**Differences in Gene Expression and Microbiome in the sea star *Pisaster ochraceus* growing in two different environments**

Lauren Ashlock, Laura Caicedo-Quiroga, Kattia Palacio-López, Charles Kilpatrick

Since the onset of the most recent outbreak of Sea Star Wasting Disease, sea star populations from Alaska to Baja have suffered massive population declines1. Although a densovirus has been associated with wasting symptoms, the causative agent of the disease is not definitively known1. Both variation in susceptibility to wasting among species and conspecific variation attributed to differences in temperature regime, wave exposure, age, size class, and microhabitat have been reported1,2.

One species impacted by this epidemic is the rocky intertidal keystone predator, *Pisaster ochraceus*1. *P. ochraceus* is commonly found in the mid to low intertidal zone and in subtidal waters to depths of ~90m3. The intertidal zone is characterized by high wave action and organisms living in this zone are subject to long periods of emersion, where they are susceptible to desiccation, high UV irradiation, and rainfall. The subtidal is a more stable environment, with less wave action and no periods of emersion. These habitats are also characterized by differing compositions of algae and invertebrates. These differences in habitat type may confer different stress levels, general health, and microbiome composition of *P. ochraceus* living in the intertidal versus living in subtidal environments.

In this study, we aim to identify the differences in host gene expression and microbiome composition among intertidal and subtidal *P. ochraceus* collected from Monterey Bay, California. Sea stars used in this study varied in their expression of wasting symptoms. Some organisms remained healthy throughout the duration of observation, while others succumbed to the disease. Interestingly, a larger proportion of animals collected from the intertidal developed wasting symptoms as compared to animals collected from a subtidal environment. Understanding the role of habitat type in defining host gene expression and microbiome composition will provide further information regarding the dynamics of this enigmatic disease.

We ask how the environment affects gene expression and epidermis microbiome in *P. ochraceus*. Specifically we propose a mechanistic approach using genomic techniques to test two hypotheses: (1)there is a difference in gene expression across the whole genome of *P. ochraceus* that grows at the intertidal and subtidal zone of the Monterey Bay,  particularly on genes that activate immune responses, thus individuals growing on the intertidal zone will have higher levels of expression in those genes. (2) there is a difference in diversity and/or taxon abundance of the epidermis microbiome communities of the organisms that grow on the two different environments. Individuals that are less sensitive to the pathogen (healthier) are characterized by a more diverse microbiome community.

For this study, we will use RNA sequences for 24 individuals of *P. ochraceus* after three days of acclimation. We have selected to use this day as a snapshot of the individuals at the start of the experiment when all sea stars are in the health state they arrived in but after acclimation to the aquaria. Our analysis will use day 3 16S rRNA sequences obtained with RNA-seq to categorize the microbiome into taxa using BLAST searches for sequence similarity. We will estimate the relative abundance and diversity of the microbiota for an assessment of the community structure for both groups using multivariate analyses.

Transcriptome sequences will be used to analyze differences in the level of gene expression. We will define a threshold to identify significant expression. Genes expressed in common and differentially will be identified, particularly those related to immune response. To parse out the effect of handling in gene expression, we will focus on expression related to immune response and discard any expression related to stress response. Additionally, healthy and sick individuals from the Intertidal and Subtidal zone will be compared. Matrices of the host’s gene expression and changes in the microbiome will be compared using a Mantel Test.

References

1. Menge, B et al. (2016). Sea Star Wasting Disease in the Keystone Predator Pisaster ochraceus in Oregon: Insights into Differential Population Impacts, Recovery, Predation Rate, and Temperature Effects from Long-Term Research. PLOS One, 11(6), E0157302.
2. Eisenlord, M et al. (2016). Ochre star mortality during the 2014 wasting disease epizootic: role of population size structure and temperature. Philos Trans R Soc Lond B Biol Sci, 371(1681).
3. Lambert, P. (2000). Sea Stars of British Columbia, Southeast Alaska and Puget Sound. Royal BC Museum.
4. Lauren…2015