The Dissertation Committee for Carly Danielle Kenkel Certifies that this is the approved version of the following dissertation:

Investigating Local Adaptation in a Reef-building Coral

Committee:

Mikhail V. Matz, Supervisor

Daniel I. Bolnick

Hans A. Hofmann

Timothy H. Keitt

Camille Parmesan

Kimberly B. Ritchie

Investigating Local Adaptation in a Reef-building Coral

by

Carly Danielle Kenkel, B.Sc.

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Dedication

To my father the epitome of work ethic, my mother the soul of courage, and my husband whose patient love knows no bounds.

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This work would not have been possible without the encouragement, guidance and assistance of mentors, lab-mates, students, family and friends, field stations, government agencies and funding agencies. At the risk of sounding cliché, it did indeed take a village to raise a Ph.D.

Eight years ago I met a crazy Russian man on the R/V DANTE FASCELL. My REU advisor, Dr. Kim Ritchie, had invited me to extend my 2006 summer internship to participate in the inaugural NOAA coral spawning cruise in the Florida Keys National Marine Sanctuary. I sewed coral spawning nets, organized supplies and bossed my fellow interns. I had previously sampled various careers in marine science, but this summer internship inspired me to pursue a career in coral research. This decision was cemented by the antics of my future advisor, Dr. Mikhail "Misha" Matz, a cruise collaborator. He showed me how to view fluorescence underwater and encouraged me to apply to the graduate program at the University of Texas at Austin where he would be moving later that year. Misha's mentorship undoubtedly helped me to grow into the scientist I am today. He taught me to always strive for perfection, even if it means having to re-analyze the data AGAIN. He taught me to stand up for what I believe in and challenged me to always know my material. He taught me to aim high. My relationship involved more arguing than a court of law, but if I had the choice to do it all over again, I wouldn't change a thing.

During my first year at UT, I was fortunate enough to rotate in the lab of Dr. Hans Hofmann. Hans has been an invaluable mentor and had I not been so committed to my coral career, I would undoubtedly have joined his lab instead. Thankfully, he has never held this decision against me and has always provided the most sound advice and insight.

I officially joined the Matz lab in May of 2009 and for the next two years I floundered and pondered and managed to come up with an idea for a thesis. I engaged the support of a stellar group of scientists to help me polish this idea. In addition to the brilliance of Misha and Hans, Dr. Dan Bolnick, Dr. Camille Parmesan, Dr. Tim Keitt, and of course, Dr. Kim Ritchie, helped me to design, execute and interpret my dissertation research. I am greatly indebted to these individuals. My dissertation would not be in its present form without their help.

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Investigating Local Adaptation in a Reef-building Coral

Carly Danielle Kenkel, Ph.D.

The University of Texas at Austin, 2014

Supervisor: Mikhail V. Matz

Environmental variation is ubiquitous in natural systems. The genetic and physiological mechanisms governing population-level responses to this variation will impact the process of speciation and the capacity for populations to persist in a changing climate. Until recently, population-level responses to environmental selection remained largely unexplored in marine systems due to the historical assumption that the inherently dispersive nature of most marine taxa would preclude their ability to specialize to local environments. This dissertation represents the first investigation of population-level responses to environmental variation in a Caribbean reef-building coral. This research integrates ecological, physiological, genetic and genomic methods to (1) determine patterns of local adaptation in the Florida Keys, (2) identify stressors driving adaptive responses, (3) distinguish the physiological and genetic mechanisms underlying coral adaptation and (4) assess the potential for future adaptation in the common reef-building coral *Porites astreoides*. Results demonstrate that corals adapt and/or acclimatize to their local habitat and that this specialization incurs fitness costs. Temperature differences between reefs likely play a selective role in differentiating inshore and offshore coral populations. Genetic and gene expression differences indicate that coral hosts play a substantial role in driving these population-level differences. Inshore corals exhibit greater gene expression plasticity, which may be involved in stabilizing physiological

responses to temperature fluctuations experienced at inshore reefs. In addition, naïve juvenile coral recruits from inshore reefs exhibit a growth rate advantage over offshore recruits under elevated temperature treatment, suggesting that thermotolerance differences observed in adult populations could continue to evolve in response to climate change. Taken together these results provide novel insight into the drivers of reef decline in the Florida Keys and the role of the host in coral adaptation capacity.

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General Introduction

PROBLEM STATEMENT

Corals are the engineers, builders and bricks of the most diverse ecosystems in the ocean, tropical coral reefs. In addition to providing habitat for reef animals, coral reefs serve as a natural breakwater for coastal regions, and support local economies through tourism and subsistence harvest (Moberg & Folke 1999; Smith 1978; Sutton 1983). In the Florida Keys alone it is estimated that reef-related activities generate $3.4 billion in sales and income annually and support 36,000 jobs (Johns et al. 2001; Johns et al. 2004). In the last 30 years coral reefs have experienced unprecedented declines (Butchart et al. 2010; Gardner et al. 2003; Hughes et al. 2003) and some authors predict their complete demise in the near future (Hoegh-Guldberg et al. 2007). However, not all corals respond to stress in the same way. Even within the same species, healthy corals can be found right next to stressed corals and on a broader scale, some reefs survive extreme stress events while others do not (Baker et al. 2008; Glynn et al. 2001; Guest et al. 2012). Identifying factors that underlie this variation in coral survival is necessary for developing effective management strategies to preserve these valuable ecosystems in the face of climate change.

In the Florida Keys, hard coral cover has declined by 44% since 1995 (Donahue et al. 2008). Yet despite decades of monitoring, I are still unable to explain why some reefs in this system are more degraded than others. Inexplicably, nearshore reefs that experience high temperature variability and elevated pollutants also exhibit higher coral cover and coral growth rates than offshore reefs that experience more stable temperatures and better water quality (Lirman & Fong 2007). A recent policy review covering coral decline in the Florida Keys suggested that the scientific community should stop debating

the causes of reef decline and initiate a blanket strategy to reduce all threats, particularly those of anthropogenic origin (Pandolfi et al. 2005). However, anthropogenic input does not seem to be the immediate cause of reef decline in this system. While action is needed, how can reef managers address threats that are undefined? Furthermore, what mechanisms enable corals to thrive in such marginal habitats as the nearshore reef environment? To address these questions, my dissertation research has focused on (1) determining patterns of local adaptation in the Florida Keys, (2) identifying stressors driving adaptive responses, (3) distinguishing the physiological and genetic mechanisms underlying coral adaptation and (4) assessing the potential for future adaptation in the common reef-building coral *Porites astreoides*.

CORAL BIOLOGY AND ADAPTATION CAPACITY

Corals are cnidarians that exist in symbiosis with dinoflagellates of the genus *Symbiodinium*. This symbiosis is considered obligate as it has been estimated that up to 95% of a coral's energy requirements are met through photosynthetically fixed carbon contributed by the endosymbiont (Muscatine 1990). Thermal stress results in the functional loss of the endosymbionts in the process known as coral bleaching, which can ultimately result in death if stressful conditions persist. While reef-building corals are found throughout warm tropical and sub-tropical waters, most exist within 1-2ºC of their temperature tolerance during summer months (Jokiel 2004). Climate change models predict that global temperatures will increase by at least 1-2ºC within the next 50 to 100 years (Hoegh-Guldberg et al. 2007), therefore placing thermally sensitive corals in jeopardy. Factors that influence thermotolerance limits of the coral holobiont (the collective unit of the coral host and symbiont) include differences in the type of *Symbiodinium* hosted (Berkelmans & van Oppen 2006; Little et al. 2004), the effect of

the host genetic background (Abrego et al. 2008; Ulstrup et al. 2006) and holobiont thermal history (Brown et al. 2002a; Brown et al. 2000, 2002b). Consequently, both adaptation and acclimatization will likely contribute to a coral's ability to track climate change.

Like other animals, corals can adapt in response to selection at the individual level (Brown et al. 2002a; Coles & Jokiel 1978) as well as at the population level, resulting in matching of coral physiology to the local environment (Clausen & Roth 1975; Meesters & Bak 1993; Oliver & Palumbi 2011a). This may either be achieved through acclimatization (plasticity/physiological adaptation), changes in the population's allele frequencies (genetic adaptation), or both. Corals also feature a unique intermediate local adaptation mechanism: some species are able to "shuffle" proportions of resident symbiont genotypes (Berkelmans & van Oppen 2006), which is essentially a plastic change in allele frequencies. Research on coral adaptation capacity has largely focused on this variation in coral-*Symbiodinium* association, as it is a potentially rapid and reversible mechanism by which corals can adapt to their thermal environment (Buddemeier & Fautin 1993). Different *Symbiodinium* types are known to confer different holobiont (the combination of host and symbiont) thermal physiologies (Abrego et al. 2008; Little et al. 2004) and coral species can associate with different symbiont types across their thermal ranges, hosting more thermotolerant types in warmer environments (Oliver & Palumbi 2009, 2011a; Ulstrup et al. 2006). However, only a minority of coral species appear capable of such flexibility in their symbiont associations (Goulet 2006). Little work has been done to explore adaptations to environmental factors other than heat (Bay et al. 2009), or to investigate host roles in the adaptive response (Baird et al. 2009a). In addition, genetic processes responsible adaptation/acclimatization

are largely unknown, particularly with respect to the potential for future adaptation (Baird et al. 2009a).

In general, adaptation is understudied in marine environments (Sanford & Kelly 2011). There are a limited number of studies that have provided experimental evidence of adaptive divergence among populations of reef-building corals and to date, all focus has been on Indo-Pacific species. D'Croz & Maté (2004) evaluated the response of *Pocillopora damicornis* from different upwelling regimes in a common garden thermal stress experiment. They found that host allozyme genotypes varied between the two environment types and that genotypes from the upwelling sites showed higher sensitivity to thermal stress than those from non-upwelling sites, suggesting genetically based adaptation, though they did not evaluate potential plastic shifts in symbiont community composition (D'Croz & Maté 2004). Ulstrup et al. (2006) used a similar experimental approach to test for variation in bleaching sensitivity in *P. damicornis* inhabiting broad latitudinal temperature gradients. As in the previous study, *P. damicornis* from different latitudes exhibited significant differences in bleaching resistance, with those from cooler latitudes bleaching earlier and at lower temperatures than those from warmer latitudes, again suggesting host-derived adaptation, though some thermotolerance variation was attributable to differing symbiont type (Ulstrup et al. 2006). A recent study by Howells et al. (2011) demonstrated that within-clade variation in symbiont physiology can also be relevant for local adaptation. They report significantly higher thermotolerance in juvenile corals hosting C1 *Symbiodinium* from a warm reef environment in comparison to C1 from a cool reef environment, that persisted even after 30 generations in culture (Howells et al. 2011). However, variation in symbiont type is known to interact with interspecific variation in the coral host (Abrego et al. 2008), therefore additional work is needed to assess the emergent properties of holobiont adaptation when symbionts interact with

differing host genotypes. In a separate reciprocal transplant of adult *Acropora millepora*, Howells et al. (2013) found that transplanted corals were unable to survive temperatures outside their native thermal regimes, likely reflecting a fixed divergence between source populations. Transplanted corals were able to shuffle *Symbiodinium* compositions, but the growth rates of transplanted corals was still substantially reduced compared to natives, suggesting a potential host role (Howells et al. 2013). Bongaerts et al. (2011) also employed a reciprocal transplant approach to investigate patterns of adaptation along depth gradients in *Seriatopora hystrix*. They observed significantly higher survival rates for native transplants, providing support for their adaptive divergence between populations at different depths, which was not attributable to symbiont shuffling (Bongaerts et al. 2011). The most comprehensive series of local adaptation studies to date were preformed on a poritid congeneric to the focal species used in this dissertation, *Porites lobata*, inhabiting different thermal environments in American Samoa. Barshis et al. (2010) found that levels of ubiquitin conjugates and host genotypes differed between individuals of *P. lobata* from different thermal environments, which was interpreted as host genotypic effects on this physiological response since symbiont populations were homogenous between sites. Though it is unclear how these physiological differences are translated into fitness effects, as overall growth patterns in a separate reciprocal transplant experiment show that corals from the backreef outgrow forereef corals in both environments (Smith et al. 2007).

Coral adaptation capacity and demography may also be strongly dependent on the reproductive strategy of the host and the mode of symbiont transmission. *Porites astreoides* is a hermaphroditic brooding coral and is the coral model chosen for my dissertation work. Brooders release only sperm, fertilization is internal and larvae develop within parental tissue (Richmond & Hunter 1990). These larvae are released

monthly, and are competent to settle within hours, often in close proximity to their parents (Carlon & Olson 1993), resulting in highly genetically structured populations (Ayre & Hughes 2000; Bongaerts et al. 2010; Maier et al. 2009; Underwood et al. 2007). In addition, many brooders transmit their symbionts vertically from parent to offspring, again, potentially reducing gene flow. The choice of *P. astreoides* as a model was partly based on this potential for reduced gene flow in both host and symbiont populations, which should facilitate local adaptation. Furthermore, this species is found in all reef environments throughout the Florida Keys and is one of the few species where populations are stable (Green et al. 2008). Finally, *P. astreoides* is a good model for functional genomics since it exhibits consistent gene expression changes in response to environmental perturbations (Kenkel et al. 2011b).

RESEARCH OVERVIEW

To date, a comprehensive study of adaptation has not been undertaken in any Caribbean reef-building coral (but see Prada & Hellberg 2013) for an excellent investigation of divergence along depth gradients in a Caribbean octocoral). This dissertation uses a multidisciplinary approach to investigate the pattern and scale of local adaptation throughout the Florida Keys, identify selective agents responsible for patterns of adaptation, explore underlying genetic mechanisms involved in the adaptive response and assess the potential for continued adaptation in the face of climate change. In chapter 1, I present results of a yearlong reciprocal transplant experiment designed to test the hypothesis that spatial and temporal variation in local adaptation/acclimatization underpins patterns of reef decline in the Florida Keys. In chapter 2, I report results of a common garden experiment evaluating the effects of elevated temperature as a selective agent on inshore and offshore coral populations in the Lower Florida Keys. Chapters 3

and 4 use gene expression profiling of corals from the common garden and reciprocal transplant experiments to understand the molecular phenotypes in host corals that may underpin population level variation in thermotolerance and patterns of adaptation/acclimatization. Finally, in chapter 5 I quantify variation in growth rate and survival in 38 families of recruits of *P. astreoides* to evaluate the potential for continued evolutionary response.

CHAPTER 1: Spatial and temporal variation in local adaptation/acclimatization may underpin patterns of coral decline in the Florida Keys

ABSTRACT

Despite decades of monitoring global reef decline, I are still largely unable to explain patterns of reef deterioration at local scales, which precludes the development of effective management strategies. Offshore reefs of the Florida Keys experience milder temperatures and lower nutrient loads in comparison to inshore reefs yet remain considerably more degraded. A year-long reciprocal transplantation experiment of the mustard hill coral (*Porites astreoides*) involving four source and eight transplantation locations was conducted to investigate this problem. Results demonstrate that corals adapt and/or acclimatize to their local habitat on a less than 10 km scale and across locations the coefficient of selection against transplants is 0.07 ± 0.02. This local specialization also incurs a trade-off, which will affect the success of assisted migration efforts. For every 1% increase in growth in the native habitat there is 0.4 ± 0.07% decline in growth upon transplantation. Specialization of corals to habitats increasingly differentiated by anthropogenic impacts may render recruits from neighboring populations unfit for re-colonization of reef sites devastated by acute disturbance events, providing a unifying hypothesis to explain patterns of reef decline in this ecosystem.

INTRODUCTION

A central problem in ecology is to understand the pattern and scale at which organisms respond to their environment (Levin 1992). This problem has received comparatively little attention in marine systems where, until recently, it was thought that most species were panmictic and unaffected by spatial and temporal variation in the environment (reviewed in Conover et al. 2006). Though it is now widely accepted that even species with planktonic larval dispersal can exhibit adaptive differentiation in response to both biotic and abiotic selection gradients, the spatial and temporal scales over which adaptation can occur remain poorly resolved (Levin 2006; Sanford & Kelly 2011).

This knowledge gap is particularly critical for reef-building corals, which constitute the foundation of the most biodiverse ecosystem in the marine environment. Coral reefs around the world have degraded significantly in recent years, particularly in the Caribbean (Gardner et al. 2003). In the Florida Keys, hard coral communities that dominated reefs in the 1970s have now largely been replaced by soft corals, sponges and macroalgae (Pandolfi et al. 2005). The environmental factors that brought about this dramatic transition are still a matter of debate. The most widely cited causes include increasing coastal development leading to physical damage and eutrophication (Pandolfi et al. 2005); coral disease (Aronson & Precht 2001); the 1983 epidemic that nearly wiped out Caribbean urchin populations, important reef herbivores (Lessios 1988); mortality from heat-induced bleaching (McWilliams et al. 2005); hurricane damage (Gardner et al. 2005); and, more recently, mortality from extreme cold events (Lirman et al. 2011).

While these factors likely contributed to the overall decline of Florida reefs, I are still largely unable to explain patterns of variation in the degree of reef deterioration at a local scale. The most prominent example of this is the contrast between inshore patch

reefs and the offshore reef tract. Inshore patch reefs are characterized by increased turbidity, sedimentation, nutrients, and temperature variation (Boyer & Briceno 2011; Lirman & Fong 2007; Lirman et al. 2011), all of which affect coral growth detrimentally in the lab (Fabricius 2005; Jokiel 2004, Fig. A1). The offshore reef tract, on the other hand, is characterized by milder temperatures and low turbidity. Generally, one would expect that buffering by the Florida Current (a part of the Gulf Stream) and remoteness from sources of pollution on shore would facilitate better coral survival there. Contrary to this expectation, corals at inshore patch reefs in the Florida Keys consistently maintain higher cover, higher growth rates and lower partial mortality rates than corals at offshore reefs (Causey et al. 2002; Lirman & Fong 2007).

This study tested the hypothesis that local adaptation and/or long-term acclimatization of corals to their local reef environments might be limiting reef recovery in this system. While both local adaptation and acclimatization can occur in response to different environmental pressures, adaptation is a heritable difference between populations that has evolved due to selection while acclimatization is a plastic response that increases fitness but is not genetically based (Conover et al. 2006; Kawecki & Ebert 2004). Given the long generation times and poorly controllable reproductive behavior of corals, it is not feasible to rear individuals and obtain an F2 population under standardized laboratory conditions in order to distinguish genetic adaptation from long-term acclimatization. Therefore, I conducted a reciprocal transplant with naturally collected *Porites astreoides* corals from four populations sourced from the Middle and Lower Keys regions to investigate the combined effects of adaptation and long-term acclimatization, which I term "specialization" (Fig. 1.1). Within both regions, fragments of the same coral colonies were transplanted between inshore and offshore reefs as well as to novel sites the same distance from land as the native reefs.

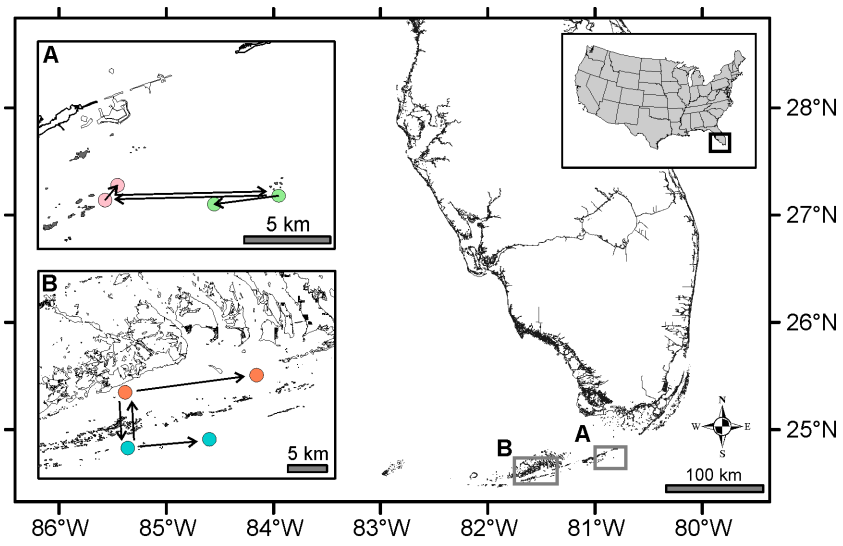


Figure 1.1. Map of the Florida Keys, USA, with insets showing reciprocal transplant sites for the (A) Middle and (B) Lower Keys.

The transplants were sampled after six months and one year to test whether populations exhibited greater fitness in their home reef environment, consistent with local specialization. While ultimately distinguishing between genetic adaptation and adaptive plasticity is important for developing effective management strategies, contemporary reef restoration methods involve transplant of naturally collected corals between reefs (Jaap et al. 2006). Therefore, the absolute response of native and foreign individuals to different environments is most relevant for informing current management practices.

METHODS

Experimental Design

For the Lower Keys transplant, fifteen colonies of *Porites astreoides* were collected on 14 October 2011 from a depth of 2–3 m from each of two sites: an inshore patch reef (Jaap Reef, 24°35.153N, 81°34.886W, Fig. 1.1B) and an offshore reef

(Maryland Shoals Rockpiles, 24°31.299N, 81°34.661W, Fig. 1.1B) 7.2 km apart near Sugarloaf Key under Florida Keys National Marine Sanctuary (FKNMS) permit 2011-115. Corals were immediately returned to Mote Marine Tropical Research Laboratory and placed in a shaded (70% photosynthetically active radiation reducing) flow-through seawater system (raceway). On 19 October, corals were cut into six pieces using a diamond blade tile saw. Coral fragments were affixed to cement pucks using marine epoxy (All Fix Epoxy Putty, Philadelphia, PA, USA) and each puck was labeled with a cattle tag designating both genotype (1-30) and replicate. Each puck was weighed in duplicate using a buoyant weighting method (Davies 1989). On 3 November, corals were reciprocally transplanted between collection sites and origin specific novel "type" sites: a neighboring inshore site (Summerland Shoals Patch, 24°36.346N, 81°25.742W) for the inshore origin corals, and another offshore site (Dave's Ledge, 24°31.887N, 81°29.013W) for offshore origin corals (Fig. 1.1B). Pucks were semi-randomly assigned to cinder blocks (n=10 pucks/block), which were cemented to the reef substrate. Blocks were cleaned of excess algal growth every 1.5 months and checked for damage and/or puck loss.

An analogous transplantation was performed in the Middle Keys. Corals were collected on 18 October 2011 under FKNMS permit 2011-115 from an inshore patch reef (East Turtle Shoal, 24°43.501N, 80°55.120W) and an offshore reef (Hunt 1-4, 24°43.618N, 80°49.680W) 9.2 km apart near Long Key (Fig. 1.1A). Corals were returned to Long Key Marine Laboratory and placed in a shaded raceway. Corals were fragmented on 25 October and outplanted to the field on 31 October. Novel transplant sites were located at E East Turtle Shoal (24°43.969N, 80°54.738W) for the inshore origin corals, and 11ft Mound (24°43.371N, 80°51.700W) for offshore origin corals (Fig. 1.1A).

In April 2012, one fragment of each coral genotype was collected from each site to represent the "six-month" time-point. Collections occurred on 24 April in the Lower Keys and 27 April in the Middle Keys. The final fragments were collected in October 2012, representing the "one-year" time-point. Collections occurred on 3 October in the Lower Keys and 5 October in the Middle Keys. Pucks were cleaned of algal growth and again buoyant weighted in duplicate. Coral fragments were then removed from their pucks and frozen on dry ice. Fragments were kept at -80°C, shipped to The University of Texas at Austin on dry ice and again stored at -80°C until processing.

Environmental disturbances, including Hurricane Issac in August 2012, resulted in the stochastic loss of some coral fragments at each site. All fragments were recovered in the Lower Keys following six months. After one year, two fragments were lost from inshore sites, and seven from offshore sites in the Lower Keys. In the Middle Keys 4 fragments were lost from inshore reefs and 17 from offshore reefs following six months. After one year, 17 fragments were lost from inshore reefs and 22 from offshore reefs in the Middle Keys.

Environmental Data

Data loggers (HOBO Pendant Temperature/Light Data Logger, Onset, Bourne, MA, USA) recorded temperatures at each of the transplant sites every 15 minutes. Additional water quality data for the Florida Keys were provided by the SERC-FIU Water Quality Monitoring Network which is supported by EPA Agreement #X994621-94-0 and NOAA Agreement #NA09NOS4260253.

Phenotypic Trait Measurements

Coral growth was assessed by calculating the percent weight gained by each coral fragment as described in (Kenkel et al. 2013a). Coral tissue surface area was quantified

using the aluminum foil method (Marsh 1970). To evaluate physiological condition of the coral, tissue was removed from the frozen samples using an airbrush with extraction buffer (50 mM phosphate buffer, pH 7.8, with 0.05 mM dithiothreitol) over ice. Tissue slurries were homogenized by vortexing with 1 mm glass beads (BioSpec, Bartlesville, OK, USA) for 1 min and left on ice for 5 min. 1 ml of this slurry was aliquoted for symbiont density analysis. *Symbiodinium* cell numbers were determined by conducting four replicate counts of 1 µl samples using a haemocytometer and a compound microscope (100x magnification). Densities were expressed as the number of symbiont cells per cm^2 of coral surface area.

The remaining slurry was centrifuged at 4°C at 3500 rpm for 5 min to separate coral and endosymbiotic algal fractions. Photosynthetic pigments were extracted from the algal pellet by 24 hr incubation in 90% acetone at 4°C. Symbiont chlorophyll content (a and c2) was assessed by triplicate measures of pigment extract absorbance at 663 nm and 630 nm on a Spectramax M2 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA). Pigment content was quantified using the equations described in (Jeffrey & Haxo 1968) and expressed as ng of pigment per symbiont cell.

The supernatant was aliquoted for host protein, carbohydrate and lipid analysis and frozen at -20°C (1 ml each). Total protein was extracted by incubating the protein aliquot 1:1 in 0.2 M NaOH for one hour at 90°C. This extract was centrifuged at 3500 rpm for 5 min to separate cell debris from the solution and 20 µl clear supernatant was assayed in triplicate using a Pierce BCA assay kit following the manufacturers instructions (Fisher ThermoScientific, Waltham, MA, USA). Blank 0.1 M NaOH samples and BSA protein standards (0.125, 0.25, 0.5, 0.75, 1, 1.5 and 2 mg/ml) were run in triplicate on each plate and absorbance was read at 562 nm on a Spectramax M2 spectrophotometer. Standard curves had an R^2 of 0.96-0.99. Total protein content per

sample was expressed per cm^2 of coral surface area. Carbohydrate was quantified using a phenol-sulfuric acid method following the protocol described in (Masuko et al. 2005). Blank samples and D-Glucose standards (0.03125,0.125, 0.25, 0.5, 1 and 2 mg/ml) were run in triplicate on each plate and absorbance was read at 485 nm on a Spectramax M2 spectrophotometer. Standard curves had an R^2 of 0.90-0.99. Total carbohydrate content per sample was expressed per cm^2 of coral surface area. Lipid analysis was completed for a subset of samples (Lower Keys, one year reciprocal samples only) at Boston University's School of Medicine Core Facility. Total lipid content per sample was expressed per cm^2 of coral surface area.

Statistical Analyses

Robustness in growth, representing the reaction norm for an individual genotype in response to transplantation, was calculated by subtracting growth in the native environment from growth in the foreign environment. Positive values indicate that the genotype grew more in the foreign environment than in the home environment while negative values indicate reduction in growth in the foreign environment. Selection against transplants was calculated according to Eq. 1 in (Hereford 2009), and reflects the difference in relative fitness of the native and non-native populations in the native populations environment at a given sampling time. The magnitude of fitness trade-offs was calculated as in (Bennett & Lenski 2007) and it describes the correlation between the fitness advantage of a focal population at its native reef site (relative fitness in the native environment) and the fitness advantage of the population at a non-native site (relative fitness in the non-native environment).

All analyses were carried out using R 2.15.3 (R Development Core Team 2013). Differences in absolute trait values (growth, symbiont density, total protein, total lipid,

total carbohydrate, Chlorophyll a and Chlorophyll c2) were evaluated with respect to time of sampling (levels: six months and one year), region (levels: Lower and Middle Keys), reef origin (levels: inshore and offshore) and transplant destination (levels: home, cross-channel and along-shore) using a nested series of linear mixed models implemented in the nlme package (Pinheiro et al. 2013). Robustness measures were evaluated with respect to time, region, origin and direction of transplant (factor levels: cross-channel and along-shore). I also explored the relationship between the primary fitness proxy, growth, and additional physiological trait measurements and robustness values in a separate series of linear mixed models. Symbiont cell density, total carbohydrate and chlorophyll content (a and c2) were log-transformed prior to statistical analyses to satisfy model assumptions. For all models, time, region, origin, transplant and trait measurements were modeled as fixed factors. Colony identity was included as a scalar random factor. For growth and robustness data, model selection was performed using Akaike Information Criterion (AIC), using the *stepAIC()* command from the MASS package (Venables & Ripley 2002). For physiological trait data, model selection was performed using the AICcmodavg package (Mazerolle 2013). I required a minimum of a ten-point difference in delta AIC (ΔAIC) to justify selection of a top model. Marginal estimates of effect sizes were obtained by running lme models with individual factors. Nominal P-values for the significance of pair-wise comparisons of fixed factor levels were derived via Markov Chain Monte Carlo (MCMC) simulations using the package MCMCglmm (Hadfield 2010). The false discovery rate (Benjamini & Hochberg 1995) was controlled at the 5% level using the function *p.adjust()*.

RESULTS

Factors affecting growth

Changes in growth were best explained by a four-way interaction model (origin x destination x region x time, ΔAIC >10, Fig. 1.2A-D, Table A1). Though the response to

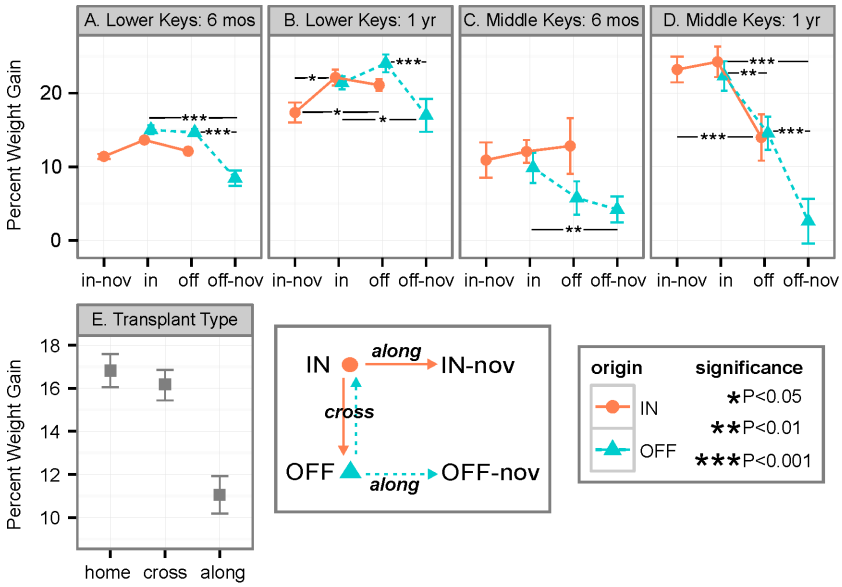


Figure 1.2. Mean growth differences ± SEM in source coral populations when transplanted to different habitats in the Florida Keys. Lower Keys populations after (A) six months and (B) one year; and Middle Keys populations after (C) six months and (D) one year. (E) Mean growth with respect to transplant destination overall. Significance of pair-wise comparisons are derived from a MCMCglmm model.

transplantation in this experiment was complex, Wald tests for terms within this model revealed some generalities in smaller-order interaction terms. A significant destination effect was observed, ($P<0.0001$, Fig. 1.2E). Corals exhibited the highest mean growth in their home environment supporting a home vs. away definition of local adaptation (Kawecki & Ebert 2004). Compared to growth in the native environment, growth was reduced by 0.1% in the cross-channel environment (inshore to offshore; offshore to

inshore). Surprisingly, transplantation along-shore to novel sites of the same habitat type resulted in a 5 ± 1% reduction in mean growth. While inshore corals exhibited higher growth rates on average in comparison to offshore corals (ß = 3 ± 1.2%, P<0.01), inshore origin corals were more affected by the cross-channel transplant than offshore origin corals (origin x destination, P<0.0001). Corals also show origin-specific patterns of growth that differed depending on the region: offshore corals tend to outgrow inshore corals in the Lower Keys (ß = 0.4%), but this pattern is reversed in the Middle Keys, with inshore origin corals exhibiting a higher mean growth rate (ß = 7 ± 2%, P<0.01). On average, corals in the Lower Keys exhibited higher growth rates than corals in the Middle Keys (ß = 3.9 ± 1.2%, P<0.01).

Corals collected after one year exhibited greater changes in growth on average than corals collected after six months (ß = 7.7 ± 0.7%, P<0.0001). The observed differences in growth between transplant destinations diminished through time (P=0.05), though this effect is better explained by higher order interactions with origin and region (Fig. 1.2A-D, Table A1).

In the Lower Keys, inshore origin corals show growth trends consistent with local specialization after only six months: growth was reduced in both non-native environments in comparison to growth at the home reef (Fig. 1.2A). While offshore origin corals show a significant effect of along-shore transplantation at this time-point, they appear unaffected by the cross-channel environment, exhibiting similar growth in comparison to their native reef. In Middle Keys corals, the growth response after six months is almost completely reversed. Inshore origin corals are unaffected by transplantation while offshore origin corals show a pattern of maladaptation: offshore origin corals grow better in the cross-channel environment though growth is still suppressed along-shore (Fig. 1.2C).

Significant differences with respect to site, origin, and transplant destination are observed after one year, which may reflect the impact of elevated summer temperatures (Fig. A1E,F). In the Lower Keys, while the overall change in growth is greater, initial trends observed after six months have magnified, save for the response of offshore origin corals at inshore reefs (Fig. 1.2B). Offshore origin corals no longer outgrow inshore origin corals at this site, satisfying the local vs. foreign criteria for local adaptation (Kawecki & Ebert 2004). In the Middle Keys, initial six-month patterns have also magnified, save for the response of inshore corals at the offshore site (Fig. 1.2D). This population is the only one to exhibit growth patterns consistent with initial predictions: inshore corals are unaffected by transplantation to a novel inshore reef site but exhibit a significant growth disadvantage at the offshore reef.

Analysis of additional phenotypic trait data revealed weak, though positive, linear relationships with growth (Fig. A2, A3). Only models that incorporated symbiont density and total protein showed AICc values that indicated an improvement over the null model. These traits were significantly positively correlated with growth, though the overall variance explained by these measures was low, only 10% and 2%, respectively, for total protein and symbiont density (Fig. 1.3).

Strength of selection against transplants

The strength of selection against transplants can be defined as the difference in relative fitness between a native and non-native population in the native population's environment (Hereford 2009). Using growth as a proxy of fitness, the overall strength of selection after one year of transplantation was 0.07 ± 0.02 SE, meaning that native populations had, on average, 7% greater fitness than foreign populations (Table 1.1). This measure also reflects the magnitude of local specialization of a given population. While

corals in the Lower Keys displayed a significant origin by transplant interaction after one year, consistent with local specialization, it must be noted that transplant destination was the dominant effect on corals in the Middle Keys. This conclusion is also contingent on the time of sampling, which, again, may reflect seasonal variation between reef environments. After six months, two of the four native populations exhibited lower fitness than that of foreign transplants: inshore corals from the Lower Keys and offshore corals from the Middle Keys.

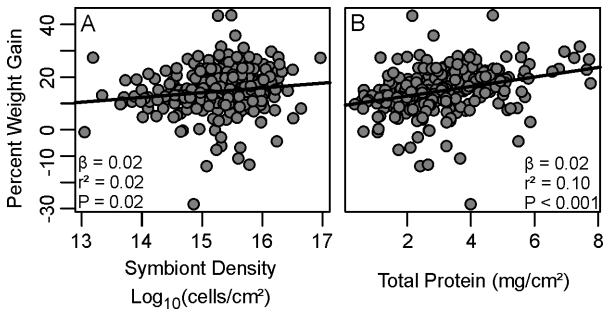


Figure 1.3. Linear models incorporating (A) symbiont densities and (B) total protein were the only ones to show AICc values that indicated an improvement over the null model.

Costs of local specialization

Maximization of growth rates in the native environment incurs a trade-off in the ability of corals to grow in a foreign environment both at the level of individual and the level of population. Growth of an individual at its home reef explained 17% of the variance in robustness, which reflects the decline in growth of a genotype when transplanted to a foreign environment compared to growth in its native environment (Fig. 1.4A). For every 1% increase in the growth of a genotype at its native reef there is a 0.4 ± 0.07% decline in growth when transplanted to a novel environment. Population-level trade-offs, defined as pair-wise comparisons between populations grown in each other's

native environments, showed a temporal effect. All fully reciprocal population pairs exhibited greater relative fitness in their native environment but only after one year of transplantation (Fig. 1.4B). No trade-offs are evident at the six-month time point.

Table 1.1. Estimate of selection against transplants into the specified population at each sampling time.

Sampling Time	Region	Site	Relative Fitness‡	Interaction P-value*
Six Months (Oct-Apr)	Lower Keys	Inshore	-0.10	NS
Six Months	Lower Keys	Offshore	0.19	NS
Six Months	Middle Keys	Inshore	0.20	NS
Six Months	Middle Keys	Offshore	-0.76	NS
One Year (Oct-Oct)	Lower Keys	Inshore	0.03	$P < 0.05$
One Year	Lower Keys	Offshore	0.13	$P < 0.05$
One Year	Middle Keys	Inshore	0.08	NS
One Year	Middle Keys	Offshore	0.04	NS

‡ Positive values indicate selection against transplants (i.e., local adaptation), and negative values indicate that transplants have greater fitness than the native population (maladaptation of native population).
* Significance value for origin x destination interaction term of reciprocal transplants

Factors affecting robustness

Differences in robustness, representing the reaction norm for an individual genotype in response to transplantation, were also best explained by a model including a four-way interaction term (origin x destination x region x time, $\Delta AIC > 10$, Fig. 1.4C-F, Table A2). Wald tests for terms within this model revealed only three additional model terms passing the 5% significance threshold: region, transplant, and the origin x transplant interaction. Interestingly, corals in the Middle Keys were more robust than corals in the Lower Keys ($ß = 3.2 \pm 1.2\%$, $P<0.05$), perhaps reflective of a regional effect of growth trade-offs as Lower Keys populations exhibited higher mean growth rates. Destination effects recapitulate patterns observed for mean growth: along-shore transplants were more stressful than cross-channel transplants on average ($P<0.0001$).

Destination also interacted with origin: offshore origin corals were more robust to cross-channel transplantation but less robust to along-shore transplantation than inshore origin corals ($P<0.05$). However, this interaction is contingent upon sampling time and region.

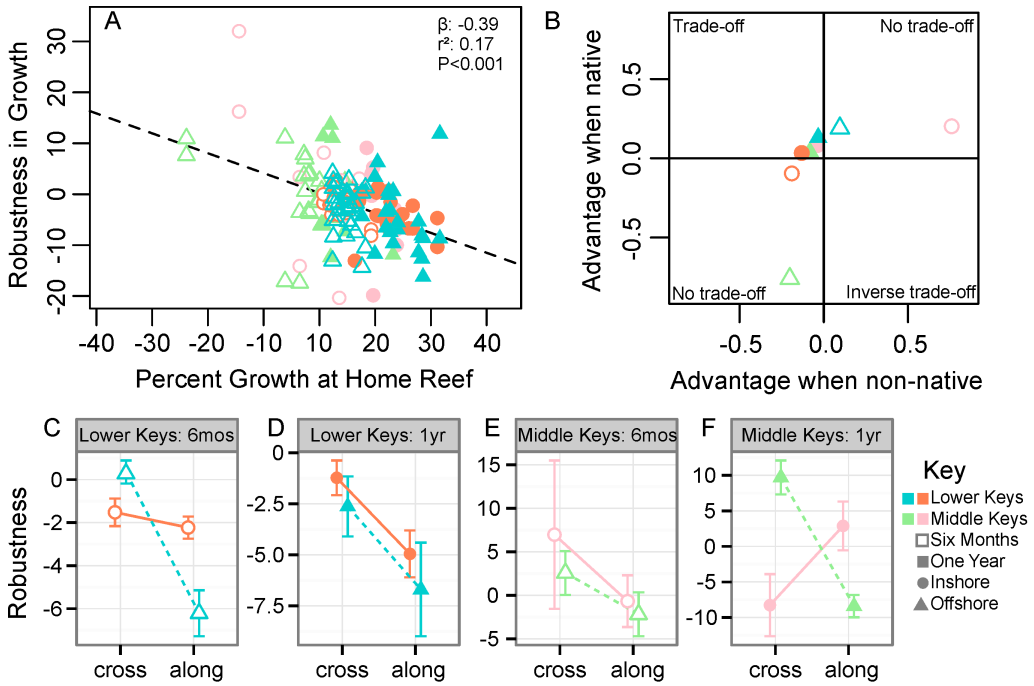


Figure 1.4. Specialization to the native reef incurs trade-offs in corals transplanted to foreign environments. (A) Growth of individual corals at their home reef site explains 17% of the variance in robustness of transplanted individuals. (B) Each point represents a comparison between mean fitness of the native population and foreign transplants at a given reef site through time. Quadrants indicate separate qualitative outcomes of transplant experiments as in Hereford (2009). (C-F) Mean robustness ± SEM of coral populations when transplanted to different habitats in the Lower Keys after (C) six months and (D) one year; and Middle Keys populations after (E) six months and (F) one year.

Lower Keys corals at the six-month time-point and Middle Keys corals at the one-year time point follow this pattern (Fig. 1.4C,F). However, for Lower Keys corals at the

one-year time point and Middle Keys corals at the six-month time point, inshore origin corals appear more robust to transplantation in general (Fig. 1.4D,E).

DISCUSSION

Highly structured local specialization suggests substantial environmental heterogeneity

Patterns of growth following one year of transplantation indicate that reef environments are more heterogeneous than abiotic gradients suggest (Fig. A1,1.2) and that corals have responded to this variation. Inshore and offshore reefs are separated by less than 10 km, yet at all sites tested native populations outcompeted foreign transplants on average (Table 1.1). Results from one-way transplantation experiments between reef environments in the Florida Keys suggest that other scleractinian coral species may exhibit similar patterns of specialization (Hudson 1981; Shinn 1966).

Evidence from controlled laboratory experiments on nutrient loading and temperature extremes dictate that the inshore reef should be more stressful for corals (Fabricius 2005; Jokiel 2004). Yet inshore corals were consistently more negatively affected by transplantation to the offshore reef tract than offshore corals were by transplantation inshore. One plausible explanation for this pattern involves physiological trade-offs in inshore corals that reduce their mean growth rate overall. Work on local adaptation to heavy metal pollution in other invertebrates shows a similar pattern: tolerant populations are more fit in contaminated environments because of the greatly reduced growth of non-adapted individuals, but tolerant individuals cannot increase mean growth in unpolluted waters and are subsequently outcompeted (Piola & Johnston 2006; van Ooik & Rantala 2010). In the Florida Keys, physiological adaptations to elevated summer temperatures may underpin differential growth patterns, as trade-offs are only evident

after one full year, which included summer months (Fig. 1.4B) and temperature stress has previously been demonstrated to be a selective agent for inshore and offshore populations (Kenkel et al. 2013a). In the Lower Keys, mean growth of inshore origin corals did not differ greatly between inshore and offshore locations (Fig. 2A,B). Offshore populations on the other hand exhibited higher mean growth in their home environment and only exhibited reduced growth at the one-year sampling time point, which included summer months (Fig 1.2B).

However, the idea of trade-offs is not a satisfactory explanation for corals in the Middle Keys where offshore corals grew more at inshore reefs than in their native reef during the summer months while inshore origin corals exhibited a major growth reduction at offshore reefs (Fig 1.2C,D). One explanation for this pattern is environmental stochasticity. Local maladaptation is surprisingly common in natural populations and can be temporally variable (i.e. present in one year but not in another, (Fraser et al. 2011; Rice & Mack 1991). In addition to seasonal temperature variation, corals in this experiment were also exposed to the stochastic effects of hurricane damage (Hurricane Issac, August 2012), a common disturbance on Keys reefs (Gardner et al. 2005). Middle Keys sites were more damaged than Lower Keys sites, and in both regions offshore transplant sites experienced the most damage as a result of this disturbance, which may have played a role in the uniformly reduced growth observed at offshore reefs in the Middle Keys.

Alternatively, or in conjunction with the previous explanations, corals at offshore reefs may be impacted by an as yet unknown stressor, which also imposes selection. One possible and thus far largely overlooked source of stress offshore might be internal tidal bores which bring sub-thermocline water to the reef (Leichter et al. 1996). These twice-daily mini-upwelling events contribute to a lower aragonite saturation state offshore

(Manzello et al. 2012), which is known to negatively affect growth of calcifying organisms such as corals (Langdon et al. 2000).

The most surprising result of this study was growth response of the along-shore transplants. Every population exhibited either a trend or a significant decline in growth at every sampling time point when transplanted to these putatively environmentally similar sites (Fig 1.2). While the effect of cross-channel transplantation on growth is seasonally variable, the consistency of growth declines upon along-shore transplantation suggests that the selective agents structuring reef habitats along-shore are likely chronic. Predominant current patterns restrict water flow between inshore and offshore reefs (Smith & Pitts 2001). However, nutrient-rich water masses from Florida Bay are capable of flowing across tidal channels (Hu et al. 2003). This source of heterogeneity may affect coral fitness, though no significant reductions in growth have been reported for corals that are found nearer to these channels (Lirman & Fong 2007). Clearly more work is needed to better understand patterns of coral response to environmental heterogeneity in this system.

Habitat specialization incurs a trade-off: conservation implications

This reciprocal home site advantage observed for all focal populations in this study indicates that specialization to the native reef comes at a cost of diminished fitness in foreign environments, implying that divergent selection is occurring between reef habitats in the Florida Keys (Hereford 2009). Both at the level of individual genotypes and with respect to the entire population, corals exhibited reduced growth when transplanted to a novel environment (Fig 1.4A,B). The absolute magnitude of the trade-off is not large, but given the longevity of reef-building corals, growth deficits can impact long-term population dynamics (Babcock 1991).

Conover et al. (Conover et al. 2006) recognized that human-induced selection that structures genotypes based on fitness can be a greater threat to genetic diversity than the direct loss of populations. This problem could be impacting reefs in the Florida Keys. Elevated nutrients and temperature extremes are not directly killing inshore populations as corals are capable of adapting and/or acclimatizing to conditions at their native reef site. However, corals must pay a cost as the underlying genetic or physiological changes necessary to maximize growth in a coral's native reef are not favored elsewhere in this ecosystem. Coral recruitment throughout the Caribbean is very low (Gardner et al. 2003), though adult populations release billions of viable gametes in annual mass spawning events (Levitan et al. 2004; Vize et al. 2005). Two explanations for this pattern are physical dispersal limitation and post-recruitment mortality of non-native juveniles, or immigrant inviability (Miller et al. 2000). The increasing frequency of stochastic mortality events, for example hurricanes (Gardner et al. 2005) or mass bleaching events (McWilliams et al. 2005), results in a loss of local populations. Concomitantly, adaptation of corals to increasingly differentiated habitats resulting from anthropogenic impacts (Pandolfi et al. 2005b) may render recruits from neighboring populations unfit for re-colonization of the devastated reef sites. Interestingly, Miller et. al. (Miller et al. 2000) reported that juvenile mortality, rather than a lack of recruitment, structures coral populations in Biscayne National Park at the northern fringe of the Florida reef tract, providing support for this hypothesis. Taken together, this phenotype-environment mismatch (Marshall et al. 2010) may explain observed patterns of decline in the Florida Keys.

Trade-offs will also impact the success of reef restoration (assisted migration) efforts which are currently ongoing in the Florida Keys (Jaap et al. 2006). The goal of assisted migration is to increase the frequency of climate adapted alleles to facilitate

adaptation in future generations (reviewed in (Aitken & Whitlock 2013). Temperature is assumed to be one of the largest climate change stressors impacting contemporary reefs (Hughes et al. 2003). While the genetic basis for temperature tolerance in corals is unknown, populations living in elevated temperature environments, such as inshore corals, are the most likely carriers of temperature tolerance alleles (Barshis et al. 2013). However, assisted migration can reduce fitness if local adaptation of populations also occurs in response to other environmental variables (Aitken & Whitlock 2013; Howells et al. 2013). The patterns of growth across reef sites observed in this experiment suggest that additional environmental variables may be structuring coral populations in the Florida Keys. Reef managers interested in implementing assisted migration should carefully evaluate selection of source populations and transplantation sites in order to ensure that target environmental gradients are properly aligned to maximize the success of transplants and subsequent effects on gene flow. Work on additional coral species is needed for the Florida Keys ecosystem, as an understanding of the extent of local adaptation to climate and other environmental factors is critical for assessing the relative risks of assisted gene flow and the potential for maladaptation (Aitken & Whitlock 2013).

CONCLUSIONS

Corals in the Florida Keys have specialized to habitats on fine spatial scales, and this specialization incurs a trade-off in the ability of corals to grow when transplanted away from their native reef site. Knowledge of abiotic gradients is insufficient for understanding coral decline in this system as reductions in growth were also observed for corals transplanted to environments with similar temperature and nutrient regimes. This pattern of highly structured local specialization will affect the success of assisted migration efforts and could prevent effective re-colonization of damaged reefs following

stochastic disturbance events, which may help explain observed patterns of coral decline in the Florida Keys. A deeper understanding of the extent of local specialization to climate and other environmental factors will be necessary for refining management plans aimed at conserving this critically endangered ecosystem.

CHAPTER 2: Evidence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments

ABSTRACT

Studying the mechanisms that enable coral populations to inhabit spatially varying thermal environments can help evaluate how they will respond in time to the effects of global climate change, and elucidate the evolutionary forces that enable or constrain adaptation. Since inshore reefs in the Florida Keys experience higher temperatures than offshore reefs for prolonged periods during the summer, I conducted a common garden experiment with heat stress as my selective agent to test for local thermal adaptation in corals from inshore and offshore reefs. I show that inshore corals are more tolerant of a six-week temperature stress than offshore corals. Compared to inshore corals, offshore corals in the 31ºC treatment showed significantly elevated bleaching levels concomitant with a tendency towards reduced growth. In addition, dinoflagellate symbionts (*Symbiodinium* sp.) of offshore corals exhibited reduced photochemical yields. Although these results suggest that between-population divergence in thermotolerance may be symbiont-driven, I did not detect differences in the genotypic composition of *Symbiodinium* communities hosted by inshore and offshore corals or genotype frequency shifts ("shuffling") in response to thermal stress. Instead, coral host populations showed significant genetic divergence between inshore and offshore reefs, suggesting that in *P. astreoides* the coral host might play a prominent role in holobiont thermotolerance variation. My results demonstrate that coral populations inhabiting reefs less than 10-km

apart can exhibit substantial differences in their physiological response to thermal stress, which could impact their population dynamics under climate change.[1]

[1] Considerable portions of this chapter were published as Kenkel CD, Goodbody-Gringley G, Caillaud D, Davies SW, Bartels E, Matz MV. 2013. Evodence for a host role in thermotolerance divergence between populations of the mustard hill coral (*Porites astreoides*) from different reef environments. *Molecular Ecology* 22: 4335-4348. Contributions – Conceived and designed experiments: CDK, MVM. Performed the experiments: CDK, GGG, EB. Developed microsatellite assays: SWD. Completed ploidy modeling: DC. Analyzed the data: CDK. All authors contributed to writing the manuscript.

INTRODUCTION

Reef-building corals are cnidarians that exist in obligate symbiosis with photosynthetic dinoflagellates of the genus *Symbiodinium* (Muscatine 1990). Thermal stress results in the functional loss of the endosymbionts in the process known as coral bleaching, which can ultimately result in death if stressful conditions persist (Glynn 1993). Increasingly frequent and severe bleaching episodes in combination with anthropogenic disturbance, eutrophication and ongoing climate change (Harvell et al. 1999; Lesser et al. 2007) have led to suggestions that these organisms, and the reefs they support, may not persist in the future (Hoegh-Guldberg 1999; Hoegh-Guldberg et al. 2007). While there is substantial inter and intraspecific variation in thermotolerance, most corals appear to exist within one to two degrees of their local thermal tolerance limit (Berkelmans & Oliver 1999), rendering them vulnerable to even slight warming. However, corals have persisted through warming episodes in the recent geological past without any noticeable decline (Pandolfi 1996, 1999), and today can be found over broad latitudinal ranges inhabiting a variety of thermal conditions (Hughes et al. 2003). This suggests that historically, corals have adapted to both spatial and temporal variation in temperature. Furthermore, evidence is mounting that suggests ongoing adaptation of coral populations to repeated bleaching events, manifested as higher bleaching resistance at sites that experienced frequent or particularly devastating bleaching in the past (Glynn et al. 2001; Guest et al. 2012; Maynard et al. 2008; Thompson & van Woesik 2009). The question remains, however, whether the particular adaptive mechanisms used by corals are efficient enough to keep up with the present rate of climate change, compounded by historically unprecedented stressors such as ocean acidification and declining water quality (Pandolfi et al. 2011; Wooldridge 2009).

Like other animals, corals can respond to elevated temperature at the individual colony level (Brown et al. 2002b; Coles & Jokiel 1978) as well as the population level, resulting in a matching of coral physiology to the local environment (Meesters & Bak 1993; Oliver & Palumbi 2011a). This process can be achieved through physiological plasticity (i.e. acclimatization), changes in population allele frequencies (i.e. adaptation, (Kawecki & Ebert 2004), or both. Corals also feature a unique intermediate response mechanism: some species are able to "shuffle" proportions of resident symbiont genotypes (Berkelmans & van Oppen 2006), which is essentially a plastic change in allele frequencies. Research into coral adaptation and acclimatization capacity has largely focused on variation in coral-*Symbiodinium* associations, as it is a potentially rapid and reversible mechanism by which corals can cope with their thermal environment (Buddemeier & Fautin 1993). However, not all coral species appear to be capable of such temporal flexibility in their symbiont associations (Goulet 2006; Stat et al. 2009), though investigation of this phenomenon is still underway (Silverstein et al. 2012). Therefore the coral host must also play a role in shaping thermotolerance variation (Baird et al. 2009a). Interactions between host coral species and symbiont types have been shown to alter the ultimate holobiont thermal physiology, providing support for the role of the host (Abrego et al. 2008). Host-specific effects have been implicated in the absence of significant symbiont genetic differences in corals from varying thermal environments that exhibit divergent thermotolerance physiologies (Barshis et al. 2010).

While it is clear that both host and symbiont are involved in shaping holobiont thermotolerance limits, it is difficult to evaluate the relative contributions of each partner without evaluating their respective physiologies within the same study system. Furthermore, when investigating population-level variation in response to temperature, genotyping both hosts and symbionts can provide an additional layer of information by

suggesting which partner may be driving the adaptive response. I employed this approach to test for local thermal adaptation or acclimatization in the mustard hill coral, *Porites astreoides*, from thermally distinct reef habitats in the Florida Keys. Since inshore reefs experience higher temperatures than offshore reefs annually for prolonged periods during the summer, I hypothesized that inshore corals are better adapted to long-term heat stress than offshore corals. I conducted a common garden experiment with heat stress as my selective agent to compare within-genotype responses of *Porites astreoides* for growth and bleaching (holobiont fitness proxies) and photosynthesis photochemical efficiency (a *Symbiodinium* fitness proxy). As a host-specific response, I profiled host gene expression, which is described in an accompanying paper (Kenkel et al. 2013b). Newly developed microsatellite assays were used for coral host genotyping. *Symbiodinium* were genotyped using a novel approach, which involved deep sequencing of the second internal transcribed spacer of the ribosomal RNA gene (ITS2) to detect shifts in symbiont community composition in response to heat stress or between populations.

METHODS

Study system

The Florida Keys are a 180 km chain of islands emerging from the southern tip of Florida that separate Florida Bay from the greater Atlantic Ocean (Fig. 2.1). Florida Bay is shallow (1.2 to 1.5 m deep on average) and current systems push water in an eastward direction across the Bay, onto nearshore Florida reefs (Smith & Pitts 2001). Temperature variation at inshore reefs is considerable, likely due to the reduced heat storage capacity of shallow nearshore waters in addition to Florida Bay inputs (Chiappone 1996). Temperature variation at offshore reefs is buffered by the along-shore current patterns of Hawk Channel, which disrupt flow from Florida Bay (Smith & Pitts 2001). In addition,

offshore reefs also experience the thermal buffering of the Florida Current (Gulf Stream), resulting in less variable annual thermal profiles (Lirman et al. 2011). Hourly temperature data from 2006-2011 for a pair of inshore and offshore reefs in the lower Keys show that on average, the inshore reef was 1°C warmer in summer and 1.4°C cooler in winter than the offshore reef (Fig. 2.1).

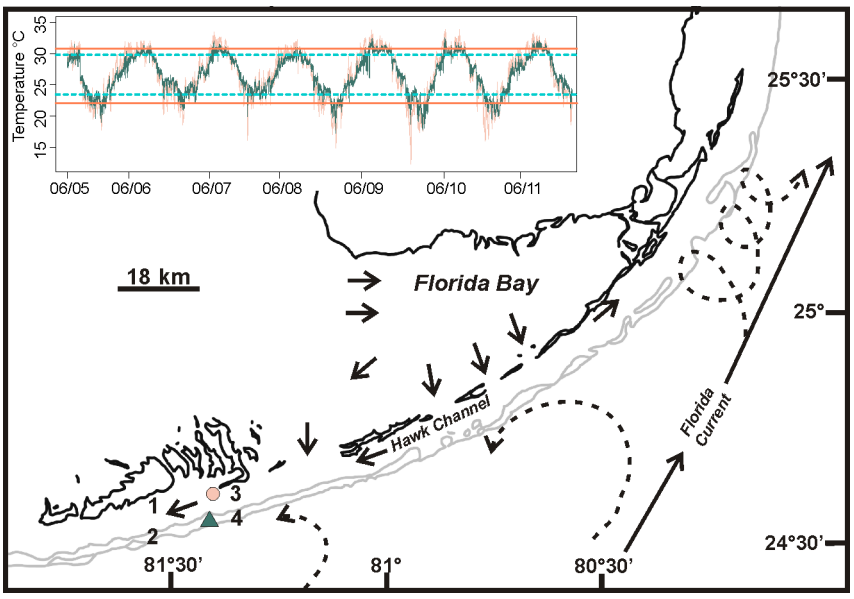


Figure 2.1. Current systems influencing reef environments in the Florida Keys (re-drawn and modified from (Klein & Orlando 1994; Smith & Pitts 2001). Bold arrows indicate predominant direction of current flow. Offshore reef tract shown in gray. Inset shows hourly temperature data for a representative inshore and offshore site from the lower Keys, marked by the red circle and blue triangle, respectively. Upper and lower solid lines indicate mean June-Aug and Dec-Feb temperatures for the inshore site, while dashed lines indicate means for the offshore site. Numbers correspond to populations sampled for genotyping as shown in Fig. 2.6.

Experimental design

Fifteen colonies of *P. astreoides* were collected on the same day from a depth of 2-3 m from each of two sites: an inshore patch reef (N 24°35.142, W 81°34.957, site 1, Fig. 2.1) and an offshore reef (N 24°31.303, W 81°34.605, site 2, Fig. 2.1) 7.1 km apart near Sugarloaf Key in August 2010 under Florida Keys National Marine Sanctuary (FKNMS) permit 2010-094. Colonies were immediately returned to Mote Marine Laboratory's Tropical Research Laboratory and halved using a hammer and chisel. Fragments were placed in a shaded (70% PAR reducing) flow-through seawater system (raceway) with an average water temperature of 28.0 ± 0.7°C and allowed to acclimate for 10 days. Post-acclimation, one half of each colony was randomly assigned to a temperature treatment, a tank within that treatment and a specific position within that tank (n=3 fragments per tank). Temperature treatment consisted of two shaded (70% PAR reducing) raceways, one control and one elevated temperature, each holding ten 40L aquaria with clear plastic lids. Control temperature treatment was achieved by completely filling the 40L tanks with seawater, equipping each tank with a 2-W aquarium pump (Hesen) and allowing water to flow-through the raceway as a water bath. The elevated temperature raceway was set up next to the control in exactly the same manner, but each individual tank was also equipped with a 200-W aquarium heater (Marineland) set to maximum heat. Temperatures were 27.2 ± 0.4°C in the control tanks and 30.9 ± 1.1°C in the heated tanks (Fig. A4). Treatment continued for six weeks (43 days) with tank cleaning and 30-50% water changes performed three times each week to maintain salinity levels at 35 ppt.

Holobiont Trait Measurements (Growth and Bleaching)

Immediately prior to turning on the heaters, all fragments were buoyant weighted in duplicate as described in (Davies 1989). Following the six-week treatment, all

fragments were cleaned using a small brush to remove any filamentous algal growth and buoyant weighted again in duplicate. Technical replicates of weight measurements for each fragment were averaged. Initial weight measurements were subtracted from final weight measurements and divided by the initial weight measurement to determine the proportion of weight gained over the six-week treatment for each fragment. Corals were photographed during the acclimation period and again at the end of the six-week treatment. To quantify color changes associated with reduced chlorophyll and symbiont densities, Corel PHOTO-PAINT was used to balance exposures across photographs using a common white standard. Mean red channel intensity was then calculated for 10 quadrates of 25 x 25 pixels within each coral fragment as a measure of brightness; higher brightness indicated reduction in algal pigments (i.e., bleaching). This analysis was performed following (Winters et al. 2009) using the MATLAB macro "AnalyzeIntensity".

***Symbiodinium* trait measurements (photochemical yield)**

The photochemical efficiency of the symbionts' photosystem II was quantified for each experimental fragment using a pulse amplitude modulated fluorometer (PAM) (Diving-PAM, Waltz). Reductions in effective quantum yield (EQY) indicate a temporary down-regulation of photochemistry under excess heat and/or light, while reduced maximum quantum yield (MQY) values are indicative of sustained damage to the photosystem. Quantum yield measurements of the symbiont photosystem were taken during the last 2 days of the acclimation period, the first 3 days of heat stress treatment and the final 2 days of the heat stress experiment. Effective quantum yield (EQY) measurements were made during the day, between 0800-1800 hours, (n=3 measurements per coral fragment during acclimation, n=5 during treatment days 1-3 and n=3 during

treatment days 42-43) while maximum quantum yield measurements were made following dark adaptation 1.5 hours after sunset (n=2 acclimation, n=1 d1-3, and n=1 d42-43).

Host genotyping

Eight *P. astreoides* microsatellites were mined from the transcriptome (Kenkel et al. 2013b) using a custom Perl script (Table A3). Additional individuals from each of the compared populations were collected for genotyping under FKNMS permit 2011-115 for a total of 33 inshore and 40 offshore individuals. In addition, 27 individuals were genotyped from a novel inshore-offshore reef pair near Summerland Key (14 inshore and 13 offshore, sites 3 and 4, Fig. 2.1) collected under FKNMS permit 2009-078. Tissue samples were preserved in RNALater and extracted using RNAqueous kits, which retained considerable amounts of high-purity DNA along with the RNA. Microsatellite markers were amplified individually following PCR conditions described in (Davies et al. 2012). Electrophoregrams were analyzed using GeneMarker Software 1.70 (Soft Genetics), and alleles were scored manually based on amplicon size. For 12 of the 15 inshore individuals and 14 of the 15 offshore individuals, genotypes were identical between halves of experimental individuals, as expected. The remaining corals, 3 inshore and 1 offshore, exhibited different genotypes between heat and control treatment halves, likely due to co-settlement of individuals at the larval stage, as has been observed in *Acropora millepora* (Puill-Stephan et al. 2009). The separate halves of these individuals were coded as novel genotypes in subsequent genetic and statistical analyses. Three pairs of corals in the additional population samples (1 inshore, 2 offshore) exhibited identical genotypes across all loci. These individuals were considered clones and one from each pair was removed prior to statistical analyses.

No amplification of expected fragment size was observed for any marker when tested using pure A4 *Symbiodinium* DNA (A4 isolate 368, LaJeunesse 2001). For 41 of the 100 unique *P. astreoides* genotypes, I observed amplification peaks indicating third alleles at one to four loci (out of eight); and across all samples, all markers exhibited occasional three-allele states. Such individuals were found in all four subpopulations. These triallelic genotypes remained despite repeated extractions and amplifications, and when observed, occurred in both halves of individuals. Triallelic genotypes were also observed in independent analyses with *P. astreoides* populations from the US Virgin Islands and Bermuda, though at a lower frequency (Xaymara Serrano, pers. comm.).

Although somatic mutation is known to produce intracolony variation (Maier et al. 2012), this process would be unlikely to generate identical novel genotypes in different colony halves. Alternative explanations include physical proximity of planula larvae during recruitment resulting in fine-scale chimerism, the presence of brooded larvae in the genotyped corals, or a ploidy greater than 2n. Since the karyotype of *P. astreoides* is unknown, I tested for higher order ploidy by modeling the expected multi-allele frequency under different ploidy scenarios given observed allelic diversity. If polyploidy (triploidy or tetraploidy) is the cause of these third alleles, then all the individuals of a population are expected to be polyploid, and diploid-looking individuals should be polyploid individuals that only have two different alleles on their three or four homologous chromosomes. Alternatively, if the presence of third alleles is caused by DNA "contamination" arising from brooded larvae or chimerism, then diploid-looking individuals should be either non-contaminated individuals or contaminated individuals that happen to have only two different alleles at each locus.

A model was built to assess which of the scenario is the most likely. This model included nine parameters, which were estimated using the maximum likelihood method.

Parameters Ti, with $i \in \{1,..,8\}$ are the probability that an individual's genotype, at locus i, is triallelic, given than the individual is polyploid or contaminated. The last parameter, denoted Q, is simply the frequency of polyploid or contaminated individuals in the population. If triallelic individuals are polyploids, Q should be equal to one. The probability P_j of an individual j with a multilocus genotype including at least one triallelic locus is:

$$P_j = Q \times \prod_i Ind(A_{i,j} > 2) \times T_i + Ind(A_{i,j} \leq 2) \times (1 - T_i)$$

where $A_{i,j}$ is the number of different alleles in individual j at locus i and $Ind(\cdot)$ is the indicator function. The probability of an individual with a multilocus genotype including only mono- or diallelic loci is:

$$P_j = Q \times \prod_i (1 - T_i) + (1 - Q)$$

The log-likelihood function for the entire population can then be calculated as:

$$L = \sum_j \log(P_j)$$

The function *constrOptim()* from software R 2.13.2 (R Developent Core Team 2013) was used to obtain maximum likelihood estimates of the nine parameters. In addition, 95% profile likelihood confidence intervals of parameter Q were also calculated. These analyses were done separately for all four coral subpopulations and also on the whole population. Estimates of Q were significantly different from 1 in the entire dataset, as well as in two of the four subpopulations (Sugarloaf inshore and offshore) and not significantly different from 1 in Summerland inshore and offshore (Fig. A5). As all four confidence intervals overlap, there is no evidence that the value of parameter Q differs among subpopulations. The overall value of the parameter is 0.45.

These models find no support for polyploidy, rather subpopulations include about 55% of diploid and "non-contaminated" colonies, and 45% of colonies that are probably diploid as well, but are "contaminated" by extraneous genetic material. Since the remaining explanations cannot be ruled out, and since the true diploid genotype of "contaminated" individuals cannot be determined, all individuals that exhibited triallelic genotypes at any loci were excluded from the allelic analyses, leaving 17 inshore and 20 offshore individuals from Sugarloaf and 11 inshore and 7 offshore individuals from Summerland.

The significance of genetic differentiation between populations was examined using a multi-locus G-test following (Goudet et al. 1996). The pairwise F_{ST} statistic was calculated after (Weir & Cockerham 1984) using FSTAT version 2.9.3.2 (Goudet et al. 2002). As a parallel alternative approach, the number of genetically differentiated clusters (K) was estimated using the program STRUCTURE (Falush et al. 2003) with an admixture model including sampling site as a prior. Log-likelihood values for each K (1-16) were computed from the multilocus genotypes with a series of 20 independent runs for each K. The most likely K was evaluated using the method of (Evanno et al. 2005) as implemented in STRUCTURE HARVESTER (Earl & vonHoldt 2011) . CLUMPP (Jakobsson & Rosenberg 2007) and DISTRUCT (Rosenberg 2004) were used to visualize results for the most likely K.

***Symbiodinium* genotyping**

Symbionts were genotyped from both halves of experimental individuals using the standard *Symbiodinium* marker, the nuclear internal transcribed spacer region 2 (ITS2). I developed a deep sequencing approach based on 454 (Roche), following the

logic of (Stat et al. 2011), to more accurately quantify the relative proportions of different ITS2 genotypes within each coral fragment.

The ITS2 region was amplified via PCR using the forward primer its-dino (5' GTGAATTGCAGAACTCCGTG 3') and the reverse primer its2rev2 (5' CCTCCGCTTACTTATATGCTT 3') (Pochon et al. 2001). An aliquot of non-DNAse treated RNA, diluted to 1ng/µl was used as the initial template. Each 25 μl reaction consisted of 6 μl (=6 ng) of template, 13.34 μl of milli-Q water, 2.5 μl of ExTaq HS 10x ExTaq Buffer (Takara Biotechnology), 2 μl of 10mM dNTPs, 1 μl of a 10 μM forward and reverse primer mix, and 0.4 U ExTaq HS Polymerase (Takara Biotechnology) and 0.2 U Pfu polymerase (Agilent Technologies).

Amplifications were verified on agarose gels following 19 cycles and additional cycles were added as necessary to determine the appropriate number of amplification cycles for each sample that yielded a faint band when 3 μl of product were loaded on a 1% agarose gel and run for 15 min at 180 V (cycle numbers ranged from 19-34 across samples). These "cycle-check" PCR's were preformed on a Tetrad 2 Peltier Thermal Cycler (Bio-Rad) using the following conditions (modified from (LaJeunesse & Trench 2000): 94°C for 5 min, followed by 19 cycles of 94°C for 15s, 62-52°C for 30s (decreasing 0.5°C per cycle), and 72°C for 30s. An additional X cycles of amplification were added as necessary, at an annealing temperature of 52°C to achieve the necessary cycle number per sample and a final extension of 8 min at 72°C completed the program. Once cycle numbers were obtained, samples were amplified once more to their specific cycle number and checked on a gel together to verify equal band intensity.

Each PCR reaction was then cleaned using a PCR clean-up kit (Fermentas), measured using Nanodrop (Thermo Scientific) and diluted to 10 ng/μl. This product was then used as template in a second series of PCRs in order to incorporate 454-RAPID

primers and unique barcodes to each sample. Each PCR contained the same forward primer, Br-ITS2-F (5'-CCTATCCCCTGTGTGCCTTGAGAGACGHCGTGAATTGC AGAACTCCGTG-3') in addition to a unique reverse primer containing an 8-bp barcode for subsequent sample identification Ar-ITS2-R-16 (5'-CCATCTCATCCCTGCGTGTCTCCGACGACTTGTAGCGCCCTCCGCTTACTTAT ATGCTT-3'). Each 30 μl reaction consisted of 1ul cleaned PCR product (=10 ng), 1 μl of 10 µM uniquely barcoded reverse primer, 23 μl of milli-Q water, 3 μl of ExTaq HS 10x ExTaq Buffer (Takara), 0.7 μl of 10 mM dNTPs, 1 μl of 10 μM Br-ITS2-F, 0.75 U ExTaq HS Polymerase (Takara Biotechnology) and 0.375 U Pfu polymerase (Agilent Technologies). PCR's were preformed on the same instrument listed above using the following conditions: 94°C for 5 min, followed by 4 cycles of 94°C for 15s, 52°C for 30s, 72°C for 30s, with a final extension at 72°C for 8 min.

All samples were verified on a gel and subsequently pooled for the final 454 sequencing run based on visually assessed band intensity. The pooled sample was then cleaned via ethanol precipitation as follows: 1/10 volume 3M NaOAc and 3x volume 100% EtOH were added to the pooled sample and incubated for 30 mins at -20°C, followed by a 30 min max speed centrifugation at 4°C. Supernatant was removed and samples were washed with 500 μl of 70% EtOH followed by a 2 min max speed centrifugation at 4°C. Supernatant was removed and the pellet was dried at room temperature for 5 minutes followed by re-suspension in 25 μl milli-Q water. 3-5 μg of this cleaned product was run on a 1% Agarose gel stained with SYBR Green (Invitrogen) for 15 min at 180V. The gel was visualized on a blue-light box and the target band excised using a clean razor blade and placed in 25 μl milli-Q water for overnight incubation at 4°C. The resulting supernatant was then submitted for 454 sequencing.

ITS2 amplicons were only sequenced from samples in which they could be amplified in less than 35 PCR cycles. In total, sequences were obtained for 49 out of the 60 fragments: 12 inshore control, 12 inshore heat, 15 offshore control and 10 offshore heat. Of the 45,192 total reads, 44,393 were left after adaptor trimming, quality filtering and discarding reads shorter than 150 bp. Remaining sequences per sample ranged from 180-2,886 (mean: 854, median: 687). Pooled reads from all samples were then clustered into 100% identical groups using the program cd-hit (Weizhong & Godzik 2006) resulting in 11,395 unique sequence clusters. 48% of all the sequence reads were contained within seven clusters each comprising >200 reads (Fig. A6). These sequences were aligned using the program SeqMan (DNASTAR) to identify SNPs and the consensus alignment was used as a query to blast against the GenBank (NCBI) nucleotide collection.

These seven unique sequences were used as reference haplotypes to which I mapped all reads using the command *runMapping* from the Newbler GS Reference Mapper program v2.6 (Roche), with the repeat score threshold (-rst) parameter set to 0. Of the 44,393 trimmed sequences across samples, 39,654 (89%) were successfully mapped to one of the seven reference haplotypes. The most frequent haplotype in my reference was assigned 31% of all mapped reads, whereas the least frequent one was assigned 2% of the reads, 5-fold lower that the typical detection limit (10%) in standard *Symbiodinium* genotyping methodologies based on electrophoresis. Rarer references were not included since smaller haplotype clusters were increasingly likely to correspond to systematic errors in sequencing rather than unique, low-abundance symbiont haplotypes, and also because the physiological impact of such rare genotypes is uncertain. The number of reads assigned to each genotype within sample were divided by the total

number of reads uniquely mapped to normalize variation in absolute read number between samples.

Statistical analysis

All analyses were carried out using R 2.13.2 (R Development Core Team 2013). Differences in the physiological response variables growth, bleaching (brightness in the red channel), EQY and MQY were evaluated with respect to treatment and population of origin using a nested series of linear mixed models implemented in the lme4 package (Bates 2005). For all models, treatment and origin were modeled as fixed factors, with levels control/heat and inshore/offshore, respectively, as well as their interaction. Colony identity was included as a scalar random factor. Significance of factors was evaluated using likelihood ratio tests (LRT). A random effect of tank was also included if model fit was significantly improved with its addition, according to the LRT. The applicability of model assumptions (linearity, normality, homoscedasticity) to the data were verified using diagnostic plots of residuals formed by fitting a linear model with the fixed factors listed above, using the functions *lm()* and *plot()*. The proportions data (EQY and MQY) were arcsin square-root transformed and independent comparisons were performed at three time points: the final two days of acclimation, the three initial days of treatment and the final two days of treatment. For symbiont sequence data, frequencies of haplotypes within each sample were arcsin square-root transformed and the frequency of each haplotype with respect to colony origin and experimental treatment was evaluated independently as a response variable. Multiple test correction was subsequently applied to LRT p-values for symbiont genotype frequency models using the function *p.adjust()*, as recommended by (Benjamini & Hochberg 1995). Symbiont genotype divergence between origin, treatment and bleaching status was also explored using the entire

haplotype frequency dataset through a principal components analysis (PCA) using the labdsv package (Roberts 2008). For the purpose of this comparison, heat-treated corals were subdivided into two "bleaching status" categories based on their brightness. The lightest-colored top 50% of heat-treated coral samples were designated "bleached", while those in the bottom 50% were designated "pale". All the control samples were designated "normal".

The divergence between the seven symbiont reference haplotypes was evaluated by constructing haplotype networks using the functions *haplotype()* and *haploNet()* from the pegas package (Paradis 2010). The four top-scoring BLAST hits from NCBI's GenBank (Pruitt et al. 2012) were included in haplotype network reconstruction, in addition to one more distantly related ITS2 outgroup. Haplotypes were manually trimmed to be of identical length and gaps were coded as a single-base change such that each indel was considered equivalent to a single point mutation. In addition, I conducted pair-wise regressions for all of my symbiont haplotype frequency data to look for positive correlations suggesting that compared ITS2 sequences might represent variants within the same genome (Thornhill et al. 2007).

RESULTS

Growth and bleaching

Growth was significantly affected by treatment, with heat stressed corals gaining significantly less weight than controls irrespective of population origin ($P < 0.001$, LRT, Fig. 2.2). Graphical trends suggest a site of origin by treatment interaction, as offshore corals gained 0.4% less weight on average than inshore corals, though this interaction was not significant ($P = 0.23$, Fig. 2.2). Prior to beginning treatment, corals did not differ in their brightness (Fig. 2.3A). At the end of the six-week experimental period, however,

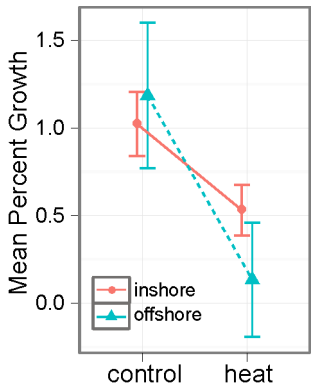


Figure 2.2. Mean percent weight gain following six weeks of experimental treatment as calculated from a buoyant weighting method (Davies 1989). Red circles: inshore population average (±SEM); blue triangles: offshore population average (±SEM) under control and elevated temperature treatment.

I observed significant differences in brightness with respect to treatment and the origin by treatment interaction. Heat treatment resulted in a major increase in brightness for both inshore and offshore corals, indicating reduced symbiont and/or chlorophyll densities (i.e. bleaching, $P<0.001$, LRT, Fig. 2.3B). In addition, offshore corals exhibited significantly higher brightness under heat treatment than inshore corals, indicating that offshore corals bleached more severely than inshore corals in response to a common thermal stress ($P<0.01$, LRT, Fig. 2.3B).

Photochemical yield of *Symbiodinium* photosynthesis

The results for both effective (EQY) and maximum quantum yield (MQY) recapitulate bleaching patterns. Prior to beginning the experimental treatment, EQY and MQY were not significantly different between population or treatment groups (Fig. 2.4A,D). After the first three days of experimental treatment, both EQY and MQY showed significant reductions under heat treatment by 7% and 11%, respectively ($P<0.001$, LRT, Fig. 2.4B,E). A significant treatment effect was also observed at the end of the experiment. Individuals under heat treatment showed a 47% reduction in EQY and a

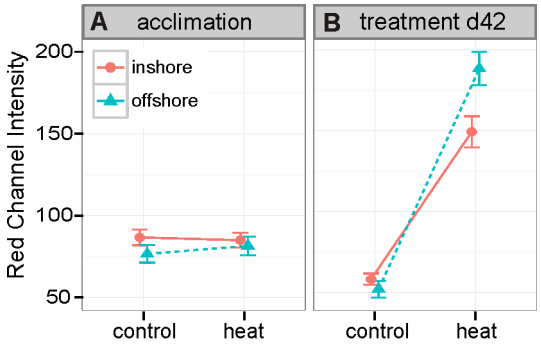


Figure 2.3. Bleaching as measured by brightness in the red channel in standardized coral photographs (A) during the acclimation period prior to beginning treatment and (B) at the end of the six-week experimental period. Red circles: inshore population average (±SEM); blue triangles: offshore population average (±SEM) under control and elevated temperature treatment.

46% reduction in MQY in comparison to their paired controls ($P < 0.001$, LRT, Fig. 2.4C,F). Final MQY measurements also differed between inshore and offshore corals, with offshore corals exhibiting slightly reduced MQY values overall in comparison to inshore corals ($P = 0.045$, LRT, Fig. 2.4F, "heat final"). MQY measurements > 0.6 can be used as an indicator of a healthy photosystem (Chalker 1983). MQY values for the duration of the experiment stayed above 0.6, except for heat-treated corals at the final time point, where inshore and offshore MQY values were 0.42 and 0.29, respectively. Final EQY measurements revealed a significant origin by treatment interaction ($P < 0.01$, LRT). Inshore and offshore controls did not differ significantly from each other, whereas under heat treatment, offshore origin individuals showed a 37% reduction in EQY, compared to inshore origin individuals (Fig. 2.4C).

To explore the correlation between symbiont photosynthesis and growth, I plotted mean percent weight gain values against the average of both EQY and MQY measurements made during the final days of the experiment. A subtle but significant positive relationship was found, where quantum yield measurements explain

approximately 12% of the variation in weight gain across individuals (adjusted $r^2 = 0.124$, $P = 0.004$, Fig. 2.5).

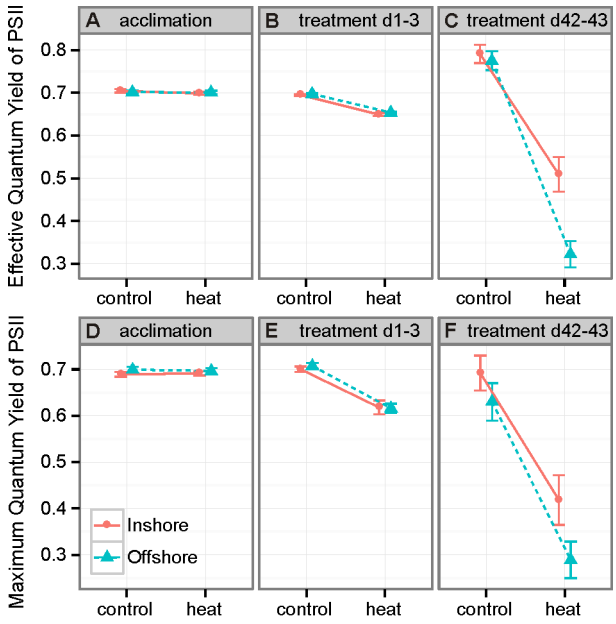


Figure 2.4. Photochemical yield of in hospite *Symbiodinium*. (A-C) Effective quantum yield and (D-F) maximum quantum yield for inshore (red circles) and offshore (blue triangles) coral fragments under control and heat treatments represent three time periods: prior to beginning the temperature treatment (A,D), during the initial 3 days of elevated temperature treatment (B,E) and at the end of the six-week temperature treatment (C,F).

Genotyping (host)

Multilocus genotyping revealed a substantial frequency of triallelic genotypes across all loci. As polyploidy was deemed unlikely (see Host Genotyping Methods), I only included individuals showing diallelic genotypes across all loci for the F_{ST} and STRUCTURE analyses. The frequencies of multilocus genotypes differed significantly between inshore and offshore origin corals for the entire dataset (n=46 inshore, n=51 offshore) as well as for the diallelic-only dataset (n=28 inshore, n=26 offshore, P<0.001,

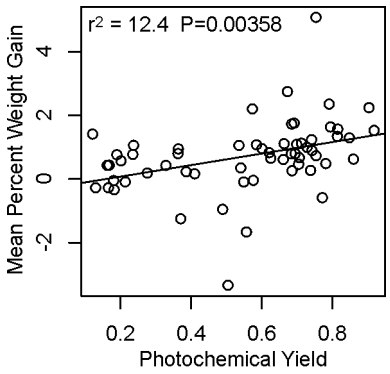


Figure 2.5. Percent weight gain as a function of symbiont photosynthetic function (mean photochemical yield measured as Chlorophyll a fluorescence) at the end of the six-week experimental treatment.

multilocus G-test). Allele frequencies, as calculated for the diallelic dataset, also differed significantly between inshore and offshore origin individuals (Summerland Key inshore:offshore $F_{ST} = 0.0402$, $P<0.05$; Sugarloaf inshore:offshore $F_{ST} = 0.0575$, $P<0.05$) as well as between the two offshore reef sites ($F_{ST} = 0.0487$, $P < 0.05$). Allele frequencies between the two inshore sites showed no significant differentiation ($F_{ST} = 0.0134$, $P = 0.15$). Plots of delta-K (Evanno 2005) from STRUCTURE indicate that the most likely number of genetic clusters is 4. Visualization of STRUCTURE results reveals that inshore individuals show a higher probability of assignment to clusters not observed at offshore sites (Fig. 2.6).

Genotyping (symbiont)

All genotyped *P. astreoides* hosted *Symbiodinium* type A4/A4a, as has been reported previously for individuals of this species in the Florida Keys (Thornhill et al. 2006). Haplotype network reconstruction clusters the seven reference haplotypes with A4 and A4a sequences from GenBank (NCBI), while the more distantly related A1 (S. microadriaticum) is a clear outgroup. GenBank sequence A4.3 aligned against reference sequence 1 with 100% identity (Fig. 2.7A).

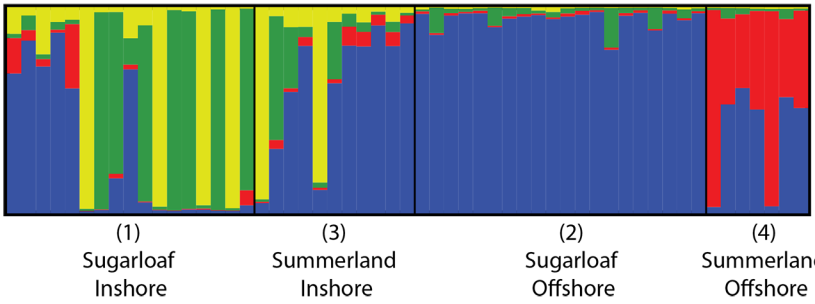


Figure 2.6. Genotypic composition of inshore and offshore *P. astreoides* populations in the lower Florida Keys based on analysis of 8 microsatellite loci using STRUCTURE (Pritchard et al. 2000). Colors correspond to distinct genotypic clusters and the proportion of colors within columns indicates the probability of an individual corals assignment to that cluster. Numbers associated with sites correspond to sampling locations indicated in Fig. 2.1.

The majority of reads were assigned to haplotypes 1, 2, 4 and 7, together comprising 89% of the mapped reads. None of these haplotypes showed enrichment with respect to origin, treatment or the interaction. Differences in symbiont genetic composition between populations were limited to two minor-frequency haplotypes. Haplotypes 3 ($P<0.05$, LRT) and 6 ($P=0.05$, LRT) together accounting for 7% of the mapped reads were significantly more represented in inshore individuals (Fig. 2.7A). A principal components analysis (PCA) suggested that the increase in representation of haplotypes 3 and 6 might result from a reduction in haplotype 1 (Fig. 2.7B,C), though this decrease was not statistically significant in the individual tests, because haplotypes 3 and 6 are the strongest positive loadings on PC1, while haplotype 1 is the strongest negative loading. Apart from this trend, there was no apparent clustering of corals for either the origin/treatment interaction (Fig. 2.7B) or bleaching status (Fig. 2.7C) with respect to total haplotype representation.

The proportion of reads mapped to haplotype 3 showed a significant positive relationship with those mapped to haplotype 6 (adjusted $r^2 = 0.41$, $P < 0.001$). ITS2 is

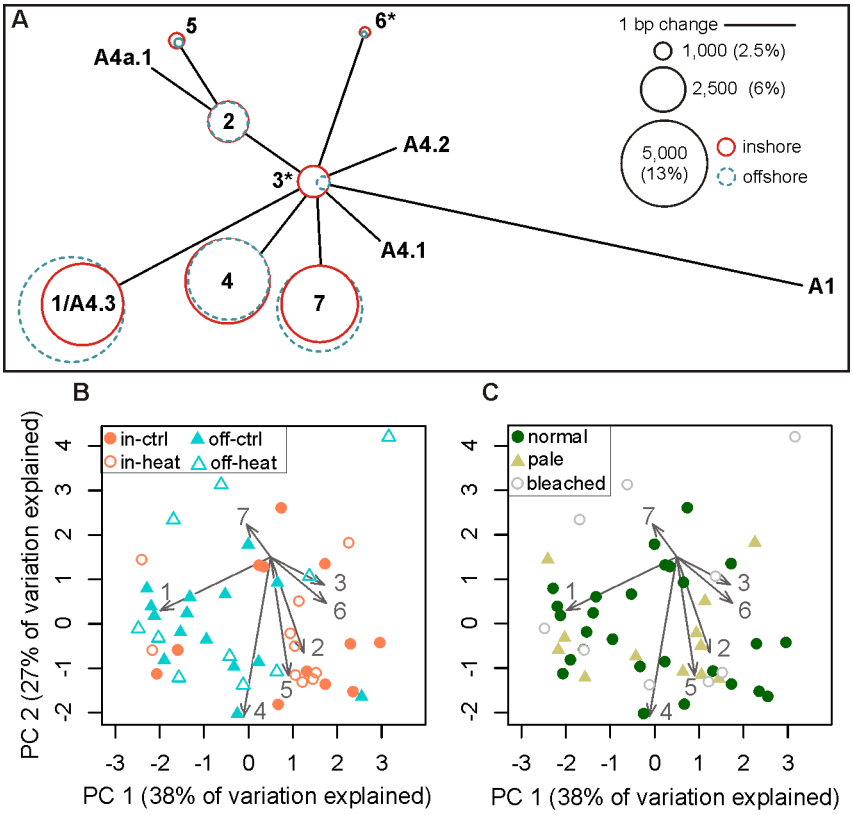


Figure 2.7. *Symbiodinium* diversity at the ITS2 locus. (A) Haplotype network built using an infinite site model for the seven major haplotypes (circles 1-7). Four additional clade A4/A4a sequences and one A1 outlier were obtained from GenBank to serve as references. Haplotype 1 and reference sequence A4.3 are identical. The linear distance between haplotypes is proportional to the number of point mutations or indels and the circle size corresponds to the proportion of mapped reads recorded from inshore corals (solid red circles) and offshore corals (dashed blue circles). Asterisks mark significant differences ($P<0.05$) between locations. (B, C) Principal components analysis of *Symbiodinium* communities as defined by relative proportions of ITS2 types. Both plots contain the same points and loading vectors, but are color-coded according to different factors. (B) Differences with respect to origin (inshore: red circles, offshore: blue triangles) and treatment (control: solid symbols, heat: open symbols). (C) Differences with respect to bleaching phenotype: normal (i.e. control, green circles), pale (tan triangles) and bleached (white circles).

multi-copy and can vary intra-genomically (Thornhill et al. 2007), therefore a positive correlation between ITS2 haplotype frequencies could indicate that they represent variants co-occurring within the same genome. To distinguish co-occurrence from enrichment of both haplotypes in inshore individuals, I ran separate regression of haplotype 3 on haplotype 6 within each population. Both showed significant positive relationships, although the correlation was stronger in inshore corals (adjusted $r^2 = 0.37$, $P = 0.001$; offshore: adjusted $r^2 = 0.26$, $P = 0.005$, Fig. A7). Relationships between the major haplotypes all show either negative or neutral relationships (Fig. A8) indicating that they likely represent different symbiont genomes.

DISCUSSION

Elevated thermotolerance of inshore *P. astreoides*

Significant differences in bleaching and symbiont photophysiology accompanied by a trend towards differential growth in inshore and offshore corals under common heat stress indicate elevated thermotolerance in inshore corals (Fig. 2.2,2.3). This could be due to local thermal adaptation but may also be the result of long-term acclimatization, the effects of which did not dissipate over the relatively short acclimation period. Coral growth and reproduction are significantly influenced by symbiont type and condition (Jones & Berkelmans 2011; Little et al. 2004; Szmant & Gassman 1989). It has been estimated that up to 95% of host energy requirements are met by symbiont-derived photosynthetically fixed carbon (Muscatine 1990), which contributes to skeletal deposition (Vago et al. 1997). Bleaching has been shown to reduce reproduction (Szmant & Gassman 1989) and growth, which can persist for months following the event (Jones & Berkelmans 2010a). The effects of heat observed at the holobiont level, (growth and bleaching,) might therefore be driven by the decrease in symbiont photosynthetic

function (Fig. 2.4). This decrease, in turn, can be attributed to higher stress susceptibility of offshore symbionts, failure of offshore hosts to mitigate the effects of heat stress on their symbionts, or a combination of the two. Below I discuss my results in light of these alternatives.

Reproductive strategy and local adaptation: general considerations

In broadcast-spawning coral species, the role of symbionts in shaping holobiont thermotolerance physiology has been more thoroughly explored. Some species are known to associate with more thermotolerant symbiont types in warmer environments (Oliver & Palumbi 2011b; van Oppen et al. 2001), and others are able to shuffle proportions of symbionts in hospite to favor more thermotolerant types in stressful conditions (Berkelmans & van Oppen 2006; Jones et al. 2008).

Given the fact that the majority of broadcast-spawning species do not receive their symbionts from parent colonies (Baird et al. 2009b; van Oppen 2004), and assuming wider connectivity ranges for coral larvae than for symbionts (Barshis et al. 2010; Baums et al. 2010; Howells et al. 2009; Kirk et al. 2009; LaJeunesse et al. 2010; Rodriguez-Lanetty et al. 2001), the driving role of symbionts in local thermal adaptation of these species is not surprising. Establishing a relationship with locally adapted symbionts should maximize holobiont fitness irrespective of the coral's own genetic background, which would allow the coral to maintain high genetic connectivity across environmental gradients while reducing the detrimental effect of gene flow on local adaptation.

Such a strategy does not seem feasible for most brooding species, like *P. astreoides*, which transmit their symbionts vertically from parent to larvae. Vertical transmission may instead potentiate long-term co-evolution of hosts and symbionts, which has been proposed previously for *P. astreoides* and other vertical transmitters

(Diekmann et al. 2003; Thornhill et al. 2006). I further propose that such a co-evolutionary scenario would result in adaptation of both symbiotic partners to the local environment as well as to each other, due to the reduced dispersal capacity typical of brooding species (Ayre & Hughes 2000; Underwood et al. 2007) that keeps successive holobiont generations within the same environmental conditions. One prediction following from this scenario is a limited flexibility in host-symbiont association; another, that host genotype should play a more prominent role in determining spatial variation in thermotolerance physiology than in broadcast-spawning species.

Genetic divergence between inshore and offshore coral hosts

I found that *P. astreoides* inhabiting reefs separated by only 7-8 km exhibit significant population genetic subdivision, consistent with previous studies of other brooding corals (Fig. 2.6, Ayre & Hughes 2000; Goodbody-Gringley et al. 2010; Maier et al. 2005; Underwood et al. 2007), which increases the potential for adaptive divergence in the coral host in response to local selection. The reduced gene flow observed in this study has three explanations that are not mutually exclusive: *P. astreoides* larvae have difficulty traversing the current systems of Hawk Channel that separate inshore and offshore reefs; larvae have an innate propensity for rapid recruitment; and/or there is selection against immigrants from different reef habitats. Empirically distinguishing between these explanations is non-trivial (Hedgecock 1986). Assuming that variation at my microsatellite loci is neutral or nearly-neutral, natural selection should not have precluded the exchange of alleles across habitats if even a small proportion of immigrants survived to reproductive age. Limited dispersal appears more likely, as brooded larvae are competent to settle within 4 hours of release (Goodbody-Gringley 2010; Stake &

Sammarco 2003) and tend to recruit locally, in some cases within 100 m of the parent colony (Underwood et al. 2007).

Similarity of *Symbiodinium* communities

The constancy of the *P. astreoides-Symbiodinium* clade A4/A4a association in the Florida Keys suggests consistent vertical transmission in this system (Thornhill et al. 2006). However, *P. astreoides* is known to host different symbiont types elsewhere throughout its range (LaJeunesse 2002b). Moreover, clade A4/A4a is by no means specific to *P. astreoides* and has been found in association with a wide variety of cnidarians (LaJeunesse 2001; LaJeunesse 2002b) as well as in a free-living state (Porto et al. 2008). This raises the possibility that *P. astreoides* might be able to associate with local sub-types of A4 found in inshore or offshore environments. My results from deep sequencing of the *Symbiodinium* ITS2 suggest otherwise (Fig. 2.7). The relative proportions of major A4-related haplotypes, together accounting for 89% of all mapped sequences, remained constant across locations. These major haplotypes likely represent different symbiont genomes, since they are predominantly negatively correlated in frequency. Both inshore and offshore corals hosted the same symbiont haplotypes at similar frequencies, indicating the lack of horizontal acquisition of novel strains. I also did not detect a change in the symbionts' proportions ("shuffling") in response to heat treatment despite pronounced paling and bleaching of the holobionts. This suggests that all haplotypes are equally susceptible to heat stress and none appears to be more heat-tolerant, at least in terms of a more dominant association with the host.

Host and symbiont roles in holobiont thermotolerance

If the predominant symbiont types are not changing in this system, where do population-level differences in holobiont fitness and symbiont photophysiology come

from? First, they might be attributable to the effect of minor *Symbiodinium* ITS2 haplotypes (3 and 6) that are significantly elevated in inshore corals. Such low-frequency *Symbiodinium* strains, together accounting for only about 7% of mapped reads, have never been demonstrated to play an important role in modulating holobiont fitness, although this possibility cannot be formally excluded. Additionally, these haplotypes do not increase in frequency as a result of heat treatment as would be expected if they conferred elevated thermotolerance to their host (Berkelmans & van Oppen 2006).

ITS2 is a single, moderately variable marker; therefore it is hardly surprising that differences in *Symbiodinium* thermotolerance can arise in different environments in isolates of identical ITS type (Howells et al. 2011). However, given that I observe multiple ITS2 haplotypes within inshore and offshore corals, it is more challenging to explain how holobiont thermotolerance varies without an accompanying change in the relative proportions of these hosted types. If ITS2 variants represent alleles segregating within a sexually reproducing panmictic population of symbionts, habitat-driven selection could act on fitness-associated loci elsewhere in the *Symbiodinium* genome without affecting the frequencies of unlinked loci such as ITS2. However, existing evidence suggesting that *Symbiodinium* reproduction in hospite is predominantly asexual (Correa & Baker 2009; van Oppen et al. 2011), arguing against this explanation. Moreover, the free-living stage in the symbionts' life cycle is likely bypassed due to the vertical mode of symbiont transmission in *P. astreoides*, so this asexual phase may last for several coral generations.

Conversely, ITS2 types could represent different non-recombining *Symbiodinium* lineages (LaJeunesse 2001, 2005; Litaker et al. 2007), but see (Correa & Baker 2009). In this case, their relative frequencies in hospite might depend more strongly on other lineage-specific traits rather than on their contributions to holobiont thermotolerance. The

evolution of higher thermotolerance could then happen within individual lineages without accompanying changes in the overall composition of the community. However, thermotolerance appears to be a key determinant of a strain's success in hospite, as evidenced by symbiont community modulation in response to temperature stress ("symbiont shuffling") at least in some coral species (Berkelmans & van Oppen 2006).

Alternatively, population level differences in holobiont performance may be attributable to divergence in host populations. Two lines of evidence support this notion: first, the coral host does show significant genetic divergence between inshore and offshore reefs; and second, host gene expression patterns suggest that inshore and offshore corals manage their energetic metabolism differently in response to thermal stress (Kenkel et al. 2013b). It must be acknowledged that the use of higher-resolution genetic markers might uncover additional variation within individual *Symbiodinium* ITS2 types (LaJeunesse & Thornhill 2011), some of it with respect to location. Even so, the lack of change in ITS2 frequency profiles between corals from different locations or during heat exposure remains a strong indication that none of these putative within-ITS2-type variants is more heat-tolerant than other *Symbiodinium* types and sub-types hosted by *P. astreoides*.

Adaptation versus acclimatization

Further work is needed to determine the mechanism underlying observed differences in holobiont thermotolerance between inshore and offshore populations. Divergent responses may be due to physiological plasticity or they could be the result of genetically-based adaptation resulting from generations of selection under different thermal regimes, aided by restricted migration between reef environments. The former implies that both inshore and offshore populations have the potential to achieve the full

range of observed thermotolerances if given enough time to acclimatize; the latter, that offshore populations might be unable to adapt to increased temperature stress without genetic input from more thermotolerant inshore populations (Sanford & Kelly 2011). Distinguishing between these scenarios is critical to understanding current population dynamics and predicting the response of populations to a rapidly changing climate.

CONCLUSIONS

My results indicate that inshore and offshore *P. astreoides* populations in the Florida Keys are either adapted or have acclimatized to local thermal conditions, with inshore corals exhibiting higher thermotolerance than offshore corals. In contrast to the typical scenario observed in broadcast-spawning coral species, this physiological divergence does not seem to be the result of differences in hosted *Symbiodinium* ITS types. Genetic divergence between coral host populations in combination with location-specific modifications to host energy metabolism (Kenkel et al. 2013b) argue in favor of a prominent role of the host in shaping holobiont thermotolerance responses in this system. Further work will aim to clarify the details of host-symbiont interactions governing these population-level differences.

CHAPTER 3: Gene expression under chronic heat stress in populations of the mustard hill coral (*Porites astreoides*) from different thermal environments

ABSTRACT

Recent evidence suggests that corals can acclimatize or adapt to local stress factors through differential regulation of their gene expression. Profiling gene expression in corals from diverse environments can elucidate the physiological processes that may be responsible for maximizing coral fitness in their natural habitat and lead to a better understanding of the coral's capacity to survive the effects of global climate change. In an accompanying paper, I show that *Porites astreoides* from thermally different reef habitats exhibit distinct physiological responses when exposed to six-weeks of chronic temperature stress in a common garden experiment. Here I describe expression profiles obtained from the same corals for a panel of 9 previously reported and 10 novel candidate stress response genes identified in a pilot RNA-seq experiment. The strongest expression change was observed in a novel candidate gene potentially involved in calcification, SLC26, a member of the solute carrier family 26 anion exchangers, which was down-regulated by 92-fold in bleached corals relative to controls. The most notable signature of divergence between coral populations was constitutive up-regulation of metabolic genes in corals from the warmer inshore location, including the gluconeogenesis enzymes pyruvate carboxylase and phosphoenolpyruvate carboxykinase and the lipid beta-oxidation enzyme acyl-CoA dehydrogenase. My observations highlight several molecular pathways that were not previously implicated in the coral stress response and

suggest that host management of energy budgets might play an adaptive role in holobiont thermotolerance.[2]

[2] Considerable portions of this chapter were published as Kenkel CD, Meyer E, Matz MV. 2013. Gene expression under chronic heat stress in populations of the mustard hill coral (*Porites astreoides*) from different thermal environments. *Molecular Ecology* 22: 4322-4334. Contributions – Conceived and designed experiments: CDK, MVM. Performed the experiments: CDK. Assembled and annotated the transcriptome: EM. Analyzed the data: CDK.

INTRODUCTION

Gene expression analyses have emerged as a powerful means of assessing variation between individuals and populations (Oleksiak et al. 2002; Whitehead & Crawford 2006b), evaluating organismal responses to both biotic and abiotic environments (Evans & Hofmann 2012; Gasch et al. 2000) and generating new hypotheses (Becker & Feijo 2007; Cui & Paules 2010). This method has proven particularly useful for nontraditional model organisms as it enables investigation of molecular pathways in ecological and evolutionary contexts without the need for traditional genetics manipulations (Aubin-Horth & Renn 2009; Ekblom & Galindo 2011; Wong & Hofmann 2010). Researchers studying reef-building corals have capitalized on this era of transcriptomics, employing gene expression analyses to improve my understanding of how these organisms respond to environmental stress (Bay et al. 2009; DeSalvo et al. 2008; Meyer et al. 2011; Polato et al. 2010; Rodriguez-Lanetty et al. 2009; Seneca et al. 2010).

Reef-building corals are obligate symbiotic organisms, consisting of a Cnidarian host and a dinoflagellate endosymbiont of the genus *Symbiodinium*. The ultimate phenotypic manifestation of a stress response in corals is the breakdown of this symbiotic relationship in the process known as coral bleaching (Glynn 1993). While bleaching can occur in response to a multitude of environmental stressors (Brown & Howard 1985), it has recently received the most attention in the context of thermal stress because of mass mortality following thermal bleaching events and the expected rise of sea surface temperatures under climate change (Brown 1997; Hoegh-Guldberg 1999). Conserved host transcriptomic responses to thermal-stress induced bleaching include up-regulation of heat shock protein (Hsp) activity and antioxidant enzymes; down-regulation of Ca2+ homeostasis and ribosomal proteins; and changes to cytoskeleton and extra-cellular

matrix proteins (Csaszar et al. 2009; DeSalvo et al. 2008; DeSalvo et al. 2010a; DeSalvo et al. 2010b; Kenkel et al. 2011a). Additional investigations into thermal stress responses in aposymbiotic juvenile life history stages also identified up-regulation of Hsp and antioxidant genes as well as cytoskeletal components, further supporting the idea of a conserved thermal stress response (Meyer et al. 2011; Meyer et al. 2009b; Polato et al. 2010; Rodriguez-Lanetty et al. 2009; Voolstra et al. 2009). In addition to these evolutionarily conserved pathways, two stress responses apparently specific to the coral lineage have been described: differential regulation of GFP-like fluorescent proteins (Bay et al. 2009; DeSalvo et al. 2008; Dove et al. 2006; Kenkel et al. 2011a; Smith-Keune & Dove 2008), and up-regulation of small cysteine-rich proteins (SCRiPs, Sunagawa et al. 2009).

Characterizing molecular changes that occur in the coral host under thermal stress is critical for understanding how that response is manifested in a symbiotic system; however, it is important to note that corals also exhibit substantial variation in bleaching responses (Marshall & Baird 2000). Some coral species are characterized as thermotolerant, such as *Porites* spp. while others, such as *Acropora* and *Pocillopora* spp., are considered more susceptible to bleaching (Gleason 1993; Glynn 1984, 1993; Hoegh-Guldberg 1999), though recent work suggests that these identities may not be fixed through evolutionary time (Guest et al. 2012; van Woesik et al. 2011). Stress responses can also vary within a species (Edmunds 1994; Jokiel & Coles 1990). Conspecifics exhibit thermal tolerance limits that vary according to the latitudinal ranges they inhabit (Hughes et al. 2003), and even neighboring individuals can display different bleaching responses to what is apparently the same thermal environment (Ogden & Wicklund 1988). Furthermore, the identity of *Symbiodinium* hosted can affect holobiont stress responses (Baker 2003; Mieog et al. 2009; van Oppen et al. 2009). However, not all of

the variation observed between individuals and species can be attributed to flexibility in the symbiotic partnership. Host-symbiont interactions can also alter the ultimate holobiont phenotype. Different host species colonized by the same symbiont type can develop different holobiont thermotolerance physiologies (Abrego et al. 2008), suggesting a host role that warrants further study (Baird et al. 2009a). Identifying transcriptomic variation between individuals and populations may help shed light on how host responses can affect the variation seen in holobiont thermal stress response phenotypes.

While considerable progress has been made in characterizing the molecular response to thermal stress in coral hosts, transcriptomic variation potentially underlying phenotypic variation observed among coral populations has not received much attention, but see (Bay et al. 2009; Morgan et al. 2005). To my knowledge, only two studies exploring population level expression variation in corals in response to thermal stress have been published. The first was undertaken in an aposymbiotic larval system (Polato et al. 2010). The authors compared gene expression responses to thermal stress between *Montastraea faveolata* larvae reared in either Florida or Mexico, obtained by cross-fertilizing gametes from 3-4 local adult corals (Polato et al. 2010). Early expression patterns in the larvae largely varied by site, with differential regulation of stress response and metabolic genes; though the later differences were primarily driven by temperature treatment (Polato et al. 2010). Given that adult *M. faveolata* are known to host a diversity of symbiont genotypes, sometimes within a single host coral, and that the identity of the symbiont can potentially influence expression patterns of the host (DeSalvo et al. 2010a), it is prudent to examine host expression in this species in an aposymbiotic state. However, it prevents identification of responses that may arise during the process of bleaching. Most recently, Barshis et al. (2013) used global gene expression profiling to

evaluate molecular responses to short-term temperature stress in adult symbiotic corals from tidal pools that experience different variability in daily temperature regimes. Previous work showed that adult corals from more thermally variable pools exhibit greater thermotolerance (Oliver & Palumbi 2011a). Barshis et al. (2013) found that these thermotolerant corals also exhibit constitutively elevated expression of stress response genes such as Hsps, antioxidant enzymes, apoptosis and tumor suppression factors, and innate immune components. They suggested that this mechanism of "frontloading" transcription might facilitate increased thermotolerance in reef-building corals.

I examined transcriptomic responses to long-term thermal stress in adult populations of the mustard hill coral, *Porites astreoides*, from thermally distinct reef habitats in the Florida Keys. In chapter 2, I showed that inshore-origin *P. astreoides* holobionts (the combination of host and symbiont) are more heat-tolerant than conspecifics from an offshore reef. Over the course of six-week heat stress experiment inshore corals maintained elevated symbiont photosynthetic function, bleached less, and tended to grow better. These results indicate the presence of local thermal acclimatization or adaptation, which is likely induced by more frequent temperature extremes experienced at inshore reefs. In highly dispersive broadcast-spawning coral species that acquire their symbionts upon recruitment to the local environment, such location-specific thermotolerances can often be attributed to differences in the acquired symbiont strains (Fabricius et al. 2004; Ulstrup & van Oppen 2003; van Oppen et al. 2001). However, variation in symbiont ITS types were not observed for *P. astreoides* populations used in the present experiment (Kenkel et al. 2013a). Instead, coral host populations showed significant genetic divergence at neutral loci between locations (Fig. 3.1), which led to the hypothesis that location-specific thermotolerances in *P. astreoides* may be due to divergence in host-specific traits (Kenkel et al. 2013a). The present chapter explores this

hypothesis by analyzing expression of a number of host genes implicated in stress response, energy metabolism, growth, and calcification, in corals from the same two-population six-week heat stress experiment.

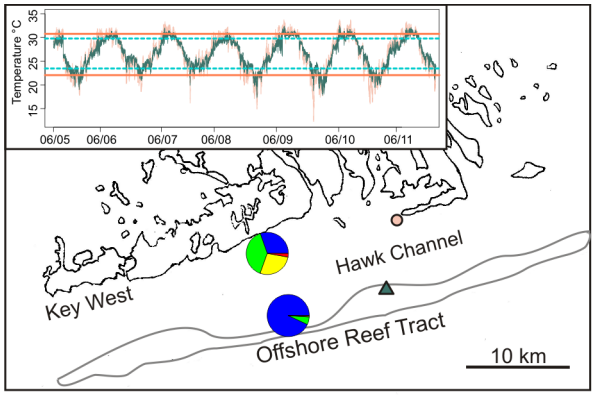


Figure 3.1. Map of the lower Florida Keys showing locations of the two sampled coral populations represented as pie charts illustrating genetic structure for the coral host, after (Kenkel et al. 2013a). Offshore reef tract shown in gray. Inset shows hourly temperature data for a representative inshore and offshore site, marked by the red circle and blue triangle, respectively. Upper and lower solid lines indicate mean June-Aug and Dec-Feb temperatures for the inshore site, while dashed lines indicate means for the offshore site.

METHODS

Heat stress experiment

The experiment is described in detail in chapter 2. Briefly, fifteen colonies of *Porites astreoides* were collected from an inshore patch reef (N 24°35.142, W 81°34.957) and fifteen from an offshore reef (N 24°31.303, W 81°34.605). Colonies were halved and allowed to acclimate in a shaded raceway for 10 days. One half of each colony was then subjected to heat stress conditions while the other half was left at ambient temperature conditions. The experiment continued for six weeks. On average, temperatures were 27.2°C (± 0.43°C) in the control tanks and 30.9°C (± 1.1°C) in the heated tanks. By the

end of the experiment, all coral fragments in the stress treatment (especially those of offshore-origin) paled or completely bleached and exhibited significantly diminished growth and symbiont photosystem function, while control fragments remained healthy (Kenkel et al. 2013a). Tissue samples for RNA isolation were removed from the skeleton using a razor blade, snap-frozen in liquid nitrogen at the end of the six-week temperature treatment and stored at -20°C for 3 days followed by storage at -80°C until extraction. Total RNA was extracted using an RNAqeous kit (Ambion). RNA quality was assessed through gel electrophoresis and evaluated based on the presence of the ribosomal RNA bands. Of the 60 original paired samples, 11 inshore/control, 11 inshore/heat, 12 offshore/control and 12 offshore/heat samples were of sufficient quality for gene expression analysis.

***Porites astreoides* transcriptome**

To facilitate gene expression profiling in *P. astreoides* I constructed a reference transcriptome assembly by sequencing (454 Titanium) cDNA prepared from a single colony unrelated to those used in the stress experiments. Library preparation, assembly, and annotation were conducted as previously described (Meyer et al. 2009a). Because *P. astreoides* transmits symbionts vertically, and therefore does not naturally occur in an aposymbiotic state, some fraction of cDNA sequences was probably derived from the symbionts. To screen for these contaminants, assembled sequences were compared against the *Symbiodinium* clade A transcriptome (Bayer et al. 2012) using BLASTN, and sequences with e-values 10-5 or lower (2,915 total) were excluded. To verify the completeness of the assembly, sequences were further classified by pathway using the KEGG KAAS tool (http://www.genome.jp/tools/kaas/) and the representation of annotated metabolic pathways was compared graphically to the metabolic map inferred

from the complete genome sequence of the sea anemone *Nematostella vectensis* using iPath2 (Letunic et al. 2008), http://pathways.embl.de/ , Fig. A9). The annotated transcriptome data was released for unrestricted use prior to this publication (http://www.bio.utexas.edu/research/matz_lab/matzlab/Data.html).

Gene expression profiling

Expression of candidate stress response genes was measured using qRT-PCR assays designed using Primer3 (Rozen & Skaletsky 2000) based on the annotated transcriptome assembly. Nine genes regulated in response to short-term heat stress (24-48 hrs) have been described previously (Kenkel et al. 2011a). Ten additional genes were chosen based on a preliminary RNA-seq experiment using five stress/control paired samples in inshore and offshore corals. The fragments used for RNA-seq came from the same common garden experiment but were different from those used in the qRT-PCR part of this study (Table 3.1). The identified candidate genes were considered long-term stress response candidates given the six-week duration of treatment used in this study. Although RNA-seq data were unsuitable for rigorous systems-level analysis due to high sampling variance most likely stemming from the insufficient amount of RNA that was available (300 ng of total RNA per sample on average), they were useful for identifying candidate genes for further in-depth validation. The unpublished RNA-seq data are available on DRYAD (doi:10.5061/dryad.1kn38). RNA samples for qPCR were DNAse treated and reverse transcribed into cDNA as in (Kenkel et al. 2011a). qPCR reactions were performed in the Roche LightCycler 480 (Roche) in 15 µl volumes using 2x LightCycler 480 SYBR Green I Master Mix (Roche), 0.1 μM forward and reverse primer and 1 ng of cDNA template. For amplification detection, SYBR Green 1 Nucleic Acid Gel Stain (Invitrogen) was diluted 200-fold and this dilution was used as a 100x

Table 3.1. Novel long-term stress candidate genes suggested from the pilot RNA-seq dataset of the six-week temperature stress experiment described in this study. Short-term stress (24-48 hrs) candidate genes were previously described in (Kenkel et al. 2011a). Abbreviations as listed in text, oligonucleotide sequences, target amplicon sizes and primer efficiencies as used for qPCR analyses and efficiency correction of raw Cp values for the double-gene assays.

Gene Name	Abbreviation	Primer Sequence (5'-3')	Size (bp)	Efficiency
Acyl-CoA dehydrogenase	ACAD	F: ATGGCCATATCAGCCAACAT R: GCATTAGAAGAGGCGACCAA	101	1.96
Alanine-glyoxylate aminotransferase	AGXT	F: GTGACATGCCTTGACGACAc R: GAAGAGCCGATCAAGATTGC	132	2.04
Apolipophorin	ALP	F: ATTTGGTGGCCTTGTACTCG R: GAAGAGCATTAGCGCCAAAC	116	2.10
Carbonic anhydrase	CA	F: AAAaCAGGCCAGTGGTCATC R: ATCGTCCACCATTGGAACTC	138	2.14
Collagen type V1 alpha 1		F: TCACCTTCTGCTCCCTCAGT R: CAAGACGGGTTTAAGGGtGA	137	1.98
Exocyst complex component 4	EXOC4	F: CTCCagTCCATCCATCCAGT R: TCCACAAGAATTGCAGCATC	123	2.03
Malate synthase		F: TCTGGCAAAAcaCAgTCAGG R: GCtgCAGTGAACATGGAAGA	131	2.00
Phosphoenolpyruvate carboxykinase	PEPCK	F: CTTTCGCAGGGATTCACATT R: CAGGCACCATCAACACTGAC	104	1.93
Pyruvate carboxylase	PC	F: TGccGCTCCAAAGTCTtAGT R: TTGAACATCAAGGTGCTTGC	131	1.95
Solute carrier family 26 member 6	SLC26	F: TCTAGTTTGGCTGCGTCCTT R: ATTTGTCTGATGGTGGCACA	149	2.06

concentrate, using 1x in each reaction. Preliminary analyses (Cp calling and melting curve analysis) were performed using the GeneScan software (Roche). Each cDNA sample was assayed in duplicate in independent qPCR runs and these technical replicates were averaged.

Double-gene assays

A double-gene qRT-PCR assay, as introduced by (Kenkel et al. 2011a), measures the fold-difference in abundances of two indicator genes exhibiting opposite responses to the factor being measured. The assay does not require any control genes since the template loading factor (sample-specific deviation due to template amount and quality) cancels out when computing the difference in expression of two genes from the same sample (Livak & Schmittgen 2001). The correction for amplification efficiency (Pfaffl 2001) is performed prior to this computation using the formula Ca = - Cp * log2(E) (Kenkel et al. 2011a), where Ca is the corrected value corresponding to log2 of the starting target amount, Cp is the raw expression measurement, and E is the amplification factor per PCR cycle measured using a dilution series as in (Pfaffl 2001). The anti-correlated responses of the two indicator genes also serve to increase the power and the dynamic range of the assay, improving detection capability (Kenkel et al. 2011a). I evaluated two pairs of genes that showed opposing expression patterns with respect to bleaching phenotype. Exocyst complex component 4 and a ubiquitin-like protein showed trends of up and down-regulation, respectively, in pale samples. Their difference values are referred to as the bleaching-in-progress double-gene assay. Phosphoenolpyruvate carboxykinase and a member of solute carrier family 26 showed respective patterns of up and down-regulation in bleached samples, and their difference values are referred to as the long-term stress double-gene assay.

Statistical Analysis

The analysis followed the procedures outlined in (Kenkel et al. 2011a). Briefly, after initial correction of Cp values for amplification efficiency and normalization using three control genes (ND5, RPL11, and EIF3H, (Kenkel et al. 2011a), the data were analyzed using linear mixed models on a gene-by-gene basis. Bleaching status, origin, and their interaction were modeled as fixed factors. Bleaching status had three levels (normal, pale, or bleached), assigned based on coral fragment brightness in the red channel of intensity-normalized photographs (Winters et al. 2009). The distribution of coral samples among bleaching status categories (Fig. 3.2) reflected the result presented in chapter 2: offshore corals were more susceptible to heat-induced bleaching than

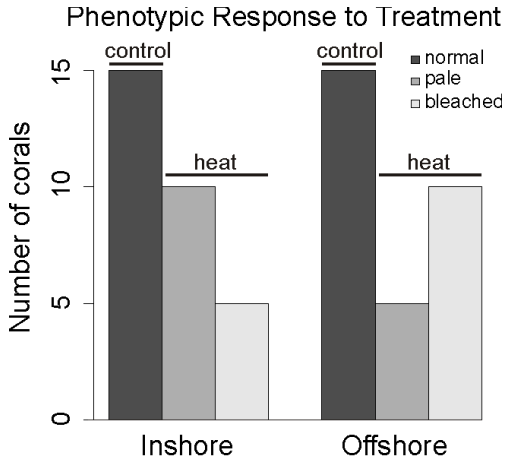


Figure 3.2. Bleaching phenotypes at the end of the six-week experimental period. All control fragments were designated "normal". Heat-treated fragments were divided into "pale" and "bleached" categories based on coral fragment brightness in the red channel of intensity-normalized photographs. Of these 60 samples, 22 inshore (11 normal, 6 pale, 5 bleached) and 24 offshore (12 normal, 5 pale, 7 bleached) yielded a sufficient quality and quantity of RNA for qPCR analyses.

inshore corals. Of the samples analyzed for gene expression, heat-treated fragments were classified as either "bleached" (top 50% brightness quantile, n = 12) or "pale" (bottom

50% brightness quantile, n = 11, Fig. A10). All fragments that were maintained under control conditions were classified as "normal" (n = 23). The origin factor had two levels, "inshore" (n= 22) and "offshore" (n = 24). The number of biological replicates for each combination of factors ranged from 5 ("bleached-inshore") to 12 ("normal-offshore"). In all models, colony identity was included as a scalar random factor. An additional random effect of tank was included if model fit was significantly improved with its addition (there were 10 heat-treated and 10 control tanks, to which the fragments were assigned randomly, n=3 fragments per tank). Significance of fixed and random factors was evaluated using likelihood ratio tests (LRT). If the effect of bleaching status was found to be significant at the $P < 0.1$ level following false discovery rate correction using the method of (Benjamini & Hochberg 1995), a post-hoc Tukey's test was used to evaluate the significance of each pair-wise comparison using the function *ghlt()* of the multcomp package (Bretz et al. 2010). All statistical analyses were carried out using R software (R Development Core Team 2013).

RESULTS

P. astreoides transcriptome

Sequencing yielded 291,044 total reads, of which 258,482 were assembled into contigs comprising 31,663 isogroups (an isogroup is a collection of sequences originating from the same gene). Most isogroups (30,393) included a single contig. The total length of the assembly, not including singletons, was 16.3 million bases (Mb). Large contigs (≥500 bp) accounted for more than half (54%) of the assembly; the average length of large contigs was 840 bp, and 90.3% of their consensus quality scores were at least 40 (i.e., an error rate of less than one in 10,000 bases). Symbiont-derived isogroups (2,915

total) were excluded from further analysis based on a blast match to the *Symbiodinium* clade A transcriptome (Bayer et al. 2012).

Pathway annotation of this dataset using KEGG KAAS tool resulted in 3,378 unique annotations, of which 2,156 could be mapped to KEGG reference pathways. Visualization using iPath2 revealed that these account for the overwhelming majority of core metabolic functions that are represented in a fully-sequenced genome of the sea anemone *Nematostella vectensis*, the only other representative of class Anthozoa currently available in the KEGG database (Fig. A9). This suggests the transcriptome produced in this study provides a relatively complete reference for analysis of metabolic changes in the coral host.

The *P. astreoides* transcriptome contained two enzymes involved in the glyoxylate cycle: isocitrate lyase (IL) and malate synthase (MS). This pathway, which is present in prokaryotes, protists, plants and fungi (but was secondarily lost in most multicellular animals (Kondrashov et al. 2006), allows for synthesis of carbohydrates from the products of lipid beta-oxidation (Voet & Voet 2004). Expression of these genes has been reported in aposymbiotic coral larvae of *Acropora millepora* (Meyer et al. 2009a) as well as in adult and larval *Acropora palmata* in response to thermal stress (DeSalvo et al. 2010b; Polato et al. 2013), in addition to being present in the *Acropora digitifera* genome (Shinzato et al. 2011). IL and MS activity is considered a signature of the glyoxylate cycle as these enzymes are specific to this pathway and essential for its function (Voet & Voet 2004). Expression of these genes in two families of scleractinian corals suggests that these animals have retained this functional pathway.

Responses of individual genes

Of the nine candidate genes chosen based on previous studies of short-term thermal stress in *P. astreoides* (24-48 hrs, Kenkel et al. 2011a), two showed significant down-regulation under heat treatment, irrespective of bleaching status: adenosine kinase (ADK, $P < 0.05$, LRT) and Hsp90 ($P < 0.001$, LRT, Fig. 3.3). Samples that paled under heat treatment exhibited 2-fold reduced expression of ADK ($P < 0.05$, Tukey's HSD) and a 2.4-fold reduction in Hsp90 ($P < 0.001$, Tukey's HSD). Samples that bleached reduced expression of ADK by 2.1-fold ($P < 0.01$, Tukey's HSD) and Hsp90 by 1.6-fold ($P < 0.01$, Tukey's HSD). Expression of these two genes did not differ significantly between pale and bleached samples (Fig. 3.3). No differences were observed with respect to origin or the status by origin interaction for these short-term stress candidates.

The ten long-term stress candidates selected from the exploratory RNA-seq analysis exhibited differential regulation with respect to both origin and treatment when tested in remaining experimental samples using qPCR. Three candidate genes exhibited differential regulation in response to heat treatment that varied with respect to bleaching status of the sample. Expression of a carbonic anhydrase decreased under heat stress ($P < 0.05$, LRT) with a down-regulation of 5.3-fold on average ($P < 0.05$, Tukey's HSD) in bleached samples relative to controls ("normal", Fig. 3.3). This result appears to be primarily driven by expression in offshore samples, though no significant interaction terms were detected for any of these long-term stress candidates. Phosphoenolpyruvate carboxykinase (PEPCK) showed a pattern of up-regulation, with a 4.8-fold difference in expression between control and bleached samples ($P < 0.01$, Tukey's HSD, Fig. 3.3). A member of solute carrier family 26 (SLC26) showed the strongest response of any gene. Pale samples were distinguishable from controls by an 8.5-fold down-regulation ($P < 0.01$, Tukey's HSD, Fig. 3.3). Bleached samples were differentiated form pale samples

by an additional 10.9-fold down-regulation ($P < 0.01$, Tukey's HSD). The full extent of SLC26 down-regulation in bleached relative to normal samples was 92-fold ($P < 0.001$, Tukey's HSD).

Three genes showed significant up-regulation in inshore origin samples in comparison to offshore origin samples: collagen type IV by 2.3-fold ($P < 0.1$, LRT), pyruvate carboxylase by 1.4-fold ($P < 0.1$, LRT) and acyl-CoA dehydrogenase by 4.9-fold (ACAD, $P < 0.05$, LRT, Fig. 3.3). Five candidate genes were selected based on their putative involvement in lipid oxidation and gluconeogenesis: ACAD, malate synthase, alanine-glyoxylate aminotransferase (AGXT), pyruvate carboxylase and PEPCK. Notably, all of these metabolic genes, except for malate synthase, showed a trend of elevated expression in inshore samples ($P < 0.05$ prior to FDR-correction), even under control conditions ("normal", Fig. 3.3). An additional metabolism-related trend in the same direction is demonstrated by apolipophorin (ALP), which was also expressed at a higher level in inshore corals under control conditions (Fig. 3.3). However, it is important to note that none of the long-term stress candidates exhibited significant differences with respect to the origin by bleaching status interaction.

Diagnostic double-gene assays

The inverse regulation observed for some pairs of candidates suggested that these genes might be used in diagnostic double-gene assays indicative of chronic thermal stress. I previously described such an assay to measure acute stress in *P. astreoides* based on the consistent up-regulation of Hsp16 and down-regulation of actin (Kenkel et al. 2011a). For a bleaching-in-progress assay, I selected the ubiquitin-like protein and exocyst complex component 4, as these genes showed a trend of anti-correlated regulation only in pale samples. The large dynamic range of SLC26 renders it an ideal

candidate for a diagnostic assay for long-term stress. As its partner, I selected PEPCK, as it was the only gene in my panel significantly up-regulated in bleached samples. Both double-gene assays were able to discriminate between samples of different bleaching status (Fig. 3.4). For the bleaching-in-progress assay, pale samples showed an

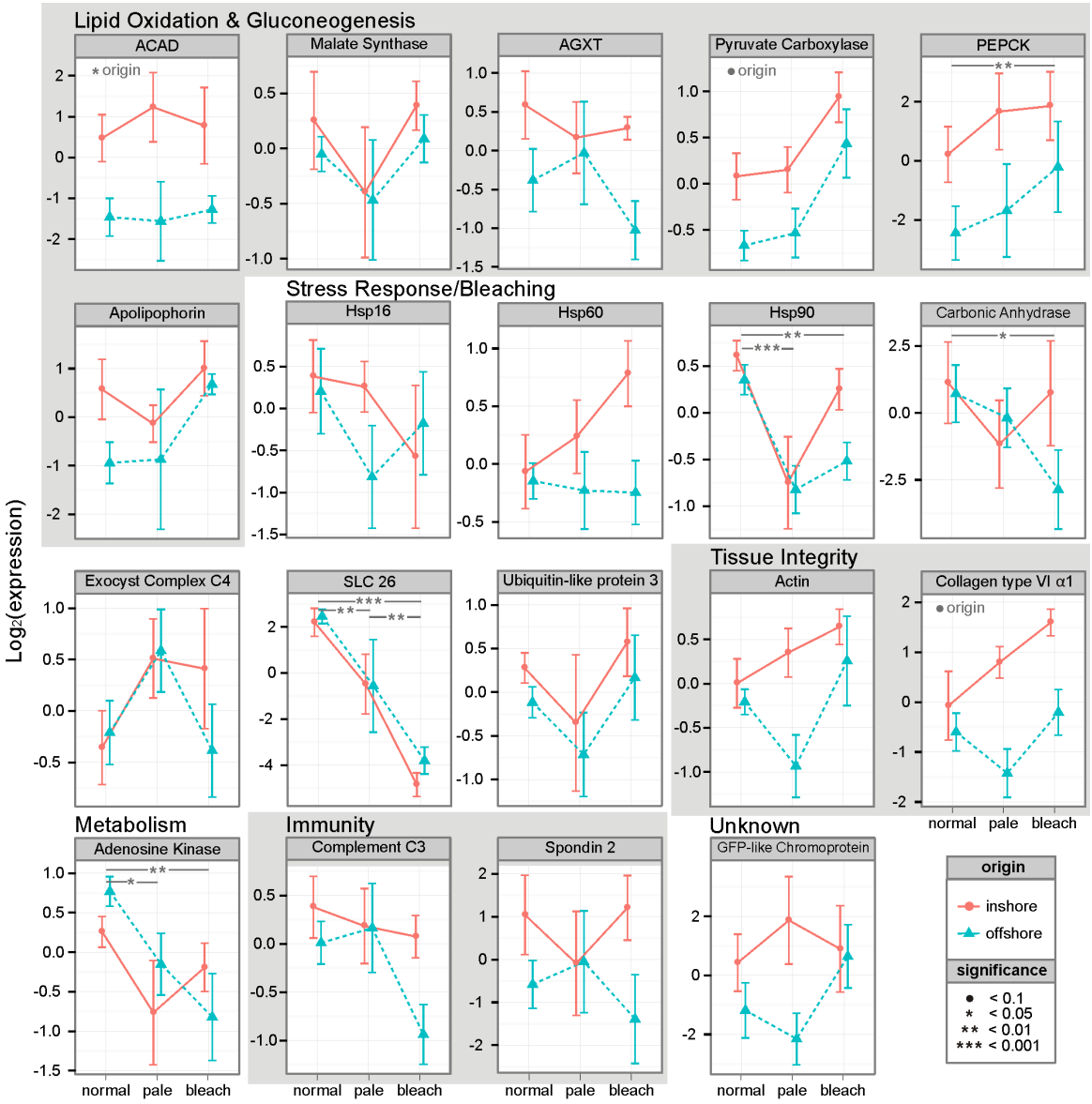


Figure 3.3. Gene expression in response to chronic heat stress in populations of *Porites astreoides* from different thermal environments. Normalized $\log_2$ transformed expression values (± SEM) of candidate genes with respect to origin (red circles = inshore, blue triangles = offshore) and bleaching status. Significance of post-hoc Tukey's HSD comparisons between bleaching phenotypes is shown for genes with $P<0.1$ after false discovery rate correction (Benjamini & Hochberg 1995). Candidates with significant origin terms following FDR correction have an "origin" designation in the panel. ACAD: Acyl-CoA dehydrogenase; AGXT: Alanine-glyoxylate aminotransferase; PEPCK: Phosphoenolpyruvate carboxykinase; Hsp: Heat shock protein; SLC26: member of solute carrier family 26.

approximately 3.6-fold increase in the assay's response in comparison to controls and a 4.1-fold increase in comparison to bleached corals (Fig. 3.4). In the long-term stress assay, pale samples were also differentiated from controls by a 13.5-fold increase in response. Bleached samples showed an additional 31-fold increase relative to pale samples and were distinguishable from controls by a 415-fold increase (Fig. 3.4).

DISCUSSION

Coral condition had the strongest effect on gene expression patterns reported in this study. In addition, accounting for phenotypic variation of corals (pale or bleached) in response to heat treatment revealed patterns of expression that would have been masked by comparing treatment levels alone (Fig. A11), providing additional insight into differential regulation during the bleaching process. I also observed significant differences in expression between inshore and offshore coral populations, suggesting that these populations have developed differential responses to thermal stress, likely because of differing temperature regimes that occur at these reef sites. Below, I discuss patterns of gene regulation in the context of candidate gene function, and relate these functions to coral phenotype and population-level differentiation.

Direct effects of heat stress

Two genes, Hsp90 and adenosine kinase (ADK) showed significant down-regulation in both pale and bleached individuals relative to controls, suggesting that they are directly modulated by thermal stress rather than by bleaching status. Though Hsp90 is a chaperone involved in maintenance of protein structure under stress, it is costly to sustain elevated Hsp expression under chronic stress (Sørensen et al. 2003). Its down-

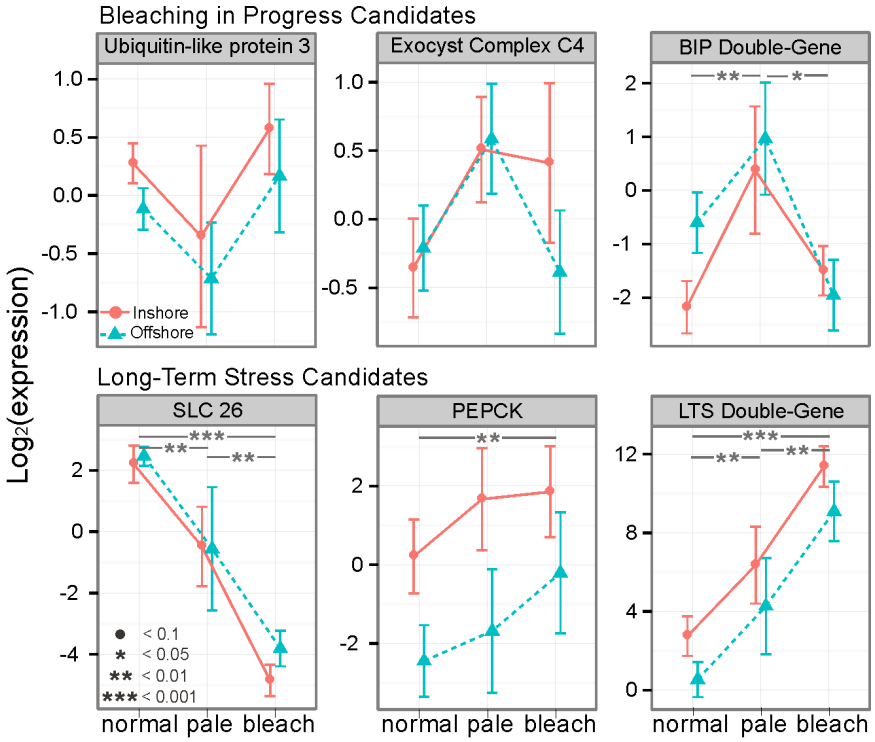


Figure 3.4. Development of diagnostic double-gene assays indicative of bleaching-in-progress (top row) and long-term stress (bottom row). The first two panels in each row show $\log_2$ transformed normalized expression values (± SEM) of candidate indicator genes with respect to origin (red circles = inshore, blue triangles = offshore) and bleaching status. The response of a double-gene assay (last panel in each row) is the $\log_2$-transformed fold-difference in expression between the two indicator genes, which can be computed without the use of additional control genes for normalization.

regulation under long-term heat stress has been observed in aposymbiotic larvae (Meyer et al. 2011) but to my knowledge, this is the first report of a similar pattern of down-regulation of Hsp90 in an adult coral. However, another chaperone, Hsp70 did show a similar pattern of down-regulation in bleached individuals of Goniopora djiboutiensis following long-term heat stress (Sharp et al. 1997). The function of ADK supports transmethylation reactions involving s-adenosine-methionine: ADK removes free adenosine, the inhibitory by-product of these reactions (Boison et al. 2002; Moffatt et al. 2002). Methylation of histones facilitates chromatin remodeling during cellular differentiation (reviewed in (Mohn & Schübeler 2009), and indeed methylation appears to play a role in the control of metamorphosis and pattern formation in hydrozoa (Berking 1986). If this is the case, ADK could be a marker of new polyp formation associated with normal growth. Therefore its down-regulation could indicate suppression of host growth under heat stress, though further research is necessary to ascertain the function of this enzyme in corals.

Calcification

A carbonic anhydrase (CA) showed significant down-regulation only in bleached individuals relative to controls. A member of solute carrier family 26 (SLC26) showed the most significant down-regulation both in pale and bleached corals, with pale individuals being intermediate between bleached and control samples. These patterns suggest that these genes do not respond to heat stress directly, but are regulated in response to symbiont loss during bleaching. Their down-regulation most likely represents a signature of decreased calcification. CAs catalyze the reversible hydration of carbon dioxide into bicarbonate and are known to be directly involved in skeletal deposition in scleractinian corals (Moya et al. 2008). Bleaching incurs substantial metabolic costs in

corals due to the loss of symbiont-derived nutrition (Muscatine 1990). Down-regulation of calcification could reflect this metabolic deficit, as less energy is available to support calcification. Alternatively, slowing down bicarbonate production may be a means of concentrating CO2 to support photosynthesis by the symbionts (Weis et al. 1989). Whatever the proximate cause, CAs are down-regulated in both adult corals (Edge et al. 2005) and aposymbiotic larvae (Meyer et al. 2011) in response to temperature stress. Since growth was depressed in experimentally heat-treated individuals (Kenkel et al. 2013a) and most pronounced in fully bleached individuals (data not shown), I hypothesize that expression of CA is reflective of reduced energetic input into skeletal growth.

Strong down-regulation of SLC26 in pale and bleached corals lends further support to this conjecture. Like the carbonic anhydrase family, SLC26 isoforms constitute a large, conserved family of anion exchangers, mediating transport of chloride for bicarbonate, hydroxyl, sulfate, formate, iodide or oxalate with variable specificity (Soleimani & Xu 2006). The best match for the SLC26 used in this study is SLC26-A6 (UniProt, Q0VA12), which functions as a bicarbonate:chloride exchanger in vertebrates (Gray 2004). Notably, the existence of such an exchanger has been postulated in pharmacological studies of coral calcification (Allemand & Grillo 1992), with the role of excreting metabolically generated CO2 (converted into bicarbonate by CA activity) to the outside of the host cells to make it available for calcification. If SLC26 plays this role, its down-regulation could be linked to a reduced production of metabolic CO2 during bleaching, and/or a reduced supply of CA-generated bicarbonate. In contrast to other metabolic enzymes discussed below, I did not detect constitutive differences in the expression of CA or SLC26 between inshore and offshore populations.

Signatures of bleaching-in-progress

The combined expression patterns of a ubiquitin-like protein (UBL) and exocyst complex component 4 (EXOC4) showed a pattern of differential regulation only in pale individuals. UBLs are part of the eukaryotic ubiquitin family of proteins that are involved in post-translational modification of macromolecules (van der Veen & Ploegh 2012). There are almost 20 members in this protein family and they are involved in a wide range of cellular processes, the most well known being proteasomal degradation. However, ubiquitination does not always lead to degradation; additional roles for this family have been described in endocytosis, cell signaling, membrane-protein trafficking and DNA repair (Hochstrasser 2009). The UBL in this study most closely resembles a membrane-anchored ubiquitin-fold protein, UBL3 (UniProt, Q7ZXN0). Such proteins have been shown to move specific components of the ubiquitination system to the plasma membrane (Dowil et al. 2011). Their down-regulation in pale individuals might therefore be indicative of a change in the plasma membrane maintenance associated with expulsion of the symbionts or membrane damage from oxidative stress. Conversely, EXOC4 showed a trend of up-regulation in pale individuals. EXOC4 is part of the multiprotein exocyst complex, which is essential for targeting exocytic vesicles to specific docking sites on the plasma membrane (Terbush et al. 1996) and therefore has an even more direct logical connection to the process of symbionts' expulsion. Differential expression of exocytosis factors has been previously observed during light-induced bleaching in *Acropora* microphthalma (Starcevic et al. 2010).

Structural proteins

Collagen, the main component of the extracellular matrix, and actin, a major cytoskeleton protein, demonstrated very similar regulation patterns: they were both up-regulated in inshore corals. In addition, they both have a tendency towards elevated

expression in bleached corals from both reef sites, though this trend was not statistically significant. Their up-regulation during stress is generally unexpected: actin is substantially down-regulated under short-term thermal stress in this species (Kenkel et al. 2011a), and collagen genes have been previously shown to be down-regulated during UV-induced bleaching in sea anemones (Moya et al. 2012). Although the trends of up-regulation of these two structural proteins are in contrast to previous reports, divergence of these responses in corals from different populations warrants their future study.

Up-regulation of gluconeogenesis in bleached corals

The breakdown of symbiosis can incur substantial metabolic costs (Muscatine 1990). Corals in this experiment were subject to six weeks of elevated temperature stress in 20 micron filtered seawater without supplemental feeding. In addition, the first signs of bleaching occurred after only two weeks of treatment, rendering nutritional deprivation likely, especially in fully bleached individuals.

Phosphoenolpyruvate carboxykinase (PEPCK) showed significant up-regulation only in bleached individuals, though a trend of up-regulation in pale individuals was also observed. PEPCK catalyzes the irreversible conversion of oxaloacetate to phosphoenolpyruvate and is a rate-limiting enzyme in gluconeogenesis (Pilkis & Granner 1992). Pyruvate carboxylase (PC), another gluconeogenesis enzyme, also showed a trend towards up-regulation in bleached individuals. PC catalyzes the conversion of pyruvate to oxaloacetate directly preceding the step catalyzed by PEPCK in the process of gluconeogenesis. Both PEPCK and PC are known to be up-regulated during starvation in mammals, to facilitate enhanced gluconeogenesis (Jitrapakdee & Wallace 1999; Tilghman et al. 1974). Increased expression of these genes in bleached individuals

suggests that coral hosts make up for the loss of symbiont-derived nutritional products by converting their internal energy stores to carbohydrates.

The role of the glyoxylate cycle

In contrast to most multicellular animals (Kondrashov et al. 2006), corals appear to possess a full complement of glyoxylate cycle enzymes (DeSalvo et al. 2010b; Meyer et al. 2009a; Polato et al. 2013), which is further evidenced by the presence of the two enzymes necessary to complete this pathway, isocitrate lyase and malate synthase, in the *P. astreoides* transcriptome. Malate synthase generates a four-carbon compound (malate) from a pair of two-carbon compounds (glyoxylate and acetyl-CoA), making it possible to channel the product of lipid beta-oxidation (acetyl-CoA) into the gluconeogenesis pathway (Voet & Voet 2004). This gives the organism an ability to synthesize carbohydrates from lipids, which is especially critical for corals since lipids comprise their main energetic stores (Davies 1991; Pearse & Muscatine 1971). The up-regulation of lipid beta-oxidation and regulation of the glyoxylate cycle has been previously proposed as a mechanism of nutritional stress mitigation in coral larvae (Polato et al. 2013). This result suggests that adult corals may also employ this mechanism and that the products of these reactions might be channeled towards gluconeogenesis. Glyoxylate cycle enzymes are induced by starvation in both yeast and plants (Dduntze et al. 1969); (Graham et al. 1994) however, I did not observe up-regulation of malate synthase in response to bleaching. Neither did I see an up-regulation of one of the key enzymes in beta-oxidation, acyl-CoA dehydrogenase (ACAD). If my hypothesis is correct, the rate-limiting regulatory step of the chain of events from lipid breakdown to carbohydrate synthesis must lie elsewhere, one probable candidate being PEPCK.

Metabolic differences between populations

Although the strongest gene expression responses observed in my study were with respect to bleaching condition, there were some notable differences with respect to coral origin. All of the metabolic enzymes mentioned in the previous two sections, with the exception of malate synthase, are either constitutively up-regulated or exhibit trends towards up-regulation during bleaching in inshore corals but not in offshore corals. It is tempting to speculate that this pattern is reflective of population-level differences in the utilization of symbiont products. Perhaps inshore corals assimilate symbiont-derived photosynthetic products into long-term energy stores (e.g. lipids) and generally operate on a storage budget involving higher lipid transport, lipid oxidation and gluconeogenesis. Offshore corals, on the other hand, may rely more directly on symbiont-supplied carbohydrates, glycerol, and other products. This might render offshore corals more energy efficient under benign conditions but more vulnerable to environmental perturbations that affect symbiont performance (such as heat stress).

CONCLUSIONS

In chapter 2, I show that inshore and offshore populations of *P. astreoides* exhibit different holobiont thermotolerances. In addition, coral host populations showed significant genetic differentiation between inshore and offshore reefs, while the genetic composition of resident *Symbiodinium* communities remained constant between inshore and offshore populations and showed no change in the relative frequencies of individual haplotypes ("shuffling", Berkelmans & van Oppen 2006) in response to heat stress (Kenkel et al. 2013a). Combined with the population-specific host gene expression patterns reported here, these results suggest that the coral host may play a substantial role in maximizing holobiont fitness under local conditions in *P. astreoides*. In particular, constitutive up-regulation of metabolic genes in inshore corals could be indicative of

transcriptional “frontloading”, a mechanism by which corals may elevate their thermotolerance in response to periodic stress events (Barshis et al. 2013). However, as the genetic marker currently used for symbiont genotyping does not fully reflect variation in their thermal physiology (Howells et al. 2011) I cannot exclude the possibility that symbionts also play a role in holobiont thermotolerance. Additional studies that involve high-resolution genotyping and global gene expression profiling of both symbiotic partners will help clarify the relative contribution of host and symbiont to population-level differences in the holobiont.

CHAPTER 4: Local adaptation in a Caribbean coral is associated with gene expression plasticity

ABSTRACT

Local adaptation is ubiquitous, but the molecular mechanisms giving rise to this ecological phenomenon are still largely unknown. Gene expression is the proximate mechanism linking genotype to phenotype, therefore correlation of transcriptomic responses with quantitative trait variation across populations can provide insight into the molecular basis of local adaptation. A reciprocal transplant experiment demonstrated that inshore and offshore populations of *Porites astreoides* corals in the Lower Florida Keys exhibit elevated growth, protein and lipid content in their home reef environment, consistent with local adaptation. Here I employ global gene expression profiling in conjunction with co-expression network analysis to explore the molecular basis of adaptation. I find that inshore corals exhibit greater gene expression plasticity in comparison to offshore corals. In addition, corals from the inshore reef more strongly regulate genes involved in the environmental stress response, and corals with the highest expression of these genes maintained the highest symbiont densities following transplantation. These results suggest that it is the ability to strongly regulate expression that is important for local adaptation of these populations rather than constitutive expression differences. Furthermore, this expression plasticity may underpin phenotypic robustness in response to environmental variation, reducing growth rates but elevating thermal tolerance in inshore corals.

INTRODUCTION

Populations can respond to spatial environmental variation by specializing to their local environments (Hereford 2009; Kawecki & Ebert 2004; Savolainen et al. 2013). When this specialization results in higher fitness of local individuals compared to foreign transplants, populations are considered to be locally adapted (Kawecki & Ebert 2004). Numerous examples of local adaptation across taxa have been described (Hereford 2009) but the genetic components that underpin these patterns are not well understood (Savolainen et al. 2013).

Gene expression is the proximate mechanism linking genotype to phenotype (Hodgins-Davis & Townsend 2009) and plays a central role in cellular adaptation to environmental change (Lopez-Maury et al. 2008). Expression regulation can be highly heritable (Brem & Kruglyak 2004; Schadt et al. 2003; Whitehead & Crawford 2006b) and recent work has suggested that expression patterns can evolve in response to differential selection between environments (Whitehead & Crawford 2006a). Once local adaptation has been inferred through higher order phenotypic traits it becomes possible to correlate transcriptomic responses to quantitative trait variation across populations (Meier et al. 2014) and thus identify the molecular phenotypes putatively facilitating adaptation to spatially varying environments.

This insight will be particularly critical for non-model organisms in which traditional genetics approaches aimed at identifying the molecular basis of adaptation are still unfeasible. Especially important are species of prime conservation concern, such as reef-building corals. Increasing evidence suggests that these foundation species are capable of adapting and/or acclimatizing to local environmental regimes (Barshis et al. 2013; Barshis et al. 2010; D'Croz & Mate 2004; Kenkel et al. 2013a; Ulstrup et al. 2006). As corals are obligate symbiotic organisms consisting of a Cnidarian host and

intracellular populations of photosynthetic dinoflagellates (*Symbiodinium* spp.) the physiological mechanism of adaptation can occur at the level of the symbiont, at the level of the host, or through some combination of host and symbiont factors. Coral species capable of inter-generationally flexible symbioses can associate with different symbiont types across their range (LaJeunesse 2002a; LaJeunesse et al. 2010). If symbionts are locally specialized this environment-specific association would increase local fitness, facilitating rapid adaptation (Oliver & Palumbi 2011b). In addition, corals that host mixed symbiont types are also able to shuffle symbiont populations favoring more thermally tolerant types in warmer conditions (Berkelmans & van Oppen 2006). However, not all species are capable of such flexibility in their symbiotic associations (Goulet 2006; Thornhill et al. 2006) and little work has been done to address the role of the host in adaptation to local environments (Baird et al. 2009a), particularly in species with largely inflexible symbioses, but see (Barshis et al. 2010).

In chapter 1, a reciprocal transplant experiment demonstrated that inshore and offshore populations of *Porites astreoides* corals in the Lower Florida Keys exhibit elevated growth, protein and lipid content in their home reef environment, and grow better than foreign transplants on average, consistent with local adaptation. Inshore corals from these same populations also exhibit greater thermal tolerance than corals from the offshore reef, though the dominant symbiont ITS type is identical for corals in each population (Kenkel et al. 2013a). Here, I employ global gene expression analyses using RNA-seq in conjunction with co-expression network analysis to explore the relationship between host gene expression patterns and phenotypic trait variation in the same corals used in the reciprocal transplant experiment described in chapter 1.

METHODS

Sample collection and processing

Experimental design details and methods for obtaining quantitative trait measures can be found in chapter 1. Briefly, fifteen genotypes (individual colonies) of *Porites astreoides* from both an inshore and an offshore reef in the Lower Florida Keys, USA, were fragmented and outplanted at native and foreign sites. Following one year of transplantation coral growth rates, energetic stores (total protein, lipid and carbohydrate content), symbiont densities and chlorophyll content were measured for each genotype. Sample collection was carried out under Florida Keys National Marine Sanctuary permit #2011-115. Immediately upon field collection of the transplant experiment, 1-cm^2 tissue samples were taken from each coral fragment using a razor blade and fixed in RNALater (Ambion, Life Technologies) on ice. Upon return to Mote Tropical Research Laboratory, samples were stored at -80ºC until processing. Total RNA was extracted using a slightly modified RNAqueous kit protocol (Ambion, Life Technologies), and DNAse treated as in (Kenkel et al. 2011a). RNA quality was assessed through gel electrophoresis and evaluated based on the presence of the ribosomal RNA bands. One μg of RNA per sample was prepared for tag-based RNA-seq as in (Meyer et al. 2011), with appropriate modifications for sequencing on the Illumina platform (the full protocol can be found at http://www.bio.utexas.edu/research/matz_lab/matzlab/Methods.html).

RNA-seq and bioinformatic analyses

A total of 45 libraries, prepared from each biological sample were sequenced on the Illumina HiSeq version 2500 at UT Austin's Genome Sequencing and Analysis Facility. The full bioinformatics pipeline can be found at (http://www.bio.utexas.edu/research/matz_lab/matzlab/Methods.html). Briefly, 527.9

million raw reads were generated, with individual counts ranging from 3.2 to 26.3 million per sample (median = 11.1 million reads). Of these, reads without the 5'-Illumina leader sequence were discarded, and this leader was trimmed from remaining reads. The *fastx_toolkit* (http://hannonlab.cshl.edu/fastx_toolkit) was then used to trim the reads after a homopolymer run of 'A' ≥ 8 bases was encountered, retain reads with minimum sequence length of 20 bases, and quality filter them requiring PHRED quality of at least 20 over 90% of the sequence. 2.3 to 14.6 million reads per sample (median = 7.9 million reads) remained after quality filtering. Filtered reads were mapped to the *Porites astreoides* reference transcriptome (Kenkel et al. 2013b) using the *gmapper* command of the SHRiMP package (Rumble et al. 2009), with –strata flag to return only best-scoring alignments of identical quality and –local flag to allow incomplete read alignments. Overall, 294.4 million reads were mapped for all 45 samples, with 1.9 to 11.7 million reads per sample (median = 6.1 million reads), resulting in an average mapping efficiency of 79%. Read counts were assembled by isogroup (i.e. groups of sequences putatively originating from the same gene, or with sufficiently high sequence similarity to justify the assumption that they serve the same function) using a custom perl script, which discarded any PCR duplicates, which were defined as reads mapping to the same starting position in the reference and aligning with 100% identity along the length of the shorter read. Reads mapping to multiple isogroups were disregarded. In total, 106,881 to 624,207 unique reads per sample (median=295,077 reads) were successfully mapped to 28,663 isogroups.

Differential expression, co-expression network and functional enrichment analyses

All analyses were carried out in the R statistical environment (R Development Core Team 2013). Of the 45 libraries, three were identified as outliers using the package

arrayQualityMetrics (Kauffmann et al. 2009). Count data for the remaining 42 samples were analyzed using the package DESeq (Anders & Huber 2010). Dispersion estimates of raw counts were obtained by maximizing a Cox-Reid adjusted profile likelihood of a model specifying origin and transplant environment effects for each sample and the empirical dispersion value was retained for each gene. Low-expression genes were excluded from subsequent analyses by removing isogroups with read count standard deviations in the bottom 40% quantile, which was identified as the filter statistic best satisfying the assumptions of independent filtering as implemented in the package genefilter (Gentleman et al.), leaving 17,198 highly expressed isogroups. Expression differences in these 17,198 isogroups were evaluated with respect to population origin, transplant environment and the interaction using a series of generalized linear models implemented in the function *fitNbinomGLMs*.

Variance stabilized count data for the top differentially expressed genes (DEGs, 2,664 genes, uncorrected FDR of 1%) was used to conduct a weighted gene co-expression network analysis using the package WGCNA (Langfelder & Horvath 2008, 2012). R scripts for my network construction were modeled after tutorials for undirected, weighted gene co-expression network analysis (WGCNA) (Langfelder & Horvath 2014). Construction of co-expression networks consisted of four general steps: (1) Pearson correlations for all genes across all treatments were used to construct a similarity matrix of gene expression, retaining the sign of the expression change ("signed networks"); (2) Expression similarities were transformed into connection strengths (connectivities) through a power adjacency function, using a soft thresholding power of 18 which best satisfied assumptions of a scale-free network topology (Fig. A12); (3) Linkage hierarchical clustering was coupled with a topological overlap matrix to identify groups of genes (network modules) whose expression was highly positively correlated, retaining

only modules with at least 30 genes and merging modules with 80% similar expression profiles (Fig. A13); and (4) External trait data was then related to network properties.

Functional enrichment analyses were conducted to identify over-representation of a particular functional groups within modules, based on Gene Ontology (GO) classification (Consortium 2000). For each GO term, the number of annotations assigned to genes within a module was compared to the number of annotations assigned to the rest of the dataset, to evaluate whether any ontologies were more highly represented within the module than expected by chance (Fisher's exact test, $P < 0.05$). The GO categories were hierarchically clustered based on the number of shared genes within the dataset to identify categories likely driven by the same genes.

To independently validate plasticity patterns observed in expression modules, a discriminant analysis of principal components (DAPC) was used to compare expression of the top 2,664 DEGs for corals in their native environments with expression patterns in foreign transplants, using the adegenet package (Jombart 2008; Jombart et al. 2010). A discriminant function was built by defining native transplants as groups (inshore corals transplanted to inshore reefs, and offshore corals transplanted to offshore reefs) and retaining the first four principal components in the expression dataset. Group memberships were then predicted for the transplant samples based on the DAPC results for the native populations.

RESULTS

Module-trait relationships: host expression is correlated with symbiont traits

Of 2,664 total host differentially expressed genes (DEGs), 2,428 were assigned to six co-expression modules which all showed strong correlations with the symbiont specific traits of total density, and chlorophyll a and c2 content per symbiont cell (Fig.

4.1A). Brown, green and black module eigengenes (the first principal component of a module, representative of gene expression profiles within that module) were significantly positively correlated with symbiont density, with correlation coefficients of 0.52, 0.41 and 0.35, respectively (Fig. 4.1A). To further validate these relationships, correlations

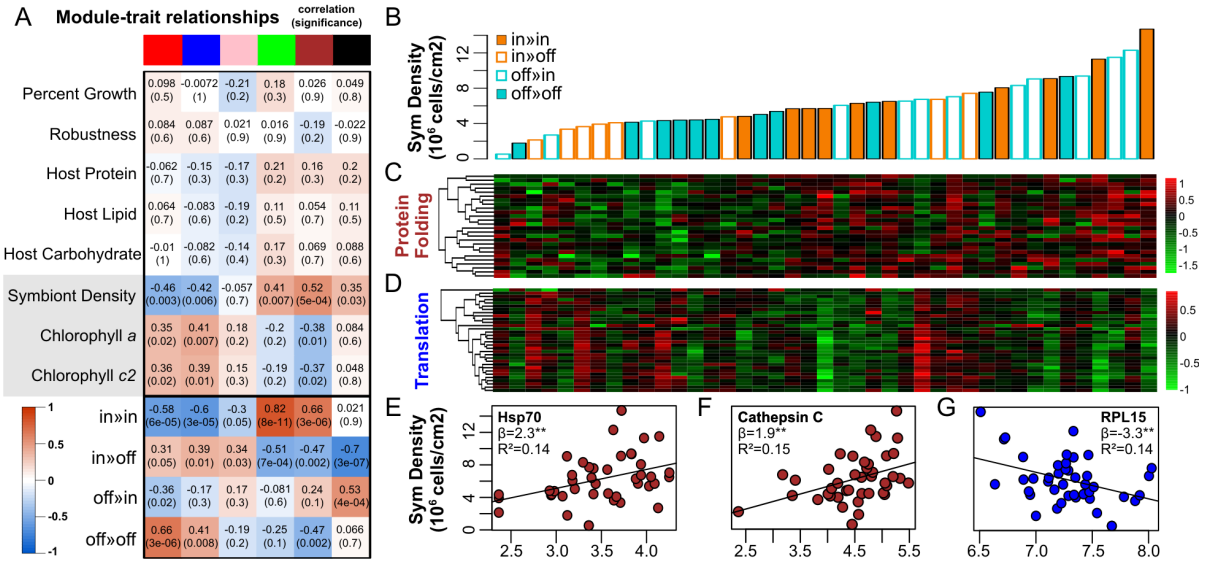


Figure 4.1. Co-expression modules show the strongest correlations with symbiont related traits. (A) Heatmap of the magnitude and sign of correlation between module eigengene (the first principal component of a module, representative of gene expression profiles within that module) and phenotypic traits (Fig. A17) or the origin by transplant interaction. (B) Barplot of symbiont density for individual corals by origin and transplant. (C) Heatmap of brown module genes in the most significant gene ontology (GO) term, 'protein folding'. (D) Heatmap of blue module genes in the most significant GO term, 'translation'. (E-F) Expression of individual genes (Log_2(fold-change)) within the blue and brown modules explain a significant proportion of the variance in symbiont density across samples.

between gene significance and module membership were analyzed for each module. Gene significance is defined as the absolute value of the correlation between individual genes within a module and the trait of interest, while module membership represents the

correlation of the individual gene's expression profile with the module eigengene. All correlations between gene significance for symbiont density and module membership were significant, but the strongest relationship was observed for brown module genes, with green and black showing less pronounced relationships (Fig. A14). The brown module eigengene was also significantly negatively correlated with chlorophyll a and c2 content, though to a lesser extent, with correlation coefficients of -0.38 and -0.37, respectively (Fig. 4.1A). As for symbiont density, correlations between gene significance for chlorophyll a and c2 and brown module membership were also strongly significant (Fig. A14).

These module eigengenes also showed strong correlations with origin and transplant environment. The brown and green modules showed origin by transplant specific expression correlations: both modules were up-regulated by inshore corals at inshore reefs and down-regulated by offshore corals at offshore reefs, with correlation coefficients of 0.66 and 0.82, and -0.47 and -0.25, respectively (Figs. 4.1A,4.2). Inshore corals transplanted to offshore reefs showed a complete shift in brown module expression, perfectly matching the magnitude and direction of the eigengene shown in native offshore corals (correlation coefficient of -0.47 for both populations at offshore reefs, Figs. 4.1A,4.2). Offshore corals transplanted to inshore reefs, on the other hand, altered the direction of eigengene expression in the brown module but were not able to fully match the magnitude of expression shown by native inshore corals (correlation coefficient of 0.24 and 0.66 for offshore and inshore populations, respectively, at inshore reefs, Figs. 4.1A,4.2). The shift was even more pronounced for green module genes: inshore corals show an even stronger down-regulation of these genes at offshore reefs than native corals with correlation coefficients of -0.51 and -0.25 for inshore and offshore

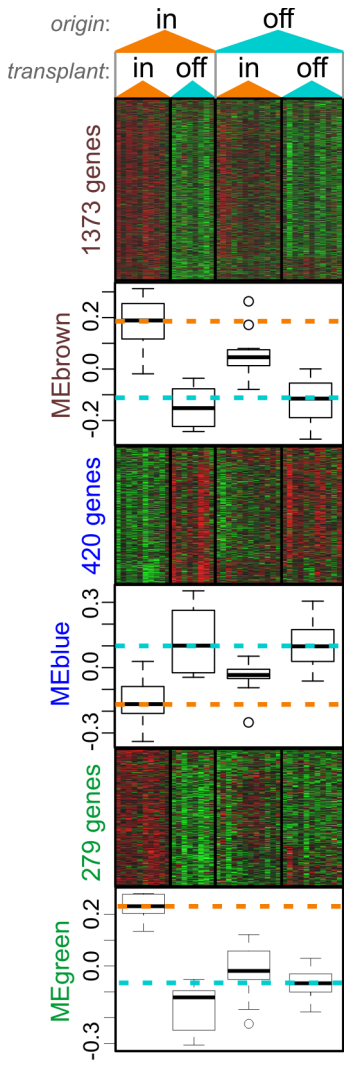


Figure 4.2. Inshore corals exhibit greater expression plasticity than offshore corals. Heatmaps of all genes assigned to the brown, blue and green modules by individual samples and boxplot distributions with respect to site of origin and transplant environment.

populations, respectively, at offshore reefs; while offshore corals transplanted to inshore reefs are unable to match the magnitude or direction of expression of native corals (Correlation coefficients of -0.081 and 0.82, respectively, Figs. 4.1A,4.2). The black module eigengene was only correlated with transplanted corals, showing a strong negative correlation (i.e. down-regulation) in inshore corals transplanted to offshore

reefs, and a strong positive correlation (i.e. up-regulation) in offshore corals transplanted to inshore reefs (Fig. 4.1A,A15).

Red and blue module eigengenes were significantly negatively correlated with symbiont density, with correlation coefficients of -0.46 and -0.42, respectively (Fig. 4.1A,A15). Strong significant correlations between gene significance for symbiont density and module membership for these modules further validates these relationships (Fig. A14). These modules were also significantly positively correlated with chlorophyll a and c2 content, again to a lesser extent than density correlations, with coefficients of 0.35 and 0.41 for red and blue eigengene correlations to chlorophyll a and 0.36 and 0.39 for correlations with chlorophyll c2 (Fig. 4.1A). Again, gene significance and module membership correlations were also significant, though the relationships were stronger for blue module genes than for red module genes (Fig. A14).

As in the brown and green modules, blue and red module genes are also strongly regulated with respect to origin and transplant environment, though the direction of expression is reversed. The blue module mirrors the brown module: genes in this module are strongly down-regulated by inshore corals at inshore reefs and up-regulated by offshore corals at offshore reefs (correlation coefficients of -0.60 and 0.41, respectively, Fig. 4.1A). Inshore corals transplanted to offshore reefs almost perfectly match the magnitude and direction of expression shown in native offshore corals, shifting the correlation coefficient of the eigengene to 0.39, while offshore corals transplanted to inshore reefs down-regulate expression, but not to the same magnitude of native corals (correlation coefficient -0.17, Figs. 4.1A,4.2). Genes in the red module are also strongly down-regulated by inshore corals at inshore reefs and up-regulated by offshore corals at offshore reefs (correlation coefficients of -0.58 and 0.66, respectively), and though transplants shift the direction of expression, they are not able to match the magnitude of

expression shown by native populations (correlation coefficients of -0.36 and 0.31, Fig. 4.1A,A15).

The pink module was not significantly correlated with any traits, though expression in this module tended to be negatively correlated with host traits of growth, protein, lipid and carbohydrate content, and positively correlated with chlorophyll content. Expression of this module also appeared to be related to environmental novelty as both inshore and offshore corals tended to up-regulate genes in this module in foreign environments, however this pattern is most likely the result of a few strong sample outliers (Fig. A15).

Population-level variation in expression plasticity

To further validate patterns of expression plasticity observed in individual WGCNA modules (Fig. 4.2) I conducted a discriminant analysis of principal components (DAPC) for all DEGs. DAPC differentiates between a priori defined groups using a subset of linear combinations of the original expression data (principal components) with the largest between-group variance and the smallest within-group variance. I constructed a function using the first four principal components to discriminate between expression of populations within their native reef environment: inshore corals transplanted to inshore reefs, and offshore corals transplanted to offshore reefs (Fig. 4.3C, solid distributions). Memberships were then derived for foreign transplants using the same discriminant function (Fig. 4.3C, transparent distributions). As in the co-expression modules, inshore corals transplanted to offshore reefs more closely match native offshore expression patterns than offshore corals transplanted to inshore reefs (Fig. 4.3C,D).

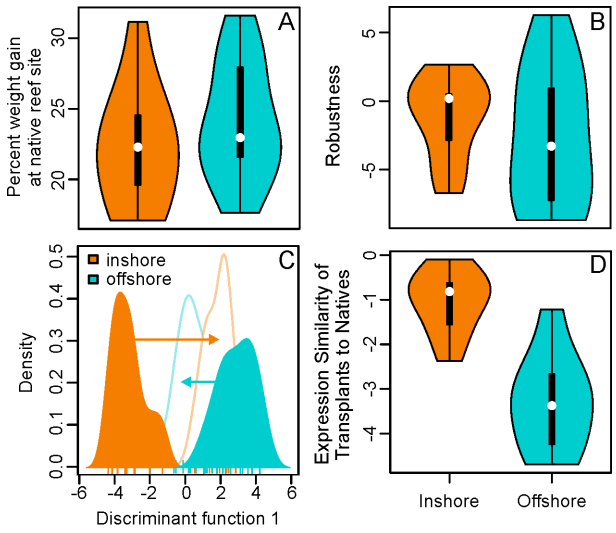


Figure 4.3. Population level variation in growth, robustness and expression plasticity. Violin plots show that inshore corals tend to grow less at their native reef site in comparison to offshore corals (A) but greater robustness when transplanted to novel environments (B). (C) Discriminant analysis of principal components for the top 2,664 DEGs for inshore and offshore corals when transplanted to their native reef (solid distributions) and non-native reef (transparent distributions) sites. (D) Violin plot showing distribution of the difference in expression values of transplants (individual coordinates along to discriminant axis) to the native population mean expression value. Violin plots display the median (white circle) and interquartile range (black rectangle) of trait values by population overlaid with a kernel density estimation.

Functional enrichment within modules

The top gene ontology (GO) terms identified for each module indicated that though expression patterns are similar for some modules, these groupings likely represent different functional processes (Table 4.1). The top GO terms for the brown module were 'protein folding' (GO:0006457), 'lytic vacuole' (GO:0000323), and 'small molecule binding' (GO:0036094, Table 1). Out of 26 total protein folding genes, 18 are molecular chaperones and overall, higher expression of these genes was associated with higher symbiont densities (Fig 1B,C). Some individual genes within this module showed strongly significant correlations, for example, expression of Hsp70 explained 14% of the

variation in symbiont density across all samples (Fig. 4.1E). The remaining genes in this group appear to be involved in protein transport, including genes putatively involved in the backward transport of proteins into the endoplasmic reticulum as part of the ubiquitin-proteasome dependent degradation pathway (translocation protein SEC63). Further supporting activation of protein repair and degradation pathways is the enrichment of genes assigned to the GO term 'lytic vacuoles', including the protease Cathepsin C, the expression of which is also significantly positively correlated with symbiont density (Fig. 4.1F). Finally, 'tumor necrosis factor-activated receptor activity' (GO:0005031) was one of the most enriched molecular function terms in the brown module (P=0.001)

The top GO terms for the blue module were 'translation' (GO:0006412), 'ribosome' (GO:0005840) and 'structural constituent of ribosome' (GO:0003735), suggesting that this group of genes represents translation. Lower expression of translation genes was associated with higher symbiont densities overall (Fig 4.1B,D), and again, some individual genes within this module also showed strong negative correlations, for example, a 2-fold decrease in expression of ribosomal protein L15 corresponds to an increase in symbiont cell density of roughly 3 $x10^6$ cells/cm^2.

For genes in the green module, the most significant GO terms were 'sodium ion transport' (GO:0006814), 'microtubule associated complex' (GO:0005875) and 'purine nucleotide binding' (GO:0017076), which may explain the weak positive relationship between expression of these genes and host traits such as growth, and total lipid, protein and carbohydrate content, though expression in this module is broadly similar to expression in the brown module (Fig. 4.1A). Red module genes were most strongly enriched for 'myelination' (GO:0042552), 'extracellular region' (GO:0005576) and 'carbohydrate transmembrane transporter activity' (GO:0015144), suggesting that though

expression patterns are broadly similar to the blue module (Fig. 4.1A), these genes are more likely involved in directly mediating the symbiosome maintenance and host-symbiont exchange across the symbiosome membrane. GO term enrichment of genes in the black and pink modules, together with foreign transplant specific expression suggests that these modules may be involved in mediating immune responses to novel environments (Table 4.1, Fig. 4.1A).

Table 4.1. Top gene ontology (GO) terms by P-value resulting from functional enrichment analysis of co-expression modules.

		Top GO enrichment (P-value*)		
Module	# genes	Biological Process	Cellular Component	Molecular Function
Brown	1373	protein folding (0.0004)	lytic vacuole (0.01)	small molecule binding (0.0008)
Blue	420	translation (2.64 x 10-15)	ribosome (2.55 x 10-19)	structural constituent of ribosome (3.37 x 10-22)
Green	279	sodium ion transport (0.001)	microtubule associated complex (0.003)	purine nucleotide binding (0.0002)
Red	185	myelination (0.0008)	extracellular region (0.015)	carbohydrate transmembrane transporter activity (0.0008)
Black	100	aminoglycan metabolic process (0.0003)	extracellular region part (0.0003)	chitin binding (0.0007)
Pink	71	cell redox homeostasis (0.014)	extracellular region part (0.005)	ligase activity (0.003)

* Fisher's exact test uncorrected p-value.

DISCUSSION

Signature of differential stress response among locations

Maintenance of homeostasis in the face of environmental variability is a physiological challenge faced by all organisms, but sessile animals, such as reef-building corals, are particularly susceptible since they cannot change habitats to escape stressors (Lopez-Maury et al. 2008). Increasing evidence suggests that corals are capable of mounting an environmental stress response (ESR) expression program similar to that first described in yeast, which adjusts cellular physiology and protects against cell damage and/or death (Barshis et al. 2013; DeSalvo et al. 2008; Meyer et al. 2011; Polato et al. 2010; Rodriguez-Lanetty et al. 2009; Voolstra et al. 2009). Functional enrichment of genes in the brown and blue modules (Fig. 4.1,4.2, Table 4.1) suggests that *Porites astreoides* are also capable of mounting this response, as up-regulation of molecular chaperones and protein degradation pathways (brown module) was concomitant with a down-regulation of ribosome biogenesis (blue module), a hallmark of the ESR (Lopez-Maury et al. 2008).

The most likely explanation for this pattern is an ongoing response to environmental stress in these populations at the time of sampling as minor thermal-stress induced bleaching was observed in corals collected at the inshore reef (Fig. A16). Bleaching is defined as a stress-induced functional loss of the endosymbionts (Vaughan 1914; Yonge & Nicholls 1931), which supply anywhere from 78-161% of a coral's energy requirements (Muscatine 1990). Their loss can be fatal (Brown et al. 2000), and even non-fatal events cause latent reductions in growth and reproductive capacity (Jones & Berkelmans 2010b; Szmant & Gassman 1990).

Further supporting the conjecture that ESR regulation is a direct response to elevated summer temperatures is the strong positive correlation between the ESR

response and symbiont density: corals with the strongest ESR responses also maintained the highest symbiont densities (Fig. 4.1B-G). This suggests that the ability of the coral host to mitigate intracellular damage resulting from environmental stress can reduce bleaching susceptibility of the holobiont (the combination of host and symbiont). This is in contrast to many other systems in which differences in symbiont type are the most parsimonious explanation for variation in stress susceptibility in the holobiont (Berkelmans & van Oppen 2006; Oliver & Palumbi 2011b). *P. astreoides* transmits its symbionts vertically from parent to offspring, and thus maintains highly stable symbioses (Thornhill et al. 2006). Previous work on corals from the same populations used in this study has shown that even hosts from different populations have highly similar symbiont complements (Kenkel et al. 2013a) and genotypes of symbionts in this study are also highly similar, both within and between populations (data not shown).

Alternatively, the causality of the correlation between higher symbiont density and elevated ESR could be just the opposite: the over-abundance of symbionts might impose higher stress as *Pocillopora damicornis* corals with higher symbiont densities have been shown to be more susceptible to bleaching (Cunning & Baker 2013). However, the temporal observations in this study are inconsistent with this explanation. Presumably, stress prior to the time of sample collection resulted in some minor bleaching at the inshore reef site (Fig. A16). If the overabundance of symbionts was the only factor influencing bleaching susceptibility, then all corals at the inshore reef site should have exhibited elevated bleaching in comparison to corals at the offshore reef site as symbiont densities were significantly higher inshore (Fig. A17, Chapter 1). However, offshore origin corals were the only ones to show phenotypic signatures of bleaching at the inshore reef site (Fig. A16A). Furthermore, if elevated symbiont densities increase bleaching susceptibility and by extension, coral stress levels, then corals with the highest

level of symbiont loss would be expected to show the highest signatures of stress response gene expression. I observe the exact opposite pattern: corals with the highest symbiont densities post-stress have the highest expression of ESR genes (Fig. 4.1B-G), suggesting that these expression patterns are indeed reflective of a host role in mediating holobiont thermotolerance, rather than a response to symbiont density.

Tumor necrosis factor receptors

It is notable that both my study and the study of (Barshis et al. 2013) highlighted tumor necrosis factor receptors (TNFRs) as prominent players in coral thermal stress response and acclimatization. These receptors, in cooperation with adaptor proteins, such as TNF receptor associated factors (TRAFs), modulate cell and tissue fate, such as inflammation and apoptosis responses (Inoue et al. 2000) and are numerous in coral genomes (33 isogroups in *P. astreoides*, 97 isogroups in *Acropora millepora*). Their involvement in thermal tolerance in such divergent coral lineages as acroporids and poritids (Fukami et al. 2008) suggests that TNFRs are key regulators of stress response, adaptation and/or acclimatization across all reef-building corals (order Scleractinia). Although such a role has never been attributed to this whole protein family in other animal models, it is interesting to note that one of the most well-studied local adaptation genes, ectodysplasin-receptor-associated protein (Eda) of threespine stickleback fish, associated with morphological, physiological and even behavioral differences between freshwater and saltwater populations is a member of the tumor necrosis family as well (Barrett et al. 2008; Barrett et al. 2009; Colosimo et al. 2005; Marchinko & Schluter 2007).

Role of gene expression plasticity in local adaptation

Inshore reefs experience greater fluctuations in temperature than offshore reefs: mean summer temperatures are approximately 1ºC warmer and mean winter temperatures approximately 1.4ºC cooler at inshore reefs (Kenkel et al. 2013a). Thermal stress is one of the greatest factors affecting the decline of contemporary reef-building corals (Hoegh-Guldberg et al. 2007). Corals live in close proximity to their upper thermal maximum, though this limit can vary across reef sites, an increase in temperature of 1ºC on a local scale is sufficient to induce bleaching (Jokiel 2004). Given the different thermal regimes between inshore and offshore reefs in the Lower Florida Keys it is perhaps not surprising that inshore corals have adapted and/or acclimated to their native reef environment (Chapter 1) and exhibit elevated thermotolerance (Kenkel et al. 2013a). The surprising result of the present study was that expression plasticity may be involved in mediating these higher-order phenotypic responses. In foreign environments inshore corals are better able to match expression profiles of the native offshore population than offshore corals are at matching inshore natives (Fig. 4.2,4.3C,D). The only evidence thus far suggesting that adaptation might involve modulation of gene expression plasticity comes from a recent study of anadromous and freshwater brown trout: laboratory reared F2 populations of anadromous fry exhibited greater expression plasticity than freshwater populations in response to elevated temperature stress, putatively resulting from the greater thermal variability experienced by the anadromous fish in life-history transitions between fresh- and saltwater (Meier et al. 2014). Similar to the results of the trout study, expression plasticity in inshore corals may be a response to the greater environmental variability experienced by this population.

This difference in expression plasticity is in contrast to another recently reported mechanism by which corals may be responding to temperature variation. Barshis et al.

(2013) found that corals from more thermally variable pools exhibited constitutive up-regulation of ESR transcripts, which they termed "frontloading". Constitutive up-regulation of metallothionein and other stress response genes was also observed in populations of springtails living in metal contaminated mining sites (Roelofs et al. 2009; Roelofs et al. 2007). It has been argued that constitutive over-expression is a common evolutionary response to abiotic stress across taxa (Roelofs et al. 2010). However, inshore corals in the Lower Florida Keys exhibit greater expression plasticity and a more thermotolerant phenotype (Kenkel et al. 2013a) suggesting that in this case, expression plasticity may facilitate adaptation to local abiotic stressors.

The difference between these strategies may be due to the frequency at which these coral populations are exposed to thermal stress events. The dominant cycle of temperature fluctuations in the Florida Keys occurs on an annual scale, with corals at inshore reefs experiencing more extreme temperatures in summer and winter, while the corals studied by Barshis et al. (2013) experience dominant cycles on a daily scale, with changes of up to 6ºC during tidal cycles (Craig et al. 2001). The scale of environmental variation is predicted to greatly influence the evolutionary strategies adopted by organisms (Gillespie 1974; Levins 1963). Theory predicts that coarse-grained selection should result in constitutive expression of an adaptive phenotype, whereas fine-grained variation should result in adaptive plasticity whereby genotypes can flexibly alter phenotypes to maximize fitness under a given condition (Banta et al. 2007). The constitutive up-regulation of ESR genes by corals in tidal pools (Barshis et al. 2013) suggests that this population integrates over the periodicity of stress events and has responded to thermal stress as a constant, analogous with a coarse grained environment. Whereas the variable expression of ESR genes in inshore coral populations suggests that these corals have adopted an alternate evolutionary solution, employing adaptive

plasticity to cope with fine grained cycles of annual temperature variation in the Florida Keys. Understanding the ability of coral populations to adapt to local thermal stress may be important for predicting coral responses to future climate change. However, corals appear to be sensitive to the grain of environmental variation. Future work should aim to investigate how the different strategies of constitutive front-loading and adaptive plasticity affect the capacity of coral populations to further adapt to a changing climate.

Is there a cost to gene expression plasticity?

Previous work on *P. astreoides* populations throughout the Florida Keys has shown that robustness in growth (the ability to maintain growth rate across reef environments) incurs a trade-off in the form of diminished growth in a coral's native reef environment (Chapter 1). Given these results, I hypothesize that gene expression plasticity may explain elevated robustness and it's trade-off: the highly plastic inshore population tends to exhibit a reduced mean growth rate at their native reef when compared to offshore corals, but displays higher robustness in growth when transplanted to a novel environment than the offshore population (Fig. 4.3A,B). Furthermore, expression of brown module genes that are positively associated with symbiont density (Fig. 4.1B,C) and undergo plastic changes in response to transplant location, with stronger regulation in inshore origin corals (Fig. 4.2), exhibit a trend of negative correlation with robustness (Fig. 4.1A). Thus far, the costs of gene expression plasticity have been investigated only in a laboratory setting. In bacteria, gratuitous expression is known to result in reduced growth (Andrews & Hegeman 1976; Dekel & Alon 2005; Stoebel et al. 2008). Furthermore, Lang et al. (2009) have shown that sterile strains of yeast achieve a growth rate advantage over non-sterile strains by eliminating basal expression of mating pathway genes. This study presents the first experimental evidence

that regulation of gene expression might incur fitness costs in a natural ecological context.

CONCLUSIONS

Overall, inshore corals exhibit more plastic regulation of expression than offshore corals. In particular, genes involved in the environmental stress response (Gasch et al. 2000) are more strongly regulated in inshore origin corals, and corals with the highest expression of these genes maintained the highest symbiont densities following transplantation. Taken together, these results suggest that the ability to regulate expression is important for local adaptation of these populations. In addition, expression plasticity may underpin phenotypic robustness in response to environmental variation, resulting in slower growth rates, but elevating thermal tolerance responses in inshore corals. Future work will aim to definitively test the causative relationships between expression plasticity, growth rates and bleaching susceptibility in these populations.

CHAPTER 5: Heritable differences in fitness-related traits among populations of the mustard hill coral, *Porites astreoides*

ABSTRACT

A population's potential for rapid evolutionary adaptation can be estimated from the amount of genetic variation in fitness-related traits. Inshore populations of the mustard hill coral (*Porites astreoides*) have been shown to be more tolerant of thermal stress than offshore populations, but it is unclear whether this difference is due to long-term physiological acclimatization or genetic adaptation. Here, I evaluated variation in growth rate and survival among 38 families of juvenile recruits of *P. astreoides* spawned by colonies originating from inshore and offshore locations. Recruits were reared in a common garden for 5 weeks and then subjected to two thermal treatments (28°C and 31°C) for 2.5 weeks. The most significant effects were detected during the first 5 weeks, before thermal stress was applied: 36-39% of variance in growth, 40-45% of the variance in bleaching and 94% of variance in recruit survival was attributable to parental effects. Genotyping of eight microsatellite loci indicated that the high early mortality of some of the recruit families was not due to higher inbreeding. Post-treatment, parental effects diminished such that only 10-18% of variance in growth rate and 17-21% of the variance in bleaching was explained, which most likely reflects the dissipation of maternal effects. However, offshore-origin recruits still bleached significantly more and grew significantly less under elevated temperature compared to inshore-origin recruits. These differences observed in naïve juvenile corals suggest that thermotolerance has a genetic basis and could represent raw material for natural selection in times of climate change.

INTRODUCTION

Determining the amount of genetic variability in traits under selection is essential for evaluating a population's potential for evolutionary change (Charmantier and Garant, 2005). The heritability of a trait is the proportion of phenotypic variation due to genetic variation between individuals (H_2 = VG/VP) and largely determines the magnitude and speed of phenotypic change in response to selection (Falconer and Mackay, 1996). Predicting response to selection is particularly important for reef-building corals, which experience substantial environmental variation across species ranges (Hughes et al, 2003) and are undergoing climate change at an unprecedented rate (Burrows et al, 2011).

Corals are cnidarians that exist in symbiosis with dinoflagellates of the genus *Symbiodinium*. This symbiosis is considered obligate as it has been estimated that up to 95% of a coral's energy requirements are met through photosynthetically fixed carbon contributed by the endosymbiont (Muscatine, 1990). Thermal stress results in the functional loss of the endosymbionts in the process known as coral bleaching, which can ultimately result in death if stressful conditions persist (Brown, 1997). While reef-building corals are found throughout warm tropical and sub-tropical waters, most exist within 1-2ºC of their temperature tolerance limit during summer months (Jokiel, 2004). Climate change models predict that global ocean temperatures will increase by 1-2ºC within the next 50 to 100 years (Hoegh-Guldberg et al, 2007), therefore putting thermally sensitive corals in jeopardy.

Thermotolerance limits of the coral holobiont (the collective unit of the coral host and symbiont) are greatly influenced by the type of *Symbiodinium* hosted. Substantial variation exists in thermotolerance among genotypes of *Symbiodinium* (Howells et al, 2011; Robinson and Warner, 2006) that affects holobiont growth (Jones and Berkelmans, 2010; Little et al, 2004) and bleaching thresholds (Oliver and Palumbi, 2011). In

addition, high heritability estimates for functional traits in *Symbiodinium* suggests that thermal adaptation of symbionts will play an important role in coral response to climate change (Csaszar et al, 2010). However, thermotolerance limits are also impacted by host genetic background (Abrego et al, 2008; Kenkel et al, 2013a; Ulstrup et al, 2006) and holobiont thermal history (Brown et al, 2002a; Brown et al, 2000; Brown et al, 2002b). Therefore, it is the adaptive potential of the coral holobiont that will truly determine the ability of coral populations to respond to climate change. Csaszar et al. (2010) report significant broad-sense heritabilities for coral growth in two populations of *Acropora millepora*. However, to my knowledge, no estimates of holobiont trait heritabilities exist for any Caribbean species.

In the Florida Keys, *Porites astreoides* corals from inshore reefs exhibit elevated temperature tolerance in comparison to corals from offshore reefs (Kenkel et al, 2013a). These populations harbor indistinguishable *Symbiodinium* genotypes but hosts are significantly genetically differentiated (Kenkel et al, 2013a) and exhibit divergent gene expression patterns (Kenkel et al, 2013b) suggesting that the coral host plays a more prominent role in thermal adaptation in this species. However, it is not clear if observed thermotolerance differences in these populations are the result of heritable genetic variation or long-term acclimatization to their native habitat. In order to isolate the contribution of genetics, it is necessary to minimize the possibility that the differences in physiology and fitness-related traits are due to prior acclimatization of the organisms to different environments. Here, I used naïve *P. astreoides* juveniles obtained from parental colonies from inshore and offshore populations, in attempt to minimize the effect of prior exposure to different environments. In addition, juvenile recruits were reared for 5 weeks in a common garden environment prior to conducting the thermal stress experiment to

reduce the impact of maternal effects, as these effects are known to dissipate over the first month of life in marine invertebrate larvae (Cruz and Ibarra, 1997; Sman et al, 2009).

METHODS

Coral collection, spawning and common rearing

P. astreoides uses a brooding reproductive strategy. In brooding corals, eggs are fertilized internally and fully-formed planulae are released in multiple reproductive cycles throughout the year (McGuire, 1998; Richmond and Hunter, 1990). Peak reproduction in *P. astreoides* occurs during the new moon, with the majority of larval release occurring in April/May (McGuire, 1998).

Twenty-five parent colonies of *P. astreoides* were collected on 19 April 2012 from a depth of 2-3 m from each of two sites: an inshore patch reef (Summerland Shoals Patch, 24°36.346N, 81°25.742W) and an offshore reef (Dave's Ledge, 24°31.887N, 81°29.013W) 9.9 km apart near Summerland Key under Florida Keys National Marine Sanctuary (FKNMS) permit 2012-028. Colonies were immediately returned to Mote Marine Laboratory's Tropical Research Laboratory and placed in a shaded (70% PAR reducing) flow-through seawater system (raceway).

Each evening, from 19 April to 22 April, parent colonies were isolated in 15 L plastic tubs filled with 0.35 μM filtered seawater (FSW) placed into the raceway. As planulae larvae of brooding corals are competent to settle within hours of release (Goodbody-Gringley, 2010; Isomura and Nishihira, 2001), parent corals were placed on top of two 7.5 cm2 pre-conditioned terra cotta tiles to obtain populations of juvenile recruits. Each morning, the remaining swimming larvae were collected by filtering the tub water gently through a 200 μM Nitex mesh filter and transferred to 1 L plastic containers filled with 0.35 μM FSW by family. Swimming larvae were subsequently

induced to settle onto pre-conditioned tiles with crustose coralline algae (Heyward and Negri, 1999).

In total, 38 corals, 19 inshore and 19 offshore, released sufficient larvae such that each coral family was represented by at least 2 tiles worth of juvenile recruits. On 25 April, parent corals were cut in half using a diamond blade tile saw and tiles were photographed to obtain baseline estimates for survival, growth and bleaching measurements. Tiles were then placed into racks in a common shaded (70% PAR reducing) raceway with parent corals and left to acclimate for five weeks prior to the common garden heat stress experiment.

Common garden experiment

On 28 May 2012, all parent colony halves were buoyant weighted in duplicate (Davies, 1989) and tiles were again photographed to obtain post-rearing measures of growth, bleaching and survival. Parent colony halves and recruit tiles were then randomly assigned to a temperature treatment, a tank within that treatment and a specific position within that tank (n=4 parent halves and 4-6 tiles per tank). Temperature treatment consisted of two shaded (70% PAR reducing) raceways, each holding eight 40L aquaria with clear plastic lids, with four control and four elevated temperature treatment tanks per raceway. Control temperature treatment was achieved by filling the 40L tanks with seawater, equipping each tank with a 2-W aquarium pump (Hesen) and allowing water to flow-through the raceway as a water bath. Elevated temperature treatment tanks were set up in exactly the same manner, but each individual tank was also equipped with a 200-W aquarium heater (Marineland) set to maximum heat. Temperatures were 28 ± 0.4°C in the control tanks and 30.9 ± 1.1°C in the heated tanks.

Treatment continued for 2.5 weeks (16 days) with tank cleaning and 30-50% water changes performed three times each week to maintain salinity levels at 35 ppt.

Trait measurements

Following treatment, all parent fragments were cleaned using a small brush to remove any filamentous algal growth and again buoyant weighted in duplicate. Technical replicates of weight measurements for each coral half were averaged. Initial weight measurements were subtracted from final weight measurements and divided by the initial weight measurement to determine the proportion of weight gained over the 2.5-week treatment for each parent coral (Kenkel et al, 2013a). Bleaching phenotypes were also recorded for each parent fragment by scoring coral color against the Coral Health Chart (Siebeck et al, 2006).

Tiles were again photographed and images were color and contrast enhanced and analyzed using ImageJ (Rasband, 1997-2014). To determine juvenile growth for the two time periods, the first 5 weeks of rearing (25 April to 28 May) and the 2.5-week common garden experiment (28 May to 13 June), individual recruits and tiles were outlined and pixel area was recorded. Recruit area was normalized by tile area and the percent change in area at each time point for each individual recruit (co-settled clumps of recruits were not included in this analysis) was calculated by subtracting initial area from final area and dividing by initial area. I used recruit brightness as a bleaching proxy, analogous to the method of (Winters et al, 2009), with the exception that tile photographs were taken without a common color standard. Though I were unable to balance photographs using an independent standard, all photographs were taken at the same time in a controlled photograph chamber, so brightness values should not vary substantially among photographs at any given time point. For brightness measures, mean RGB values,

representing mean pixel brightness, were recorded for each individual recruit (discarding co-settled clumps of recruits). Brightness values were normalized by the mean brightness of all individual recruits at each time point and the percent change in brightness was calculated for each individual recruit at each time point. Survival was assessed by calculating the difference in absolute number of recruits per tile at each time point. Co-settled clumps of recruits were considered a single individual in terms of survival.

Genotyping

DNA was extracted from 10 individual larvae from each of 10 families (5 inshore and 5 offshore) as in (Kenkel et al, 2013a). Each individual was genotyped at eight microsatellite loci (Kenkel et al, 2013a) following the PCR reaction conditions described in (Davies et al, 2012). GENEMARKER software 1.70 (Soft Genetics) was used to analyze electropherograms and alleles were scored manually based on amplicon size. One inshore family was discarded due to poor amplification across all samples. In addition, select individuals were discarded based on the presence of spurious amplification peaks. 77 individuals (n=5-10 per family; n=9 families) were used in the subsequent statistical analyses.

Statistical analyses

All analyses were carried out using R 2.13.2 (R Development Core Team, 2013). Differences in parent growth and bleaching were evaluated with respect to treatment and population of origin using a nested series of linear mixed models implemented in the lme4 package (Bates, 2005). Differences in adult bleaching score were evaluated using a nested series of cumulative link mixed models as implemented in the ordinal package (Christensen, 2012), using a probit link function. Differences in juvenile growth rate and bleaching, expressed as the $\log_2$(fold-change), were evaluated with respect to treatment,

population of origin and time-point using a nested series of linear mixed models implemented in the nlme package (Pinheiro et al, 2013). For all models, treatment, origin, time-point and their interaction were modeled as fixed factors, with levels control/heat, inshore/offshore and 5 week rearing/2.5 week experiment, respectively. For adult models, colony identity and treatment tank were included as scalar random factors. For juvenile models, individual recruit and parent identity were included as scalar random factors. For linear models, the applicability of model assumptions (normality, homoscedasticity) to the data were verified using diagnostic plots. For parent traits, likelihood ratio tests (LRT) were used to evaluate individual factor significance, while a Wald test was run to evaluate factor significance for juvenile growth rates and the change in bleaching. Relationships between sibling growth and bleaching under control and heat treatment and parent-offspring growth were evaluated with Pearson's product-moment correlations using the function *cor.test()*, after removing outliers. The relationship between bleaching under heat treatment in parents and offspring was evaluated with a Spearman's rank correlation. Population-level differences in the initial number of recruits per tile and standardized recruit size $\log_{10}$(recruit area/tile area) were evaluated with Welch's t-tests using the function *t.test()*.

A series of generalized linear mixed models was used to model recruit growth, bleaching and survival at each time point, with family as a random effect and origin and treatment as fixed effects using the package MCMCglmm (Hadfield, 2010). A binomial model was used to model count data for survival. The MCMC chain was run for 50,000 iterations, sampling parameter values every 20 iterations and discarding the first 10,000 iterations as burn-in period. Mean and quantiles of the sampled variance parameters were calculated to estimate the variance in each trait explained by broad sense heritability (H_2,

parent effects), while accounting for effects of population origin and temperature treatment, when relevant.

The adegenet package (Jombart, 2008) was used to estimate F_{IS} values for each individual with respect to population (inshore or offshore) using the function *inbreeding()*. F_{IS} values were averaged by family. The relationship between mean F_{IS} and mean percent survival by family was evaluated with a Pearson's product-moment correlation using the function *cor.test()*.

RESULTS

Reproductive output by population

Inshore corals released more larvae than offshore corals during the April 2012 spawning event, resulting in a trend of increased recruits per tile at the beginning of the 5-week rearing period ($P = 0.1$, Fig. 5.1A). In addition, inshore-origin recruits were also 1.3 times larger on average than offshore-origin recruits at the beginning of the 5-week rearing period ($P < 0.001$, Fig. 5.1B).

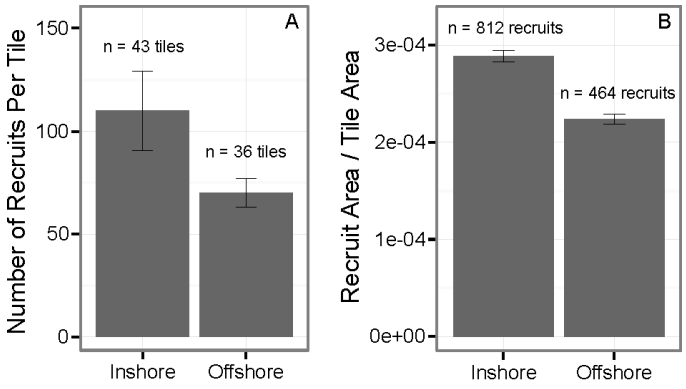


Figure 5.1. Reproductive output of parent corals by origin (A) expressed as the mean number of recruits per tile (± SEM) and size of offspring (B) expressed as mean recruit area relative to tile area (± SEM) prior to beginning the 5-week rearing period.

Parent coral response to temperature stress

Growth of parent corals was uniformly reduced in response to the elevated temperature treatment, irrespective of coral origin ($P_{LRT} < 0.01$, Fig. 5.2A). Bleaching status of parent corals was significantly influenced by temperature treatment, with heat stress causing a greater loss of pigmentation relative to controls ($P_{LRT} < 0.001$, Fig. 5.2D). Bleaching also revealed a marginally significant interaction term effect, with offshore corals bleaching more than inshore corals in response to temperature stress ($P_{LRT} = 0.054$, Fig. 5.2D).

Juvenile coral response to temperature stress

No mortality was observed as a result of the 2.5-week heat stress experiment. Juvenile growth, however, was significantly different between inshore and offshore populations in response to heat stress when compared to growth trajectories in the first 5-week rearing period ($P_{WALD} = 0.0001$, Fig. 5.2B,C; Table 5.1). Overall, juveniles grew more during the rearing period than during experimental treatment ($P_{WALD} < 0.0001$), with offshore-origin recruits exhibiting higher growth during the rearing period and inshore-origin recruits exhibiting higher growth during the heat stress experiment ($P_{WALD} < 0.0001$, Fig. 5.2C). This reversal also interacted with temperature treatment. Though inshore-origin recruits subsequently assigned to heat treatment exhibited the lowest growth rate during the rearing period, these individuals reversed their growth trajectories and substantially out-grew offshore-origin recruits during the heat stress experiment (Fig. 5.2C).

Recruit bleaching (measured by brightness as a proxy) showed a similarly complicated pattern, but again, responses were significantly different between populations in response to heat stress when compared to changes in the initial rearing

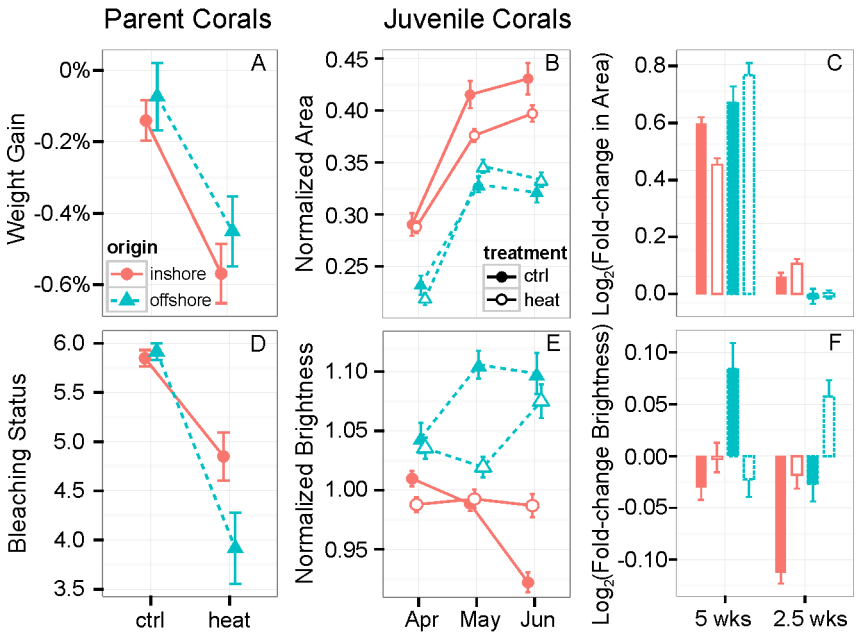


Figure 5.2. Fitness-related traits in parent and juvenile corals. (A) Weight gain of parent corals (mean ± SEM) by origin and treatment following the 2.5-week thermal stress experiment. (B) Normalized growth of juvenile corals (recruit area/tile area *1000 ± SEM) by origin and treatment (treatment only applies to May-June time period). (C) Change in growth rate of recruits during the initial 5-week rearing period and the 2.5-week thermal stress experiment, expressed as Log_2(Fold-change in normalized area) ± SEM for each time period. (D) Bleaching status of parent corals (mean ± SEM). (E) Bleaching of juvenile corals (recruit brightness/mean recruit brightness per measurement period ± SEM) by origin and treatment (treatment only applies to May-June time period). (F) Change in bleaching (Log_2(Fold-change in normalized area) ± SEM) of recruits during the initial 5-week rearing period and the 2.5-week thermal stress experiment.

period (P_{WALD} = 0.002, Fig. 5.2E,F; Table 5.2). Though tiles were randomly arranged, offshore juveniles destined for control treatments did show a small, but significant increase in bleaching during the rearing period. No other groups showed substantial changes in bleaching during the 5-week rearing (Fig. 5.2E,F). Significant changes in bleaching were observed among other groups during the 2.5-week experimental period. Inshore juveniles under control treatment showed a decrease in bleaching, whereas

Table 5.1. Wald test statistics for juvenile coral growth rate, Log_2(Fold-change in normalized area), with respect to population origin (inshore/offshore), treatment condition (control/heat) and time point (5 weeks rearing/2.5 weeks stress expt).

Factor	numDF	denDF	F-value	P-value
(Intercept)	1	1272	93.3	0.000
Origin	1	36	2.8	0.10
Treatment	1	1272	0.2	0.68
Time	1	1272	1001.4	0.000
Origin x Treatment	1	1272	4.3	0.04
Origin x Time	1	1272	67.8	0.000
Treatment x Time	1	1272	6.9	0.009
Origin x Treatment x Time	1	1272	15.0	0.0001

Table 5.2. Wald test statistics for change in brightness of juvenile corals, Log_2(Fold-change in normalized brightness), with respect to population origin (inshore/offshore), treatment condition (control/heat) and time point (5 weeks rearing/2.5 weeks stress expt).

Factor	numDF	denDF	F-value	P-value
(Intercept)	1	1272	5.1	0.02
Origin	1	36	0.7	0.41
Treatment	1	1236	10.9	0.001
Time	1	1272	9.1	0.003
Origin x Treatment	1	1236	8.8	0.003
Origin x Time	1	1272	6.2	0.01
Treatment x Time	1	1272	31.0	0.000
Origin x Treatment x Time	1	1272	9.4	0.002

inshore juveniles under heat treatment showed no significant change in bleaching during the 2.5-week experimental period (Fig. 5.2E,F). Interestingly, offshore corals in the control treatment did not continue to bleach during the 2.5-week experimental period,

suggesting that it was indeed conditions in the rearing tanks that resulted in their bleaching during the previous 5-week time period (Fig. 5.2E,F). Offshore corals under heat treatment, on the other hand, were the only group to exhibit an increase in bleaching (Fig. 5.2E,F).

Heritability estimates for fitness related traits

The most pronounced heritabilities were detected during the initial rearing period. Origin had no effect, but family explained almost all of the variance in survival, with a calculated H_2 of 0.94 (95% credible interval: [0.86, 0.99], Fig. 5.3). Though substantial, the variance in survival among larval families during the rearing period was independent of recruit density, and does not predict subsequent differences in growth during the common garden experiment (Fig. A18). Significant heritability was also detected for initial juvenile growth rates and bleaching. The H_2 of initial growth rate was 0.39 (95% CI [0.28,0.52], Fig. 5.3), of which 3% could be attributed to the effect of origin based in the decrease in heritability after accounting for the parental origin (origin-corrected H_2 = 0.36, 95% CI [0.25, 0.49]). The H_2 of bleaching was 0.45 (95% CI [0.32, 0.58], Fig. 5.3), of which 5% could be attributed to the effect of origin (origin-corrected H_2 = 0.40, 95% CI [0.28, 0.53]).

Heritability estimates diminished following the 2.5-week common garden experiment. The family effect on growth was cut in half, with an H_2 of 0.15 (95% CI [0.08, 0.23], Fig. 5.3). While some variance was still attributable to parental origin (2%), heat treatment reduced the overall heritability of growth, though this difference was not significant (Fig. 5.3). Control treatment yielded a growth H_2 of 0.18 (95% CI [0.09, 0.28]), while heat treatment yielded a growth H_2 of 0.11 (95% CI [0.05, 0.18]). For bleaching, H_2 during the heat stress experiment was reduced to 0.22 (95% CI [0.12, 0.31],

Fig. 5.3). Origin accounted for a much smaller proportion of the variance in brightness (2%). Accounting for the effects of temperature treatment reduced heritability

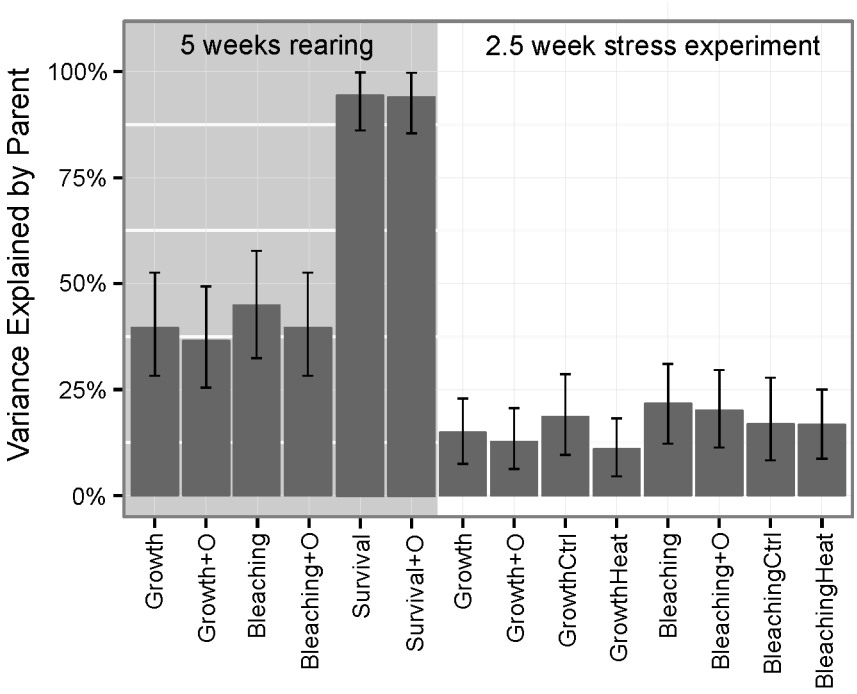


Figure 5.3. Broad-sense heritability estimates for survival, growth and bleaching, expressed as the mean variance (± 95% CI) explained by parental effects, accounting for the effects of parental origin (+O) and temperature treatment (Ctrl, Heat) across time points when meaningful.

estimates overall, but estimates did not differ between treatments (Fig. 5.3). A bleaching H_2 of 0.17 was estimated for both control and heat treatment, with credible intervals being slightly narrower for the heat-treatment estimate (control: 95% CI [0.08, 0.28]; heat: 95% CI [0.09, 0.25]).

As a secondary validation of trait heritability, I evaluated trait correlations between parents and offspring as well as for recruit families under different treatments. Significant positive relationships were observed for recruit growth under different

treatments (r = 0.49, P = 0.005, Fig. 5.4A) and between weight gain of parent colonies and mean offspring growth rate (r = 0.28, P = 0.04, Fig. 5.4C), further supporting the observation of heritable variation in growth (Fig. 5.3). No relationship was observed between recruit bleaching under control and heat treatment, suggesting that bleaching under heat is not explained by the initial bleaching level due to parental effects (Fig. 5.4B). However, there was a marginally significant relationship between the bleaching status of parents and bleaching of juveniles: juveniles with the greatest bleaching values

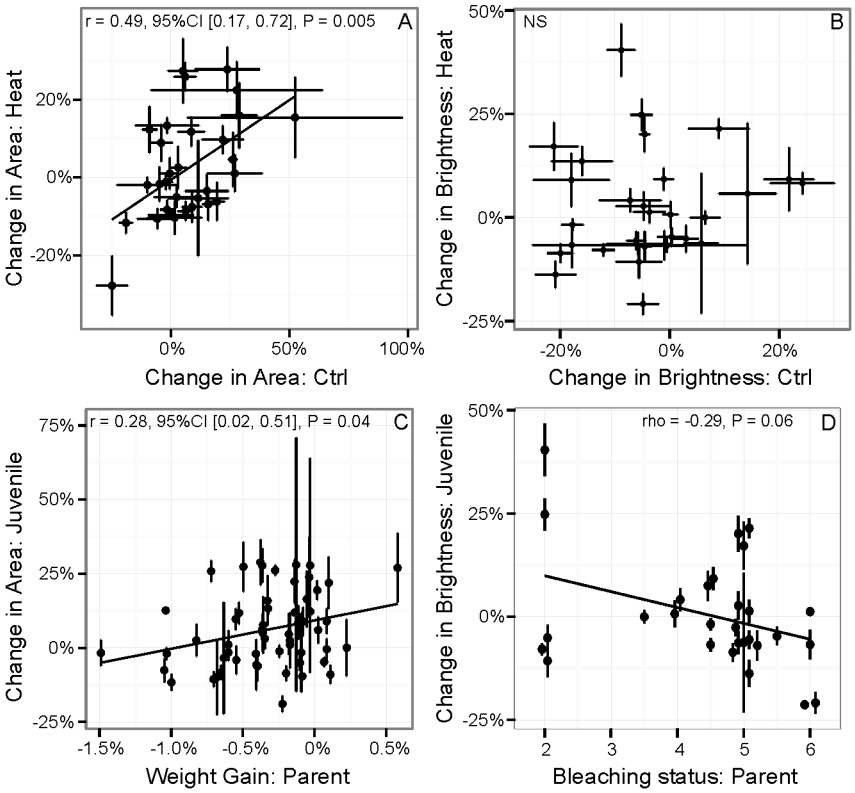


Figure 5.4. Heritability of fitness traits illustrated by differences in mean (± SEM) growth (A) and mean (± SEM) bleaching (B) among recruit families under both control and heat treatment and by parent-offspring regressions for mean (± SEM) growth (C) and mean (± SEM) bleaching (D).

were offspring of parents that exhibited the strongest bleaching under heat stress, as evidenced by low bleaching status scores (*rho* = -0.29, P = 0.06, Fig. 5.4D).

DISCUSSION

Population-level variation in temperature tolerance

Adult corals recapitulate previously reported population-specific responses to common temperature stress (Kenkel et al, 2013a): growth was uniformly depressed and offshore *P. astreoides* corals bleached sooner and more severely than inshore corals (Fig. 5.2A,D). Juvenile bleaching (measured by brightness as a proxy) are similar to adult phenotypes: offshore juveniles under heat treatment were the only individuals to show an increase in bleaching under heat stress (Fig. 5.2E,F). In addition, bleaching phenotypes were correlated between parents and offspring (Fig. 5.4D). However, naïve juvenile offspring exhibited strikingly different growth phenotypes when challenged in the same temperature stress environment. Initially, offshore juveniles exhibited higher growth rates, and individuals destined for heat treatment showed a significant growth advantage over inshore individuals assigned to the same treatment (Fig. 5.2C). This pattern was completely reversed during the common garden stress experiment. Following this 2.5-week period, inshore-origin juveniles displayed an overall growth advantage and significantly outgrew offshore-origin juveniles under heat treatment (Fig. 5.2C).

The differences in juvenile growth profiles between populations may be due to different maternal investment in inshore and offshore corals and its subsequent dissipation during recruit development. Adult corals from offshore reefs tend to out-grow inshore origin corals under control laboratory conditions (Kenkel et al, 2013a). A trend of elevated growth in offshore corals was also observed in the present study, further supporting these earlier reports (Fig. 5.2A). This pattern of elevated growth in both adult

corals and offspring appears to contradict the expected life-history trade-off between fitness and reproduction, where presumably a high investment in parental growth should preclude strong investment in offspring provisioning (Stearns, 1992). This discrepancy could be resolved by accounting for the absolute numbers of offspring produced by inshore and offshore corals: the higher growth rate of offshore recruits could have come at a cost of fewer larvae per parent overall. Indeed, offshore corals released fewer larvae than inshore corals during the spawning period, resulting in fewer total recruits (Fig. 5.1A). Still, the higher growth rate of offshore recruits did not seem to be due solely to higher maternal provisioning, as offshore-origin recruits were significantly smaller than inshore-origin recruits (Fig. 5.1B). However, recruit size is not necessarily a good proxy of energetic content. Larval size is correlated with energetic content in bryozoans of the genus *Bugula* (Wendt, 2000), but not in intertidal mussels (Phillips, 2007). The relationship between larvae and recruit size and energetic stores has yet to be established for *P. astreoides*. Furthermore, *P. astreoides* are capable of multiple spawning events annually (McGuire, 1998). Therefore additional data are needed to determine if inshore and offshore populations truly engage in alternative reproductive strategies.

Alternatively, the variation in growth among families of naïve juveniles before and after exposure to thermal stress may reflect genetically based thermotolerance responses. Adult inshore *P. astreoides* are more tolerant of thermal stress than offshore *P. astreoides*, as evidenced by differences in bleaching phenotype (Fig. 5.1D, Kenkel et al, 2013a). If this difference is genetically based, naïve inshore juveniles should display higher fitness under temperature stress than offshore juveniles. It must be noted that larvae were exposed to the parental environment when corals were at their native inshore and offshore reefs during the brooding period. *P. astreoides* release male gametes around the full moon and brooded larvae are released around the new moon (Chornesky and

Peters, 1987; McGuire, 1998), indicating that larvae developed in different environments for at least two weeks in situ. However, it is unlikely that developing larvae experienced different temperature environments during this period. Though inshore reefs in the Florida Keys experience warmer summer temperatures and colder winter temperatures, fall and spring water temperatures are similar between inshore and offshore reefs (Kenkel et al, 2013a) and were not significantly different in April 2012 when adult corals were collected (Briceno and Boyer, 2012). Therefore, elevated bleaching in offshore recruits under heat stress and the observed growth advantage of naïve inshore juveniles under heat stress (Fig. 5.1C,F) strongly suggests that thermotolerance in this species is indeed genetically heritable.

One possible mechanism underlying such heritable variation is differential expression of metabolic genes. Inshore and offshore adult *P. astreoides* display gene expression patterns that may reflect different energy allocation strategies, with offshore corals relying directly on symbiont-derived products and inshore coral operating mostly on stored reserves (Kenkel et al, 2013b). Similar variation in recruit populations could underpin the differences in growth rates through time and in response to temperature stress, but additional transcriptomic studies are needed to validate this hypothesis.

Yet one more possible explanation for the variation in growth and bleaching between populations is a difference in the hosted symbiont genotypes. As vertically transmitting brooding corals, *P. astreoides* are assumed to inherit their maternal symbionts (Thornhill et al, 2006) and early life growth trends may reflect this heritable symbiont component. Although previous studies demonstrated that symbiont complements in inshore and offshore populations of *P. astreoides* are indistinguishable at the level of internal transcribed spacer 2 of the rRNA gene (Kenkel et al, 2013a), the possibility of functionally relevant variation elsewhere in the symbiont's genome cannot

be excluded. Such genetic variation would be relevant for understanding evolutionary adaptation in this coral species as long as the evolutionary histories of host and symbiont remain linked through vertical transmission. The extent to which this relationship can be maintained in natural coral populations in the long term remains unknown and merits an in-depth investigation in the future using high-resolution genetic markers.

Heritability of fitness-related traits in corals

Broad-sense heritability, H_2, describes the summed contribution of genetic, epigenetic, and maternal effects in generating phenotypic variation (Falconer and Mackay, 1996). While this metric is a good approximation of adaptive potential in long-lived, clonal organisms, such as corals (Csaszar et al, 2010), it is a fraction of phenotypic variance attributable to additive genetic effects (termed narrow-sense heritability, h_2) that determines the immediate response to selection (Falconer and Mackay, 1996). Since h_2 is part of H_2 my estimates of broad-sense heritability for growth and survival in two populations of *P. astreoides* (Fig. 5.3) over-emphasize the true adaptive potential in these traits. However, the sheer magnitude of broad-sense heritability (36-39% of the variation in growth, 40-45% of the variation in bleaching and 94% of the variation in early survival, Fig. 5.3) suggest that the additive genetic component is likely to be significant as well.

Alternatively, these large broad-sense heritabilities can be attributed to strong maternal effects. Brooded larvae of *P. astreoides* depend on the resources of the maternal coral for completion of their development, nutritional provisioning and even their *Symbiodinium* complement (Richmond and Hunter, 1990). Large maternal effects have been inferred for other brooding coral species. Size variation in the larvae, presumably reflective of developmental time and/or rate within the maternal colony, correlates with

differential larval survival (Isomura and Nishihira, 2001). Furthermore, variation in the timing of larval release by the maternal colony can also affect subsequent recruitment success (Cumbo et al, 2012; Nozawa and Harrison, 2005), though this effect is known to dissipate with time (Goodbody-Gringley, 2010). Growth rates of brooded *Favia fragum* recruits exposed to different pCO2 treatments only begin to diverge after six weeks of treatment, possibly as maternal provisions are depleted (C. Lowery, pers. comm.). The disparity between heritability estimates for growth rate and bleaching during the rearing and experimental periods is most likely attributable to the dissipation of the maternal effects due to similar depletion of maternally derived resources (Fig. 5.3), indicating that heritability estimates obtained during the 2.5-week experimental period are likely more reflective of the true additive component.

Though few estimates of trait heritability are available for any coral species, the H_2 of 0.10-0.18 reported here for later growth and 0.17-0.22 for bleaching (Fig. 5.3) is comparatively low, given that the additive component is some fraction of these values. Meyer et al. (2009) used a diallel crossing design to evaluate the contribution of additive genetic variance to variation in a suite of traits under thermal stress in aposymbiotic larval families of *Acropora millepora*. They found that larval settlement rates and expression of a small heat shock protein (ßγ-crystallin) both had significant additive genetic components of 0.49 and 0.38, respectively (Meyer et al, 2009). A later study on the same species reports a similar estimate, with additive parental effects explaining 47% of the variance in larval settlement (Kenkel et al, 2011). Csaszar et al. (2010) estimated broad-sense heritabilities of symbiont, host and holobiont traits in adult *A. millepora*. Contrary to the results of Meyer et al. (2009), they report primarily non-significant heritabilities for host antioxidant gene expression (Csaszar et al, 2010). However, reported heritabilities for symbiont and holobiont traits are almost all significant and H_2

estimates for growth range from 0.19 to 0.59 (Csaszar et al, 2010). Still, it is not unusual to find heritability estimates that vary substantially among populations and species (Visscher et al, 2008), and considerable differences can be expected between species that differ in their reproductive strategy. *A. millepora* is a broadcast spawning coral, which releases egg-sperm bundles during annual reproductive events, and larval fertilization and development is independent of the maternal colony. Given the greater maternal investment of brooding species, strong maternal effects may be a more successful strategy for maximizing juvenile fitness. However, it must be noted that heritability is dependent on population specific parameters (Falconer and Mackay, 1996). Additional heritability data for both brooding and broadcasting coral species is needed to fully evaluate this conjecture.

Variation in early-life mortality: inbreeding and genetic load

The incredibly high heritability (H_2=0.94) of early juvenile mortality (Fig. 5.3) implies that nearly all of the variation in mortality, which ranged from 97 to 0 % among families, is attributable to some form of parental effects. Many marine larvae experience uniformly high mortality rates early in life, exhibiting the Type 3 survivorship curve typically taught in population ecology courses (Rauschert, 2010). In Pacific oysters, this pattern has been attributed to genetic load and reflects the effect of deleterious alleles segregating within the natural population (Launey and Hedgecock, 2001). In the case of *P. astreoides*, indiscriminate early-life mortality in some, but not all, families could be explained by the unmasking of recessive deleterious alleles as a result of inbreeding. Inbreeding has been observed in other brooding coral species (Ayre and Hughes, 2000; Ayre and Miller, 2006) and likely results from short-range larval dispersal typical of many brooding species (Hellberg, 1996; Nishikawa et al, 2003; Underwood et al, 2007)

generating highly related local populations. I estimated inbreeding for a subset of individual larvae from 9 families spanning a range of different survival values. I find no relationship between mean F_{IS} and percent survival across families (Fig. 5), suggesting that some other mechanism may be driving variation in juvenile mortality in these populations.

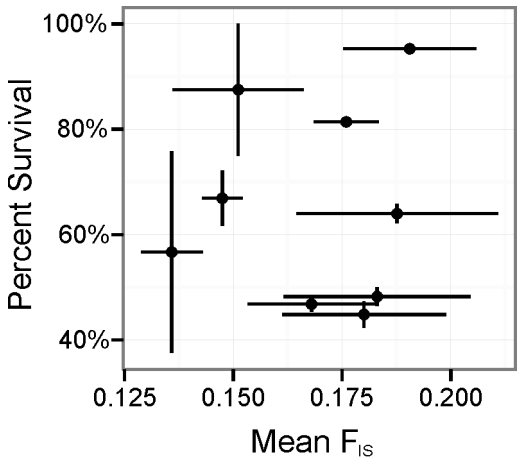


Figure 5.5. Mean (± SEM) percent survival as a function of mean (± SEM) F_{IS} (a measure of inbreeding) by family.

Heritability and selection: implications for "human-assisted evolution"

The worldwide decline of coral populations in this era of rapid climate change (Gardner et al, 2003; Hoegh-Guldberg et al, 2007) has prompted an interest in "human-assisted evolution" as potential means to breed more tolerant coral populations capable of coping with future climate warming and ocean acidification (Mascarelli, 2014). Understanding heritabilities and the underlying genetic architecture of targeted traits will be critical for evaluating the potential of such selective breeding programs. The breeder's equation was developed to improve animal and plant yields under artificial selection by providing a prediction of the phenotypic response to selection on a quantitative trait

(Falconer and Mackay, 1996). The product of the narrow sense heritability of a trait (h_2) and the strength of selection (s) predict the mean change in trait value per generation (ΔZ, expressed in units of standard deviations of the initial group), though more accurate predictions are obtained when genetic covariances of additional traits correlated with the trait of interest are taken into account (Lande, 1979). Given the heritabilities estimated here and assuming strong selection (only individuals in the top 10% of trait values are allowed to reproduce), growth rate will increase by at most 0.04 σ and bleaching tolerance will increase by at most 0.22 σ per generation. Though there are many caveats associated with estimating the effects of artificial selection (Hill and Caballero, 1992), this rough calculation suggests that, at least for this species, several generations of selective breeding will be necessary to evolve populations beyond the range of growth and bleaching phenotypes presently observed. As oceans are predicted to warm by 1-3°C within the next century (IPCC, 2013), such an implementation of "human-assisted evolution" would not be very efficient. The prospects might be better if greater genetic variation is accessed by bringing in individuals from other parts of the species' range (i.e., by combining human-assisted evolution with human-assisted migration, (Aitken and Whitlock, 2013).

CONCLUSIONS

My results represent the first estimates of broad sense heritabilities in fitness-related traits for a Caribbean reef-building coral. In addition, naïve juvenile corals representing inshore and offshore populations exhibited divergent growth and bleaching patterns in response to heat stress. These results support the hypothesis that previously observed divergence in thermotolerance between adult coral populations is due, at least in part, to genetic effects. The high heritabilities reported here for temperature tolerance

may explain the relative ecological success of this species in the face of severe environmental disturbances (Green et al, 2008). However, additional heritability estimates are needed for other Caribbean species to gain insight into the potential for genetic adaptation of these endangered organisms in the face of climate change. Future work will aim to understand the molecular basis of population-level variation in temperature tolerance and evaluate the role of natural selection in producing it.

Summary and Implications

This dissertation integrates across multiple levels of biological organization to investigate the pattern and scale of local adaptation in populations of *Porites astreoides* corals throughout the Florida Keys. This study is the first to investigate local adaptation in a Caribbean reef-building coral. This research presents a novel explanation for anomalous patterns of reef decline in the Florida Keys and provides insight into the role of the coral host in adaptation capacity.

In chapter 1, I present results of a yearlong reciprocal transplant experiment designed to test the hypothesis that spatial and temporal variation in local adaptation/acclimatization underpins patterns of reef decline in the Florida Keys. Although major differences in coral fitness were expected when comparing inshore-offshore coral populations, my results demonstrate that corals adapt and/or acclimatize to their local habitat on considerably finer scales and that this specialization incurs fitness trade-offs. This represents the first demonstration of local adaptation/acclimatization in a Caribbean coral. In addition, these results have broad implications for management of reefs in this ecosystem. Strong adaptation/acclimatization of corals to their local reef environment could affect the success of assisted migration efforts currently being employed in the Florida Keys (Jaap et al. 2006) since assisted migration could reduce fitness if local adaptation occurs in response to multiple environmental variables (Aitken & Whitlock 2013). Finally, adaptation of corals to increasingly differentiated habitats resulting from anthropogenic impacts (Pandolfi et al. 2005b) may render recruits from neighboring populations unfit for re-colonization of reef sites damaged by natural disturbances. Taken together, this work provides evidence for a novel hypothesis of

human-induced selection that structures coral populations in the Florida Keys, uniting disparate causes of reef decline and the observed lack of reef recovery in this ecosystem.

In chapter 2, I report the results of a common garden experiment evaluating the effects of elevated temperature as a selective agent on inshore and offshore coral populations in the Lower Florida Keys. I found that inshore corals are more resilient to temperature stress than offshore corals, consistent with the temperature extremes they experience in their natural reef environments. In addition, dinoflagellate symbionts (*Symbiodinium* spp.) of offshore corals exhibited reduced photochemical yields, suggesting that between-population divergence in thermotolerance may be symbiont-driven. However, I did not detect differences in the genotypic composition of *Symbiodinium* communities hosted by inshore and offshore corals or genotype frequency shifts ("shuffling") in response to thermal stress. Instead, coral host populations showed significant genetic divergence between inshore and offshore reefs, suggesting that in *P. astreoides*, the coral host might play a prominent role in holobiont thermotolerance variation.

Chapters 3 and 4 use gene expression profiling of corals from the common garden and reciprocal transplant experiments to understand the molecular phenotypes in host corals that may underpin population level variation in thermotolerance and patterns of adaptation/acclimatization. In chapter 3, I report on the construction of the first transcriptomic reference for *P. astreoides* and describe candidate gene expression profiles obtained from the same corals used in the common garden thermal stress experiment. The most notable signature of divergence between coral populations was constitutive up-regulation of metabolic genes in corals from the warmer inshore location, including the gluconeogenesis enzymes pyruvate carboxylase and phosphoenolpyruvate carboxykinase and the lipid beta-oxidation enzyme acyl-CoA dehydrogenase. These results highlight

several molecular pathways that were not previously implicated in the coral stress response and suggest that host management of energy budgets might play an adaptive role in holobiont thermotolerance. In chapter 4, I employed global gene expression analyses using RNA-seq in conjunction with co-expression network analysis to explore the relationship between host gene expression patterns and phenotypic trait variation in corals from the reciprocal transplant experiment. I found that inshore corals exhibit more plastic expression than offshore corals. In particular, genes involved in the environmental stress response (Gasch et al. 2000) are more strongly regulated in inshore origin corals, and corals with the highest expression of these genes maintained the highest symbiont densities following transplantation. Taken together, these results suggest that the ability to regulate expression is important for local adaptation of these populations, and that expression variation may underpin phenotypic robustness to environmental variation.

Finally, in chapter 5 I quantify variation in growth rate and survival in 38 families of *Porites astreoides* coral recruits to evaluate the potential for continued evolutionary change. I find significant broad-sense heritability for growth, though estimates diminished through time, suggesting the influence of a strong maternal component early in life. In addition, naïve juvenile coral recruits from inshore reefs also exhibited a growth advantage over offshore recruits under elevated temperature treatment, suggesting that thermotolerance differences observed in adult populations have a genetic basis and that this trait could continue to evolve in response to climate change.

Appendix

Table A1. Wald test statistics for individual factors and interaction terms within the top model for growth response.

Factor	num DF	denDF	F-value	P-value	Corrected* P-value
(Intercept)	1	211	792.4	0.000	0.000
Origin	1	56	10.4	0.002	0.004
Destination	2	211	40.7	0.000	0.000
Region	1	56	11.3	0.001	0.003
Time	1	211	182.0	0.000	0.000
Origin x Destination	2	211	15.7	0.000	0.000
Origin x Region	1	56	11.8	0.001	0.003
Destination x Region	2	211	2.9	0.057	0.076
Origin x Time	1	211	1.8	0.183	0.209
Destination x Time	2	211	3.5	0.031	0.045
Region x Time	1	211	0.3	0.596	0.599
Origin x Destination x Region	2	211	6.4	0.0019	0.004
Origin x Destination x Time	2	211	3.9	0.022	0.035
Origin x Region x Time	1	211	3.4	0.067	0.082
Destination x Region x Time	2	211	1.0	0.383	0.409
Origin x Destination x Region x Time	2	211	17.5	0.000	0.000

* Significance value corrected for multiple testing using the method of (Benjamini & Hochberg 1995).

Table A2. Wald test statistics for individual factors and interaction terms within the top model for change in robustness.

Factor	numDF	denDF	F-value	P-value	Corrected* P-value
(Intercept)	1	93	7.0	0.010	0.031
Origin	1	50	0.8	0.374	0.665
Destination	1	93	28.7	0.000	0.000
Region	1	50	9.1	0.004	0.016
Time	1	93	3.9	0.051	0.136
Origin x Destination	1	93	10	0.002	0.011
Origin x Region	1	50	0.04	0.834	0.975
Destination x Region	1	93	0.7	0.418	0.669
Origin x Time	1	93	0.007	0.936	0.975
Destination x Time	1	93	0.005	0.943	0.975
Region x Time	1	93	0.001	0.975	0.975
Origin x Destination x Region	1	93	1.8	0.187	0.374
Origin x Destination x Time	1	93	2.3	0.135	0.310
Origin x Region x Time	1	93	0.4	0.520	0.756
Destination x Region x Time	2	93	0.2	0.630	0.840
Origin x Destination x Region x Time	2	93	29.4	0.000	0.000

* Significance value corrected for multiple testing using the method of (Benjamini & Hochberg 1995).

Table A3. Summary of eight polymorphic *Porites astreoides* SSR markers assessed across 21 individuals from the offshore site of Sugarloaf Key, Florida.

Locus (Repeat)	Primer Sequence 5'-3'	Observed (bp)	N	Na	Ho	He	GenBank Accession no.	HWE
Past_17 (ATTG)x	F: FAM-accaaaatgcttcctcgttg R: agcggccactttcttctgta	272-316	21	8	0.833	0.866	GW267284	0.065
Past_21 (ATGx)x	F: FAM-ttggagatcagtcgcacaaa R: tctctcacttgcgggttctt	180-222	20	10	0.800	0.798	GW254471	0.825
Past_3 (CAT)x	F: FAM-cagttgttctaagctcgccc R: gggttttgaagtgccagaaa	429-459	21	6	0.810	0.790	GW257722	0.130
Past_8 (TTA)x	F: FAM-acgtgaaggcaaggaaaatg R: caacaacaacaattcgccac	384-414	21	8	0.714	0.787	GW255333	0.513
Past_10 (TTA)x	F: HEX-caacgatgtgggtgtagacg R: ctgcggaccaacttaagagc	387-474	20	13	0.600	0.866	GW248775	0.000*
Past_13 (CAT)x	F: HEX-ttgcaggctaggtacaggct R: ccctgaacactgagggtcat	424-460	21	6	0.649	0.664	GW250389	0.000*
Past_16 (CAT)x	F: NED-ggtcggtatggtcgaagaaa R: ccttggcctccgttaagata	264-279	21	5	0.714	0.743	GW249713	0.598
Past_2 (TAA)x	F: NED- cccttccccaaaaattctgt R: tgactgggtcgatgttgtgt	363-414	21	14	0.952	0.872	GW275092	0.052

N: number of individuals, Na: number of alleles, Ho: observed heterozygosity, He: expected heterzygosity, HWE: Hardy-Weinberg Equilibrium
(a) "FAM," "HEX" or "NED" at the 5' –end of the primer indicate FAM, HEX or NED-labeled fluorescent primer
* Deviates significantly from Hardy-Weinberg Equilibrium (HWE) after bonferroni correction ($\alpha = 0.006$)

Figure A1. Inshore and offshore reefs in the Florida Keys exhibit differences in water quality and temperature. Mean ± SE for measures of (A) Benthic dissolved inorganic nitrogen, (B) total organic carbon, (C) total phosphorous and (D) turbidity from 1995-2011, provided by the SERC-FIU Water Quality Monitoring Network which is supported by EPA Agreement #X994621-94-0 and NOAA Agreement #NA09NOS4260253. Benthic temperature profiles for the (E) Lower and (F) Middle Keys were obtained by in-situ data loggers set to record every 15 minutes. Horizontal bars represent the mean winter (Dec-Feb) and summer (Jun-Aug) temperatures at each reef site.

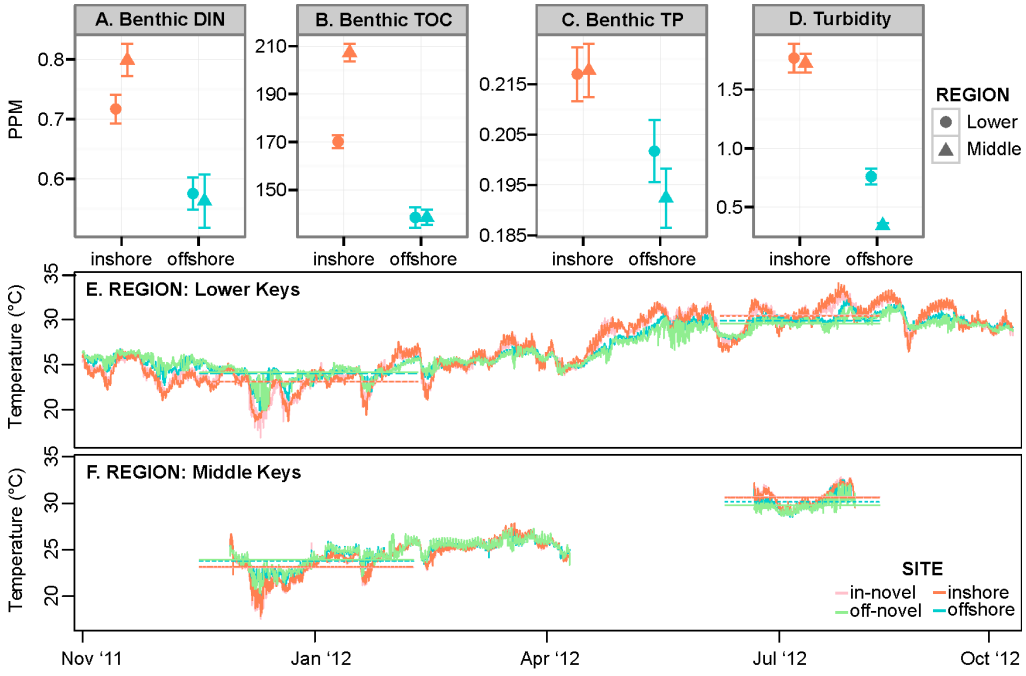


Figure A2. Mean phenotypic trait values ± SE of corals at each site and time point. Significance of pair-wise comparisons are derived from MCMCglmm models.

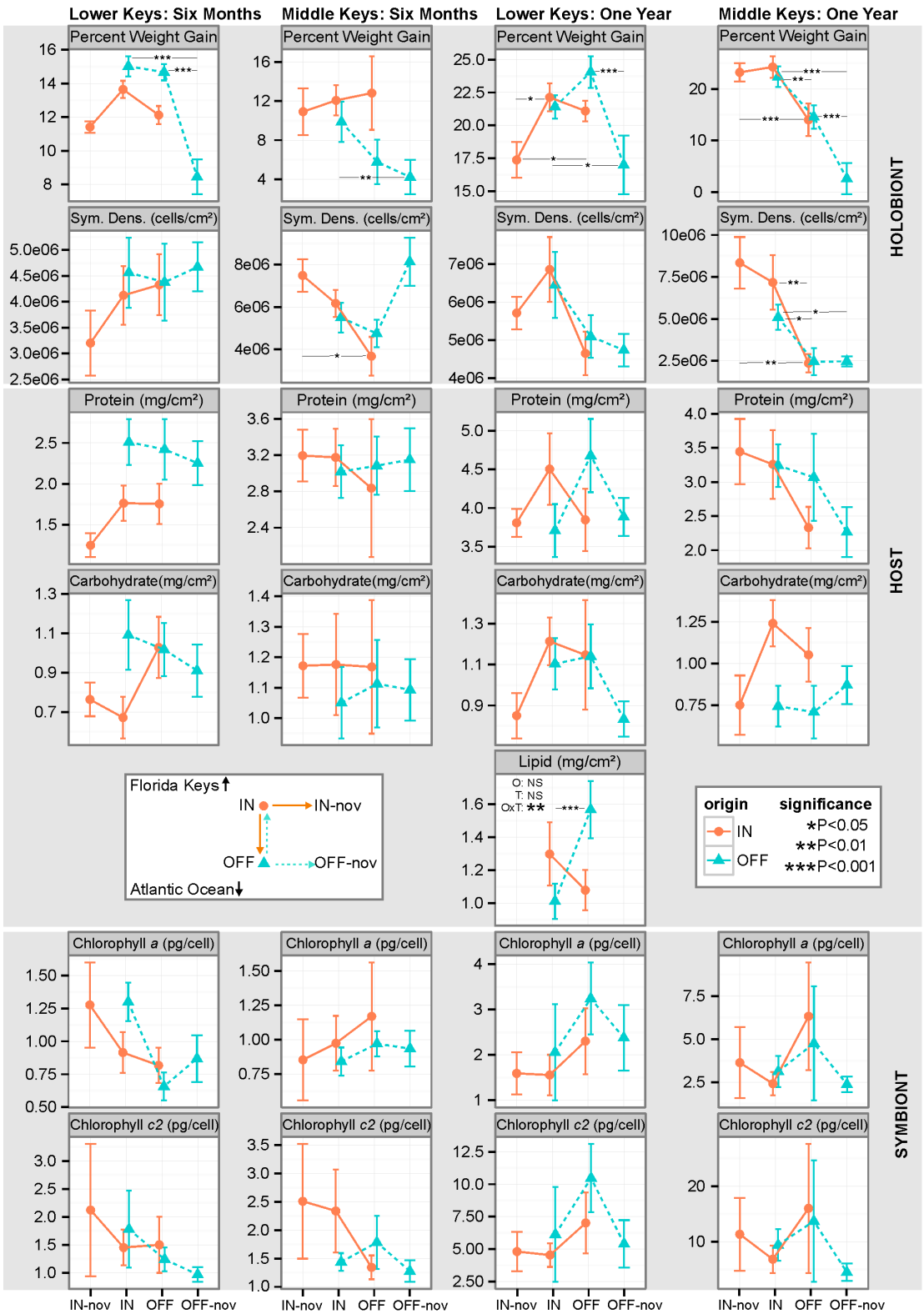

Lower Keys: Six Months
Middle Keys: Six Months
Lower Keys: One Year
Middle Keys: One Year
HOLOBIONT
HOST
SYMBIONT
Percent Weight Gain
Sym. Dens. (cells/cm²)
Protein (mg/cm²)
Carbohydrate(mg/cm²)
Lipid (mg/cm²)
Chlorophyll a (pg/cell)
Chlorophyll c2 (pg/cell)
IN-nov
IN
OFF
OFF-nov
Florida Keys
Atlantic Ocean
O: NS
T: NS
OxT: **
origin
significance
*P<0.05
**P<0.01
***P<0.001

Figure A3. Correlations between phenotypic trait data and percent growth indicate weak positive relationships overall.

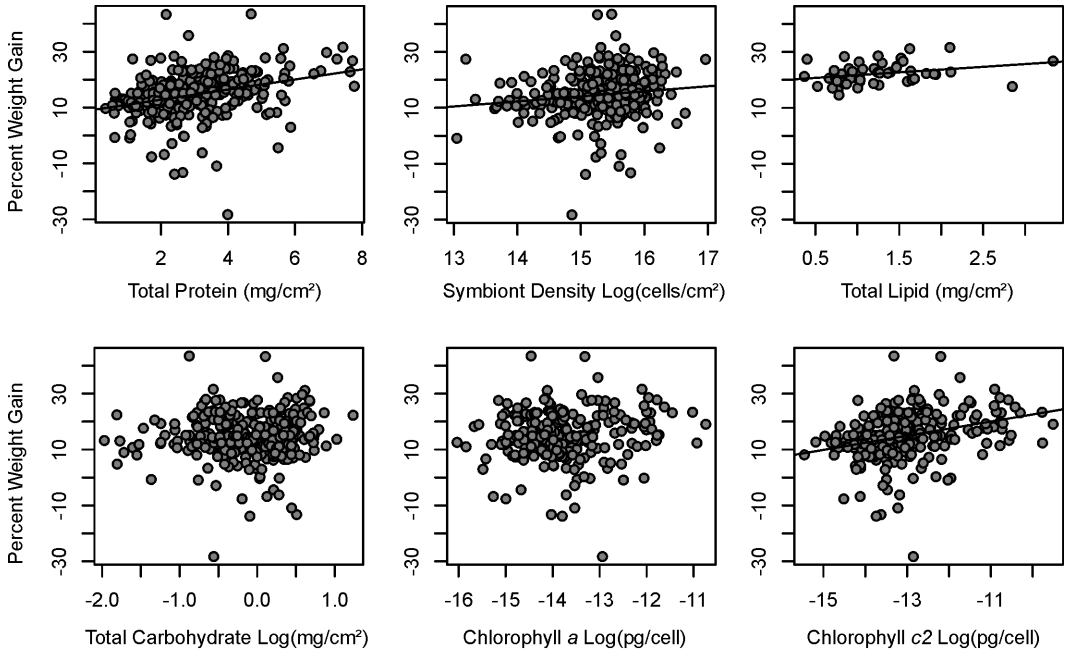


Figure A4. (A) Mean temperature (± one standard deviation) in each treatment tank for the duration of the six-week experiment. (B) Continuous temperature data (measurements taken every 8 min) for the one control and the one heat tank equipped with HOBO (Onset) data loggers for the duration of the six-week experiment.

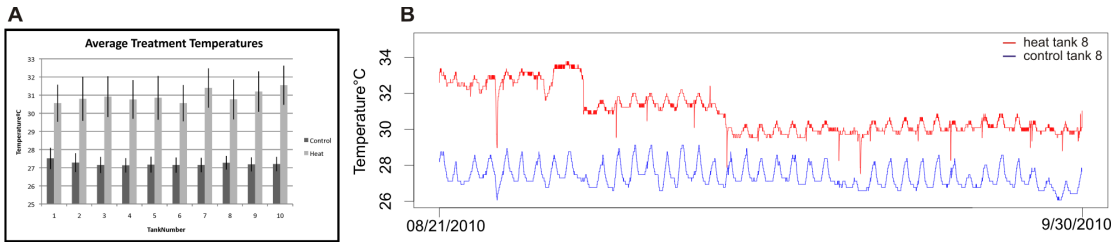


Figure A5. Mean parameter Q ± 95% profile likelihood confidence intervals for individual sub-populations and the entire dataset.

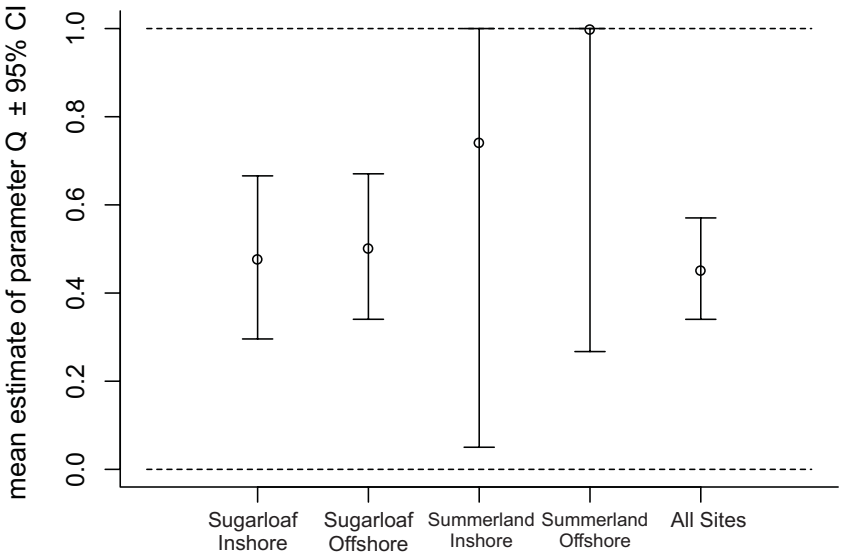


Figure A6. (A) Histogram of high-frequency 100% identity sequence clusters indicating size of the cluster, e.g. the ">25" bar indicates the number of clusters that were assigned at least 25 individual sequence reads. (B) The proportion of total read data represented in the high-frequency clusters.

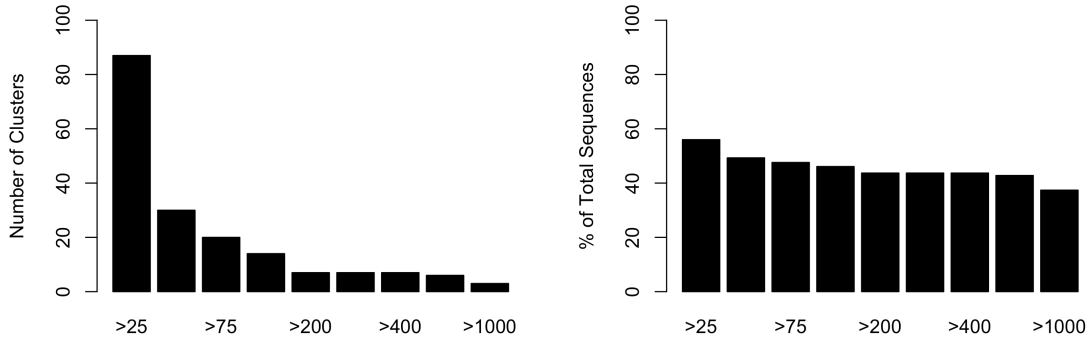


Figure A7. Correlations of frequencies of haplotypes 3 and 6 for inshore and offshore populations.

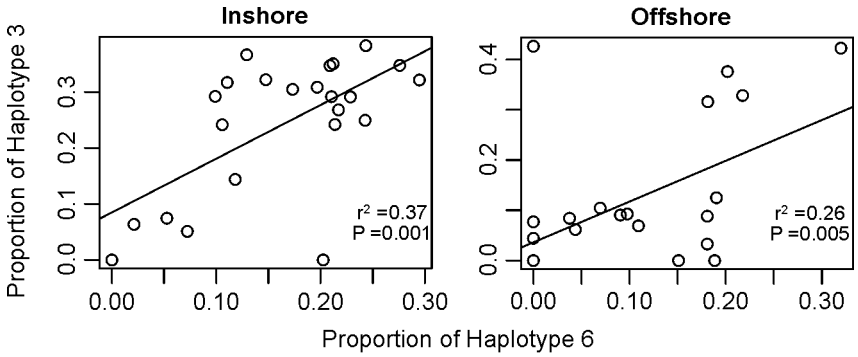


Figure A8. Pairwise correlations between frequencies of dominant haplotypes (1, 2, 4 and 7) among individual colonies across all populations and experimental treatments.

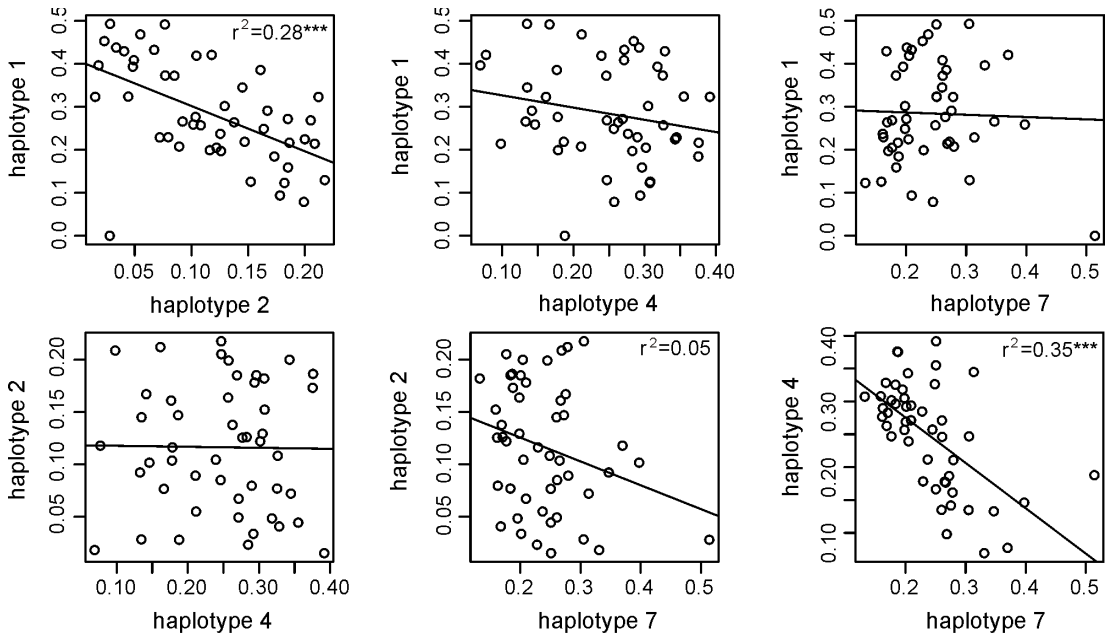


Figure A9. Mapping of the KEGG-annotated metabolic pathways from the *P. astreoides* transcriptome to pathways annotated in the fully sequenced genome of the sea anemone *Nematostella vectensis*. The grey lines correspond to pathways found in the *N. vectensis* but absent in the *P. astreoides* transcriptome, which might indicate absence in the *P. astreoides* genome or a lack of expression. Extensive overlap between the two maps (red lines) indicates that the *P. astreoides* transcriptome represents a relatively complete database for analysis of metabolism-related gene expression changes.

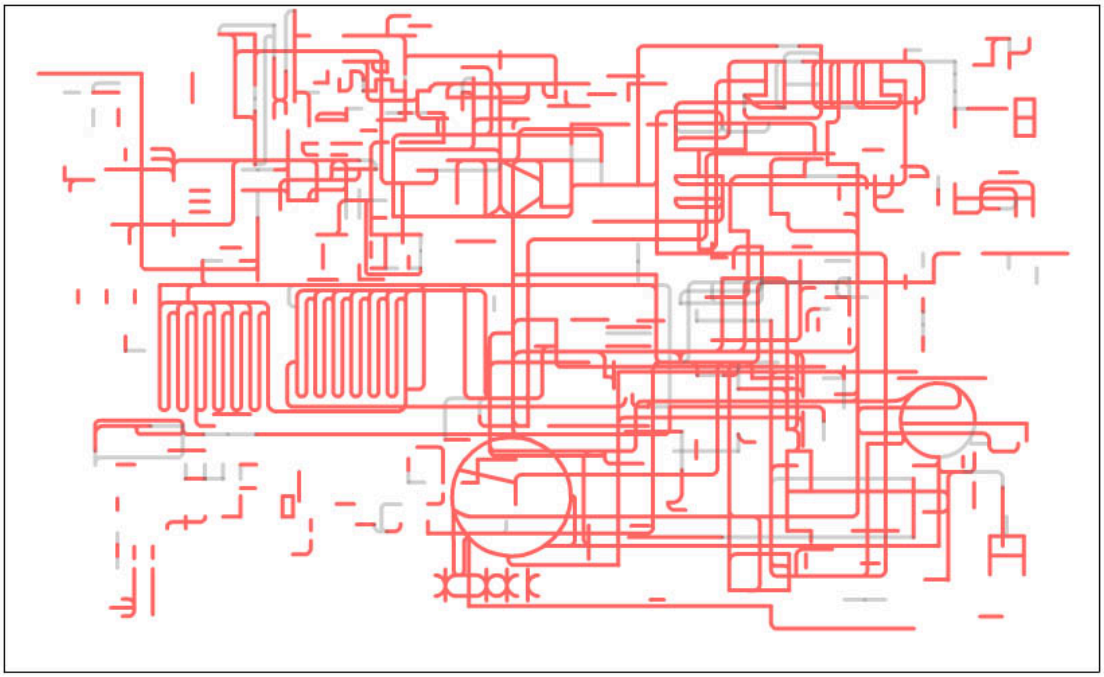


Figure A10. Red channel brightness values in intensity-normalized photographs of heat-treated coral fragments illustrating their classification into “pale” and “bleached” categories.

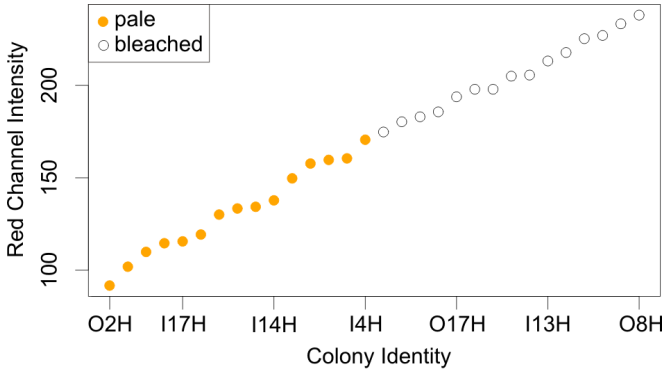


Figure A11. Gene expression in response to chronic heat stress in populations of *Porites astreoides* from different thermal environments. Normalized log2 transformed expression values (± SEM) of candidate genes with respect to origin (red circles: inshore, blue triangles: offshore) and experimental treatment. Significance of post-hoc Tukey’s HSD comparisons between bleaching phenotypes is shown for genes with P<0.1 after false discovery rate correction (Benjamini& Hochberg 1995). Candidates with significant origin terms following FDR correction have an “origin” designation in the panel. ACAD: Acyl-CoA dehydrogenase; AGXT: Alanine-glyoxylate aminotransferase; PEPCK: Phosphoenolpyruvate carboxykinase; Hsp: Heat shock protein; SLC26: member of solute carrier family 26, LTS: Long-term stress double-gene assay, BIP: Bleaching-in-progress double-gene assay.

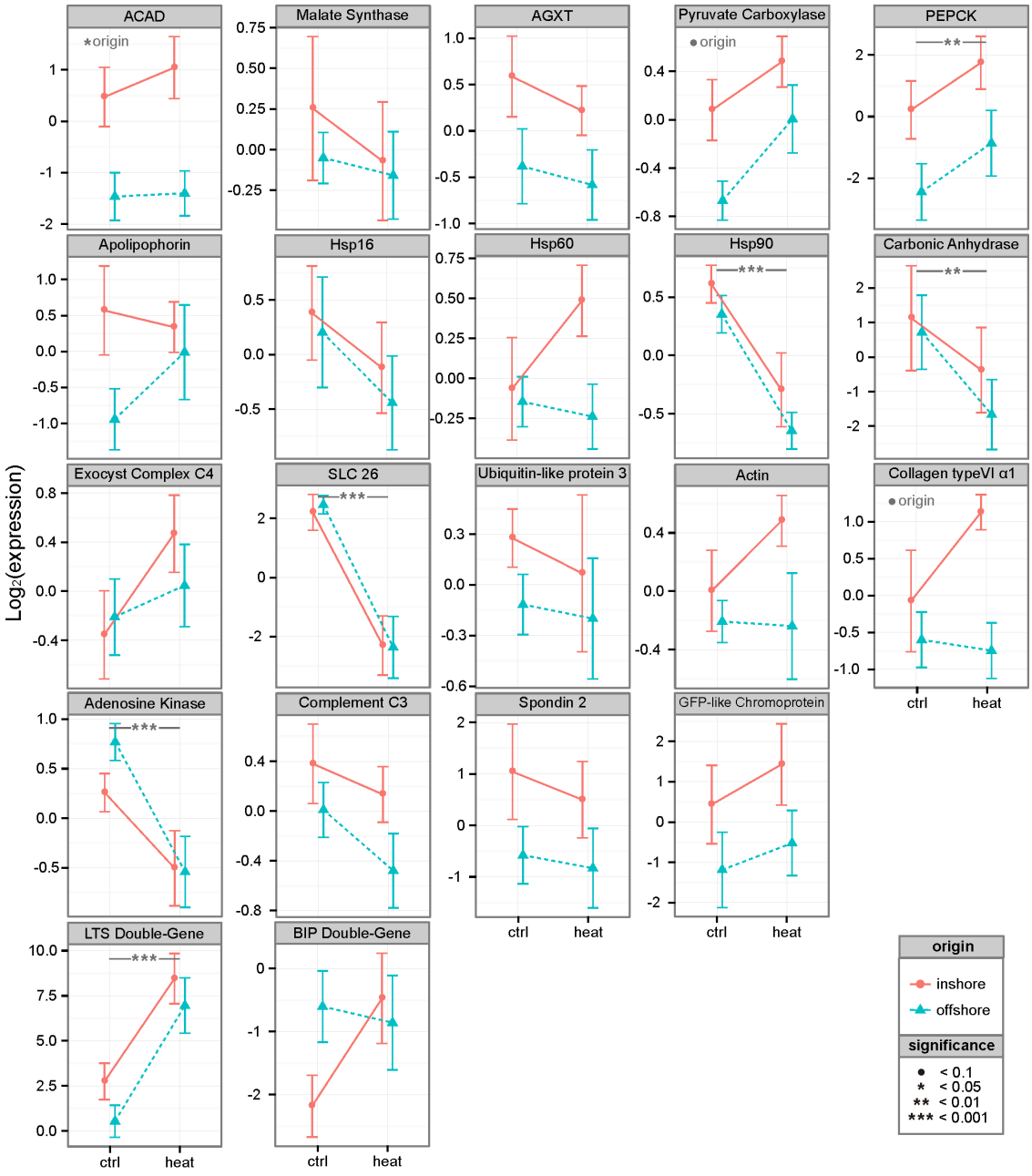

ACAD
*origin
Malate Synthase
AGXT
Pyruvate Carboxylase
•origin
PEPCK
**
Apolipophorin
Hsp16
Hsp60
Hsp90
***
Carbonic Anhydrase
**
Exocyst Complex C4
SLC 26
***
Ubiquitin-like protein 3
Actin
Collagen typeVI α1
•origin
Adenosine Kinase
***
Complement C3
Spondin 2
GFP-like Chromoprotein
LTS Double-Gene
***
BIP Double-Gene
Log2(expression)
ctrl
heat
origin
inshore
offshore
significance
• < 0.1
* < 0.05
** < 0.01
*** < 0.001

Figure A12. Analysis of network topology for select soft-thresholding powers. The scale-free fit index as a function of the soft-thresholding power is shown on the left, while mean connectivity as a function of the soft-thresholding power is on the right. I selected the power 18, the lowest value for which the scale-free topology fit index curve plateaus upon reaching a high value.

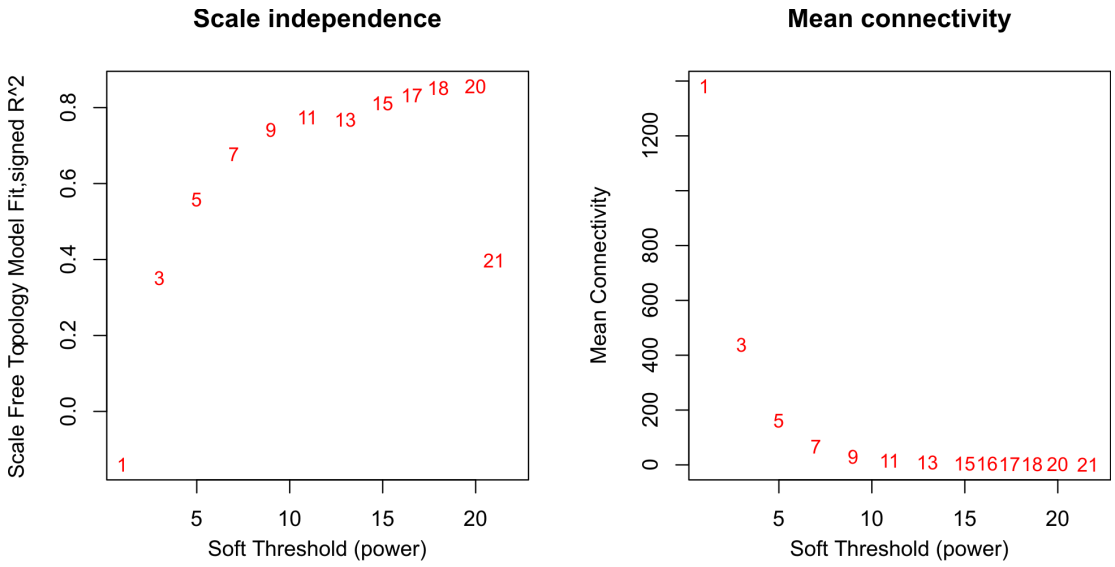


Figure A13. Clustering dendrogram of top DEGs, with dissimilarities based on topological overlap shown with assigned module colors (Dynamic tree cut) and upon merging modules whose expression profiles were 80% similar (Merged dynamic).

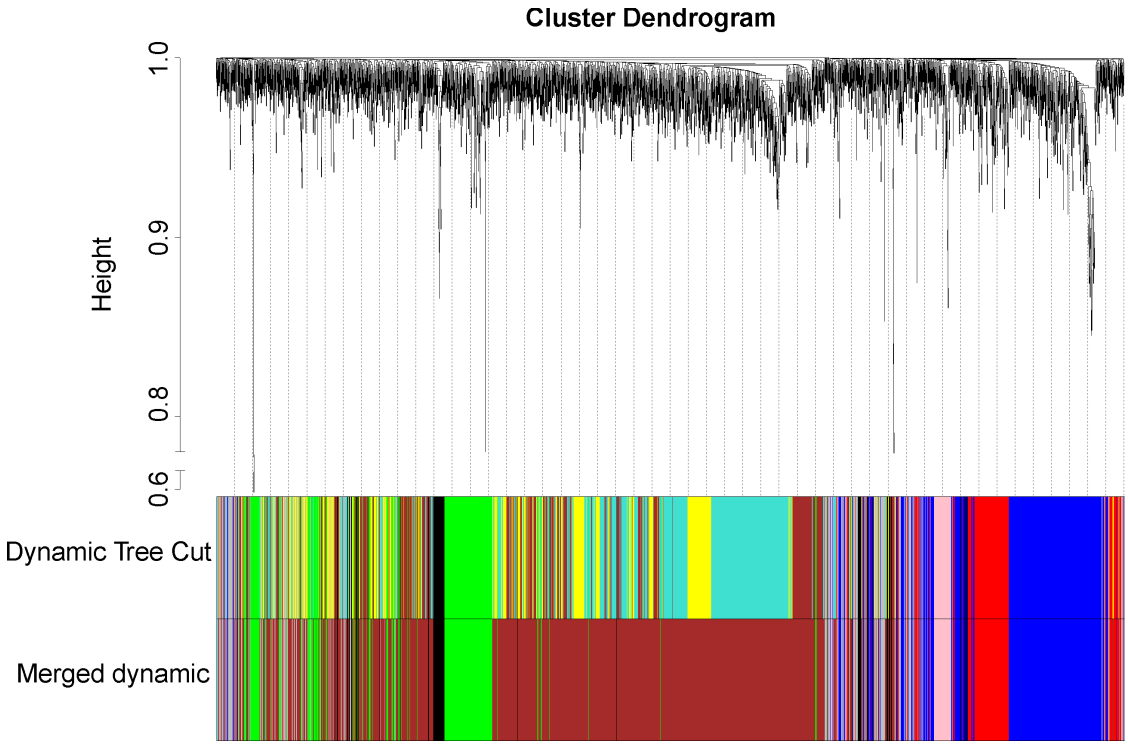


Figure A14. Correlations between gene significance for specified traits and module membership in the brown, blue, green, red and black modules.

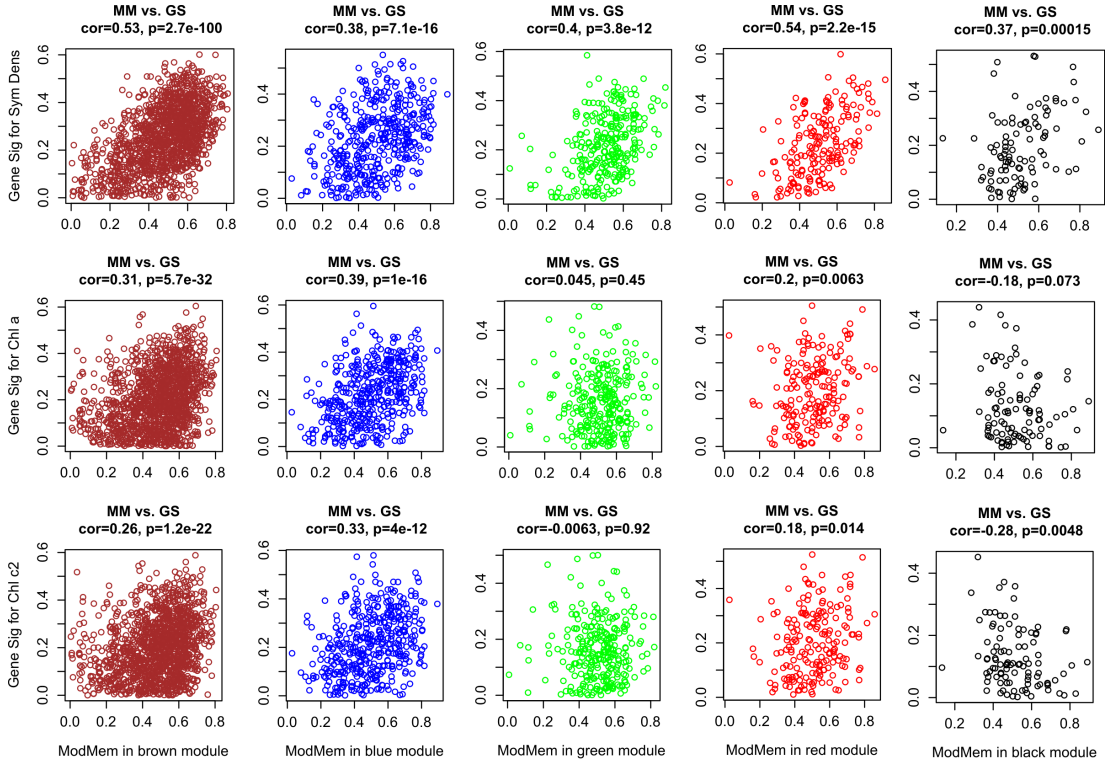


Figure A15. Heatmaps of all genes assigned to the red, black and pink modules for individual corals by site of origin and transplant destination.

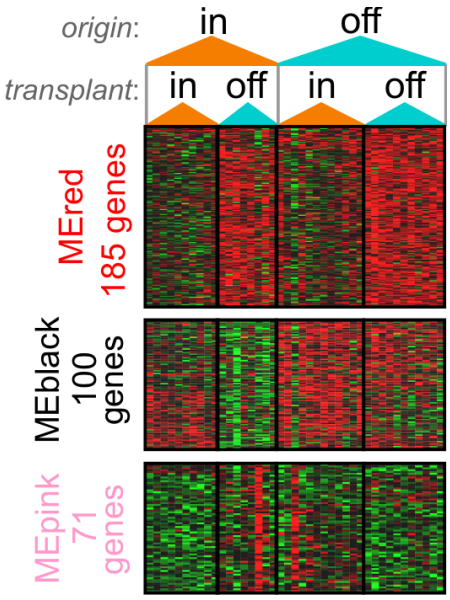


Figure A16. Photographs of coral fragments collected at the inshore site (A) and the offshore site (B) following one year of transplantation. Genotypes 1-15 are inshore origin; 16-30 are offshore origin. Four corals in (A) show signs of bleaching: genotypes 16, 17, 21 and 27.

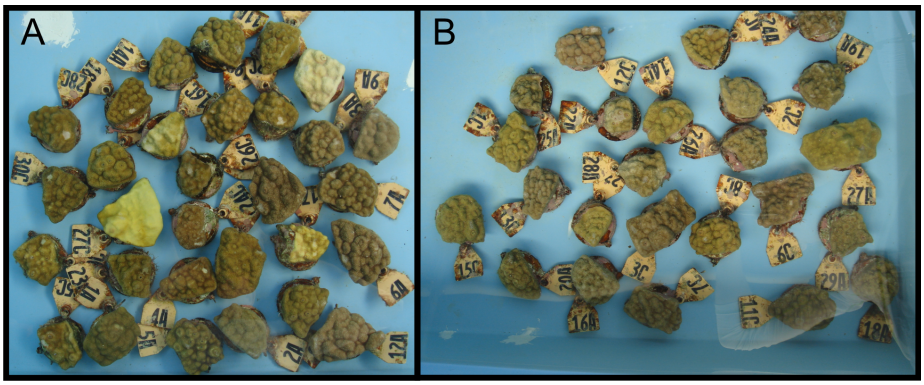


Figure A17. Mean ± SE of individual trait measurements with respect to origin (inshore=circles, offshore=triangles) and transplant location (x-axis).

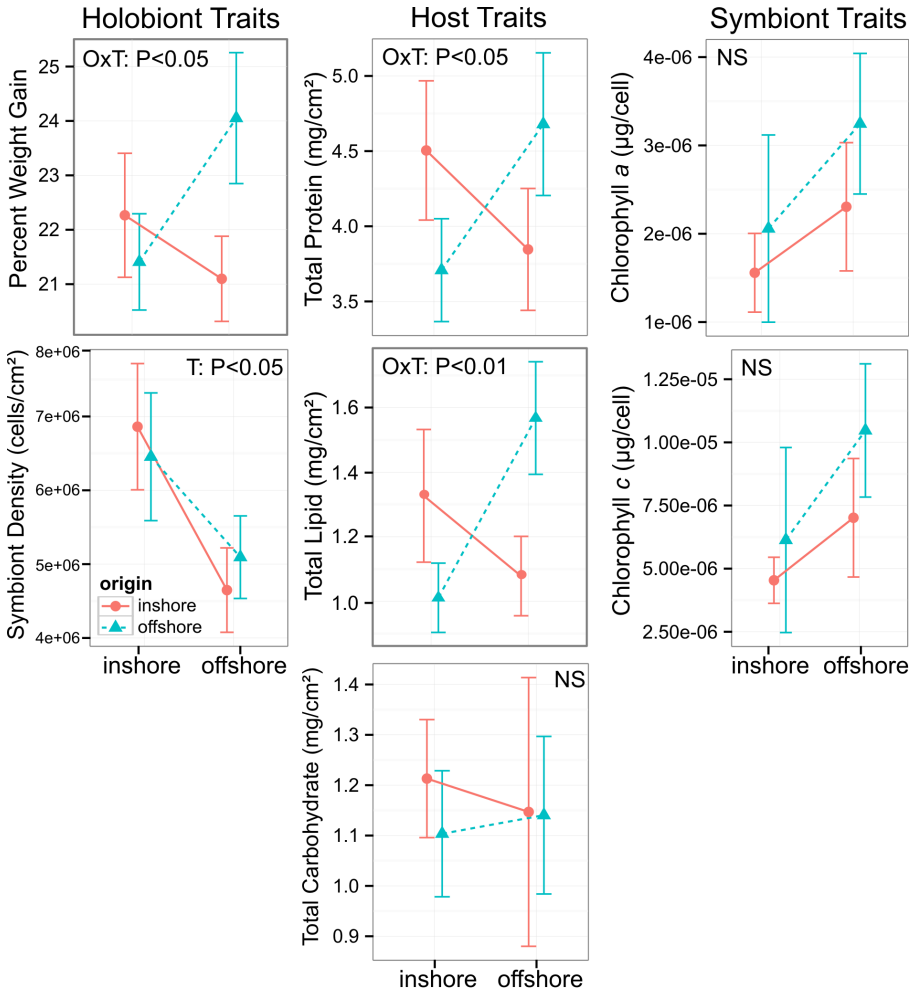
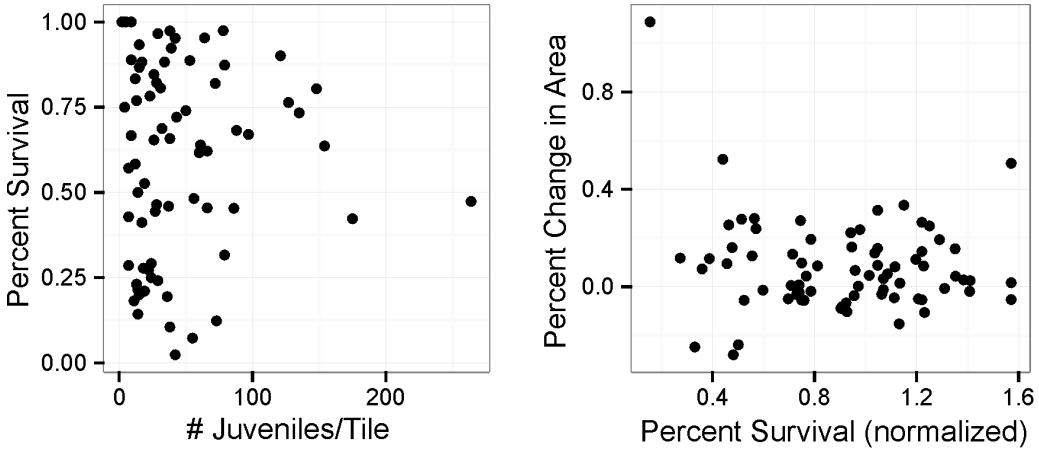


Figure A18. Survival among recruit families during the rearing period as a function of recruit density and differences in growth during the common garden experiment as a function of normalized survival among recruit families.



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