Determining Optimal Conditions for Aquaculture of *Strongylocentrotus droebachiensis* through Nutritional, Behavioral, and Larval Studies

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Dietary and behavioral studies were conducted to determine the viability of the green sea urchin, *Strongylocentrotus droebachiensis*, as a value-added product in existing bivalve aquaculture sites in the Gulf of Maine. Experiments were run to determine which of three different macroalgal species *S. droebachiensis* grew best on. In another dietary study, growth rates were measured of urchins provided with an artificial wafer diet and *Saccharina latissima*. In the behavioral study, urchins were given different types of substrate to observe what they preferred to use for camouflage. During *S. droebachiensis*' breeding season, three larval spawnings took place to demonstrate the potential of raising urchins from eggs and sperm for use in aquaculture. *Saccharina latissima* was the preferred algal species of the three provided. Both the *S. latissima* and the wafers were successful in sustaining urchins. In the camouflage study, the only observed difference was between the number of urchins that were uncovered and those eating. Of the three spawnings performed only one produced any larvae that reached the juvenile stage. Raising *S. droebachiensis* for use in aquaculture is a time consuming and challenging task that would benefit from further research.

Introduction

During the 1970s, in the Canadian Maritimes, there was a rapid increase in populations of the green sea urchin, *Strongylocentrotus droebachiensis*. This led to overgrazing of the benthic community and caused what is known as urchin barrens (Eddy et al. 2015). Urchin barrens are macroalgal areas, such as kelp forests, that have been consumed and leave behind rocky substrate and crusts of coralline algae (Eddy et al. 2015; Harris and Eddy 2015; Steneck et al. 2013). By the 1980s this exponential growth was also being observed in the Gulf of Maine and causing similar destruction to macroalgal beds (Eddy et al. 2015; Steneck et al. 2013; Witman, 1985). Due to the creation of the urchin barrens, many lobstermen started to view *S. droebachiensis* as a pest for two reasons: 1. they destroyed the habitats that lobsters utilize (Scheibling and Hatcher 2013) and 2. because large quantities of urchins would be brought up in their traps (Eddy et al. 2015). Occurring at the same time in Japan they had overharvested their own stock of sea urchins that they use for the Japanese delicacy known as uni. Japan then started to receive its supply of sea urchins from other countries, including the United States (Eddy et al. 2015; Steneck et al. 2013; Sun and Chiang 2015).

One of the areas that started to supply Japan, as well as the growing interest in uni right here in the United States, was the Gulf of Maine where *S. droebachiensis* naturally occurs (Eddy et al. 2013; Steneck et al. 2013; Sun and Chiang 2015). *Strongylocentrotus droebachiensis* is one of the highest quality, and most popular, species of sea urchins used to make uni (Hagen 1996; Steneck et al. 2013; Sun and Chiang 2015). Of the many species of sea urchins, only a limited number are used to make uni, this includes other species of *Strongylocentrotus* and a few other genera, such as *Heterocentrotus* and *Pseudocentrotus* (Hagen 1996). Uni is the Japanese term

used for the gonads, male or female, of sea urchins. There are five gonads in total per animal, and they resemble slices of an orange in both shape and coloring (Hagen 1996; Sun and Chiang 2015). Quality of the gonads is judged by color, shape, consistency, and taste (Hagen 1996).

The explosion of *S. droebachiensis* continued to increase throughout the 1980s (Eddy et al. 2015), and in 1987 commercial-scale harvesting of *S. droebachiensis* began to gain attention in the economy (Eddy et al. 2015; Steneck et al. 2013; Williams and Harris 1996). Harvesting continued to increase and peaked around 40 million pounds with an approximate dollar value of $35,000,000 in 1993. However, due to there being few regulations on the fishery, such as closed seasons and size or catch limitations, fishermen were starting to notice a decline in the populations. This caused Maine to create a set of regulations for the fishery in 1993 (Eddy et al. 2015) and soon after other New England states followed suit. Even with the regulations in place, by 1997 the catch had been cut almost in half to about 20 million pounds bringing in about $20,000,000 (Eddy et al. 2015). By 2010, this number had been reduced to about 3 million pounds with a dollar value of $5,000,000 (Eddy et al. 2015; Sun and Chiang 2015). For the past several years, and continuing today, the Maine fishery has stabilized at about 2 million pounds.

As the population continued to decline the Sea Urchin Zone Council (SUZC) was created in 1996, which makes recommendations for management of the sea urchin population to the Maine Department of Marine Resources (MDMR) that manages the fishery (Chen and Hunter 2003; Eddy et al. 2015; Johnson et al. 2012). The SUZC is a team consisting of scientists, including an aquaculture scientist, and industry members (Eddy et al. 2015). The MDMR now manages the fishery based on limited entry, a minimum and maximum size limit, and a limited time frame in which sea urchins can be harvested (Chen and Hunter 2003). The declining fishery

also sparked interest in ways of restocking the fisheries, via recruitment or restocking, as well as creating aquaculture of *S. droebachiensis* to assist the fishery (Eddy et al. 2015; Sun and Chiang 2015; Williams and Harris 1998). The aquaculture research is looking into both onshore and offshore attempts (Scheibling and Hatcher 2013). With recruitment not being successful as of yet, the potential of introducing sea urchins into aquaculture remains an active area of research but is still young and needs further study for sea urchin aquaculture in the Gulf of Maine to be successful (Böttger et al. 2004; Eddy et al. 2015; Williams and Harris 1998). As of late, the research has mainly been focused on introducing *S. droebachiensis* into existing aquaculture lease sites that utilize cages and other materials for the suspended aquaculture of bivalves. The reasoning behind this is aquaculture of the sea urchin alone does not have the potential to be as profitable due to many varying factors such as export costs, facility expenses, and slow growth of the urchins (over two years to commercial size).

Before it is possible to begin the integration of *S. droebachiensis* into these aquaculture facilities, a better understanding of their life history is needed. *Strongylocentrotus droebachiensis* has an important ecological role as grazers of macroalgae (Eddy et al. 2015; Harris and Eddy 2015; Scheibling and Hatcher 2013; Nestler and Harris 1994). This allows them to control the growth of macroalgae, but as stated before, can be detrimental to macroalgal beds when the population of *S. droebachiensis* is not controlled by predators. While macroalgae is a large part of their diet, they are omnivores (Nestler and Harris 1994; Williams and Harris 1998). One important nutrient they receive from the animal protein is calcium which urchins use to build their tests. They acquire animal protein from the consumption of small animals such as bryozoans, mussels, and some crustaceans. (Böttger et al. 2004; Nestler and Harris 1994; Harris

and Eddy 2015; Scheibling and Hatcher 2013). In multiple studies, the importance of a mixed diet has been demonstrated. Nestler and Harris (1994) demonstrated that *S. droebachiensis* had increased growth rates when on a diet of *Saccharina latissima* with the bryozoan *Membranipora membranacea* compared to just *S. latissima* alone. Böttger et al. (2004) also mentioned that sea urchins will change how much food they are consuming depending upon the ratio of protein to vegetable in prepared diets.

This study aimed to examine several different characteristics of the life history of *S. droebachiensis*. The objectives were to 1. examine growth on other natural and artificial diets and compare them to growth on *S. droebachiensis*' common diet of *S. latissima* with an animal protein, 2. observe behavioral aspects involving camouflage and "rapid growth" in a high versus low water current, and 3. further study the spawning and rearing of larvae to gain more knowledge about how to successfully raise them without advanced scientific facilities or intensive labor.

Societal and Global Impacts

In any type of aquaculture, there is going to be an impact generally on both a local and global scale. One of the potential effects of introducing *S. droebachiensis* into aquaculture is reducing the stress on the natural populations by supplementing some of the fisheries with aquaculture-grown urchins. This will allow urchin populations in the wild to increase. This increase will not only benefit the populations of *S. droebachiensis* but will have a societal impact on the fishing community in the Gulf of Maine as well. Increasing population size could allow for more urchins to be harvested over time which will increase profit for fisherman in an industry

that is difficult to work in since they rely on nature and animals that essentially live in another world. As a by-product of this societal impact, this will also have a global impact on the market for *S. droebachiensis*. With both the aquaculture of *S. droebachiensis* and increased catch rate for fisherman, this will ultimately expand the international market allowing the exporters of *S. droebachiensis* to increase their profit gain. In summary, aquaculture of *S. droebachiensis* has the potential to be profitable on both a societal and global scale while also helping the natural populations stay afloat. This could keep costs lower instead of having them skyrocket and result in the market (mainly the export market) crashing due to such high costs.

Materials and Methods

Most of the urchins were collected from ledges off Smuttynose Island in Gosport Harbor at the Isles of Shoals in the Gulf of Maine, USA. Throughout this study, the urchins were spread among three recirculating, open sea tables (Figure 1) to allow for optimal space for them to move around and studies to be carried out with cages at the same time. A cooling system was used to keep the water at approximately 50° F and a salinity of about 32 ppt was maintained. Salinity was monitored using a refractometer and distilled water was added if salinity increased too much. Filter bags were changed weekly and the system siphoned of debris on the bottom of the sea tables weekly to bi-weekly depending upon the quantity of urchins in the sea table and what study was underway. When the water became too dirty partial water changes would be done using clean sea

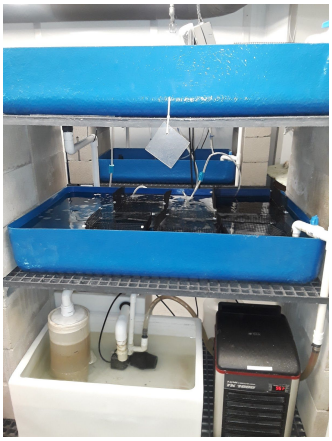

Figure 1. Example set up of the sea tables used in the course of the study.

water from storage tanks. Urchins being used in studies were kept in mesh cages of approximately 24 cm long by 25 cm wide by 16.5 cm high. For the spring growth studies, four of these cages were divided in half lengthwise to allow for two groups in one cage. This was done to optimize space and number of groups. The cages were made from a plastic mesh used for the siding and a piece of polycarbonate for the bottom.

Fall 2018 Growth Studies and Dietary Preference

Three species of macroalgae were used to evaluate the growth of *Strongylocentrotus droebachiensis*. *Saccharina latissima* with *Membranipora membranacea, Gracilaria vermiculophylla* (an invasive species), and *Dasysiphonia japonica* (an invasive species) were individually fed to three groups of urchins separated into cages. A total of 156 urchins were initially used and separated into three size groups: small (0.7-1.1 cm; n=38), medium (1.1-1.6 cm; n=80) and large (1.7-2.5 cm; n=38). The small group was fed a diet of *D. japonica*, the medium group was fed a diet of *S. latissima* with *M. membranacea*, and the large group was fed a diet of *G. vermiculophylla* (Figure 2). Food was checked a few times a week and replenished as needed. During this study there was troubleshooting (due to apparent toxicity) with one of the systems and all urchins were in one of three cages depending upon size. Eventually, another system was utilized, and the three size groups were split up into smaller groups within each size to allow for replicates. Urchins were measured once every two weeks by the widest part of the test. The study ran from October 2018 to December 2018 and analysis of data was qualitative only.

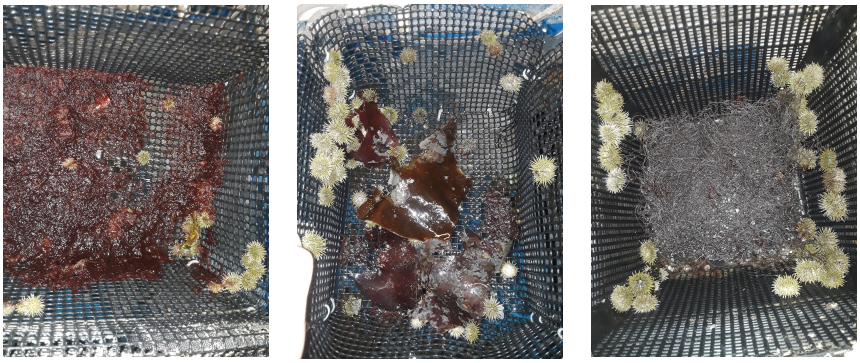

Figure 2. From left to right: small group on *D. japonica*, medium group on *S. latissima* with *M. membranacea*, and large group on *G. vermiculophylla*.

Part way through the study the small group was taken off the diet of *D. japonica* and switched from the growth study to a preferential diet study. This study examined the preference between *S. latissima* with *M. membranacea* and *G. vermiculophylla*. The wet weight of the food supply was taken every other day Monday through Friday. Each week, food supply was emptied and seven grams of food were weighed and replaced in each cage on Friday to ensure a steady supply of food throughout the week. Qualitative analysis via differences in the food consumption within each week was used to assess diet preference.

Spring 2019 Growth Studies

This study started with a total of 40 urchins that were divided into eight groups of five urchins each (average size 2.4 cm) and were placed in cages that were divided in half lengthwise. Four groups were fed a diet of *S. latissima* without *M. membranacea* this time, as it does not grow in the colder months. Part way through the study a few crushed juvenile mussels were added to each study group on *S. latissima* as an animal protein substitute. The other four groups were fed a diet of Ken's Premium Veggie Wafers, an artificial pellet made for benthic feeding organisms. Presentation of the pellets to the urchins started with whole wafers but much of it

went unconsumed and the procedure was changed to wafers chopped into approximately same sized pieces using a razor blade. The number of pieces given to the urchins depended upon the number of urchins in the group as some urchins were lost along the way (due to mortality). Feeding of the groups on *S. latissima* was again checked a few times a week and replenished as needed. The wafer groups varied a bit with feeding occurring, on average, every three days due to how the wafers interacted with the water and how the urchins interacted with the wafers. Measurements of the test were taken bi-weekly again. This study ran from the beginning of February to the end of March. Initially, an independent samples t-Test utilizing both JMP Pro 14 and Excel were performed, but after comparing the results and qualitative examination of the data the test was switched to an one-way ANOVA utilizing the open-source program R.

Behavioral: Camouflage Tendency

This study specifically looked for a potential preference between the use of pieces of shell versus pieces of *S. latissima* (still without *M. membranacea*) as camouflage material. A total of 87 urchins were used and broken into three groups of size classes (based on the diameter of the test). Both the pieces of *S. latissima* and broken shell were approximately equal to the average size of the urchins (Figures 3a, b, and c), which allowed for easy manipulation of the materials presented. The size classes were small (average=1.3 cm; n=30), medium (average=1.8 cm; n=30), and large (average= 2.8cm; n=27). Before the large group was utilized they were allowed to sit in a holding area for a week with pieces of *S. latissima* as the majority of them had just come out of the wafer versus *S. latissima* study. This was done to decrease the chance of those urchins that had been on the wafer diet showing a preference for *S. latissima* over shell.

After division by size, they were further broken down into groups of ten (nine in the instance of the large class) and placed in separate mesh cages.

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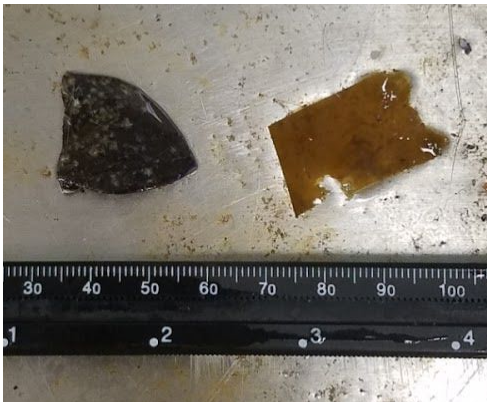
b) 

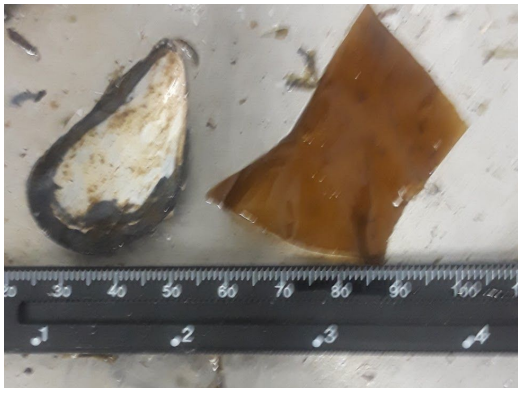
c) 

Figure 3. Approximate sized pieces of *S. latissima* and mussel shell used for the a) small group, b) medium group, and c) large group.

About ten minutes was given to allow the urchins to settle themselves in the cages at the beginning of the study to limit the influence of behavior as the pieces of *S. latissima* and shells were going to be randomly dispersed in the cages. During this time shells were broken up and *S. latissima* was cut into pieces. Ten pieces of *S. latissima* and ten pieces of shell were randomly distributed in each cage to provide one of each material per urchin and limit competition. The urchins were left alone for 24 hours and the data was recorded the next day by placing each

urchin in one of five categories: uncovered, kelp, shell, both, and eating. A piece of material was considered being used as camouflage by whether the individual was using its tube feet to hold onto the material above it and/or to the sides of it. A Friedman test followed by a Tukey test were used for statistical analysis in the open-source program R.

"Rapid Growth" in High versus Low Current

This aimed to examine an abnormal swelling of the test of the urchins when placed in high current waters. This was observed once by Harris and previous students and has not been formally tested. After the urchin swells it does not grow for a while as it takes time to build in the rest of its test. Two trials were done: one in early March and the other in mid-April. Both times thirty urchins were used and the test diameter measured before the study started (1st trial average=1.5 cm; 2nd trial average=1.6 cm). The thirty urchins were divided into groups of ten urchins and placed in one of three cages with ample *S. latissima* for food supply. One container was retained in the lab as the control and the other two were placed out at the University of New Hampshire's Judd Gregg Coastal Marine Lab in New Castle, NH, USA.

One of the containers was tied to the floating dock in a high current area and the other was tied to the floating dock in a low current area. In the first trial the cages were allowed to move freely but the second trial they were weighted down by using a short length of rope to tie bricks to the bottom of the cages. High currents were defined as cages directly exposed to tidal influence and low current cages were placed in areas with a barrier between the cage and the direct tidal flow (a concrete support wall for the UNH Coastal Marine Lab pier). For the first trial growth was recorded at one-week intervals for two weeks. On the second recording, they were

collected then brought back to the lab after three days and measured. This was because Harris and previous students initially saw swelling of the test within three days of being put out in the high current.

Larval Culture

Mature urchins measuring greater than 5 cm in test diameter were used for gamete collection. To do this less than 1 mL of a 0.55M solution of potassium chloride (KCl) was injected into the coelomic cavity through the perioral membrane surrounding the Aristotle's lantern (Figure 4). This was done in two to three different spots to ensure the full release of the gametes from the gonads (Foltz et al. 2004; Whiteley et al. 1987). Individual urchins were then placed in small bowls with the mouth up to allow gametes to be released into the dish from the gonopores that open near the anus. After 5-10 minutes, urchins were removed and either sperm (white) or eggs (yellow) were observed (Figure 5). Sperm and eggs were then mixed in a dish with filtered seawater to cause fertilization. They were then observed under a dissecting microscope until it was confirmed that eggs were fertilized by the formation of a fertilization membrane around the egg to prevent further fertilization (Figure 6). A total of 3 spawning trials were carried out.

Figure 4. Example of injecting a mature sea urchin with KCl to induce spawning of gametes.

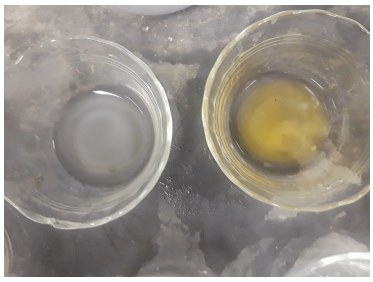


Figure 5. Dish of sperm (left) and eggs (right)

Figure 6. Fertilized eggs with the fertilization membrane.

Fertilized eggs were distributed amongst approximately four 3L beakers (depending upon the density of fertilized eggs) filled with the same filtered seawater the eggs were fertilized in. A glass pipette attached to an air system was placed in each of the 3L beakers with a gentle supply of air to provide oxygen and mix the seawater. The beakers were kept in the back of one of the open sea tables to regulate the temperature of the sea water in the beaker. The beakers and air supply system were set up at least 24 hours in advance to allow the temperature of the water to acclimate to the sea tables. Larval development was monitored daily via a dissecting microscope and about 3-5 days in, when they had developed a gut, they were then fed a diet of *Dunaliella tertiolecta* (Wray et al. 2004) daily (40-100mL of approximately 5.0 x 10^5 cells/mL depending on the stage of growth and larval density). About 21 days into larval development they started to settle on the bottom of the 3L beakers and food supply was reduced as *D. tertiolecta* is a mobile green alga. After metamorphosis young urchins were transferred to modified plastic containers that had cutouts in the wall with inserted mesh. They were supplied with a diet of diatoms as they now ate like adult urchins.

Algal Cultures for Larvae

Cultures of *Dunaliella tertiolecta* were maintained as a food supply for spawned urchin larvae, as urchins are unable to digest macroalgae until much later in their development. Algae was cultured in two glass vials and two 1L Erlenmeyer flasks with an ample, constant supply of

light (UV grow lights) and stirring (via air system). Density was measured weekly via hemocytometer and maintained at 5.00 x 10^5 cells/mL. The formula for the growth medium for *Dunaliella* species was received from Dr. Lee Jenkhe and is as follows:

Solution	Compound/Amount	Final Conc.
T1	$CaCl_2 \cdot 2H_2O$, mw 147 1.5 g in 500 ml DI water	0.2 mM
T2	Na_2HPO_4, mw 142 2.8g in 500 ml DI water	0.4mM
T3	H_3BO_4, mw 62 0.4g KNO_3, mw 101 26.5g $MgSO_4 \cdot 7H_2O$, mw 247 60.3g	0.13 mM 5.2mM 5mM
Ka	EDTA, Na_3, mw 358 8.1g/150ml titrated to pH 6-7	75 µM
Kb	$FeSO_4 \cdot 7H_2O$, mw 278 0.84 g/150ml + 2 drops 10% H_2SO_4	10 µM
Kc	$ZnSO_4 \cdot 7H_2O$, fw 288 86mg $NaMoO_3 \cdot 2H_2O$, fw 242 36mg $CuSO_4 \cdot 5H_2O$, fw 250 23mg $CoCl_2 \cdot 6H_2O$, fw 238 7mg $MnCl_2 \cdot 4H_2O$, fw 198 446mg	1 µM 0.5 µM 0.3 µM 0.1 µM 7.0 µM

To prepare the solutions 10 mL each of T1, T2, and T3 and 0.5mL of Ka, Kb, and Kc were added to distilled water per liter of culture medium (31.5mL of nutrients + 968.5mL of DI water)

and salinity was adjusted to 34 ppt. New medium was added to the culture tube each time the culture was utilized to feed the larvae. This was done to maintain the algal cultures at the same volume. The algal culture tubes were also rotated after each feeding to allow them to recover to a healthy supply.

Results

Fall 2018 Growth Studies and Dietary Preference

Strongylocentrotus droebachiensis was observed to have a higher growth rate on the diet of *Saccharina latissima* with *Membranipora membranacea* rather than a diet of *Gracilaria vermiculophylla*. A large increase in growth was seen between the November 7th and November 20th measurements (Table 1) of the *S. latissima* diet that occurred at the same time as the troubleshooting with one of the other sea tables that was having toxicity problems. Urchins were used to test this system and the sample number was reduced from 73 (some urchins had been lost earlier) to 40 between these two measurement dates. Another observation is that in some weeks the mean test diameter of the urchins seemed to decrease. After qualitative analysis, it was determined that *S. droebachiensis* grew approximately 69% more on *S. latissima* than on *G. vermiculophylla* (overall growth on *S. latissima*=0.280 cm vs. *G. vermiculophylla*=0.138 cm; Figure 7).

	S. latissima with *M. membranacea*		*G. vermiculophylla*	
Date	Mean Growth	SD	Mean Growth	SD
October 10	1.313	0.150	1.995	0.238
October 24	1.332	0.173	2.039	0.262
November 7	1.330	0.165	2.017	0.243
November 20	1.500	0.175	2.169	0.263
December 5	1.400	0.183	2.200	0.217
December 19	1.593	0.119	2.133	0.115

Table 1. Mean growth was taken from all groups and was measured in centimeters. SD is the standard deviation.

Figure 7. The growth rate of *S. droebachiensis* on diets of *S. latissima* with *M. membranacea* and *G. vermiculophylla*. As can be seen, the *S. latissima* diet has a larger increase in growth than the *G. vermiculophylla* diet.

The small group of urchins (n=38) was switched from the *Dasysiphonia japonica* diet, due to the observance of cannibalistic behavior. They were switched to a preferential diet study of *S. latissima* with *M. membranipora* versus *G. vermiculophylla*. On average more *S. latissima* was consumed, in total, than *G. vermiculophylla* per week. This occurred in all but the week of November 30th and the last week both in which at least some data was not collected (Figure 8).

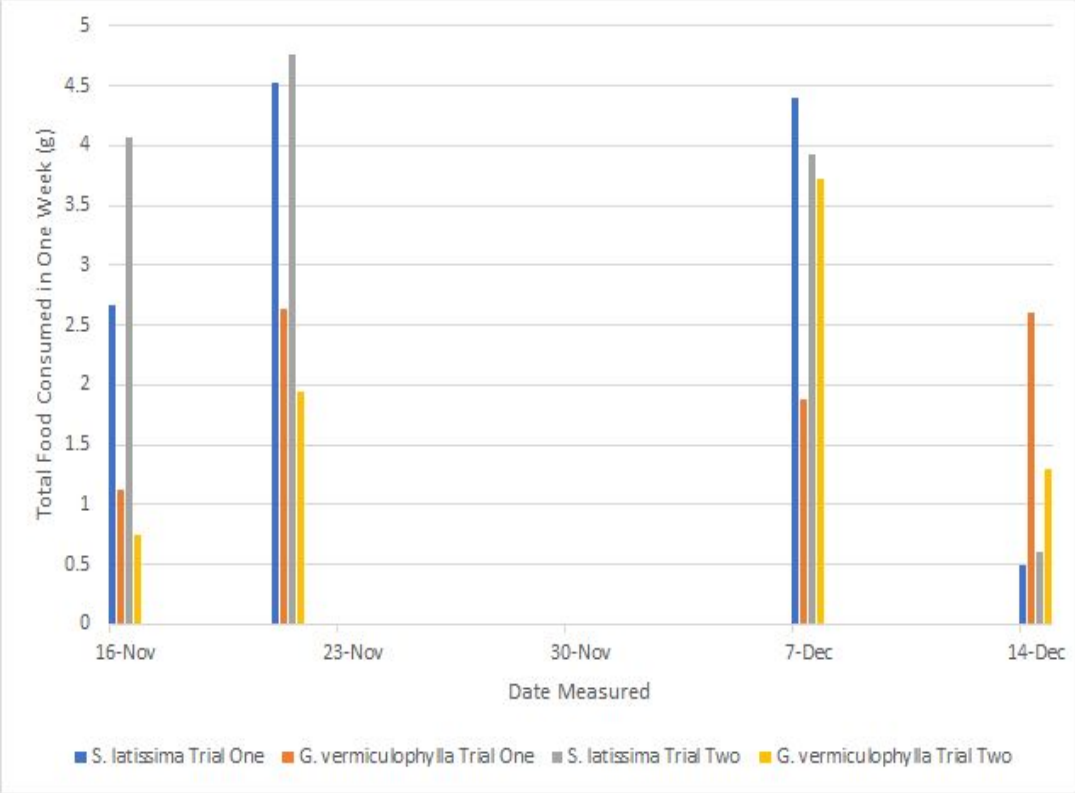


Figure 8. The total amount of *S. latissima* and *G. vermiculophylla* consumed per week by *S. droebachiensis*. One week of data is not included due to an increase in the wet mass of algae present after initial measurement.

Spring 2019 Growth Studies

About mid-way through this study cannibalism was observed in one of the four groups on the diet of *S. latissima*. In order to prevent further cannibalism crushed juvenile mussels were broken and added to each of the four replicates once a week for the urchins on the *S. latissima* diet only. In the statistical testing the t-test, both in the JMP Pro 14 statistical software and

Microsoft Excel, produced a p-value of 0.467. However, upon further qualitative analysis of the data (Table 2) and examination of the graph (Figure 9), it was decided that with how close the p-value was to the alpha value of 0.05 something was occurring that was not being accounted for in the test.

	S. latissima		Artificial Wafer	
Date	Mean Growth	SD	Mean Growth	SD
February 6	2.460	0.127	2.430	0.098
February 15	2.629	0.205	2.473	0.088
March 1	2.721	0.267	2.606	0.161
March 15	2.631	0.229	2.569	0.158
March 29	2.677	0.235	2.650	0.203

Table 2. Shows the mean growth across the four replicates within each diet. Measurements taken in centimeters.

It was determined that this result may have been influenced by the variation seen in the second and third week (Figure 9). To determine whether there actually was a difference between the two diets an one-way ANOVA, with the error set as the dates within the diets, was run on the open-source program R. This time the results concluded that there was no significant difference in growth (p-value=0.185) between the diet of *S. latissima* and the artificial wafers. However, while the results were not significant, growth was slightly higher every week on the diet of *S. latissima*.

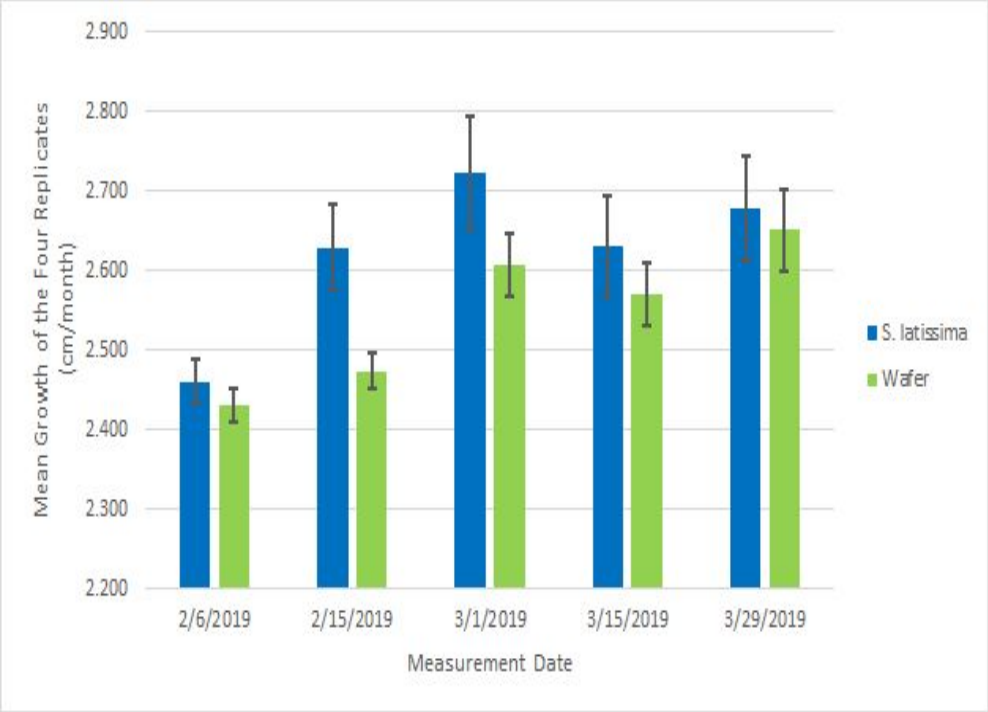


Figure 9. The mean growth of *S. droebachiensis* on diets of *S. latissima* and artificial wafers. The mean was calculated across the four replicates within each diet. The error bars show the standard error.

Behavioral: Camouflage Tendency

Saccharina latissima and mussel shells were chosen as the materials to be used due to the observance of urchins, that were free in the sea tables, naturally holding onto material they found (mainly *S. latissima* and shell bits). In one observation a small urchin (approximate test diameter 1.9 cm) had enclosed itself within two halves of a mussel shell. In the data, it was qualitatively observed that 52.2% of the small urchins and 41.1% of the medium urchins did not utilize camouflage but 43.2% of the large urchins utilized shells as camouflage. If camouflage was used by the small and medium urchins, *S. latissima* was chosen over shells (small: 26.7% and medium: 28.9%; Figure 10). In the statistical analysis, the Friedman test returned that there was a significant difference (p-value=0.023) between at least one of the groups. The Tukey test determined that this difference occurred between the number of urchins that were uncovered and

the number of urchins that were eating kelp. However, in some of the cages (mostly the large-sized urchins), it had been observed that the majority, if not all, of the pieces of *S. latissima*, had been consumed by the urchins.

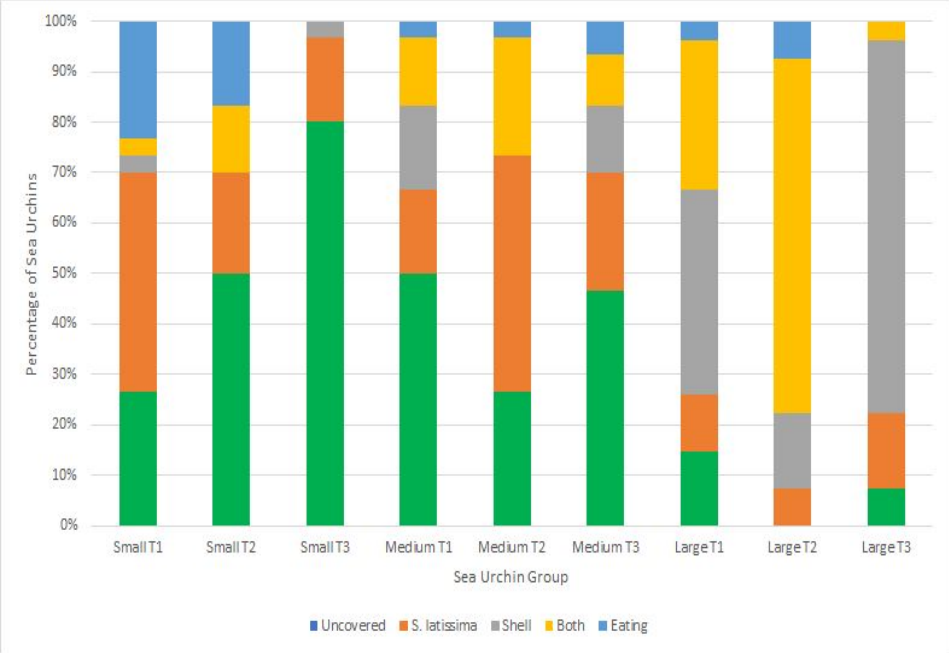


Figure 10. The percentage of *S. droebachiensis* in each category within each of the size groups during each trial. The percentage represents the mean of the three replicates within each trial.

"Rapid Growth" in High vs. Low Current

In the first trial, there seemed to be a decrease in test diameter in the urchins that were put out at the Coastal Marine Lab in both the high and low currents. However, as they were being measured it was observed that the spine length had been greatly reduced in all of them due to broken spines. In the second trial, where a weight had been added to the cages, it was observed that there was less spine damage. Still, there was no "rapid growth" as the urchins did not grow in any measurable quantity.

Larval Culture

Very few adults were lost due to the injections of potassium chloride during this study. In all three spawnings that were performed some fertilized eggs reached the larval stage. Only a few larvae in the first spawning trial reached metamorphosis and were transferred to a separate container in the open sea table. The metamorphosed larvae never successfully achieved recruitment, and the culture was lost. The last spawning attempt contained larvae that were developing incorrectly. The algal cultures of *Dunaliella tertiolecta* became contaminated for a short period of time towards the end of the larval culturing. An unidentified cyanobacteria was initially found in the water but was then determined to be growing above the waterline on the inside of the tube. Upon finding the contamination the culture tubes were cleaned out and cultures remade with uncontaminated culture. Another contamination observed was in the larval cultures themselves which was ciliates, a copepod, and a fairy shrimp. The copepod and fairy shrimp were only observed once each.

Discussion

Fall 2018 Growth Studies and Dietary Preference

It is suspected that the toxicity problems with the sea table were due to at least one of two factors. The first could have been the microbial community was not adequately established to handle the ammonia (one of the waste products) given off by urchins. The second could be the cages initially made to keep the urchins in were constructed out of a plastic coated metal wire mesh. It is possible that a chemical in the plastic coating or ions from the metal leached into the

water and made the system toxic to the urchins. This is when the materials were switched to a plastic mesh siding and polycarbonate bottom.

In the diet study to observe if the urchins grew faster on *Saccharina latissima* with *Membranipora membranacea* or *Gracilaria vermiculophylla*, it is not possible to conduct any statistical analysis because the diets were tested on different size groups of urchins. This causes too many confounding variables that make it unclear if the change in growth rate is from the diet provided or related to the fact that larger urchins will likely not grow as fast because the growth rate is inversely correlated with age (Russell et al. 1998; Ebert 2013). In Figure 7, the general trend of the data for *S. latissima* and *G. vermiculophylla* are roughly the same with the exception of the data collected from December 5th to December 19th. In this two week span, the medium urchins fed *S. latissima* grew approximately two times as fast as the large urchins that were fed *G. vermiculophylla*. The reason for this is most likely due to the presence of *M. membranacea* which fulfills the requirement for animal protein in the diets of *S. droebachiensis* (Böttger et al. 2004; Nestler and Harris 1994; Harris and Eddy 2015; Scheibling and Hatcher 2013; Williams and Harris 1998).

This growth study initially included a third diet of small-sized urchins being fed *Dasysiphonia japonica*. However, *D. japonica* was determined to be a poor diet for the urchins because they would not consume it and resorted to cannibalism. This could have occurred due to one of two reasons. First, they may not have eaten it strictly due to *S. droebachiensis* being selective about what algae is consumed (Böttger et al. 2004; Harris and Eddy 2015). Second, they may not have eaten it due to the potential of *D. japonica* possessing anti-herbivory qualities that many red alga are known to have (Hay and Fenical 1988). Being that *D. japonica* is now

common it may have negative implications for urchin population recovery. In the preferential study, that resulted due to these observations, there appears to be a slight preference for *S. latissima* with *M. membranacea* based on the data. There were only four data collection weeks in this study because on the week of November 30th data was collected but the ending wet mass of the *G. vermiculophylla* was greater than the beginning mass. There are two possibilities as to how this happened; either it was not measured correctly at the beginning of the new week or at some point more was mistakenly added to the wrong cage when the urchins on the growth study were fed.

Another problem is seen in Figure 8 which shows that on December 14th both *G. vermiculophylla* trials had greater total consumption than the *S. latissima* trials. However, when the new food was measured out for the new week it was not recorded and is, therefore, missing some data. This causes these data points to not support the rest of the data collected in this preferential study. Overall in the preferential study, it is seen that *S. latissima* with *M. membranacea* is the preferred food over *G. vermiculophylla*. This preference is most likely due to the presence of *M. membranacea* which provides animal protein to the urchins, a crucial nutritional requirement of building their tests. These results line up with numerous other works that address the importance of omnivory in the diet of *S. droebachiensis* (Böttger et al. 2004; Harris and Eddy 2015; Nestler and Harris 1994; Scheibling and Hatcher 2013; Williams and Harris 1998).

Spring 2019 Growth Studies

As was observed in the results there were problems with cannibalism for the urchins fed *S. latissima*. This was likely due to the urchins not having enough animal protein which they normally acquire from *M. membranacea* that covers *S. latissima* in the warmer months. Cannibalistic behavior allowed them to find alternative ways to acquire necessary animal protein which is what largely supplies them with calcium to build their tests for growth (Nestler and Harris 1994). This was not a problem in the fall growth study because there was *M. membranacea* coating the *S. latissima* fed to the urchins. This behavior is why juvenile mussels were incorporated into the diet of *S. latissima*. Generally, this is not a problem seen in the ocean during the colder months as *S. droebachiensis* will find animal protein to feed on such as mussels, barnacles, and dead fish (Scheibling and Hatcher, 2013). In one study by Nestler and Harris (1994) they were even seen to consume amphipods when offered.

In the statistical analysis of the study, the initial results using JMP Pro 14 and Microsoft Excel was most likely influenced by the lack of overlap between the error bars of the diets seen on February 15th and to a lesser extent March 1st (Figure 9). However, utilizing the open source program R this error was able to be accounted for and the artificial wafer diet appears to be just as capable as *S. latissima* at supporting the growth of juvenile urchins. This was similarly seen by Williams and Harris (1998). As seen in the results though, growth on a diet of *S. latissima* with some kind of incorporated animal protein is still slightly higher in every week than the artificial wafer. This demonstrates a need to find an artificial diet that is comparable to the nutritional makeup of *S. latissima* with animal protein.

Behavioral: Camouflage Tendency

Strongylocentrotus droebachiensis, like many other sea urchins, is known to rely on camouflage or cracks and crevices in hard substrates to hide themselves (Lawrence 2013). Not much is known as to why but many factors have been considered in both biological and physical factors (Dumont et al. 2007; Lawrence 2013). In this study, the majority of the small (average=1.3 cm) and medium (average=1.8 cm) urchins did not use camouflage but when they did they utilized *S. latissima* instead of shells. This may have been because it is a food source and can, therefore, serve a dual purpose. Dumont et al. (2007) noted that urchins smaller than 2 cm typically stay in a single area and remain camouflaged in some manner not foraging for food until needed. However, urchins that are larger than 2 cm will forage openly. While the majority of the urchins less than 2 cm remained uncovered they still had plenty of *S. latissima* in their cages potentially showing that they did not move to any great extent. This could also explain why most of the kelp had been eaten in the trials with the large urchins (average=2.8 cm) since they would be more likely to move around to acquire food. Dumont et al. (2007) also showed that physical factors such as wave action can play a large role in the use of camouflage material. Since there was no wave action in the sea tables and the small and medium groups have been demonstrated to move very little it is likely that these two factors combined explain as to why the majority of the urchins less than 2 cm remained uncovered even though they would be the more susceptible sizes to predation in the wild.

The larger urchins used shells and sometimes also *S. latissima* because shells provide more protection from predators and they are stronger than the small urchins and able to carry heavier and more camouflage materials around. Along with wave action, Dumont et al. (2007)

also observed that *S. droebachiensis* will cover itself to a degree in light and larger urchins seem to react more strongly to the presence of light. This and the unhindered foraging of urchins larger than 2 cm could explain why the large group was mostly using shells as camouflage material. Overall the Friedman and Tukey test agreed with the results that there was no statistically significant difference between the two materials under study as the only statistical difference was between the number of urchins uncovered and the number eating. These two factors were not strictly under study but were behavioral characteristics that were accounted for.

"Rapid Growth" in High vs. Low Current

The first trial, when the cages were free to move with the current, seemed to result in a decrease of the test diameter of the urchins. However, it is believed that this is due to the cages moving with the current and breaking the spines of the urchins. It was shown by Dumont et al. (2007) that urchins exposed to wave action typically cover themselves with materials to avoid breaking of their spines. Also, if the urchins truly did decrease in size that would be in opposition with a previous study that saw growth rates increase dramatically when urchins were transferred into open sea water (Williams and Harris 1998). The second time this study was repeated the cages were weighted which kept the cages from moving as much to prevent breaking of the spines, so the difficulty of measuring the test size remained the same instead of getting easier like in the first trial. This allowed the results to be more reliable even though they showed no "rapid growth" as seen by Harris and previous students.

Larval Cultures

The adults lost after spawning could have been because too much KCl was injected during spawning or they could have become stressed from the injections. Larval cultures are extremely sensitive to the environment around them and any number of reasons could explain why larval cultures died. While the fertilized eggs were split among 3L beakers it is still possible that densities were too high which makes them more prone to infection by ciliates (a problem often observed in this study) and other microbes (Whiteley et al. 1987; Wray et al. 2004). Of all the spawnings, only the first one was successful at rearing the urchins from fertilized eggs through metamorphosis. This may be due to the mortality of larvae being naturally high or there not being a substrate with biofilm for them to eat present at the time of settlement (Metaxas 2013; Whiteley et al. 1987). The final spawning occurred in early spring when the urchins were at the end of their spawning season. This resulted in many eggs that were fertilized but did not develop and larvae that were developing slowly with shorter appendages than were previously observed in other more successful spawning attempts. Based on this, raising *S. droebachiensis* from an egg with the intention for use in aquaculture does not seem feasible. Additionally, even if these trials were more successful, natural mortality of some larvae would still occur, requiring that large quantities of gametes would be necessary for larger scale aquaculture.

Possible uses of natural recruitment

Studies performed in Japan support the concept that utilizing natural recruitment of sea urchins is a more successful and lower mortality option compared to raising urchins exclusively in a laboratory. Many different materials, depths, and configurations were tested but urchins

seemed to prefer materials that were textured rather than smooth because it gave them something to hold onto (Department of Mariculture, Hokkaido Central Fisheries Experimental Station 1984; Tegner 1989). Using suspended recruitment plates worked best when the plates were deployed about three months before the start of the breeding season likely to allow time for diatoms, detritus, and other food sources to accumulate. Once the urchins (*Strongylocentrotus intermedius*) grew to between 3-5mm they would drop off of the culture plates during wave action. That combined with predation of young urchins by other small animals resulted in net baskets being installed around the plates (Department of Mariculture, Hokkaido Central Fisheries Experimental Station 1984; Tegner 1989). The urchins would then drop off into the net baskets where algae and other food could be added instead of falling to the seafloor and likely being eaten. The scientists succeeded in using these methods to grow urchins for 5-6 months to a releasable size of 15 mm. It was also suggested that keeping the plates on longer ropes at greater depths would decrease wave oscillations causing fewer urchins to drop off or to grow out the urchins in a laboratory right before they were at the size to begin dropping off. Based on these studies it feasible that this type of natural recruitment system could work in the Gulf of Maine.

Possible Future Studies

Much work is still needed beyond what this study encompassed. The second dietary study showed what could be promising results with the urchins on the artificial wafers growing in a comparable manner to those on *S. latissima*. This study looked at only a small time frame and should be expanded to a longer time period allowing for growth to be more extensively tracked. Also other aspects that can be observed are test color and spine length which Williams and

Harris (1998) had observed to differ between juvenile urchins fed an artificial versus natural diet. Furthermore, in this study *S. latissima* did not have *M. membranacea* as it does in the warmer months. Growth during this time span could also differ as they have constant access to *M. membranacea* instead of crushed juvenile mussels being a periodic substitute for animal protein.

More behavioral studies would also be needed to understand how they interact with their surrounding environment. Aspects that could be studied more in-depth are reactions to the presence of predators such as *Cancer borealis* (Jonah crab), reaction to light versus dark conditions, and a preference of having substrate to camouflage themselves with versus hiding in crevices. Dumont et al. (2007) observed that behavioral aspects of camouflage not only varied with what environmental conditions the urchins were put in but also with whether they were adult or juvenile urchins. Lastly, the study looking into "rapid growth" of *S. droebachiensis* in high and low currents warrants further research as Harris and previous students had observed large increases in test diameter. Many other studies should still be performed on the life history of *S. droebachiensis* but these few recommendations are a starting point that builds off the studies of this research and others similar to it.

Acknowledgments

We would like to largely thank New Hampshire Sea Grant for funding of this project which allowed us to put our science skills to use and learn much more along the way. Also a special thank you to Dr. Larry Harris for his guidance, knowledge, use of his lab, and, most importantly, his patience with all of our questions as we learned to work with the animals. We would like to thank Dr. Lee Jenkhe for his quick assistance when our algal cultures became contaminated as well as Dr. Christopher Neefus for his insights into the macroalgal world on short notice. Thank you to Seth Goodnight as well for his assistance and initial teachings of creating and maintaining the algal cultures. Finally, we would like to thank Jenny Gibson and Dr. Patty Jarema for their unending patience and guidance in helping us understand the rough side of statistics in biology.

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