

**Introduction:** Coral reefs are one of the most diverse ecosystems on the planet, harboring over 25% of all marine life.<sup>1</sup> They provide invaluable services by protecting shorelines from storm surge, supplying food sources, and promoting eco-tourism.<sup>2</sup> However, coral populations around the world are exhibiting an alarming decline due to climate change, specifically from ocean warming (OW). OW has severely diminished coral health through increased disease susceptibility and bleaching.<sup>3</sup> To fully understand how corals might fare under future OW conditions, the potential for coral acclimatization over generations and life history stages must be assessed. Previous research on coral acclimatization has focused primarily on intra-generational acclimatization (IGA), which investigates if corals can adjust to new conditions within their lifetime. For example, Brown et al. (2002) found that when *G. aspera* were exposed to higher levels of solar radiation, they were less susceptible to bleaching.<sup>4</sup> While IGA is crucial in elucidating coral resilience, the study of trans-generational acclimatization (TGA) in corals is essential to understanding the persistence of coral reefs in a warmer future. TGA occurs when the phenotype of the offspring is influenced by the environmental conditions experienced by the parents and/or previous generations.<sup>5</sup> Epigenetic modifications, or heritable alterations in gene expression and cellular functions that do not involve changes to the original DNA sequence, are thought to play a role in TGA.<sup>6</sup> Most studies on epigenetic modifications and TGA have focused on exclusively DNA methylation; for example, Strader et al. (2019) found that parental environments of *S. purpuratus* affected patterns of DNA methylation in offspring.<sup>7</sup> However, other epigenetic markers, such as histone modification and chromatin remodeling, may be relevant to TGA in marine invertebrates, but have been seldom studied. I will address this knowledge gap by investigating multiple epigenetic mechanisms and outcomes of TGA in corals, over multiple generations and life history stages, in the context of OW. Additionally, I will examine coral physiological processes to better understand all aspects of coral TGA. Discerning the influence of epigenetics on TGA will contribute to the knowledge of coral resilience and susceptibility in an evolutionary and ecologically relevant context. The coral *Pocillopora damicornis* was chosen for this study, as it is an important reef-building coral in the Indo-Pacific region and is commonly used in laboratory experiments as a model coral.

**Aims and Hypotheses:** *Aim 1:* Assess physiological effects of TGA in offspring at several developmental stages (larval, juvenile). *Hypothesis 1:* Both larval and juvenile offspring whose parents were exposed to OW conditions will have a higher tolerance to OW conditions than larval and juvenile offspring whose parents experienced ambient conditions. *Aim 2:* Compare the epigenetic modifications, specifically DNA methylation patterns and histone modifications, of coral parents and offspring during several developmental stages (larval, juvenile) to evaluate the acquisition and stability of TGA. *Hypothesis 2:* Parents exposed to OW conditions will produce offspring with differentially methylated genes and modified histones compared to offspring whose parents experienced ambient conditions.

**Research Methods:** I will collect reproductively viable adult *P. damicornis* from the fringing reefs of Kaneohe Bay, Hawaii and experimentally expose them to OW conditions. To simulate current and future environmental parameters, experimental ambient/high temperatures will be defined as 26/30°C.<sup>8</sup> Exposures will take place in mesocosm tanks with flow-through experimental treatment water in the Hawaiian Institute of Marine Biology's seawater system for one month. Following the adult exposure, I will evaluate the photosynthetic efficiency ( $F_v/F_m$ ) of adult corals to assess their capacity to photosynthesize. Additionally, I will preserve adult tissue samples for later epigenetic analysis (see below). After the exposure and physiological assessment, I will induce adults from all treatments to spawn. I will collect larvae post-spawn

and experimentally expose them to ambient/OW conditions for one week. At the beginning and end of the larval exposure, I will measure lipid content and oxygen consumption from a subset of larvae in each treatment to determine energy reserves needed for metamorphosis and metabolic rate, respectively. Following the exposure, I will preserve another subset of larvae from each treatment for epigenetic analysis (see below). I will transfer the remaining larvae from each treatment into 10 L tanks with ambient flow-through seawater and plugs for settlement. After 6 months, I will experimentally expose the now-juvenile offspring to ambient/OW conditions for one month. At the end of the exposure, I will measure juvenile  $F/F_m$ ; following photosynthetic analysis, I will preserve juvenile tissue for epigenetic analysis (see below). *Epigenetics:* At the end of all exposures, I will collect tissue from juveniles/adults and a subset of larvae for DNA methylation and histone modification analyses. Using extracted genomic host DNA, I will assess whole genome DNA methylation using the MeDIP-seq approach. This method utilizes DNA immunoprecipitation and next-generation sequencing to estimate methylation levels of specific DNA regions. I will also use genomic host DNA for histone modification analysis. Histone modifications will be analyzed through the ChIP-seq method, which combines chromatin immunoprecipitation and next-generation sequencing to identify regions of the genome associated with these modifications.

**Intellectual Merit:** Not only will this research considerably enhance knowledge of physiological and epigenetic processes in coral biology, but it will be one of the first studies to provide a deeper understanding of coral resilience over multiple generations and life history stages. The utilization of cutting-edge epigenetic analyses will help to define the contribution of DNA methylation and histone modifications to TGA, which is currently understudied in corals. Additionally, the cognizance of coral TGA potential in the face of anthropogenic stressors will allow scientists and reef managers to make more informed predictions about future reef health and population evolution. An increased knowledge of epigenetic mechanisms in corals will also supply a starting point to investigate TGA potential in other marine invertebrates who may be susceptible to climate change.

**Broader Impacts:** The Graduate Research Fellowship will enable me to pursue important research opportunities and will equip me with the knowledge and abilities needed to succeed as a future governmental or non-profit research scientist. Moreover, the GRF will enhance my skills as a scientific educator and mentor to younger students. I intend to partner with local high schools in the greater University of Hawaii area to connect students with marine science and research. Through this partnership, I will provide opportunities for students to undertake independent projects within the context of my research. I will guide students through an integrated overview on how to conduct research projects from the initial proposal to the final written product. More specifically, I want to include low-income high school students during the research partnership. Low-income students can often be excluded from fully pursuing their interests in science, due to lack of financial and academic support. I hope to provide those students with research opportunities and support, so that they can receive an enriching experience.

**References:** <sup>1</sup>Reaka-Kudla (1997) *Biod. II*. <sup>2</sup>Constanza *et al.* (2014) *Glob. Env. Chan.* <sup>3</sup>Hoegh-Guldberg *et al.* (2007) *Sci.* <sup>4</sup>Brown *et al.* (2002) *C. Reefs*. <sup>5</sup>Torda *et al.* (2017) *Nat. Clim. Chan.* <sup>6</sup>Eirin-Lopez and Putnam (2019) *Ann. Rev. Mar. Scie.* <sup>7</sup>Strader *et al.* (2019) *J. Exp. Mar. Bio. Eco.* <sup>8</sup>IPCC (2013) *AR5*.