DISTRIBUTION OF SEA STAR WASTING DISEASE SYMPTOMS IN *PISASTER OCHRACEUS* IN THE ROCKY INTERTIDAL ZONE

By

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ABSTRACT

DISTRIBUTION OF SEA STAR WASTING DISEASE SYMPTOMS IN *PISASTER OCHRACEUS* IN THE ROCKY INTERTIDAL ZONE

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Beginning in 2013, many species of sea stars (phylum Echinodermata) along the Pacific coast experienced severe mortality due to sea star wasting disease (SSWD). The ochre sea star, *Pisaster ochraceus,* experienced one of the highest mortality rates during this outbreak. To test the hypothesis that the intertidal distribution of ochre sea stars influences the incidence and progression of SSWD symptoms, I documented the occurrence of symptoms and survivorship in adult and juvenile stars in the upper and lower portions of the mid-intertidal zone. I also chronicled the progression of SSWD symptoms among individually tagged adult stars to assess changes in symptoms relative to intertidal location and the extent and direction of movement. I predicted that because the higher intertidal zone is more physiologically stressful, there would be a higher proportion of symptomatic stars at higher tidal elevations relative to lower elevations. Because symptoms of SSWD include loss of turgor and individual rays, I predicted that stars in higher tidal elevations would have decreased rates of movement relative to those lower in the intertidal zone. I also predicted that juveniles would have a lower incidence of disease compared to adults. During the spring and summer of 2015-16, I surveyed ochre sea star population at False Klamath Cove in Del Norte County, CA using a 360 m^2

permanent grid and recorded the presence of symptoms, tidal elevation, size, location within the grid, and microhabitat location over seven surveys. I used a logistic regression model to test associations between intertidal elevation, microhabitat, life stage, and disease symptom expression over time. I found no significant effects of elevation or microhabitat; however, time and size were significant predictors of disease symptoms. I also conducted a transplant experiment at Palmer's Point Beach in Humboldt County, CA where I recorded the presence of symptoms, tidal elevation, and movement patterns of individually tagged adult stars in an 8,568 m^2 permanent grid. I found no significant differences among the main effects of time or treatment or their interactions. I also assessed whether the predictors of time and treatment were associated with the presence of disease symptoms in tagged stars and found no significant effect. Individually tagged ochre sea stars observed over time in the field exhibited both symptom progression and remission and showed greater movement rates compared to previous studies. In summary, life stage was a significant predictor of symptom presence with adults showing symptoms more frequently than juveniles; however, symptom expression showed no relationships to vertical distribution, microhabitat, or movement rates, suggesting that stress associated with vertical gradients in the intertidal zone might not influence the expression of SSWD disease symptoms.

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Big thanks to Yvonne Kugies and Grant Eberle for a smile, a laugh, a sounding board, and unlocking the conference room for me every week for three years. I also need to thank Dave Hoskins for his advice and, along with Pamela Ward, literally saving my life one scary, slippery, huge-swelled night in the intertidal.

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INTRODUCTION

Increased incidence of disease in marine environments poses a significant threat to the health of the world's marine ecosystems (Harvell 1999, Lafferty et al. 2004). Multiple stressors, including rising ocean temperatures, pollution, and overfishing, may have additive or synergistic effects that increase the frequency and severity of disease outbreaks and transmission. Subsequently, as these anthropogenic stressors increase, so may the prevalence of marine diseases (Harvell 1999, Lafferty et al. 2004, Baskin 2006).

Outbreaks of disease can have substantial negative impacts on populations, often by changing individuals' behavior. For example, a herpes virus that infects sea turtles affects their foraging behavior as the virus causes overgrowth of tissue around the eyes and mouth (Aguirre & Lutz 2004, Lafferty 2017). Disease outbreaks can also influence the interactions between the infected species and other species. When a predator's ability to forage is reduced due to infection, prey populations may increase as a result (Harvell 1999, Ward & Lafferty 2004, Tompkins et al. 2011). A parvovirus outbreak among wolves on Isle Royal led to a decline in their population. The wolves were not numerous or strong enough to cull their moose prey, which resulted in the proliferation of their population on the island (Peterson et al. 1998).

In addition to disease outbreaks due to direct infection from a pathogen, physical stressors can interact to create opportunities for pathogens to infect individuals in a population and trigger outbreaks of disease. Changes in physical stress such as temperature, moisture, and salinity can have deleterious effects on the health of

organisms and may occur as acute stress (e.g., a low tide during an unusually warm day) or chronic stress (e.g., warming ocean temperature) (Harley 2011, Freedman 2015, Poloczanska et al. 2016, Hewitt et al. 2016). Symptoms of stress response in organisms can be expressed in different ways, including changes in feeding rates, movement, and reduced fecundity (Menge 2002, Petes et al. 2008), but they can also manifest as lower immune response, which can lead to increased susceptibility to disease (Rapport et al. 1985, Steudel et al. 2012, Freedman 2015).

The rocky intertidal zone is a dynamic environment that is subject to a number of physical stressors, including gradients in wave action, large temperature fluctuations, and prolonged desiccation during low tides. Many of these abiotic stressors occur along a vertical stress gradient created by sequential emersion and immersion between tides and varying wave height (see Figure 4 in Bird et al. 2013). Indeed, intertidal communities are organized vertically (i.e., vertical zonation) in part by species-specific tolerance to daily fluctuations in abiotic stressors like temperature, desiccation, salinity, and wave forces, with those organisms located higher on the shore generally exhibiting higher stress tolerance (Tomanek 2002, Helmuth et al. 2006, Harley 2011).

During low tides, mobile intertidal organisms may seek refuge from increased thermal and desiccation stress by seeking sheltered habitats (e.g., under rocks). Such microhabitat selection may be an important indicator of potential temperature and desiccation stress (Garrity 1984, Burnaford & Vasquez 2008, Monaco et al. 2015). For example, an individual sea star located in a tide pool or hiding under surf grass may be

sheltered from direct sunlight and kept wet and cool, while an individual lying on a rock would be open to direct sunlight, and more exposed to desiccation and heat stress.

The ochre sea star, *Pisaster ochraceus,* is an abundant keystone predator in intertidal zones along the west coast of the United States (Paine 1974, 1976, Menge et al. 1994). As a voracious, generalist predator (Feder 1959), ochre sea stars play an important role in maintaining biodiversity in intertidal zone habitats (Paine 1966, 1980). Subsequently, they can have an effect on the health of the community, as increased biodiversity can increase the resilience of the ecosystem (Elmqvist et al. 2003).

Sea star wasting disease (SSWD) symptoms have been observed in sea star mortality events worldwide for decades. SSWD is characterized by areas of epidermal necrosis, which forms superficial lesions (Figure 1a). Other symptoms include loss of turgor (Figure 1b) and “posturing” or a pretzel-like twisting of the rays, which typically precedes ray autotomization (Figure 1c). These symptoms often lead to death (Figure 1d). The progression of the disease can be categorized using stages based on the number of lesions present and the area of the body covered by lesions (PISCO 2014).

Outbreaks have occurred in the Eastern Pacific from the Gulf of California to British Columbia, and in the South Pacific on the Great Barrier Reef (Zann et al. 1990, Pratchett 1999, Eckert et al. 2000, Bates et al. 2009, Gudenkauf & Hewson 2015). The current outbreak of SSWD symptoms was first recorded along the Pacific coast in June of 2013 in Washington (Hewson et al. 2014, Moritsch 2018, Miner et al. 2018). However, I noticed symptoms of the disease earlier in Trinidad, CA during April of 2013. Since the

outbreak, many populations of sea stars including *P. ochraceus* have experienced severe mortality (Hewson et al. 2014, Moritsch 2018, Miner et al. 2018).

Much is still unknown about the mode of transmission as well as the distribution of SSWD symptoms among sea star populations. Although the likely causative agent for SSWD in sunflower stars (*Pycnopodia helianthoides*) has been identified as a densovirus (Hewson et al. 2014), it is still unclear what causes SSWD in other asteroid species, including ochre sea stars (Hewson et al. 2018). Understanding the epidemiology of this disease requires elucidating factors that are influencing disease initiation and progression. Accordingly, documenting the distribution of symptomatic individuals is key to identifying environmental correlates and potential exacerbating factors.

The goal of this study was to test the hypothesis that an ochre star's location in the intertidal zone influences the incidence and progression of SSWD symptoms. I predicted that stars in the physiologically more stressful higher intertidal zones are more likely to be susceptible to the disease, exhibit more symptoms, and move less than individuals lower on the shore. To test this prediction, I documented the occurrence of SSWD symptoms and survivorship among individuals across their distribution in the mid-intertidal zone. I also quantified the progression of SSWD symptoms among tagged individuals in the field to assess changes in symptoms relative to their intertidal location and amount and direction of movement.

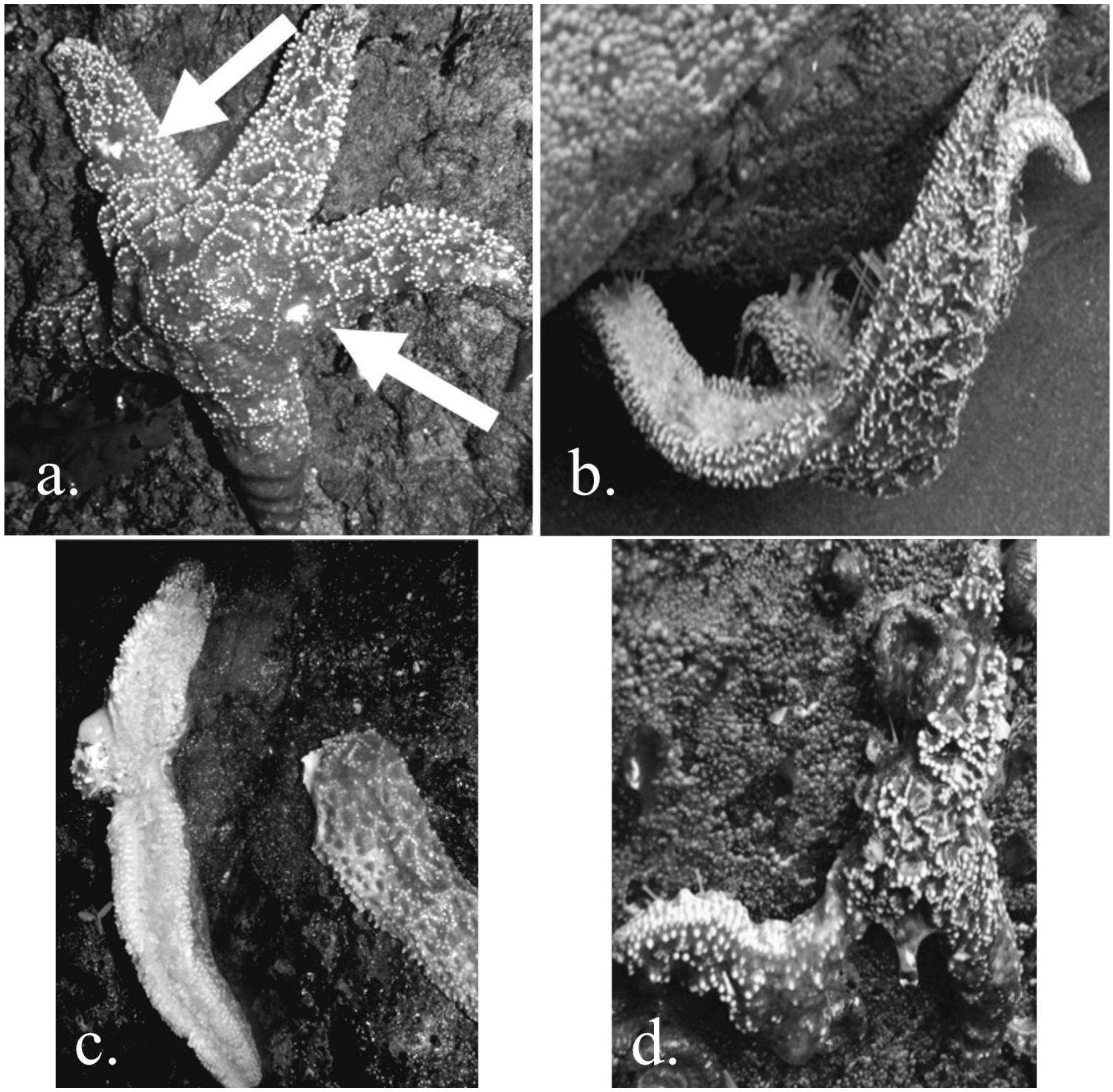

Figure 1. Obvious symptoms of SSWD beginning with lesion formation (a), loss of turgor (b), ray autotomization (c), which often leads to the death of the ochre sea star (d).

MATERIALS AND METHODS

Study Sites

I conducted observational surveys on ochre sea stars at False Klamath Cove in Del Norte County (41.59 N, -124.10 W), and tagging studies at Palmer's Point inside Patrick's Point State Park in Humboldt County (41.13 N, -124.16 W) (Figure 2). Study sites were dominated by boulders that provided abundant habitat for multiple species of sea stars.

I chose the study plot locations because they spanned a large portion of the mid- and low-intertidal zones where ochre stars were abundant, and they were large enough to ensure a sufficient amount of individuals for study. The plots consisted of barnacle-covered boulders and included natural boundaries of deep tide pools colonized by purple urchins (*Strongylocentrotus purpuratus*) and kelp that limited sea star movement outside the study area.

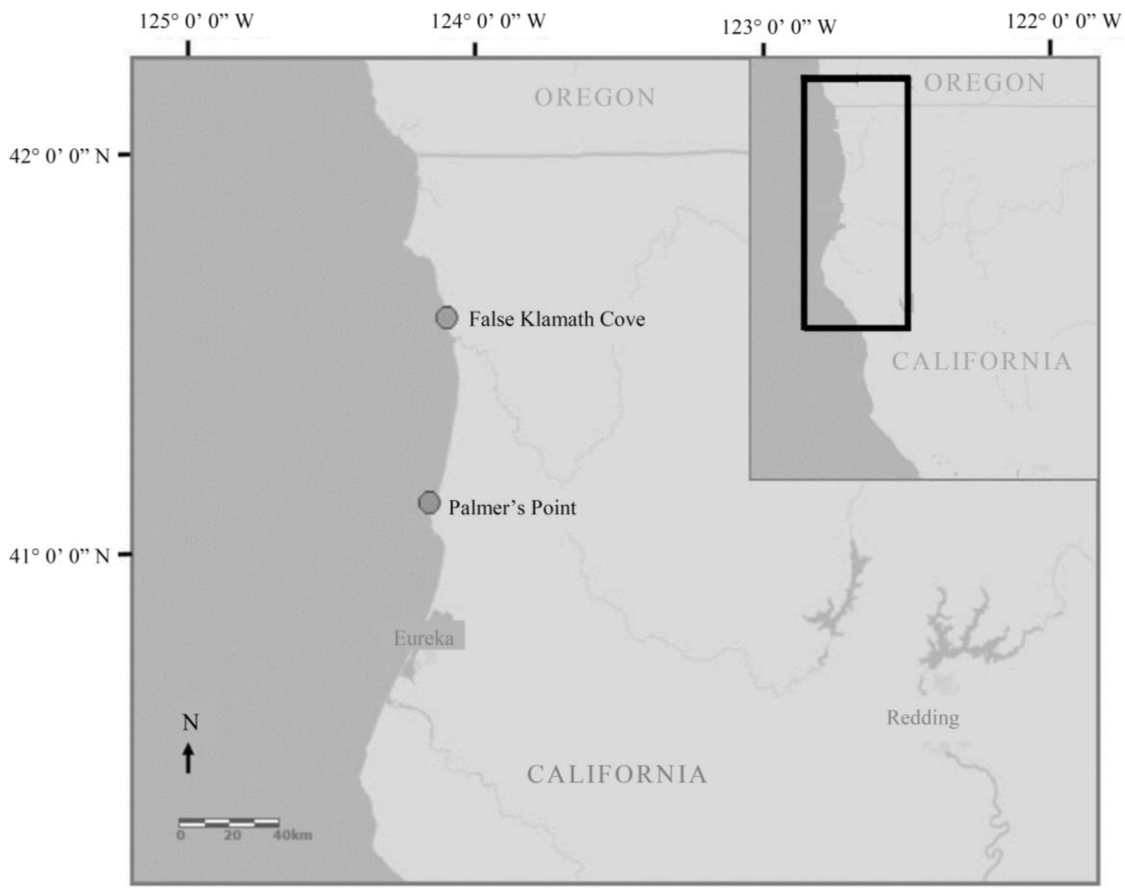

Figure 2. Location of study sites in Del Norte (False Klamath Cove) and Humboldt (Palmer's Point Beach) Counties, California, USA.

Observational Surveys

I conducted monthly observational surveys during low tide in the spring and summer over two years to identify patterns in the location and distribution of asymptomatic and symptomatic ochre sea stars (May-August 2015; May-September 2016). I created a permanent study grid using five bolts spaced 3 m apart and attached to a 30 m transect running perpendicular to shore (Figure 3a). I searched the area between transects for ochre stars and recorded each star's location along the transect perpendicular to the shoreline (y- also referred to as "swath location,") and parallel to the shoreline (x) to the nearest 0.05 m. I also measured each star's vertical elevation relative to Mean Lower Low Water (MLLW), to the nearest 0.05 m using a Northwest NCL 22x auto level builder's sight survey laser leveler. MLLW was determined at each site by measuring the vertical elevation of the water line at the NOAA published time of the day's low tide.

I measured each ochre sea star's radius to the nearest 1.0 mm by measuring from the central disk to the tip of the ray that was the most visible or accessible. Based on Morris et al. (1980) and Lamb & Hanby (2005), adults are distinguished from juveniles based on size (>30 mm in radius) and color. In this study, I defined juvenile ochre sea stars by size (generally <30 mm in radius) and color (juveniles exhibited grey mottling). I also categorized stars as symptomatic or asymptomatic. Symptomatic stars were identified by the presence of visible lesions in conjunction with the loss of turgor or automatized rays. Asymptomatic stars displayed no discernable signs of disease. I

recorded each ochre star's microhabitat location as sheltered or unsheltered from exposure to direct sunlight (Table 1).

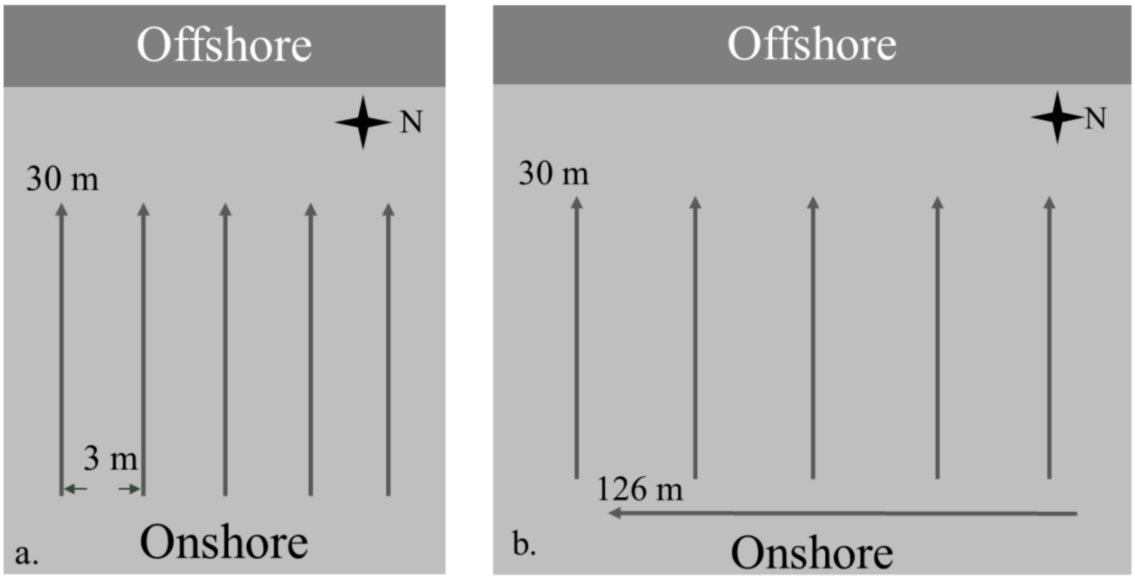

Figure 3. Depiction of study area transect grids established at False Klamath Cove (a) and Palmer's Point (b).

Table 1. Microhabitat codes and definitions used in the study.

Microhabitat Code	Definition	Classification
ORO	On Rock Open –on the top of a rock, exposed to air and sun	unsheltered
SRO	Side Rock Open – on the side of a rock, exposed to air and sun	unsheltered
ORS	On Rock Sheltered –on the top of a rock, sheltered by algae	sheltered
TP	Tidepool –in a tide pool, covered by water	sheltered
SRS	Side Rock Sheltered – on the side of a rock, sheltered by algae or neighboring rock (i.e., located in a crevice)	sheltered
UR	Under Rock – on the underside of a rock, not exposed to direct sun	sheltered
UA	Under Algae – not on a rock or in a tide pool, but sheltered under algae layer	sheltered

Transplant Experiment

The results of my preliminary tagging study indicated that a non-invasive method was the best approach for tagging individual ochre sea stars. All ochre sea stars subjected to invasive techniques during the preliminary study expired, while individuals tagged with the vital stain “neutral red” remained alive for the duration of the preliminary study. However, because the vital stain was only conspicuous on orange individuals, I did not use brown or purple ochre sea stars in the transplant experiment (see Appendix for a full description of the preliminary tagging study and results).

In May of 2016, I collected 55 orange-colored asymptomatic ochre sea stars between 40-50 mm in radius from a permanent plot at Palmer’s Point (Figure 3b) and recorded their location in my study grid (x, y, and elevation) and their microhabitat type. I assigned each star an individual number corresponding to their collection location, then marked them with masking tape identifying their number. I transported the stars in buckets filled with 3 L of seawater to the marine lab, approximately six miles away. I photographed each star, then stained its rays with an individually identifiable pattern, and placed them in a quarantined flow-through seawater tank at the marine lab until low tide on the following day. After undergoing the staining procedure, the stars were individually identifiable through a combination of unique stain pattern, size, and a visual comparison of aboral spine patterns on their central disks.

For the experiment, I returned 41 stained ochre sea stars to the exact location from where they were collected (control). The remaining 14 stars (treatment) were placed at a

different tidal height from where they were initially collected. Stars collected from the high-mid intertidal zone were placed in the low-mid intertidal zone, and those collected in the low-mid intertidal zone were placed in the high-mid intertidal zone. I did not change the microhabitat type, which remained the same for all individuals. I intended for an additional 13 stars to be used for the treatment block, however, due to a labeling error when collected, those individuals were not transplanted and instead served as further controls.

I searched for tagged individuals twice during each monthly low tide series for three consecutive months (June – August 2016), for a total of six surveys. I removed all individuals from the substrate for identification during each recapture event. Photographs of each recaptured ochre sea star were used to assist in identification.

Statistical Analysis

I examined associations between predictive factors (time, elevation, swath location, and size) and the binary response variable of disease symptom expression (symptomatic or asymptomatic) using a logistic regression model. I evaluated the data for multicollinearity by comparing variance inflation factors (VIF), and used a Pearson's correlation to measure the strength of the linear relationship between those predictors with high VIF scores. I removed swath location from the model as it was highly correlated with elevation.

Ochre sea stars were counted more than once throughout the transplant experiment, so I used a repeated measures two-way ANOVA to account for this among-subject variance over time. I investigated both the change in lateral movement and the change in vertical elevation (response variables) using two levels of disease symptom expression (asymptomatic, symptomatic), two levels of experimental treatment (transplant, control), and time (four recapture events) as predictor variables. I determined the change in lateral movement (distance) for each ochre sea star by calculating the Euclidean distance between the star's x and y coordinates in the swath grid at each time of recapture. I also used a logistic regression model to assess whether transplant location was associated with the presence of disease symptoms using disease as the binary response variable, and time and experimental treatment (control or transplant) as predictors.

I examined the data for normality and homogeneity in all analyses, and used log transformation in cases where assumptions were not met. I performed all statistical analyses using Minitab (v 18, State College, PA).

RESULTS

Distributional Patterns

I counted 1,788 ochre sea stars over seven sampling events spanning two years. Of the total counted, 46% were adults (n = 830) and 54% were juveniles (n = 958). Only 5% of juveniles were symptomatic (n = 48), while 31% of adults were symptomatic (n = 257) (Figure 4). The number of symptomatic stars increased sharply (46%) over the first sampling season between June-August 2015 and then dropped in the second season, decreasing by 16% between April-July 2016 (Figure 5).

Elevation and swath location were moderately negatively correlated (Pearson's $r = -0.33$), so I removed swath location as a predictor variable. The resulting logistic regression model showed a significant association between time and symptom expression ($P = 0.01$), and size and symptom expression ($P < 0.001$) (Figure 6). None of the interactions were significantly associated with symptom expression (Table 2).

Average ochre sea star size decreased by 5% (3.20 mm) per month throughout the study. When examining the data by symptom expression, symptomatic stars were larger than asymptomatic stars (Figure 6), and had a smaller decrease in size over time (2.26 mm per month), when compared to asymptomatic stars (4.13 mm per month). The average vertical elevation also decreased over time by 9% (2.60 cm) per month with asymptomatic stars again showing a slightly larger decrease (2.90 cm per month) than symptomatic stars (2.26 cm per month) (Figure 7).

Table 2. Logistic regression model examining the presence of disease symptoms as predicted by time, elevation, size, microhabitat, and their interactions.

Source	df	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	15	187.60	12.51	187.60	0.00
Time	1	6.80	6.80	6.80	0.01
Elevation	1	0.76	0.76	0.76	0.38
Size	1	102.13	102.13	102.13	0.000
Microhabitat	1	2.29	2.29	2.29	0.13
Time*Elevation	1	0.02	0.02	0.02	0.90
Time*Size	1	0.66	0.66	0.66	0.42
Elevation*Size	1	1.43	1.43	1.43	0.23
Time*Microhabitat	1	0.28	0.28	0.28	0.60
Elevation*Microhabitat	1	0.00	0.00	0.00	0.96
Size*Microhabitat	1	1.49	1.49	1.49	0.22
Time*Elevation*Size	1	0.00	0.00	0.00	1.00
Time*Elevation*Microhabitat	1	1.49	1.49	1.49	0.22
Time*Size*Microhabitat	1	0.23	0.23	0.23	0.63
Elevation*Size*Microhabitat	1	0.02	0.02	0.02	0.89
Time*Elevation*Size*Microhabitat	1	3.85	3.85	3.85	0.08
Error	1767	1431.38	0.81		
Total	1782	1618.98			

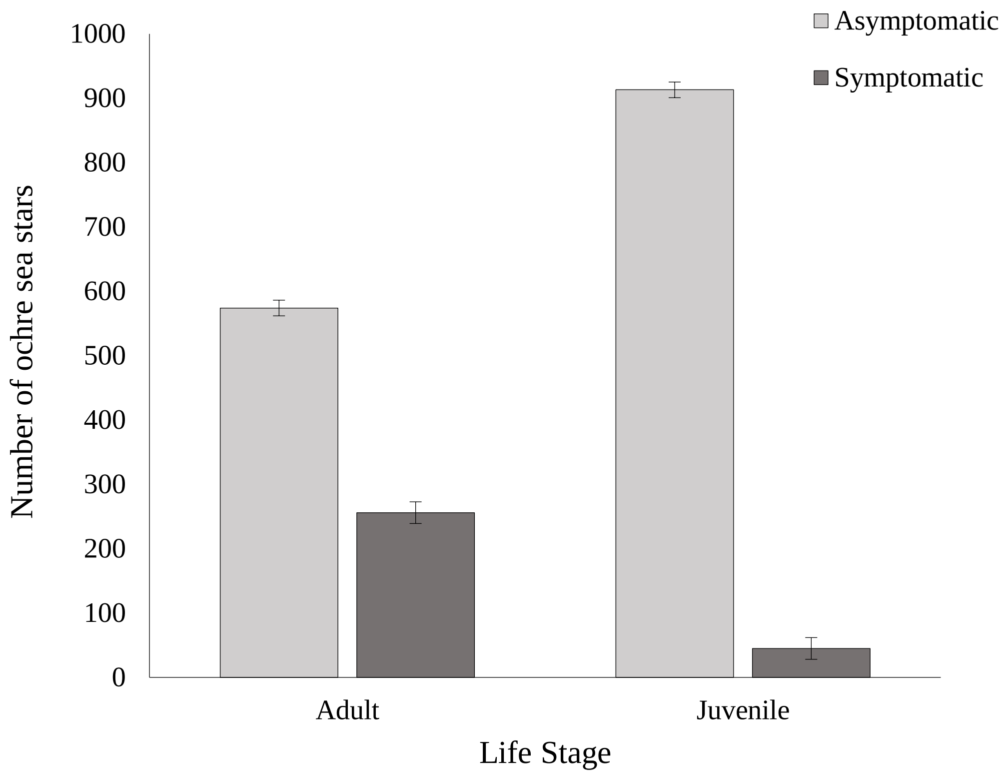

Figure 4. Total number of ochre sea stars counted in seven observational surveys showing a higher number of juveniles (n = 958) than adults (n = 830). Juveniles also showed a higher proportion of asymptomatic individuals compared to adults.

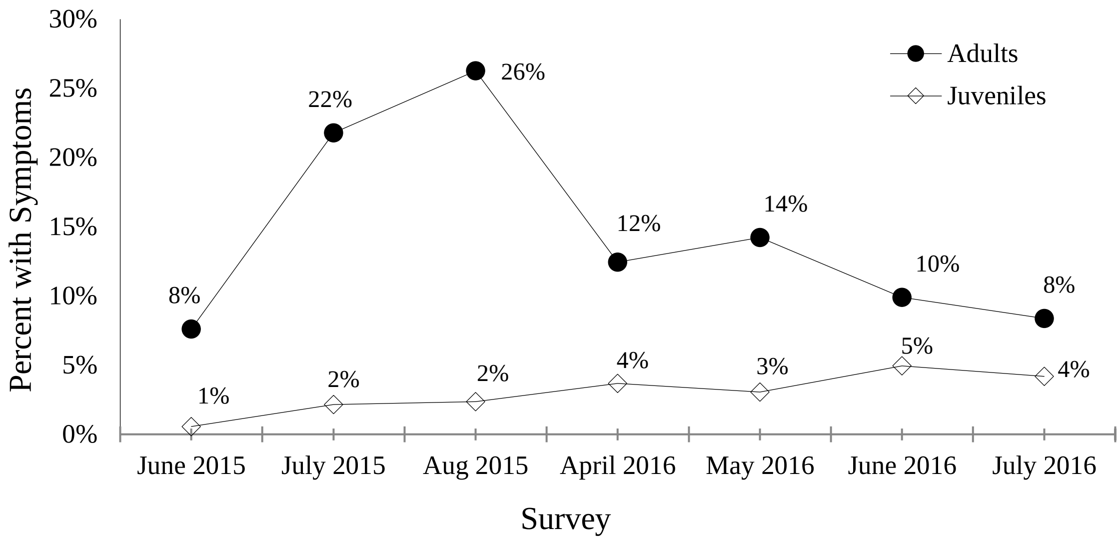

Figure 5. Percentage of symptomatic sea stars over seven surveys from 2015-2016, showing a sharp increase in symptomatic adults in the first sampling season (2015) and generally low symptomatic juveniles throughout all surveys.

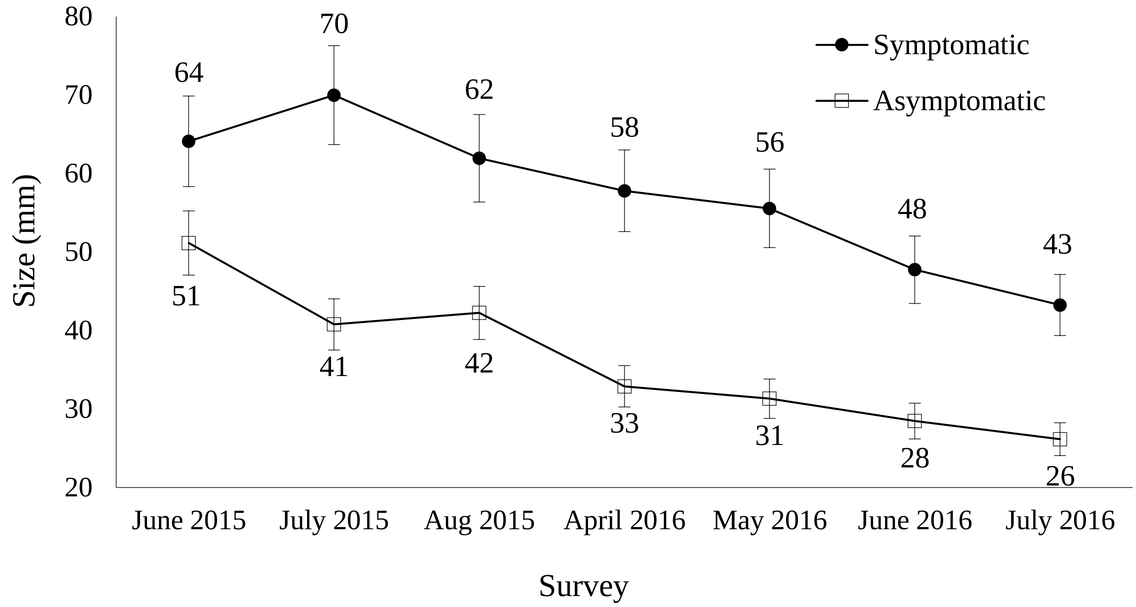

Figure 6. Interval plot of mean sea star size over time, grouped by disease symptom presence and showing that average size decreases over time, with symptomatic ochre sea stars exhibiting average larger sizes than asymptomatic ochre sea stars, and a smaller decrease in size over time (2.26 mm per month), when compared to asymptomatic ochre sea stars (4.13 mm per month).

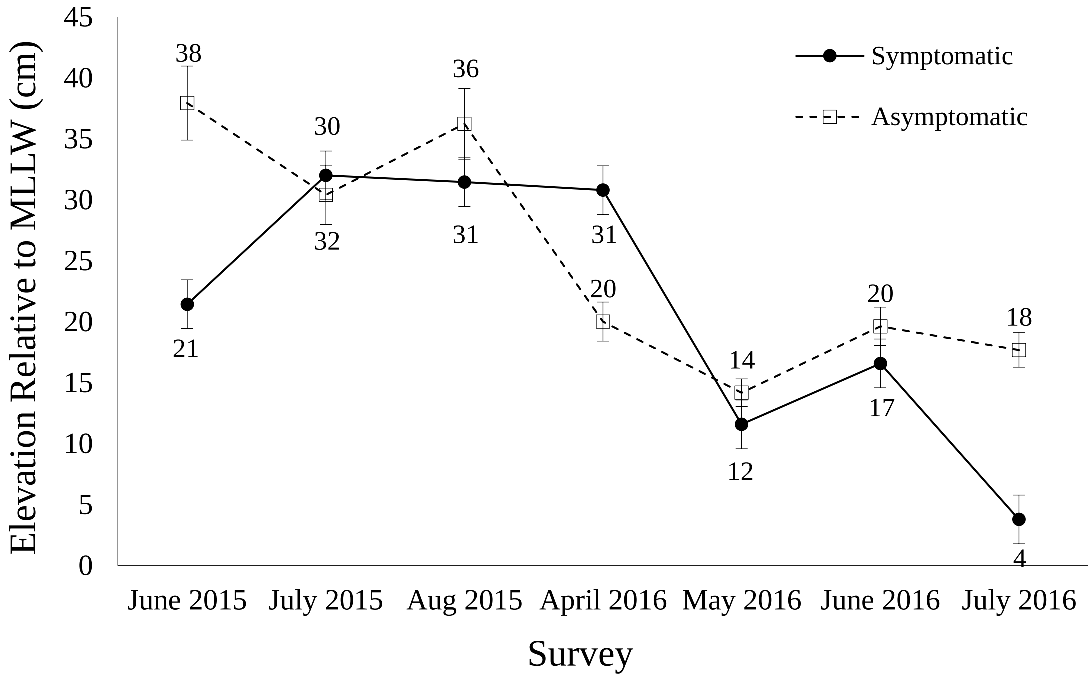

Figure 7. The average vertical elevation per month, relative to MLLW, during seven sampling events showing a decrease over time with asymptomatic stars showing a slightly larger decline in elevation (2.90 cm per month) when compared to symptomatic stars (2.26 cm per month).

Effects of Transplantation on Symptom Progression

With regard to recapture rate, 75% of the total number of tagged ochre sea stars (control and treatment, n = 55), were recaptured once over the three-month experiment and 29% were recaptured twice. The number of recaptured stars decreased at each recapture event from 47% at the first recapture, 33% at the second recapture, 16% at the third recapture and 13% at the fourth and final recapture (Figure 8).

When evaluating disease symptom progression, all ochre sea stars were asymptomatic at the time of tagging; however, 42% were symptomatic at the time of first recapture (n = 11) (Figure 9). Comparison between control and transplanted stars over time showed 56% of controls (n = 32) and 78% of transplanted stars (n = 9) became symptomatic (Figure 10). Three of the stars that began the study as asymptomatic were observed to exhibit symptoms on their first recapture and then returned to an asymptomatic state on their second recapture. On average, stars showed symptoms after one month, although 35% remained asymptomatic for the duration of the three-month study (n = 19).

When comparing treatment effects on disease symptom expression, a logistic regression model showed there was no significant association between the control/treatment ($P = 0.29$) or time ($P = 0.22$) and disease symptom expression (Table 3) (Figure 11).

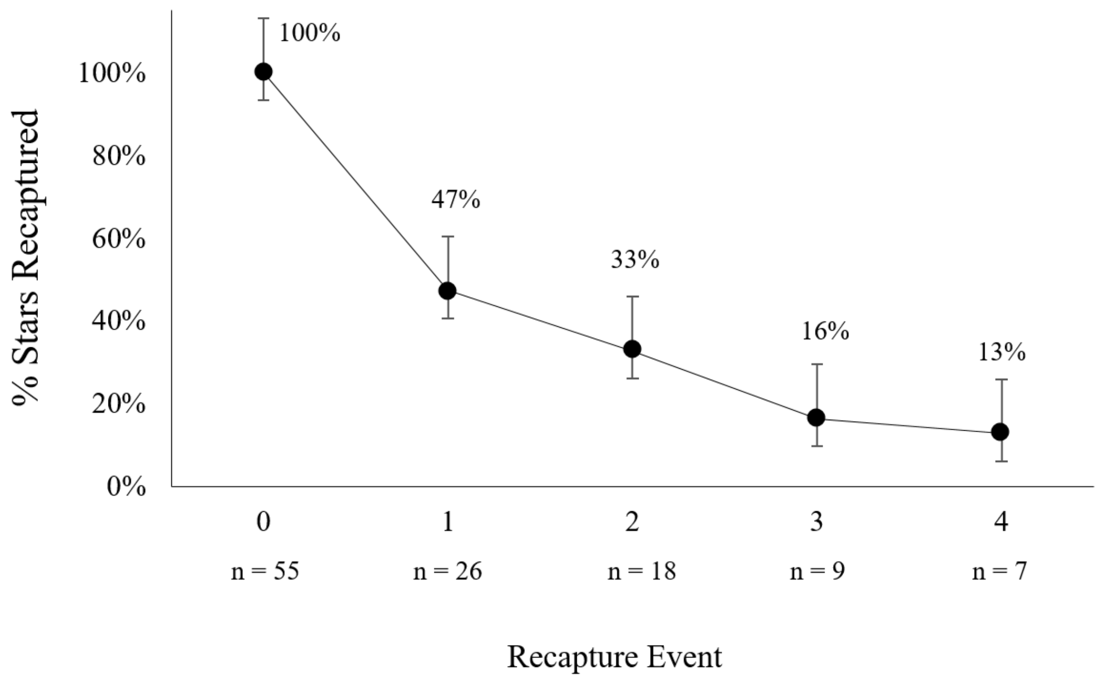

Figure 8. Percentage of all tagged ochre sea stars recaptured at each of four recapture events, showing a decrease over each recapture event.

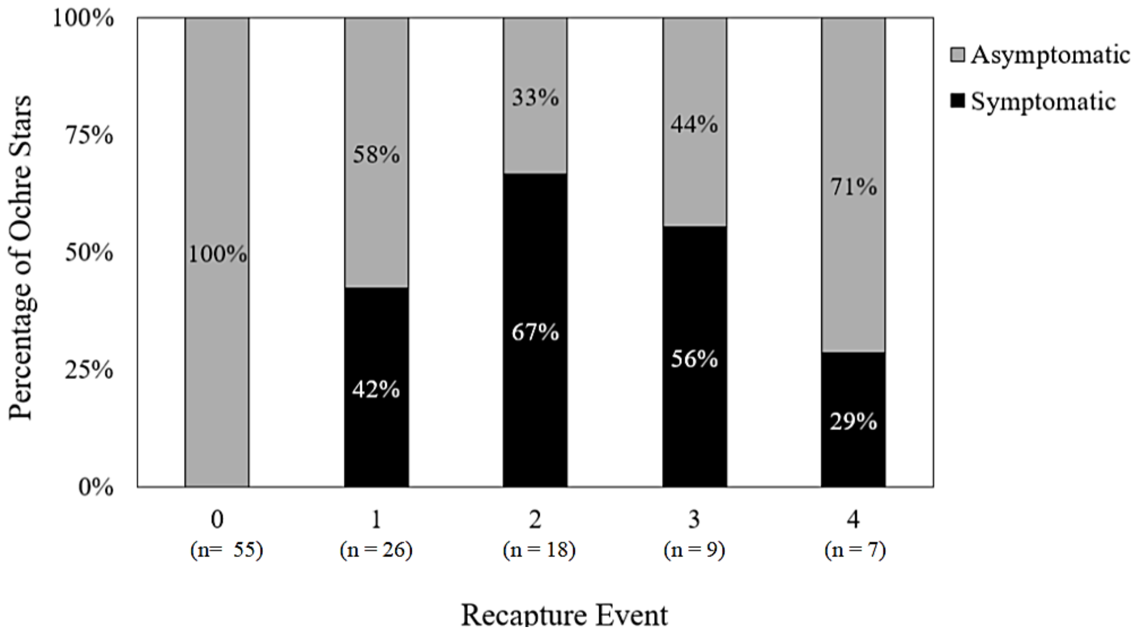

Figure 9. Transplant recapture results showing the proportion of asymptomatic and symptomatic ochre sea stars at recapture events in May-July, 2016.

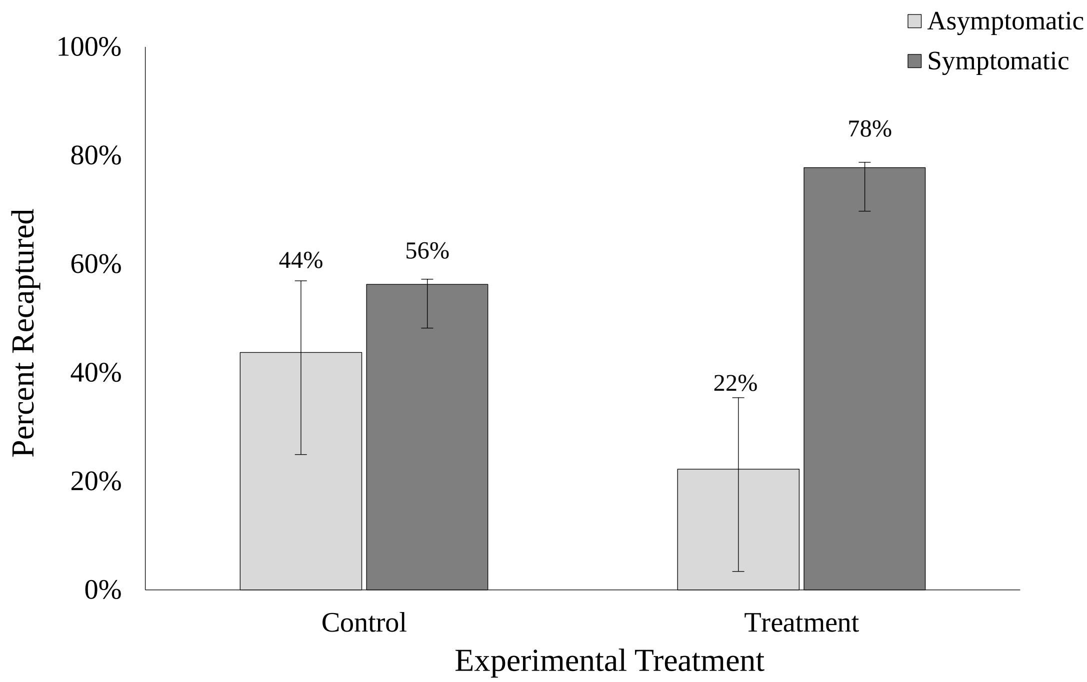

Figure 10. Comparison of disease symptom expression between control (n = 32) and treatment groups (n = 9) for recaptured ochre sea stars.

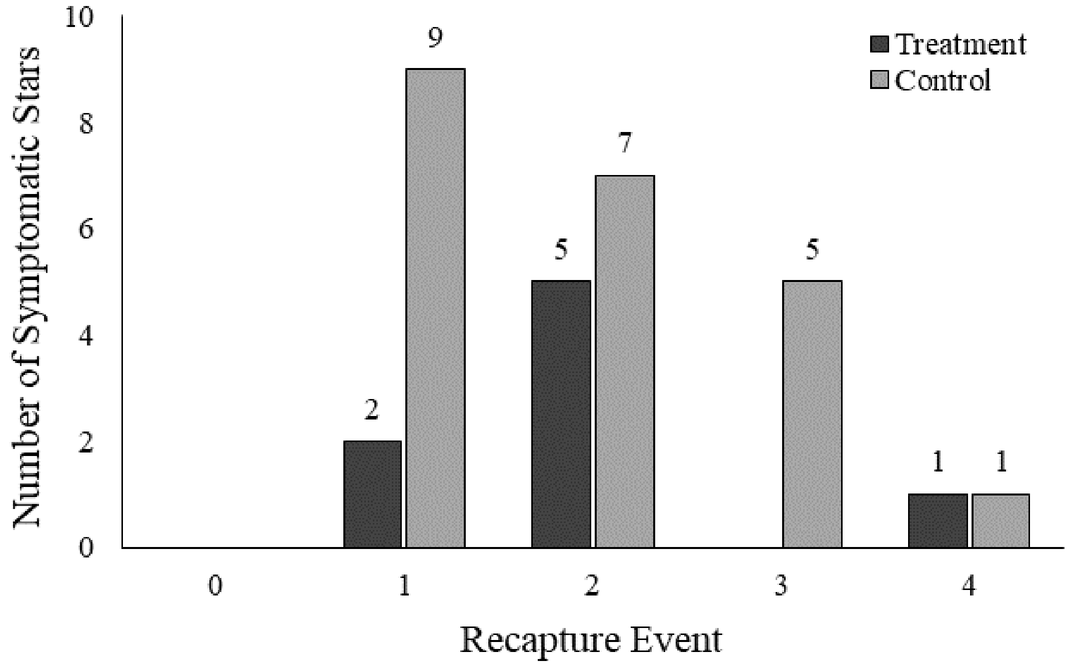

Figure 11. The number of symptomatic ochre sea stars in transplanted control and treatment groups over time (May-July 3 2016). There were no significant differences between treatments or time.

Table 3. Logistic regression on the effects of transplantation on disease symptom expression for recapture events (time).

Source	df	Adj Dev	Adj Mean	Chi-Square	*P*-Value
Regression	4	6.115	1.529	6.11	0.191
Control/Treatment	1	1.135	1.135	1.14	0.287
Time	3	4.435	1.478	4.43	0.218
Error	57	79.836	1.401		
Total	61	85.950			

Effects of Transplantation on Movement

Overall, recaptured ochre sea stars moved a minimum average of 45.6 m per month and increased their vertical elevation by 0.8 m (Figure 12). There was no significant effect of disease symptom expression on distance traveled ($F_{[1, 55]} = 0.01$, $P = 0.93$) or change in elevation ($F_{[1, 55]} = 0.09$, $P = 0.76$). Whether or not a star was transplanted also had no significant effect on the average distance traveled ($F_{[1, 55]} = 1.35$, $P = 0.25$), or on the change in elevation ($F_{[1, 55]} = 1.25$, $P = 0.27$), however, time was significant for both the distance traveled ($F_{[3, 55]} = 2.68$, $P = 0.05$), and the change in elevation ($F_{[3, 55]} = 13.56$, $P = 0.00$) of recaptured stars (Tables 4 and 5).

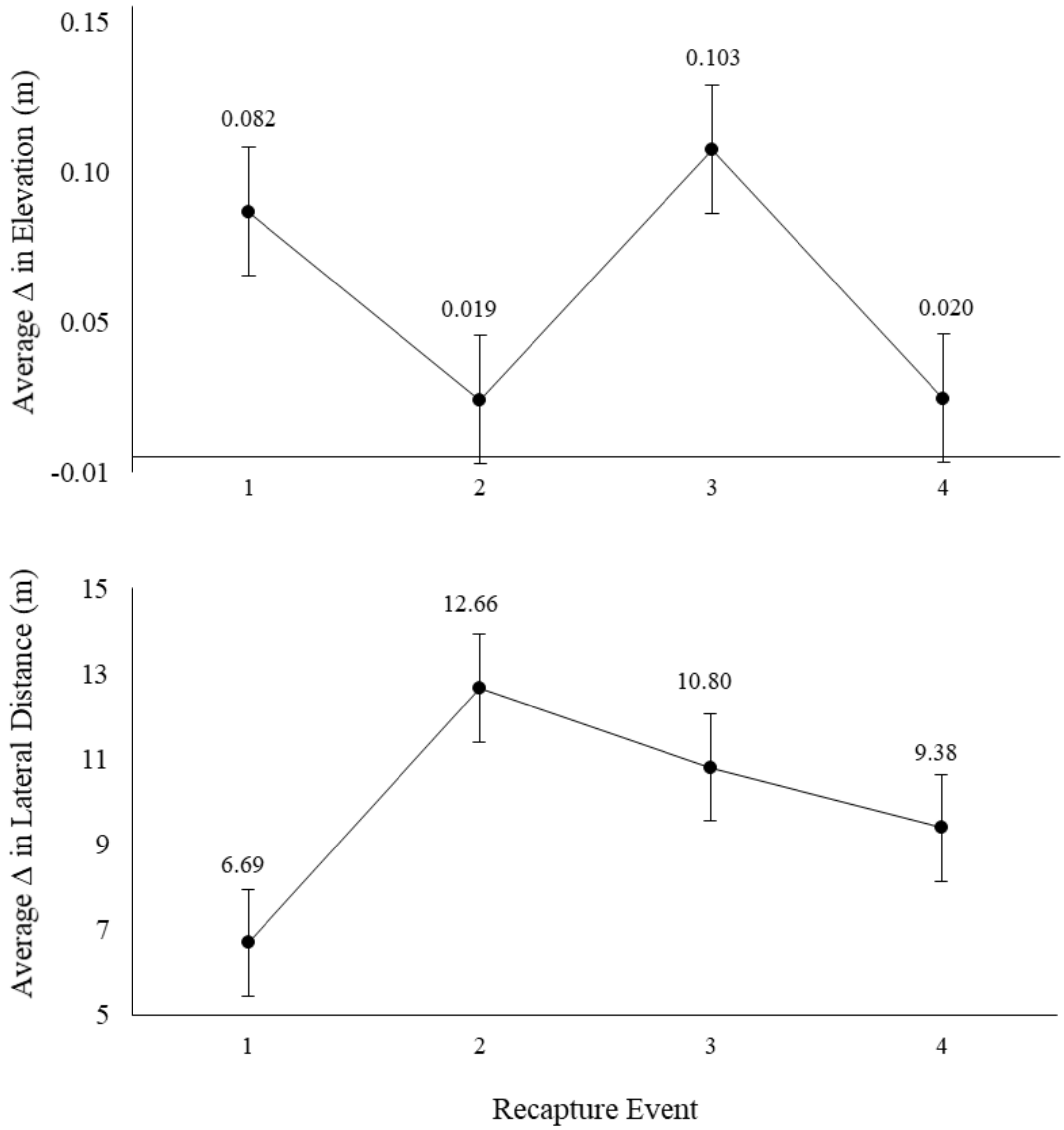

Figure 12. Average change in elevation and mean minimum distance traveled for all recaptured ochre sea stars at each recapture event.

Table 4. Two-way repeated measures ANOVA comparing the effects of disease symptoms and treatment on average distance traveled over time.

Source	df	Adj SS	Adj MS	*F*-Value	*P*-value
Time	3	352.41	117.47	2.68	0.05
Control/Treatment	1	59.01	59.01	1.35	0.25
Disease	1	0.34	0.34	0.01	0.93
Control/Treatment*Disease	1	2.69	2.69	0.06	0.81
Error	55	2406.39	43.75		
Total	61	2812.59			

Table 5. Two-way repeated measures ANOVA comparing the effects of disease symptoms and treatment on average change in elevation over time.

Source	df	Adj SS	Adj MS	*F*-Value	*P*-value
Time	3	42.61	14.20	13.56	0.00
Control/Treatment	1	0.82	0.82	0.78	0.38
Disease	1	0.09	0.09	0.09	0.76
Control/Treatment*Disease	1	1.31	1.31	1.25	0.27
Error	55	57.63	1.05		
Total	61	103.50			

DISCUSSION

My initial prediction was that sea stars in higher tidal locations would be more likely to exhibit symptoms of SSWD, yet my regression results showed that intertidal location did not influence the expression of disease symptoms as neither elevation nor microhabitat were significant predictors. However, time and life stage were important predictors of disease susceptibility, which indicates that while abiotic stress may not contribute to symptom expression, SSWD may have temporal patterns or variations of symptom expression that change over time, as opposed to responses to environmental stressors. My transplant results showed that symptom expression is dynamic and that sea stars can alternate between symptomatic and asymptomatic states, which adds to the notion that abiotic stressors alone may not be eliciting symptom response.

Transplanted ochre stars did not show significantly decreased movement or shifts in elevation after transplant as I initially anticipated. Although not significant, those stars that were transplanted to higher tidal locations did move more overall than those transplanted to lower tidal locations. Stars transplanted to higher tidal locations had more access to mussel and barnacle beds which may explain this increase in movement. Stars that were transplanted to lower tidal locations were subjected to more wave action which may explain why they had less overall movement when compared to those stars that were transplanted to higher tidal locations.

In summary, I expected that ochre sea stars in higher tidal locations would experience more physical stress and lead to an increase in expression of the symptoms of

SSWD. However, my results do not support this conclusion, suggesting that common intertidal stressors such as temperature and desiccation may not be contributing to the progression of disease symptoms and that SSWD may be stochastic in this population and/or environment.

Distributional Patterns

Survey time was a significant predictor, which may suggest that the expression of disease symptoms can change over time as ochre sea stars experience fluctuations in temperature and desiccation. During the spring and summer months, the lowest tides in northern California decrease in height and shift from evening to morning, leaving the intertidal zone exposed for more extended periods during daylight, which may affect the expression of symptoms. Additionally, based on the results of my transplant experiment, which showed disease symptoms could resolve and resurface in the same individual, I expected to see these fluctuations in disease symptom expression over time, especially in a population that is experiencing an active outbreak.

Sea star size was a significant predictor of disease symptom expression, and the majority of asymptomatic ochre sea stars were smaller than symptomatic ochre sea stars. In addition, the mean size of asymptomatic stars decreased over time, which suggests that larger individuals were being removed from the population. However, juvenile recruitment in 2015 was exceptionally high at False Klamath Cove when compared to other Northern California sites monitored by Miner et al. (2018, Figure 4). This large recruitment pulse may have influenced the number of overall juveniles surveyed

throughout my 2015-2016 observational surveys at False Klamath Cove. Future studies should include tagged juveniles to provide a better understanding of juvenile survivorship.

Ochre sea stars generally exhibit risk-avoidance behavior to avoid thermal stress and desiccation by utilizing sheltered habitat (Monaco et al. 2015); however, I found no significant associations between microhabitat and the expression of disease symptoms. Future studies should record aspect details such as whether or not an ochre sea star is on the north, south, east, or west-facing portion of a rock when collecting microhabitat location. This can affect the amount of direct sunlight the ochre sea star receives during different seasons in different locations and will assist in more accurately assessing the potential of this predictor for disease symptom expression.

Effects of Transplantation on Symptom Progression

The progression of SSWD symptoms in ochre sea stars has only been examined in the field by one other study (Bates et al. 2009) which found that increased temperatures affected the presence of disease symptoms but did not report recovery or remission from symptoms. During my transplant experiment, I observed stars that developed lesions that healed in subsequent surveys– but even after recovery, some developed symptoms again, although whether or not a star was transplanted did not affect the incidence of disease symptom expression. This observation suggests that the lesions used to identify infected ochre sea stars are part of a dynamic process and may heal and reform over time.

Therefore, external SSWD symptoms, like lesion formation, may not represent the extent of infection in a population. A more in-depth method, such as tube feet sampling, should be utilized to more accurately estimate disease presence in a population.

Echinoderms have been shown to have both a cellular and a humoral immune response (Leclerc 2015). This enables their immune system to recognize and respond to foreign bodies, and also provides the elements required for adaptive immunity and immunological memory, both of which can help respond to specific antibodies once previous exposure has occurred (Leclerc 1996). These immunological capabilities may explain the ochre sea star's ability to recover from symptoms; however, more research is needed to understand why the adaptive immune response may not be producing antibody protection from SSWD, as evidenced by the reemergence of SSWD symptoms in the same individual.

The physical stress caused by the tagging procedure may have contributed to the high number of lesions seen in recaptured ochre sea stars over the duration of the study. The staining procedure required that stars were transported to the lab after being removed from the substrate, which resulted in the tearing of tube feet. This procedure was likely stressful and may have contributed to an increase in the incidence of symptomatic stars during subsequent recaptures. Stars selected for tagging appeared healthy but may have been diseased. There was no way to know if tagging induced symptoms or the physical stress of removal from the substrate in addition to tagging caused an increased in the expression of symptoms and subsequent mortality. I found that all tagging methods were

disruptive to the animal, and future attempts should devise a way to stain the stars in the field, which does not require removal.

Effects of Transplantation on Movement

In general, the change in distance traveled and in elevation was significantly different over time for recaptured ochre sea stars. The low tide events during the spring and summer in Northern California are during the morning hours, which expose the rocky intertidal zone to more sunlight when compared to low tides during the winter months, which are during the evening hours. The prolonged exposure to thermal and desiccation stress during the spring and summer sampling events of my study may explain the changes in movement for ochre sea stars actively foraging and seeking shelter during these exposed periods.

I found that the average minimum distance traveled of ochre sea stars transplanted to higher or lower locations in the intertidal zone was not significantly different compared to the control stars that were returned to their same location. While tidal location had no significant effect on disease symptom expression, the effects of removal from the substrate at recapture may have contributed to disease symptom expression and increased mortality resulting in decreased degrees of freedom. I did find that ochre sea stars have a higher movement rate than previously observed by Paine (1976).

Paine (1976) found that tagged ochre sea stars moved 3.1 and 3.3 m per month in two different populations. The ochre sea stars in my study moved 40.2 and 51.0 m per

month on average between my control and transplanted stars, respectively. These substantially higher rates could be due to habitat differences, tagging procedures, or the presence of SSWD. The mid-intertidal boulder-field habitat at my study site was a relatively protected north-facing cove, while Paine's Washington site is a more wave-exposed intertidal bench. Wave forces can inhibit movement in order to remain attached to the substrate (Denny 1985, Hayne & Palmer 2013). Furthermore, Paine (1976) tagged his stars in the field by threading a fishing line through the distal portion of the star's ray and was able to observe them for 4-8 months without removal from the substrate after the initial transplant. His transplanted stars did not migrate outside of the study plot and were recaptured multiple times. The ochre sea stars in my study were removed from the substrate multiple times in order to tag and identify individuals. I believe the stress of my tagging procedure, coupled with the stress of removal from the substrate at each recapture, may have provoked a high movement rate in the ochre sea stars in this study. Finally, Palmer's Point is dominated by algae, which can reduce the amount of available substrate for large mussel or barnacle patches, which serve as food for *Pisaster*. In habitats like these where food for ochre sea stars is sparse, they may have had to travel farther distances in search of prey items.

The higher movement rates observed in my study may have also influenced the lower recapture rates of individuals in the transplant study. Thus, reduced survivorship cannot be directly attributed to death, but also to movement outside of the study area. I also observed two tagged ochre sea stars outside of the plot attached to rocks in deeper,

inaccessible water. Therefore, subtidal monitoring of individuals would be useful to understand movement patterns and improve estimates of mortality.

SUMMARY AND CONCLUSIONS

My study did not find a relationship between the presence of disease symptoms and intertidal elevation or microhabitat. Moreover, transplantation of individuals to higher or lower intertidal locations did not affect the occurrence of symptoms. The lack of significant effects of these factors may indicate that variations in thermal, desiccation, and wave stress along intertidal gradients do not influence the expression of SSWD symptoms. However, the high percentage of symptoms observed in recaptured ochre sea stars suggests that disease progression may have occurred from stress related to the tagging and recapture procedures. Moreover, not every star remained symptomatic or asymptomatic, and some individuals showed healing of lesions over time. Finally, ochre sea stars in my study traveled considerably farther than previously documented (Paine 1976).

Although we have yet to discern the mode of transmission, these new insights on the rate of movement of ochre sea stars may provide further understanding into the spread of the disease through the population, especially since I have demonstrated that not all symptomatic stars experience mortality, and asymptomatic stars may not be free of disease. While we may not be able to prevent future epidemics, a better understanding of the underlying dynamics may help inform what causes the outbreaks and how we can monitor the overall health of marine ecosystems.

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APPENDIX

Methods: Preliminary Tagging Study

During January 2015, I conducted a study to test the effectiveness of three invasive, and one non-invasive, method of tagging ochre sea stars. I collected 20 asymptomatic ochre sea stars from Trinidad State Beach in Humboldt County, CA (41.06 N, -124.15 W) and kept them in flow-through seawater tanks at Humboldt State University's Telonicher Marine Lab in Trinidad, CA.

For the first method, I used preloaded syringes similar to those utilized for domestic pet identification and injected a Radio-Frequency Identification (RFID) tag (Figure 13a) into the distal portion of the first ray of five ochre sea stars. In the second method, I attached RFID "dot" tags (Figure 13b inset) to an additional five ochre sea stars using stainless steel wire and fishing line threaded through the aboral side of the ochre sea stars to attach small housings containing the tags (Figure 13b). Previous studies (Paine 1976, Lamare et al. 2009, Chim & Tan 2013) used similar procedures using fishing line and colored beads. For the final invasive tagging technique, I inserted a dermal anchor via incision into five ochre sea stars just below the epidermal layer to which a dot RFID tag was epoxied (Figure 13c). I recorded changes in health such as loss of turgor, ray splitting, lesion formation, and abscessing at the incision or attachment site, every 24 hours following the tagging procedure.

I tested one non-invasive tagging method on five additional ochre sea stars using the vital stain "neutral red" applied to the ochre sea stars' rays in order to create individual patterns for each (Figure 14a). I prepared the staining solution using 4 g of powdered dye dissolved in 10mL of distilled water, then added to seawater to create a 1L bath (Figure 14b), which was kept aerated throughout the staining process as described in Feder (1955). I applied this staining technique to three orange and two brown ochre sea stars.

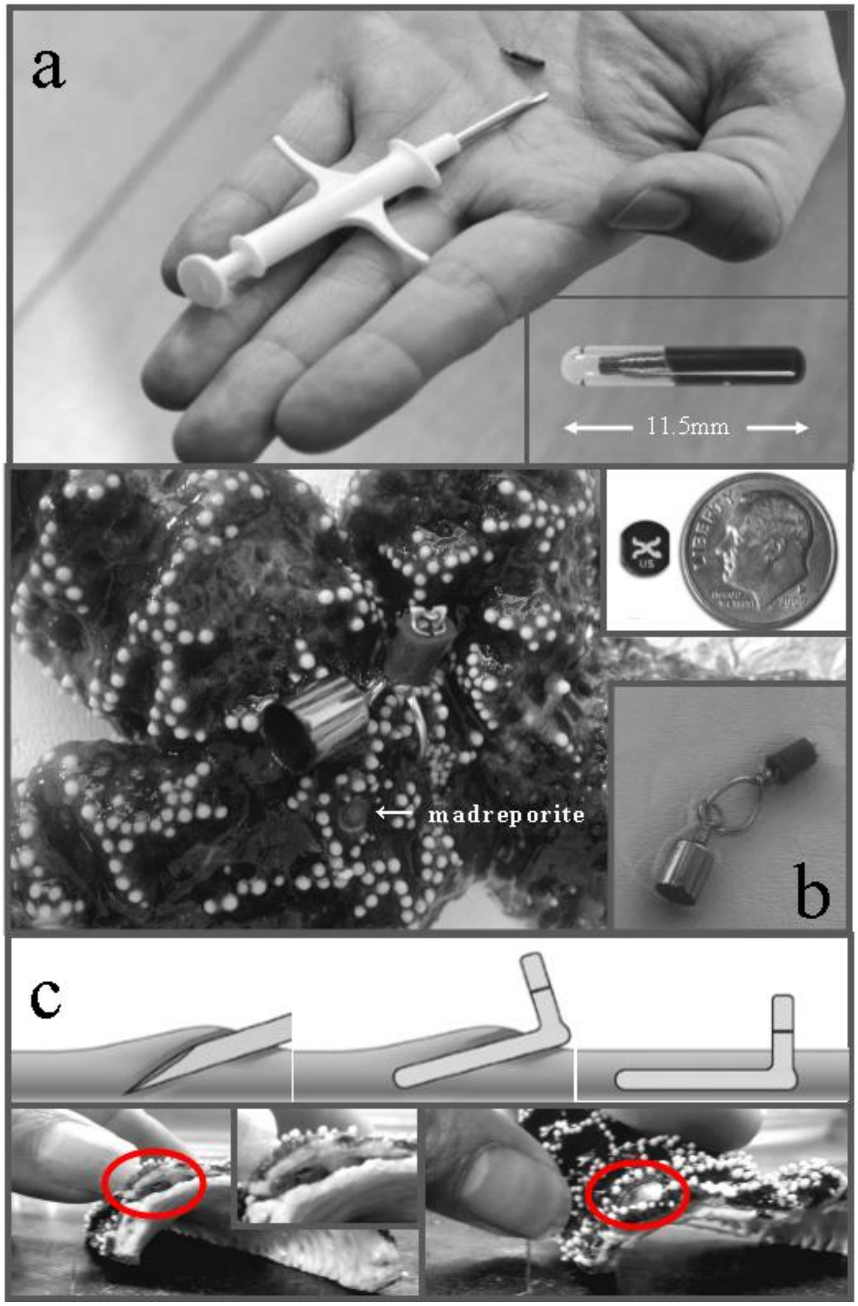

Figure 13. Invasive tagging techniques: (a) Syringes preloaded with the RFID tags (inset). Tags were injected into the distal portion of the first ray; (b) Stainless steel wire through the central disk for RFID housing attachment; (c) Dermal anchor inserted via incision.

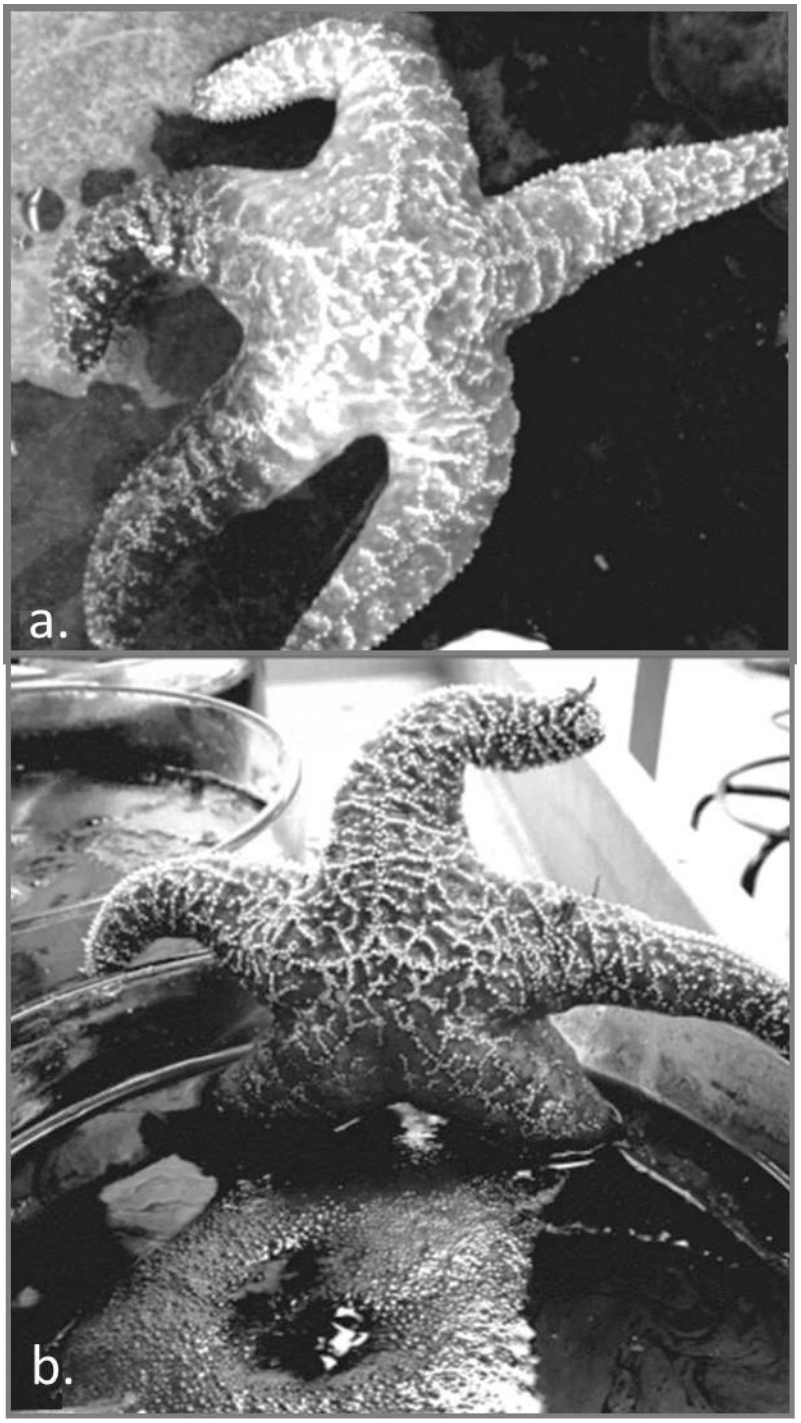

Figure 14. An ochre sea star post-stain with two rays marked (a), and an aerated bath of neutral red showing an ochre sea star positioned for staining of two rays (b).

Results: Preliminary Tagging Study

Both the RFID injection and the external attachment methods were not effective in monitoring ochre sea star survivorship. The five stars that I injected with RFID tags either ejected the tags from their bodies or died. The two stars that retained their RFID tags showed epidermal decay at the injection site that proliferated into the central disk, leading to death within 72 hours. The ten ochre sea stars tagged with external housing units or dermal anchors had a very high mortality rate. In addition, the procedure was time-consuming. On average, it took 17 minutes to successfully attach the wire to one ochre sea star and 25 minutes to create an incision and place a dermal anchor. Seven of the stars showed signs of ulceration and epidermal decay at the attachment or incision site within 24-48 hours. An additional two stars autotomized rays within 24 hours. Ultimately, these physical responses resulted in the loss of rays, perforated body wall, and the expiration of nine of the ten stars within five days (Figure 14).

After ten days, the stain applied to the brown ochre sea stars was only visible in the ambulacral grooves on the oral side of the star, versus the stain on the three orange stars, which remained readily visible through 24 days of observation in the lab. In general, the stain remained conspicuous on orange stars for the duration of the field study. One of the pilot study stars was located in the field approximately one year later with their stained pattern still clearly discernible.

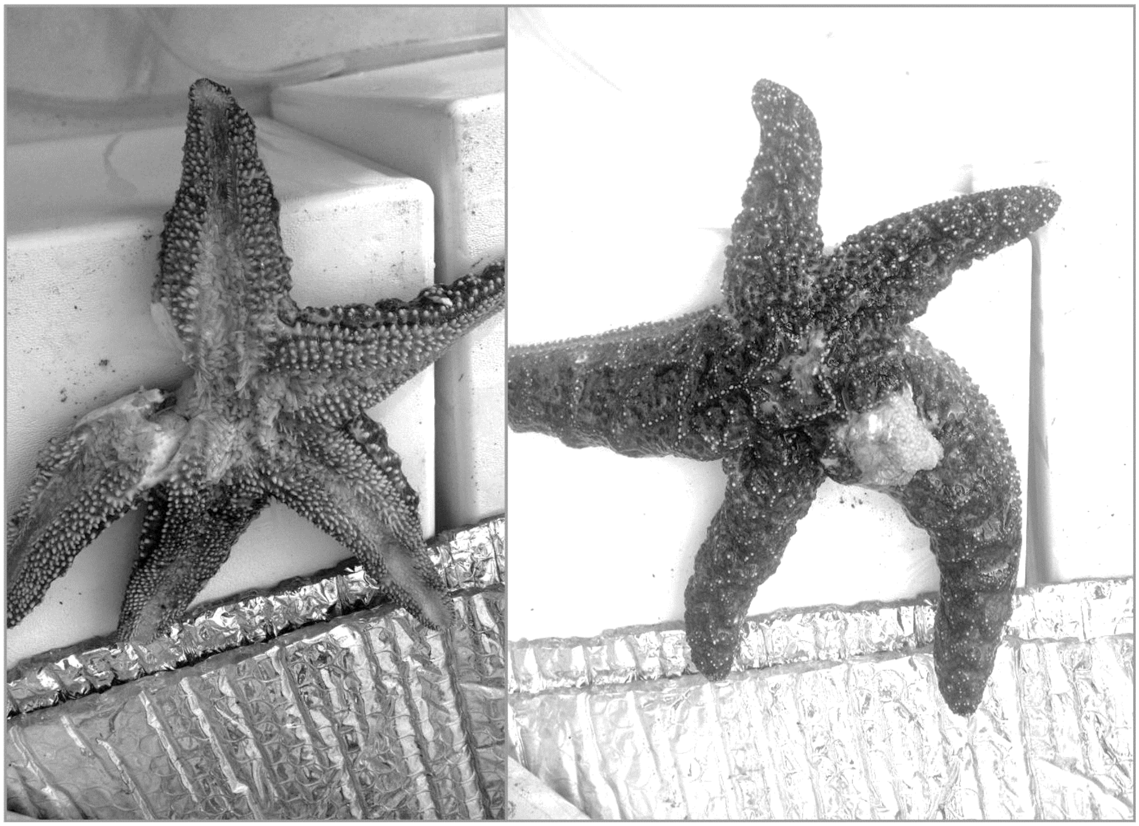

Figure 15. Aboral side of a star in the process of autotomization 48 hours after stainless steel wire attachment to central disk (a). Perforated body wall and gonad exposure 24 hours after RFID tag injection into ray (b).

Discussion: Preliminary Tagging Study

Perhaps because ochre sea stars are quite efficient at recognizing and eliminating foreign bodies (Leclerc 1996, Matranga 1996, Smith et al. 2010, Olsen et al. 2015), all invasive tagging techniques I investigated failed. It is also possible that the active SSWD outbreak in this ochre sea star study population, coupled with the stress of invasive tagging techniques was enough to cause the death of an animal that has historically been described as ‘indestructible’ due to their regenerative capabilities (Lawrence 1992, Chesler 2017, Wells 2017). In my tagging study, stars either expelled their tags or ulcerated rapidly at the sight of injection/incision, which ultimately led to death, not regeneration.