Effects of diet, temperature, salinity and season on wasting disease in ecologically important predatory sea stars

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Abstract: This study investigates sea star wasting disease seen in ecologically important predatory sea stars on intertidal coasts of San Juan Island, Lopez Island and Orcas Island (Washington, United States). Three experiments were conducted to analyze the effects of diet, temperature, salinity on the vulnerability of sea stars to wasting disease. To test the hypothesis that higher pathogen-dosed food would result in heightened infection intensities and also different diet types would result in different infection intensities, we fed *Pycnopodia helianthoides* with healthy and pathogen-dosed clams and mussels separately. There is no significant difference in either different diet types or in different pathogen-dosed levels treatments to the prevalence of sea star wasting disease. To test the hypothesis that temperature influences the prevalence of sea star wasting disease, we kept *P. helianthoides* in normal temperature tanks and higher temperature tanks. The prevalence of sea star wasting disease was always higher in warmer temperature treatments. To test the hypothesis that lower salinity environment would lead to higher prevalence of sea star wasting disease, we kept *P. helianthoides* under normal and lower salinity treatments. More symptomatic sea stars were found in the lower salinity experiment group. Furthermore, disease effects showed seasonal changes in field surveys. Compared with survey data from last winter, sea stars showed higher prevalence of disease in late spring.

INTRODUCTION

Climate warming is an important factor that increases the occurrence of marine diseases because pathogens tend to grow faster under warmer temperatures, and hosts are more susceptible under heat stress (Harvell et al. 2002). The decimation of populations of ecologically important species by pathogen infections can strongly influence entire marine communities. Mass mortality, and reduced growth and recruitment due to disease outbreaks have recently affected echinoderm populations in the Pacific Ocean, providing compelling examples of major shifts in ecosystem state and cascading community effects following disease-induced die-offs (Uthicke et al. 2009, Harvell et al. 1999).

Warmer ocean temperatures played a critical role in the intertidal pathogen-induced decimation of the purple sea urchin *Strongylocentrotus purpuratus* (Lester et al. 2007). Thus, pathogen-mediated decline of local marine species, and subsequent changes to marine ecological communities, can be an undesirable effect of global warming.

One type of disease, named 'wasting disease', has caused tens of thousands of deaths of multiple species of sea star on North America's Pacific coast (Eckert et al. 1999, Lester et al. 2010). The wasting disease has been documented in 10 Asteroidean species from the northeast Pacific since 1972 (Eckert et al. 1999). For example, large-scale devastation in the population of the ochre sea star *Pisaster ochraceus* has been caused by wasting disease (Bates et al. 2009). *P. ochraceus* was identified as a keystone predator in the intertidal zone, based on their critical consumption of competitively dominant prey, which has a large effect on marine communities (Menge and Freidenburg 2001). Thus, the infectious diseases outbreaks in the keystone species can have large effects on the structure and function of marine ecosystems (Ward and Lafferty 2004).

The etiological agent of wasting disease has not been determined and may vary among different species and localities (Bates et al. 2009). Nevertheless, the progress of wasting disease is predictable. Curling arms are the first indication of sea star wasting disease, which is an abnormal behavior for healthy sea stars. Then, a 'deflated' appearance was observed on symptomatic sea stars, due to loss of turgor. White lesions were then noted on the aboral side of the body surface, which initially appeared at the junctions of the arms with the central disk. Arms crawling away from central disk was found during disintegration, then body parts dissolved before death (Fig. 1). Our main objective was to test the effects of diet, temperature, salinity and season on the prevalence of wasting disease in predatory sea stars.

MATERIALS AND METHODS

Study location and materials. Twelve *Pycnopodia* were collected from localities near the dock of the Friday Harbor Laboratory (FHL) (Friday Harbor, WA: 48°32'43.8"N 123°00'50.8"W) on April 23rd 2014 for diet and temperature tests. Ten *Pycnopodia* were collected from the same localities on May 24th 2014 for salinity test. Clams and mussels were collected from Orcas Island (Crescent Beach) and kept in a sea table with sick sea stars for two days before the diet experiment for pathogen-dosing; these were used as sick food. Clams and mussels from San Juan Island (Cattle Point) were stored in clean tanks and used as clean healthy food.

Experiments. Experiments were conducted at FHL with visibly healthy *Pycnopodia*. In all cases, experimental duration for diet test was 34 days, for temperature test was 34 days and for salinity test was 8 days. Temperature and salinity data were collected with a YSI conductivity, salinity and temperature meter.

Field surveys. Three sea star species (*Pisaster ochraceus, Pycnopodia helianthoides* and *Henricia leviuscula*) were inspected for indication of sea star wasting disease in May at San Juan Island (Colin's Cove, FHL Dock, Point Caution-Strathmann's Beach, Reuben Tarte County Park, Cattle Point Lighthouse, Dead Man's Bay and San Juan County Park), Orcas Island (Crescent Beach, Indian Island, Rosario Point, West Sound and East Sound Waterfront) and Lopez Island (Richardson Pier) (Fig. 2). Disease incidence rates (percentage of the population with visible lesions) were calculated. Temperature of the shallow ocean water at Point Caution-Strathmann's Beach, Reuben Tarte County Park, San Juan County Park, Indian Island, Rosario Point, West Sound, East Sound Waterfront and Richardson Pier were recorded (Table 1).

Diet. To quantify the influence of different pathogen-dosed levels of food and different types of diet on the morbidity and intensity of wasting disease, we conducted a laboratory experiment from April 23rd 2014 to May 26th 2014. Twelve *P. helianthoides* were kept in twelve separate tanks in 4 treatments (3 replicate sea stars in each treatment), temperatures were maintained around 11 °C by circulated cold sea water and sun shields covered the tops of tanks. The sea stars were fed either healthy mussels, sick mussels, healthy clams or sick clams. Symptom appearances were recorded daily except for field trip period (from May 16th 2014 to May 21st 2014).

Temperature. To examine the effects of warmed-up water temperature on the prevalence of sea star wasting disease, the temperature was manipulated in the diet experiment. Sea stars were kept in ambient temperature (around 11.70 ± 0.03 °C) sea water for 16 days. Circulated sea water was then shut down and sun shields were removed to increase temperature. Water in the tanks was heated up for 6 hours per day for 6 days. Temperatures were mostly maintained at 17.7 ± 0.2 °C, except for a brief extreme when temperature reached 21.0 ± 0.2 °C. Water was then cooled down to ambient temperature again for 12 days to test the recovery ability of sick sea stars. Symptom appearances were recorded daily except for field trip period (from May 16th 2014 to May 21st 2014). At the end, sick and dead sea stars were stored in freezers until dealt as trash disposal, while visibly healthy sea stars were returned to the ocean near FHL dock.

Salinity. To investigate the influence of lowered salinity on the prevalence of sea star wasting disease, ten *P. helianthoides* were kept in two treatments (5 sea stars in each treatment, 1 tank per treatment). One treatment was kept at ambient salinity (about 30.63 ± 0.38 ppt.) and the other treatment was kept at lower salinity (22.62 ± 0.75 ppt.) by mixing ocean water with fresh water. Appearance of symptom was recorded every day for 8 days (from May 24th 2014 to May 31st 2014). One sea star in the ambient salinity tank has lesions appear on the second day, and was removed from the experiment. Wasted body tissue was found and removed from the lowered salinity tank on May 27th 2014. Temperature and salinity were recorded daily.

Season. Field surveys were conducted to compare of wasting disease between late spring (May 2014) and winter (January 2014). Disease prevalence data from the same sites in different seasons were compared with temperature measurements.

Statistical analyses. Chi-square tests were used for data in diet experiment. T-test was used for data in temperature, salinity experiment and field surveys.

RESULTS

Diet. There is no significant difference (Table 2) ($\chi^2 = 1.6667$, P = 0.1967, two-tailed) in disease prevalence between sea stars fed pathogen-dosed food versus clean food, or between the two types of species of bivalves they ate.

Temperature. In the high temperature treatment, *Pycnopodia* were more vulnerable to pathogens and showed higher prevalence of sea star wasting disease (Fig. 3) (t = 9.093, P < 0.0001). Arm regeneration and lesion recovery were observed at post high temperature manipulation (17.7 ± 0.2 °C). However, one sea star was not able to regenerate from disintegration, and one sea star was totally decayed and dead.

Salinity. Under reduced salinity, there was a higher disease prevalence on *Pycnopodia* than under normal salinity treatment (Fig. 4) (t = 7, P = 0.0198, two-tailed). In our salinity experiment, since sea water has a higher density than fresh water, the stress test tank has higher salinity at the bottom than the top, a phenomena of avoiding low salinity behavior was detected on all sea stars in lowered salinity tank (they stayed at the bottom of the tank all the time).

***Season* and field surveys.** Wasting disease was observed in *Pisaster ochraceus*, *Pycnopodia helianthoides* and *Henricia leviuscula* populations in May 2014 (Table 1). The disease incidence rates shows a significant positive linear correlation with seawater temperatures of the localities (Fig.5) (*Pisaster*: t = 14.4380, d. f. = 7, P < 0.0001, two-tailed; *Pycnopodia*: t = 3.6558, d. f. = 4, P = 0.0217, two-tailed; *Henricia*: t = 4.1302, d. f. = 3, P = 0.0257, two-tailed). Seasonal effects on the disease severity were also closely related to the temperatures changing; there was significant difference between the survey data from last winter to late spring (Fig. 6) (t = 2.4648, p = 0.0488, two-tailed), with an increase of symptomatic sea stars found at multiple locations.

DISCUSSION

Our temperature experiment showed that high temperature stress can lead to a large mortality to the sea star populations, although most of them were able to regenerate when returned to cooler waters. Observation about sea stars avoiding lower salinity water layer in our salinity stress test indicates that sea stars felt stressed under lower salinity. In our field surveys, populations at the higher temperature localities displayed consistently high disease prevalence than populations in lower temperature localities for all three sea star species. Thus, the changes in environment, such as heightened temperature and lowered salinity, appear to be playing important roles in the prevalence of sea star wasting disease.

Since sea stars are ecologically important species, the decline of their populations can cause disruption in the local food web, and thus can have a huge effect on the marine communities. Our research results suggest the sea star wasting disease may be caused by the impaired immune system due to the variations in environment which are acting as stressors. Understanding stressful human impacts on the environment is thus necessary to reduce the prevalence of sea star wasting disease.

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FIGURES

Figure 1. A) Healthy *Pycnopodia helianthoides*. B) Curly and Twisted arms are initial indication of wasting disease. C) Lesions between arm joints and central disc. D) Body decayed.

Figure 2. Survey sites. San Juan Island (Colin's Cove, FHL Dock, Point Caution-Strathmann's Beach, Reuben Tarte County Park, Cattle Point Lighthouse, Dead Man's Bay and San Juan County Park), Orcas Island (Crescent Beach, Indian Island, Rosario Point, West Sound and East Sound Waterfront) and Lopez Island (Richardson Pier).

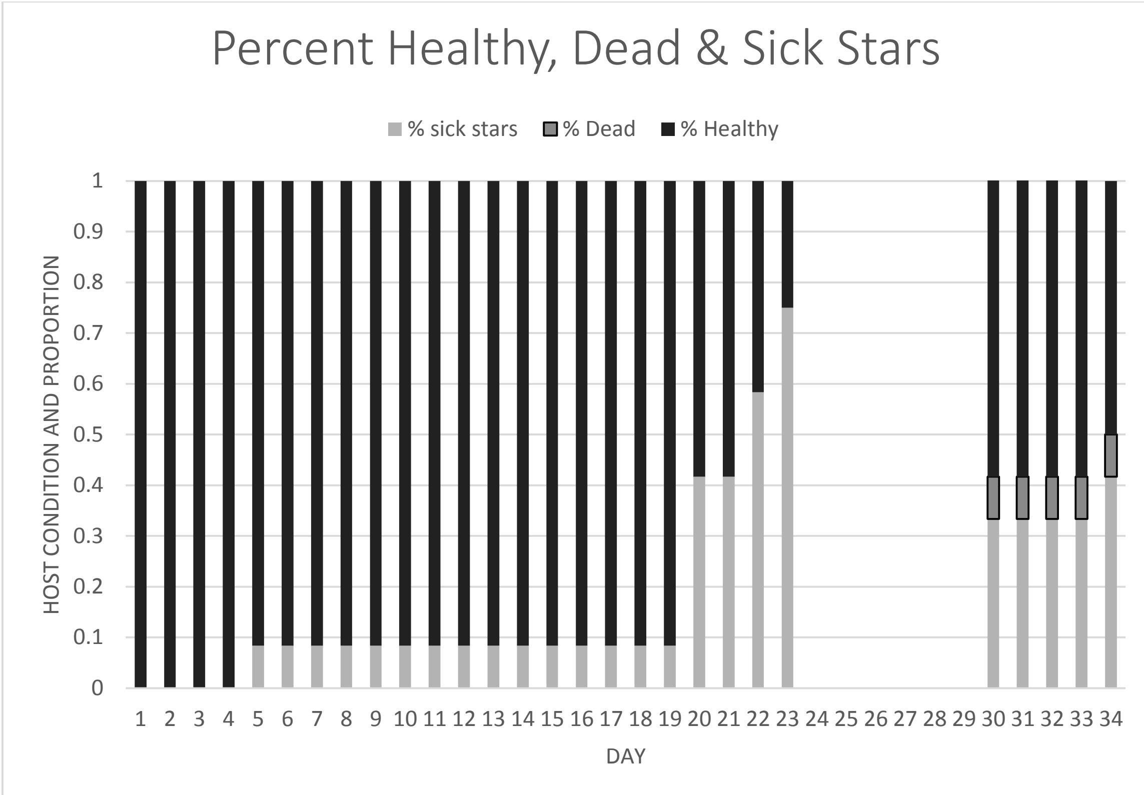


Figure 3. Graph of host physical condition relative to different temperature treatments. From day 1 to day 16, sea stars were under the ambient temperature treatment. From day 17 to day 22, sea stars were under the heated-up treatment. Water was cooled down to ambient temperature again on day 23, data were missing from day 24 to day 19 since a field trip was conducted in Canada, and data were not collected. Data were collected again after day 30 to day 34.

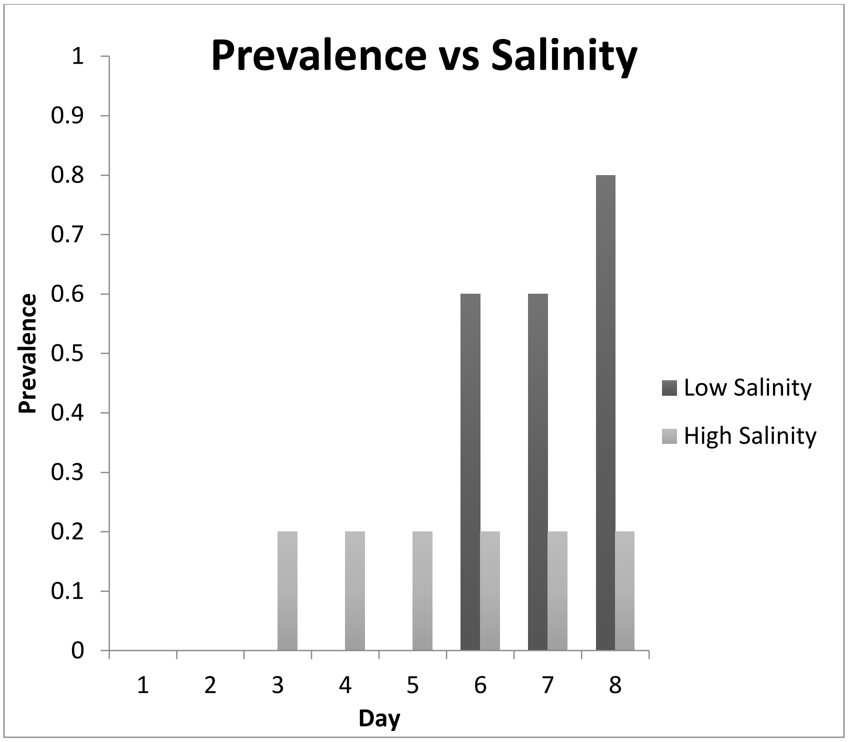
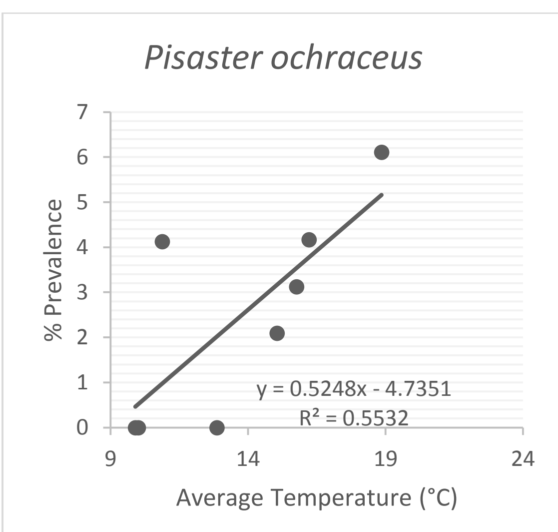
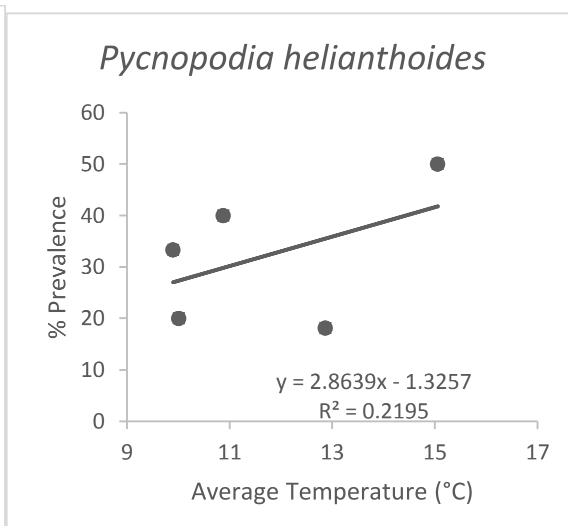


Figure 4. The prevalence rate of *Pycnopodia* relative to the different salinity treatments. Lower salinity led stress and a higher disease prevalence.





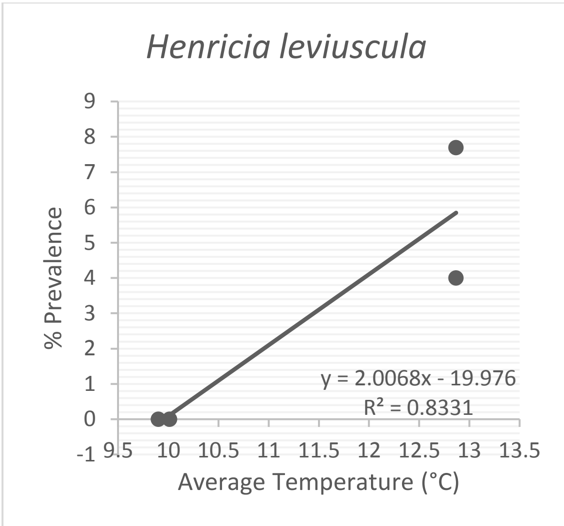


Figure 5. Three graphs of the significant linear relationship of wasting disease prevalence rates among three keystone species (*Pisaster ochraceus, Pycnopodia helianthoides* and *Henricia leviuscula*) and average temperatures from each localities.

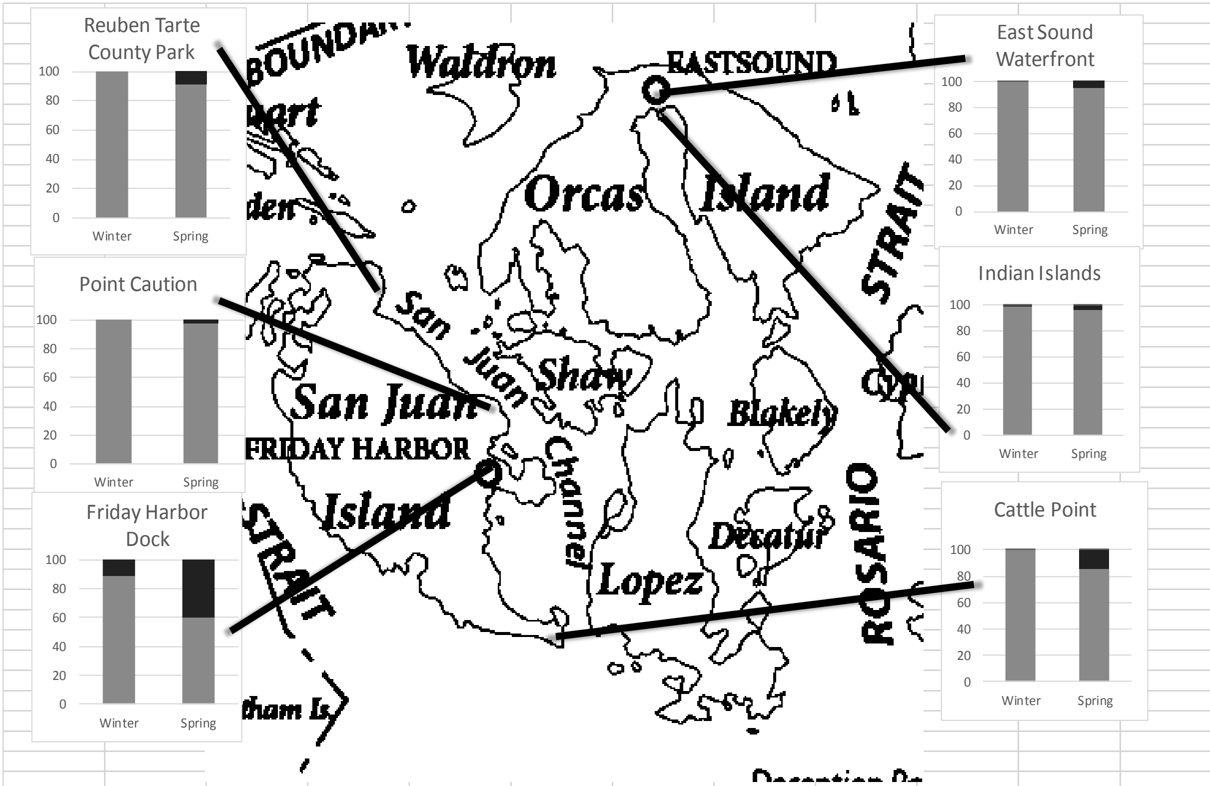


Figure 6. Comparison of disease incidence percentages for three sea star species (*Pisaster ochraceus*, *Pycnopodia helianthoides* and *Henricia leviuscula*), data collected from winter survey (January 2014, left bar) and late spring survey (May 2014, right bar). Darker portion indicates the sick sea stars, lighter portion indicates the health sea stars.

TABLES

	Localities	Average Temperature (°C)	Average Salinity (ppt)	Heathy (all species)	Sick & Dead (all species)	% Prevalence
5/15/2014	Crescent Beach			115	0	0.0
5/16/2014	Indian Island	16.2	31.03	161	7	4.2
5/16/2014	Colin's Cove			25	0	0.0
5/24/2014	FHL Dock			10	15	60.0
5/27/2014	Point Caution-Strathmann's Beach	9.9		82	2	2.4
5/27/2014	Reuben Tarte County Park	12.9	30.60	72	7	8.9
5/28/2014	West Sound	15.8	30.63	31	1	3.1
5/28/2014	Rosario Point	15.1	30.60	283	8	2.7
5/28/2014	Cattle Point Lighthouse			6	1	14.3
5/29/2014	Dead Man's Bay			12	1	7.7
5/29/2014	San Juan County Park	10.0	31.30	40	3	7.0
5/30/2014	Richardson Pier	10.9	31.40	103	6	5.5
5/31/2014	East Sound Waterfront	18.9	30.63	123	8	6.1

Date	Localities	Average Temperature (°C)	Average Salinity (ppt)	*Pycnopodia* Healthy	*Pycnopodia* Sick & Dead	% Prevalence
5/15/2014	Crescent Beach			5	0	0.0
5/16/2014	Indian Island	16.2	31.03	0	0	
5/16/2014	Colin's Cove			3	0	0.0
5/24/2014	FHL Dock			10	15	60.0
5/27/2014	Point Caution-Strathmann's Beach	9.9		4	2	33.3
5/27/2014	Reuben Tarte County Park	12.9	30.60	27	6	18.2
5/28/2014	West Sound	15.8	30.63	0	0	
5/28/2014	Rosario Point	15.1	30.60	2	2	50.0
5/28/2014	Cattle Point Lighthouse			2	1	33.3
5/29/2014	Dead Man's Bay			1	1	50.0
5/29/2014	San Juan County Park	10.0	31.30	8	2	20.0
5/30/2014	Richardson Pier	10.9	31.40	3	2	40.0
5/31/2014	East Sound Waterfront	18.9	30.63	0	0	

Date	Localities	Average Temperature (°C)	Average Salinity (ppt)	*Pisaster* Healthy	*Pisaster* Sick & Dead	% Prevalence
5/15/2014	Crescent Beach			110	0	0.0
5/16/2014	Indian Island	16.2	31.03	161	7	4.2
5/16/2014	Colin's Cove			19	0	0.0
5/24/2014	FHL Dock			0	0	
5/27/2014	Point Caution-Strathmann's Beach	9.9		74	0	0.0
5/27/2014	Reuben Tarte County Park	12.9	30.60	21	0	0.0
5/28/2014	West Sound	15.8	30.63	31	1	3.1
5/28/2014	Rosario Point	15.1	30.60	281	6	2.1
5/28/2014	Cattle Point Lighthouse			1	0	0.0
5/29/2014	Dead Man's Bay			7	0	0.0
5/29/2014	San Juan County Park	10.0	31.30	20	0	0.0
5/30/2014	Richardson Pier	10.9	31.40	93	4	4.1
5/31/2014	East Sound Waterfront	18.9	30.63	123	8	6.1

Date	Localities	Average Temperature (°C)	Average Salinity (ppt)	*Henricia* Healthy	*Henricia* Sick & Dead	% Prevalence
5/15/2014	Crescent Beach			0	0	
5/16/2014	Indian Island	16.2	31.03	0	0	
5/16/2014	Colin's Cove			3	0	0.0
5/24/2014	FHL Dock			0	0	
5/27/2014	Point Caution-Strathmann's Beach	9.9		4	0	0.0
5/27/2014	Reuben Tarte County Park	12.9	30.60	24	1	4.0
5/28/2014	West Sound	15.8	30.63	0	0	
5/28/2014	Rosario Point	15.1	30.60	0	0	
5/28/2014	Cattle Point Lighthouse			3	0	0.0
5/29/2014	Dead Man's Bay			4	0	0.0
5/29/2014	San Juan County Park	10.0	31.30	12	1	7.7
5/30/2014	Richardson Pier	10.9	31.40	7	0	0.0
5/31/2014	East Sound Waterfront	18.9	30.63	0	0	

Table 1. Survey data shows average temperatures, average salinities, number of healthy sea stars, number of sick and dead sea stars, and percentages of prevalence, categorized by localities and species.

	Mussels	Clams	Total
Clean	O=3, E=2	O=2, E=3	5
Pathogen-dosed	O=1, E=2	O=4, E=3	5
Total	4	6	10

Table 2. Observed and expected wasting disease infected sea stars under different types of diets. O = observed data, E= expected data.