

**A Historical Intertidal Study on the Effects of Increasing Temperature on *Katharina tunicata*
Abundance and Related Population Dynamics**

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Abstract

The intertidal zone is a stressful environment putting forth both terrestrial and marine stressors. Factors of climate change, specifically increases in temperatures, are expected to exaggerate these stressors. *Katharina tunicata* is a keystone species in the intertidal that contributes greatly to community dynamics, where even a change in body size frequency of *Katharina* caused by increasing temperatures could influence other species' populations within the community. This research involves measuring body sizes of *Katharina*, the changing *Katharina* populations, and their interactions with limpet density and algae coverage.

Introduction

The intertidal zone is one of the most stressful environments on Earth. Experiencing abiotic factors of both terrestrial and marine environments, species living here must be well adapted to constant change (Helmuth, 2006). Organisms must be able to endure increases in air temperatures, direct and intense sunlight, and low levels of moisture to avoid desiccation (Sara, 2017). All of these factors are dependent on decreased water levels, which is dependent on tide cycles. Because of the dynamic abiotic factors, the intertidal zone is an ideal place to perform experiments and observational surveys when researching the resilience of organisms. The intertidal community structure represents how species react to extreme conditions over short periods of time, because more resilient species will be in areas that could be flooded or sun exposed depending on the tides, while more sensitive species will be found in areas with more constant environments (Sara, 2017). Furthermore, researching species and community responses to extreme conditions can contribute to understanding the potential effects of climate change (Ohlberger, 2013).

Climate change involves many factors such as the increase in average air temperature, an increase in carbon dioxide in the sea, and more extreme seasonal changes, all of which are attributed to an increase in greenhouse-gas production and natural resource depletion (Betts et al., 2016; Helmuth, 2006; IPCC, 2013). As climate change involves the fluctuation of mostly constant variables in a stable environment, the same stressors that are normally changing in the

intertidal zone will show even extreme effects where similar variables are changing anyway (Sara, 2017).

Increasing temperature is an effect of climate change that is a limiting factor on many processes of animals (Gardner, 2011). One of these processes is body growth, as smaller body sizes are associated with warmer water and air temperatures (Gardner, 2011; Ohlberger, 2013). One general rule with animal growth is “Hotter is Smaller,” where hotter temperatures play a role in the plasticity of phenotypical growth (Huey, 2008). Growth is affected by environmental conditions, where a predicted body growth rate curve has a negative slope with final size against relatively high temperatures (Huey, 2008). With warming temperatures, metabolic demands of animals increase, and research is lacking in showing that animals will be able to meet their nutrient quota, on which growth is dependent (Sara, 2014). This idea would be exacerbated with larger sized animals. Body size of individuals in a population drives ecological processes and structures; if a decrease in mean body size of individuals were to occur due to an increase in temperature, this could potentially lead to a decrease in mean body size of populations and an increased proportion of species that are historically smaller (Daufresne, 2009; Lindmark, 2018). As the result of different optimum growth temperatures and acclimation abilities, some species live in more extreme and changing conditions than others (Ohlberger, 2018).

Katharina tunicata is a keystone species in the intertidal zone and could cause such an effect if its population experienced shifts in the intertidal zone (Duggins and Dethier, 1985). *Katharina* structure the intertidal habitat as a relatively large herbivore that grazes and controls diatom and algal coverage and interacts with smaller grazers such as limpets (Duggins and Dethier, 1985). More specifically, by feeding on brown algae, *Katharina* control such kelp populations (Duggins and Dethier, 1985), and thus enabling limpets to recruit more successfully (Lewis and Bowman, 1975). Conversely, when *Katharina* feed on less kelp, kelp grows more abundantly, making the habitat less hospitable and lacking in nutrient availability for limpets, possibly leading to a decrease of individuals in a limpet population, known as a topdown effect (Defraunse, 2009).

In this study, we compared current body sizes of *Katharina tunicata* at Pile Point, San Juan Island, WA, to those measured by Duggins and Dethier (1985). Associated with increases

in temperature, we expected to see: (1) a decrease in the average body size (2) a shift in the *Katharina* to limpets ratio; and (3) a change in the community structure due to a top-down effect. While we may not be able to pinpoint a single mechanism that causes these changes, we will consider our findings in a broader context, including temperature increases. This research will investigate the strength of direct and indirect effects by observing the population dynamics of *Katharina* populations and body sizes, limpet populations, and algal coverage.

Methods

Location

Pile Point on San Juan Island has relatively low levels of local anthropogenic stressors like trampling, recreational activities, and commercial harvesting of any organisms. Direct anthropogenic effects are minimized, while indirect effects, such as those tied to climate change, are more apparent. Detrimental effects on organisms we see are indirect anthropogenic effects that are commonly known as climate change, specifically surface level sea temperature increases, sea level rise, and dissolved calcium carbonate levels.

Contemporary Procedures

Using Duggins and Dethier's 1985 published research, we re-surveyed the same areas that were used as experimental areas for *Katharina* removals and control to study the trophic effects. In Duggins and Dethier's 1985 paper, four experimental zones are referred to as "Removal Zone A", "Removal Zone B", "Control Zone A", and "Control Zone B". These area names were used for the sake of accuracy. As this is not an experimental study, no organisms were removed from their original location. I will refer to each zone as Area RA, RB, CA, or CB, respectively. We consulted the authors of the historical study to confirm the exact locations of these zones at Pile Point, San Juan Island, WA (latitude, longitude). See Fig. 1 for coordinates of each site.

Duggins and Dethier's (1985) methods included delineating an area and choosing a quadrat haphazardly by throwing a 0.1 meter square quadrat in the area. To avoid a "throwing"

bias, we divided each zone of 25 meters square into three areas, perpendicular to the shoreline, using transect tape. To determine where the 0.1 meter square quadrat would be placed, we used a random number generator along the transect tape. If the randomly generated number was even, we went right, and left for odd numbers, from the transect tape. After determining the direction, we again used the random number generator to determine a distance to go between a distance of zero and 1.67 meters. This process was repeated along both transect tapes and in all four zones, so each area in each zone had four quadrats, making a total of eight quadrats. This was to ensure we had an equal chance of surveying a quadrat in the upper, mid, and lower intertidal zones of each area.

In each quadrat, we measured *Katharina tunicata* abundance, limpet abundance, and algae coverage. Limpets were counted in a single category for simplicity, as the reference study (Duggins and Dethier 1985) organized them in one group, and as they are difficult to differentiate between the several species. Algae coverage was specified into predetermined species to observe, such as *Saccharina sessilis*, *Phyllospadix scouleri*, various polysiphonous red algae, coralline algae, and crusts. In addition to measuring *Katharina* abundance, we also measured body lengths without removing the chiton to impose as little disturbance as possible. We collected body lengths of *Katharina* in Area RA and Area CB and went along the two 25 meters transects with a one meter square quadrat, rounded to the nearest whole centimeter, replicating Duggins and Dethier (1979, unpublished data).

We took tidal heights of every quadrat, relative to the waterline. We recorded the time for every measurement we took. Out of the field, we looked up the tide heights on NOAA for Kanaka Bay and converted our measurements to result in how high the quadrat location was relative to MLLW.

Data Analysis

From Duggins and Dethier's published paper (1985), we used Table 1 to compare algal coverage. Using the raw data of Megan Dethier's files, we used unpublished data on the initial surveys of abundance per quadrat that included algal coverage and number of limpets.

We used RStudio for statistical analyses and figure designing. We installed the “tidyverse” and “ggplot2” packages and used the libraries “tidyverse”, “tidyr”, and “dplyr”, and “ggplot2”. We calculated summary statistics for algal coverage and configured boxplots to visualise the changing medians of data over time. The boxplots we created have whiskers that represent 1.5 times the interquartile range, where the interquartile range is shown by the box. The outliers are anything greater than 1.5 times the interquartile range. We made a size frequency distribution using a scatter plot instead of a traditional histogram to better demonstrate the trends between 1979 and 2018 side by side.

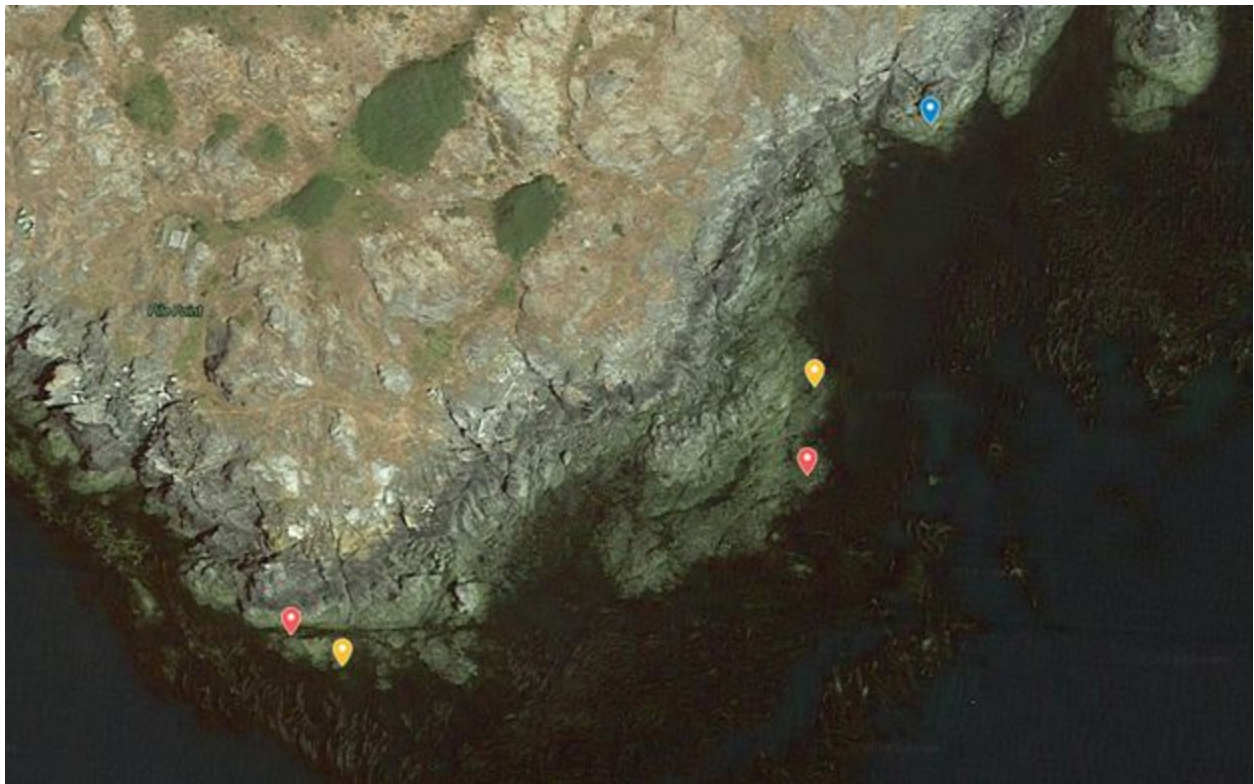


Figure 1: This is a map of study areas at Pile Pt., San Juan Island, WA. This map was made using Google Earth.

Blue = addition

Red = removal

Yellow = Control

Right to left: Area RA, Area CB, Area RB, Area CB, Addition Area (where we did not perform any surveys).

Table 1: Here we have coordinates of each area listed in the paper. We found these with David Duggins and Megan Dethier in April 2018. A mobile cellular app Gaia was used to report coordinates, and Google Earth was used to make the map.

Location name/Color	Latitude	Longitude
Removal Zone A (Red)	48.48169	-123.09373
Control Zone A (Yellow)	48.48163	-123.09358
Removal Zone B (Red)	48.48200	-123.09222
Control Zone B (Yellow)	48.48217	-123.09220
Addition Zone (Blue)	48.48268	-123.09186

Results

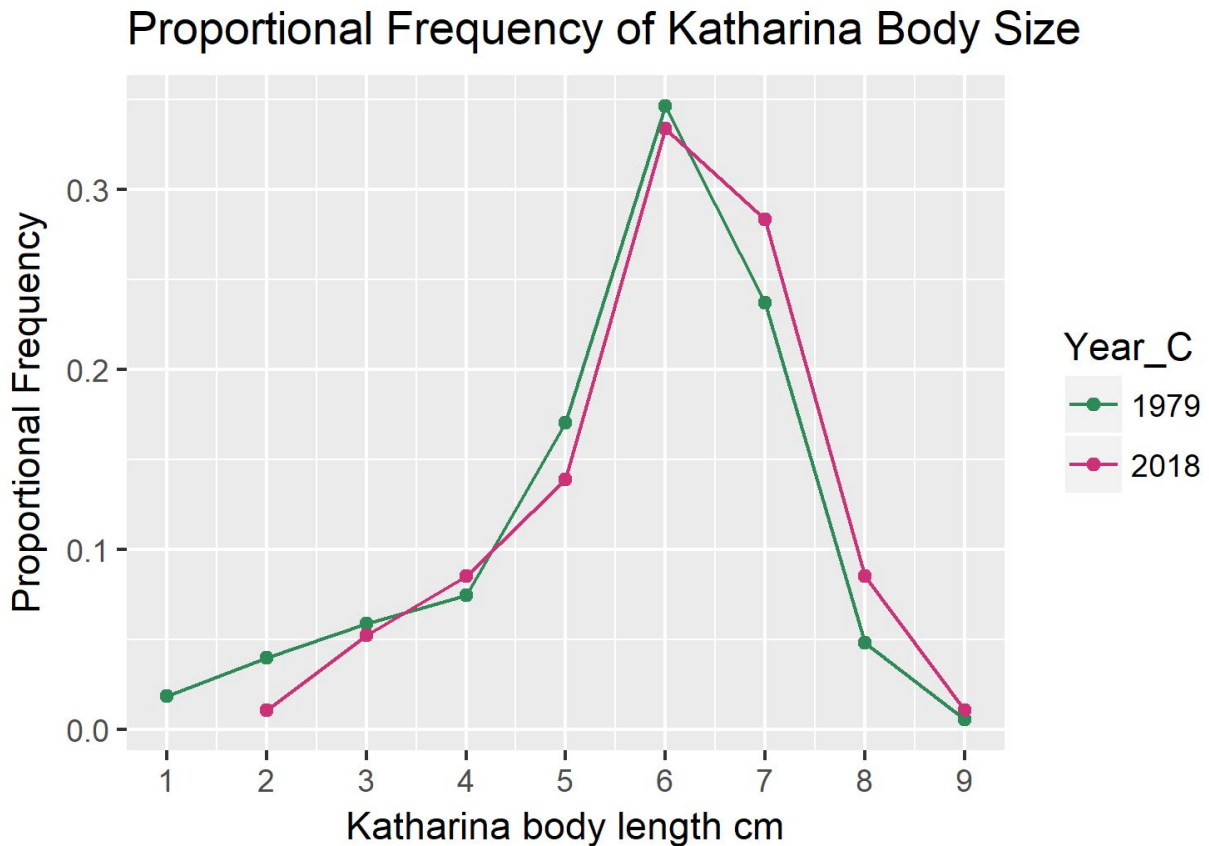


Figure 2: We compared the proportional distribution of *Katharina* body lengths in each area, by era. $n = 575$ and 375 for 1979 and 2018, respectively.

Fig. 2 shows the pattern in frequency of body lengths among *Katharina* is the same in 2018 as in 1979. The y-axis shows body lengths we measured to the nearest centimeter. The x-axis is the proportion of individuals of all the *Katharina* we sampled. A single point on the graph is a representation of the proportion of *Katharina* that are a certain length. We see that the most frequent measurement is six centimeters in both 1979 and 2018. Measuring a *Katharina* of nine centimeters and two centimeters or less seemed to be as rare in our 2018 measurements as they were in 1979. We can see a little fluctuation between historical or present body lengths being consistently larger or smaller, but the overall pattern remains unchanged.

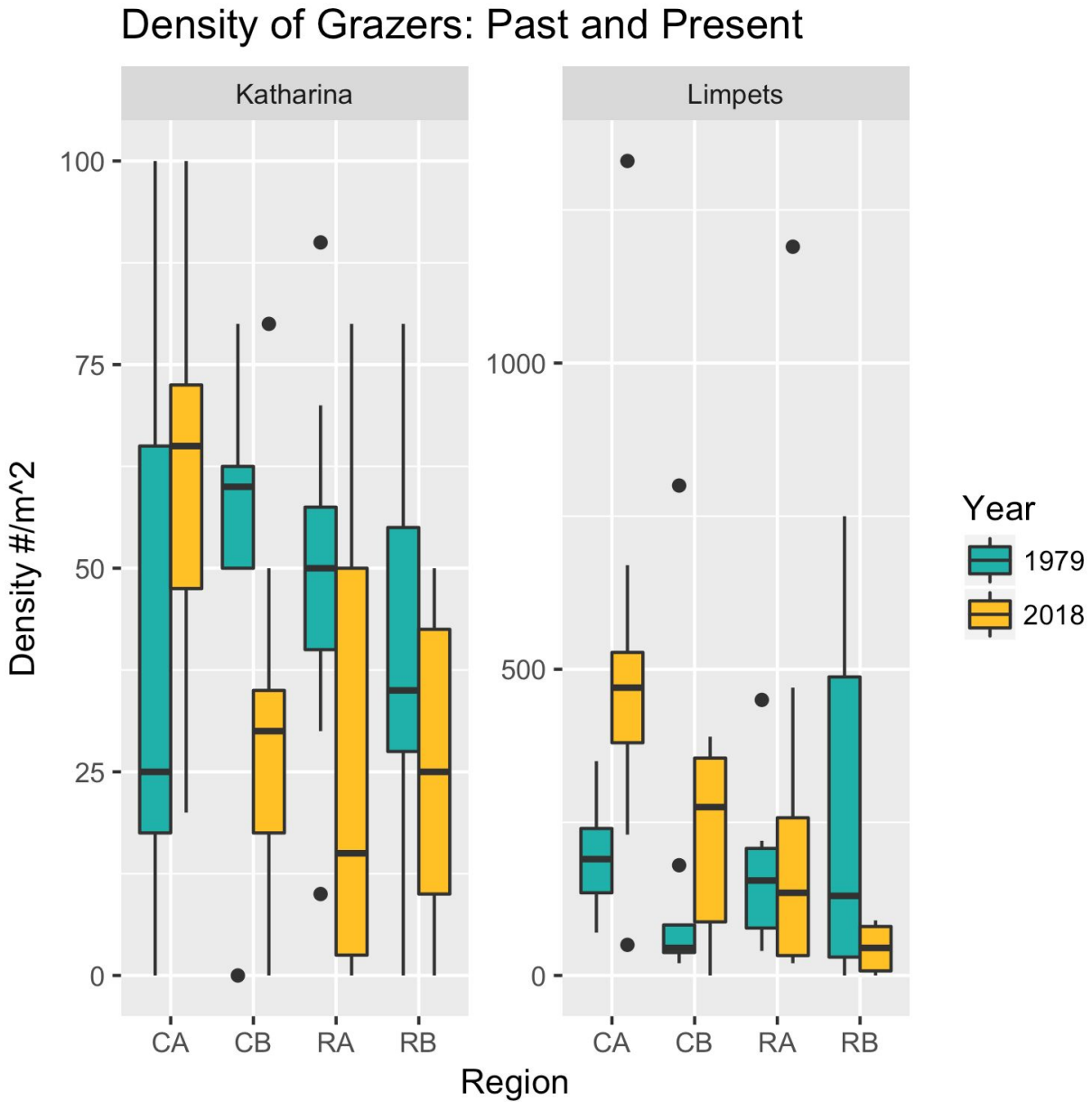


Figure 3: This graph demonstrates the density of grazers per meter square, where grazers are *Katharina* and limpets, and density per meter square has been normalized from 0.1 meter square quadrats. Compared by year.

Across our four study areas, we observed an overall decline in *Katharina* population density between 1979 and 2018. With a mean density of 45.8 *Katharina* per meter square (standard deviation = 7.07) in 1979, the decreased trend showed us that now the mean density is

only 45.8 (standard deviation = 70.7) by 2018. There was a decrease in *Katharina* density in Areas CB, RA, and RB from 1979 to 2018. Limpet density experienced a decrease only in Areas RA and RB during the same time period.

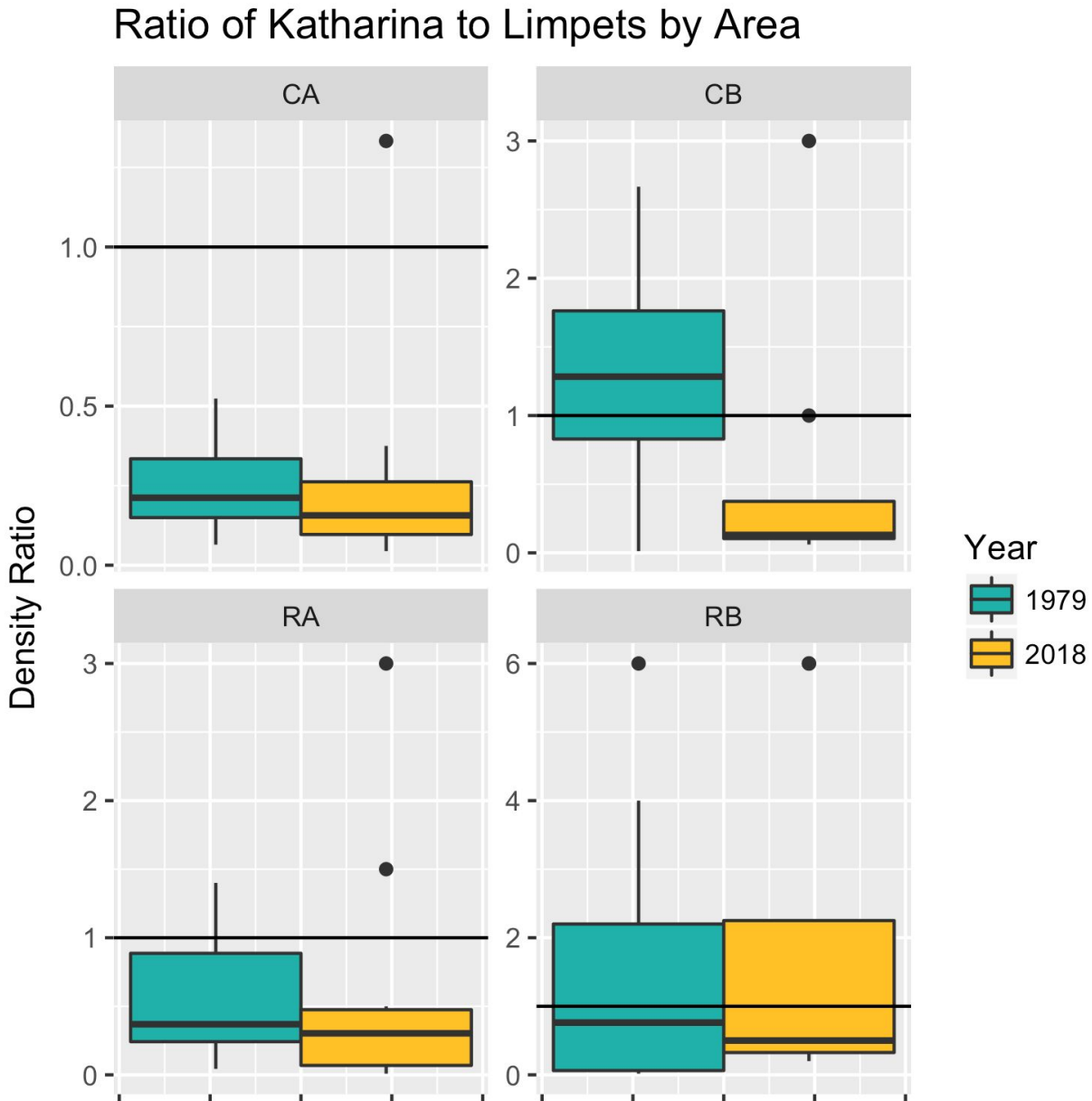


Figure 4: Ratio of *Katharina* to limpet populations plotted by year and area, where the boxplots represent the median ratio of each area.

Derived from our raw data, we made this boxplot to show the ratio of densities of *Katharina* to limpets, and overall we see that the median density ratios have stayed mostly the

same across the sampled areas. According to Fig. 3, we saw a decrease in the median of *Katharina* to limpets in every area we sampled. The horizontal line at $x = 1$ shows that a ratio equal to one would mean equal amounts of *Katharina* and limpets. A boxplot above the line shows that the *Katharina* population outnumbered the limpet population in the given area. If both boxplots are below the line, then limpets outnumbered *Katharina*.

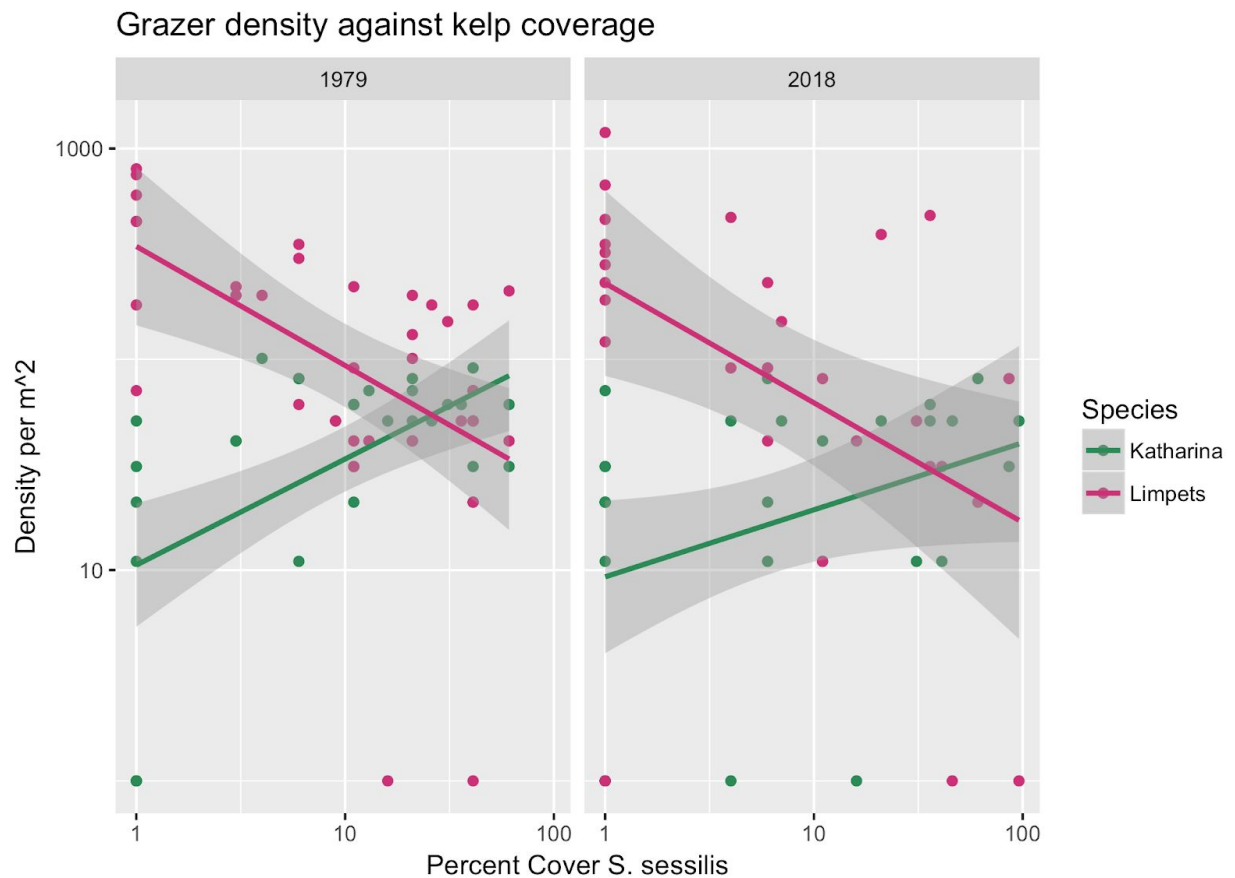


Figure 5: *Katharina* and limpet densities compared to each other against *Saccharina sessilis* coverage in 2019 and 2018.

We saw an opposite trend for *Katharina* and limpets in the areas more dense in *Saccharina*. Fig. 5 demonstrates an overall decrease in individual limpets while *Saccharina* increases, while the opposite occurs for the increasing numbers of *Katharina* in areas populated heavily by *Saccharina*. The x-axis shows the percent coverage of *Saccharina* we measured, and the y-axis shows density of grazers per meter square that has been normalized from the

individual grazers we counted in each 0.1 meter quadrat. Both axes are on a logarithmic scale to better read the data. We used log-scales on both axes to better visualize the data and normalize large outliers.

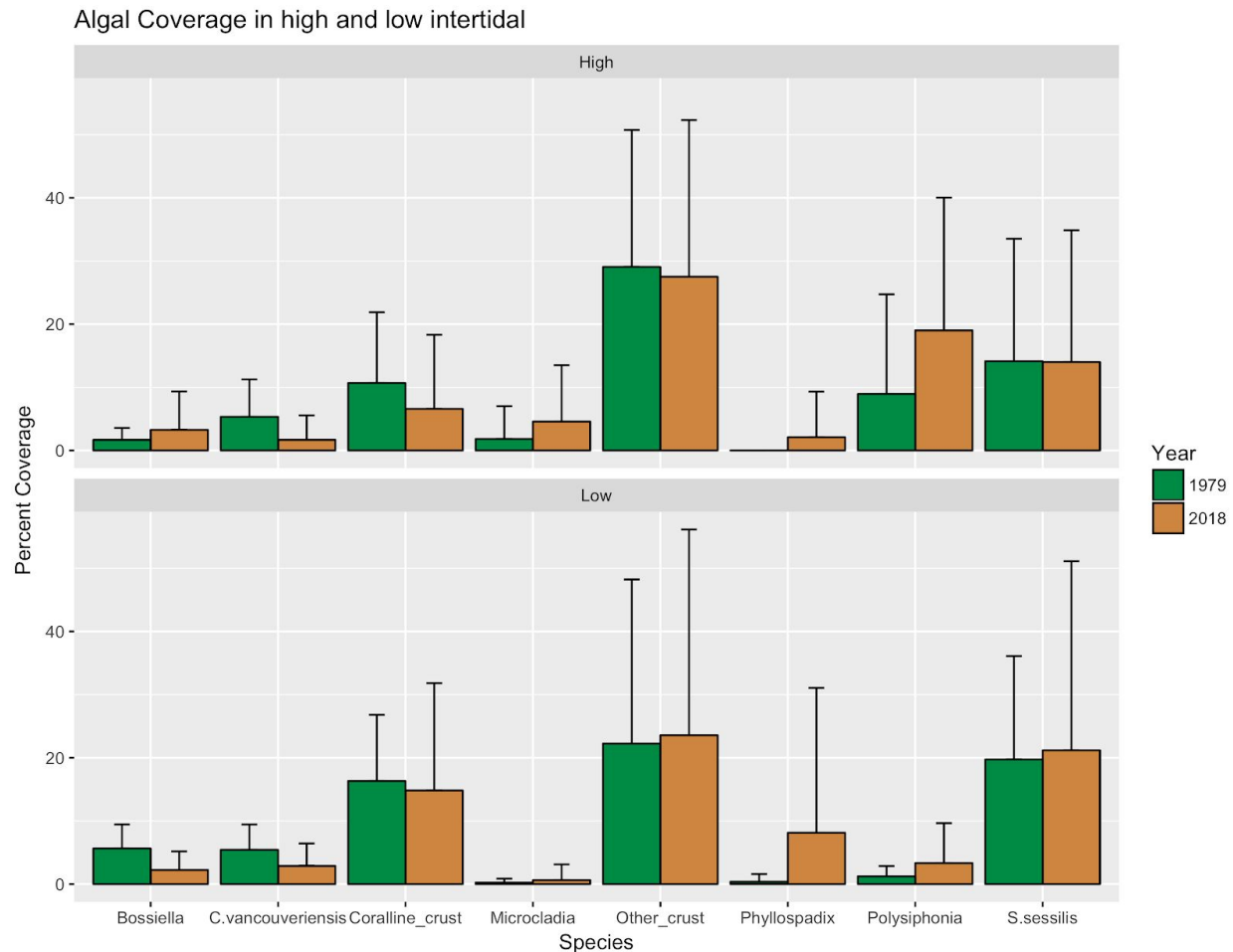


Figure 6: Comparing percent coverage of algae in quadrats of upper and lower tidal heights in years 1979 and 2018, where upper tidal height is above 0 MLLW and lower is below 0 MLLW.

Figure 6 shows an overall consistency in algal coverage from 1979 to 2018. We saw variation in the tidal zones, but this was mostly consistent with the year. In Fig. 6, we saw an seven percent increase in *Phyllospadix* in all around the areas, in upper and lower quadrats. All other algae remained approximately the same over time. Algae that seemed to vary the most between upper and lower is *Saccharina*, which was more prevalent in lower quadrats, and *Microcladia*, mostly present in upper quadrats.

Discussion

As Fig. 2 shows, the body size of *Katharina* in the study areas have remained unchanged throughout the forty years. The graph shows both years impressively with the same shape, leading me to believe that increases in temperature do not affect *Katharina* on this small temporal and spatial scale. According to National Centers for Environment Information, the yearly average air temperature has increased by 3.0° F from 1979 to 2016. This increase, however, is not shown as a constant increase, but rather inconsistent fluctuation throughout the near forty years. We should look further into the monthly patterns of air temperature, specifically in April and May when Duggins and Detheir did their initial surveys in 1979 and when we have performed our surveys this year.

An idea to consider is that *Katharina* are known for their durable shell, which has been researched to be resilient in the face of climate change in terms of ocean acidification (Sigwart et al., 2015). *Katharina* show to have a strong growth process to be able to secrete an equally strong shell in conditions with heightened carbon dioxide concentrations (Sigwart et al., 2015). Therefore, I hypothesize that *Katharina* are expected to withstand factors like increased temperatures with their strong growth process in future research.

Katharina densities have decreased in three of the four areas we sampled. This could be due to a population shift in density, where larger body herbivores are no longer favored in population sizes (Daufresne, 2009). Limpet densities have increased in two of the areas, but decreased in the other two areas. Acknowledging a trend and attributing a reason to that trend is difficult here. This could be due to geographical differences, making the area less accessible, or differences in algal coverage that limpets do not favor. A study with a larger temporal scale would be beneficial in identifying a strong pattern of limpet populations far past this two week survey has.

The ratio of *Katharina* to limpets has decreased in every area we sampled from 1979 to 2018, with a much more noticeable decrease in Area CB. In 1979, Area CB had more *Katharina* than limpets, but experienced a shift to more limpets than *Katharina*. This demonstrates Daufresne's published ideas that individuals of smaller sizes experiencing increases in temperature will be favored for, thus showing a population shift in favor for their species. We

may have also expected to see a decrease in limpets as *Katharina* populations have decreased. *Katharina*'s presence is important to a population of limpets because *Katharina* are able to graze down the macroalgae that the limpets cannot eat or move around (Duggins and Dethiers, 1985). However, we did not observe this pattern, but would potentially see it in the future if replicating the experimental design by removing *Katharina*.

When comparing grazer density to coverage of *Saccharina sessilis*, we see a positive correlation with *Katharina*. *Katharina* rely on young *Saccharina* as a major food source and older, larger *Saccharina* as protection from large predators or environmental stress, explaining why we see a positive correlation in *Saccharina* coverage and *Katharina* densities in the same area (Duggins and Dethier, 1985). Burnaford (2004) attributed findings of greater *Katharina* densities in highly covered *Saccharina* areas during spring and summer months during an experiment, where the “open” areas where, conversely, heavily populated only during months without sun exposure. To observe these conclusions, we would have needed to document the sun exposure in the quadrats, which would be an area of improvement for this study if it were repeated.

Unlike *Katharina* in an area of *Saccharina*, we observed a negative correlation with limpet density and *Saccharina* coverage. Limpets have a reduced variety of algae prey they can eat, unlike chitons that have a radula that allows for macro-algae consumption (Duggins and Dethier, 1985). Research is sparse in suggesting that limpets use *Saccharina* as protection from predators and environmental stressors. Taking only their dietary needs into consideration, our findings that limpet density decreases with *Saccharina* coverage makes sense. However, our observations included notes about seeing higher densities of limpets in areas with increasing coverage of encrusting algae, which would be a more habitable area based on the microalgae needs of limpets (Duggins and Dethier, 1985).

This information about Fig. 5 does not support our third hypothesis, where we predicted a decrease in *Katharina* sizes would cause a top down effect, ultimately causing a decrease in limpet populations. While we did see negative correlation between limpets and *Saccharina*, our information did not show an increase in *Saccharina* coverage (Fig. 6). Therefore, we cannot conclude that limpet populations were decreasing across Pile Point because *Saccharina* was

increasing. We would need to further a study more intensely focused on a greater spatial scale that can sample more areas, in addition to the four we sampled.

The algal coverage of areas across Pile Point seemed to be unaffected by temperature increase, with few exceptions. *Phyllospadix scouleri* is one of the few species that has had a noticeable increase at Pile Point. Anecdotally, the lower intertidal had far less *Phyllospadix* in 1979 as in 2018 (M. Dethier, personal communication). Due to a sampling affect, we cannot see the true amount in the lower intertidal, but its presence was still significant enough to see a difference from the historical data. In the quadrats with high percentages of *Phyllospadix* we did not find a high abundance of *Katharina* or limpets. As mentioned earlier, *Katharina* have a tendency to populate kelp-filled areas. *Phyllospadix* shades the area that brown algal species grow in, making the habitat inhospitable for the algae, therefore lacking in nutrients for *Katharina* (Hori, 2006). Furthermore, the *Phyllospadix* shoots require a generous amount of space for such large populations, possibly creating competition for space with the *Katharina*.

Overall, the environment at Pile Point remains unchanged from 1979 to 2018. Increasing temperatures is a trend in the world (IPCC 2013), but average temperatures have fluctuated greatly at Pile Point. This could be the reason why we see such a stable environment. If temperatures are not steadily increasing, the chance of body sizes decreasing, population proportions shifting, and top down effects changing community dynamics are less likely. This research is important in seeing the resilience of *Katharina* and understanding their role as a keystone species in a stressful environment. We have also noticed a great inconsistency with limpet populations and drivers of population dynamics, so a study with a focus in their community, possibly long-term, experimental research, would provide more insight.

Recognizing the unique environment at Pile Point is important. Under stressful conditions, that fluctuate on yearly averages and are increasing overall, individual organisms are resilient. We see this result in strong and stable populations. Further research in this area would be beneficial in understanding conservation aspects. In that, ensuring that rare, untouched environments should continue to be separated from direct anthropogenic changes to cause as little disturbance to intertidal communities.

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