The Rising Sea Stars

Proposal

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Since June 2013, millions of sea stars in at least 20 different species (Asteroidea), from Alaska to Mexico, have perished due to a suite of symptoms called sea star wasting disease (SSWD), which involves loss of turgor pressure, lesions, loss of limbs and ultimately, death (Hewson et al. 2014). As keystone species in many of marine ecosystems, mass mortality of sea stars could cause dramatic alteration and biodiversity loss of benthic communities (Lessios et al. 1984; Paine 1966). To avoid trophic cascades that would upset the balance of marine ecosystems and potentially cause irreversible damage, it is imperative to obtain information on both the pathology of SSWD and on the biology of the seastars. We are currently focusing on *Pisaster ocraceus*, a keystone predator involved in the regulation of mussel populations as the seastar species have exhibited severe and unprecedented population declines due to the 2013 SSWD outbreak.

The cause of SSWD is still unknown. A previous study has identified the sea star associated densovirus (SSaDV) as a potential causative agent of the disease when comparing gene expression and immune response to SSWD between sick and healthy individuals (Hewson et al. 2014). However, our data show that the echinoderms displayed symptoms and became infected even in the absence of the densovirus, indicating the possibility that another pathogen (or multiple pathogens) cause SSWD. Many environmental factors have also been shown to affect  the onset of the disease both directly and indirectly, rendering the task of finding its causes even more challenging. Previous sea star wasting events have been associated with warmer ocean temperatures (Bates et al. 2009; Staehli et al. 2009) and Eisenlord et al. (2016) exhibited in their lab experiment a faster progression of SSWD in ochre stars subjected to warmer temperatures. Recently, work was published on the effect of warmer temperatures facilitating the increase in abundance of dangerous bacteria such as *Vibrio* spp. that is another potential causative agent (Vezzulli et al. 2016). These studies indicate that we have very little knowledge as to what pathogen or pathogens cause(s) SSWD, how those respective pathogen(s) work, and how environmental factors including temperature and microbiota can facilitate its spread through altering the resistance or susceptibility of sea stars to the disease, which can consequently yield to disease outbreaks.

In this study, we seek to determine whether 1) there is genetic basis for resistance to SSWD in sea stars and/or 2) if the microbiomes of the sea stars are responsible for the disease’s progression. Based on previous findings by Hewson *et al.* (2014), we hypothesize that sea stars free of the disease are potentially doing so through genetic means such as having more suitable alleles of immune response genes. We will test our hypothesis by first comparing healthy and sick individuals from the same intertidal population under controlled conditions for 15 days to observe whether there are significant allele frequency differences between the healthy and sick individuals by looking at SNP data from the population. If our hypothesis is true, we predict to see a difference in allele frequencies between healthy and sick individuals at certain distinct loci. We will also compare our healthy intertidal individuals with healthy individuals from a neighboring subtidal population at these loci to observe whether the healthy individuals from both populations use the same mechanism to resist the disease. If so, we will include the healthy subtidal individuals in our initial analysis to increase the sample size of healthy individuals, which is currently rather small.

The second part of our study will be comparing the microbiomes of healthy and sick individuals to investigate the roles, if any, that microbiota plays in susceptibility or resistance of sea stars to the pathogen. This will be particularly helpful if genotypes of individuals have little to no effect on likelihood of being infected. We hypothesize that an individual’s microbiome does play a role in the onset and progression of SSWD and to test this, we will use 16S analyses on epidermal tissues of the sea stars at different times to 1) determine If individuals affected by the pathogen(s) harbor a different suite of microorganisms than resistant ones and 2) to detect the changes, if any in microbiome of individuals over time. If our hypothesis is true, we predict to see a significant difference in microbiome composition between healthy and sick individuals and also a significantly more stable microbiome over time in healthy individuals than in sick ones. To distinguish whether the change in microbiome composition is causing/exacerbating the disease or if it is simply a side-effect of the disease, we will look closely at individuals that were initially healthy but then turned sick during the course of our study; if a drastic change in microbiome precedes individuals showing any symptom of the disease, there is a possibility that the change in microbiome is the cause of the disease or at least partly responsible for the disease affecting the sea star.

Regardless of whether we find the actual cause of SSWD, our study will undoubtedly advance the knowledge of the scientific community in understanding this threat that echinoderms (and consequently marine communities) are facing and will help in determining conservation initiatives that may be required in the near future. Our study will furthermore contribute to the genomics of both *Pisaster ocraceus* and its microbiome as well as help us understand the interactions between echinoderms and their symbionts, that will aid other scientists such as marine biologists, ecologists, pathologists and epidemiologists in their research.

Rising Seastars,

Great ideas in this proposal. I especially like the focus on allelic differences between HH and SS individuals. As we move through the course and you gain proficiency with methods for working with the data, you’ll want to think about the best way to test for these differences. Absolute difference in SNP frequency? Fst? Treating SNPs within the same transcript as independent, or grouping them into haplotypes first, and then testing on the latter? We can help when you’re ready to dig in. -–Steve

Hi Rising Seastars,

Great ideas here. I like the focus on SNPs and the microbiome – this is unique relative to the other group projects, but as I mentioned in a comment above, you could potentially use the immune genes identified by Team Sherlock. If you do find SNP differences between the sick versus healthy, you could see if those genes are also differentially expressed – Expression QTL. Other suggestions include grouping the subtidal in if there aren’t strong genetic differences. I also note some other hypotheses regarding microbial diversity that you can test. Let us know how we can help as you get into analyses!

My best,

Melissa