Improving Symbiodiniaceae Cell Density Measurements Using Flow Cytometry

Introduction: Climate change impacts, including rising temperatures and ocean acidification, are threatening the survival of coral reef ecosystems worldwide (Obura 2005; Hoegh-Guldberg et al. 2007). Higher temperature and pCO_2 conditions cause cellular damage in both the symbiont and host cells, which over time can lead to breakdown of the vital coral holobiont symbiotic relationship (Gates et al 1992; Baker et al 2008). This dysbiosis between the coral host and intracellular Symbiodiniaceae, termed "coral bleaching", is a physiological stress response leaving the host without its primary nutritional resource that can cause starvation and mortality. Coral bleaching events are increasing in both frequency and intensity (Hughes et al. 2018), leaving coral reef ecosystems decimated worldwide. Quantifying the intensity of coral bleaching is, therefore, a crucial component of understanding the coral holobiont stress response.

A common method of measuring coral bleaching is by Symbiodiniaceae cell counts. The current procedure uses manual cell counting under a compound microscope with a specialized counting slide, or hemocytometer. This is an accurate method but an extremely tedious and ineffective use of laboratory time. Because cell density is such a critical measurement for addressing knowledge gaps in mechanisms that underlie the coral bleaching process, there is a need for a more efficient method. Due to symbionts naturally having a red fluorescent glow, using the flow cytometer will be easier, as there is no need to add fluorescent dye. Flow cytometry has higher precision, accuracy, and efficient use of time (due to the instrument's automated sample handling). With this method, algal counts can be quantified accurately over a wide range of densities and in small volumes of tissue homogenate (Krediet et al 2015). The use of flow cytometers for Symbiodiniaceae counts has been proven to be successful in several laboratories, but has yet to be used here at URI in the Putnam Lab. In light of this, I propose to develop a new flow cytometry protocol that will provide more effective and efficient cell count measures to further investigate the effects of coral bleaching on the coral-symbiont relationship.

Aim 1: To improve current cell density quantification by developing a flow cytometry protocol, and to optimize this process for the four prominent coral species in the Putnam Lab: *Astrangia poculata, Pocillopora acuta, Montipora capitata,* and *Porites astreoides.*

***Methods 1*:** First, we will airbrush collected coral fragments from each species and create a tissue homogenate consisting of host and Symbiodiniaceae cells in phosphate-buffered saline (PBS). Next, we would aliquot 500 uL of the homogenate and centrifuge to concentrate the algal pellet. After removing the supernatant (coral host portion) the algal pellet will be resuspend in PBS. Each sample will be counted on a haemocytometer in six replicates.. Then I will optimize side and forward scatter, and fluorescence settings on the flow cytometer (BD FACSVerse) to isolate Symbiodiniaceae cells from debris. I will repeat this process for all four species, as each species has varying types of symbionts, so we can assume the settings will be different. To ensure the accuracy of this new method, I will create linear regression models to compare the flow cytometer counts to the haemocytometer. After the new method is verified, I will publish the new protocol on Github to be an open source and reproducible resource for the rest of the field. This will allow my new protocol to be used by other

professionals across the country, and helping other scientists on research is a huge goal of mine for not only my undergraduate career but the entirety of my future career as a research scientist.

Aim 2: To measure cell density using the newly developed flow cytometer protocol to quantify coral bleaching intensity in response to multiple stressors over time.

***Methods* 2:** Two common reef-building corals, *Montipora capitata* and *Pocillopora acuta*, were exposed to two months of stress (increased temperature, increased pCO_2 concentrations, or a combined temperature and pCO_2 treatment) and two months of ambient recovery conditions in 2018. Corals were physiologically sampled during both acute (1 and 2 days) and chronic (1, 2, 4, 6, and 8 week) stress, and recovery (12 and 16 week) time points. I had previously airbrushed numerous *Montipora capitata* and *Pocillopora acuta* in the Spring 2020 semester and saved the homogenates for later downstream processes. With the funding, we would have the ability to measure the cell densities of these samples. Symbiodiniaceae cell density measurements will be analyzed with a two factor analysis of variance (ANOVA) statistical test to assess the significance of the effect of treatment through time using R and RStudio.

With the funding from the Biological Sciences Undergraduate Research Grant, I will be able to strengthen and broaden my ecophysiology research skillset. I would be able to expand my laboratory work and protocol development abilities, improve on data analysis methods and statistical methods using R, and continue to develop scientific writing and data visualization skills. I would also be given the opportunity to present my research at the Spring 2021 undergraduate research symposium. Throughout my time at URI, I have become incredibly interested in marine ecophysiology in ecosystems like coral reefs and intertidal zones and how they are affected by the ever-changing environment. I want to explore how environmental conditions affect the functionality of organisms in high-tolerance ecosystems such as intertidal zones and what conservation efforts can be made to help deter these effects. This proposed project would allow me to take lead on my own research in the Putnam Lab and allow me to learn on my feet what it truly is like to perform personal research, collect data, and write scientific papers. As I look forward to my future career and graduate programs I know mastering these skills would put me one step ahead of other applicants in my desired field.

I have been continuously working with Emma Strand, a Ph.D. student in Dr. Putnam's Lab, for a semester already, and plan on continuing to work under her throughout the summer and into my last year as an undergraduate at URI. I have had the pleasure of getting to know everyone in the lab and have been able to establish a strong foundation with my fellow scientists. I will be given the opportunity to work alongside Dr. Putnam as well as Emma next year on this project if chosen.

References

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