Elucidating the Physiological and Genomic Mechanisms of Environmental Memory and Acclimatization in the Coral Holobiont

Overview

Populations of critical reef-building corals worldwide have been decimated as a result of increasing thermal pressure driving coral bleaching, disease outbreaks, and local human impacts (Hughes et. al. 2003). Conservation efforts have included restoration practices like coral gardening and assisted evolution, but the long-term efficacy is not well defined. In order to guide effective long-term conservation, this study investigates both the physiology and genomic mechanisms that underlie the process of acclimatization of the coral holobiont. Particularly, the potential for “environmental memory” after reoccurring stress events. Here we propose an environmental memory by reduced reactions theory, which hypothesizes the change in physiological and genomic response will decrease with each stress event as the organisms moves towards an optimum performance for their local environment. We propose to investigate the validity of this theory and elucidate the relationship between gene expression and epigenetic markers that have downstream impacts on phenotype and function. This study will also assess the influence of each symbiotic partner within the coral holobiont on the organism’s change in phenotype over the process of acclimatization. The knowledge gained about the process of acclimatization and potential for environmental memory will be instrumental in advising the most effective long-term restoration practices.

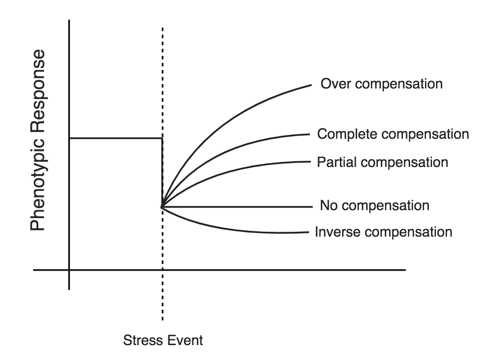
Intellectual Merit

The proposed study is an integrative approach to assessing acclimatization that presents a novel framework of the mechanisms behind “environmental memory” that would be applicable to all invertebrates, not solely corals. Given the rapidly changing environment surrounding coral reefs, the timing of this study is imperative to guiding effective restoration efforts. Using genomic methods for conservation is at the fore-front of climate change research and is quickly gaining merit in predicting potential survival of populations in future climate change conditions. This proposed work will further knowledge in *both* academic fields and applied conservation. The projects proposed in this study have a strong foundation of preliminary field work to expand on. In addition, the research teams involved have the experimental, physiology, and molecular experience necessary as well as strong, existing collaborations with the proposed field sites.

Broader Impacts

The research team has extensive experience in and holds a strong emphasis on science communication and outreach. All data, code, and results will be uploaded to GitHub to promote open science and reproducibility. Infographics and short films depicting the methods and analyses of the projects, are made for science communication. The team frequently leads education-based activities including SkypeAScientist, Letters to a Scientist, and neighboring school and community visits (near home university *and* field sites). Public speaking about this project and application to conservation will include seminars at universities, local aquariums, and communities at the field sites. Members of the team partner with The Ocean Agency and the International Coral Reef Society Student Chapter to organize outreach events world-wide as well as marine institutions near heavy diving communities. As a result of this women-led study, several graduate and undergraduate students from underrepresented groups will gain research experience vital to their early careers in this field. The proposed work applies cutting edge genomic and physiological methods to effective long-term conservation and science communication efforts.

**1. Introduction**

The rapidly changing environments that surround coral reefs are forcing organisms to either migrate, acclimatize, or adapt in order to survive their new, stressful conditions. Adaptation refers to changes in allele frequencies over several generations within a population (Wilmer et al 2009). Whereas acclimatization has been defined as a long-term change in phenotype within the lifetime of an organism in response to environmental change that does not involve a change in gene sequence, but rather physiological or biochemical (van Oppen et al 2015; Wilmer et al 2009). These reactions can range from a response greater than the original phenotype (over compensation), to a lack of response after the stress event (no compensation), and to a further decrease in response (inverse compensation) as seen in Figure 1 on the left (Phenotypic responses to a single stress event; modified from Edmunds and Gates 2008). Whether complete compensation is beneficial to the organism or not is context-dependent. However, the physiological response to reoccurring stressors and the mechanisms that underlie this response is likely to be much more complex (Edmunds and Gates 2008). For example, prior exposure before a stress event occurs has been suggested to be critical in shaping the phenotypic response of organism to that stressor. Preceding sublethal thermal exposure has been shown to reduce the negative impacts of a subsequent, intense thermal stress event on coral reefs (Ainsworth et al 2016; DeCarlo 2019). Naturally occurring high-frequency temperature variability reefs have experienced less severe bleaching events (Sully et al 2019; Safaie et al 2018), supporting the essential role thermal history plays in physiological response and the potential for an “environmental memory”. However, the mechanisms underlying this potential for environmental memory are not well understood.

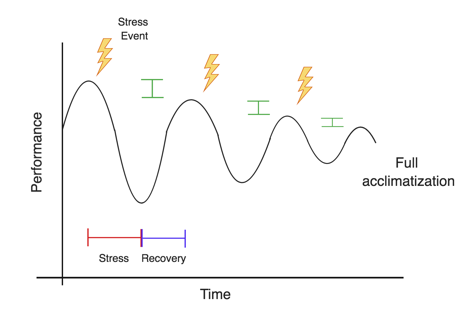
A recent study showed corals from an environment with high thermal variability, compared to corals from environments with lower thermal variability, had a smaller change in gene expression in response to acute heat stress, but had higher levels of expression to start with in genes involved in stress response (Barshis et al 2013). This reduced reaction response suggests that the more resilient corals could be “front-loading” genes needed for thermotolerance (Barshis et al 2013). Regulation of gene expression, especially in genes involved in an overall stress response, is likely to be vital in the process of acclimatization and beneficial phenotypic response.

Another potential mechanism of environmental memory is epigenetic markers. These markers include the heritable post-synthesis modification of DNA, without a change in the DNA sequence itself, which enable the genome to produce multiple outcomes from the same genetic material, via changes in gene expression, induced by environmental triggering (Eirin-Lopez and Putnam 2018). The best-studied mechanism to date is DNA methylation, the addition of a methyl group to DNA nucleotides, most commonly on cytosine in the sequence CpG in animals. The environment can trigger *de novo* DNA methylation, which can be heritable by way of maintenance DNA methyltransferases that propagates hemi-methylated DNA during cell replication and even during meiosis (Eirin-Lopez and Putnam 2018). In this way, the environment an organism experiences can shape its response to stress within a generation and across generations. Epigenetic modifications affect the accessibility of genes to transcription factors and thus alter gene expression and consequently function and phenotype. **The genomic mechanisms, such as gene expression regulation and epigenetic markers, underlying an organism’s phenotypic response and potential for environmental memory need to be fully elucidated in order to evaluate an organism’s ability to acclimatize and thus predict survival potential in future climate change driven conditions.**

In addition to the responsiveness of the coral host due to genetic mechanisms, corals also harbor a diverse range of eukaryotic and prokaryotic microbes that are essential for coral function (Glasl et. al. 2016). Anthropogenic-driven climate change has disrupted the symbiotic relationship with this microbiota (Hong et. al. 2009), which leads to negative effects on the coral host’s physiological performance (Littman et. al. 2011). Diversity in the symbiotic microbiome (e.g., dinoflagellates and bacteria) can play a driving role in coral performance as these symbionts provide critical nutritional and metabolic provisions to the host (Bourne et. al. 2016). For example, Symbiodiniaceae in the genus *Cladocopium* (Clade C) produce and translocation more carbon than those in the genus *Symbiodinium* (Clade A) (Stat et al. 2008). The bacterial microbiome is also associated with coral propensity to bleach (Morrow et al. 2018). **Therefore, clarifying the microbiota’s role in host performance in response to stress will be critical moving forward with evaluating acclimatization and effective restoration efforts.**

In a targeted effort to re-populate and sustain coral reef ecosystems, restoration has been implemented in a variety of locations worldwide. These restoration nurseries take advantage of the ability of reef building coral to asexually reproduce as clonal fragments, in which a single fragment can be split, grown, and split again to produce an exponentially growing population. Past genetic research suggests conservation of corals should be focused on sustaining local populations based on the limited genetic exchange across regions (e.g., in the Caribbean) (Hemond et. al. 2010). Coral genotype has been shown to strongly influence a coral’s ability to acclimate and survive (Drury et. al. 2017) and the importance of standing genetic variation has been reiterated in several studies regarding a population’s predicted ability to adapt to future climate change scenarios (Jordan et al 2017; Bay et al 2018). More recent conservation approaches have advocated more invasive measures such as selective breeding, assisted evolution, and assisted migration (van Oppen et. al 2015, Anthony et al. 2017). **In order for these approaches to be successful, genetic and epi-genetic diversity of the coral holobiont needs to be maintained and its role in physiological performance needs to be quantified.**

Overall, many gaps in knowledge remain regarding the mechanisms underlying phenotypic responses during the process of acclimatization. Both physiological and genomic changes are made in the coral holobiont, but to what degree each mechanism and each symbiotic partner plays a role is largely unknown. This study aims to assess the patterns of phenotypic and genomic change over reoccurring stress and recovery events in an effort to further understand the process of acclimatization and potential for environmental memory. As well as characterize shifts in holobiont (symbiont and microbiome) diversity to evaluate its role in the changes that occur throughout stress and recovery. Investigating this phenotypic, genetic, and epi-genetic diversity will aid in guiding effective conservation practices like restoration nurseries.

Figure 2. Here we propose an environmental memory theory in the form of reduced reactions. Over time, with reoccurring stress events, an acclimatized organism will display a decrease in change of performance, gene expression, and DNA methylation. The phenotypic response will not necessarily return to the original level because full compensation as the most beneficial response assumes that the organism was performing at optimum prior to the stress. A partial compensation reaction could be the most beneficial and efficient in their environment. We hypothesize that the change in performance, gene expression, and DNA methylation at the end of the recovery periods (green bars) will decrease with each stress event because of the prior thermal history and the potential for environmental memory. The study aims to support this theory with a series of experiments over a three-year period.

**2. Aims**

The overall goal of this study is to characterize the coral holobiont’s environmental memory response to environmental change by examining physiological performance, (epi)genetics, and microbiome communities to inform more sustainable coral restoration plans. This study focuses on four main aims described below. Associated projects are indicated in colored superscript.

Aim 1: To test the validity of the environmental memory by reduced reactions theory proposed above.

*Sub-aim 1.1* **1,2,3**: To investigate the patterns of change in DNA methylation, gene expression, and physiological performance in response to repeat stress exposures. We hypothesize that the changes in the three mechanisms stated above will decrease with repeat exposure (Figure 2).

*Sub-aim 1.2* **2**: To evaluate how much of an influence seasonal variation in methylation plays a role in the patterns of change.

*Sub-aim 1.3* **2***:* To measure the recovery window associated with environmental memory and the potential to lose acclimatization if stress is not encountered for an extended period of time.

Aim 2 **1,2**: To investigate the relationship between gene expression and methylation by identifying genes involved in the coral holobiont stress response and measuring methylation patterns of those genes in response to multivariate stressors over time.

Aim 3 **1,3**: To assess the influence of microbiome and symbiont diversity on the holobiont physiological response to stress, and therefore the overall potential for acclimatization.

Aim 4 **3**: To incorporate the acquired knowledge from Aims 1, 2, and 3 to guide effective restoration practices and further research-based conservation efforts.

**3. Project Design**

Project 1, Year 1: **1**

This study proposes to expand the experimental work we have done at Hawaii Institute of Marine Biology (HIMB) in the Fall of 2018. We completed a 2-month stress and 2-month recovery time series of two species of adult corals, *Montipora capitate* and *Pocillopora Acuta*, under elevated temperature and pCO2 conditions. Over the course of 4 months, we measured adult performance (calcification, photosynthesis, respiration, bleaching) at 10 timepoints and collected molecular samples at 12 timepoints to measure gene expression, DNA methylation, and the genetic identity of the endosymbiotic dinoflagellates and the bacterial microbiome. At the end of the experiment, coral fragments were placed back out in a coral nursery next to HIMB.

The goal of this proposed study is to re-expose the fragments that are currently recovering on the reef to a second stress event and recovery in order to directly compare the change in gene expression, DNA methylation, and physiological response (performance measures stated above) to the first stress and recovery period. Fragments will be randomly allocated to either elevated temperature or ambient conditions for 2 months and allowed to recover in ambient conditions for 2 months. Both molecular analysis (defined above) and physiological performance parameters (defined above) will be sampled at Time 0, Day 1, 2, and 7, and Weeks 2, 4, 6, 8, 10, and 12. For later molecular analysis, samples will be snap frozen and stored at -80C. Molecular analysis from both stress event and recovery time periods will include *Symbiodinium* types determined by amplicon sequencing of ITS2 and the bacterial microbiome determined by amplicon sequencing of 16s. Restriction site-associated DNA sequencing (RADSeq and EpiRAD) and whole genome bisulfite sequencing will be used to determine genetic and epigenetic polymorphism differences across the entire genome. Gene ontology (GO) term enrichment will be used to identify the functional group of expressed genes.

Project 2, Year 2,3 **2:**

This goal of this project is to investigate further details of the environmental memory by reduced reactions theory proposed above, specifically 1.) the maximum amount of recovery time before the next stress event without losing acclimatization and memory, and 2.) the effects of seasonal methylation on the pattern changes. *Aiptasia sp.* individuals collected from the coast of Narragansett, Rhode Island will be kept in an aquaria system at URI. After 3 weeks of acclimation, individuals will be randomly allocated to either ambient or increased temperature conditions for 3 months during Summer 1 and then allowed to recover in ambient conditions. Individuals will then be randomly allocated to one of three exposures: 1.) 9 months of recovery, and 3 months of stress in Summer 2, 2.) 12 months of recovery and no stress in Summer 2, or 3.) 3 months of recovery, 3 months of stress, 3 months of recovery, and 3 months of stress in Summer 2. All individuals will be allowed to recover for 9 months after Summer 2, and then exposed to a final stress period of 3 months in Summer 3. Physiological parameters (defined in Project 1) and molecular analysis (defined in Project 1) samples will be taken at the following timepoints: stress periods: Day 0, 1, 2, and 7, and Weeks 2, 4, 8, and 12; and recovery periods: every 3 months up until next stress event. Exposure paths 1 and 2 will address the first goal of this project to identify if an extended recovery period is detrimental to their acclimatization and if there is potential to lose any stress memory gained. Exposure paths 1 and 3 will address the second goal to address the potential cofounding effect of seasonal variation in methylation patterns.

Project 3, Year 3 **3**:

This study proposes to expand on experimental work done in coral nurseries as a part of our collaborative workshop in Moorea, French Polynesia in 2018. The goal of this projected is to investigate coral (epi)genetics, and microbiome genetic diversity within 1) adults of a vital species, *Acropora hyacinthus*, and to link these metrics to physiological performance at sites with differing environmental characteristics 2) test offspring performance due to adult exposure, and 3) track the offspring through time to look for changes in coral epigenetics, and microbiome genetic diversity in the different family crosses. Specifically, we will characterize mechanisms that contribute to holobiont variation in performance based on host genotype, epigenotype, or microbiome community. This will help to inform restoration practices at both the adult fragmentation and larval scales in a time of climate change and warming waters.

Part 1: Corals will be collected from 4 sites with different environmental characteristics identified in the CRIOBE-URI FACE PUF collaboration and the Moorea Coral Reef Long Term Ecological Research site data. Ten adults genotype will be fragmented into clonal replicates. Fragments from each genotype will be collected for baseline measurements of DNA methylation. The remaining fragments will be exposed to ambient and increased temperature for 14 days. Following exposure, corals will recover in ambient conditions for 10 days and samples will be collected after. Then, the corals from each initial treatment will be exposed to experimental conditions again in a reciprocal fashion and samples will be collected after 14 days.  Following exposure, corals will be recover in ambient conditions for 10 days and final samples will be collected after. This design will provide samples prior to treatment, after treatment, after recovery, and after second treatment and second recovery to determine the role of host genetics, host DNA methylation, and microbiome community identity and diversity in acclimatization.

Part 2: To test for trans-generational acclimatization, adult corals will be conditioned at ambient and high temperatures for 4 weeks. Coral eggs and sperm will be crossed to generate family crosses of their offspring. The offspring at the swimming larval stage will be exposed to stress tests of temperature ramping from ambient to ambient + 5°C to determine maximum thermal tolerance. Samples will be taken of the adults, eggs, sperm, and swimming larvae before and at each degree of temperature change to assess the similarity in response variables described below across the generations and at different life stages.

Part 3: Larval samples will be settled, grown for 1 month and will be exposed to stress tests of temperature ramping from ambient to ambient + 5°C to determine maximum thermal tolerance. Samples will be collected every 1 week and photographed for growth and saved for molecular analysis (defined in Project 1). This proposed project will contribute to the development of a more sustainable restoration plan that will determine which genotype, epigenotype, and *Symbiodinium* type are most likely to survive future conditions, and therefore which would be most beneficial and successful to populate nurseries. Results will advance recent work in assisted evolution and selective breeding for conservation measures(van Oppen et. al 2015, Anthony et al. 2017).

References:

Anthony, K., Bay, L. K., Costanza, R., Firn, J., Gunn, J., Harrison, P., ... & Mumby, P. J. (2017). New interventions are needed to save coral reefs. *Nature ecology & evolution*, *1*(10), 1420.

Ainsworth, T. D., Heron, S. F., Ortiz, J. C., Mumby, P. J., Grech, A., Ogawa, D., ... & Leggat, W. (2016). Climate change disables coral bleaching protection on the Great Barrier Reef. *Science*, *352*(6283), 338-342.

Barshis, D. J., Ladner, J. T., Oliver, T. A., Seneca, F. O., Traylor-Knowles, N., & Palumbi, S. R. (2013). Genomic basis for coral resilience to climate change. *Proceedings of the National Academy of Sciences*, *110*(4), 1387-1392.

Bay, R. A., Harrigan, R. J., Le Underwood, V., Gibbs, H. L., Smith, T. B., & Ruegg, K. (2018). Genomic signals of selection predict climate-driven population declines in a migratory bird. *Science*, *359*(6371), 83-86.

Bourne, D. G., Morrow, K. M., & Webster, N. S. (2016). Insights into the coral microbiome: underpinning the health and resilience of reef ecosystems. *Annual Review of Microbiology*, *70*, 317-340.

Bowden-Kerby, A. (2008, July). Restoration of threatened Acropora cervicornis corals: intraspecific variation as a factor in mortality, growth, and self-attachment. In *Proceedings of the 11th International Coral Reef Symposium* (Vol. 2, pp. 1200-1204). Nova Southeastern University National Coral Reef Institute Davie.

Chisholm, J. R., & Gattuso, J. P. (1991). Validation of the alkalinity anomaly technique for investigating calcification of photosynthesis in coral reef communities. *Limnology and Oceanography*, *36*(6), 1232-1239.

DeCarlo, T. M., Harrison, H. B., Gajdzik, L., Alaguarda, D., Rodolfo-Metalpa, R., D'Olivo, J., ... & McCulloch, M. T. (2019). Acclimatization of massive reef-building corals to consecutive heatwaves. *Proceedings of the Royal Society B*, *286*(1898), 20190235.

Drury, C., Manzello, D., & Lirman, D. (2017). Genotype and local environment dynamically influence growth, disturbance response and survivorship in the threatened coral, Acropora cervicornis. *PloS one*, *12*(3), e0174000.

Edmunds, P. J., & Gates, R. D. (2008). Acclimatization in tropical reef corals. *Marine Ecology Progress Series*, *361*, 307-310.

Eirin-Lopez, J. M., & Putnam, H. M. (2018). Marine environmental epigenetics. *Annual review of marine science*, (0).

Jordan, R., Hoffmann, A. A., Dillon, S. K., & Prober, S. M. (2017). Evidence of genomic adaptation to climate in Eucalyptus microcarpa: Implications for adaptive potential to projected climate change. *Molecular ecology*, *26*(21), 6002-6020.

Hemond, E. M., & Vollmer, S. V. (2010). Genetic diversity and connectivity in the threatened staghorn coral (Acropora cervicornis) in Florida. *PLoS One*, *5*(1), e8652.

Hong, M. J., Yu, Y. T., Chen, C. A., Chiang, P. W., & Tang, S. L. (2009). Influence of species specificity and other factors on bacteria associated with the coral Stylophora pistillata in Taiwan. *Applied and environmental microbiology*, *75*(24), 7797-7806.

Hughes, T. P., Baird, A. H., Bellwood, D. R., Card, M., Connolly, S. R., Folke, C., ... & Lough, J. M. (2003). Climate change, human impacts, and the resilience of coral reefs. *science*, *301*(5635), 929-933.

Glasl, B., Herndl, G. J., & Frade, P. R. (2016). The microbiome of coral surface mucus has a key role in mediating holobiont health and survival upon disturbance. *The ISME journal*, *10*(9), 2280.

Littman, R., Willis, B. L., & Bourne, D. G. (2011). Metagenomic analysis of the coral holobiont during a natural bleaching event on the Great Barrier Reef. *Environmental Microbiology Reports*, *3*(6), 651-660.

Morrow, K. M., Muller, E., & Lesser, M. P. (2018). How Does the Coral Microbiome Cause, Respond to, or Modulate the Bleaching Process?. In *Coral Bleaching* (pp. 153-188). Springer, Cham.

Safaie, A., Silbiger, N. J., McClanahan, T. R., Pawlak, G., Barshis, D. J., Hench, J. L., ... & Davis, K. A. (2018). High frequency temperature variability reduces the risk of coral bleaching. *Nature communications*, *9*.

Stat, M., Morris, E., & Gates, R. D. (2008). Functional diversity in coral–dinoflagellate symbiosis. *Proceedings of the National Academy of Sciences*, *105*(27), 9256-9261.

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

Torda, G., Donelson, J. M., Aranda, M., Barshis, D. J., Bay, L., Berumen, M. L., ... & Miller, D. J. (2017). Rapid adaptive responses to climate change in corals. *Nature Climate Change*, *7*(9), 627.

Willmer, P., Stone, G., & Johnston, I. (2009). *Environmental physiology of animals*. John Wiley & Sons.

van Oppen, M. J., Oliver, J. K., Putnam, H. M., & Gates, R. D. (2015). Building coral reef resilience through assisted evolution. *Proceedings of the National Academy of Sciences*, *112*(8), 2307-2313.