ECOLOGICAL CONSEQUENCES OF SEA STAR WASTING DISEASE: NON-CONSUMPTIVE EFFECTS AND TRAIT-MEDIATED INDIRECT INTERACTIONS FROM *PISASTER OCHRACEUS.*

By

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ABSTRACT

ECOLOGICAL CONSEQUENCES OF SEA STAR WASTING DISEASE: NON-CONSUMPTIVE EFFECTS AND TRAIT-MEDIATED INDIRECT INTERACTIONS FROM *PISASTER OCHRACEUS.*

Timothy I. McClure

Consumptive effects (CEs) of predators are an important factor in structuring biological communities, but further work is needed to understand how the interaction between spatial and temporal differences in predator density affects non-consumptive effects (NCEs) on prey. NCEs can cause indirect effects on food resources, known as trait-mediated indirect interactions (TMIIs), and thus can also affect community structure. However, few studies have considered the relationships between spatial and temporal predator density variation and the strength of NCEs and TMIIs in the natural environment. The ochre star *Pisaster ochraceus* is common predator of the herbivorous black turban snail *Tegula funebralis*, imposing both CEs, but also NCEs and TMIIs by inducing *Tegula* avoidance behavior and suppressing *Tegula* grazing. *Pisaster* density differences along the eastern North Pacific have been exacerbated by the onset of Sea Star Wasting Disease (SSWD), resulting in a gradient of *Pisaster* abundance along California's North Coast. I hypothesized that *Tegula* growth and grazing would be increased at sites with decreased *Pisaster* density via release from NCEs, and that temporal *Pisaster* density variation would elicit stronger anti-predator responses from *Tegula* at sites with lower background levels of *Pisaster* density. To test these

hypotheses, I used a cage-exclusion experiment at sites comprising a gradient of *Pisaster* density, introduced temporal *Pisaster* density variation in experimental plots, and measured *Tegula* growth and grazing in cages at unmanipulated and experimental plots. I also used a laboratory experiment to confirm the association between *Tegula* growth and grazing across field-relevant concentrations of *Pisaster* cue. My results indicate that decreased *Tegula* soft tissue growth and grazing were associated with increased *Pisaster* density, and that *Tegula* anti-predator responses to temporal variation in *Pisaster* density were strongest at sites with low background *Pisaster* density. These results suggest that NCEs and subsequent TMIIs were predator density-dependent, highlighting the interactive effect of spatial and temporal variation in predator density on NCE and TMII strength. My lab experiment suggested that the NCEs and TMIIs observed in my field experiment were induced by *Pisaster* cues, and also highlighted the importance of designing realistic laboratory experiments. Finally, my thesis indicates that variation in *Pisaster* density associated with Sea Star Wasting Disease (SSWD) could affect NCEs and resulting TMIIs in a site and context-specific manner, contributing a novel finding to the growing body of literature considering the ecological impacts of SSWD in intertidal communities.

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INTRODUCTION

Since the beginnings of modern ecological research, a considerable body of work has investigated the importance of predation in regulating characteristics of populations and communities (Paine 1966, Menge and Sutherland 1976, Sih et al. 1985). Consumption of prey by predators is an important structuring force in natural communities. An example of this is the "Keystone Species" concept (Paine 1966), whereby the consumptive effects of predators can have disproportionately large effects on the community via the depletion of prey abundance (Paine 1995). Recently, focus has shifted from examining only the effects of prey consumption ("consumptive effects" or CEs) by predators, to include the "non-consumptive effects" (or NCEs) of predators. With NCEs, the non-lethal presence of predators alone alters the traits of prey, increasing prey survival in the presence of predators, but coming at the cost of reduced feeding, growth, and reproduction (Lima 1998, Peacor and Werner 2001, Trussell et al. 2003). Whereas research has historically focused on CEs reducing prey density, a meta-analysis of NCEs across a variety of ecosystems found that they comprise up to 63% of total predator effects on prey (see Preisser et al. 2005); thus representing the majority of net predator effects on prey.

Direct effects of predators on their prey (i.e. CEs and NCEs) can also impose indirect effects on food resources used by prey. Indirect effects via CEs are termed "Density-Mediated Indirect Interactions" (DMIIs) as they act via prey density reductions (Abrams et al. 1996). For instance, in the classic example of a trophic cascade, increased

sea otter predation results in decreased sea urchin abundance, which imposes a DMII by decreasing grazing pressure on kelp and other habitat-foming macrophytes (Estes and Palmisano 1974). Whereas DMIIs act via prey density reductions, indirect effects via NCEs are termed "Trait-Mediated Indirect Interactions" (TMIIs) as they act via changes in prey traits or behaviors (Abrams et al. 1996). A well-known example of a potential TMII is the interaction between wolf, elk, and aspen in Yellowstone, where the threat of wolf predation causes elk to reduce grazing and increase vigilance, thus benefitting aspen populations by reducing grazing pressure (Ripple et al. 2001, but see Kauffman et al. 2010). Since NCEs have cumulative impacts during a prey's lifetime, TMIIs are also likely a major component of net indirect effects of predators (Abrams et al. 1996, Peacor and Werner 2001, Trussell et al. 2003).

Theory predicts that NCEs and TMIIs should strengthen with increasing predator density, because increased predation risk will increase anti-predator responses in prey (Peacor and Werner 2001). Further, the interaction between spatial and temporal variation in risk can influence the magnitude and duration of NCEs on prey and resultant TMIIs. For example, the risk allocation hypothesis predicts that prey will exhibit their greatest anti-predator behavior during periods of high, or "acute" risk, while constraining risky behavior such as grazing to periods of low, or "background" risk (Lima and Bednekoff 1999). Additionally, increasing the "attack ratio" (the ratio between predation probabilities in high and low-risk situations), rather than absolute risk levels, increases anti-predator effort in high risk situations (Lima and Bednekoff 1999). Since anti-predator effort will be greater in situations with acute-risk pulses at low background risk,

the strength of NCEs (i.e. reduced feeding opportunity resulting from prey avoidance behavior) and TMIIs (reduced feeding pressure on food resources) should also increase as functions of increased spatial predator density variation, temporal predator density variation, and their interaction (i.e. the attack ratio). Surprisingly, few studies have directly manipulated predator density in space and time, or used natural gradients in predator density, to test these theoretical predictions (but see Matassa and Trussell 2011, Trussell et al. 2011).

Much of what is known regarding NCEs and TMIIs comes from laboratory-based studies (Peacor and Werner 2001, Trussell et al. 2003, Bourdeau 2009, Matassa and Trussell 2011, Gosnell and Gaines 2012) or field experiments with artificially-created predator density differences (Okuyama 2002, Trussell et al. 2004, Griffin 2006, Gravem and Morgan 2016, Morgan et al. 2016). For example, in marine systems, many species rely on waterborne chemical cues to detect the presence of their predators, and thus the risk of predation (Tollrian and Harvell 1998, Trussell et al. 2003, Keppel and Scrosati 2004, Trussell et al. 2004, Dalziel and Boulding 2005, Bourdeau 2009, Matassa and Trussell 2011, Gosnell and Gaines 2012, Morgan et al. 2016). However, laboratory studies often over-saturate experimental venues with predator cues at concentrations far higher than experienced in the natural environment (Bourdeau 2009, Gosnell and Gaines 2012, Murie and Bourdeau *in review*). Predator cue oversaturation in the laboratory and field experiments, could then result in overestimating the importance of NCEs and TMIIs. Further, over longer time periods, prey can become less responsive to high background levels of predation risk (Murie and Bourdeau *in review*), consistent with the

risk allocation hypothesis and the eventual necessity of feeding (Lima and Bednekoff 1999).

The interaction between *Pisaster ochraceus* and *Tegula funebralis* (hereafter *Pisaster* and *Tegula*) is an excellent system for examining the effects of spatial and temporal variation in predator density on NCE and TMII strengths in the natural environment. *Pisaster* is a voracious intertidal predatory sea star that commonly preys on *Tegula*, a herbivorous gastropod (Paine 1969, Nielsen 2001). *Pisaster* has the potential to exert NCEs on *Tegula* because its chemical cues induce strong anti-predator behavioral responses, such as crawling out of tide pools, or vertically migrating to higher elevations on the shore (Markowitz 1980, Schmitt 1981, Watanabe 1984, Pruitt et al. 2012, Jellison et al. 2016, Morgan et al. 2016). Previous experimental work has shown that *Pisaster* additions cause predation stress and energetic demands that are associated with *Tegula* zonation and vertical migration in the intertidal (Markowitz 1980). Further, *Pisaster* predation on *Tegula* reduces grazing pressure on food resources, indirectly affecting algal communities. Evidence of the potential for indirect effects in the intertidal from *Pisaster* are clear (Morgan et al. 2016), but to date studies have not investigated the strength and importance of NCEs and TMIIs from *Pisaster* under natural predator densities and cue concentrations. Indeed, more extensive and long-term field experiments are needed to determine the strength of TMIIs imposed by *Pisaster* in natural populations (Morgan et al. 2016).

The recent onset of Sea Star Wasting Disease (SSWD) has produced mass die-offs of many asteroid species along the eastern North Pacific, exacerbating existing

differences in *Pisaster* density (Hewson et al. 2014, Kohl et al. 2016, Miner et al. 2018). SSWD has been associated with a novel densovirus (*Parvoviridae*), however infected populations were also associated with microbial communities dominated by the bacterial genus *Vibrio* (Hewson et al. 2014). Mass mortality associated with SSWD has the potential to disrupt direct effects from *Pisaster* on *Tegula*, and indirect effects on algal communities. However, the severity of mass mortality associated with SSWD has not been constant, exacerbating existing spatial and temporal variation in *Pisaster* density along the northeastern Pacific coast, particularly the North Coast of California (Miner et al. 2018).

Here, I combine a field manipulation and laboratory experiment to quantify how spatial and temporal variation in *Pisaster* density affects the magnitude and relative importance of NCEs on *Tegula* and subsequent TMIIs on macroalgae. I hypothesized that reductions in *Pisaster* density would lead to density-dependent increases in *Tegula* soft tissue growth, reductions in shell growth, and increases in grazing behavior (i.e., reductions in NCEs), and that reduced grazing pressure would reduce TMIIs on macroalgae. I hypothesized that introducing temporal *Pisaster* density variation to simulate pulses of acute risk would lead to greater reductions in NCEs and TMIIs in areas with lower background levels of *Pisaster* predation risk.

METHODS

Field Experiment - Effects of Spatial and Temporal Variation in *Pisaster* Density on *Tegula* Growth and Grazing

In the summer of 2017, I used *Pisaster* additions (Markowitz 1980) with a cage exclusion experiment measuring *Tegula* grazing rates and morphological changes, to quantify how spatial and temporal variation in *Pisaster* density affects NCEs on *Tegula* growth, and TMIIs via grazing on *Pteryogophora californica*, a robust low-intertidal and subtidal kelp on the northeast Pacific coast.

Site and plot selections

In May, I selected three rocky shore field sites in Northern California: Pt. St. George, near Crescent City, CA (PSG); Baker Beach, near Trinidad, CA (BB); and Devil's Gate, near Cape Mendocino, CA (DG; Fig 1). These sites represent a gradient of *Pisaster ochraceus* density that is positively associated with *Tegula funebralis* vertical height on the shore (Murie and Bourdeau, *in review*), which is consistent with previous studies demonstrating the association between *Pisaster* presence, and *Tegula* zonation and migration in the intertidal zone (Markowitz 1980). I use three pre-established 50m transects at PSG, 21m, 22m, and 37m transects at BB, and four 75m transects at DG to maximize coverage of the intertidal zone at each site, and create an area in which to select experimental plot locations. I measured the height of each transect endpoint above Mean Lower Low Water (MLLW) using a laser leveler during quiet water. I then selected

two plot locations between 0.15 and 0.6m above MLLW, on the wave-protected faces of large boulders, and marked the center and corners of each plot. These plot locations were selected to minimize wave exposure on my cages, but *Tegula* were naturally abundant in these locations amongst red turf algae (*Endocladia muricate)*, and *Pisaster* were present in the vicinity (McClure, *pers. obs.).*

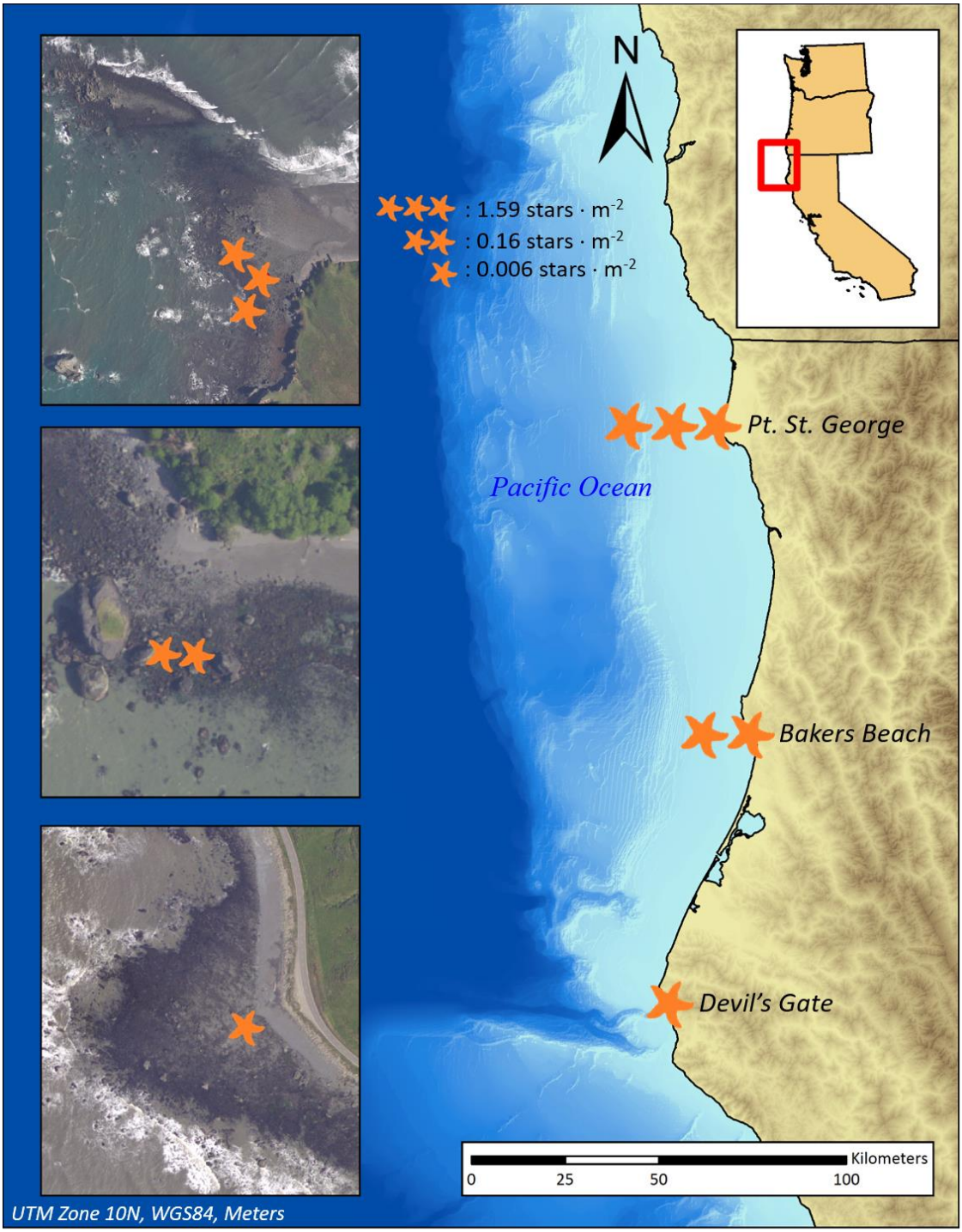

Figure 1. Map of the north coast of California showing locations of my study sites and the natural *Pisaster* abundance gradient. Sea star icons indicate relative *Pisaster* densities, as shown (Source datasets: NOAA/NCEI; Imagery: NOAA/NGS IOCM; Boundaries: USGS/National Map).

Collection and maintenance of study organisms

Tegula differ in their behavioral responses to *Pisaster* risk cues based on their size (Vermeij 1972, Markowitz 1980), so I collected 32 small (<16mm) and 32 large (>21mm) *Tegula* from each site (N_{total}=192). At the lab, I starved all *Tegula* for one week, scraped off shell epiphytes, and individually labelled each with a bee tag (Beeworks, Canada); assigning common tag colors for each collection site. For each individual, I measured total (dry) mass and buoyant mass. To estimate shell mass, and subtracted buoyant mass from total mass to non-destructively quantify soft tissue mass. Then, I validated and corrected these estimates by destructively sampling 24 random individuals, measuring their shell mass, comparing estimated and actual shell masses with a linear regression (R^2= 0.97, P< 0.001; Appendix A), and calculating revised estimates for shell masses using the regression equation (Palmer 1982). Then, I corrected my soft tissue masses by subtracting my corrected estimates for shell mass from the total mass.

Cage construction and installation

Using 0.635cm opening woven stainless-steel cloth, I constructed a total of twenty-four 15 x 15 x 6 cm fence enclosures for my caging experiment. I sealed fences along the bottom with a gasket formed from split 0.794cm rubber tubing, and created cages using 0.635cm opening Vexar™ plastic mesh and reusable zipties to construct removable tops to the fences (Miller 2006; Fig 2). For each plot within each site, I also constructed an additional *Tegula*-free control cage, made entirely of Vexar™ mesh, for a

total of six mesh cages. Because the tops for experimental cages were also Vexar™, control cages should not have experienced shading differences from the experimental cages. Further, since Vexar™ is slightly less rigid than stainless steel mesh, any resultant increases in degradation in control algae due to cage deformation would ultimately lead to more conservative estimates of *Tegula* grazing in my experimental cages. I drilled drywall anchors into wave-protected faces of boulders in each 5x5m plot and installed four experimental cages and one control cage, for a total of five cages, in each plot in early June, 2017. I mounted cages as closely together as possible within the center of each plot to minimize variation in tidal height and potential wave exposure. During my experiment from July 24 through August 20, I also mounted iButton temperature loggers in the center of each plot and recorded temperature every 20 minutes (Appendix B).

Figure 2. Stainless steel mesh fences with removable Vexar™ mesh tops (left) and rubber tubing gasket seal along base (right).

Experimental design

After installing cages within plots, I randomly assigned 6 large and 6 small *Tegula* from each collection site, into each of the four experimental cages in each plot.

Incorporating *Tegula* from all sites into my cage populations was critical to demonstrate that effects in my field experiment were associated with *Pisaster* density differences, and not differences among local *Tegula* populations at each site, as a growing body of literature suggests that individual variation in prey state can alter TMII strength (see Morgan et al. 2016). From 22 June until 21 September 2017, I stocked each cage bi-monthly with 48 g of *Pterygophora californica* blade, trimmed from the top of the stipe, which served as a food source for *Tegula*. *Pterygophora* has hearty blades that do not break down in cages over multiple weeks, but is also readily consumed by *Tegula* in the lab (McClure, *pers. obs.)*. To eliminate potentially confounding effects of site-specific variation in kelp quality and expressed anti-herbivory defenses, I hand-collected all *Pterygophora* for the experiment from the shallow subtidal zone in Trinidad Bay, near the Telonicher Marine Laboratory. I measured the amount of *Pterygophora* grazed by *Tegula* by re-weighing the segments after each two-week interval. After re-weighing grazed *Pterygophora* from each cage and calculating the change in mass during the experimental interval, I also imaged each blade to visualize grazing marks and qualitatively assess grazing damage from *Tegula.*

At one of the two plots within each site, I simulated acute pulses of predation risk by increasing local *Pisaster* density above the site-level 'background' density (Fig 3). I accomplished this by stocking each plot bi-monthly with *Pisaster,* to bring the plot total up to 25 individuals (1.1 *Pisaster* $\cdot$ m^{-2}), which approximated the average *Pisaster* density at PSG from 2016-2017. For each re-stocking, I first surveyed the plot and identified how many *Pisaster* were required, and haphazardly selected and removed asymptomatic

Pisaster from locations within each site, attempting to maximize the distance between the plot and the locations from which I collected *Pisaster*. I haphazardly placed the relocated stars within the plot, avoiding intentionally clustering stars within plots.

Figure 3. Schematic of plot layout at Baker Beach, Trinidad, CA. The yellow dashed square indicates the un-manipulated 5x5m "background risk" plot, the solid red square indicates the experimental addition "acute risk" plot. I placed cages at the centers of each plot. Larger, purple stars indicate existing stars within and around the plots, and smaller, orange stars indicate areas outside the plots where I selected stars for stocking plots.

I also used manual removals of caged *Tegula* to directly compare the strength of TMIIs (i.e., reduction in *Tegula* grazing induced by the non-lethal presence of *Pisaster*) to overall indirect effects, or "total predator" effects (TMIIs + DMIIs; i.e., the combined effects of non-lethal *Pisaster*-induced grazing suppression, and reduction in grazing due to the manual removal of caged *Tegula*). I randomly assigned manual-removal and un-manipulated treatments (hereafter Total Predator and TMII-only treatments) to each cage pair within each plot. In the Total Predator treatment, I simulated *Pisaster* predation on

Tegula by haphazardly removing one snail monthly from each cage. At the conclusion of the experiment, 9 *Tegula* remained in each cage, representing a 25% decrease in the initial population size (12 *Tegula*). *Pisaster* annually consume approximately 25-28% of the *Tegula* standing stock each year at some locations (Paine 1969), and other experiments examining CEs simulated population reductions between 4% to 25% over the course of their experiments (Peacor and Werner 2001, Trussell et al. 2003). Thus, my manual removals were similar to those used by other studies. Since manual removals applied the same simulated CE and DMII at all sites, the growth and grazing differences between Total Predator and TMII-only treatments at low *Pisaster* density (i.e. DG) provided a 'control' for comparing the presence of NCEs and TMIIs at intermediate and high *Pisaster* densities (i.e. BB, PSG). After 91 days of field exposure, I re-measured *Tegula* shell dimensions, whole mass, and buoyant mass to assess *Pisaster* NCEs on *Tegula* growth.

Since changes in kelp mass in my experimental cages could be due to factors other than *Tegula* grazing, I applied a correction for non-grazing changes in kelp mass by subtracting the change in control cage kelp mass from the change in each treatment cage kelp mass. To compare differences in grazing between acute and background risk treatments (TMIIs), I calculated per-capita cumulative grazing during the course of the experiment by dividing the grazing amount (corrected change in kelp mass) in each sampling interval by the number of snails present during that interval, and summing the per-capita amounts grazed per interval as follows:

$$\sum_{i=1}^{5} \frac{grazing\ amount\ (g)_i}{\#\ of\ \text{Tegula}_i} \ .$$

I also calculated cumulative grazing amounts to compare differences in grazing (TMIIs) between *Tegula* removal treatments. DMIIs are based on *Tegula* density reductions, so per-capita cumulative grazing is not an appropriate grazing metric, as it eliminates differences due to *Tegula* density within each cage.

To compare differences in soft tissue and shell growth (NCEs), I estimated soft tissue and shell masses from measurements taken before and after the experiment, as described above (Palmer 1982). I corrected the buoyant masses using the derived regression equation to estimate shell mass, and subtracted the new estimates for shell mass from total mass to estimate soft tissue mass. I calculated soft tissue and shell growth as the differences between estimated mass measurements before and after the experiment. Then, I calculated average soft tissue and shell growth for each cage, which I used in my statistical analyses of growth differences between risk and removal treatments.

Laboratory Experiment – Effects of Field-Relevant *Pisaster* Chemical Cue Density on *Tegula* Anti-Predator Behavior

After completing my field experiment, I did a laboratory experiment measuring *Tegula* grazing differences and other behavioral responses to confirm that trends I observed in the field were consistent with behavioral responses elicited by natural *Pisaster* cue concentrations.

Experimental Design

Using the previously described, pre-established transects at each of my field sites, I converted tidal elevations from meters above MLLW to meters below MHHW. Then, making the conservative assumption that *Pisaster* cue is homogenously mixed in the water column, I used transect areas and depths to estimate the volume of seawater above the transects. Finally, I used those volumes to convert *Pisaster* areal density to volumetric density, as follows:

$$\frac{\text{Pisaster } count\ (ind)}{area(m^2) \times depth(m) \times (1000l \cdot m^{-3})}.$$

To create representative cue densities that reflected *Pisaster* volumetric densities in the field, I used the above equation to estimate natural *Pisaster* cue densities from PSG (2.63×10^{-4} stars $\cdot$ L^{-1}), BB (1.31×10^{-4} stars $\cdot$ L^{-1}), and DG (2.39×10^{-5} stars $\cdot$ L^{-1}). These natural cue densities were combined with a positive control (2.5×10^{-2} stars $\cdot$ L^{-1}), and a *Pisaster*-absent negative control to serve as the range of cue treatments in my laboratory experiment. I used header tanks each containing one *Pisaster* in 40L of seawater ($2.5\times10^{-}$

^{2} *Pisaster* · L^{-1}), connected to a system of flow-through cascade cups with fresh seawater inputs (Fig 4, Fig 5), to create the gradient of cue concentration treatments (2.5×10^{-2}, 2.5×10^{-3}, 2.5×10^{-4}, and 2.5×10^{-5} *Pisaster* · L^{-1}). Using this dilute range of cue densities allowed me to test whether *Tegula* were capable of responding to natural *Pisaster* cue densities in a density-dependent manner. Further, these calculations assume that cue was mixed homogeneously throughout the water column. This was an intentionally conservative approach, so that any observed differences would highlight the low cue concentrations at which *Tegula* can respond differently to *Pisaster* predation threat. I ensured that each cascade cup was a 1:10 dilution by measuring cup outflow rates (mL · sec^{-1}), and set untreated seawater inputs immediately downstream 9 times higher than outflow rates. Since contamination of downstream aquaria with upstream-treated seawater could greatly affect the cue concentration, I enclosed the header tanks and cascade dilutions with plastic sheets to eliminate the possibility of concentrated *Pisaster* cue splashing into aquaria. The Telonicher Marine Laboratory operates on a recirculating seawater system with a total volume of 175,770 liters, which can be operated without fresh seawater input, thus introducing the potential for recirculating *Pisaster* cue. During my experiment there were approximately 12 *Pisaster* housed in the recirculating system, which represents the potential for a residual concentration of 6.8×10^{-5} *Pisaster* · L^{-1} in recirculating seawater. However, any residual cue would have been present in all experimental header tanks (including the *Pisaster*-absent negative control treatment), and

seawater is also passed through a sand and charcoal filtration system, so the potential for any confounding effects in my experiment due to recirculating cue is minimal.

I constructed two replicate header tanks and cascade dilution systems for each cue concentration treatment. I gravity-fed *Pisaster*-treated and diluted seawater from the cascades through manifolds to 4 downstream aquaria, each containing 8 *Tegula* that were provided 24g of *Pteryogphora* and a PVC tile shelter (Fig 4). Over a period of one week, I assessed *Pisaster* NCEs on *Tegula* by observing *Tegula* behavior twice daily, counting the number of *Tegula* above the waterline in each aquarium. If *Tegula* were above the waterline or on the aquaria lid before observation and fell off while handling aquaria, I reset them aperture-down in on the bottom of the aquaria, as upside-down *Tegula* often will not right themselves in the presence of *Pisaster* cue (Murie, *pers. obs.*) To assess TMIIs from *Pisaster* on *Pterygophora* via *Tegula* grazing, I weighed the remaining *Pterygophora* in each aquarium at the conclusion of the experiment to calculate cumulative grazing amounts, an analogous measurement to my field experiment. Lastly, I calculated the average number of *Tegula* above the waterline, and the average grazing amount, for each header tank, as my response variables.

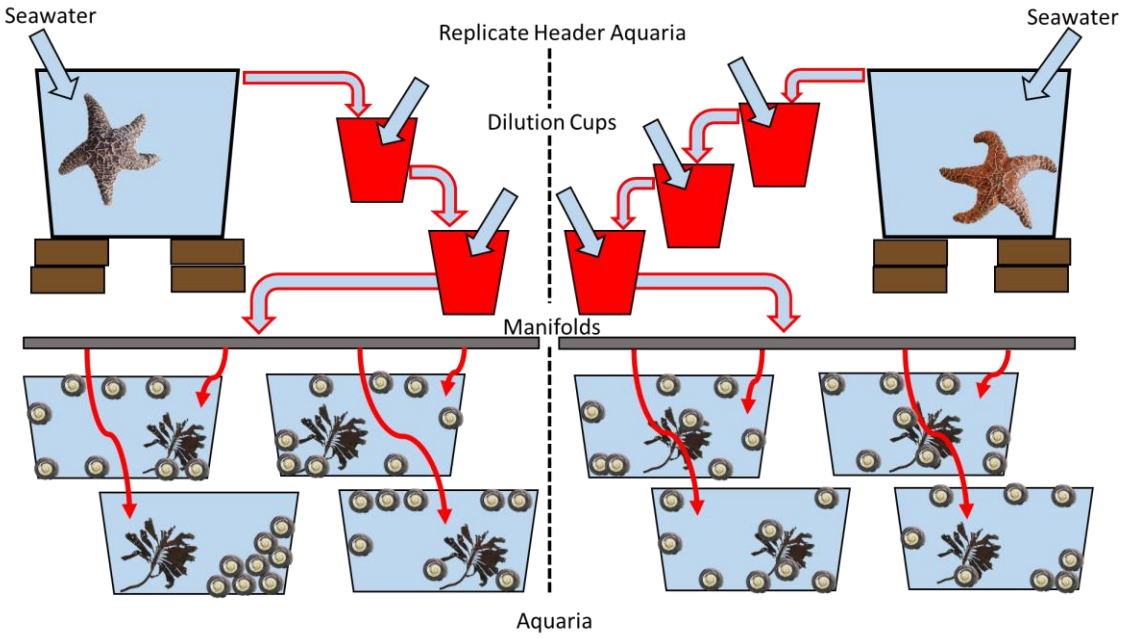

Figure 4. Schematic of laboratory experiment setup. Header tanks, dilution cups, and experimental aquaria are labelled, red arrows represent *Pisaster*-conditioned seawater flow, and light blue arrows represent untreated seawater inputs. I varied the number of cascading 1:10 dilution cups to create different *Pisaster* cue concentration treatments.

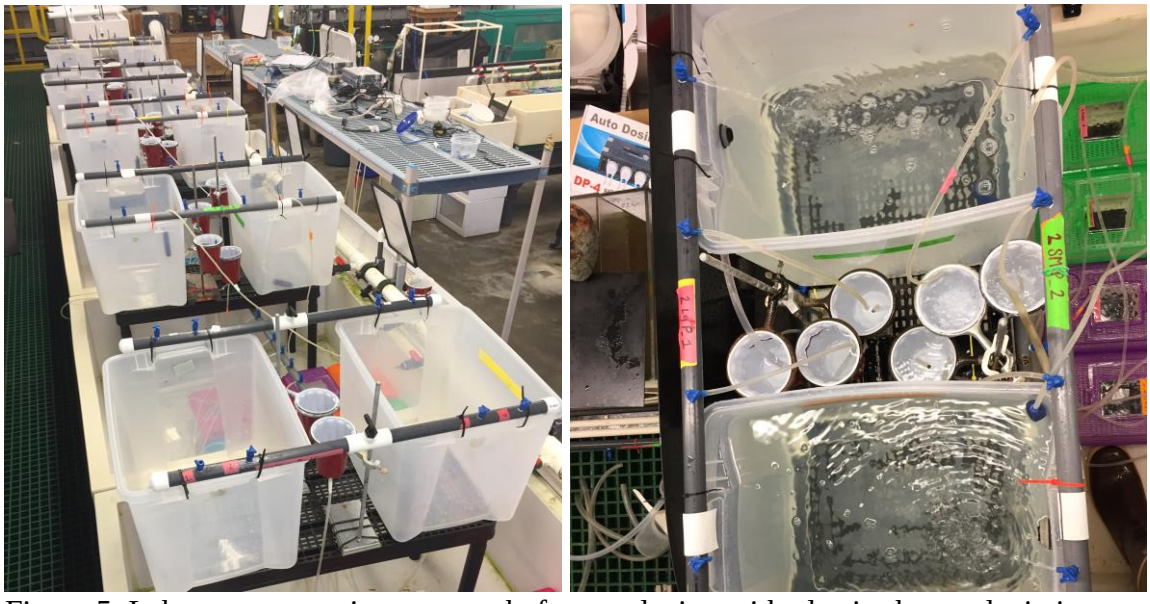

Figure 5. Laboratory experiment setup before enclosing with plastic sheets, depicting header tanks and cascade dilution system (left); and flowing with seawater before *Pisaster* addition (right).

Statistical Analyses

Field experiment - effects of spatial and temporal *Pisaster* density variation on *Tegula* growth and grazing

Effects of background levels of predation risk on NCEs and TMIIs. To compare *Tegula* growth differences among background risk treatments at each site, I used a pair of ANOVAs with average soft tissue growth and shell growth from each cage, pooling across removal treatments, as the response variable, and log-transformed *Pisaster* density as the predictor variable (N_{site}=4). To compare *Tegula* grazing differences among background risk treatments, I used an analogous ANOVA with log-transformed *Pisaster* density as the predictor variable and per-capita cumulative grazing from each cage as the response variable (N_{site}=4). For each ANOVA, I assessed the significance of individual pairwise differences using post-hoc Tukey HSD tests.

Effects of acute pulses of risk and background levels of risk on NCEs and TMIIs. I calculated acute risk effect sizes using standardized differences between acute and background treatments for soft tissue growth, shell growth, and per-capita cumulative grazing. I did this by subtracting the average background treatment value from each acute treatment replicate value, before taking the overall average, indicated by the following formulae:

$$NCE\ effect\ size\ = Average\left[Growth_{acute} - Average\left(Growth_{background}\right)\right],$$

$$TMII\ effect\ size\ = Average\left[Grazing_{acute} - Average\left(Grazing_{background}\right)\right].$$

This is important as a means of standardization, because differences in background levels of risk also affect *Tegula* growth and grazing. To test for associations between acute risk effect sizes and site-level 'background' risk, I used a series of ordinary least squares (OLS) regressions with log-transformed *Pisaster* density as the predictor variable, and standardized effect sizes as response variables. To assess whether effect sizes among sites were significantly different from one another, I calculated 95% confidence intervals for effect sizes using the following formulae:

$$NCE\ 95\%\ CI\ =\ SE\left[Growth_{acute} - Average\left(Growth_{background}\right)\right] \times T_{3,0.05},$$

$$TMII\ 95\%\ CI\ =\ SE\left[Grazing_{acute} - Average\left(Grazing_{background}\right)\right] \times T_{3,0.05}.$$

To assess whether pairwise differences between effect sizes were significant, I assessed the overlap of confidence intervals and means between each site (Zou and Donner 2008).

Relative contributions of consumptive and non-consumptive effects on *Tegula* growth and grazing. I calculated differences in soft tissue growth, shell growth, and cumulative grazing amounts between Total Predator and TMII-only cages at each site, and calculated 95% confidence intervals by bootstrapping two random cage pair combinations from each removal treatment (4 cages total) for 10,000 iterations. Then, I tested for a relationship between log-transformed *Pisaster* density and differences in soft tissue growth, shell growth, and cumulative grazing amounts with a series of OLS regressions. I also assessed the significance of differences between Total Predator and TMII-only treatments within each site by comparing whether the 95% confidence intervals associated with these differences overlapped zero. Since I hypothesized that

NCEs and TMIIs are minimal at low *Pisaster* density, the difference between Total Predator and TMII-only treatments should be positive for soft tissue growth (NCE), and negative for shell growth (NCE) and cumulative grazing (TMII). Thus, I would use non-significant growth and grazing differences to detect the presence of NCEs and TMIIs at intermediate and high *Pisaster* between Total Predator and TMII-only treatments. To eliminate confounding effects from acute risk treatment when assessing the relative contributions of consumptive and non-consumptive effects, I only used data from plots without *Pisaster* additions (background risk treatment).

Temperature differences between plots. I summarized my iButton temperature data for each plot into daily maximums and minimums, subtracted minimums from maximums to calculate the temperature range for each day, and calculated average temperature ranges for each site. I tested for differences in thermal ranges within each plot using an ANOVA with site as the predictor variable and average temperature range as the response (Appendix B).

Laboratory experiment – effects of field-relevant *Pisaster* chemical cue density on *Tegula* anti-predator behavior

To examine the effects of *Pisaster* cue concentration on TMIIs on *Pterygophora* via suppression of *Tegula* grazing, I assessed the relationship between *Pisaster* cue concentration and average grazing amounts with a log-log regression. Similarly, I compared *Pisaster* NCEs on *Tegula* via increases in behavioral responses, using a log-log regression with *Pisaster* cue concentration as the predictor variable and average number

of *Tegula* above the waterline as a response variable. In both regressions, I added 1^{-20} to all *Pisaster* cue concentrations, grazing amounts, and above waterline counts, since log-transforming zeros result in undefined values.

I did all statistical analyses of growth and grazing described above using R and RStudio (R Core Team 2018, RStudio Team 2018). For among-treatment growth and grazing differences, I assessed significance by comparing the overlap of 95% confidence intervals and means (Zou and Donner 2008), and for ANOVAs and regression models I used an alpha-level of 0.05 for assessing significance.

RESULTS

Field Experiment - Effects of Spatial and Temporal *Pisaster* Density Variation on *Tegula* Growth and Grazing

Effects of Background Levels of Predation Risk on NCEs and TMIIs

I did not observe significant differences in soft tissue growth or shell growth with increasing background *Pisaster* density (soft tissue: $F_{2,9}$=1.21, P=0.342; shell: $F_{2,9}$=0.21, P=0.820; Fig 6). However, I observed significant decreases in grazing amounts with increasing background *Pisaster* density (Fig 7). The highest per-capita cumulative grazing occurred at Devil's Gate, the lowest *Pisaster* density site, with decreasing grazing amounts at Bakers Beach and Pt. St. George, the intermediate- and high-density sites, respectively ($F_{2,9}$=38.25, P<0.001; 95% CIs, Appendix C).

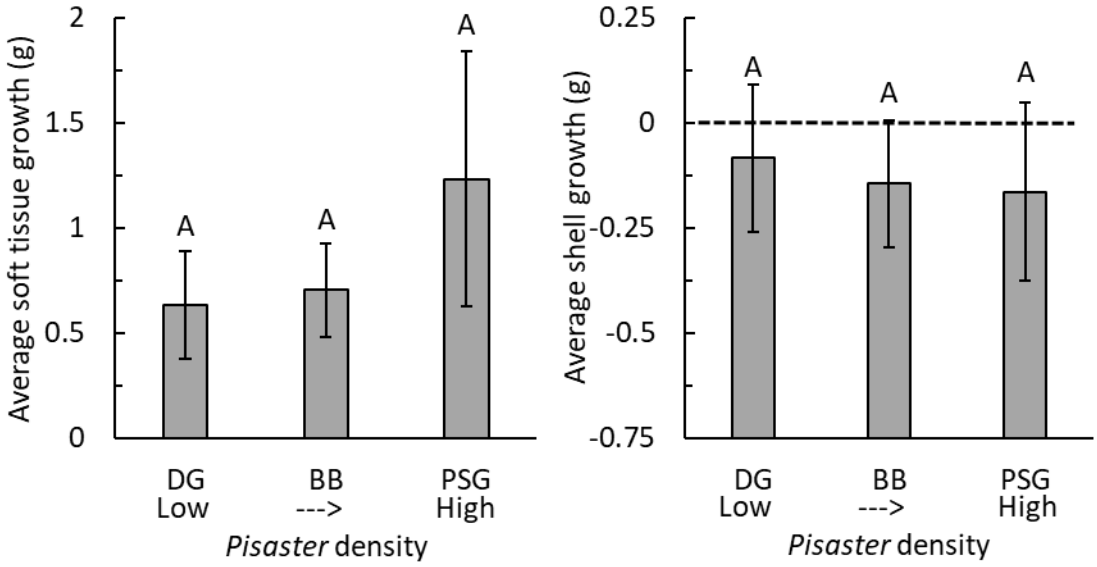

Figure 6. Soft tissue growth (left) and shell growth (right) for background-only risk treatments at each site (soft tissue: $F_{2,9}$=1.21, P=0.342; shell: $F_{2,9}$=0.21, P=0.820). Significant differences among treatments are indicated with letters, error bars represent 95% confidence intervals.

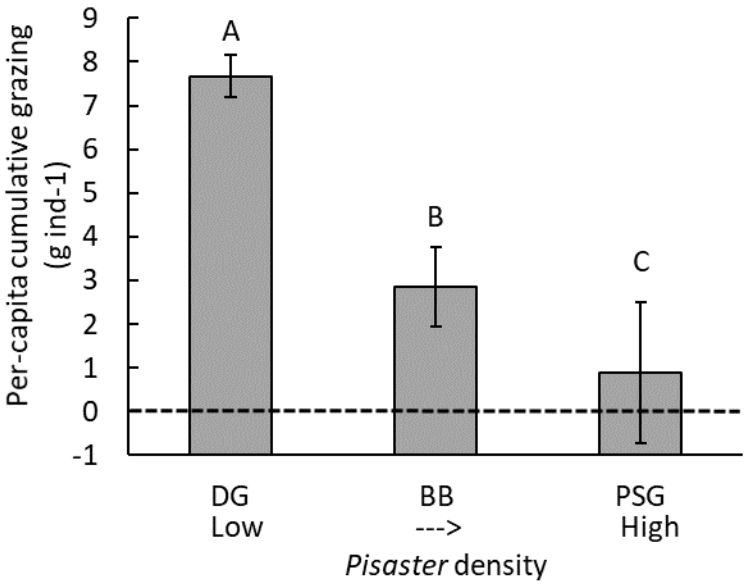

Figure 7. Per-capita cumulative grazing ($F_{2,9}$=38.25, P<0.001) for background-only risk treatments at each site. Significant differences among treatments are indicated with letters, error bars represent 95% confidence intervals. Dashed lines indicate zero lines in order to display negative values extents of error bars.

Effects of acute pulses of risk and background levels of risk on NCEs and TMIIs

I did not observe a significant relationship between log-transformed *Pisaster* density and soft tissue or shell growth effect sizes (soft tissue: R^2=0.244, P=0.843; shell: R^2=0.543, P=0.641; Fig 8). However, I observed a significant negative effect of acute *Pisaster* risk on *Tegula* soft tissue growth at intermediate background *Pisaster* density, but not at low or high background density (95% CIs, Appendix 3). Lastly, I observed significant positive effects of acute *Pisaster* risk on *Tegula* shell growth at low and intermediate background *Pisaster* density, but not at high background density (95% CIs, Appendix D).

I observed a significant relationship between *Pisaster* density and cumulative grazing amount effect sizes (R^2=0.997, P=0.029, Fig 9). Grazing reductions in acute risk treatments were significantly different from zero at low and intermediate background *Pisaster* density, but I observed a significant grazing increase in acute risk treatment at the highest background density (95% CIs, Appendix D).

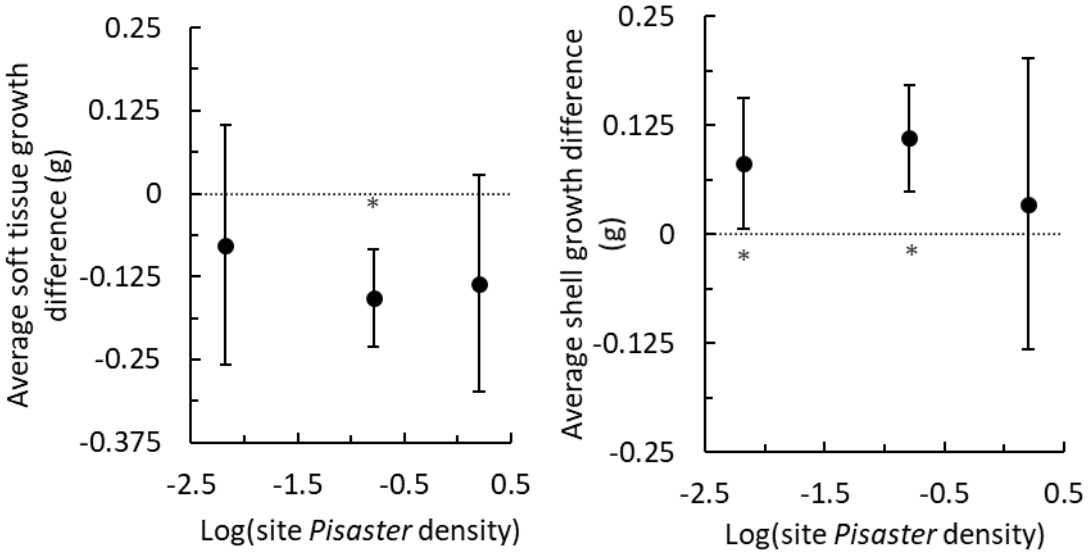

Figure 8. Relationships between soft tissue growth differences (left) and shell growth differences (right) between acute and background-only risk treatments, and log-transformed *Pisaster* density (soft tissue: R^2=0.244, p=0.843; shell: R^2=0.543, p=0.641). Significant differences from zero are indicated with asterisks, error bars represent 95% confidence intervals. Negative soft tissue growth values (left) and positive shell growth values (right) indicate reductions and increases due to acute risk, respectively.

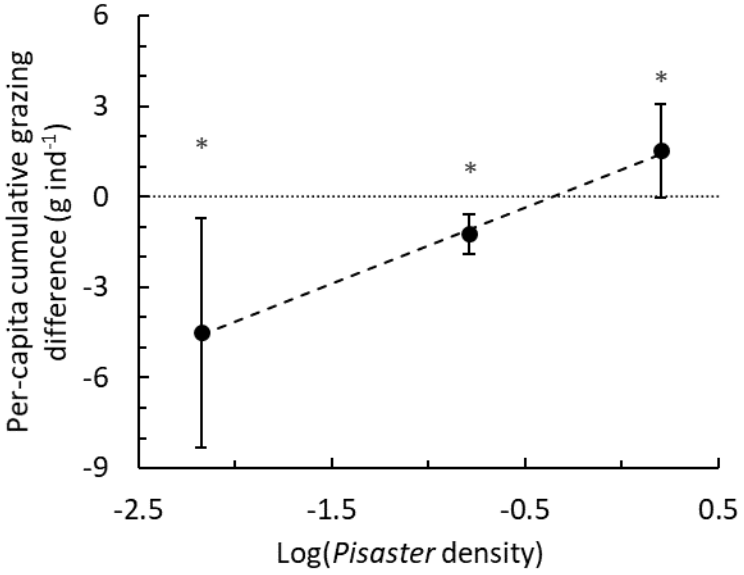

Figure 9. Relationship between per-capita cumulative grazing differences between acute and background-only risk treatments and log-transformed *Pisaster* density (R^2=0.997, P=0.029). Significant differences from zero are indicated with asterisks, error bars represent 95% confidence intervals. Negative values indicate reductions in grazing due to acute risk.

Relative contributions of consumptive and non-consumptive effects on *Tegula* growth and grazing

I did not observe a significant relationship between log-transformed *Pisaster* density and soft tissue growth differences between Total Predator and TMII-only treatments (R^2=0.960, P=0.183; Fig 10 left). The soft tissue growth increase in the absence of NCEs was significantly greater at low *Pisaster* density (95% CIs, Appendix E); thus, non-significant soft tissue growth differences at intermediate and high density were associated with NCE-driven decreases in soft tissue growth. Likewise, I did not observe a significant relationship between log-transformed *Pisaster* density and shell growth differences (R^2=0.972, P=0.159; Fig 10 right). However, I did observe a significant decrease in shell growth due to CEs at low *Pisaster* density, indicating that that non-significant shell growth differences at intermediate and high density were associated with NCE increases in shell growth.

I did not observe a significant relationship between log-transformed *Pisaster* density and cumulative grazing amount differences between Total Predator and TMII-only treatments (R^2=0.853, P=0.251; Fig 11). However, the DMII grazing reduction was significantly greater at low *Pisaster* density (95% CIs, Appendix E), indicating the absence of TMIIs, and that non-significant grazing differences at intermediate and high density were associated with TMIIs.

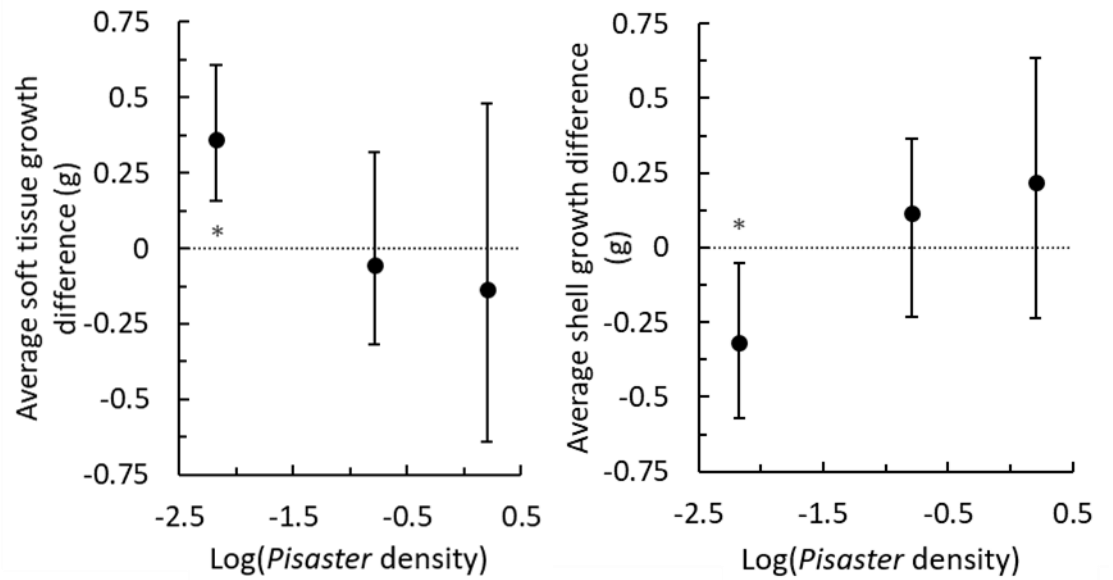

Figure 10. Relationships between soft tissue growth differences (left) and shell growth differences (right) between Total Predator and TMII-only paired-cage treatments, and log-transformed *Pisaster* density (soft tissue: R^2=0.960, P=0.183; shell: R^2=0.972, P=0.159). Significant differences from zero are indicated with asterisks, error bars represent bootstrapped 95% confidence intervals. Differences represent Total Predator – TMII only, so non-significant values at intermediate and high *Pisaster* density represent decreases in soft tissue growth, and increases in shell growth, associated with NCEs.

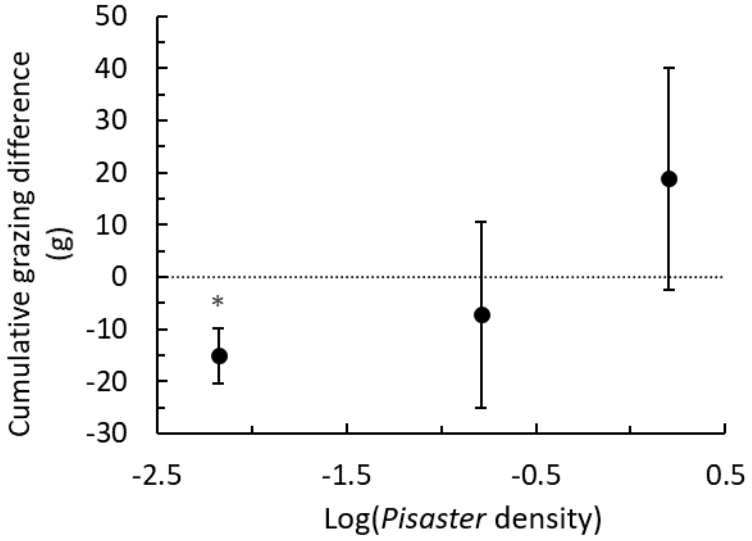

Figure 11. Relationship between cumulative grazing differences between Total Predator and TMII-only paired-cage treatments and log-transformed *Pisaster* density (R^2=0.853, P=0.251). Significant differences from zero are indicated with asterisks, error bars represent bootstrapped 95% confidence intervals. Non-significant values at intermediate and high *Pisaster* density represent grazing reductions associated with TMIIs.

Characteristics of *Tegula* grazing behavior on *Pterygophora* blades and other observations of *Tegula* defensive behavior

In addition to measuring changes in *Pterygophora* mass, I also visually assessed *Tegula* grazing on *Pterygophora,* which left distinct, visible radular scrapes and grooves in the outer layers of the blades (Fig 12). Over the duration of my field experiment, I also observed *Pisaster* foraging in the immediate vicinity of my cages at all field sites, and caged *Tegula* responding to the threat of predation by huddling, climbing to the top cage corner opposite of approaching *Pisaster,* and hiding amongst *Pterygophora* blades (Fig 13). Despite the qualitative nature of these observations, they present strong evidence that, besides a reduction in growth and grazing, *Tegula* were exhibiting avoidance behaviors in response to *Pisaster* presence during my field experiment.

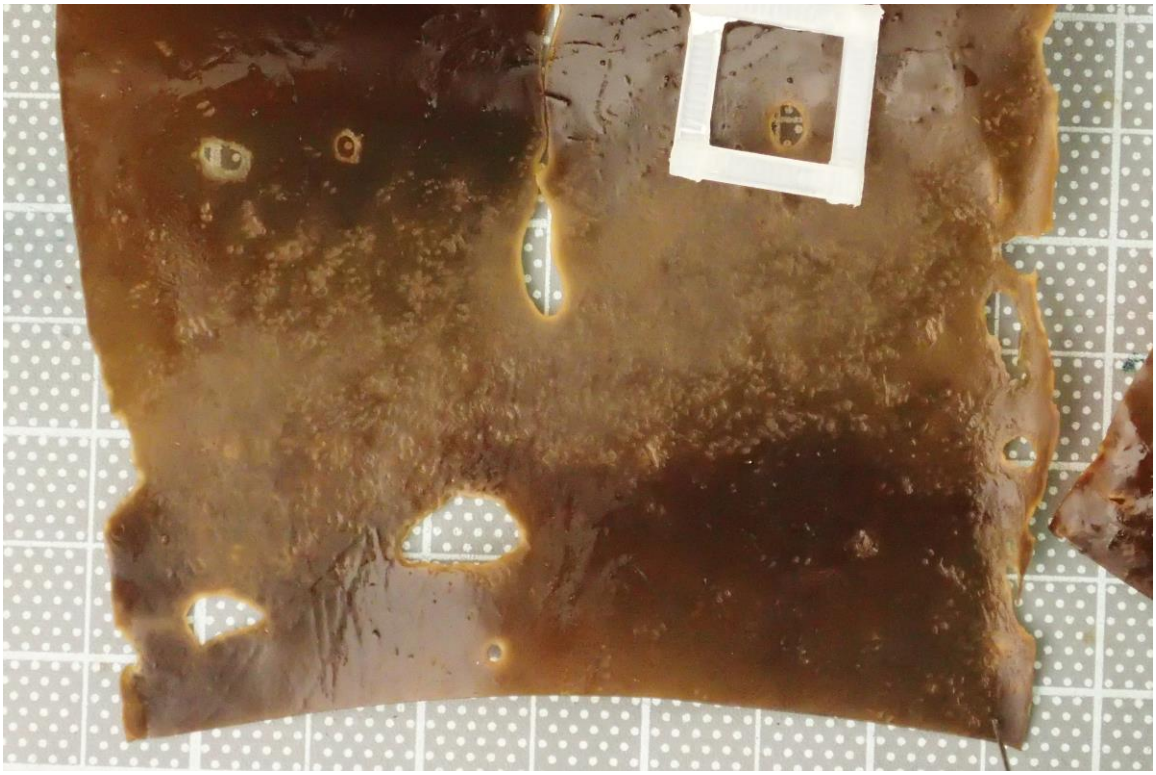

Figure 12. Example of *Tegula* grazing marks on *Pterygophora.* 1x1cm “quadrat” shown for scale.

Figure 13. *Tegula* avoidance responses from perceived *Pisaster* threat at Baker Beach (top left, right) and Devil's Gate (bottom). Red circles highlight groups of *Tegula* exhibiting anti-predator behavior, fleeing from encroaching *Pisaster*.

Laboratory Experiment – Effects of Field-Relevant *Pisaster* Chemical Cue Density on *Tegula* Anti-Predator Behavior

As with my field experiment, I observed significant decreases in *Tegula* grazing with increasing *Pisaster* cue density (log-log regression: R^2= 0.591, P=0.009; Fig 14). I also observed a significant increase in the number of *Tegula* out of water with increasing *Pisaster* cue concentration (log-log regression: R^2= 0.379, P<0.001; Fig 15).

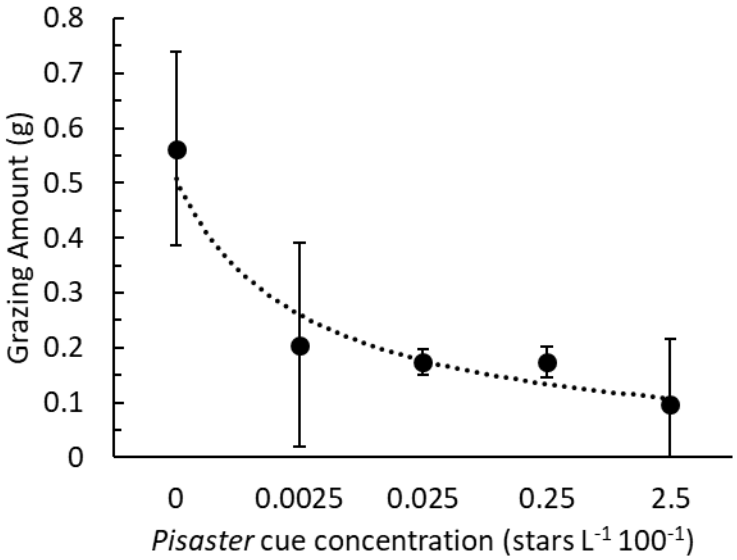

Figure 14. The relationship between average amount grazed by *Tegula* and *Pisaster* cue density (log-log regression: R^2= 0.591, P=0.009). Error bars represent 95% confidence intervals.

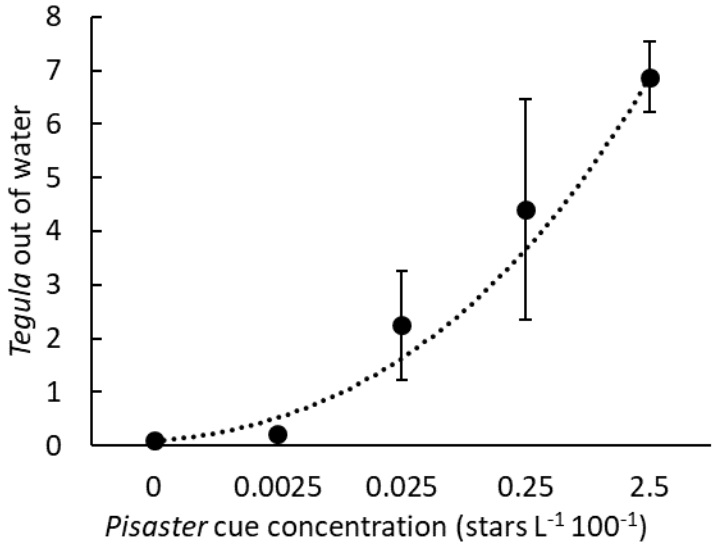

Figure 15. The relationship between average numbers of *Tegula* out of water and *Pisaster* cue density (log-log regression: R^2= 0.379, P<0.001). Error bars represent 95% confidence intervals.

DISCUSSION

In the field, natural variation in *Pisaster ochraceus* density (Miner et al. 2018) led to increases in *Tegula funebralis* grazing, representing reductions in TMIIs; though growth NCEs were not reduced with decreasing *Pisaster* density. Further, NCEs and resulting TMIIs were only detectable at intermediate and high *Pisaster* densities. When comparing growth and grazing differences between acute and background-only risk treatments, I found support for the risk allocation hypothesis (Lima and Bednekoff 1999) in that increased risk asymmetries between acute and background-risk situations increased *Tegula* anti-predator effort. *Tegula* grazing and anti-predator behavior in the lab also depended on *Pisaster* density at field-relevant cue concentrations, suggesting that the NCEs and TMIIs observed in my field experiment were indeed induced by *Pisaster* chemical cues, and that NCEs and TMIIs do operate under natural conditions and not just unrealistically high-risk conditions created in most lab studies. Lastly, the results from my two experiments contribute a novel finding to the growing body of literature considering the ecological impacts of SSWD, indicating that variation in *Pisaster* density can affect NCEs and TMIIs in affected rocky shore systems.

NCEs and TMIIs have mostly been studied through laboratory experiments, as well as some field experiments with artificially-created predator density differences, but my field experiment quantified density-dependent differences in grazing NCEs and TMIIs in the natural environment. Increases in background *Pisaster* density increased NCEs via *Tegula* per-capita grazing reductions, leading to increased TMIIs from *Pisaster*

on *Pterygophora californica*. This result is important because it is consistent with existing theory that NCEs and resultant TMIIs are density-dependent, varying proportionally to predation threat, and demonstrates that NCEs from predators are important factors in structuring communities.

The Risk Allocation Hypothesis suggests that increasing predator density increases predation risk, so the strength of NCEs and TMIIs should likewise increase. While a growing body of literature has considered whether NCE strength is affected by prey or resource density (Bolnick and Preisser 2005), considerably less work has addressed how predator density affects NCE strength, as well as the strength of resultant TMIIs. A study manipulating refugia used by invertebrate predators in farmed grasslands increased predator survivorship, thus inducing predator density differences, and found increased growth by primary producers, suggesting the presence of an indirect effect (Thomas et al. 1991). However, this study did not differentiate whether the increases in primary production were associated with DMIIs or TMIIs. Another study comparing field manipulations of invertebrate predators in ephemeral and permanent ponds found that increased desiccation, which limits the density of predatory fish typically found in permanent ponds, increased the strength of NCEs and TMIIs by reducing trophic complexity and competition amongst predators (Greig et al. 2013), though predator density was not directly manipulated. Lastly, increased blue crab (*Callinectes sapidus*) biomass increased NCEs on mud crabs (*Panopeus herbstii*), resulting in increased oyster (*Crassostrea virginica*) survivorship (Hill and Weissburg 2013). While this study considered predator biomass rather than density, it is consistent with the mechanism that

increased predator cue increases NCE and TMII strength. These studies indicate predator density variation, or variations in perceived predation risk, can affect NCE and TMII strength in terrestrial, aquatic, and marine systems. However, less is known about the effects of natural variation in predator density on NCE and TMII strength specifically. As such, my thesis is a novel contribution to the growing body of literature studying NCEs and TMIIs in natural environments.

Tegula responded more strongly to acute pulses of *Pisaster* risk at low background *Pisaster* density, supporting a prediction of the risk allocation hypothesis that anti-predator effort increases with an increasing attack ratio (i.e., the difference in predation probability between high and low risk situations; Lima and Bednekoff 1999). The risk allocation hypothesis also states that with increasing durations of high risk, anti-predator effort in low-risk situations may drop to low levels as an animal allocates as much feeding as possible to these brief, unpredictable periods (Lima and Bednekoff 1999). While I did not experimentally manipulate the duration of high risk, I found that grazing in the acute risk treatment at high background *Pisaster* density (a situation with a low attack ratio) was actually greater than grazing in the background-only treatment. This result suggests that *Tegula* exposed to a situation with consistent high risk and infrequent low risk further increased their grazing activity above normal levels during any periods of relatively lower risk they experienced, anticipating long durations of high risk to come. Taken together, these results from my field experiment are consistent with both predictions from the risk allocation hypothesis.

Tegula grazing and soft tissue growth both decreased with increasing attack ratios in my analyses of acute and background risk differences, indicating that longer-term NCEs can be a by-product of energetic consequences from short-term anti-predator behavior. Previous experiments investigating the effects of green crab risk cues on littorine snail consumption of fucoid algae have highlighted that short and longer-term NCEs, such as reduced grazing and soft tissue growth, could be linked or separate mechanisms (Trussell et al. 2003). In my field experiment, the inclusion of temporal variation (i.e. acute risk) resulted in soft tissue growth decreases, shell growth increases, and grazing reductions dependent on predator density. This is an important result, as it demonstrates that temporal variation in predator density is a key component of any mechanistic link between growth NCEs and anti-predator behavior reducing energy reserves. Therefore, incorporating temporal predator density variation to our mechanistic understanding of NCEs and TMIIs may be necessary for detecting potential interactions between shorter and longer-term NCEs.

Observations in the field indicated that *Tegula* were often "hiding" on folds in the *Pterygophora* blades, potentially grazing. I also observed *Tegula* hiding underneath but on *Pterygophora* while also leaving grazing marks in the laboratory experiment. One premise of the risk allocation hypothesis is that feeding behavior is inherently risky, creating a tradeoff between gaining energy and reducing vulnerability to predators. However, *Tegula* are known to sometimes hide among macroalgae (Watanabe 1984), so simultaneous feeding and defensive behavior can be possible. This is consistent with my observations of *Tegula* hiding amongst *Pterygophora* blades while feeding in both

experiments. While some studies have examined whether prey select the resources they consume based on predation risk (Matassa and Trussell 2011), presumably prey could also select refugia based on the potential for resource availability. Future studies should consider the potential for overlap between resource-gaining and defensive behaviors, the resulting implications regarding assumed tradeoffs between energy acquisition and risk allocation in prey, and examine whether resource availability affects refugia selection by prey experiencing predation risk.

My study indicated that *Pisaster* initiate TMIIs on *Pterygophora* by suppressing *Tegula* grazing; however, *Tegula* also graze on microalgal communities. Although I replaced *Pterygophora* blades biweekly (thus preventing microalgae from accumulating atop the blades) it is possible that alternative microalgal resources became available in the cages since I could not clean them out during each interval without risking displacing *Tegula* enclosed in the cage. The presence of unmeasurable resource availability and consumption could have resulted in the equitable growth observed across background densities, despite variation in grazing rates on *Pterygophora*. However, decreased soft tissue growth and increased shell growth suggests that an energy tradeoff occurred: soft tissue growth reductions were associated with shell growth increases under acute risk at medium and low background *Pisaster* densities (higher attack ratios), and soft tissue growth reductions were associated with shell growth increases due to NCEs at intermediate and high *Pisaster* density. Furthermore, I mostly observed *Tegula* on the cage walls or on *Pterygophora* (I had to remove most individuals from the *Pterygophora* blades when replacing them), not on the bare rock where microalgae could have grown,

and visual examination of *Pterygophora* blades indicated that radular scrapes from *Tegula* dug into the blades. Taken together, it is unlikely that *Tegula* acquired additional energy from undetected microalgal grazing in my experiment, as it likely would have resulted in both soft tissue and shell growth increases. Strictly controlling and measuring the resources available to prey in aquatic systems can be challenging even in laboratory and mesocosm experiments; previous studies have used similarly indirect methods for estimating prey feeding effort, due to challenges in directly measuring microalgal and periphyton consumption (Peacor and Werner 2001). Thus, while *Pterygophora* was a viable method of estimating *Tegula* grazing effort, future studies should consider also assessing the TMIIs from *Pisaster* via *Tegula* grazing on microalgal communities.

That *Tegula* responded to field-relevant *Pisaster* cue concentrations in a density-dependent manner, indicates that the NCEs/TMIIs observed in my field experiment were induced by *Pisaster* cues. Further, it highlights the importance of designing laboratory and mesocosm experiments with conditions comparable to those in the natural environment. Flow-through seawater systems are an effective and commonly-used experimental design (numerous, but see Trussell et al. 2003, Bourdeau 2009, Matassa and Trussell 2011, Gosnell and Gaines 2012, Morgan et al. 2016, Murie and Bourdeau *in review*) as they spatially isolate predator and prey but allow continuous introduction of predator cue to a downstream aquarium. The field-relevant predator cue concentrations I used in my experiment indicate a departure from many previous laboratory studies. Cue concentrations in experiments with similar flow-through designs have ranged from 0-2 *Pisaster* in one aquarium (Gosnell and Gaines 2012), 0.197 stars $\cdot$ L^{-1} (Bourdeau 2009),

or 0.05 stars · L^{-1} (Murie and Bourdeau, *in review*), but these concentrations are at least 3 orders of magnitude higher than the estimates for volumetric cue concentration derived from my field surveys (between $2.63x10^{-4}$ stars · L^{-1} and $2.39x10^{-5}$ stars · L^{-1}). Over increasing periods of exposure, *Tegula* can become less responsive to high levels of perceived predation risk (Murie and Bourdeau, *pers. obs)*, a result that is also consistent with the risk duration component of risk allocation hypothesis (Lima and Bednekoff 1999). Taken together, these findings indicate that future laboratory studies of NCEs and TMIIs should strive to ensure that the cue concentrations used are indicative of conditions experienced in the field.

Sea Star Wasting Disease has exacerbating existing differences in *Pisaster* density along the northeast Pacific coast (Miner et al. 2018). My results indicate that SSWD can introduce site-specific differences in community structure via *Tegula* NCEs and resulting TMIIs from *Pisaster* on the macroalgal community. Since the onset of SSWD, researchers have begun to consider the potential for impacts to community structure in in the intertidal via consumptive effects (Menge et al. 2016a, 2016b), and compensatory predation in multiple-predator systems (Hull and Bourdeau 2017, Gravem and Morgan 2019). Some studies have attempted to assess the relationship between *Leptasterias* spp. and *Pisaster* declines associated with SSWD (Gravem and Morgan 2017), and the potential for TMIIs from *Leptasterias* spp. on *Tegula* (Gravem and Morgan 2017, 2019). However, these studies have not assessed the potential for TMIIs from *Pisaster* via *Tegula* responses, or considered the potential for temporal variation in local *Pisaster*

density to affect the relative magnitudes of NCEs and TMIIs. My results indicate that *Pisaster* impose density-dependent NCEs on *Tegula* and TMIIs on their food resources in the natural environment. However, decreases in background *Pisaster* density will increase the attack ratio experienced by prey when they do encounter *Pisaster*, so low spatial *Pisaster* variation effectively increases temporal variation, which increases the magnitude of NCEs and TMIIs. Thus, as the northeast Pacific coast experiences patchy *Pisaster* abundance after the onset of Sea Star Wasting Disease, and recent recovery at some sites (Miner et al. 2018), NCEs and TMIIs from *Pisaster* will become more site and context-specific.

In conclusion, my thesis indicates that *Pisaster* density variation can affect NCEs and resulting TMIIs in a site and context-specific manner, contributing a novel finding to the growing bodies of literature considering the role of NCEs and TMIIs regulating community structure in natural environments, and the ecological impacts of SSWD in intertidal communities.

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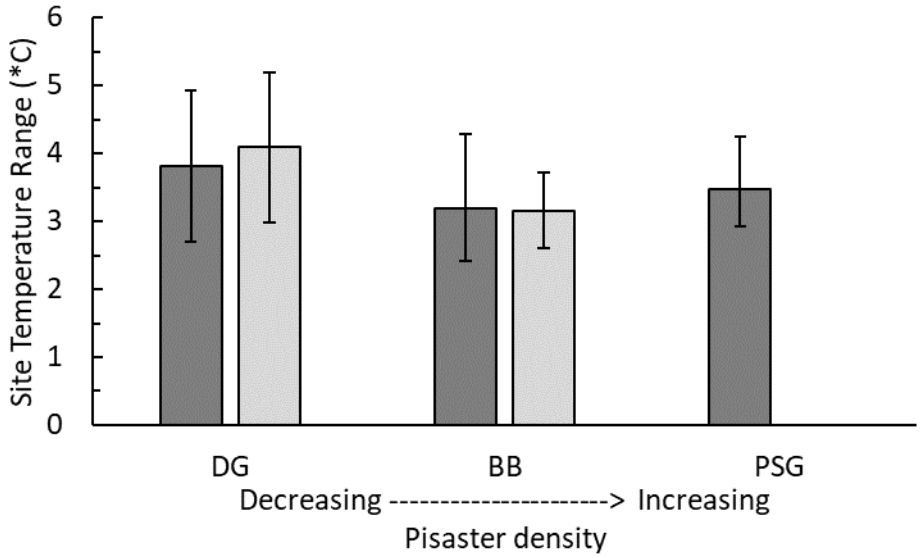
APPENDICES

Appendix A. Summary of regression results used to calculate non-destructive estimates of shell masses (Palmer's Method) used in field experiment analyses.

Response:	**Estimated Shell Mass (g)**			
Predictors:	*Estimate*	*SE*	*T*	*P*
(Intercept)	0.014	0.215	0.069	0.946
Buoyant Mass	1.871	0.075	24.99	$< \mathbf{2x10^{-16}}$
Observations	24			
R^2 / adjusted R^2	0.967/ 0.965			
Regression Equation	$Mass_{estimate} = 1.871(Mass_{buoyant}) + 0.014$			

Appendix B. Average daily temperature ranges for each site collected between 24 July and 20 August, 2017. Dark bars represent acute-pulse plots, light bars represent background-only plots, error bars represent 95% confidence intervals. Daily max temperatures ranged from 14.79-16.22*C, and min temperatures ranged from 11.63-12.75*C. Unfortunately, I did not recover the iButton from the PSG background-only plot.



Appendix C. Table of 95% confidence intervals for per-capita cumulative grazing for background-risk only plots at each site.

Site	Lower bound	Upper Bound
Pt St George	-0.714	2.51
Bakers Beach	1.95	3.76
Devil's Gate	7.18	8.15

Appendix D. Table of 95% confidence intervals for per-capita cumulative grazing, soft tissue growth, and shell growth differences between acute and background-risk treatments at each site.

	Per-Capita Cumulative Grazing		Soft tissue growth		Shell growth	
Site	Lower bound	Upper Bound	Lower bound	Upper Bound	Lower bound	Upper Bound
Pt St George	0.14	2.92	-0.27	0.004	-0.11	0.18
Bakers Beach	-1.79	-0.69	-0.22	-0.10	0.06	0.16
Devil's Gate	-7.74	-1.28	-0.23	0.08	0.02	0.15

Appendix E. Table of bootstrapped 95% confidence intervals for cumulative grazing, soft tissue growth, and shell growth differences between Total Predator and TMII-only treatments at each site.

	Cumulative Grazing		Soft tissue growth		Shell growth	
Site	Lower bound	Upper Bound	Lower bound	Upper Bound	Lower bound	Upper Bound
Pt St George	-2.37	40.17	-0.35	0.39	-0.07	0.44
Bakers Beach	-25.04	10.65	-0.15	0.19	-0.13	0.20
Devil's Gate	-20.34	-9.72	0.08	0.37	-0.33	-0.07