The staghorn coral, *Acropora cervicornis,* grows faster than any known Western Atlantic coral and is a crucial reef-building coral. Due to mass mortality from white band disease (WBD) in the Caribbean, however, *A. cervicornis* is currently designated as critically endangered by the IUCN Red List of Threatened Species. While the etiological agent of this disease is unknown, my lab recently discovered that a bacterium associated with the disease is strongly stimulated by nutrient pollution. In early 2017, I assembled the genome of this obligate intracellular parasite of *A. cervicornis* and, based on its phylogenetic position and genome content, I hypothesize that it is responsible for WBD and the destruction of Caribbean *Acropora.* Yet we do not yet know its mechanisms of transmission and disease development (pathogenesis) and further experiments are needed to confirm its role as the agent of disease. Using a combination of comparative genomics and field experiments, I aim to evaluate the gene expression, biogeography, and evolution of this bacterium to help prevent and manage this disease on coral reefs in the future.

Background: WBD has been observed in the Caribbean, Red Sea, and the Pacific, but its mechanisms of pathogenicity are uncharacterized. Transmission experiments suggest that WBD is caused by bacteria,[1] with species of Rickettsiales implicated as possible etiological agents,[2,3] yet these taxa are present in both healthy and diseased coral microbiomes. Recent studies[4,5] led by my advisor at Oregon State, Dr. Rebecca Vega Thurber, indicated that exposing corals to nitrogen stimulates the growth of an intracellular bacterium (order Rickettsiales). A strong negative correlation was found between the abundance of this taxon and coral growth (r^2=0.9987, $p<0.001$) in the presence of nutrients.[5] In *A. cervicornis*, this taxon increased from <11% of the microbial community to ~88% after 8 weeks of enrichment. In an unpublished study, tissue homogenates were generated from diseased and healthy *A. cervicornis*. The diseased homogenate, which caused a sixfold increase in mortality of exposed corals, was found to have a relative abundance of >50% Rickettsiales, while these species comprised only 0.1% of the healthy homogenate.

While known pathogens in the genus *Rickettsia* clustered together in a 16S rRNA phylogenetic tree, I discovered that the intracellular parasite of *A. cervicornis* clustered most closely with uncultured symbionts of marine invertebrates, primarily corals and sponges.[6] Based on strong statistical support for the distinction of these intracellular symbionts of marine invertebrates from other Rickettsiales, I proposed a new genus, *Marinoinvertebrata* gen. nov., in a publication in preparation for the ISME Journal.[6] I named the newly-discovered parasite *M. rohwerii* sp. nov. after the lab that first observed it.[3] Given their abundance in corals exhibiting reduced growth and signs of WBD, **I hypothesize that: (1)** *Marinoinvertebrata* spp. are members of the healthy coral microbiome but have evolved mechanisms of pathogenesis (encoded by virulence genes) that are modulated by environmental factors. **(2)** The increased expression of these genes due to nutrient exposure, in tandem with weakened host immune function caused by pollution, leads to coral disease. I will address these hypotheses through two central questions:

Q1: How do mechanisms of pathogenesis and environmental response differ in species of *Marinoinvertebrata* from distinct regions? (Aim 1) I will compare the genome of *M. rohwerii* to genomes of disease-causing *Rickettsia* to identify homologs to virulence genes. I will also compare the pathogenicity of this organism with that of related species from different regions.

Q2: How does concurrent exposure to nutrients and infection by *Marinoinvertebrata* spp. alter host physiology and induce disease? (Aim 2) I will conduct nutrient enrichment experiments on six genotypes of *A. cervicornis* to induce growth of *Marinoinvertebrata* spp. I will track parasite abundance using quantitative PCR as well as changes in microbiome composition, host/pathogen gene expression, growth rate, and disease progression. Lastly, I will use nanoscale secondary ion mass spectrometry (NanoSIMS) to trace parasite nutrient assimilation.

Research Plan: Aim 1: Via the KAAS server,[7] I will use reference genomes of pathogenic *Rickettsia* to guide my search for homologous virulence genes in the assembled genome of *M. rohwerii*. Using this method, I previously uncovered a complete Type IV secretion system in this genome, which is involved with host infection and genetic exchange in related bacteria[8], as well as the NtrY-NtrX two-component system involved in sensing extracellular nitrate levels. However, additional virulence and environmental response genes present in *Rickettsia* may have more distant homologs in *Marinoinvertebrata* spp. As part of the Global Coral Microbiome Project and Tara Pacific, I have access to microbial community data from hundreds of Caribbean and Indo-Pacific coral samples and have identified species of *Marinoinvertebrata* in samples from Australia, Saudi Arabia, and Colombia. I will assemble the genomes of these species and determine whether they also possess virulence genes, and whether these are modulated by environmental-sensing genes.

Aim 2: I will expose six different genotypes of *A. cervicornis* (raised in nurseries at Mote Marine Lab (MML)) to various levels of inorganic nitrogen to stimulate Rickettsiales proliferation as shown previously.[4,5] Three of these genotypes are sensitive to WBD and three are resistant, as determined by Dr. Erinn Muller, who will be my host at MML. From the genome of *M. rohwerii*, I will generate quantitative PCR markers to track its absolute abundance throughout the enrichment experiments. Coral fragments will be sampled weekly with the help of students from MML's after-school program, and high-throughput RNA sequencing will be used to assess Rickettsiales and host gene expression. I will track coral health through growth, photosynthesis, and respiration rates and changes to the host microbiome using 16S rRNA amplicon analysis. Finally, I will spend the summer of 2019 working with Dr. Xavier Mayali of Lawrence Livermore National Laboratory, using NanoSIMS to isotopically trace whether *M. rohwerii* scavenges nutrients from the host, from symbiotic algae, or from the environment.

Intellectual Merit: Our ability to directly alter host-microbe interactions using nutrient enrichment provides a reliable model to ascertain which genes play a role in disease initiation and host response. Beyond its implications for coral disease, the opportunity to reconstruct the genome of a novel pathogen is rare and may uncover new mechanisms of transmission, especially when this pathogen is traced throughout different regions and coral hosts. As coral reef fish are crucial to the economy of many tropical regions, it is critical to combat the rapid destruction of their habitat by disease. The PCR primers I develop for this experiment can be used to quantify disease progression and track the spread of *M. rohwerii*, and our understanding of the host and pathogen transcriptomes will contribute to further studies on antibiotic treatment of WBD. Lastly, as MML's coral nurseries were damaged by Hurricane Irma, my research will inform recovery efforts as genotypes most resistant to *Marinoinvertebrata* infection will be used in new nurseries.

Broader impacts: Given my extensive advising and leadership experience, the robust educational programs already established by MML will provide an excellent framework to involve the local community in my research. Through MML's Research-Based After-School Program for Students, I will work directly with high school students passionate about ocean conservation to introduce them to lab- and field-based research by helping them develop short-term experiments on how nutrient dose effects parasite growth. Dr. Muller and I will lead volunteer teams to propagate new nurseries and learn about the effects of coral disease. These teams will "adopt" their own corals to observe over time, increasing their personal investment in the reef. I will also work with the Oregon Coast Aquarium as a Scientific Interpreter to lead demonstrations showing how nutrient pollution negatively effects the health of all marine organisms, not just coral systems.

Citations: [1] Gignoux-Wolfsohn *et al.* (2012) *Sci. Rep*. **[2]** Miller *et al.* (2014) *PeerJ.* **[3]** Casas *et al.* (2004) *Environ. Microbiol.* **[4]** Zaneveld *et al.* (2016) *Nat. Commun.* **[5]** Shaver *et al.* (2017) *Ecology* **[6]** Klinges *et al*. (in prep.) *ISME J*. **[7]** Moriya *et al.* (2007) *Nucleic Acids Res.* **[8]** Cascales *et al.* (2003) *Nat. Rev. Micro.*