### **Using reverse pathology to characterize the pathogen associated with seastar wasting disease**

**Team Sherlock: S. Alger, L. Ash, A. K. Brody, P. A. Burnham, A. Das, & E. Keller**

**Introduction.** Since 2013, sea star (asteroid) populations of the Pacific Coast from Mexico to Alaska have experienced severe population declines caused by a rapidly spreading disease identified as sea star wasting disease. Putatively caused by a virus, this highly virulent marine epizootic disease causes lesions, loss of turgor, limb autonomy, and death (Hewson et al. 2014). Surveys have documented up to 100% prevalence in some sites and outbreaks of the disease have been identified as the largest documented marine epizootic disease of non-commerical marine animals (Eisenlord et al. 2016).

A densovirus was associated with the sea star wasting disease (Hewson et al. 2014). In later experiments, artificial inoculation with a viral sized fragment of diseased tissue elicited a suite of immune system related gene responses as well as differential expression of genes responsible for nervous system processes and tissue regeneration in *P. helianthoides* (Fuess et al. 2015). Although the implication of a densovirus seems clear from these experiments, recent work suggests that other causal agents may be involved. In particular, numerous sea star individuals exhibited classic symptoms of the disease but were found negative for densovirus (Pespeni et al, unpublished work). A first step in preventing future outbreaks is to characterize the causal agent(s). Thus, further work is critically needed.

We propose to take a “reverse pathology” approach to elucidate what is driving the disease. Using genomic tools we will characterize the immune response of healthy versus sick *Pycnopodia helianthoides* individuals, the latter of which presented with symptoms classically associated with sea star wasting disease. We will attempt to identify the pathogens associated with sea star wasting disease by measuring differences in the expression of immune-related genes between healthy and sick sea star individuals. In addition, we will measure and compare immune system gene expression to a randomly chosen set of genes in both healthy and sick individuals, since it is the *difference* in gene expression from others that is the response of interest.

We hypothesize: 1. There are differences in immune related gene expression between healthy and sick individuals and there is upregulation or downregulation of those genes as individuals transition from healthy to sick, and 2. differences in expression of immune related genes can help identify the causative agent of sea star wasting disease.

**Methods.** First, we will identify candidate genes of known importance in immune response to particular pathogens (viral, bacterial, fungal, parasitic, and autoimmune) from the literature. These genes will be the focus of our investigation.

From the individuals collected by Pespeni (Year), we identified 5 seastar individuals that were healthy throughout the study (HH), 5 that presented as healthy initially but then became sick (HS) and 5 that were identified as sick, remained sick and eventually died (SS). From those that were healthy and then became sick, we identified the time steps of sampling that spanned this transition and the corresponding HH and SS days (**Table 1**). Thus for 5 individuals/class (HH, HS, and SS) we have 2 sampling dates (before and after the transition) for a total of 30 datasets.

Using available software (e.g. DESeq, edgeR, baySeq, NOIseq), we will evaluate the differential gene expression of our genes of interest (by mapping the reads with the reference) between the three groups (HH, HS, SS). To assess for differences in gene expression between groups, we will also compare gene expression in a group of random genes. Differentially expressed genes can be matched with other homologs and orthologs in various organisms from which they are known to play a key role in immune response using a multiple sequence alignment and blast approach.

**Expected Outcomes.** Upon conducting a literature review, we expect to identify multiple genes among echinoderm species associated with mounting an immune response to different pathogen types (ie. viral, bacterial, fungal, parasitic, and autoimmune). We may also broaden our search to include genes conserved across invertebrates. Upon analyzing our sequence data, we expect to identify differences in gene expression for several immune related genes between healthy and sick individuals. We expect to see similar trends in gene expression for healthy individuals that became sick. If we are unable to identify genes specific to a pathogen type, we will instead examine species specific differences in immune response to sea star wasting disease by comparing the immune response of our focal species, *P. ochraceus*, to that of *P. helianthoides* (Fuess et al. 2015).

**References**

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**Potential resources**

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**Table 1. Samples we plan to use in our experiment. We will choose 5 individuals from each of the three groups: healthy individuals that became sick (HS), healthy individuals that remained healthy (HH) and sick individuals that remained sick (SS). IDs of each sample are listed in the table.**

|  |  |  |  |
| --- | --- | --- | --- |
| **HS ID** | **HS Transition Days** | **Matched HH ID** | **Matched SS ID** |
| **08** | 3-6 | 10 | 26 |
| **09** | 3-9† | 24 | 22 |
| **15** | 9-12 | 27 | 28 |
| **19** | 12-15 | 31 | 23 |
| **20** | 12-15 | 33 | 36\* |

**† Missing data on day 6, matched with individuals with data available on days 3 and 9**

**\*Missing SS individual with data on days 9 and 12, so this individual has data only for days 3-9**

Guys,

This looks quite interesting, and I especially like aspects of your experimental design (choosing candidate genes a-priori, controlling for background expression levels using a matched set of randomly chosen genes, matching temporal samples across your HH, HS, and SS categories). The one thing that isn’t clear to me from your plan is how you will treat the temporal reps in comparisons of the disease status categories. I can envison a couple different ways of proceeding here:

1. Compare HH v. HS v. SS at each time step, so you have a 1-way ANOVA type analysis that you do twice (before and after H>S transition period). What predictions would you make for how the differential expression (DE) results might change between the 2 time periods?
2. Compare HS samples for before and after transition to identify DE genes associated with the transition. Then compare HH v. SS samples at a single time point to determine a second set of DE genes associated with consistent differences between healthy and diseased individuals. One could then make the Venn diagram between these 2 analyses to see which of the HS DE genes overlap with the systematic differences observed between HH and SS individuals.
3. Other possibilities for using your temporal and disease status reps exist as well…worth brainstorming about this as a group.

Good luck. Let us know how we can help. –Steve

Team Sherlock,

This study will be particularly interesting as there is a fair amount know about the echinoderm immune system and there is another RNAseq study with which to compare results. As I mentioned in the text comments, it will be particularly interesting to compare to the sunflower star results (Ruess et al.) since those were experimentally infected with virus-sized particles and our animals showed symptoms without virus-particle injection. How consistent are the immune responses (a) across different experiments when the symptoms are the same, and (b) between different species?

For your analyses, I was thinking of something along the lines of option 2 that Steve describes. Three tests for differential expression: for the HS individuals when they’re H vs. S, and the respective “controls” matched for days, H vs. H, and S vs. S. Your expectation could be that you would see differential expression of your a priori identified immune genes in this first test, and they wouldn’t show up in the second or third tests. This would be an interesting hypothesis, however, several factors could make the results not match the predictions, e.g., if the HH are launching an immune response but not presenting with detectable symptoms, and that the SS ones could be differentially regulating their immune genes through time.

Let us know how we can help!

My best,

Melissa