The Urch- to Eat: Effect of encrusting bryozoans on the growth and feeding behavior of green urchins

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Abstract

Symbiotic relationships are a fluid part of all ecosystems; two species' biological relationship contributes to their role in shaping the dynamics of the ecosystem. Sometimes a symbiotic connection can help both species (mutualism), one species can benefit at the expense of another (predation or parasitism), or in some cases, one species can benefit while the other is unaffected (commensalism). Kelp forests are biodiverse habitats that are home to a multitude of vertebrate and invertebrate species. One of the most common and dominant invertebrate grazers of kelp forests is *Strongylocentrotus droebachiensis*, the green sea urchin. *S. droebachiensis* feed on various seaweed species, including the kelp *Agarum fimbriatum*. The epiphytic bryozoan, *Membranipora membranacea*, often lives on the same blades of the kelp on which green sea urchins feed. We quantified *S. droebachiensis'* herbivorous relationship with kelp, investigating the symbiotic interaction between *A. fimbriatum* and *M. membranacea*. We used laboratory feeding experiments to assess urchin growth and feeding on kelp with and without encrusting bryozoans. In no-choice feeding experiments, the urchins consumed more of the bryozoan and kelp diet than those offered a kelp-only diet. This observation of increased urchin feeding on bryozoan-encrusted kelp blades suggests that *M. membranacea* may have a parasitic relationship with the kelp *A. fimbriatum*, possibly deriving benefit at the kelp's expense by making it more likely to be consumed. Still, we did not detect a statistically significant difference in urchin growth between the two diets. Continued analysis of the interaction between *A. fimbriatum* and *M. membranacea* will help further explain this symbiotic relationship and how it has affected and may continue to influence kelp forest ecosystems in a changing ocean.

Introduction

All ecosystems have symbiotic relationships, which arise when the biological connections between two species support their ecological roles in the ecosystem. Symbiotic relationships are often shared between two or more organisms of different species and can generally be defined by three types of relationships: mutualism, in which both species benefit; commensalism, where one species benefits and the other is unaffected; parasitism, in which one species benefits at the other's expense. These symbiotic relationships help to format the function and biodiversity in an ecosystem (Chomicki et al., 2022).

Kelp are keystone species and kelp forests significantly support primary production, act as strong carbon storage, and are also hosts for rich biodiversity in the marine environment (Pfister et al. 2018). A variety of invertebrate and fish species can find food and refuge in kelp beds, which are species-rich ecosystems with many symbiotic relationships. Species interactions in kelp beds can influence the health of the kelp itself as well as other species that live in these biodiverse habitats. It is important to better comprehend urchin grazing preferences and their relationship to the health of kelp beds because considerable grazing by urchins can negatively impact kelp beds (Scheibling & Hatcher, 2007).

The green sea urchin, *Strongylocentrotus droebachiensis*, has a very broad distribution across both the North Atlantic and Pacific oceans, through North America, Iceland, Scotland, Norway, Denmark, Sweden, Siberia, and the Aleutian Islands, among others (Scheibling & Hatcher, 2001). For the purpose of this paper, we focused on *S. droebachiensis'* range on the western coast of North America in the Salish Sea in

Washington State. Green sea urchins are omnivores, eating mostly kelp and having a significant effect on the overall composition of marine communities (Scheibling & Hatcher, 2013). Along the edges of kelp beds, urchins can form enormous mobile feeding aggregations that destructively graze kelps and any accompanying bryozoa (Knip, 2007). Urchins are often found in areas with limited access to food, making populations of macroalgae even more scarce (Suskiewicz, 2017). *S. droebachiensis* can play a significant role in determining the quantity and range of kelp species.

Membranipora membranacea, a type of encrusting bryozoan, also has a very large geographic range spanning both the Northern and Southern hemispheres (Schwaninger, 1999, 2008). We concentrated on *M. membranaceas'* range on the western coast of North America in the Salish Sea in Washington State for the purposes of this research. Pelagic *M.membrancea* larvae colonize kelp blades and rapidly grow to consume as much space as is availible on the blade. Recruitment of larvae happens during late spring and colonies continue to grow rapidly throughout the summer (Seed, 1976). The overgrowth of the *M. membranacea* weakens the kelp blade, making the kelp more susceptible to deforestation (Scheibling & Gagnon, 2009). If we are to better understand the environmental implications of the symbiotic relationship between *A. fimbriatum* and *M. membranacea*, observation of green sea urchin feeding and growth behaviors when given a diet with the encrusting bryozoan is essential.

Understanding the nuances of the kelp-bryozoan relationship can illuminate potential harm or benefit to the marine environment. If green sea urchins grow faster when consuming blades encrusted with bryozoan colonies or prefer to consume kelp with bryozoan-encrusted blades, it may influence an area's overall urchin and kelp populations, possibly providing insight into ecosystems where kelp, green sea urchins, and this encrusting bryozoan interact. In this study, we utilize *S. droebachiensis'* herbivorous relationship as a way to better understand the symbiotic relationship between *A. fimbriatum* and *M. membranacea*. Monitoring green sea urchins' feeding behavior, we tested if the presence of the encrusting bryozoan affected *S. droebachiensis*' choice in their diet and also if this difference in diet also affects the urchin's growth.

Methods

Growth Experiment

This experiment focused on *S. droebachiensis'* growth in test size and weight over a 27-day experimental period as they consumed sieve kelp (*Agarum fimbriatum)* with and without encrusting bryozoan colonies. We used 12 *S. droebachiensis* with test diameters between 18-27 mm and collected all urchins and kelp (*A. fimbriatum*) from the floating docks at Friday Harbor Laboratories in Friday Harbor, Washington.

We divided four 40.64 cm x 27.94 cm x 17.78 cm plastic tanks into four separate compartments of equal size,("wells"), to provide individual housing for three green sea urchins with one well set aside as a control. To create the wells inside of the plastic tank, we sized four pieces of 60.96 cm x 60.96 cm aquarium egg crate to fit snuggly at the bottom of each plastic tank. We placed four plastic tanks inside a 106.68 cm wide x 30.48 cm deep outdoor sea table to utilize a natural light schedule of 17 hours of daylight in Friday Harbor, Washington. On each side of the plastic tank about 25.4 mm from the top, I evenly placed three drainage holes to help create even drainage from each of the wells. We placed a 15.24 cm x 27.94 cm lid on each tank to prevent any wildlife or debris from possibly affecting the experiment. We used a system of PVC tubing to distribute water flow evenly in the sea table, creating a tubing system to ensure each urchin well received a similar flow of seawater. (Figure 1). To bring up the water level outside the tanks to regulate overall tank temperature, we installed a 19.05 mm PVC pipe cut to the height of 12.7 cm and ground down about 25.4 mm all around.

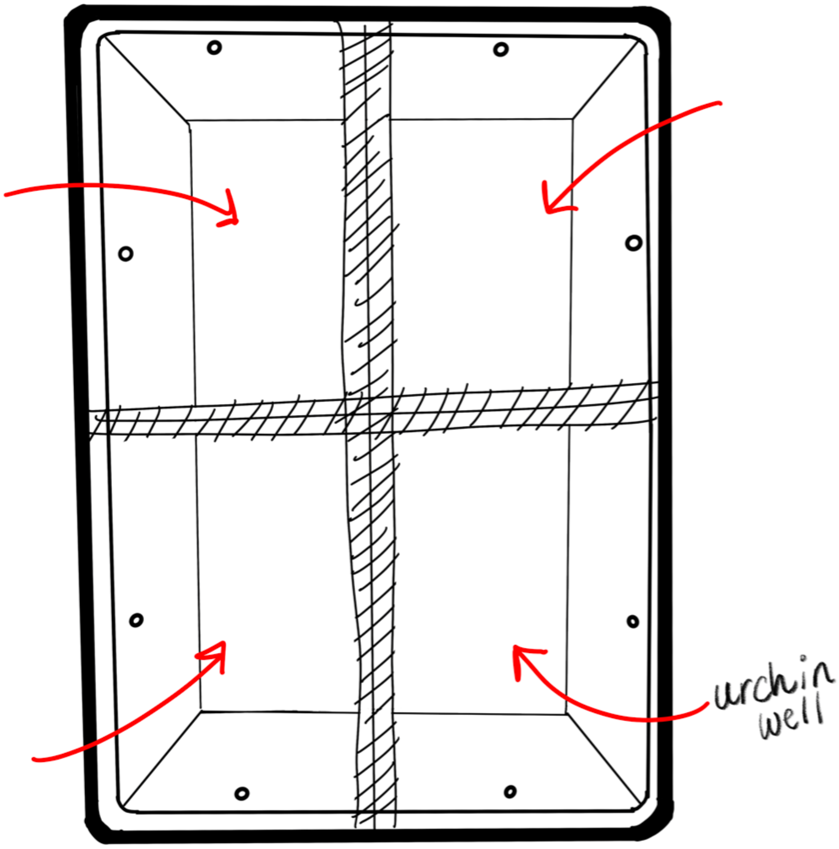

Figure 1; plastic tank; divided into four separate wells with mesh to monitor urchin growth on differing kelp diets.

We monitored the tank temperature daily using a digital aquarium thermometer (Vivosun). We also collected daily air temperature and ocean temperature data, utilizing live streaming data from the Northwest Association of Networked Ocean Observing Systems (NANOOS) Visualization System (NVS) website, the Friday Harbor Laboratories Ocean Observatory (FHLOO), and the UW Friday Harbor Laboratories Cantilever Pt station.

To mitigate any potential source of assignment bias, at the beginning of experimentation each urchin was randomly assigned a well number and a diet of *A. fimbriatum* with or without encrusting *M. membranacea* (Figure 2). To calculate the weight of the kelp, we patted it dry to eliminate as much excess water as possible and then weighed it on an electronic balance (OHAUS); the target weight for each sample was 25.5 g.

Figure 2; *Agarum fimbriatum*, fringed sea kelp, with *Membranipora membranacea* (a.) and without (b.).

Before placing an urchin into its assigned well, we recorded the wet weight (g) of the urchin by gently removing it from the holding tank and allowing the water to drip off for ten seconds. Utilizing this method allows for consistency between urchins and any change in growth during the experiment to be recorded accurately. We used a digital caliper to measure both the diameter and height of the urchin's test, gently maneuvering around the urchin's spines and placing it as close to the urchin's test as possible (Pearce et. al, 2004). We labeled urchins by placing a laminated 6.35 mm x 38.1 mm card with an identifying name in their respective well as a safeguard of keeping track of where each urchin would be. We also photographed each individual urchin with white waterproof paper as a background, a small ruler for scale, and the urchin's laminated name tag.

Every 3rd day of the experiment, we re-weighed urchins and measured to monitor any change. Every 6th day, we re-weighed and re-measured the urchins, replenished or refreshed the kelp, and changed/randomized the well assignments of the urchins. We rotated each urchin from its previous well and into a new well/tank to mitigate any possible effects of remaining in the same well/tank for the duration of the experiment. Each urchin's respective diet did not change during rotation, as we selected their specific diet at the beginning of the experiment and kept it consistent throughout. When urchins rotated, each urchin never visited the same well again while also changing the control wells, always having one control in each tank at all times.

Preference Experiment

Focusing on feeding behavior, we monitored 16 *S. droebachiensis* with test sizes between 25-36 mm over multiple six-day experiments to explore the preference of *S. droebachiensis* on kelp (*A. fibriatum*) with or without *M. membranacea*.

We used six 21.59 cm x 15.87 cm x 10.16 cm plastic tanks to house four *S. droebachiensis*, individuals, at a time with two containers being used as control (Figure 3). To best utilize the natural light schedule of 17 hours of daylight in Friday Harbor, Washington, we placed six plastic tanks inside a 106.68 cm wide x 30.48 cm deep outdoor sea table. I evenly distributed three drainage holes on each side of the plastic tank approximately 25.4 mm from the top to produce even drainage from each of the wells. To protect the experiment from any wildlife or debris, each tank had a 21.59 cm x 15.87 cm lid, we placed a lid on top. We utilized PVC tubing to equally distribute water flow in the sea table, and we also created a tubing system that we designed to ensure that each urchin received a similar flow of seawater. We fitted a 19.05 mm PVC pipe cut to a height of 76.2 mm and ground down around 25.4 mm on all sides to raise the water level outside the tanks and adjust the overall tank temperature.

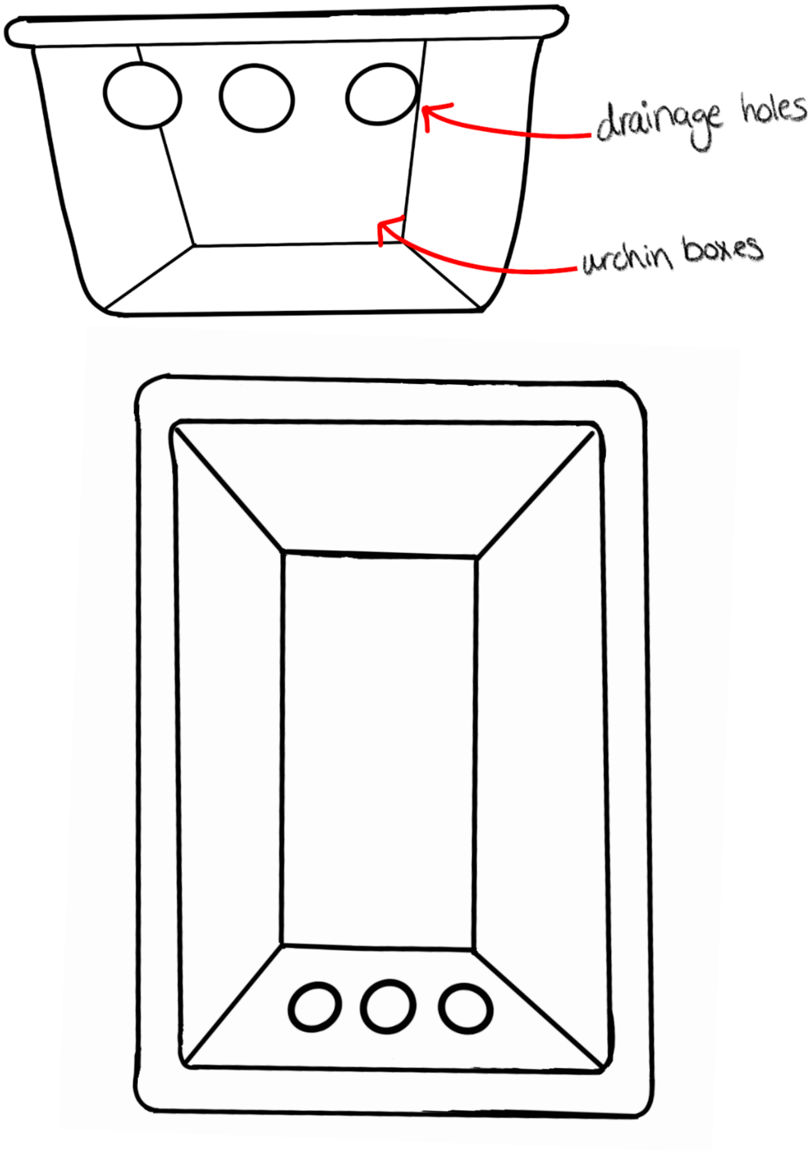

Figure 3; individual tanks for urchins preference experiment.

The urchin wet weight (g), test size (mm), and photographs, as well as kelp weighing methods, remained the same as in the growth experiment methodology. We also duplicated the daily tank, air, and ocean temperature data from the growth experiment data. We again collected all *S. droebachiensis* and all *A. fimbriatum* from the floating docks at Friday Harbor Laboratories in Friday Harbor, Washington.

Each preference experiment occurred in a "cycle"; each cycle consisted of 6 days in which we divided four urchins into four individual tanks and each fed their assigned kelp diet. Once the six-day cycle ended, I returned the four urchins to the collection site and a new set of four urchins entered the next 6-day cycle. The two other available containers served as controls with just kelp with or without *M. membranacea*. Each urchin had a laminated 6.35 mm x 38.1 mm card with an identifying name on it as a safeguard for keeping track of where each urchin would be.

The six-day cycle consisted of weighing, test measurements, photographs, rotation from one tank to another, and a 48hr starvation period (Figure 4). On days 1, 3, and 6, we weighed, measured, and photographed the urchins. On day 3, we also rotated the urchins into different tanks to mitigate any potential bias from remaining in the same container during the six-day experiment. On day 4, we set the next group of four urchins aside for a brief 48-hour starvation period to encourage the urchins to empty their stomachs and be more willing to eat the arranged kelp diet. On day 6, we promptly returned the urchins to the floating dock at Friday Harbor Laboratory. On this day, we also weighed, measured, and photographed the four urchins that we set aside to starve for 48 hours. We repeated the process for the four cycles of the experiment (n=16 urchins total). We duplicated the growth experiment methodology's urchin diet selection and rotation strategy.

	Weight (g)	Test Measurement (mm)	Photograph	Rotation of Current Group	48hr Starvation for New Group

Day 1	✔	✔	✔		
Day 3	✔	✔		✔	
Day 4					✔
Day 6	✔	✔	✔		
↪		**Begin**	**Cycle**	**Again**	↩

Figure 4; table exemplifying the 6-day cycle preference experiment routine. ✔ signifies that this task must be completed on the selected day. ↪ and ↩ signify the end of the cycle; we completed the cycle four times.

Results

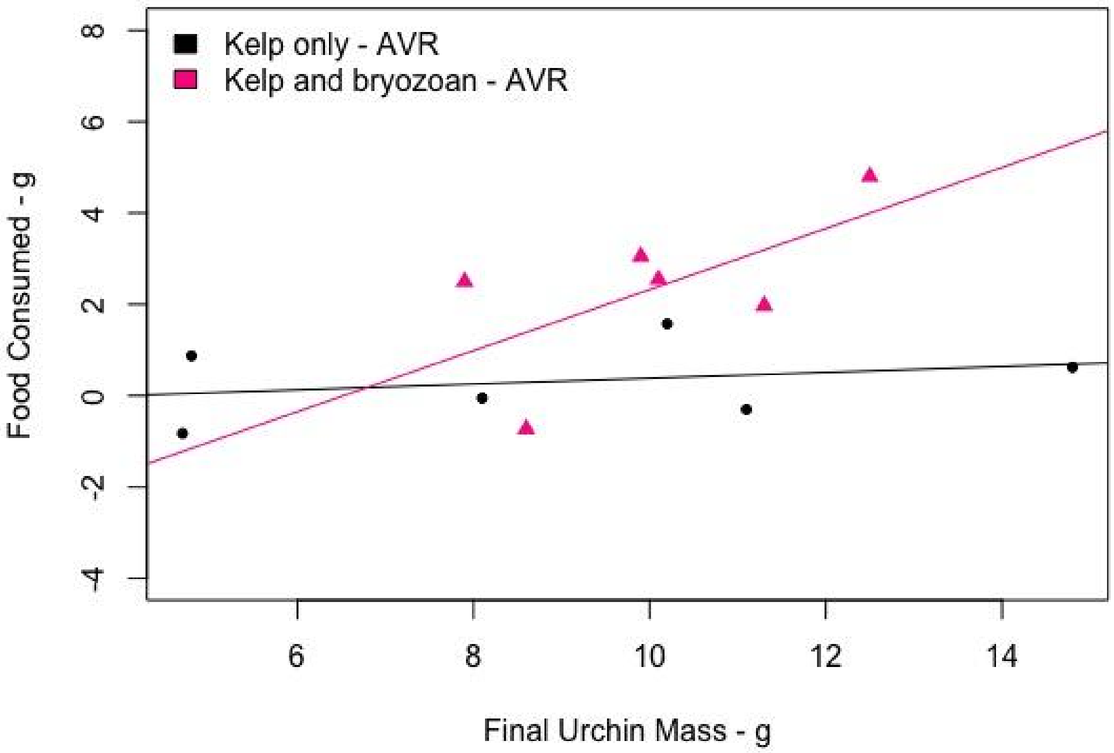

Figure 5; the size of the final urchin mass is plotted against the average total food consumed graph comparing the average of the diet consumed and urchin mass. The average kelp-only diet consumed is represented by the black circles ($r^2 = 0.3965$) accompanied by a black line that follows its trend. The average kelp and bryozoan diet consumed is represented by the pink triangles ($r^2 = 0.08242$) accompanied by a pink line that follows its trend.

In the growth experiment, we found a relationship between the average kelp consumption with bryozoan diet and urchin mass ($r^2 = 0.3965$). We also found no significant relationship between the average consumption of the kelp-only diet and urchin mass ($r^2 = 0.08242$).

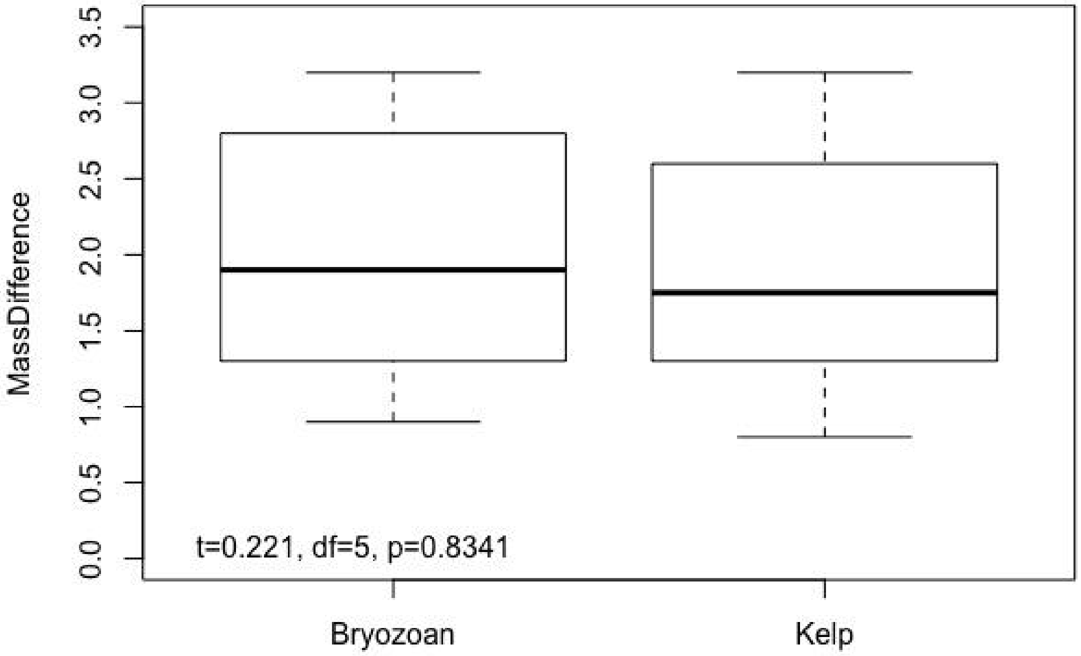

Figure 6; boxplot showing medians and interquartile ranges of mass (g) of *S. droebachiensis* of either diet-kelp or kelp with encrusting bryozoans (Bryozoan treatment). Urchins show no significant difference in mass when given a different kelp diet (paired t-test: t= 0.221, df=5, p-value=0.8341).

When measuring the growth of the urchins after the 24-day experiment, we found no significant relationship between diet and change in urchin mass ($p>0.01$). There is little to no difference in difference in urchin mass in relation to diet.

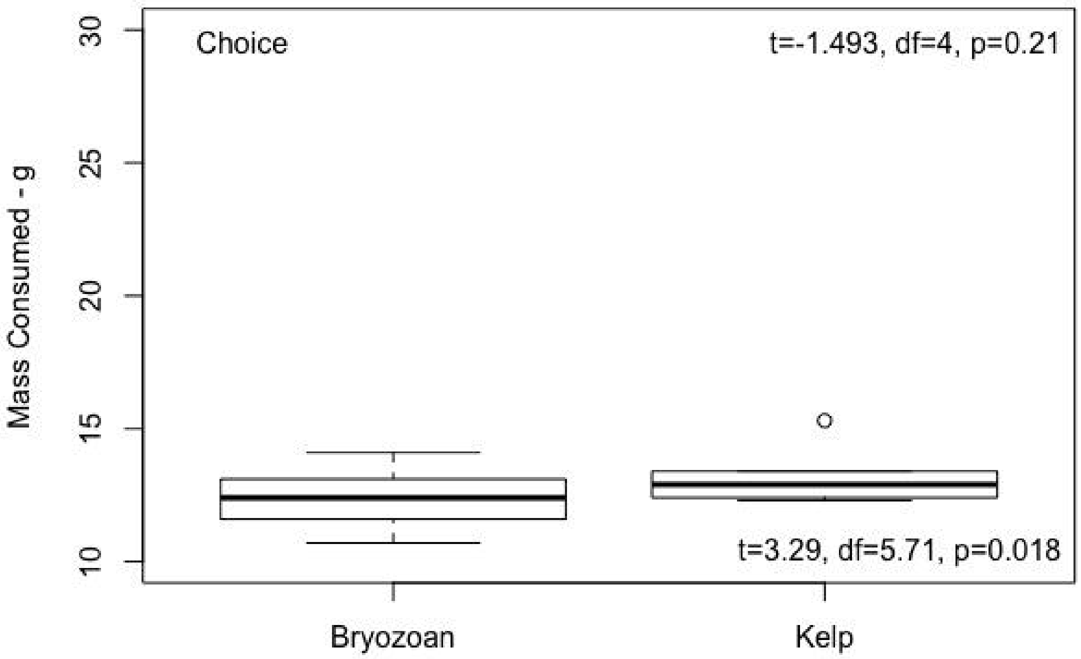

Figure 7; boxplot showing medians and interquartile ranges of mass (g) of kelp diet consumed (adjusted for the controls) by *S. droebachiensis* in choice experiments. Urchins consumed a small amount of both diets, each with slightly varying ranges (paired t-test: t= -1.493, df=4, p-value=0.21).

In choice experiments, when we offered urchins both food options to choose from, urchins showed no preference for one diet over the other (t= -1.493, df=4, p=0.21) as urchins consumed both diets. The bryozoan diet showed a more variable range (of mass consumed) and while the kelp diet has a less variable range, it did include one suspected outlier (which we included in the analysis).

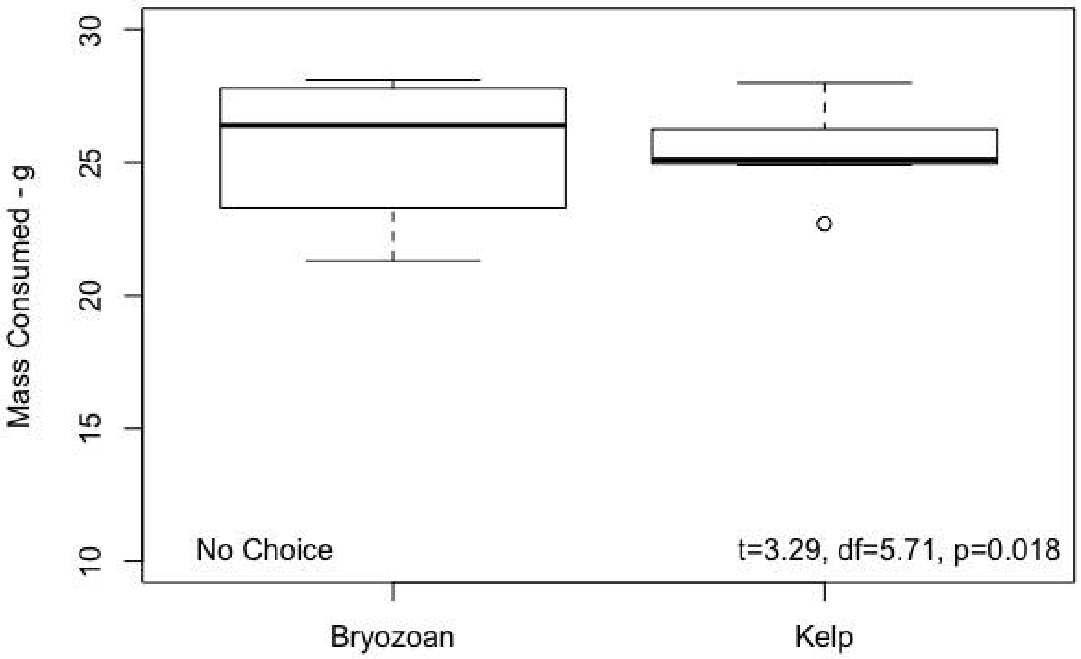

Figure 8; boxplot showing medians and interquartile ranges of mass (g) of kelp diet consumed (adjusted for the controls) by *S. droebachiensis* in no-choice experiments. Urchins consumed significantly more kelp with bryozoan (welch two-sample t-test: t=3.29, df=5.71, p=0.018). The unfilled dot represents a suspected outlier.

In the no-choice experiments, when we offered urchins only one food option, the urchins offered kelp with encrusting bryozoan colonies consumed more mass than the urchins that we offered the kelp-only diet (t=3.29, df=5.71, p=0.018).

Discussion

When given a choice, urchins will eat both kelp with and without bryozoans. When given no choice, urchins will eat whichever diet they are offered but the urchins given kelp with encrusting bryozoans will eat more mass on average. When measuring growth based on urchin diet, we found no significant change in mass between urchins that consumed a kelp diet with or without bryozoans utilizing the metrics we measured.

Analyzing diet consumed and urchin mass highlighted a potential relationship between the kelp with bryozoan and urchin mass (Figure 5). As the size of the urchin tests increased, we observed that larger urchins ate more kelp with bryozoan on average. With the kelp-only diet, urchin mass does not strongly correlate to food consumed. The increased mass consumed by urchins eating kelp with encrusting bryozoans brings into question the effect that consuming the encrusting bryozoan may have on urchins. The combination of the two food sources, or even the preferential consumption of the animal food source that just happens to live on kelp as a substrate, as the additionally added animal tissue provides a greater nutritional benefit (Knip, 2007).

To explore the possible effects of urchins consuming more kelp with bryozoan, we measured the test size and weight of each urchin and found no significant correlation between urchin mass and the offered diet (Figure 6). Utilizing the metrics we quantified for measurement of urchin growth and mass, the data collected did not help to explain the urchin's larger consumption on average of the diet of kelp with bryozoans. The data collected show no difference in urchin mass in relation to either diet.

As highlighted in Figure 7, when we provided urchins with a choice in diet between kelp with bryozoan presence or without, we found no significant preference in diet. When looking at the consumption of each kelp diet, we found a slight difference between diets. In kelp with bryozoan present, we found there is more variability in its consumption while the kelp-only diet has a noticeably less variable range. The data for the urchin choice diet experiment highlights that urchins could and did consume both diets; urchins consumed less food from each food option because they consumed both food options.

When given no choice, urchins consumed more of the single diet provided. In Figure 8, we observed a potential relationship between diet and food consumed. When comparing the consumption of one diet or the other, urchins offered the kelp with bryozoan diet consumed more. The urchins offered the kelp-only diet had less variation when compared to a larger range in food consumed with the urchins that we offered the kelp with bryozoan diet. This may be because consuming kelp with encrusting bryozoans possesses some benefit to the urchin. Certain algal-only diets may be preferred by urchins solely due to palatability and have no relation to available energy or protein in their food (Dworjanyn et al., 2007).

Overall, our data generates further questions about why exactly urchins are consuming more kelp with bryozoans when compared with kelp without bryozoan colonies. If urchins eat more kelp with bryozoans, this may mean that kelp with bryozoans is not as nutritionally favorable (so they must consume more) OR that it is more nutritionally favorable, leading to increased consumption. Other scientists have studied the effects of various diets on the gonad yield of *S. droebachiensis* and found that prepared diets are more nutritionally favorable and can increase the urchin's gonad yield. These prepared diets have consisted of dried kelp, soybean meal, corn grain, and calcium carbonate, among others (Lawrence et. al, 1997; McBride et. al, 1999). With these findings, we instead focused our study on urchin diet preference with the kelp *A. fimbriatum* with the presence of *M. membranacea* while also monitoring any test/mass effects.

The research we conducted aimed to better understand the symbiotic relationship between *A. fimbriatum* and *M. membranacea*. Our no-choice experiments suggest that green urchins eat more kelp with bryozoans, indicating that the relationship between the kelp and encrusting bryozoan may be parasitic. Bryozoans benefit from being able to colonize and flourish while living on the kelp, while the kelp is harmed by experiencing more consumption because of bryozoan presence. Additionally, kelp is increasingly stressed by warming water temperatures, and invertebrate consumers of kelp (like urchins) may experience increased metabolic rates as ocean temperatures rise, so this preference for encrusted blades may be some cause for concern. Increased herbivory in an increasingly abiotically and biotically stressful environment could endanger kelp populations and kelp forest health in the future.

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