**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 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 diversity and/or taxon abundance of the epidermis microbiome communities of the organisms that grow on the two different environments and (2) 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. Previous results in sea star have shown that sick individuals express higher immune responses than healthy individuals4. Given this result and base on our observation on the difference in the health condition of the animals among the two zones, we expect that individuals growing on the intertidal zone present higher levels of expression in genes that activate immune responses.

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 three 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 responses. To parse out the effect of handling in gene expression, we will discard any expression related to stress response. In order to identify differences in gene expression and in the epidermal microbiome of the individuals growing in the subtidal and intertidal zones, we will create gene expression and microbiome matrices that will be compared using statistical techniques such as a Mantel Test. With this approach we aim to understand the influence of the environment on the sea star *P. ochraceus,* particularly on their disease susceptibility. Our results will generate a solid background for future research in this model system and the effect of pathogens on their life period.

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. Fuess LE et al. (2015) Up in Arms: Immune and Nervous System Response to Sea Star Wasting Disease. PLoS ONE 10(7), e0133053.

Guys,

Very nice study as outlined. I have 2 suggestions for possible follow-up analyses if time permits:

1. Think about testing for genetic divergence (at the DNA level, using SNPs) between the inter- and sub-tidal groups. If you saw now genetic divergence, but significant differential expression between groups, this would provide a nice confirmation that this is being driven by habitat differences and how they affect physiology.
2. I like the overall approach of the Mantel’s test idea to associate expression with microbiome, but I think it deserves some more thought about the “how’s and why’s” of doing it. One might conceive of outputting a separate expression matrix for each gene, and then testing each for association with the microbiome. This could potentially identify which gene’s expression is associated with microbiome responses, and then could see if this was non-random with respect to the genes showing differential expression between your tidal groups. I think you need some kind of approach like this to connect it back to differences between your tidal groups.

Let us know how we can help as you move through your analyses. –Steve

Hello Team,

Nicely motivated study. I like the focus on Day 3 and the goal to integrate gene expression and microbiome composition data. The program we will use in class for these types of analyses is Qiime – there may be a module within that package that tests for an association with gene expression data. If not there, there then there are other studies that have done such analyses. Let us know if you want some papers suggested and/or if you want to think through these analyses. Definitely, there is also the opportunity to integrate the SNP data as Steve suggests.

My best, Melissa