Introduction: Elevated nutrients and herbivore loss are two of the major local threats driving global coral reef mortality. These stressors may reduce reef resilience by stimulating macroalgal growth and competition [1]. Parrotfish control macroalgae through herbivory but often predate corals to supplement their diets. This causes some coral tissue damage in the form of individual lesions, but rarely causes total colony mortality [2]. However, Zaneveld [1] surprisingly found that in waters enriched with nitrogen and phosphorous, colony mortality increased from zero to ~65% in *Porites* colonies after parrotfish predation, but why this occurred was unknown. For my dissertation project I aim to study if and how the combined stressors of predation and nutrient enrichment disrupt coral physiology and/or their microbiomes to cause this increase in mortality.

The coral holobiont is a dynamic assemblage of the coral animal and its associated microorganisms such as bacteria and algae which collectively make up the microbiome. Elevated nutrients can alter the abundance and types of coral-associated mutualistic algae in the genus *Symbiodinium* [3]. Environmental stressors cause shifts in healthy coral-associated bacteria [4], that may provide antibiotic activity against invasive microbes and pathogens [5]. Zaneveld [1] found that the combination of predation and nutrient enrichment increased the amount of potentially opportunistic bacteria when compared to proposed coral mutualists. My project will determine if parrotfish are a vector for bacterial opportunism and/or if nutrients drive an increase in host susceptibility to infection following wounding by predation.

Hypothesis: I hypothesize that nutrient enrichment and predation interact to cause two major changes to the coral holobiont that result in coral death: reduced host immunity and the proliferation of pathogens. My work will focus on the following questions:

Q1. How do bacteria in the coral mucus protect against predation-mediated mortality in water with ambient nutrient levels? I hypothesize that coral mucus already possess specialized microbiota that protect corals from pathogens via several testable mechanisms such as antibiotic production, competitive exclusion, or predation.

Q2. How do nitrogen and phosphorous alter coral and algal symbiont physiology and the microbiome? I hypothesize that a decrease in host immunity and destabilization of the *Symbiodinium* community will combine to reduce the holobiont's ability to regulate its microbiota. I also predict that the microbiota with anti-pathogen activity identified in Q1 will be reduced and opportunistic bacteria will increase in nutrient enriched treatments.

Q3. Is the microbiome-dependent route to coral death driven by physical wounding or predator specific corallivory in nutrient enriched waters? I hypothesize that parrotfish serve as vectors for the proliferation of pathogens in the mucus around the wound site. Alternatively, I hypothesize that any wounding in the presence of elevated nitrogen and phosphorus provides a route to enhanced bacterial infection.

Research Plan: These questions will be addressed at the Gump South Pacific Research Station on Moorea, French Polynesia, through two complementary experiments: on the reef and in controlled tanks. While I expect to see similar changes in microbial communities and host health between the two experiments, each will provide a specific component to my investigation. Using SCUBA, individual *Pocillopora* colonies will be transplanted to saltwater mesocosm tanks. In the tanks, I will pre-expose corals to an antibiotic mix [6] to deplete the bacterial community. The types, concentrations, and exposure length will be determined the year prior to the experiment. Then I will move the treated and untreated corals to new tanks with natural seawater or to the field for monitoring. I will simulate predation in the tanks by physically wounding the coral and track host immunity to determine if the host alone is capable of preventing mortality or if associated microbiota are necessary for defense and recovery (**Q1**). On the reef, a subset of

corals will be exposed to parrotfish predation while others will be shielded from predation with herbivore exclosures (**Q3**). A subset of the colonies in both experiments will be maintained at ambient nutrient levels while others will be enriched using slow-release fertilizer diffusers (**Q2**). N and P concentrations will be comparable to those on reefs impacted by nutrient pollution [1]. **Phase 1.** Simulate the effects of predation, nutrient loading, or a combination of these stressors with manipulative experiments on the reef and in tanks. At regular intervals, 1) photographically monitor coral tissue growth/loss and coral mortality, 2) record dissolved organic nitrogen and soluble reactive phosphorus concentrations via autoanalyzer, 3) measure *Symbiodinium* density with Pulse Amplitude Modulation, 4) measure bacterial respiration with oxygen probes, 5) count mucus associated bacteria with epifluorescence microscopy, and 6) sample coral tissues for DNA/RNA, taking care to minimize any serious damage to the coral. **Phase 2.** Track changes in the holobiont. For bacterial community dynamics, extract DNA from mucus to generate microbial 16S amplicon libraries [1] and metagenomes [4] for bacterial functional analysis. For *Symbiodinium* and host gene expression changes, extract RNA and DNA from tissue for RNAseq as well as for ITS-2 amplicon libraries [3]. **Phase 3.** Sequence the libraries on Illumina platforms at OSU's Center for Genome Research. **Phase 4.** Use bioinformatics (e.g. QIIME [7], Shotmap [8]) and statistical pipelines (e.g. STAMP [9]) to analyze changes in structure and function of microbial communities and in gene expression patterns of innate holobiont immune responses.
Predictions: Antibiotic producing bacteria, not host immunity, will be the primary defense against coral tissue loss or mortality from predation or wounding. Nutrient enrichment in combination with predation or wounding will lead to coral mortality. Coral mucus will exhibit an increase of one or more pathogenic strains, either found in low abundance in the communities of control colonies or absent from control colonies and therefore introduced by parrotfish predation. Coral mucus will also exhibit a decrease of one or more strains with antibiotic capabilities. I will identify the proliferated pathogenic strain(s) and the reduced defensive strain(s) thereby identifying the microbial route to colony mortality.
Intellectual Merit: The Zaneveld study [1] is the first to document increases in predator-mediated mortality in the presence of elevated nutrients. Parrotfish herbivory is accepted as beneficial to coral reefs, and parrotfish predation is accepted as normally benign. My study will pin down the mechanism(s) in which these herbivores become agents of mortality and will transform how we approach the conservation of coral reefs, and more specifically, trophic interactions on a reef. Restoration of parrotfish populations may have negative consequences for coral health if efforts are not simultaneously made to combat water quality issues.
Broader Impacts: During the field season in Moorea, I will design and conduct interactive teaching workshops for the local community similar to my outreach as an undergraduate. Using resources at the Gump Station and connections with the Atitia Center for outreach, I will print 2-D reef replicas of my nutrient-enriched and ambient level *in situ* corals over time for use in citizen science training. Local schoolchildren and adults will use the photos along with quadrats, transect tape, identification guides, and whiteboards to 'become' a marine biologist for a day. I will guide participants in using quadrats to quantify metrics of reef change, such as percent live coral cover. Children and adults will experientially observe how nutrients such as fertilizers affect the health of marine species. Through this citizen science initiative, I hope to transform how local communities understand and interact with their coral reefs.
Citations [1] Zaneveld *et al.* (2016) *Nat Commun*, **[2]** Rotjan & Lewis (2008) *Mar Ecol Prog Ser*, **[3]** Correa *et al.* (2009) *Coral Reefs,* **[4]** Vega Thurber *et al.* (2009) *Environ Microbiol,* **[5]** Ritchie (2006) *Mar Ecol Prog Ser,* **[6]** Glasl *et al.* (2016) *The ISME Journal*, **[7]** Caporaso *et al.* (2010) *Nat Methods,* **[8]** Nayfatch *et al.* (2015) *PLoS Comput Biol,* **[9]** Parks *et al.* (2014) *Bioinformatics*.