protein	2.10E+00	743
1820	*Tribolium castaneum*	predicted uncharacterized protein	6.00E-06	5618
2503	*Nelumbo nucifera*	predicted YTH domain-containing family protein	6.00E+00	779
7785	*Poecilia formosa*	predicted: choline transporter-like protein1	6.00E+00	577
6453	*Brassica napus*	predicted: uncharacterized protein	3.00E-05	1077
3473	*Cyprinus carpio*	predicted:rna-directed dna polymerase	0.26	435
*6881	*E. pallida*	THAP domain-containing protein 1	0.001	595
*195	*A. digitifera*	uncharacterized protein	0.55	1564
816	*A. digitifera*	uncharacterized protein	3.00E-07	1175
8960	*O. faveolata*	uncharacterized protein	7.00E-09	652
495	*Plutella xylostella*	uncharacterized protein	7.3	319
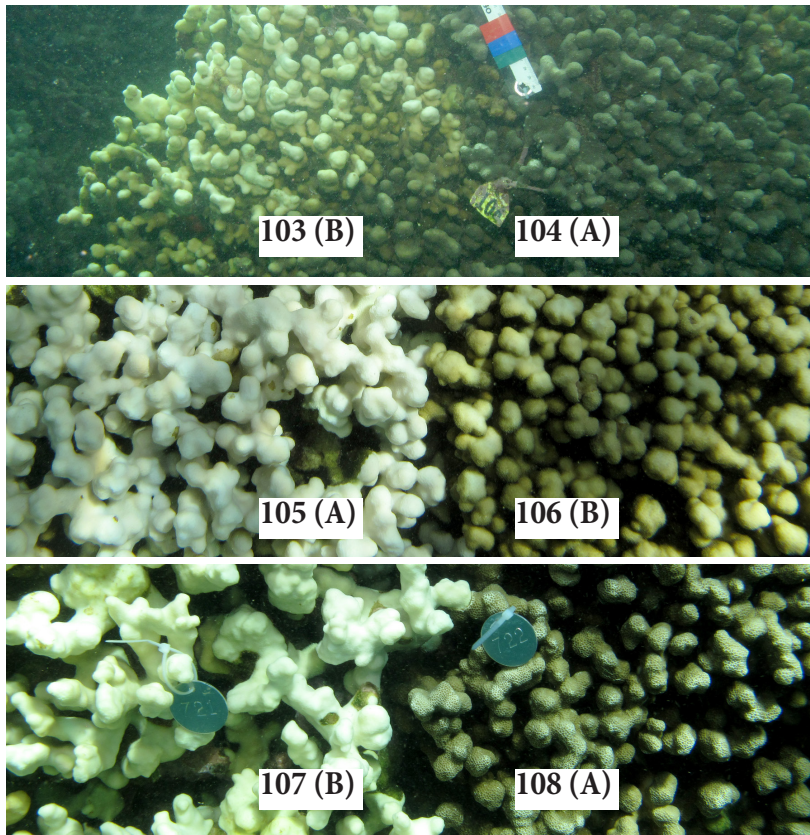
Figure 4.1. Photos of six representative *Porites compressa* colonies that were sequenced. The colony number is indicated directly on each photograph and its genomic group is indicated in parentheses. Note that each genomic group includes colonies that are bleached or not and although not shown the same genomic group was sometimes located next to each other.

Figure 4.2. A PCA plot of the two groups of *P. compressa* that are organized by their reef of origin, red is reef 44 and blue is reef 25. The blue dot in the middle of the two groups is colony number 86 which had low quality sequences.
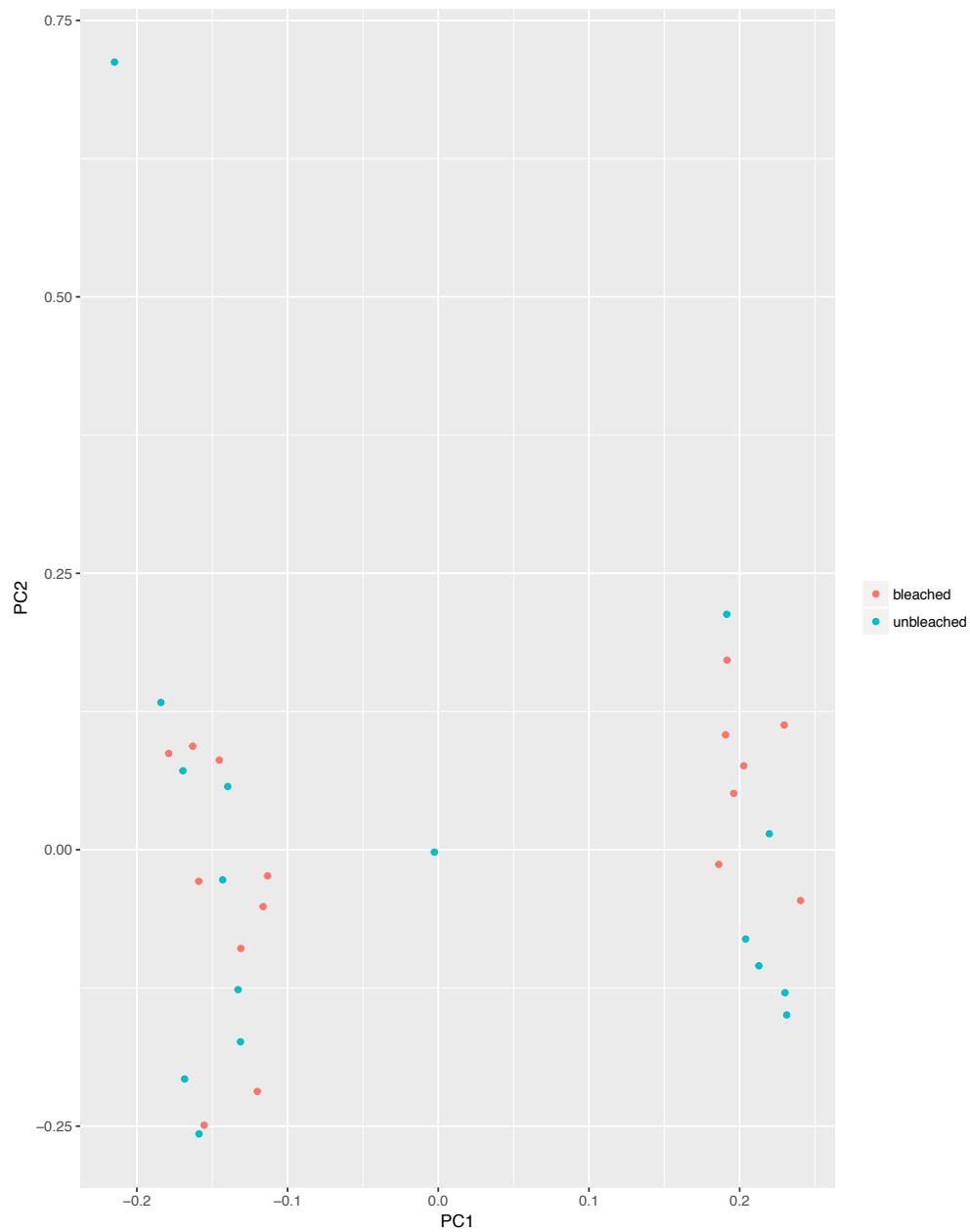
Figure 4.3. A PCA plot of the two groups of *P. compressa* that are organized by their bleaching history. Red represents those colonies that bleached in 2014 and blue represents those colonies that did not. The blue dot in the middle represents colony 86 which had low quality sequences.
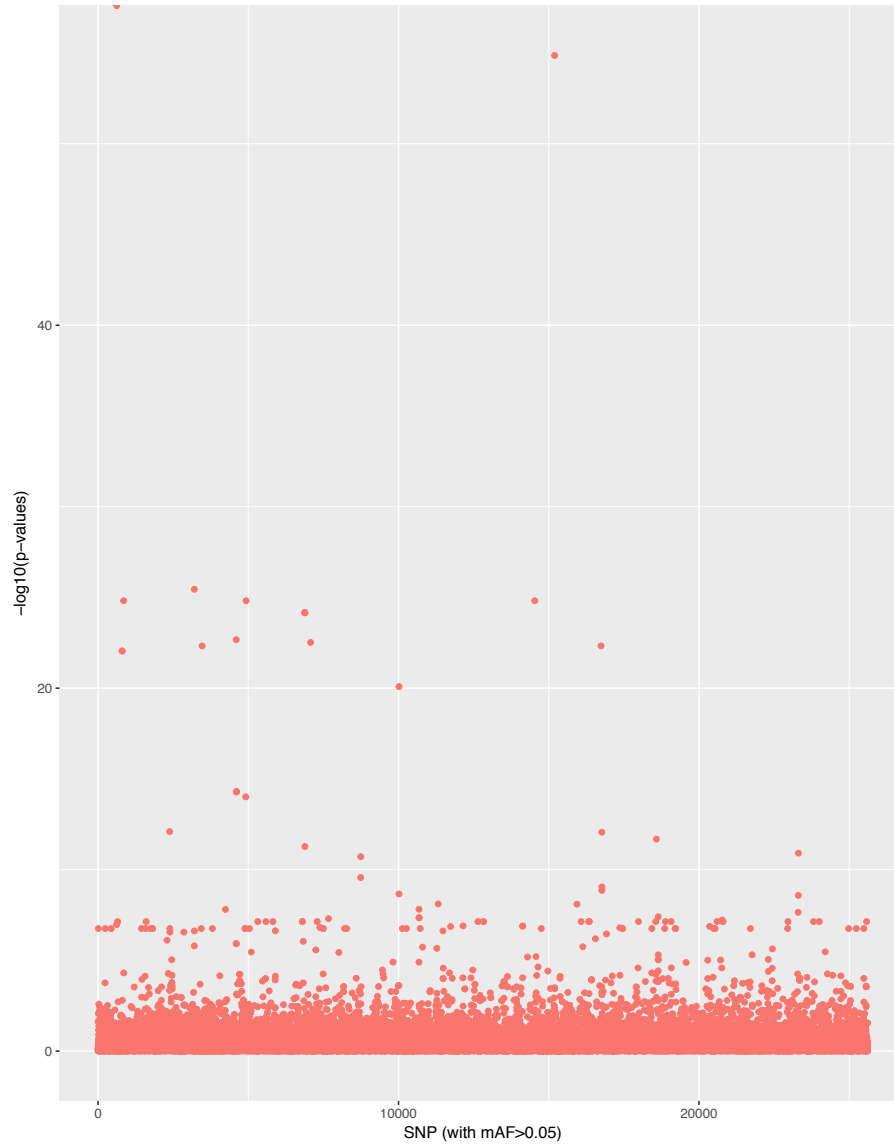
Figure 4.4. A manhattan plot of the outlier loci identified in pcadapt. The x-axis includes every detected SNP, and the y-axis indicates the alpha value for that individual SNP, with higher alpha values representing the outlier SNP's depending on the alpha value chosen as a cut off.

CHAPTER 5

The Importance of Variability for Coral Persistence: What's Known and What's Next

Synthesis

Throughout this dissertation the role of variability has been highlighted, especially variability at scales from individual genotypes to coral communities. Too often researchers ignore variability or attribute it to random 'noise', but there is a critical role for variability in the ecology, evolution, and conservation biology of coral reefs.

Variability in genotypes is the raw material for natural selection, and the observable phenotypes are a combination of genetic diversity interacting with the environment. For corals acclimatization and adaptation to modern stress events is critical to preserve the standing genetic variability already present on reefs. Through physiological plasticity many corals can withstand high temperatures, fluctuating abiotic conditions and ocean acidification. But these corals represent the exception not the norm. Most corals are susceptible to a +1-2 °C elevation in seawater temperature, which causes bleaching and mortality. However, there are signs that some corals are resilient, including a few exceptional coral individuals within some species and different tolerance among species (Chapter 3). To understand the role of this variability in the adaptation of corals it is critical to document the current genotypic diversity on reefs (Chapter 4). In concert there is a great need to understand the effective population size of coral populations, since population size is known to have a large impact on rates of adaptation (Charlesworth 2009).

While human influences promise to change many of the abiotic characteristics of near-shore habitats, there are multiple examples of corals that have adapted to "poor" habitats. These corals provide evidence that adaptation to stressors can happen. Researchers have suggested that we can use assisted migration to move these resilient corals into degraded habitats. However, a few studies that have tried transplantation (Howells et al. 2013, D'Angelo et al. 2015) have found that these resistant corals often do not survive in a novel habitat. This local adaptation is probably quite common, and while local adaptation might be critical for the persistence of refuges of resilient corals, it also calls in to doubt the potential to use these refuges as source material for transplantation.

There is a recent push to determine the genomic sites of selection using next generation sequencing methods. While multiple techniques have been developed to measure selection in non-model organisms (Stapley et al. 2010), the application of these techniques to coral research is still in it is infancy. Epigenetics, small RNA's, heritability, and quantitative trait loci are all potential mechanisms of coral adaptation that are rarely studied (Chapter 1). All of these mechanisms hold great promise to determine the underlying genomic components of coral resilience. Critical to advancing this field will be to describe more coral genomes, which would greatly increase our ability to map QTL's, measure heritability of traits and understand the regions of the genome under selection in degraded habitats. As our understanding of coral genomics increases it will greatly increase our knowledge of the genetics of coral resilience.

Importantly, coral resilience is driven by multiple demographic processes. Resilience incorporates both resistance to stress and an ability to rebound after a stress event. The ability of a reef to rebound is often attributed to coral recruitment. Coral recruitment can be impacted by multiple ecological interactions at any of three life

history stages; larval supply, larval settlement and post-settlement survival and growth (Ritson-Williams et al. 2009). But fundamental to resilience is larval dispersal and settlement, which combined result in connectivity among reefs.

Many studies have looked at the larvae of individual coral species to better understand their planktonic dispersal stage. However, much of the literature on coral larvae describes a set competence time ignoring all of the larvae that might settle later. But variability in time until settlement could be an important strategy to settle both close to parent colonies and also disperse further in the plankton to other reefs. When comparing a variety of species for the variance in time until settlement it is evident that for many species there are inherent levels of variability in the duration until settlement (Chapter 2). Even though the settlement habitat was kept consistent in all of these experiments, individual colonies produced larvae with a range of competence times. After tracking larvae for at least three weeks, those larvae that swam for a longer time were capable of settlement and growth.

Ecologically this settlement variability might allow corals to settle in a variety of habitats. Due to the variability in the availability of appropriate settlement habitat on coral reefs, this variability in competence time might be a strategy for corals to ensure that some larvae will survive and persist, both in natal and in distant reefs. But more experiments are necessary to determine the fate of the larvae that have longer planktonic durations. Is there cost to a long dispersal time, especially in the coral larvae that don't contain *Symbiodinium*? These larvae might consume their energy impacting their ability to survive and grow after settlement. In general there needs to be a stronger linkage of multiple life history stages to understand the role of dispersal and connectivity in coral population recover after a disturbance. Models of connectivity need to incorporate variable planktonic durations to better match predicted with realized connectivity. Critically if we are going to manage reefs for recovery there must be a greater effort to measure connectivity among reefs and to protect those reefs that provide larvae for coral recovery.

Implicit in resilience from a disturbance is that the corals can resist a stress. This is critical to ensure the persistence of corals, some corals must survive to reproduce and drive recovery. There is evidence that some corals can resist many different types of stresses. In Hawaiʻi, in September of 2014 and 2015 there were extensive bleaching events driven by seawater temperatures above 30 °C. In Kāneʻohe Bay it was clear that some corals were more susceptible to this thermal stress than others. By tagging individual corals we can better understand the variance in bleaching within a species, and the variance in rate of recovery from bleaching (Chapter 3). It is clear that some species bleach more than others, and this differential susceptibility provides us with data to predict the future community composition of reefs that undergo more frequent and extreme thermal stress.

While there is a range of susceptibility to thermal stress it seems that these stress events will increase in frequency and duration. Critical to understanding the persistence of corals is understanding the longer-term impact of stress events on individual, populations and communities of corals. Random surveys of % coral cover bleached are adequate to describe the extent of a thermal stress event but they do not provide critical

information on the demographic impact of differential mortality. By tracking individually tagged coral colonies we can elucidate not only the long-term trajectory of individuals, but we also set a baseline for understanding the genetic diversity of reefs, and how that diversity is changing after acute stress events (Chapter 3). This type of monitoring effort is much more intense than rapid surveys, but it holds the key to a long-term understanding of thermal stress and the persistence of corals that show resilient phenotypes (Chapter 3). Tracking the fate of individual colonies is invaluable and this type of monitoring effort should be expanded to include many different sites. With a better understanding of the variable response to stress among locations, we gain a better understanding of local adaptation and the different or common mechanisms that drive resilience in corals.

One such mechanism could be that corals contain genetic modifications that might contribute to resilience to thermal stress. This hypothesis was tested using RADseq methods to determine if the tagged *P. compressa* had any gene regions that were associated with bleaching susceptibility (Chapter 4). This is a powerful scenario to detect differences in genetic architecture since these corals showed differential susceptibility to thermal stress even though they live in the same microhabitat, the colonies were right next to each other. Additionally, *P. compressa* are known to host primarily *Symbiodinium* C15, which may eliminate some of the bleaching response due to different clades of *Symbiodinium*. However, there were no genetic loci that corresponded to bleaching resistance. It is possible that the RADseq methods used did not target the genes that contribute to bleaching susceptibility. This method is a random sampling of the genome, and it would be much more powerful to compare the genomes of these 32 coral individuals, but that was cost prohibitive. Alternatively susceptibility to bleaching could be driven by other aspects of these corals, such as gene expression (Barshis et al. 2013), microbiome (Ainsworth et al. 2010), and differential energy reserves or feeding behavior (Grottoli et al. 2006). All of these hypotheses will be tested with these corals to gain a better understanding of the factor(s) that drive bleaching resistance.

Even though the genomic data did not provide evidence for differences in bleaching susceptibility, there was evidence for a larger standing genetic diversity than was expected. The analysis found two different groups within the thirty-two individual *P. compressa* colonies (Chapter 4). These two clades were segregated by one hundred and two genes that contained SNPs. Approximately a third of these genes could be reliably annotated, three of which were mitochondrial gene regions, and the others were nuclear gene regions. The types of genes found in this analysis are consistent with genetic markers for different species. It may be that there are cryptic species within *Porites* that were never observed before. Critically this diversity might be important standing genetic variation that could contribute to the evolution of corals to climate change stressors. More work needs to be conducted on the reproductive biology of *P. compressa* to determine if there are any reproductive barriers that might separate these two groups. Additionally more work needs to be done to understand the genetic distance among species in *Porites*, as this genus may have extensive hybridization among the species (Forsman et al. 2017). *Porites* has always been a challenge to identify due to very small morphological

differences, but with NGS methods advances can be made in understanding the ecology and evolution of *Porites* spp.

The science of coral reefs is at a crossroads, there are numerous techniques and advances in technology that can help us to understand corals at multiple scales from genomics to individuals, and from species to communities, but reefs are some of the most susceptible habitats to climate change. There is a pressing need to document coral reefs as a baseline to understand the impact of current and future stress events. Additionally, there is a pressing need to conserve coral reefs as we are already experiencing extensive stress and mortality in response to bleaching events in the past three years. These bleaching events are not isolated, they are global in scale.

Conserving corals should be a priority for researchers currently studying coral reefs. With modern methods, we can now integrate long-term monitoring with tracking individual genotypes *in situ*. This gives us great power to observe corals in their native habitats and how they resist and recover from stress events. To ensure coral persistence in the future we must understand which reefs are resilient, and whether these reefs can provide larvae for future recruitment. Critical to conserving corals is establishing a baseline not only of coral cover and diversity, but also the genotypic and genomic richness that is currently present on coral reefs. If we wait to long to gather this fundamental data we will lose much of the standing genetic diversity of reefs, greatly inhibiting our ability to manage coral reefs for persistence in the future.

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