Proposed Plan for Completion of Dissertation

Corals contain abundant and diverse communities of microorganisms within their tissue such as bacteria, archaea, viruses, and algae which collectively make up the microbiome. Coral-associated bacterial communities contribute to coral health through nutrient cycling and antimicrobial activity, but they can also function as pathogens. In fact, environmental stress resulting in coral bleaching, disease, or death often coincides with changes in the composition of the microbiome. The aim of my dissertation is to evaluate how and to what extent human impacts drive changes in coral animals at microbial scales. Specifically, my published and unpublished work has characterized microbiome dysbiosis of Moorea corals under stressful environmental states that are increasingly occurring around the globe. This research is instrumental in understanding how coral microbiomes respond to these environments and how this response will shape the corals that survive into the future. In brief, my dissertation consists of three chapters assessing compositional changes in the bacterial community living in corals experiencing increasing seawater temperature, nutrient pollution, and predation. To this aim, I showed that stressed coral microbiomes are generally characterized by an increase in alpha and beta diversity, in community dissimilarity and variability, and in the abundance of potentially pathogenic bacteria. Although these increases coincide with a decrease in bacteria associated with healthy corals, I showed that some corals can recover these healthy bacteria and exhibit microbiome flexibility as well as resilience to moderate thermal stress. Importantly, my work is the first to characterize how coral reef stressors interact to drive microbiome dysbiosis. Local stressors like nutrient pollution were often assumed to worsen the effects of global climate change. However, my research shows that efforts to curb pollution on reefs at the local scale may be less impactful if international efforts to combat climate change are not simultaneously taken.

Methodology: The following methods are similar for all three chapters. In brief, corals under experimentation or monitoring in Moorea, French Polynesia, were sampled and frozen for preservation. In the lab, samples were prepared for DNA sequencing using standard techniques. Samples were sequenced at Oregon State University's Center for Genome Research and Biocomputing. DNA sequences of microorganisms were then analyzed using bioinformatic and statistical open-access pipelines (GitHub.com) I personally developed as a PhD student. I am proficient in all phases: fieldwork, sampling, lab work, data analysis, manuscript writing, and publication.

Chapter 1: *Multiple stressor tank experiment.* In September 2016, corals from a single species were collected from the reef in Moorea, French Polynesia and fragmented into pieces. Coral fragments were distributed among tanks and stressed over 21 days with increased seawater temperature, nutrient enrichment, mechanical tissue scarring or a combination of these treatments. After sampling, lab work, and sequencing, the bacterial communities within each coral fragment were analyzed for changes in community composition. Results were published in Maher et al (2019) *Sci. Rep.* and Rice & Maher et al (2019) *PeerJ*.

Chapter 2: *Multiple stressor field experiment and timeseries.* In the summer of 2016, corals in Moorea experienced a heat stress event during which seawater temperatures increased past levels that induce coral bleaching. Throughout the heat stress event, three species of corals on the reef were enriched with various nitrogen sources to simulate human pollution. We sampled these corals before, during, and after the summer stress event. Following lab work, sequencing, and analysis, I have prepared a manuscript summarizing the results for submission by January 1st, 2019.

Chapter 3: *Field experiment and timeseries.* In the summer of 2019, corals in Moorea experienced an extreme heat stress event surpassing that of 2016 in terms of coral bleaching and mortality. To determine the functional role that bacteria play in moderating the coral response to this bleaching event, I will generate metagenome assembled genomes (MAGs). This project differs from Chapters 1 & 2 in that these samples contain all DNA present including coral, bacteria, algae, fungi, etc. (metagenomic sequencing) whereas previously I only analyzed bacterial DNA (16S sequencing). From this sequencing, I will use databases to assign functions to genes found in the bacterial genomes. This will allow me to use different analysis tools and expand my -omics skillset. I expect to quickly master these techniques, having used them in coursework, and I will develop metagenomic analysis pipelines for public access on GitHub.com. When I return to Oregon State University in

April 2020, these samples will have been shotgun sequenced and will be ready for metagenomics analysis. At that time, I will begin to analyze, statistically validate, and interpret the data. These data will form the basis of a manuscript for submission for peer review and publication in Spring 2021 and inclusion in my dissertation for my defense in June 2021. I do not foresee any barriers to defending in June 2021 as I have already completed two of the three required dissertation chapters. Twelve months is also a conservative allocation of time to analyze the metagenomic datasets considering my expertise in analyzing large -omics datasets.

I received funding through the NSF Graduate Research Internship Program to work with the National Oceanic and Atmospheric Administration (NOAA) for a six-month internship. I am currently analyzing data on the genetics of Dungeness crab under ocean acidification with the Northwest Fisheries Science Center in Seattle, WA, from which we expect to produce a publication. With this work, I am developing new skillsets in genetic and genomic analysis of another marine invertebrate under threat of climate change. This internship was carefully planned to increase my professional network of collaborators, provide an introduction to the realm of government research, and expand my research portfolio while not hindering progress towards my degree.

My ambition is to be the principal investigator of a lab and to frequently publish novel and interdisciplinary science through collaboration. While I have already formed professional and personal relationships with numerous scientists around the world, my ultimate goal as a researcher is to continue to expand my growing network of international collaborators, to master cutting-edge molecular and analytical techniques, and to ask novel and provocative questions that challenge our understanding of how marine ecosystems and organisms function. My collective experiences in academia and government (NOAA) will make me a competitive applicant for post-doctoral research and, ultimately, faculty positions at universities. This fellowship will give me the financial support to devote my total efforts to completing my dissertation and applying for future positions. Publications and timely completion of my dissertation will be invaluable to securing future research positions at competitive institutions.