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Sea Star Wasting Disease in Pisaster Ochraceus on the Washington Coast and in Puget Sound

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SEA STAR WASTING DISEASE IN *PISASTER OCHRACEUS* ON THE WASHINGTON COAST AND IN PUGET SOUND

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ABSTRACT

SEA STAR WASTING DISEASE IN *PISASTER OCHRACEUS*

ON THE WASHINGTON COAST AND IN PUGET SOUND

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Pisaster ochraceus is a common North American west coast sea star whose predation of *Mytilus californianus* (the California mussel) increases the biodiversity of its intertidal community. Sea star wasting disease is an illness that causes sea star tissues to become necrotic until the creature wastes away and dies. In 2013, a coast-wide outbreak of sea star wasting disease caused a mass mortality event in *P. ochraceus.* The goals of this study were to try to identify some of the possible causes for the outbreak, as well as analyze the impact that sea star wasting disease has on biodiversity. In this study, forty tide pools in four different regions of Washington State (northern coast, southern coast, northern Puget Sound, and southern Puget Sound) were surveyed for Shannon's diversity, species richness, abundance of *P. ochraceus*, percentage of *P. ochraceus* infected with sea star wasting disease, total dissolved solids, pH, alkalinity, and salinity. Four separate statistical models were performed testing percentage of *P. ochraceus* with sea star wasting disease, presence of sea star wasting disease, Shannon biodiversity, or species richness as the response variable. Nothing was statistically significant for presence of sea star wasting disease, percentage of *P. ochraceus* with sea star wasting disease, and species richness. However, Shannon biodiversity had a positive correlation with

abundance of *P. ochraceus* and a negative correlation with percentage of *P. ochraceus* with sea star wasting disease. This negative correlation suggests that the mere presence of sea star wasting disease is enough to negatively influence *P. ochraceus* populations by disabling their ability to eat, which drives down biodiversity. The data suggests that even before a sea star dies; it is not functioning as a predator. However, neither location nor water quality appeared to influence biodiversity or sea star wasting disease, which suggests that further research should be conducted on these variables and others to try to identify a causal agent for these outbreaks.

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CHAPTER I

LITERATURE REVIEW

PISASTER OCHRACEUS

The ochre star (*Pisaster ochraceus)* is a common sea star found along the rocky intertidal zone of the North American Pacific coast (Menge et al. 1994). It is a relatively large sea star, averaging about 25 centimeters in diameter with five rays that protrude from a central disk (Kozloff 1993). The species reproduces via broadcast spawning and reaches sexual maturity around five years old (Menge 1975). The ochre star is either purple or orange, but it can also appear to be yellow, ochre red, or brown (Harley et al. 2006).

P. ochraceus has a water vascular system, which is unique to echinoderms and essential for feeding, locomotion, gas exchange, and sensory reception (Binyon 1972; Khanna and Yadav 2005). Water enters a large pore on the dorsal side of the creature called the madreporite. This is connected to a duct called the stone canal, which leads to the circular ring canal. From the circular ring canal, the water is transported to the radial canals which extend to the rays. The radial canals are connected to the lateral canal, which lead to the ampullae and the tube feet. The sea star fills entirely with water. The tube feet of *P. ochraceus* contain suckers, which enables them to cling to the sides of rocks and endure the waves of the rocky intertidal zone (Binyon 1972; Khanna and Yadav 2005).

P. ochraceus lack a central brain but do possess a central nerve ring and eyespots on the end of each ray. The eyespots are primitive; they can only sense changes in light levels. Nerves radiate out from the central ring to the rays, which coordinate the movement of the tube feet (Binyon 1972). *P. ochraceus* have sensory cells in their epithelium, which enable them to sense touch, temperature, and orientation. The tube feet are sensitive to touch and chemical changes in the water, which allows them to detect food nearby (Binyon 1972). Like many species of sea star, *P. ochraceus* can regenerate lost limbs (Edmondson 1935). To regrow tissue, they employ two different strategies: epimorphosis or morphallaxis regeneration (Suzuki et al. 2006; Agata et al. 2007). In epimorphosis, stem cells form a blastema and create new tissue. Morphallactic regeneration uses existing tissues and reshapes them into the new tissue (Suzuki et al. 2006; Agata et al. 2007).

P. ochraceus is a keystone species, meaning it exhibits an inordinate amount of influence over its community, specifically through predation (Paine 1966, 1969, 1974; Mills et al. 1993; Menge et al. 1994). *P. ochraceus* preys on the California mussel (*Mytilus californianus*) and without this predation, the California mussel would completely dominate its community, which would drive down biodiversity (Paine 1966; 1969, 1974; Mills et al. 1993; Menge et al. 1994). *P. ochraceus* have a wide variety of prey; in addition to mussels, they will also feed on barnacles, snails, and some species of Crustacea. They feed using their tube feet to handle the prey, and open shells. *P. ochraceus* will evert its cardiac stomach and engulf prey, using digestive enzymes to process the food. The digested food is absorbed by the pyloric ceca, which is then transported to the rest of the body (Kozloff 1993; Khanna and Yadav 2005). Because of

its profound effect on mussel populations and biodiversity, *P. ochraceus* is also said to be an indicator species, meaning its presence is indicative of a healthy intertidal zone (Paine 1976; Feder 1970).

SEA STAR WASTING DISEASE

Sea star wasting disease is a marine disease believed to be caused by the sea star associated densovirus (Hewson et al. 2014). While outbreaks of sea star wasting disease have been recorded since the 1970s (Eisenlord et al. 2016), little is known about this alleged virus. Methods of transmission remain a mystery, and no causal agent has been found. Some research suggests that these outbreaks are caused by an increase in the ocean's temperature, and that outbreaks are only going to become more severe as the temperature rises (Bates 2009; Eisenlord 2016; Kohl 2016). One study suggests that sea star wasting disease outbreaks in the Channel Islands coincided with the increase of water temperature due to El Nino (Blanchette et al. 2005). Another study conducted showed, under controlled conditions, that prevalence of sea star wasting disease increased as the temperature increased (Bates, et al. 2009). However, other research claims that neither temperature nor pH affect the spread of sea star wasting disease. A study published by Menge et al. in 2016 posited that increased temperature did not lead to an increase in sea star wasting disease, but that cooler temperatures increased the spread of this disease. The same study suggests that pH did not affect the spread of sea star wasting disease.

While the role of temperature remains unclear, research suggests that adult sea stars are more susceptible to temperature changes than juveniles are. It is also suggested

that juveniles succumb to the disease faster than adults do once they become symptomatic (Kohl et al. 2016). Aside from few studies on the effects of temperature, there is very little research about possible vectors or modes of transmission.

This disease presents itself in four stages or categories (UCSC 2018). The first stage is characterized by the appearance of rapidly spreading white lesions on the sea star. The next stage occurs when the spreading lesions cause the water vascular system to fail. Because of this failure, the sea star loses its grip and can no longer cling to rocky substrate. The sea star often looks deflated as the water vascular system fails. The third stage occurs when the arms begin to twist and fall off, due to the body tissue becoming necrotic. The sea star becomes even more limp and lifeless as the rays fall off and disintegrate. The final stage of this disease occurs when the lesions spread completely, all tissue becomes necrotic, and the sea star disintegrates, or wastes away, into a pile of white tissue (Eckert 1999; Fuess et al. 2015).

There have been several outbreaks of sea star wasting disease in the past (Gudenkauf and Hewson 2015), however, only the 2005 outbreak had a significant impact on *P. ochraceus*. The first notable outbreak occurred in 1972 on the east coast of the United States (Meyer 2015). Researchers noticed that the common sea star, *Asterias rubens*, was disappearing from the area, and, after further research, was disappearing due to a disease that caused them to become limp and waste away. In 1978, the predatory sea star *Heliaster kubiniji* fell victim to the disease, causing a drastic drop in the population (Dungan et al. 1982). Because they are a predatory species, their disappearance had a dramatic impact on the sea star community in the Gulf of California. In 1997, another outbreak occurred in the Gulf of California on the Channel Islands. Monitoring of several

sea star populations revealed that sea star abundance was at an all-time low after this outbreak (Eckert et al. 1999). The most famous of these outbreaks is the 2013 mass mortality event. Sea star wasting disease spread all along the west coast, instead of being contained to one area as it had been in the past. Several species experienced a mass die-off, including *P.* ochraceus (Jurgens et al. 2015; Eisenlord et al. 2016; Menge et al. 2016).

HISTORICAL MARINE DISEASE EPIDEMICS

Sea star wasting disease is not the only infectious disease that has caused mass mortality events (Fey et al. 2015). Disease outbreaks are not uncommon in oceanic species, and the consequences of a large-scale epidemic can be dire, both ecologically and economically (Groner et al. 2016).

One of the earliest recorded mass mortality events is the plague of wasting disease that struck *Zostera marina*, the common eelgrass. Ninety percent of *Z. marina* populations in the French Atlantic coast were struck with this disease from 1931-1932 (Ralph and Short 2002). The sudden disappearance of the seagrass disrupted the ecosystem: migratory birds used sea grass for food and several marine species used it as habitat (Groner et al. 2016). A study published in 2008 resurveyed the area and discovered that common eelgrass beds are still only half of what they were before this outbreak (Godet et al.).

The black sea urchin, *Diadema antillarum*, experienced a mass die off in 1983 caused by an unknown pathogen (Lessios 1995). After the initial outbreak, the Caribbean

reefs were surveyed and it was discovered that, as of 2016, only 12.5% of the original population has returned. Not only that, but the dynamics of Caribbean reefs have completely changed; what used to be a coral dominant area is now dominated by algae (Lessios 1985, 2016). The structure of the Caribbean reefs had changed entirely due to the sudden emergence of a pathogen and the resulting absence of the black sea urchin.

When a commercially important species experiences an outbreak, the results can be financially devastating. In 1992, several species of penaeid shrimp were infected with white spot syndrome in northern Taiwan (Chou et al. 1995). The movement of infected shrimp among fisheries, as well as the vulnerability of the hosts to this pathogen exacerbated the severity of this outbreak. This pandemic caused shrimp farmers to lose billions of dollars (Lafferty et al. 2015). Eco-tourism is a hugely profitable business that can also be damaged by disease. In the early 1980s, Caribbean coral reefs were struck with an outbreak of white band disease. The sudden loss of coral affected not only the fisheries who farm reef-dependent fish, but also the tourism industries that made money off the vibrant coral (Aronson and Precht 2001).

Sea star wasting disease is not the only outbreak that has had devastating consequences to marine life. The previous examples illustrate the significant impact mass mortality events can have on aquatic communities as well as humans. By studying marine pandemics, management strategies have been developed to try to stem the emergence of an outbreak (Harvell et al. 2004). Early detection is key when trying to mitigate an outbreak. By performing regular surveys, diseases can be found and an attempt to suppress their emergence can be made before they spread beyond our control (Groner et al. 2016). For diseases affected by seasonality (like many pathogens that affect tropical

coral), seasonal forecasts and real-time assessments can be used to predict the likelihood of an outbreak (Heron et al. 2010; Maynard et al. 2010). Other strategies have been proposed to stop an outbreak before it even begins, such as dispensing vaccines in fisheries and to marine mammals, as well as treating populations with a chemical that reduces pathogen intensity (Subasinghe 2009).

A key factor to successful disease management is having as much information about the pathogen as possible. Disease management strategies can be successful, but they cannot be developed if the pathogen itself remains a mystery. Sea star wasting disease is a devastating illness that could have catastrophic impacts on tide pool communities, but little is known about how this pathogen operates, which makes mitigation efforts next to impossible.

The goals of this study were to examine possible factors that affect the spread of sea star wasting disease in tide pool communities, as well as determine the effect that sea star wasting disease has on the biodiversity of tide pool communities. By studying both the impact that sea star wasting disease has on its community as well as the possible factors affecting the spread of this disease, this study aims to further our understanding on the spread and impact of sea star wasting disease. The main questions are: 1) Does the prevalence of sea star wasting disease impact the biodiversity and species richness of a tide pool? 2) Do pH, salinity, alkalinity, and total dissolved solids affect the spread of sea star wasting disease in a tide pool? And 3) Does the location of the tide pool affect the spread of sea star wasting disease?

CHAPTER II

SEA STAR WASTING DISEASE IN *PISASTER OCHRACEUS* ON THE WASHINGTON COAST AND IN PUGET SOUND

SEA STAR WASTING DISEASE IN *PISASTER OCHRACEUS* ON THE WASHINGTON COAST AND IN PUGET SOUND

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Abstract

Pisaster ochraceus is a common west coast sea star whose predation of *Mytilus californianus* (the California mussel) increases the biodiversity of its intertidal community. Sea star wasting disease is an illness that causes sea star tissues to become necrotic until the creature wastes away and dies. In 2013, a coast wide outbreak of sea star wasting disease caused a mass mortality event in *P. ochraceus.* The goals of this study were to try to identify some of the possible causes for the outbreak, as well as analyze the impact that sea star wasting disease prevalence has on biodiversity. In this study, forty tide pools in four different regions of Washington State (northern coast, southern coast, northern Puget Sound, and southern Puget Sound) were surveyed for Shannon biodiversity, species richness, abundance of *P. ochraceus*, percentage of *P. ochraceus* infected with sea star wasting disease, total dissolved solids, pH, alkalinity, and salinity. Four separate statistical models were performed testing percentage of *P. ochraceus* with sea star wasting disease, presence of sea star wasting disease, Shannon biodiversity, and species richness as the response variable. Nothing was statistically significant for presence of sea star wasting disease, percentage of *P. ochraceus* with sea star wasting disease, and species richness. However, Shannon biodiversity had a positive correlation with abundance of *P. ochraceus* and a negative correlation with percentage of *P. ochraceus* with sea star wasting disease. This negative correlation suggests that the mere presence of sea star wasting disease is enough to negatively influence *P. ochraceus* populations by disabling their ability to eat, which drives down biodiversity. The data suggests that even before a sea star dies; it is not functioning as a predator. However, neither location nor water quality appeared to influence biodiversity or sea star wasting

disease, which suggests that further research should be conducted on these variables and others to try to identify a causal agent for these outbreaks.

Keywords:

Sea star wasting disease *Pisaster ochraceus* *Mytilus californianus*

Shannon biodiversity

Introduction

One of the biggest threats facing oceanic species is the increase in oceanic disease outbreak, which evidence suggests is a consequence of climate change (Kordas et al. 2011; Lafferty et al. 2015; Groner et al. 2016; Miner et al. 2018). This increase has dire implications, especially when a keystone species is affected, due to the large influence they have over diversity of their communities (Paine 1966; Monaco et al. 2014; Miner et al. 2018). One of the species most affected by these outbreaks is the ochre star (*Pisaster ochraceus),* which has suffered a mass mortality event due to sea star wasting disease (Hewson et al. 2014).

P. ochraceus is a keystone predator, meaning that it exerts an inordinate influence in its community when compared to its relative abundance (Paine 1966; Power et al. 1996). More specifically, its predation on certain species of bivalves (chiefly the California mussel, *Mytilus californianus)* allows for greater biodiversity in its community (Paine 1966, 1969, 1974; Mills et al. 1993; Menge et al. 1994). Without *P. ochraceus*, the California mussel would overwhelm the other species in the tide pool, completely taking over and driving down biodiversity. Because of this, *P. ochraceus* is vital to tide pool communities, and is an indicator of the overall health of the community (Paine 1976; Feder 1970).

Sea star wasting disease is an illness which affects sea stars, causing their tissue to become necrotic until the sea star wastes away (Eckert 1999; Fuess et al. 2015). This disease is characterized by four distinct stages. First, the sea star develops white lesions on their body. The white lesions then spread, causing the limbs to twist and the sea star to lose its grip (it cannot hold onto the side of rocks due to its water vascular system

failing). The limbs eventually fall of as the lesions spread. Finally, total disintegration occurs once the lesions spread completely and the tissue becomes necrotic (Eckert 1999; Fuess et al. 2015). Currently, the cause for this disease remains unknown, although the most likely cause is Sea star associated densovirus, which was found in greater abundance in diseased sea stars than in healthy sea stars (Hewson et al. 2014). The vector and mechanism of this disease also remain unknown, although evidence suggests that a mutation in the elongation factor of 1-alpha locus in *P. ochraceus* could be associated with reduced mortality (Wares and Schiebelhut 2016).

In 2013, an outbreak of sea star wasting disease occurred and unlike previous outbreaks, this one spread across the West Coast and devastated several sea star populations. Because of this outbreak, the number of *P. ochraceus* found along the west coast plummeted (Jurgens et al. 2015; Eisenlord et al. 2016; Menge et al. 2016). At the Natural Bridges State Marine Reserve in California, the ochre star was a common resident in the rocky intertidal tide pools. However, in November of 2013, *P. ochraceus* was reported to have completely vanished from the area (Gong 2013). This outbreak has not only impacted *P. ochraceus,* but other species of sea star as well. In September of 2013, a mass die-off of the sunflower star (*Pycnopodia helianthoides*) was reported in Howe Sound, British Columbia. Large numbers of *P. ochraceus* were also found dead in Howe Sound (Shultz et al. 2016).

Previous research is split on the effects certain water quality metrics have on the spread of sea star wasting disease. Some studies indicate that pH and temperature do not affect the spread of this disease (Menge 2016). However, other studies support the idea that temperature affects the spread of this disease and that climate change is to blame for

the severity of the 2013 outbreak (Bates 2009; Eisenlord 2016; Kohl 2016). As of 2018, there is no evidence to identify the vector of this disease, making efforts to stem its spread extremely difficult.

Recent reports indicate that the number of *P. ochraceus* has been slowly rising, but they are nowhere near their old population numbers (Miner et al. 2018). While this is positive news, another outbreak could happen, which would be devastating for the sea star population, and without a comprehensive understanding of how this disease works, we are helpless to stop it.

The goals of this study were to examine possible factors that affect the spread of sea star wasting disease in tide pool communities, as well as determine the effect that sea star wasting disease has on the biodiversity of tide pool communities. The main questions included, 1) Does the prevalence of sea star wasting disease impact the biodiversity of a tide pool?, 2) Do pH, salinity, alkalinity, and total dissolved solids affect the spread of sea star wasting disease in a tide pool? And 3) Does the geographic location of the tide pool affect the spread of sea star wasting disease?

Methods

Study site

Forty tide pools were randomly sampled in Puget Sound and on the Washington coast, twenty at each location (Figure 1). Data collection took place June and July of 2017.

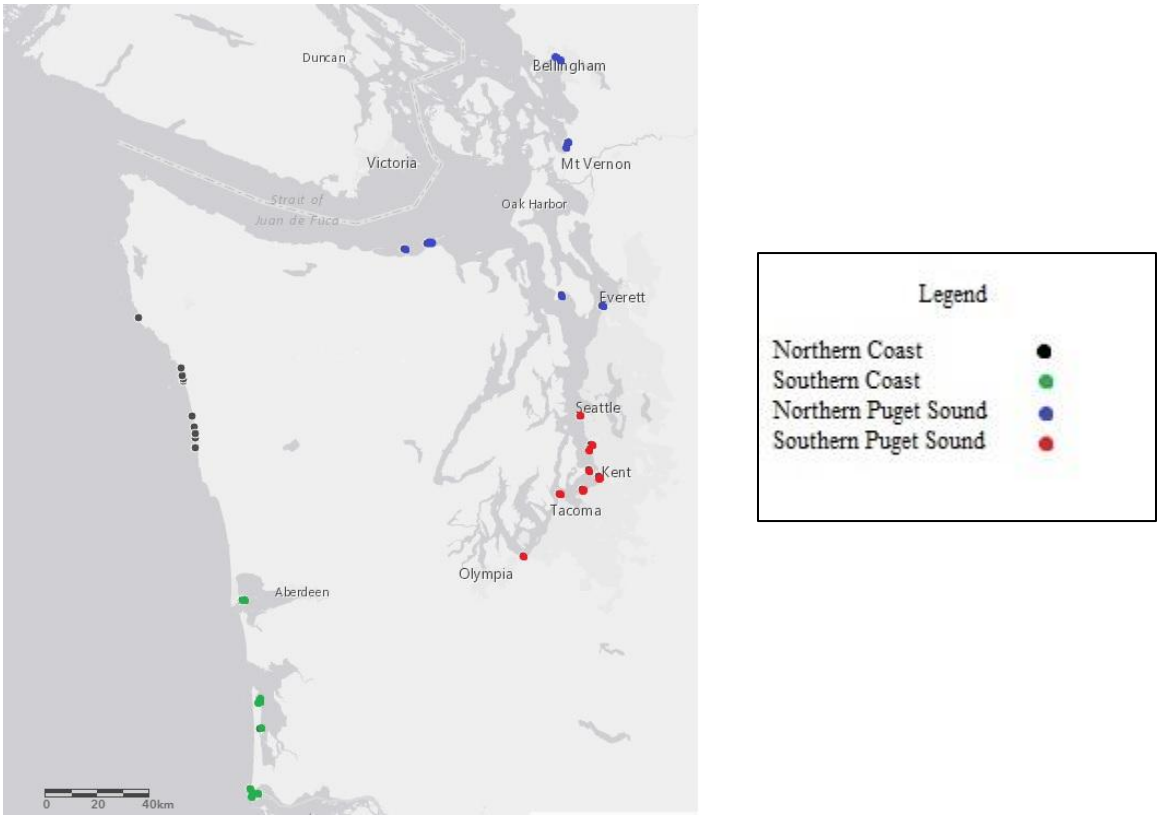

Figure 1. Map of study sites along the Washington coast and in Puget Sound

The Washington coast and Puget Sound were divided into northern and southern halves, for a total of four regions. The data sites were divided to compare the coast to Puget sound and the north and south components were added to account for the difference between Northern and southern Puget sound water quality. The line between north and south was chosen based on halfway point of the coast and Puget sound. At each

of the four regions, ten tide pools containing *P. ochraceus* were randomly selected and surveyed. Tide pools were chosen haphazardly, with the only criteria being that there must be *P. ochraceus* present. Locations of the tide pools within each region were also random, to ensure that the data was representative of the whole region, instead of one section. Locations of the tide pools were recorded using GPS (Figure 1).

Tide pool surveys

At each tide pool, a visual census of sessile marine macroinvertebrates was conducted, along with sea stars and sea urchins (Table 1), and each species identified and cataloged (Kozloff 1993).

Table 1: List of species observed and counted for Shannon biodiversity counts

Scientific Name	**Common Name**
Pisaster ochraceus	Ochre star
Mytilus californianus	California mussel
Anthopleura elegantissima	Aggregating anemone
Anthroplera xanthogrammica	Giant green anemone
Mytilus edulis	Blue mussel
Clavelina huntsmani	Lightbulb tunicate
Cucumaria pseudocurata	Tar spot sea cucumber
Henricia leviuscula	Pacific blood star
Patiria miniata	Bat star
Strongylocentrotus purpuratus	Pacific purple sea urchin
Collisella digitalis	Fingered limpet

A tide pool was defined as a rocky pool with standing water ranging in size from 4 m^2 to 9m^2. For populations of mussels that were too large to count, the number was estimated. Three 0.25 m^2 quadrats were randomly placed on the mussel population. The number of mussels in each quadrat was counted and those three numbers were averaged. This was randomized by turning away from the wall, pointing a laser pointer over the shoulder, and using that point as the upper left-hand quarter of the quadrat. The total area of mussel coverage in the tide pool was measured and, using the average number of mussels per 0.25 m^2, total number of mussels in the tide pool was calculated (Krebs 1998). Species richness and abundance was recorded for each species at each location and Shannon biodiversity was calculated off site. Species richness was calculated by tallying each individual species present and identifying them using Kozloff's field guide. Abundance was calculated by counting the individuals of each species present. The *P. ochraceus* population at each tide pool was also surveyed for the presence of sea star wasting disease. This disease is distinctive, so the chance of misidentification is low (Figure 2). The number of *P. ochraceus* infected with sea star wasting disease was also recorded, as well as the number of healthy ones. University of California – Santa Cruz has a protocol for collecting data on sea star wasting disease (2018). The protocol for timed (non-permanent study sites) surveys was followed except stage of sea star wasting disease was not recorded, nor was size of each infected sea star. These steps were omitted

because this study was only concerned with the presence or absence of the disease in tide pools, not specific stage nor was age a variable in this study, so size was also omitted.

Water quality was also measured. The YSI EcoSense® EC300A meter was used on site to measure conductivity (millisiemens), salinity (ppt) and TDS (total dissolved solids) (g/L). The conductivity measurement was standardized to 25 degrees Celsius to avoid differences due to temperature fluctuation. Samples for testing were taken from adjacent ocean and filtered through an Omicron Glass Fiber Filter 1.6µm to rid samples

Figure 2. Example of each stage of sea star wasting disease in *P. ochraceus*. The top left picture is stage 1, with characteristic lesions. The top right picture shows stage 2, as the lesions spread, and the sea star loses grip. The bottom left picture shows stage 3, when the lesions have completely spread, and the water vascular system fails. The bottom right picture shows stage 4, when the sea star has lost several limbs and the tissue has severely deteriorated. (UCSC 2014)

of suspended materials. At each tide pool, a filtered sample of approximately 125 mL was brought back and immediately frozen. The samples were then thawed in the lab to test pH

and alkalinity. and alkalinity were measured using the Mettler Toledo mp220 pH meter, which was calibrated using 4.00 pH and 7.00 pH buffers after each titration. After pH was recorded, 1 mL of 0.01 M HCl was added and the pH was recorded again. This process was repeated until the pH was at or below 3.00. From pH, the number of protons added to the sample was calculated. With these values, the gran function was graphed for each sample and the Gran extrapolation was plotted using the Gran Function graph. From the Gran extrapolation, the milliliters of acid added was calculated which was then converted to alkalinity (mg $CaCO_3$/L).

Data Analysis

In total, forty tide pools were sampled using the same sampling methods at each one. To analyze the data, four separate models were performed to test four separate response variables: percentage of *P. ochraceus* with sea star wasting disease, Shannon biodiversity, species richness, and presence of sea star wasting disease. For this study, the predictor variables are random, however, in statistics, there is much debate about what random and fixed mean in terms of model selection (Gelman and Hill 2007; Clark et al. 2010; McGill 2015). Ultimately, the variables were performed as fixed effects. The factor of location has only four levels (four different locations), and when a model has less than five levels, multilevel modelling adds little over classical models (Gelman and Hill 2007). All of the other predictor variables are continuous variables, which cannot be treated as a random effect in R (McGill 2015). This data also had zero values, and many distributions in mixed models do not allow for zero values (Zuur et al. 2009). Because of these reasons, the variables, even though they are philosophically random, were performed as fixed effects. Generalized linear models were chosen for all tests because

they allow for different distributions (Poisson, gamma, binomial) (Zuur et al. 2009). The statistics program R version 3.5.1 was used to performed all four of the models, using the nlme package to performed the generalized linear models and the package bblme was used for the backward regression. Generalized linear models require that the data meet these assumptions: each data point is independent, the residuals are normally distributed, and the variance is homogenous (Zuur et al. 2009). Standardized residuals were compared to their theoretical quantities to check for homogeneity in variance and each model went through a transformation to eliminate any heteroscedasticity (see below for details on each transformation). For each model, the residuals were plotted against the fitted values to ensure that there was normality amongst the residuals. After the appropriate transformations were performed on the data, all assumptions for the models were met.

To compare differences between the four study regions, a one-way ANOVA was performed on each variable using the base package in R. For ANOVA tests with a significant p value, a Post-hoc Tukey test was performed to determine which pairings of regions are significantly different (Zuur et al. 2009). Box plots were constructed using the ggplot2 package in R studio to visualize the data. The University of California-Santa Cruz (UCSC) has data of recorded sighting of sea star wasting disease at several locations on the Washington Coast and Puget Sound. Most of the data points only state if sea star wasting disease was sighted in the area, so comparison was limited to comparing the number of SSWD sightings in 2017. The total number of tide pools surveyed was collected and data was divided into presence vs absence of SSWD. A chi-squared analysis was performed to compare how many surveyed tide pools were infected with

SSWD UCSC's data to mine (Whitlock and Schluter 2004). Chi-squared analysis was also used to compare the data collected by UCSC in 2017 to data collected the previous years, going back to 2013 (Whitlock and Schluter 2004). This was done to see if there was a significant difference in the spread of SSWD since the outbreak first started.

To further analyze the data, the process of backward elimination regression was performed on each model. Backwards regression takes the model, with all the predictor variables, and eliminates them one by one, strengthening the model each time. This regression keeps eliminating predictor variables until the model cannot be improved (Dunkler et al. 2014). Stepwise regression eliminates predictor variables from the model, like backward elimination regression, but will add in previously eliminated variables as well as subtracting them to find the strongest model (Draper and Smith 1998). Backward elimination regression was chosen over stepwise regression for a variety of reasons. While stepwise regression can analyze more variants of the original model, it is more often associated with inflated regression coefficients and increased bias in analysis (Mantel 1970; Mickey and Greenland 1989; Maldonado and Greenland 1993; Sun et al. 1996; Dallal 2012). Also, according to Royston and Sauerbrei (2008), it is extremely rare for the addition of previously excluded variables to strengthen the model.

In this study, to determine which model is the "best", three values were found for each: the second order information criterion (AIC_C), the AIC_C differences ($\mathbf{\Delta_i}$), and the weight (W_i). The AIC_C score is based on the AIC, the Akaike's information criterion (Burnham and Anderson 2002). Akaike's information criterion estimates the distance between the fitted model and the unknown mechanism that is responsible for the observed data (Akaike 1973). Each model gets an AIC score, and the results are

compared. However, an assumption of the AIC is a large sample size (Sugiura 1978, Sakamoto et al. 1986), which this study does not have. Because of the relatively small sample size, the second order information criterion was used. The AIC_C includes a term that corrects for the bias that can occur with a small sample size (Sugiura 1978, Hurvich and Tsai 1989). However, the AIC_C score by itself is useless; it must be compared with other scores to determine which model is statistically supported. In general, the smallest AIC_C score of the models is the most supported. To compare scores, the Δ_i is found for each model. The Δ_i is the difference between the model with the lowest AIC_C score, and the AIC_C score of the model it is being compared to. The model with the lowest AIC_C score has a Δ_i of zero (Conner and Seborg 2004). However, although zero is the best Δ_i score, any Δ_i score from 0-2 is considered having substantial empirical support. This means that if a model includes more variables, and has a Δ_i that is < 2, it would be appropriate to pick that model over the model with a Δ_i of zero, if the variables included in the model were of interest (Burnham and Anderson 2002). The final value is the weight of the model. The weight is the evidence that this particular model is the best model for this data, when compared to the other models (Bozdogan 1987, Kishino et al. 1991). All of the weights of a set of models add up to one, and the model with the largest weight is considered the most supported. Weight is calculated using the Δ_i score.

To analyze the factors that influence sea star wasting disease prevalence in a population of sea stars, a generalized linear model with a Gaussian distribution was performed using percentage of *P. ochraceus* population infected with sea star wasting disease as the response variable, and total dissolved solids, salinity, Shannon biodiversity, pH, alkalinity, and location as predictor variables. A Gaussian distribution of error terms

was chosen because the response variable is numerical, continuous data containing zero values in the response variable (Cohen and Cohen 2002, Zuur et al. 2004). Location is represented by the three regions (north Puget Sound, southern coast, southern Puget Sound), the fourth location (northern coast) was used as the baseline to compare the other three locations for any statistically significant difference. The estimate (slope) of the northern coast is the same as the estimate of the intercept. A log 10 (x +1) transformation was chosen for this model because it significantly normalized the data (Whitlock and Schluter 2004).

The second model analyzed what factors, if any, affected the biodiversity of tide pool communities. For the second model, a generalized linear model was performed using a gamma distribution of error terms and Shannon biodiversity as the response variable. A gamma distribution of error terms was chosen because the response variable is continuous, numerical data with no zero values (Cohen and Cohen 2002, Zuur et al. 2004). As with the first model, location was tested as a factor and the other variables were performed as fixed, numerical values. A $\log_{10}(x+1)$ transformation was performed on the data for maximum normality (Whitlock and Schluter 2004).

For the third model, species richness was analyzed using a generalized linear model with a Poisson distribution of error terms. As with the previous models, location was performed as a factor and the other variables were performed as numerical values. A Poisson distribution of error terms was chosen because species richness is count data (discrete, numerical numbers) (Zuur et al. 2004). A square root transformation was performed on the data to increase normality (Whitlock and Schluter 2004).

For the final model, presence of sea star wasting disease was tested in a generalized linear model with a binomial distribution. A binomial distribution of error terms was chosen because presence and absence of sea star wasting disease was represented as ones and zeros (Zuur et al. 2004). As with the previous models, location was analyzed as a factor and all other variables were performed as fixed, numerical values. A square root transformation was chosen for this data set for maximum normality (Whitlock and Schluter 2004).

Results

According to the one-way ANOVA (Table 2), the only variables that are not significantly different between the regions are the number of infected *P. ochraceus* and the percentage of infected *P. ochraceus* (Figures 9 and 10). Boxplots comparing the locations for each of the variables were constructed to visualize the data (Figures 3-10), and a compact letter display was added to each to indicate which groups are significantly different from each other. Any group sharing a letter means they are not significantly different, while different letters indicate that they are. Northern Puget sound had significantly higher pH, TDS, salinity, and alkalinity when compared to other locations (Figures 3-6). The northern coast had greater biodiversity (Figure 7) and the northern and southern coasts had a greater number of *P. ochraceus* (Figure 8), both of which were statistically significant (Table 2).

Table 2: Results of One-Way ANOVA test comparing response variables between the four regions

Variable	**F statistic**	**p-value**
pH	4.85	0.006*
Shannon Diversity	10.14	$6.53e^{-5}$*
TDS	26.74	$1.10e^{-5}$*
Salinity	32.88	$3.19e^{-6}$*
Number of *P. ochraceus*	27.03	$1.97e^{-5}$*
Number of Infected *P. ochraceus*	0.94	0.432
Percentage of Infected *P. ochraceus*	8.52	0.359
Alkalinity	4.80	0.006*

* indicates statistical significance ($p \leq 0.05$)

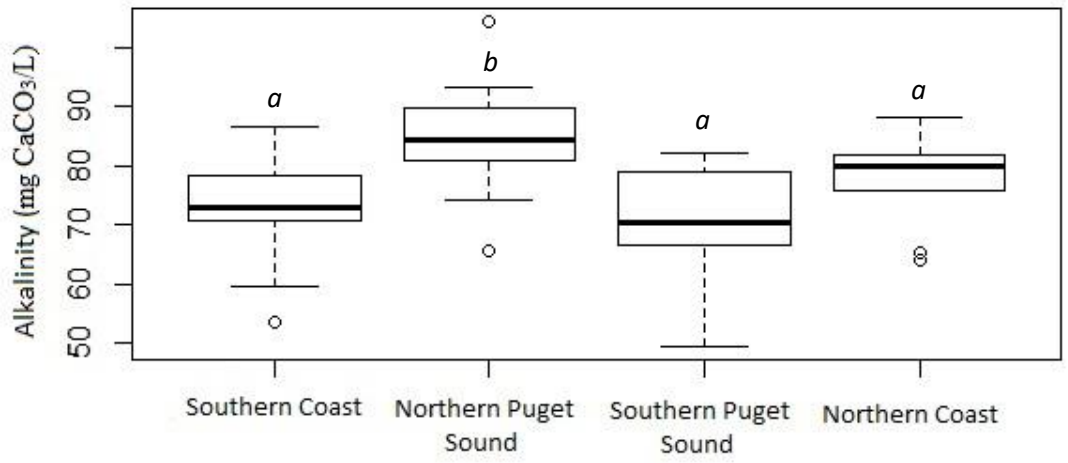

Figure 3: Boxplot comparing the alkalinity of the four locations; groups sharing the same letter are not statistically significantly different (p = 0.05)

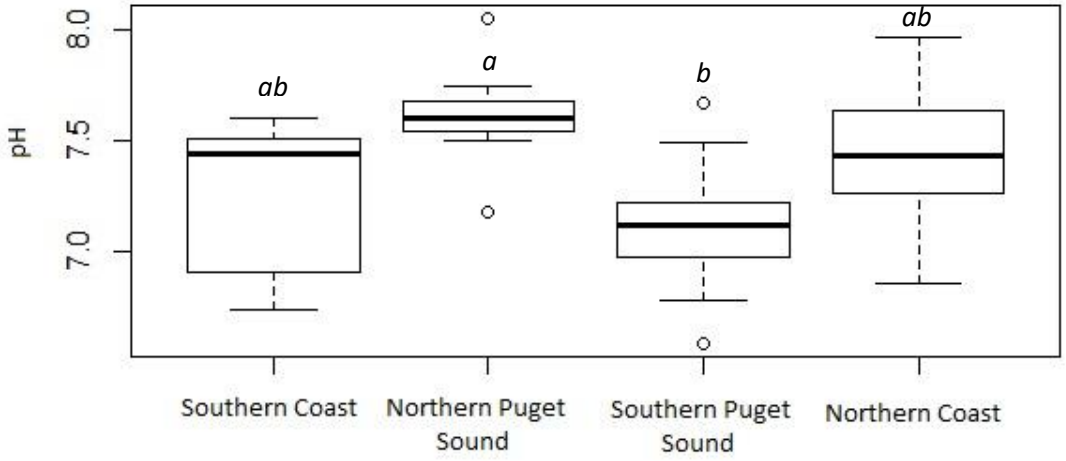

Figure 4: Boxplot comparing the pH of the four locations; groups sharing the same letter are not statistically significantly different (p = 0.05)

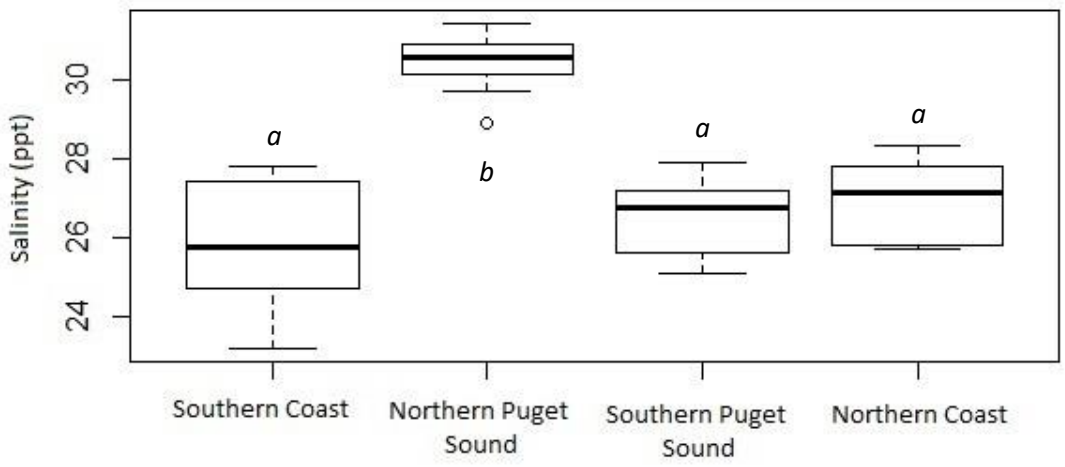

Figure 5: Boxplot comparing the salinity of the four locations; groups sharing the same letter are not statistically significantly different (p = 0.05)

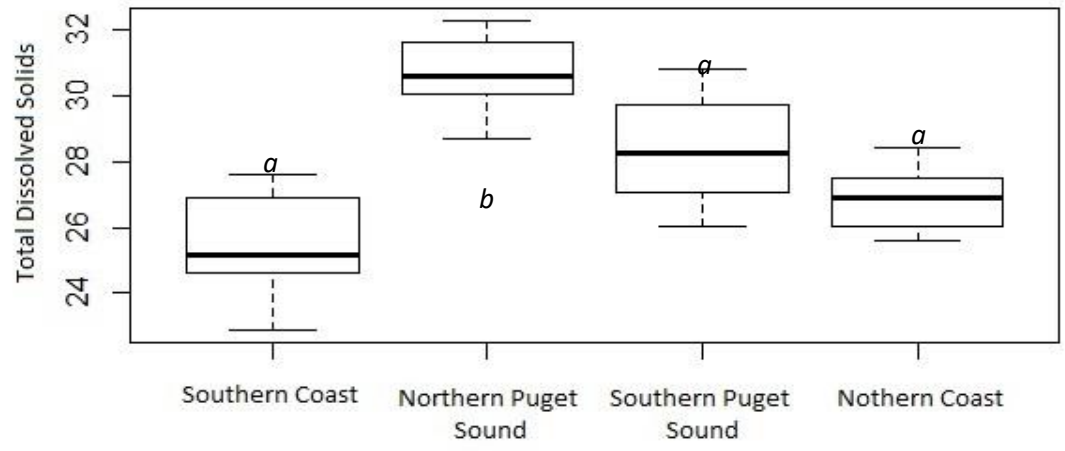

Figure 6: Boxplot comparing the dissolved solids of the four locations; groups sharing the same letter are not statistically significantly different (p = 0.05)

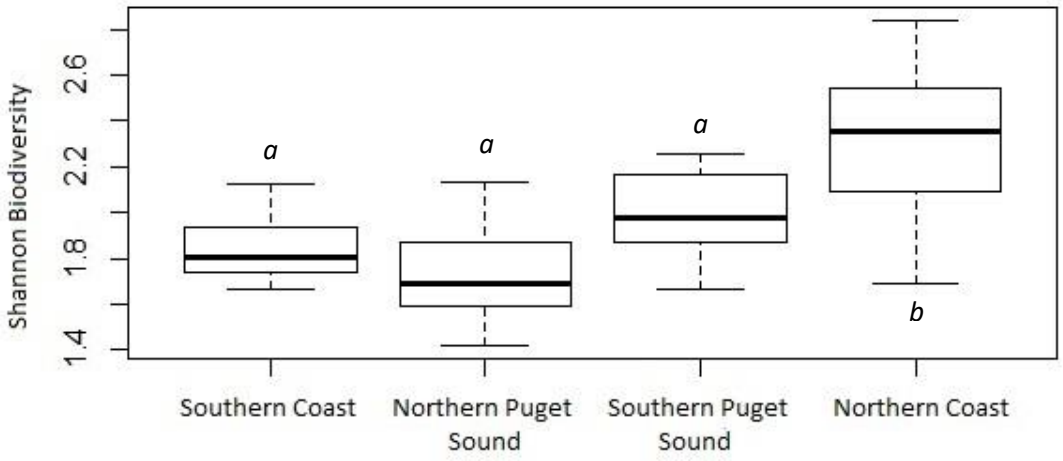

Figure 7: Boxplot comparing the Shannon Biodiversity of the four locations; groups sharing the same letter are not statistically significantly different (p = 0.05)

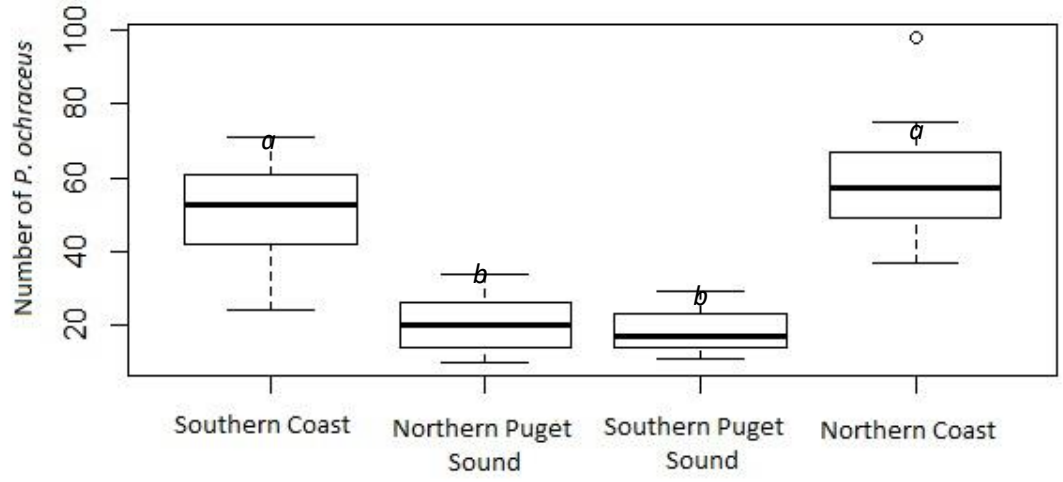

Figure 8: Boxplot comparing the Number of *P. ochraceus* of the four locations; groups sharing the same letter are not statistically significantly different (p = 0.05)

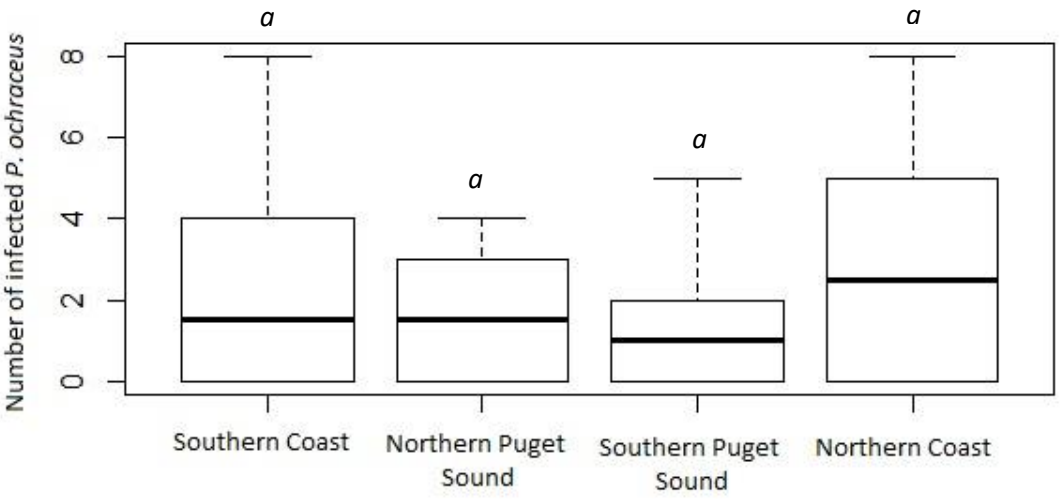

Figure 9: Boxplot comparing the number of infected of *P. ochraceus* of the four locations; groups sharing the same letter are not statistically significantly different (p = 0.05)

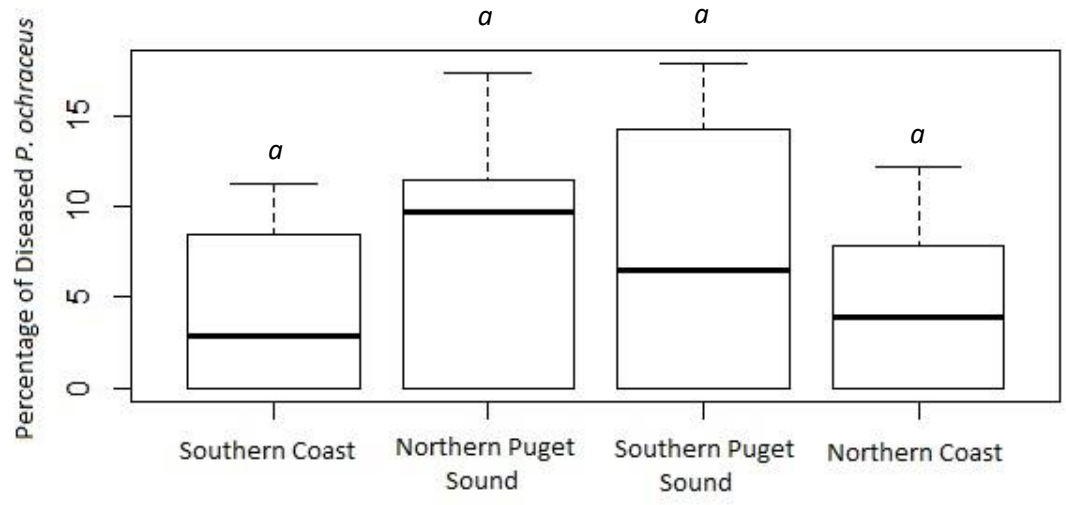

Figure 10: Boxplot comparing the percentage of *P. ochraceus* of the four locations; groups sharing the same letter are not statistically significantly different (p = 0.05)

A chi-squared analysis was used to compare the presence of sea star wasting disease data collected by UC Santa Cruz to the data collected for this study. In 2017, UC Santa Cruz surveyed forty-three tide pools, thirty of which had sea star wasting disease. This study surveyed forty tide pools, twenty-eight of which had sea star wasting disease. The chi-squared test had a chi-square statistic of 0.0468 and a p-value of 0.9816. The data collected by UC Santa Cruz is not significantly different from the data collected by this study.

Chi-squared analysis was also used to compare the data collected by UC Santa Cruz in 2017 to the previous years (Table 3). All years but 2015 had significantly higher instances of sea star wasting disease from 2017.

Table 3: Results of Chi-squared test comparing UC Santa Cruz's 2017 data of SSWD presence to previous years

Year	**X^2**	**p-value**
2013	4.69	0.030*
2014	6.65	$9.89e^{-3}$*
2015	0.53	0.465
2016	5.27	0.022*

*indicates statistical significance ($p \leq 0.05$)

Table 4. Results of backward elimination regression preformed on model with Percentage of *P. ochraceus* with sea star wasting disease as the response.

Step 1 Model Variables: TDS, Salinity, Shannon Biodiversity, pH, Alkalinity, and Location			
Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	72.7	8.9	0.0094
Location	63.9	0.0	0.7926
Alkalinity	69.2	5.4	0.0542
TDS	69.3	5.4	0.0530
Salinity	70.0	6.1	0.0380
pH	70.3	6.4	0.0328
Shannon Biodiversity	71.3	7.4	0.0198
Step 2 Model Variables: TDS, Salinity Shannon Biodiversity, pH, and Alkalinity			
Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	63.9	2.9	0.0701
TDS	61.0	0.0	0.2924
Alkalinity	61.0	0.0	0.2924
pH	62.1	1.0	0.1743
Salinity	62.5	2.9	0.1398
Shannon Biodiversity	65.3	4.3	0.0346
Step 3 Model Variables: Salinity, Shannon Biodiversity, pH, and Alkalinity			
Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	61.0	2.7	0.1092
Alkalinity	58.3	0.0	0.4258
pH	59.3	0.9	0.2602
Salinity	60.2	1.9	0.1636
Shannon Biodiversity	62.6	4.3	0.0494
	69.3	10.9	0.0017
Step 4 Model Variables: Salinity, Shannon Biodiversity and pH			
Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	58.3	0.7	0.2496
Salinity	57.6	0.0	0.3553
pH	58.6	1.0	0.2177
Shannon Biodiversity	60.1	2.4	0.1064

Table 4 (CONTINUED)

Step 5 Model Variables: Shannon Biodiversity and pH			
Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	57.6	0.8	0.2418
pH	56.8	0.0	0.3628
Shannon Biodiversity	57.7	0.9	0.2337

Percentage of *P. ochraceus* with sea star wasting disease

According to the backward elimination regression that was performed (Table 4), the best model has only Shannon biodiversity as its explanatory variable, with percentage of *P. ochraceus* with sea star wasting disease as its response (Table 5). According to the final model, Shannon biodiversity did not have a statistically significant relationship with the percentage of the *P. ochraceus* population infected with sea star wasting disease.

Table 5. Results of generalized linear model with percentage of *P. ochraceus* infected with sea star wasting disease as a function of the variables selected by the stepwise regression.

Variable	**Estimate**	**Standard error**	**t statistic**	**p-value**
Intercept	-4.0067	3.0771	-1.321	0.232
Shannon Biodiversity	0.6754	0.6759	1.122	0.179

Shannon Biodiversity

A backward elimination regression was performed on the second model (Table 6) to determine which variables should be included in the final model. According to the regression, the model with the most statistical support only had the variables location and pH removed. This model was chosen for analysis.

Table 6. Results of backward elimination regression preformed on model with Shannon biodiversity as the response variable.

Step 1 Model Variables: TDS, Salinity, Percentage of *P. ochraceus* with sea star wasting disease (% with SSWD), *P. ochraceus* abundance, pH, Alkalinity, and Location

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	1.2	4.2	0.0456
Location	-2.9	0.0	0.3660
pH	-2.2	0.8	0.2491
Alkalinity	-0.7	2.2	0.1213
TDS	-0.5	2.4	0.1096
Salinity	-0.2	2.7	0.0939
% with SSWD	3.8	6.7	0.0127
Abundance	7.5	10.5	0.0019

Step 2 Model Variables: TDS, Salinity, Percentage of *P. ochraceus* with sea star wasting disease (% with SSWD), *P. ochraceus* abundance, pH, and Alkalinity

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	-2.9	3.1	0.1209
pH	-6.0	0.0	0.5694
Alkalinity	-3.9	2.2	0.1935
TDS	-2.4	3.1	0.1209
% with SSWD	0.6	6.7	0.0201
Salinity	5.2	4.3	0.0346
Abundance	15.5	21.5	<0.001

Step 3 Model Variables: TDS, Salinity, Percentage of *P. ochraceus* with sea star wasting disease (% with SSWD), *P. ochraceus* abundance, and Alkalinity

TABLE 6 CONTINUED

Variable removed from model	AIC_c	Δ_i	W_i
None	-6.0	0.0	0.3854
Alkalinity	-5.8	0.3	0.3384
TDS	-5.0	1.1	0.2241
% with SSWD	-1.9	4.1	0.0485
Salinity	3.3	9.4	0.0035
Abundance	12.6	18.6	<0.001

In this model (Table 7), the abundance of *P. ochraceus* is positively correlated with Shannon biodiversity, while the percentage of sea stars infected with sea star wasting disease is negatively correlated. Salinity is also statistically significant, showing a negative relationship between salinity and biodiversity.

Table 7. Results of generalized linear model with Shannon Biodiversity as a function of the selected variables after backward elimination regression

Variable	Estimate	Standard error	T statistic	p-value
Intercept	0.879	0.460	1.908	0.065
Number of *P. ochraceus*	0.181	0.036	5.015	$1.64e^{-5}$*
Percentage of *P. ochraceus* with SSWD	-0.043	0.017	-2.571	0.015*
TDS	0.690	0.358	1.926	0.063
Salinity	-1.428	0.406	-3.514	0.001*
Alkalinity	0.229	0.188	1.224	0.230

*indicates statistical significance ($p \leq 0.05$)

Species richness

A backward elimination regression was performed on the third model (Table 8) to determine which variables should be included in the model. The model with the lowest Δ_i had only salinity as the explanatory variable. However, according to the analysis, salinity did not have a statistically significant influence over species richness (Table 9).

Table 8. Results of backward elimination regression preformed on model with species richness as the response variable.

Step 1 Model Variables: TDS, Salinity, Percentage of *P. ochraceus* with sea star wasting disease (% with SSWD), *P. ochraceus* abundance, pH, Alkalinity, and Location

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	199.0	8.0	0.0114
Location	191.0	0.0	0.6102
Alkalinity	195.4	4.4	0.0673
pH	195.4	4.4	0.0673
TDS	195.4	4.5	0.0668
Salinity	195.5	4.5	0.0645
Abundance	195.6	4.6	0.0618
% with SSWD	195.8	4.8	0.0564

Step 2 Model Variables: TDS, Salinity, Percentage of *P. ochraceus* with sea star wasting disease (% with SSWD), *P. ochraceus* abundance, pH, and Alkalinity

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	191.0	2.9	0.0553
Alkalinity	188.0	0.0	0.2188
pH	188.2	0.2	0.2184
Abundance	188.9	0.8	0.1596
TDS	189.1	1.0	0.1442
% with SSWD	189.5	1.4	0.1164
Salinity	190.5	2.5	0.0703

TABLE 8 CONTINUED

Step 3 Model Variables: TDS, Salinity, Percentage of *P. ochraceus* with sea star wasting disease (% with SSWD), *P. ochraceus* abundance, and pH

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	188.0	2.5	0.0798
pH	185.5	0.0	0.2801
Abundance	186.1	0.6	0.2106
TDS	186.3	0.8	0.1857
% with SSWD	186.7	1.2	0.1544
Salinity	187.8	2.2	0.0913

Step 4 Model Variables: TDS, Salinity, Percentage of *P. ochraceus* with sea star wasting disease (% with SSWD), and *P. ochraceus* abundance

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	185.5	1.6	0.1227
Abundance	183.9	0.0	0.2796
% with SSWD	184.1	0.3	0.2431
TDS	184.1	0.3	0.2430
Salinity	186.5	2.6	0.0754

Step 5 Model Variables: TDS, Salinity, and Percentage of *P. ochraceus* with sea star wasting disease (% with SSWD)

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	183.9	1.3	0.1702
% with SSWD	182.6	0.0	0.3225
TDS	183.5	1.0	0.2004
Salinity	184.5	2.0	0.1213

Step 6 Model Variables: TDS, and Salinity

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	182.6	0.2	0.2806
TDS	182.3	0.0	0.2801
Salinity	183.3	0.9	0.1754

Table 9. Results of generalized linear model with species richness as a function of the selected variables after backward elimination regression

Variable	Estimate	Standard error	T statistic	p-value
Intercept	1.212	1.988	1.754	0.3125
Salinity	0.509	0.441	1.243	0.0945

Presence of sea star wasting disease

A backward elimination regression was performed on the final model (Table 10) to determine which variables should be removed for the most statistical significance. After five steps, the model containing pH and salinity as the explanatory variables was found to be the best, when compared to the other models. The variables that were included are of interest when presence of sea star wasting disease is the response variable, so this model was chosen to be analyzed. According to the analysis (Table 11) there is a positive correlation between pH and presence of sea star wasting disease, but salinity has no effect on presence of sea star wasting disease.

Table 10. Results of backward elimination regression preformed on model with presence of sea star wasting disease as the response variable.

Step 1 Model Variables: TDS, Salinity, *P. ochraceus* abundance, pH, Alkalinity, and Location

Variable removed from model	AIC_c	Δ_i	W_i
None	59.5	4.1	0.0442
Location	55.4	0.0	0.3457
Salinity	56.2	0.8	0.2308
Alkalinity	56.7	1.3	0.1836
TDS	57.0	1.6	0.1582
pH	60.5	5.1	0.0279
Abundance	62.2	6.7	0.0125

TABLE 10 CONTINUED

Step 2 Model Variables: TDS, Salinity, *P. ochraceus* abundance, pH, and Alkalinity

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	55.4	2.4	0.0914
Alkalinity	53.0	0.0	0.3012
Abundance	53.6	0.6	0.2235
TDS	53.6	0.6	0.2223
Salinity	54.6	1.6	0.1368
pH	57.8	4.8	0.0277

Step 3 Model Variables: TDS, Salinity, *P. ochraceus* abundance, and pH

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	53.0	1.9	0.1368
Abundance	51.2	0.0	0.3432
TDS	51.3	0.1	0.3266
Salinity	52.9	1.7	0.1471
pH	56.3	5.1	0.0263

Step 4 Model Variables: TDS, Salinity, and pH

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	51.2	2.1	0.1895
TDS	49.1	0.0	0.5324
Salinity	51.2	2.1	0.1907
pH	55.0	5.9	0.0284

Step 5 Model Variables: Salinity and pH

Variable removed from model	**AIC_c**	**Δ_i**	**W_i**
None	49.1	0.0	0.3736
Salinity	49.4	0.3	0.3152
pH	52.6	3.5	0.0644

Table 11. Results of generalized linear model with presence of sea star wasting disease as a function of the selected variables after backward elimination regression

Variable	Estimate	Standard error	T statistic	p-value
Intercept	-23.083	16.443	-1.404	0.1604
Salinity	-3.589	2.364	-1.518	0.1289
pH	15.817	7.134	-2.217	0.0266*

*indicates statistical significance ($p \leq 0.05$)

Discussion

Prevalence of sea star wasting disease and its impact on biodiversity

The main purpose of this study was to test whether the prevalence of sea star wasting disease effected the Shannon diversity and species richness of the tide pools. Dr. Robert Paine identified the concept of keystone species by pulling *P. ochraceus* out of tide pools in Washington State and watching the adverse effect their absence had on the biodiversity of their tide pool communities (1966). This study hypothesized that when a sea star is infected with sea star wasting disease, it cannot function as a keystone species. Essentially, even in the early stages of the disease, an infected sea star might as well be absent, because sea star wasting disease is effectively removing it from the tide pool.

Shannon biodiversity was analyzed as a function of the water quality metrics, location, abundance of *P. ochraceus*, and percentage of infected *P. ochraceus.* While most of the water quality metrics and location proved to be insignificant, abundance of *Pisaster* and prevalence of sea star wasting disease were not (Table 7). Abundance of *P. ochraceus* was shown to be positively correlated with Shannon biodiversity. This relationship supports the studies conducted by Dr. Paine illustrating the importance of *P. ochraceus* in its tide pool communities (1966, 1969, 1974, 1976). While these findings are not surprising, it is encouraging to have this data reflect a known biological phenomenon. Salinity was also negatively correlated with Shannon biodiversity (Table 7). According to the results, as salinity increases, Shannon biodiversity decreases. Salinity can increase due to greater evaporation rates caused by higher temperatures (Panin and Brezgunov, 2007). These results could illustrate the impact climate change has on tide pool communities.

According to the model (Table 7), the percentage of *P. ochraceus* infected with sea star wasting disease is negatively correlated to Shannon biodiversity, meaning that the more *Pisaster ochraceus* that are infected in the tide pool, the lower the biodiversity of that tide pool. These results support the hypothesis that sea star wasting disease disables a sea star long before it finally wastes away; at a certain point between contracting the disease, and succumbing to the necrotic tissue, the sea star's ability to feed is either greatly reduced or completely gone. While the disease spreads inside of the sea star, it is essentially the living dead: unable to eat, unable to move, but still technically alive. This makes sea star wasting disease even more insidious; not only does it kill the sea star; it incapacitates it before it dies.

Water quality and its effects on the spread of sea star wasting disease

However, although abundance of *P. ochraceus* and prevalence of sea star wasting disease were shown to be related to Shannon biodiversity, none of the water quality metrics, location, abundance of *P. ochraceus,* or percentage of infected sea stars proved to be significantly correlated to species richness in tide pools (Table 9). However, species richness only tells part of the story. What this insignificance shows is the importance of abundance in biodiversity; while species richness by itself proved to have no significance, when combined with abundance and transformed into Shannon biodiversity, significance is found. This is supported by the previous research that states *P. ochraceus* abundance increases biodiversity, and *M. californianus* abundance decreases biodiversity (Paine 1966, 1967, 1974). Abundance is clearly an important factor when it comes to measuring *P. ochraceus'* impact on its tide pool community.

One of the goals of this study was to test the multiple factors that could influence the spread of sea star wasting disease in the tide pools of Washington State and it does not appear that the percentage of sea star wasting disease in *P. ochraceus* populations is influenced by the location of the tide pool, or the various water quality metrics that were tested (Table 5).

The results did show a positive correlation between pH and presence of sea star wasting disease in tide pool communities (Table 11). The range of pH for these samples was 6.7 and 8.1, which skews more acidic than the average pH of 8.1 for the Pacific Ocean and 7.8 for Puget sound (Hickey 2019). This result conflicts with both previous research (Menge et al. 2016) and the previous model (Table 5) in this study. It is also curious that the relationship appears to be positive, as in, when pH increases, so does the presence of sea star wasting disease in tide pools. This relationship would not be caused by ocean acidification because sea star wasting disease appears to be more prevalent as the water becomes more alkaline. Because this result seems to contradict previous literature, and the low sample size of this study, it is impossible to ignore the possibility of a type 1 error. pH was the only water quality metric that had a significant relationship with presence of sea star wasting disease.

Location's effect on the spread of sea star wasting disease

The one-way ANOVA was performed to determine if there was a significant difference in the predictor variables between the four regions. The results show that there is a difference in pH, Shannon diversity, TDS, salinity, and alkalinity between the four

regions (Table 1). The two variables analyzing sea star wasting disease were not significantly different, which could suggest that sea star wasting disease is not affected by regional differences in water quality. A Post-hoc Tukey test was performed on all significant variables to determine which regions are statistically different from the others and box plots were created to compare the locations. Northern Puget sound was the most unique in terms of water quality; this region had a significantly higher alkalinity (Figure 3), pH (Figure 4), salinity (Figure 5), and TDS (Figure 6) than the other three. The northern coast had a higher Shannon biodiversity (Figure 7), which could be a result of the lack of an industrial presence in the state parks. The northern and southern coast had a greater amount of *P. ochraceus* when compared to the Puget sound regions, which could also be explained by a decreased industrial presence, as well as more water mixing. However, this study wanted to see the effects that location had specifically on the spread of sea star wasting disease, which was ultimately not affected by the location (Figures 9 and 10).

Analysis of UC Santa Cruz Data

A chi-squared analysis was used to compare the data collected by UC Santa Cruz to the data collected for this study. The data used was collected in 2017 and states a location and whether sea star wasting disease was observed. UC Santa Cruz observed 43 tide pools, 30 of which had sea star wasting disease. The test had a chi-square statistic of 0.0468 and a p-value of 0.9816, showing that the data collected by UC Santa Cruz is not statistically different from the data collected for this study. While this doesn't shed new light on the data, it is corroborating evidence for the data collected. The 2017 UC Santa Cruz data was also compared to data collected by UC Santa Cruz in previous years, going

back to 2013 (Table 3). Chi-squared analysis was used to determine if there is a difference between 2017 and the previous years. According to the test, 2013, 2014, and 2016 are significantly higher from 2017; to fully discuss these results, it is important to look at the raw data. In 2013, nineteen tidepools were observed, eighteen of which were infected with SSWD. In 2014, twenty-five tidepools were observed, twenty-four of which were infected. Comparing these numbers to the data from 2017 (forty-three total tidepools, thirty infected), it appears that the significant difference comes from the number of uninfected tidepools. For both 2013 and 2014, only one tidepool was uninfected, compared to the thirteen uninfected tidepools in 2017. This could be evidence of the severity of the initial outbreak, which started in 2013 because the vast majority of tidepools had SSWD in 2013 and 2014 (Menge et al. 2016). However, in 2016, only twelve tidepools were observed; four were infected and eight were not. The lack of data for this year could have many causes: It is worth noting that UC Santa Cruz's data is crowd sourced; anybody can make an observation and submit it on the UC Santa Cruz website. It is possible that research groups that were making observations in previous years were not active in 2016. It is also possible that infected sea stars died before they could be observed, which would increase the number of uninfected tidepools. This could also illustrate a lull in the outbreak, which raises further questions about what caused the resurgence in 2017.

Possible shortcomings

The goals of this study were to test the various factors that influence sea star wasting disease in *P. ochraceus* as well as determine the effect sea star wasting disease has on Shannon biodiversity. To analyze the data, regression analysis was performed on

each of the four models. Backward elimination regression is one of the more popular methods of data analysis in ecological papers (Whittingham et al. 2006), however, there is a growing number of scientists who believe this method of data analysis is flawed, and can produce unreliable results (Whittingham et al. 2006, Lewis 2007, Hegyi and Garamszegi 2010, Knapp and Sawilowsky 2010). According to the literature, there are three leading issues that can arise with this regression: bias in model selection, inaccurate parameter estimation, and model overfitting (Thompson 1995, Burnham and Anderson 2002, Whittingham et al. 2006, Lewis 2007).

In many backward elimination regression software packages, the models created display their test statistic and the p-value for each variable as soon as the model is performed. This can create bias in choosing the model; the researcher might be tempted to pick the model with the most significant p-values, not necessarily the one that best represents the data (Anderson et al. 2000, Burnham and Anderson 2002). In this study, this problem was avoided by using AIC_C values to compare models. By choosing a model based on a score that relays the relative strength of the model, but not the significance, the issue of bias in model selection is eliminated (Whittingham et al. 2006). Only after choosing which variables to include was the model performed for statistical significance, making it impossible to determine the p-values for the variable until the regression was completed and the model was chosen. However, bias can also be involuntary. A small sample size can cause the AIC score to be incorrectly calculated (Sugiura 1978, Sakamoto et al. 1986). When $n/K < 40$ (where n is the sample size and K is the number of parameters), the AIC score can perform poorly, leading to inaccurate model selection (Burnham and Anderson 2002). This study avoided this type of error by using the AIC_C

score, instead of the AIC. The AIC_C score, or second order information criterion, is similar to the AIC score, except that it has an extra term that corrects for a small sample size (Hurvich and Tsai 1989). By using the AIC_C score, the bias inherent in a small sample size is greatly reduced (Burnham and Anderson 2002).

Another type of error that can occur in backward elimination regression is inaccurate parameter estimation. In statistics, a parameter is a numerical quantity that describes or characterizes a population or a sample of that population (Everitt and Skrondal 2010). The number of parameters in a statistical model is used to calculate the AIC_C score, which in turn is used to select the most appropriate model (Hurvich and Tsai 1989). Several regression software packages performed multiple steps at once: the data is entered, and the program will remove as many variables as necessary to arrive at the best model (Lewis 2007). By taking the data through multiple steps, as if no previous testing had occurred, the number of parameters can be miscalculated; the program will use the same number of starting parameters for each step, as opposed to recalculating when a variable has been removed (Wilkinson 1979, Thompson 1995, Lewis 2007). By miscalculating the parameters, the AIC_C score is also miscalculated, causing the results to be unreliable (Burnham and Anderson 2002). To correct this potential error, this study ran the regression models one step at a time. At the start of each regression, each possible model (one variable removed from each) was created, and compared using the bblme package in R studio, which produced the AIC_C score, weight, and Δ_i for each model. The best model was chosen using the given criteria, the variable removed, and then the process of creating each possible model and taking them for comparison was repeated.

By testing the models one step at a time, the chance of parameter estimation errors was greatly reduced, because the parameters were recalculated for each model at each step.

A large amount of predictor variables can also cause model inaccuracy (Burnham and Anderson 2002). In models, there is a "signal" and "noise". The signal is the underlying mechanism that accurately explains the data; ideally, this is what the model is representing. The noise is extra data points and anomalies that occur in the data, but do not explain the data as a whole (Babyak 2004). When the amount of predictor variables increases, the amount of noise that occurs in a model also increases. When this noise increases, the model runs a risk of being overfit. Overfitting occurs when the noise of a model, instead of the signal, is fitted (Babyak 2004, Hawkins 2004). The idiosyncrasies of the predictor variables are included in the model, as opposed to the underlying mechanism. This is dangerous because, while the model does fit this particular data set, it is nearly impossible to use the same model for new data, because the new data will not have the same noise (Babyak 2004, Hawkins 2004). Overfitting becomes a danger when the number of predictor variables exceeds the number of observations. However, this study used forty observations and seven predictor variables for data analysis. The relatively low amount of predictor variables greatly decreases the chance of model overfitting, making this particular error very unlikely (Burnham and Anderson 2002).

Backward elimination regression, when done haphazardly, can lead to an increase in error and inaccurate data analysis (Whittingham et al. 2006, Lewis 2007, Hegyi and Garamszegi 2010). However, when done carefully and respectfully, backward elimination regression is a useful form of analysis that can provide researchers with strong, supported models (Derksen and Kesselman 1992).

Conclusion and possible further research

Several studies support the idea that an increase in temperature causes the disease to spread at a quicker rate (Bates 2009; Eisenlord 2016; Kohl 2016). However, another study concluded that cooler temperatures were related to an increase in sea star wasting disease (Menge et al. 2016). Temperature is a hotly contested topic, and a far reaching study over several seasons focusing on the effect temperature has on sea star wasting disease could significantly further our understanding of this disease. If temperature is found to increase the spread of sea star wasting disease, then climate change could be to blame for the devastating effects the 2013 outbreak had on *P. ochraceus* populations. If climate change is the culprit, this could mean catastrophe for *P. ochraceus.* The temperature of the ocean is steadily climbing, which could mean even more devastating outbreaks.

When it comes to sea star wasting disease, scientists are almost completely in the dark. While there is some promising research suggesting a link between temperature and the spread of the disease, it is not nearly enough to suppress the spread of this disease or any future outbreaks. While temperature might make matters worse, there is no research that suggests how an outbreak begins, and it is theorized that each outbreak might have a different causal factor (Dungan et al 1982). *P. ochraceus* is just starting to return to its normal population numbers after the 2013 outbreak and they are by no means free of the disease (Miner et al. 2018).

In 1983, the Caribbean sea urchin *Diadema antillarum* suffered a catastrophic mass mortality event, also caused by an unidentified pathogen. 10 years and 30 years after the initial outbreak, the area was surveyed to find that only 3.5% (10 years) and 12%

(30 years) of the original population had returned. Not only were their numbers drastically decreased, the entire community had shifted from a coral abundant area, to an alga-dominated area. The Caribbean reefs are forever changed because of an echinoderm mass mortality event (Lessios 1995, 2016). Without any way to stop or at least stifle sea star wasting disease, another echinoderm mass mortality event seems inevitable, this time with *P. ochraceus* as the victim. If previous outbreaks are any indication, they are only going to get worse (Dungan et al. 1982; Eckert 1999; Pratchett 1999; Montecino-Latorre et al. 2016). Another outbreak could be catastrophic for these creatures, and in turn, the intertidal zone community itself.

Sea star wasting disease is a threat to intertidal communities, and it could seemingly occur at seemingly any time. While this study did not shed any new light on possible factors that cause the disease to spread, it did provide proof that the mere presence of sea star wasting disease is a threat to tide pool communities. It took four years for *P. ochraceus* populations to return after the 2013 outbreak. Depending on the severity, the next outbreak could permanently deplete their numbers, if they even come back at all.

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Appendixes

Appendix A – Chemical Titration Equations

NUMBER OF PROTONS ADDED TO SAMPLE

$$(V + v) * 10^{-pH}$$

V = initial volume

v = volume added to sample

NUMBER OF PROTONS ADDED TO 100 MILILITERS

$$0.1 * (((I * -1)/S) - 100)) * 10^{-3}$$

I = intercept of Gran extrapolation plot

S = slope of Gran extrapolation plot

MILIGRAMS OF $CaCO_3$ PER LITER

$$((P * 10{,}000) / 2) * 100.08$$

P = number of protons added to 100 milliliters

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