The effects that diet, salinity and temperature contribute to Seastar Wasting Disease in *Pycnopodia helianthoides* and field surveying *Pisaster ochraceus*

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Abstract:

This study investigated Seastar Wasting Disease (SWD) in both *Pycnopodia helianthoides* and *Pisaster ochraceus*, involving field surveying, diet, temperature and salinity experiments. SWD describes a set of symptoms that has been seen in sea stars and it is most easily recognized by the presence of white lesions, tissue deterioration and eventually death. The pathogen and disease mechanism is still poorly understood and there have been scattered incidences of SWD reported for the last 50 years, however the current epizooitic has persisted for nearly a year, and has afflicted sea stars on over 3000 miles of coastline. Our study showed that increasing water temperature and decreasing salinity both independently correlate with increasing rates of symptoms appearing on *Pycnopodia helianthoides*. Field surveying of *Pisaster* displayed a strong correlation between temperature at the field site and percentage of sea stars showing lesions. Prevalence of SWD increases with seasonally warming temperatures and it is imperative to explore the mechanisms of this relationship and what ecological implications this entails.

Introduction:

Marine infectious diseases are one of the driving forces that shape marine ecosystems (Harvell et al. 2009) and their rate of occurrence has been correlated with increasing temperatures (Harvell et al. 1999). Wasting diseases have been seen across a variety of species, including coral reefs, bivalves, oysters, and abalones (reviewed in Burge et al. 2013). The current epizootic affecting sea stars has decimated populations for over a year and the consequences have yet to be realized. Mass mortalities have been associated with SWD as early as 1978 leading to total eradication of *Heliaster kubiniji*

from some California beaches (Dungan et al. 1982). Mass mortalities have also been occurring with the current epizootic, however, past wasting events have been confined to small communities and lasted less than a season (Eckert et al. 2000). This current epizootic has been found in sea stars from Alaska to Mexico (http://www.eeb.ucsc.edu/). Sea stars are keystone predators and play a top-down controlling role in intertidal communities (Paine 1966), thus declines in their population could have far-reaching consequences. Our investigation looks at how various stressors correlate to symptoms of SWD.

Asteroid diet is variable but a significant portion consists of bivalves (Jangoux & Lawrence, 1982). Bivalves as filter feeders are capable of bioaccumulation, the process of concentrating organic chemicals (Jangoux & Lawrence, 1982). Investigation into whether bivalves are capable of concentrating the pathogen responsible for SWD has yet to be studied. Our hypothesis is that *Nuttalia*, a genus of clams that are invasive to the Salish Sea and *Mytilus*, a genus of mussels, are capable of concentrating the pathogen. Our study involves incubating these bivalves with infected Asteroid specimens and then feeding infected food to healthy specimens and theoretically delivering a "heavy dose" of pathogen. This is expected to lead to an increase in symptoms when thermally stressed.

Other stressors are speculated to contribute to the vulnerability of Asteroids to infectious diseases. With increasing CO_2 emissions, salinity fluctuations are expected to increase (Burge et al. 2014). It is important to consider this implication, as a fluctuating salinity could play a contributing role to marine infectious systems. Asteroids are poor osmoregulators (Dethier Lecture, Marine Zoology, 2014) and susceptible to perturbations in salinity. *Pisaster ochraceus* locomotion is inhibited at low salinity levels, and can lead

to death at 15 psu (Held and Harley 2009). To investigate whether salinity stress would cause symptoms of SWD we compare two salinity levels, one that is consistent with levels around the Salish Sea and a lower level that causes stress but not death to healthy individuals.

To understand geographical distribution of SWD in the Salish Sea we conducted field surveys around the San Juan Island area. We looked at numbers of sea stars at beaches with suitable habitat; three on San Juan Island, four on Orcas Island, and one on Lopez Island. Our question was whether there would be any variation among the sites in the percentage of sea stars with symptoms of SWD and what factors may have contributed to these variations.

Methods:

This project was composed of three parts: *a)* An investigation into the relationship between diet, temperature and symptoms of SWD, *b)* An investigation to the effects of salinity on the rates of symptoms, and *c)* Surveying beaches and compiling data to investigate the ratios of *Pisaster ocrhraceus* showing symptoms of SWD.

a) Clams – *Nuttalia* – were obtained from Crescent Beach, Orcas Island WA (North 48.6951, East -122.8980) on April 19th, 2014 - an area with reported SWD – by digging in the intertidal. Mussels - *Mytilus* - were obtained from Crescent beach as well and were harvested during low tide from oyster beds. The clams and mussels were then kept in a sea tank with 4 infected *Pycnopodia* individuals for the duration of the experiment (34 days), with a four-day pre-incubation period. Deteriorating infected Asteroids within the tank were removed out and replaced with new infected specimens showing lesions to ensure hypothesized pathogen delivery to the clams and mussels.

These clams and mussels will now be referred to as "Hot Clams" and "Hot Mussels."

On April 23rd 2014, *Nuttalia* clams were harvested from Argyle spit – a beach with no reported SWD - by digging in the intertidal and *Mytilus* mussels were taken from Argyle creek (North 48.5217, East -123.0136). The specimens harvested from Argyle were considered "Clean Food." The clean food was placed into a sea tank and served as food to our control specimens.

Twelve *Pycnopodia* specimens were collected from Friday Harbor Biological reserve from the dock pilings using SCUBA and placed into individual sea-tables with an average temperature of 10.9 ± 0.73 °C. Three specimens were fed clean mussels, three were fed clean clams to serve as a control. Three were fed hot mussels and three were fed hot clams – thought to be carrying the pathogen. The specimens were left undisturbed for 17 days and were fed either two clams every two days or three mussels every two days. This diet continued for the duration of the study. Daily evaluations of the status of the *Pycnopodia* specimens were made. Individuals were deemed "Sick" based on the presence of lesions.

Temperature stress began on day 17. The water flow into the *Pycnopodia* sea tables was turned off and the resulting temperature increase was monitored and adjusted using minimal flow to maintain 16 °C. The temperature increase lasted for six hours and then flow was returned to normal, along with the temperature. Heat treatment duration was six hours every day for six days.

For 12 days following heat treatment the sea tanks were left with normal flow and normal temperature. On the 34th day, a final assessment was made of the *Pycnopodia* status.

b) Ten *Pycnopodia* specimens were collected from the subtidal dock pilings at Friday Harbor Laboratories and placed into two sea tanks (Five into each). Tank A had a water hose in addition to the saltwater flow, and flow was adjusted to an average salinity of 22.9 ± 1.79 ppt. and temperature of 12.8 ± 0.8 °C. Tank B had normal saltwater flow and an average salinity of 30.6 ± 0.8 ppt. and average temperature of 11.3 ± 0.2 °C. The sea stars were fed clean mussels and clams. Daily evaluation on the status of the specimens was performed and they were deemed "Sick" based on the presence of lesions. One specimen became sick immediately and was removed from tank B on the third day.

Part Three: Intertidal starfish surveys were conducted at eight sites, Point Caution, Reuben Tarte, San Juan County Park, West Sound, Rosario, Richardson, Indian Island, and East Sound Waterfront. Transects were decided based upon suitable Asteroid habitat and accessibility at low tide. Each survey site was divided into either one, two or three transects. Transect length ranged from 25 feet to 125 feet and width ranged from 5 feet to 15 feet. Each Asteroid in the transect line was recorded, and status having lesions or not was also recorded. Using a thermo probe we recorded three seawater temperatures approximately a meter from shoreline at each transect and three salinities.

Results:

The status (Lesions versus Dead versus Healthy) of the 12 total *Pycnopodia* individuals is summarized in Figure 1. Heat treatment began on Day 17 and continued until day 22. Normal temperature was maintained from day 23 until day 34. There is an obvious increase in the percent showing lesions following heat treatment.

Figure 1

Percent Healthy, Dead & Sick Stars
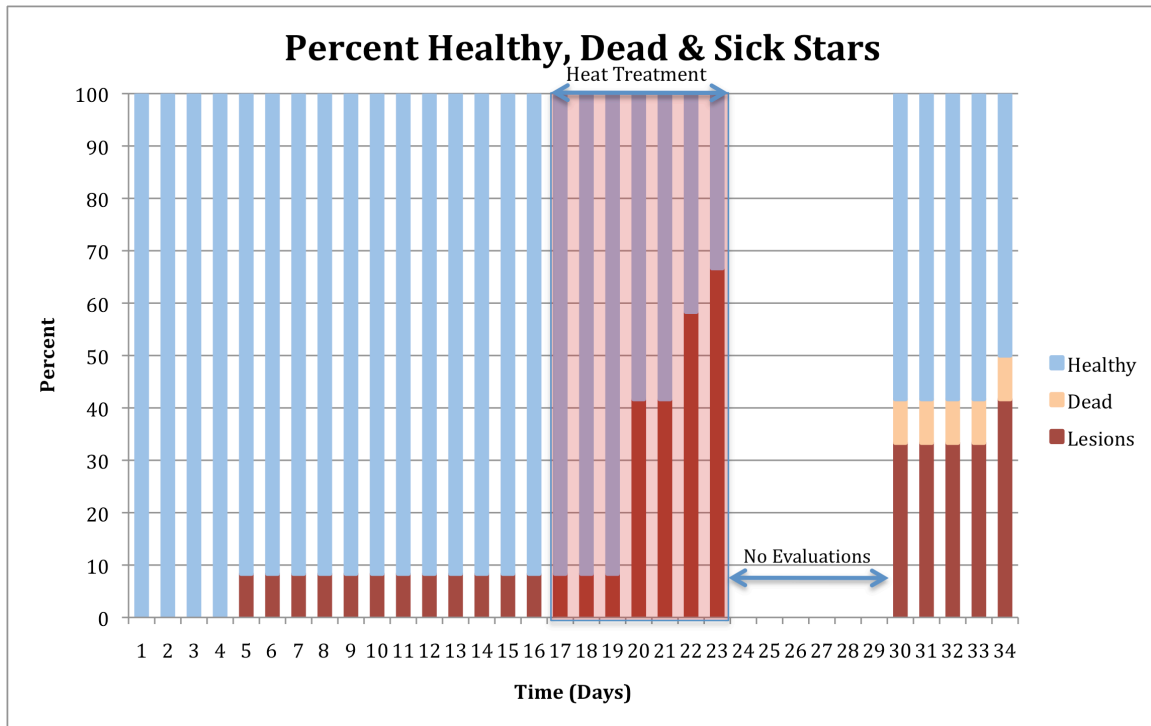
Day 17-23 signifies the time of the temperature treatments. No evaluations of the Asteroid statuses were performed between day 24 and 30.

The results of Clean Food versus Hot Food are shown in figures 2 and 3. We found no strong correlation between Hot Food and percent showing lesions. However, when comparing a clams versus mussel diet, summarized in Table 1, we found that clam was significant to symptoms of SWD (Chi Square test, p-value = 0.078).
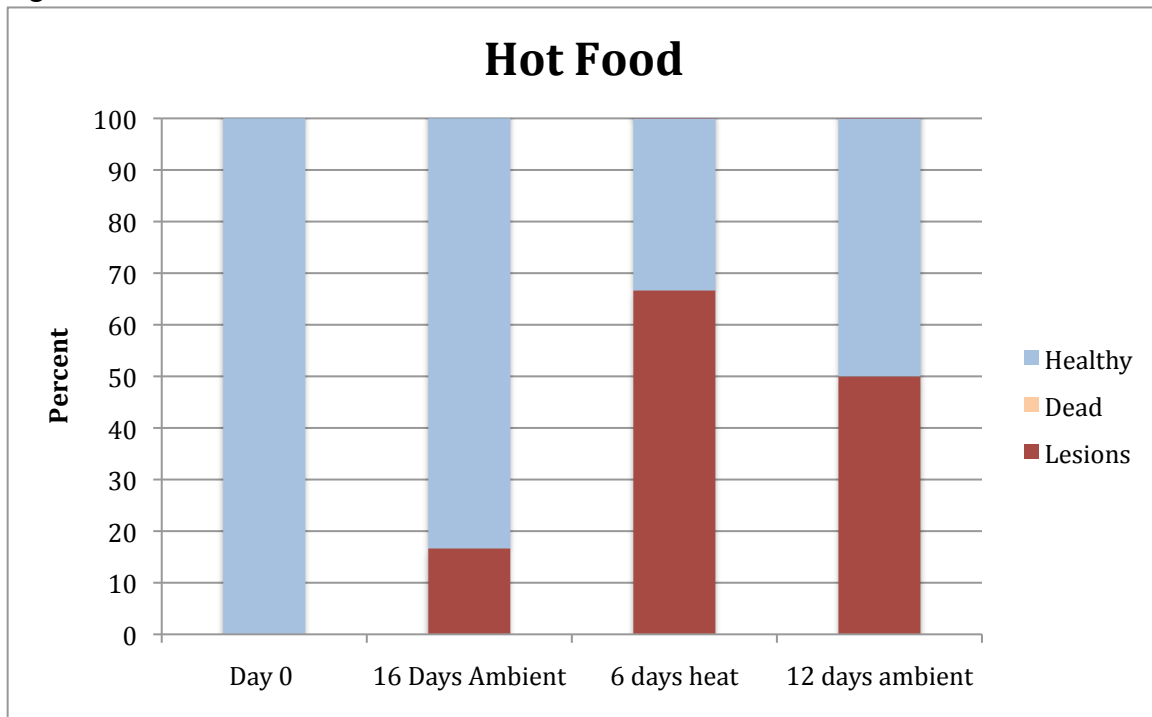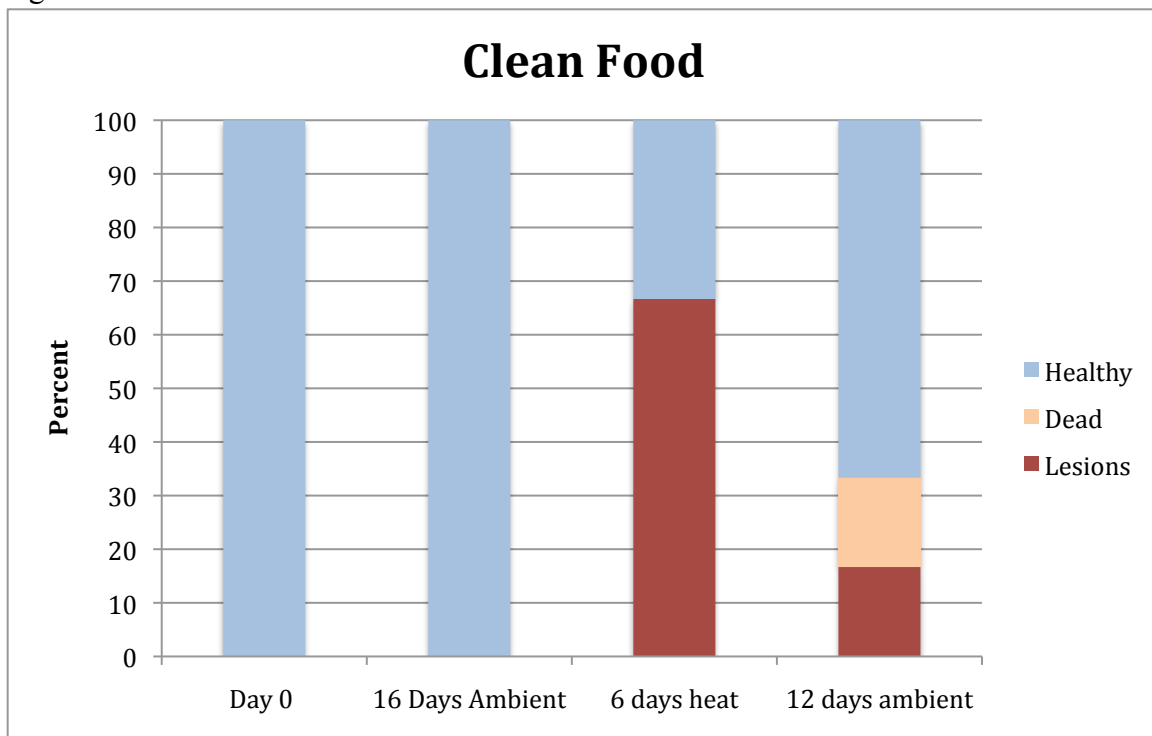
Figure 2

Hot Food

Figure 3

Clean Food

Figures 2 and 3 demonstrate how similar the percentage of sick sea stars is between Hot Food and

Healthy Food

Table 1

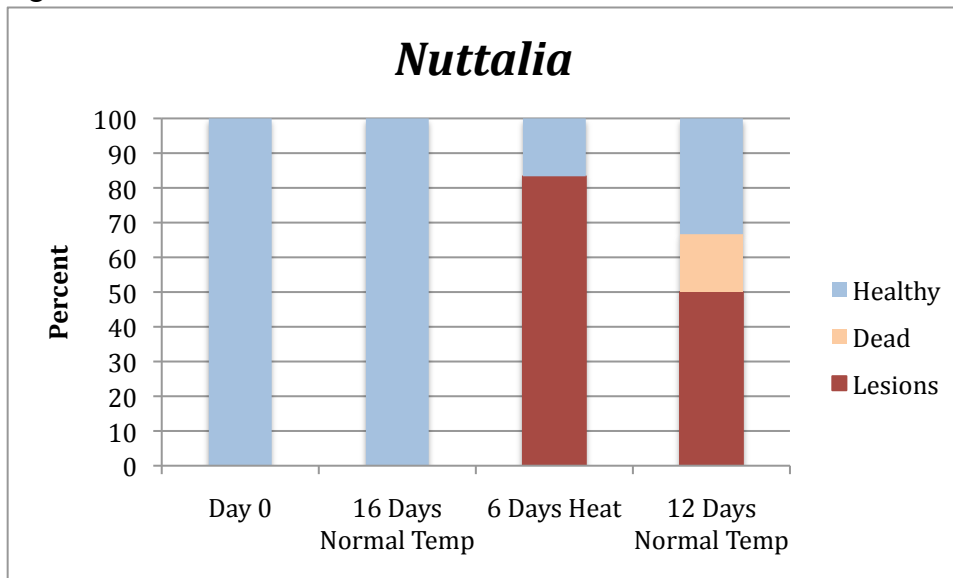| Day 34 Totals	Healthy	Lesions and/or Death
Diet Mussels	5	1
Diet Clams	2	4

Final day numbers of individuals showing lesions based on diet is significant (Chi Square test, p-value 0.078, one degree of freedom)

Figure 4
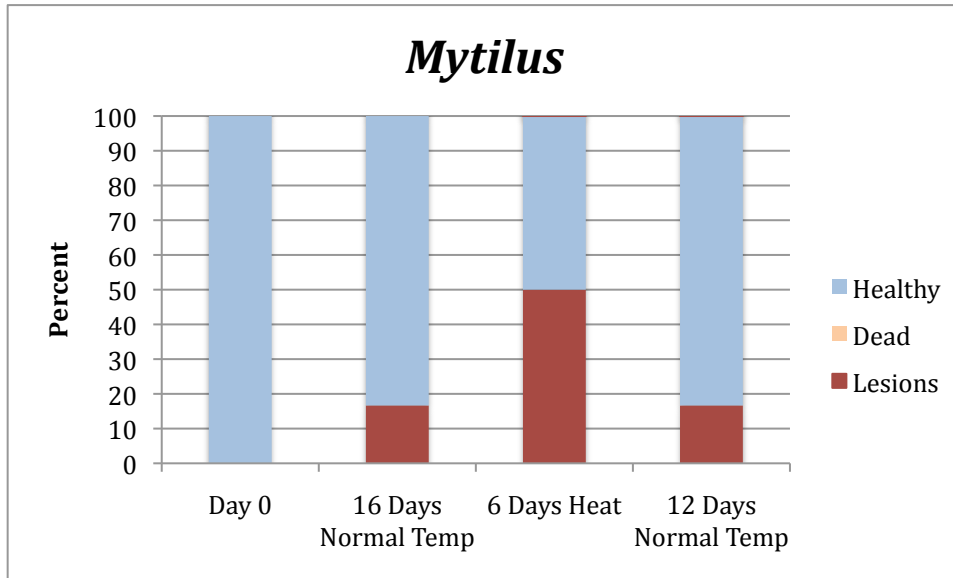
Final period assessments of sea star health are represented based on a mussel diet.

Figure 5

Final period assessments of sea star health are represented based on a clam diet

The salinity experiment led to a significant percent of sea stars with lesions in the low salinity tank versus the high normal salinity tank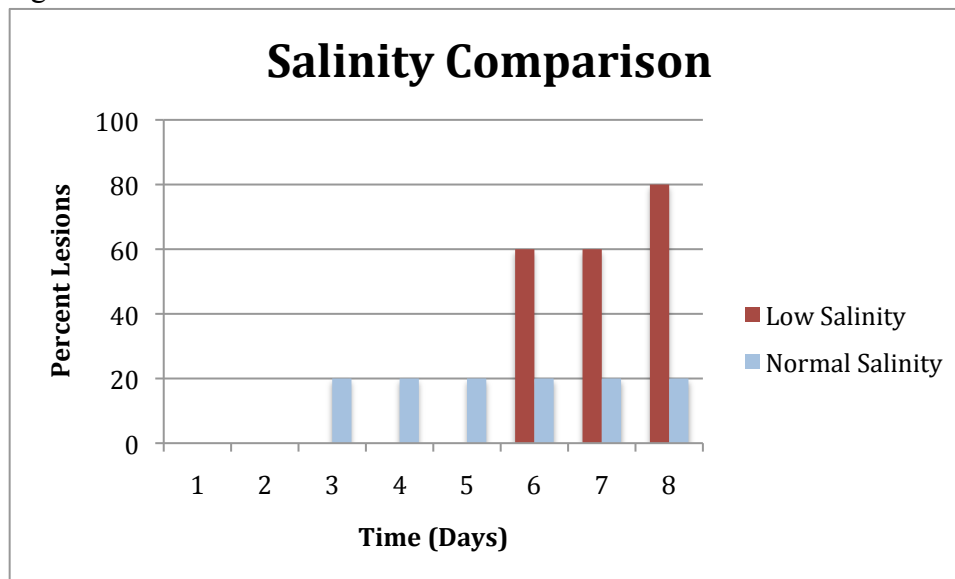 (Chi Square test, p-value = 0.058). Figure 6 shows the side-by-side percents of the low salinity versus high salinity tank.

Table 2:

Day 9 Totals	Healthy	Lesions and/or Death
A: Salinity: 23ppt	1	4
B: Salinity: 30.6	4	1

Presence of lesions based upon salinity is significant, Chi Square Test p-value = 0.0578

Figure 6

The low salinity tank shows a clear difference in final percent with lesions versus the normal salinity tank.

The field surveys revealed a very significant pattern between percent of *Pisaster* showing lesions and ocean temperature at the site (Figure 9). Data of other species of sea star are not included, as the numbers of individuals at each site were not large enough to produce unbiased results.

Table 3

Pisaster Intertidal Survey

Site	Avg Temp	Percent Lesions	Total Healthy	Total Lesions
Richardsons	10.9 ±0.64	4.12	93	4
San Juan County Park	11.0±0.67	0	20	0
West Sound	15.8±0.63	3.125	31	1
Rosario	15.1±0.37	2.09	281	6
Reuben Tarte	12.9±1.2	0	21	0
East Sound Waterfront	18.9±0.75	6.1	123	8
Indian Island	16.2±1.5	4.3	161	7
Point Caution	9.9±0	0	74	0

Figure 7

Pisaster Intertidal Survey

$y = 0.54x - 5.03$
$R^2 = 0.54$
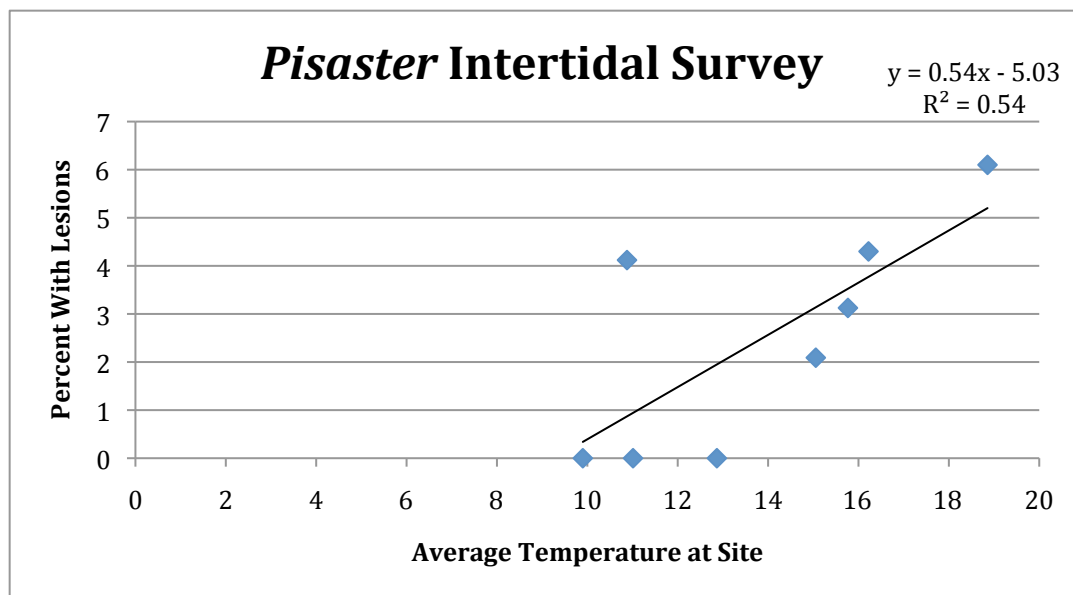
Temperatures were measured for each survey site and averages are shown on the x-axis, with percent of specimens sick shown on the y-axis.

Discussion:

The recent epizootic, SWD results in a complex set of symptoms whose many contributing factors make the underpinning mechanisms difficult to unravel. However, as demonstrated with several marine infectious diseases one stressor - ocean temperature - continues to be linked to occurrences of disease outbreaks. Our study revealed a clear correlation between ocean temperature and onset of the symptoms, namely lesions, in both the *Pycnopodia helianthoides* specimens in our diet/temperature experiment as well

as in the *Pisaster ochraceus* specimens that we surveyed. An a priori assumption about our *Pycnopodia helianthoides* specimens was that each had been exposed in some way to the SWD pathogen. The specimens used in our experiments were collected from the Friday Harbor Labs dock pilings, where incidences of SWD have been shown to be greater than 50% in late May (Trevor White & Edward Ni, subtidal survey, data unpublished), one month following the collection period. This is important to consider when evaluating the results of diet, temperature, and salinity experiments. Diet and the stressing factors employed were designed to expedite the symptoms of the pathogen that may have already been in place in the seastars. Additionally, our findings hint at a possible additional stressor, salinity, which led to an onset of symptoms in our specimens. These stressors are speculated to impair immune response in sea stars, though we have no molecular work to back this claim.

Our hypothesis was that shellfish, namely *Nuttalia* (clams) and *Mytilus* (mussels) would serve as bioaccumulators, organisms that could concentrate marine pathogen to deliver high doses to their predators as seen with Harmful Algal Blooms. Following the diet and temperature experiment there was no significant difference between Hot Food and Clean Food (Figures 2 & 3). Principal among the explanations to this relationship are: *a)* All marine shellfish have relatively equal exposure to pathogens for accumulation, therefore site and incubation do not have an impact on relative pathogen accumulation level, *b)* The time length of our experiment was not sufficient to allow for the discrepancy in diet to take effect, and *c)* perhaps the shellfish location is not as important as the shellfish species.

Though no strong relationship between Hot Food and Clean Food was discovered,

there is an interesting pattern occurring between food types, that is, between clams and mussels. Our data revealed that there is a near-significant relationship (Chi Square Test, p-value = 0.078 one degree of freedom) between rate of symptoms of SWD and diet (clams versus mussels). Perhaps clams are more perform more bioaccumulation of the pathogen.

Marine infectious diseases can be brought on by a combination of stressors. Abnormal salinity levels have impaired the ability in *Pisaster* to right itself when flipped on its back (Held & Harley 2009). Additionally, their specimens were shown to die off when salinity levels reached extreme lows (≤15psu). Our findings indicate that the expression of SWD is not independent of salinity level. Figure 6 demonstrates the relationship between percent showing lesions and average salinity. The one specimen in the normal salinity tank experienced such a rapid onset of symptoms that it may have been compromised prior to beginning the experiment.

Our survey data provided a very interesting insight into the relationship between field site and symptomatic sea stars. The sites' low tide average temperatures are correlated with the percent of lesioned *Pisaster* specimens. The results of this survey are invaluable. Predictions about vulnerability of sea stars to symptoms of SWD can be made based off of temperature. This is just the beginning of what could be a more geographically significant study.

SWD first began in early summer of 2013 and has not only persisted through the winter but the number of sites with sea stars showing symptoms have increased. Loss of keystone predators due to wasting disease will occur and we can only predict what the final consequences will be. Our study highlights the fact that stressors to sea stars impact

their vulnerability to marine infectious diseases. To counteract future epizootics we must understand the mechanisms of these diseases and to what extent our human activity oceanic environments. Only then can policies be made to prevent or change our behaviors.

Acknowledgements:

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