PROJECT SUMMARY

Overview:
Valued over trillions of dollars, coral reefs prevent shoreline erosion, aid fisheries, promote marine biodiversity, and increase eco-tourism (Costanza et al. 2014). Recently, global coral cover has decreased due to the increased frequency and intensity of disturbance events (Hughes et al. 2018). Increased seawater temperature above the annual maximum disrupts the symbiotic relationship between corals and Symbiodiniaceae, leading to severe energetic reduction and increased mortality (Glynn 1996). Recent literature has described the potential for marine invertebrates, such as corals, to acclimatize to future global change conditions through epigenetic modifications. Changes to the epigenome, such as DNA methylation, histone modifications, and non-coding RNAs, can regulate gene expression, potentially leading to alterations in phenotype. The epigenome can be influenced by environmental conditions, resulting in phenotypic differences between identical genomes (Dixon et al., 2014; Flores et al., 2013). Previous exposures to an environmental stressor within a generation may also influence coral performance through within-generational plasticity (WPG). For example, conspecifics from sites differing in temperature fluctuations respond differently to heat stress, thus resulting in differential bleaching thresholds (Brown et al., 2002). In addition to WGP, offspring may also experience trans-generational plasticity (TGP) due to parental conditioning (Putnam & Gates, 2015, Donelson et al., 2018). While DNA methylation is a potential driver of WGP and TGP in corals (Putnam et al., 2017), the mechanisms and duration and magnitude of benefits are still unknown. This study aims to determine the WGP and TGP potential in corals to thermal stress, and describe what mechanisms are involved. Through enhanced nutrition and metabolic performance, we postulate that corals may have the ability to rapidly acclimatize to thermal stress.

Intellectual Merit:
The proposed work for this project will demonstrate the potential for corals to acclimatize to projected climate change conditions. By integrating concepts and methodology from other marine invertebrate systems, it is hypothesized that nutrition and metabolic interactions play a role in epigenetic modifications, which influence the capacity for an organism to acclimatize to a change in environment. We are using a multi-faceted approach to understand now the metabolome, epigenome, transcriptome, and phenome interact in corals. Additionally, we are applying these methods in a trans-generational aspect, which has been understudied in scleractinian corals. Elucidating the role of metabolism on epigenetic modifications will be novel in the coral field, which will provide further insight on how reefs will persist under global climate change conditions.

Broader Impacts:
The data created by this study will be publicly available on open source platforms, such as GitHub. All analyses and scripts will be fully reproducible to enforce full transparency during publication and enable teaching opportunities. Additionally, the highlights of this project will be published in peer-reviewed journals. To gather the data for this project, we will include local Bermudian students through the BIOS-Bermuda Program Internship, which enables local student to have a fully paid research internship while assisting in our research. Integrating the community is an important mission statement for our research, therefore we will further their understanding of the importance of coral reefs through facility tours at the Bermuda Institute of Ocean Sciences and hold publicly available research seminars directed towards the community. At the University of Rhode Island, we will select students from the Society of Women in Marine Science (SWIMS) and underrepresented minorities to assist in the experiments and sample processing, which will allow them to gain vial research experience throughout their undergraduate degree.

1. Background

Recent studies have identified the potential for acclimatization through WGP and TGP can help marine organisms to rapidly acclimatize to environmental change, as parental history can 'precondition' offspring to survive and maintain homeostasis in adverse conditions (Donelson et al. 2018; Putnam & Gates, 2015). The mechanisms that drive environmental phenotypic variation through TGP are postulated to involve maternal provisioning of offspring (Shama & Wegner, 2014), epigenetic inheritance (Ryu et al., 2018), and mitochondrial plasticity (Gibbin et al., 2016). These mechanisms are hypothesized to be linked; for example environmental stimuli can impact mitochondrial metabolism and have potential downstream impacts on epigenetic regulation leading to phenotypic variation (Castegna et al., 2015). Studies focusing on other marine ectotherms suggest that mitochondrial efficiency is a crucial factor in TGP against ocean warming (Gibbin et al., 2016), as less efficient mitochondria will leak electrons leading to reactive oxygen species (ROS) accumulation and potential tissue damage (Schmidlin et al., 2015). Byproducts produced by mitochondrial metabolism (e.g. ATP, acetyl-CoA, NADH, etc.) can regulate DNA methylation patterns and histone structure (Castegna et al., 2015), leading to dynamic changes in the epigenome and a potential pathway for altered physiological responses to environmental stressors (Eirin-Lopez & Putnam, 2019).

The study of metabolic-epigenetic interactions elucidate the potential pathways relating the change in metabolites produced by mitochondrial respiration to altered epigenetic states (Wallace & Fan, 2010; Figure 1). One metabolic epigenetic pathway discussed in the literature is the influence of the S-adenosylmethionine (SAM) cycle byproducts on DNA methylation potential (Donohoe & Bultman, 2012). DNA can be methylated by DNA methyltransferase (DNMT) enzymes DNMT1, DNMT3, and DNMT3b (reviewed in Bergman & Cedar, 2013). In eukaryotes, methylated cytosines create a repressed transcriptional mark, potentially leading to a change in physiological state (Flores et al., 2013). SAM is a methyl-donor for DNMT enzymes and subsequently produces the byproduct S-adenosylhomocysteine (SAH), which can be biosynthesised by the methionine cycle (MAT) and adenosine triphosphate (ATP) back into SAM (reviewed in Etchegaray & Mostoslavsky, 2016; Figure 1). Additionally, serine, an amino acid derived from glycolysis, and the essential vitamin folate (reviewed in Gut & Verdin, 2013) are fundamental compounds that regulate the MAT cycle. As serine and folate cannot be biosynthesized and only supplied by diet, nutrition may play a crucial role in regulating the MAT and, subsequently, the SAM cycle (reviewed in Feil & Fraga, 2012). **Therefore, mitochondrial efficiency (the production of ATP) and nutritional state (theronine and folate availability) of an organism may regulate DNA methylation patterns that determine the acclimatization potential in scleractinian corals**.

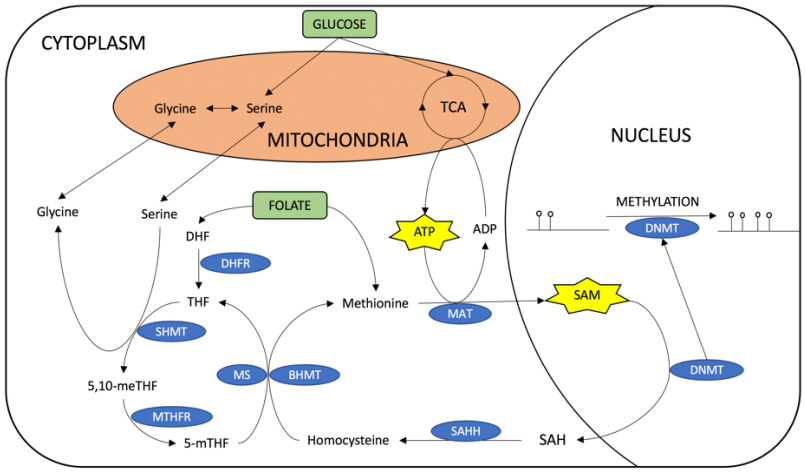

Figure 1. One pathway of the metabolic-epigenetic interaction. Green boxes indicate external sources, blue circles indicate enzymes, and yellow stars indicate metabolites of interest in this study.

2. Specific Aims
The objective of this study is to outline a potential pathway for TGP in corals to increased temperature conditions and will be addressed through the following aims:

Aim 1. To understand the links between nutritional state and mitochondrial physiology on epigenetic patterning.

Aim 2. To determine if alterations in nutrient availability and mitochondrial efficiency increase the potential for WGP and TGP in corals, leading to a buffered response to increased temperatures.

In respect to Aim 1, we hypothesize that corals with a higher nutritional availability will have higher folate concentrations to stimulate cycles that produce DNMT, leading to a higher methylation potential. Additionally, more efficient mitochondria within the cells will increase the production of metabolites and ATP, facilitating DNMT abundance and DNA methylation. In respect to Aim 2, we hypothesize that nutrient availability and mitochondrial performance increase the potential for changes in DNA methylation patterning, leading to an acclimatory response to increased temperatures and a similar buffered response to the F1 generation.

3. Linking nutritional state and mitochondrial physiology to epigenetic patterning (Aim 1)
To address Aim 1, 48 *Astrangia poculata* corals from Snugg harbour, Rhode Island, will be collected, fragmented, and glued to numbered acrylic plugs at the University of Rhode Island greenhouses. After a 2-week acclimation period, 8 corals will be randomly selected and placed into one of the three treatment conditions: no light conditions, natural light conditions, and natural light conditions with an additional food sources, with replicate tanks for each treatment. Treatment corals will be fed 0.5 mL of frozen brine shrimp mixture [0.3 g/mL] three days a week, for two weeks. After the treatment period, all corals will be snap frozen in liquid nitrogen and stored at -80°C. Before and after the treatment period, all corals will be buoyant weighted to measure growth, measured for dark adapted photosynthetic yield using Pulse Amplitude Modulation (PAM) fluorometry, and color change will be monitored as a proxy for Symbiodinaceae densities. All colonies will be airbrushed, and aliquots will be stored at -80°C for SAM, citrate synthase (a proxy for mitochondrial density), and bulk DNA methylation analyses. All physiological data (growth, dark adapted photosynthetic yield, color) will be compared to each initial timepoint to get a relative change for each colony in each treatment. Therefore, the physiological change can be related to the different nutritional treatments. The SAM, citrate synthase, and bulk methylation data will be plotted against nutritional treatment and correlated with each other to understand the interactive effects of nutritional state, which can be related to the altered physiological responses. Therefore, we can monitor enzymatic activities to describe the overall changes in bulk DNA methylation which may reflect phenotypic responses. By understanding how nutrition impacts coral physiology through the metabolism and epigenetic pathways, we can platform further experiments to understand its role in acclimatization to thermal stress.

4. Enhanced nutrition during development enhances mitochondrial activity and the potential for epigenetic change for TGP
Forty *Porites astreoides* colonies will be collected from Bailey's Bay, Bermuda and brought to the Bermuda Institute of Ocean Sciences (BIOS). For two weeks before the anticipated larval release, 20 colonies will be subjected to simulated "bleaching event" conditions (31°C) while the remaining 20 colonies will be maintained at ambient conditions (28°C; Figure 2). Within each temperature treatment, 10 corals will be fed live brine shrimp culture every other day, and 10 will not be fed. Samples and metabolism (photosynthesis and respiration rates) will be taken from all treatments prior to and after the treatment period. Larval release will be monitored from 10 days before the

new moon to 5 days after the new moon, as this is when anticipated peak release occurs for this *P. astreoides* in Bermuda (de Putron & Smith, 2011). Larvae will be pooled each day by treatment and settled onto pre-conditioned aragonite plugs. Settlement rate will be quantified after 3 days and then reared in a common garden tank under ambient conditions for 2 months where half of the juveniles from each parental history will be fed, while the other half will remain unfed. After two months, 20 juveniles from each parental history and juvenile feeding group with be sampled for physiological and molecular analyses. The remainder to the juveniles will be subjected to a 2-week high temperature (31°C) treatment. At the end of the 2-week temperature treatment, 20 juveniles from each treatment will be sampled for physiological and molecular analyses. Photosynthesis and respiration rates will be measured in the remainder of the corals. For all adult and juvenile physiological samples, electron transport system (ETS; a proxy for mitochondrial efficiency), citrate synthase (CS; a proxy for mitochondrial density) and SAM activities will be quantified in all samples. RNAseq and MBD BS (methyl-CpG-binding domain bisulfite sequencing) will be used to describe changes in DNA methylation and relative gene expression patterns in response to parental history and exposures. With this experimental design, we are able to address Aim 2 by understanding mitochondrial physiology and methylation dynamics in 2 different life stages under similar temperature stressors. This allows us to observe (1) the impacts of parental temperature and feeding on the re-exposure of juveniles to temperature stress, and (2) the role of mitochondria and nutritional state on epigenetic inheritance and buffered responses to temperature.

To analyse this data, physiological adult metrics will be compared before and after the treatment. The juvenile physiological and survivorship data will be compared before and after the juvenile treatment period in respect to parental treatment (temperature treatment and feeding regime) and juvenile feeding regime. Differential gene expression (DGE) and differential methylated gene (DMG) patterns will be compared to relate changes in DNA methylation and gene expression within and between life stages. Additionally, DMG patterns will be compared between the offspring and parents to determine if the changes in epigenetic patterning between parental treatments are inherited. Cumulatively, we will have the capacity to outline the importance of metabolic performance and enhanced nutritional states at both parental and juvenile life stages in response to increased temperature events.

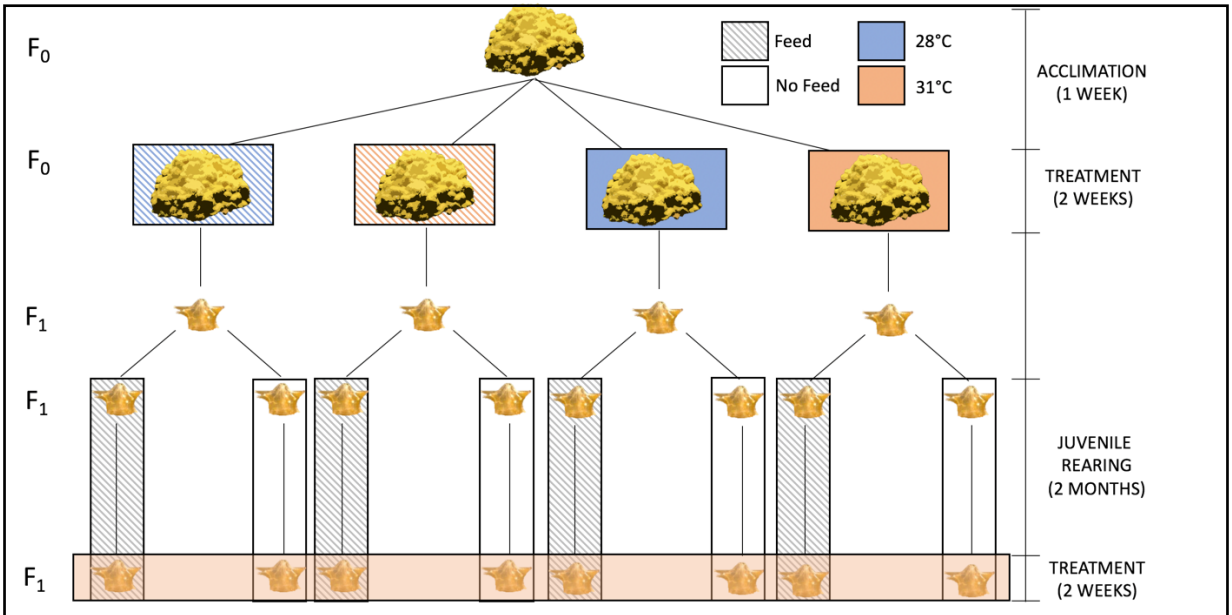

Figure 2. The experimental design of Aim 2.

5. Intellectual Merit

This study is will provide novel insight into the potential of TGP in scleratinian corals through: (1) understanding how the epigenome and gene expression can result in a beneficial acclimatory response to increased temperature, (2) that metabolic performance and nutrition can alter the epigenetic state of corals, and (3) epigenetic inheritance could potentially elicit TGP responses in brooding corals. By elucidating the mechanisms driving phenotypic plasticity in corals, we can further integrate trans-generational aspects into climate change models to inform policy and management of marine protected areas accordingly.

6. Broader Impacts

Accessibility of results

All data will be made publicly available on GitHub with reproducible formats (e.g. Rprojects) to facilitate open access analyses for publication or teaching purposes. Additionally, this project will be presented at multiple conference proceedings, such as the International Coral Reef Symposium (2020) and the Society of Integrative and Comparative Biology Meeting (2020). The main findings from each aim will be constructed into at least two publications.

Outreach

Education, outreach and communication are important aspects of a marine biologist's career, as it develops a framework to ask appropriate questions that relate to anthropogenic change to marine ecosystems. My experiences at BIOS have shown me the importance of teaching students about the ocean sciences. I gave an introduction to Bermuda's coral reefs, and ran a coral identification workshop, for the Bermuda Society for the Prevention of Cruelty to Animals (SPCA) summer camp. Leading this camp, once a week for 8 weeks, allowed me to assess the information necessary to engage 9-12 year old students and to provide an introductory understanding of coral reefs. I believe that teaching youth about marine science encourages a deeper respect for the ocean, which may translate into increased public engagement in issues of ocean policy. I plan to continue to integrate the community and young students into my research through public guest lectures and tours of the BIOS facilities.

This project will provide opportunities for undergraduate students to perform coral reef research. We will select local Bermudian interns through the BIOS-Bermuda Program to involve and engage the local community. To promote undergraduate research at URI, we will utilize students involved with the Society of Women in Marine Science (SWIMS).

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