Enhancing Thermal Tolerance in Native Hawaiian Corals through Genetic Modification of Algal Symbionts

Purpose and Significance

Coral reefs, vital ecosystems supporting marine biodiversity and coastal economies, are facing unprecedented declines due to climate change. Heat-induced coral bleaching, driven by elevated ocean temperatures, disrupts the symbiosis between corals and their algal symbionts, leading to widespread coral mortality. This project aims to investigate whether genetically modifying algal symbionts can enhance coral resilience to thermal stress by introducing the *HSF1* gene from thermally tolerant *Durusdinium glynnii* algae from the *Pocillopora* coral species into the algal symbionts of native Hawaiian corals.

Pocillopora spp. in the eastern tropical Pacific have demonstrated high thermal tolerance, attributed to their association with the thermotolerant symbiotic alga *D. glynnii*. This alga has facilitated the persistence of these reefs through multiple mass bleaching events by reducing bleaching severity and mortality (Palacio-Castro et al., 2023). Advances in CRISPR-Cas9 technology now enable precise genetic modifications in *Symbiodiniaceae*, offering the opportunity to directly test and enhance gene functions critical for thermal resilience (Cleves et al., 2018; Cleves et al., 2020). By leveraging the genetic heat tolerances observed in *D. glynnii*, this research seeks to enhance the thermal tolerance of native Hawaiian corals.

Objectives

- Determine if the replacement of the *HSF1* gene in algae *Cladocopium* enhances *Montipora capitata* coral resilience to heat stress.
- Investigate the physiological and molecular mechanisms behind enhanced thermal resilience.

Hypotheses

- Corals hosting genetically modified (GM) *HSF1* gene from *D. glynnii* will have higher survival rates and reduced bleaching under elevated temperatures (27–31°C) compared to corals hosting wild-type algae.
- There will be no significant difference in survival rates and bleaching severity between corals hosting GM *HSF1* and those hosting wild-type symbionts under thermal stress.

Previous studies have shown that the association of *Pocillopora* species with *D. glynnii* contributes to enhanced heat tolerance during thermal stress events (Palacio-Castro et al., 2023). This provides a foundation for the hypothesis that introducing a thermally resilient *HSF1* gene from *D. glynnii* into the *Cladocopium* algae will confer similar benefits to native Hawaiian corals.

Methods

This study will be conducted over a 9-month period, with genetic modification, acclimation, and thermal stress experiments occurring over the first 8 months. The final 4 weeks will focus on data analysis and the continuation of thermal stress experiments. This research requires approval from Hawaii Institute of Marine Biology's (HIMB) IACUC, a marine species collection permit from NOAA, CITES permits, and genetic modification permits.

To genetically modify the algal symbionts, the *HSF1* gene from thermally tolerant *D. glynnii* will be synthesized and optimized for expression in the *Cladocopium* algae. The CRISPR-Cas9 technique will be employed to replace the native *HSF1* gene in *Cladocopium* with the *D. glynnii* homolog. Single-guide RNAs (sgRNAs) will be designed to target the native *HSF1* locus, and a

homology-directed repair (HDR) template containing the thermally tolerant *HSF1* sequence will be introduced. After successful gene replacement is confirmed through PCR and sequencing, the expression of the *HSF1* gene will be validated using quantitative PCR and immunoblotting. After modification, GM *Cladocopium* will be introduced to coral larvae of the native Hawaiian species, *M. capitata*. The larvae will be exposed to GM algae under controlled laboratory conditions at HIMB. A control group will be paired with wild-type algae for comparison. Algal densities in coral tissues will be monitored weekly using microscopy and chlorophyll fluorescence measurements over a 10-week acclimation period to ensure stable symbiosis. After the acclimation period, the coral-algal symbionts will be subjected to thermal stress in aquaria. Water temperatures will be gradually increased to the 27–31°C range over three weeks to simulate heat stress conditions. Coral survival rates will be monitored daily, bleaching severity will be assessed visually and through quantification of algal densities, photosynthetic efficiency will be measured using PAM fluorometry, and ROS production will be quantified with fluorescence-based assays to evaluate oxidative stress.

RNA sequencing will be conducted to compare gene expression in both GM and wild-type symbionts under heat stress. Focus will be on stress-response genes related to antioxidant activity and chaperone functions. In addition, the coral hosts' heat-shock proteins (HSPs) and other stress-related pathways will be analyzed. Functional Pathway Analysis will be used to investigate changes in photosynthesis-related processes and cellular stress responses attributable to the *HSF1* replacement. Data will be analyzed using statistical methods such as ANOVA to compare survival rates, bleaching severity, photosynthetic efficiency, ROS production, and gene expression profiles between GM and wild-type symbionts. This will provide insights into the effectiveness of the *HSF1* gene replacement in enhancing coral resilience to heat stress.

Challenges

Achieving high efficiency in gene replacement and ensuring stable symbiosis with GM algae will be challenging. These issues will be mitigated by optimizing CRISPR-Cas9 protocols and maintaining high-quality aquarium conditions. Additionally, controlling environmental factors such as ammonia, nitrate, and temperature will be critical to preventing confounding effects.

Necessary Resources

The project will require access to facilities at HIMB and Stanford University, along with equipment for genetic editing (CRISPR-Cas9), algae cultivation, and molecular analysis (e.g., PCR machines, RNA-seq equipment). Coral larvae of *M. capitata*, and *Cladocopium* and *D. glynnii* cultures will be sourced, along with necessary reagents for genetic editing, microscopy, and fluorescence assays.

Outcomes, Evaluation, and Dissemination

This project is expected to provide valuable insights into the role of genetic modification of algal symbionts in enhancing coral resilience to heat stress. The evaluation of the project's outcomes will be based on a combination of physiological measurements, such as survival and bleaching rates, as well as molecular data from RNA sequencing and gene expression analyses. This will be a successful experiment if the algal symbionts are successfully genetically modified and introduced to the corals. The findings from this research will be disseminated through submission to peer-reviewed scientific journals, such as *Science Advances* or *Coral Reefs*, and sent to local and global

conservation organizations, including the Nature Conservancy, the Maui Ocean Center Marine Institute, and the Ocean Alliance Project.

Timetable

Start date: 04/2025

End date: 01/2026

Week	Task	Estimated Hours
1-4	Gene Synthesis and Optimization: 1. Synthesize the *HSF1* gene from *D. glynnii* for genetic modification of *Cladocopium* 2. Design CRISPR-Cas9 constructs and order necessary reagents 3. Set up the genetic modification lab and prepare for gene editing	12
5-8	CRISPR-Cas9 Gene Editing: 1. Perform CRISPR-Cas9 gene editing on *Cladocopium* to replace the native *HSF1* gene with the *D. glynnii* homolog 2. Use electroporation to introduce the CRISPR-Cas9 complex into the algal cells 3. Confirm successful gene replacement through PCR and sequencing	20
9	Gene Expression Validation: 1. Extract RNA from modified algae 2. Validate expression of the modified *HSF1* gene using quantitative PCR 3. Perform immunoblotting to verify protein expression	8
10-12	Culturing GM *D. glynnii*: 1. Cultivate genetically modified *Cladocopium* algae for introduction into coral larvae 2. Monitor algae growth and health under controlled conditions	12
13-14	Coral Larvae Collection: 1. Collect larvae of *M. capitata* 2. Prepare larvae for exposure to GM algae under laboratory conditions	8
15-18	Symbiont Integration: 1. Expose *M. capitata* coral larvae to GM *Cladocopium* algae 2. Monitor algae integration and stability in coral tissues 3. Conduct weekly monitoring of symbiont densities using microscopy and chlorophyll fluorescence	16
19-22	Acclimation Period: 1. Continue acclimating coral-algal holobionts 2. Maintain proper tank conditions (salinity, temperature, light, etc.) 3. Take weekly progress photos of coral samples	12
23-26	Thermal Stress Experiment: 1. Gradually increase water temperatures to 27–31°C over three weeks 2. Monitor survival rates, bleaching severity, and general health of the corals 3. Measure photosynthetic efficiency using PAM fluorometry	18
27-28	ROS Production Monitoring: 1. Quantify ROS production using fluorescence-based assays to evaluate oxidative stress in coral 2. Continue monitoring coral health under thermal stress conditions	10
29-32	RNA Sequencing: 1. Extract RNA from GM and wild-type symbionts for RNA sequencing 2. Analyze gene expression related to stress-response pathways, including antioxidant activity and heat-shock proteins (HSPs)	16
33-36	Functional Pathway Analysis: 1. Conduct functional pathway analysis to study changes in photosynthesis-related processes and stress responses due to the *HSF1* replacement 2. Perform statistical analysis (e.g., ANOVA) to compare results between GM and wild-type symbionts	16

37-40	Final Data Analysis: 1. Analyze data using one-way ANOVA tests to compare survival rates, bleaching severity, photosynthetic efficiency, ROS production, and gene expression profiles between GM and wild-type symbionts	20

Annotated Bibliography

Buerger, P., et al. “Heat-Evolved Microalgal Symbionts Increase Coral Bleaching Tolerance.” *Science Advances*, 13 May 2020, www.science.org/doi/10.1126/sciadv.aba2498.

This paper explores how heat-evolved algal symbionts enhance coral resilience to thermal stress by reducing oxidative stress and improving photosynthetic efficiency. It provides key insights into the physiological and biochemical pathways that support thermal tolerance in symbiotic algae. The findings directly support the hypothesis that genetically modifying *Cladocopium* can similarly enhance thermal resilience in native Hawaiian corals.

Chakravarti, Leela J., et al. “Rapid Thermal Adaptation in Photosymbionts of Reef-Building Corals.” *Wiley Online Library*, 27 Apr. 2017, onlinelibrary.wiley.com/doi/epdf/10.1111/gcb.13702.

Cleves, Phillip A., Amanda I. Tinoco, et al. “Reduced Thermal Tolerance in a Coral Carrying CRISPR-Induced Mutations in the Gene for a Heat-Shock Transcription Factor.” *PNAS*, 9 Nov. 2020, www.pnas.org/doi/full/10.1073/pnas.1920779117.

This study investigates how the disruption of specific stress-response genes, such as heat shock proteins (HSPs), affects coral resilience to elevated temperatures. It emphasizes the critical role of molecular pathways regulated by genes like *HSF1* in coral thermal tolerance. This research is directly relevant to the project, as it provides a baseline understanding of the molecular mechanisms the modifications aim to enhance

Cleves, Phillip A., Marie E. Strader, et al. “CRISPR/Cas9-Mediated Genome Editing in a Reef-Building Coral.” *PNAS*, 26 Mar. 2018, www.pnas.org/doi/epdf/10.1073/pnas.1722151115.

This study demonstrates the successful application of CRISPR-Cas9 technology in corals, marking a breakthrough in the genetic engineering of reef-building species. The paper provides detailed methodologies for gene editing and highlights challenges specific to corals, such as the need for precise sgRNA design and HDR optimization. This research is foundational for the project as it outlines the tools and techniques required for editing the *HSF1* gene and introduces the feasibility of genetic modification in symbiotic systems.

Dilworth, Jenna, et al. “Host Genotype and Stable Differences in Algal Symbiont Communities Explain Patterns of Thermal Stress Response of Montipora Capitata Following Thermal Pre-Exposure and across Multiple Bleaching Events - Coral Reefs.” *SpringerLink*, Springer Berlin Heidelberg, 10 Nov. 2020, link.springer.com/article/10.1007/s00338-020-02024-3#:~:text=Hawai’i).-,M.,2016%3B%20Innis%20et%20al.

Hartl, Daniel L. *Essential Genetics: A Genomics Perspective*. Jones & Bartlett Learning, 2014.

“Marine Invertebrates: All Stony Corals.” *Department of Land and Natural Resources (.Gov)* , 1 Oct. 2015, dlnr.hawaii.gov/wildlife/files/2019/03/SWAP-2015-Stony-Corals-Final.pdf.

Palacio-Castro, Ana M., et al. “Increased Dominance of Heat-Tolerant Symbionts Creates Resilient Coral Reefs in near-Term Ocean Warming.” *PNAS*, 13 Feb. 2023, www.pnas.org/doi/10.1073/pnas.2202388120.

This paper highlights how the association of *Pocillopora* species with *Durusdinium glynnii* contributed to reef survival during mass bleaching events. It underscores the ecological importance of thermotolerant symbiotic algae in mitigating the effects of climate change on coral reefs. This study supports the decision

to use the *HSF1* gene from *Pocillopora*, showcasing the natural advantages of this symbiosis and its potential to confer similar resilience to native Hawaiian corals.