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

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Since the onset of the most recent outbreak of Sea Star Wasting Disease, sea star populations ranging from Alaska, USA to Baja California, Mexico have suffered massive population declines.1 Though a densovirus has been associated with wasting symptoms, the causative agent of the disease is not definitively known.1 There is variation in susceptibility to wasting among impacted species. Additionally, variation among conspecifics has been attributed to differences in temperature regime, wave exposure, age, size class, and microhabitat.1,2

One of the 20+ 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 as well as in subtidal waters to depths of ~90m.3 The intertidal zone is characterized by high wave action. Additionally, organisms living in the intertidal are subject to long periods of emersion, where they are susceptible to desiccation, high UV irradiation, and rainfall. In contrast, the subtidal is a more stable environment, with less wave action and no periods of emersion. These two 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 *P. ochraceus* living in subtidal environments.

In this study, we aim to identify the differences in host gene expression and microbiome composition among intertidal and subtidal sea stars collected from Monterey Bay, California. Sea stars used in this study varied in their intensity of wasting symptoms. Some organisms used in this study remained healthy throughout the duration of observation, while others succumbed to the disease. 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 growth 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 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 tissue sampled 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 used 16S of the ribosomal RNA of the microbiome that was amplified and subsequently sequenced with RNA-seq on day 3 to categorize into taxa at the genus level or higher using BLAST search for sequence similarity.  RNA of the host was sequenced for assembly into transcriptomes. We will make estimates of relative abundance and diversity of the microbiota for an assessment of the community structure for both groups using multidimensional scaling or discriminant analysis. Matrices of the host’s gene expression and changes in the microbiome will be compared using a Mantel Test.

Transcriptome sequences will be used to analyze differences in the level of gene expression. We will define a threshold to identify significant expression between the two zones. 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.

References

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