Effects of temperature and body size on covering in green sea urchin, *Strongylocentrotus droebachiensis*

by

Jacob Mercer

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ABSTRACT

Green sea urchin, *Strongylocentrotus droebachiensis*, is common in shallow subtidal rocky reef habitats in the northwestern North Atlantic. It is an important ecosystem engineer, capable of overgrazing on kelp beds to form urchin barrens. Green sea urchin often exhibits a 'covering' or 'hatting' response, whereby it adorns its test with various materials available in the habitat. Covering is presumably a response to an environmental cue, however, definitive reasons for covering have not yet been described in the literature. We carried out a 2-week laboratory experiment to test the predictions that green sea urchin covers (1) less in cold (2°C) and warm (14°C) seawater, as it is outside of thermal optima; (2) more with live rhodolith fragments than with blue mussel shell fragments or denatured rhodolith fragments; and (3) more when small (1 to 2 cm in test diameter, t.d.) than large (4 to 5 cm t.d.) in still water conditions. Sea urchins were acclimated and exposed to one of three temperatures (2, 8, or 14°C) in containers within water baths. Each container, containing one sea urchin, was given a covering material type (live rhodoliths, denatured rhodoliths, or blue mussel shells), whereby the resultant degree of covering exhibited by sea urchins was assessed. Model outputs supported the predictions that temperature and sea urchin size affect covering in green sea urchin, while rejecting the prediction that covering material type affects the degree of covering in green sea urchin. Our results help establish a baseline for temperature-induced covering thresholds in green sea urchin under still water conditions, values which are not currently covered in the literature.

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CO-AUTHORSHIP STATEMENT

Jacob Mercer was responsible for assessing sea urchin percent test cover and data analysis, with assistance from Patrick Gagnon. Jacob Mercer carried out the laboratory experiments, which were co-designed and funded by Patrick Gagnon. The thesis was written by Jacob Mercer, with intellectual and editorial input by Patrick Gagnon in a format compatible with the publication of research articles. This explains the use of the first-person plural pronoun ("we") throughout. Any publication in the primary literature resulting from this work will be co-authored by Jacob Mercer and Patrick Gagnon.

1. INTRODUCTION

The creation of biological communities is heavily dependent on how organisms interact with each other and their environment (Bolker et al. 2003). Shaping these communities are factors such as predation, competition, symbiosis, reproduction, and disease (Krebs et al. 1997). When coupled with environmental conditions and genetics, these factors can influence animal behaviour (Nagelkerken & Munday 2015). Marine organisms from varying phyla exhibit multiple behaviours. In echinoderms, the common sea star, *Asterias rubens,* and brittle star *Ophiura robusta,* typically mass aggregate prior to broadcast spawning (Himmelman et al. 2008). Environmental cues may trigger aggregation (Harrison et al. 1984), more specifically, the first seasonal influx of warm water in the predominantly cold water systems characterizing the species' distribution range (Himmelman et al. 2008). Another common echinoderm behaviour is crypsis, the process of avoiding detection by other species (Ziegenhorn 2016). Many echinoderms exhibit crypsis through what is known as a 'covering' or 'hatting' response (Adams 2001).

Covering behaviour, hereafter termed "covering", presumably is a response to some environmental cues, whereby the organism adorns itself with materials available in the habitat. A few studies suggest that factors such as wave action and solar radiation trigger or affect covering (Adams 2001, Dumont et al. 2007). Sea urchins, including *Lytechinus variegatus* and *Tripneustes gratilla,* use their tube feet to grasp and hold fragments of bottom material to themselves (Brothers & McClintok 2015, Millot 1955, Ziegenhorn 2016). Green sea urchin, *Strongylocentrotus droebachiensis*, is common in shallow subtidal rocky reefs in the northwestern North Atlantic (Filbee-Dexter & Scheibling 2017, Himmelman 1986). Green sea urchin is ubiquitous along Newfoundland's subtidal rocky shores, where it plays a key ecological role by forming large feeding aggregations that remove kelp biomass upon which a number of ecologically and

economically important invertebrate and fish species depend for reproduction, feeding, and hiding from predators (Frey & Gagnon 2015, Schuster et al. 2022). Green sea urchin also covers with materials, including empty bivalve and mollusks shells, rhodolith fragments, pebbles, various seaweeds, and even fragments of tests from conspecifics (Rullens 2016, personal observations). Yet, the frequency, intensity, and drivers of covering in green sea urchin remain largely unknown and may vary with depth given the heterogeneous distribution of covering materials and shift in environmental conditions across the species' vertical distribution range (Frey & Gagnon 2015, Gagnon et al. 2003, Gregory & Anderson 1997, Nemani 2022).

A few studies suggest that in green sea urchin, ultraviolet radiation (UVR, 290-400 nm), in particular UVB or a combination of UVA and UVB, elicits covering to avoid UV damage (Adams 2001). Covering in response to photosynthetically active radiation (PAR, 400-700 nm) was on average four times less than that of UVR, suggesting that if there is a light effect, it is mainly from UVR and not PAR (Adams 2001). Further, green sea urchin is relatively insensitive to PAR (Matheson & Gagnon 2021) and covers in shade or dark (Levin et al. 2001), which complements other studies suggesting that PAR either plays a minimal, or comparatively less important, role in covering (Adams 2001, Dumont et al. 2007). Other factors such as wave action and body size may have a greater influence on covering than UVR and PAR (Dumont et al. 2007). When testing for the factors of wave action, sea urchin body size, and UVR, wave action and sea urchin body size had a larger effect on the degree of covering than UVR. Small (juvenile) individuals covered more than large (adult) ones, doing so in increasingly wavy conditions (Dumont et al. 2007). This trend suggests that *S. droebachiensis* covers primarily to avoid dislodgement from higher wave action, and that small individuals do so more frequently, presumably to increase body weight to better cope with wave-induced drag.

Brothers & McClintok (2015) investigated the effects of seawater temperature on neuromuscular-mediated behaviours (like covering) in *L. variegatus,* a common sea urchin along the coast of Florida. *Lytechinus variegatus* covered less when exposed to 32°C seawater, a 4°C increase from their 28°C baseline (Brothers & McClintok 2015). In another study, the grazing rates of green sea urchin increased with temperature between 3°C and 12°C (Frey & Gagnon 2015), a range that may reflect their thermal optima for covering too. However, within a range of 3°C to 11°C, temperature was unlikely to have altered covering in green sea urchin (Rullens 2016). Several factors could explain these different outcomes, such as differences in sea urchin species' thermal optima (Portner 2002). Tropical species (e.g. *L. variegatus*) can exhibit a wider thermal tolerance when compared to temperate and polar species (e.g. *S. droebachiensis*). Chemical cues in a water column can alter behaviour (Steller & Caceres-Martinez 2009). Invertebrate larvae settle on live rhodoliths, which act as nursery grounds (Costa et al. 2020). These larvae may rely on settlement cues that draw them to the rhodoliths (Pereira & Bahia 2021, Steller & Caceres-Martinez 2009). Consequently, green sea urchin may show a preference for one covering material over another based on these chemical cues, either from live (i.e., rhodoliths) or nonliving materials (i.e., blue mussel shells and denatured rhodoliths).

A preliminary study by Rullens (2016) investigated the spatial variation of green sea urchin covering in a natural habitat, along with the combined effect of light conditions and wave surge under controlled laboratory conditions. In the field, small (1 to 2 cm in test diameter, t.d.) sea urchins covered mainly with rhodoliths, whereas large (4 to 5 cm t.d.) individuals covered mostly with macroalgae (red filamentous algae and kelp). Under laboratory conditions, wave surge and sea urchin size had a significant effect on covering, whereas light conditions did not significantly affect covering.

The present study builds upon Rullens (2016) by investigating factors affecting percent cover of green sea urchin tests under two laboratory experiments. One experiment explored covering material preference for either fragments of live rhodoliths, fragments of denatured rhodoliths, or fragments of blue mussel shells by sea urchins with a 1 to 2 cm t.d., at three water temperatures (2, 8, and 14°C). The other experiment explored covering in sea urchins of 4 to 5 cm t.d. with fragments of live rhodoliths at 8°C. Based on literature and preliminary trials, we carried out a 2-week laboratory experiment to test the predictions that green sea urchin covers (1) less in cold (2°C) and warm (14°C) seawater, as it is outside of thermal optima; (2) more with live rhodolith fragments than with blue mussel shell fragments or denatured rhodolith fragments; and (3) more when small (1 to 2 cm in test diameter, t.d.) than large (4 to 5 cm t.d.) in still water conditions. These predictions stem from studies suggesting that (1) sea urchin (grazing) behaviour was positively correlated with temperature from 3°C to 12°C (Frey & Gagnon 2015); (2) small sea urchins covered more with live rhodoliths than other available materials in a natural habitat (Rullens 2016, personal observations of sea urchin covering in natural habitats); and (3) covering in large sea urchins was lower than covering in small sea urchins in still water (Dumont et al. 2007, Rullens 2016).

2. MATERIALS AND METHODS

2.1 Collection and preparation of organisms

In December, 2022, 400 and 500 green sea urchins with a test diameter of 1 to 2 cm and 4 to 5 cm, respectively, were hand collected by divers from the sea urchin barrens in Flatrock Cove (Fig. 1). Rhodoliths and kelp (*Alaria esculenta*) were also collected, where the former was provided as covering material for the sea urchins during the trials, and the latter as food for the sea

urchins while in the holding tanks. Organisms were transported to the Ocean Sciences Centre (OSC) of Memorial University of Newfoundland (MUN) in coolers filled with seawater.

Upon arrival at the OSC (less than 4 hours after collection), sea urchins were transferred to holding tanks, supplied with ~0.7 L min^{-1} of seawater pumped in from a depth of ~37 m in the adjacent Logy Bay. Kelp was placed in one 330-L holding tank, supplied with approximately the same flow rate as the sea urchins holding tanks. Rhodoliths were cleaned of epibionts and broken into fragments similar in weight to broken mussel shell fragments. We assumed that boiling the rhodoliths was sufficient to denature their chemical cues. The rhodoliths and mussel shell fragments were weighted to gain an average weight value that was similar for each covering material type. This procedure ensured no type of covering material was heavier than another, a factor that could have altered sea urchin covering material preference. If one covering material type was heavier than another, sea urchins could have selected the lighter material for covering because of the lower energy cost required to move the latter.

We exposed sea urchins to natural daylight cycles from the sunlight entering the lab windows facing the bay. We kept all sea urchins in the holding tanks for three days without providing kelp to standardize hunger levels prior to experimentation. Sea urchin sizes were also hand measured with a calliper (with a precision of 0.1 mm), ensuring they fit the 1 to 2 cm and 4 to 5 cm t.d. size criteria prior to use, where they were redistributed into the holding tanks based on size class. Sea urchins of the same size class were distributed among several holding tanks to avoid overcrowding. Sea urchins were also weighted using an electronic balance with a precision of 0.01 g (Precision Scale, HZY-B3200).

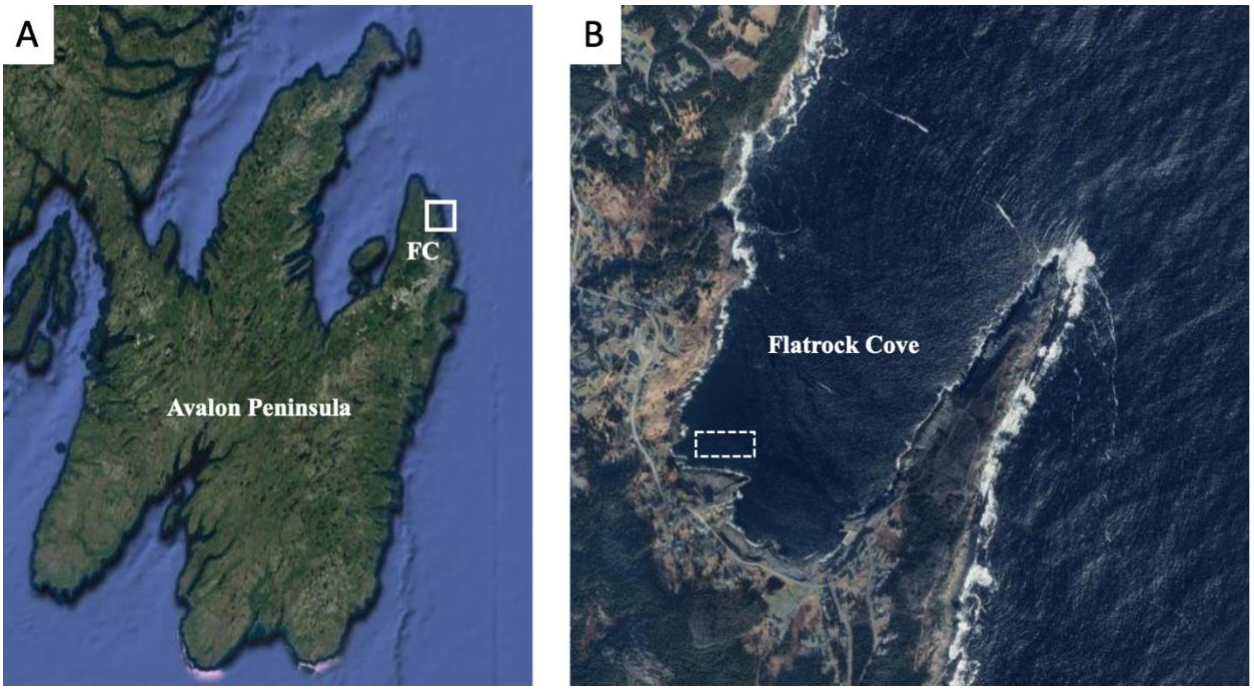

Figure 1. (A) Avalon Peninsula (southeastern Newfoundland) showing (B) the location (dashed rectangular inset) of the green sea urchin and covering materials collection and survey site in Flatrock Cove (FC).

2.2 Experimental design

Three main factors were tested: sea urchin body size, seawater temperature, and covering material on the covering of green sea urchins, which was achieved across two experiments, hereafter named Experiment 1 and Experiment 2. In both experiments we used three water baths that each measured 70 L x 30 W x 20 H cm (Grant Instruments, GR150) and allowed a fine control over temperature. In Experiment 1, we set each bath to either a low (2°C), intermediate (8°C), or high (14°C) temperature. In Experiment 2, we set all three baths to an intermediate (8°C) temperature. Temperature assignments were done through a randomization command in excel (Appendix A).

There is no published literature that has tested for seawater temperature effects on covering in green sea urchin. The low, intermediate, and high temperature values were selected based on relevant findings from Frey & Gagnon (2015), who explored a 3-to-18°C temperature range in the context of feeding behaviour rather than covering in *S. droebachiensis*. Since sea urchin feeding activity increased with increasing temperature values from 3 to 12°C (Frey & Gagnon 2015), we used these temperature values as proxies to set our thermal extremes. We expanded both endpoints to set the thermal extremes at 2 and 14°C to increase the likelihood of observing lower covering values that these new endpoints. We chose 8°C as the optimal temperature since it is halfway between these endpoints and is the temperature at which sea urchins covered the most during preliminary trials.

Each bath was filled with refrigerant solution that was circulated evenly by a pump, ensuring temperature was homogenous in all containers. Nine 1.25-L containers were filled at ~%75 capacity with the same source seawater pumped in from Logy Bay, arranged in a 3 x 3 grid pattern within all three baths. Before both experiments, sea urchins were taken haphazardly from

the holding tanks. One sea urchin was placed in each of the nine containers within the baths for the start of Experiment 1 and Experiment 2. These containers were the experimental units, and 3 extra/backup containers (1 container per bath, with no covering material in it) each containing four sea urchins were present if problems occurred concerning the sea urchins tested during both experiments.

Sea urchins were put in all containers for a 20-h acclimation to their respective water bath temperature. Water bath temperatures were set after sea urchins were placed in the containers, whereby the water bath temperatures changed on average 1°C every 18 min. During the acclimation period, sea urchins were not given any covering material. Instead, containers had air stones placed in them (1 air stone per container) for aeration, since we found that dissolved oxygen levels dropped significantly in the containers during this time (Appendix B). To ensure the air stones provided sufficient dissolved oxygen to the containers during both experiments, dissolved oxygen was measured using a multiparameter meter (YSI, with a precision of 0.01%) immediately before and after sea urchin covering assessments were made (i.e., at the start and end of each set of trials).

For Experiments 1 and 2, after the 20-h acclimation period, the air stones were removed. In Experiment 1, each container (except the backup sea urchin container) then received fragments of either live rhodoliths, denatured rhodoliths, or blue mussel shells introduced in a 3x3 Latin square grid arrangement (Appendix A), entirely covering the bottom of the containers. This particular arrangement helped address potential issues of pseureplication by ensuring no covering material appeared more than once in each row and column of containers. In Experiment 2, each container (except the backup sea urchin container) received fragments of live rhodoliths only. For both experiments, sea urchins were removed from their containers for no more than 10 s while the

covering materials were being placed into the containers. Removing the sea urchins ensured they would not simply grab the covering material as it was introduced. Reintroducing the sea urchins to the containers marked the start of each trial (i.e., t = 0).

Sea urchin covering was measured at t = 0, 5, 10, 15, 20, 40, 60, 90, 120, 150, and 180 min. Visual assessments of sea urchin covering percent were made for each container in each bath for every time interval listed above, and proceeding from left, to centre, to right bath containers. These visual assessments were made in 10% increments, resulting in 11 possible covering percent values for each sea urchin (0 to 100%, inclusive). From t = 40 min onward, pictures were taken (with an iPhone XR camera) to supplement visual assessments. Pictures were later analyzed for sea urchin test percent cover values (see section 2.3). The percent test cover values obtained from these images were compared to the visual percent test cover assessments to give an idea of the error ensuing from visual assessment (Appendix C, see Fig. 2 for complete setup).

We expected to see an overall lower degree of covering by the large sea urchins of Experiment 2 compared to the corresponding treatments of small sea urchins in Experiment 1. This expectation came from a preliminary study by Rullens (2016) in which the mean percent test cover of large sea urchins was significantly lower than in small sea urchins under still water conditions. We observed a similar trend in our preliminary trials, with small sea urchins covering more than large sea urchins under still water conditions. Since our experiments had sea urchins in still water conditions, we expected this observation to remain true.

We ran 8 replicates in Experiment 1. Each replicate contained 9 containers with 3 treatment types: 1 to 2 cm t.d. sea urchins with live rhodoliths, denatured rhodoliths, or blue mussel shells at either 2, 8, or 14°C. We ran 9 replicates in Experiment 2. Each replicate contained 9 containers with the same treatment type: 4 to 5 cm t.d. sea urchins with live rhodoliths at 8°C. Both

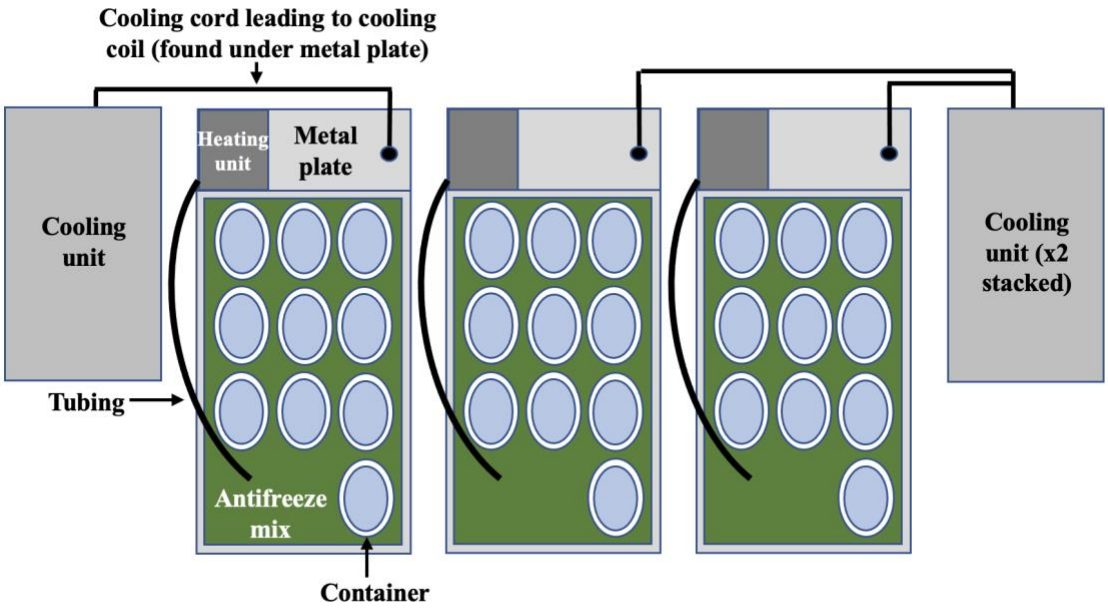

Figure 2. Experiment setup. The three baths have a pump system that circulates refrigerant solution evenly throughout the bath, ensuring even heat distribution among the containers within the baths. Each of the three baths contain 9 containers, set up in a 3x3 grid (i.e., in a Latin square design). Each container in the 3x3 grid receives 1 sea urchin. The additional container shown at the bottom/foremost section of each bath holds 4 sea urchins each. We included the extra sea urchin containers in case problems occurred concerning the sea urchins tested during both experiments.

Experiment 1 and Experiment 2 were run in tandem from 12 December, 2022 to 22 December, 2022. For every 2 days spent conducting replicates for Experiment 1, we alternated and spent 1 day conducting replicates for Experiment 2 to ensure both experiments would be completed within the same time frame. This procedure allowed us to compare covering values of large and small sea urchins between Experiment 1 and Experiment 2 without risking influence from uncontrolled changing temporal factors.

2.3 Image processing

ImageJ v1.8 was used to evaluate covering in green sea urchin. In ImageJ, we set the image scale based on a reference scale within the picture. We proceeded to analyze the image for sea urchin test and covering material surface area values. Each image consisted of 1 sea urchin covering with fragments of 1 type of covering material. We obtained a percent cover value for the sea urchin test by dividing the covering material surface area by the sea urchin test surface area. This process was repeated for each image.

We analyzed images from field data on green sea urchin covering in the sea urchin barrens of Flatrock Cove (Rullens 2016) to support our lab-based data on green sea urchin covering (Appendix D). Field data consisted of 20 images taken by a diver at a depth of 5 m in the sea urchin barrens in Flatrock Cove on 10 July, 2016. The diver photographed a quadrat, where each of the 20 images was taken roughly 1 metre apart. In ImageJ, we assessed the covering material selection and percent test cover of sea urchins within the photographed quadrat. The procedure used to assess sea urchin percent test cover was the same as outlined above, except multiple sea urchins were present within the quadrat (Fig. 3).

2.4 Statistical analysis

We used linear models to assess the effects of covering material, seawater temperature, and

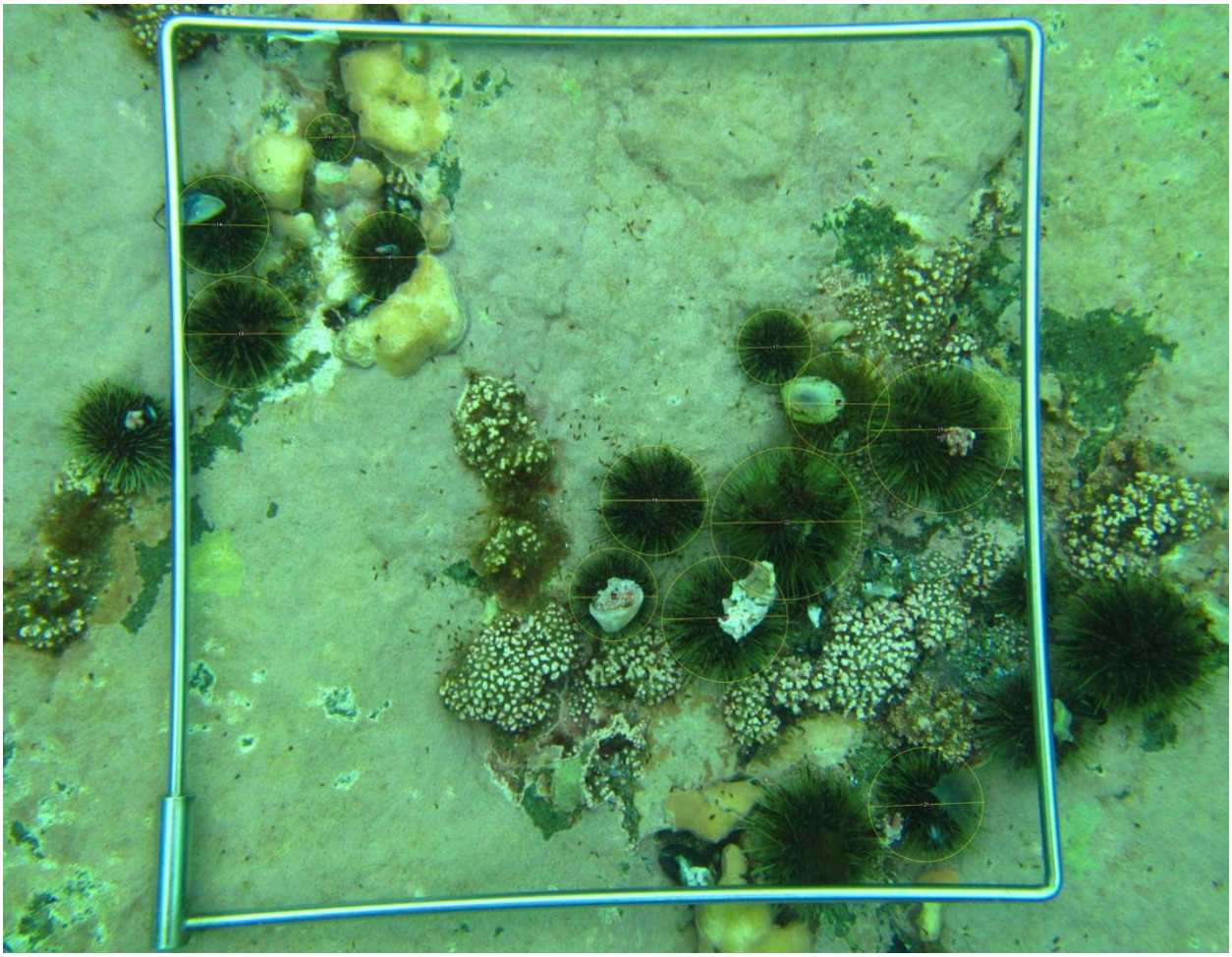

Figure 3. Sample field data image analyzed with ImageJ. The image was taken by a diver at a depth of 5 m in the sea urchin barrens in Flatrock Cove on 10 July, 2016. The large square is a quadrat (30 x 30 cm). Circles around the sea urchins indicate their outlined aboral surface, used to calculate the percent cover of each sea urchin test. Sea urchins with at least 50% of their test within the quadrat were evaluated for percent test cover.

sea urchin body size on percent test cover in green sea urchin. We used 2 linear models to test for the significance of our factors on percent test cover in green sea urchin, hereafter referred to as model 1 and model 2. The t = 60 min percent test cover values from our visual assessment were used for model 1 and model 2 because sea urchins covered the most at this time interval for Experiment 1 and Experiment 2 (Appendix E).

Model 1 was a mixed-effect (factorial ANOVA) model, containing 2 fixed factors (temperature and covering material) and 2 random factors (row and column in the 3 x 3 grid arrangement of containers). All percent test cover values used for this model were from the t = 60 min interval from Experiment 1. In accordance with the Latin square design, row and column were included as factors in this model to test for their level of significance. All interaction terms were considered between these four factors.

Model 2 was a t-test containing 1 fixed factor (sea urchin size). Sea urchin size varied between, but not within Experiment 1 and Experiment 2. Therefore, we selected and compared sea urchin percent test cover values from the same treatment type (sea urchins at 8°C covered with live rhodoliths) at the same time interval (t = 60 min) between Experiment 1 and Experiment 2. This comparison evaluated any percent test cover differences between the small (1 to 2 cm t.d.) sea urchins from Experiment 1 and the large (4 to 5 cm t.d.) sea urchins from Experiment 2.

Homogeneity of the variance and normality of the residuals were verified by assessing the distribution of the residuals and the normal probability plot of the residuals, respectively (Galecki & Burzykowski 2013). Residuals that follow a diagonal trendline without plateauing or creating a conical dispersion are deemed normal (Beckerman & Petchey 2012). R version 4.2.1 (R Core Team 2022) was used to complete all analyses, with a significance value of 0.05.

3. RESULTS

3.1 Temperature and covering material type

At the t = 60 min interval in Experiment 1, 60.2% of the sea urchins covered, while 39.8% did not. Our statistical analysis showed that temperature significantly affected percent test cover in green sea urchin. Covering material did not significantly affect percent test cover in green sea urchin. (Table 1). Sea urchins exhibited the highest percent test cover at 8°C (37.5±4 (SE) %), followed by 2°C (25.6±3.6%), then 14°C (20.0±2.9%). There was a significant difference between percent test cover values of 8°C and 2°C treatments, and between 8°C and 14°C treatments. There was no significant difference between sea urchin percent test cover values of 2°C and 14°C treatments (Fig. 4).

In proportion to all covered sea urchins, those covering with denatured rhodoliths covered the most (36.9±4.1%), followed by live rhodoliths (34.6±3.9%), then blue mussel shells (28.5±4%). There was no significant difference between the proportion of sea urchins covering with denatured rhodoliths, live rhodoliths, or blue mussel shells. Sea urchins exhibited the highest percent test cover with denatured rhodoliths (30.8±3.8%), followed by blue mussel shells (27.5±3.8%), then live rhodoliths (24.7±3.3%). There was no significant difference in sea urchin percent test cover values between denatured rhodoliths, live rhodoliths, and blue mussel shell (Fig. 5). Neither row nor column had significantly affected sea urchin percent test cover. The non-significance of these two model terms suggested that experimental conditions were similar within and between containers, which supports the notion that the experimental design was sound.

3.2 Sea urchin size

Our statistical analysis showed that size significantly affected percent test cover in green sea urchin (Table 2). We examined the proportion of sea urchins covering at each level within

Table 1. Summary of model 1 factorial ANOVA examining differences in percent test cover of green sea urchin (*Strongylocentrotus droebachiensis*) under factors temperature (T), covering material type (CMT), row (R), and column (C) in Experiment 1 (n = 216).

Source of variation	***df***	**SS**	**MS**	**F value**	***p***
T	2	11515	5757.4	6.1207	0.002839
CMT	2	629	314.4	0.3342	0.716496
R	2	4401	2200.5	2.3342	0.100204
C	2	210	104.8	0.1114	0.894656
T x CMT	4	2126	531.4	0.5649	0.688513
T x R	4	2219	554.6	0.5896	0.670692
CMT x R	4	5453	1363.3	1.4493	0.221096
T x C	4	1756	438.9	0.5666	0.760173
CMT x C	4	2981	745.3	0.7924	0.532035
R x C	4	1743	435.7	0.4632	0.762636
T x CMT x R	8	9913	1239.2	1.3174	0.239743
T x CMT x C	8	2898	362.2	0.3851	0.927114
T x R x C	8	5726	715.8	0.7609	0.637690
CMT x R x C	8	11381	1422.6	1.5124	0.158164
T x CMT x R x C	13	9685	745.0	0.7920	0.667547
Residuals	138	129808	940.6		

df degrees of freedom, SS sum of squares, MS mean square, *p* p-value

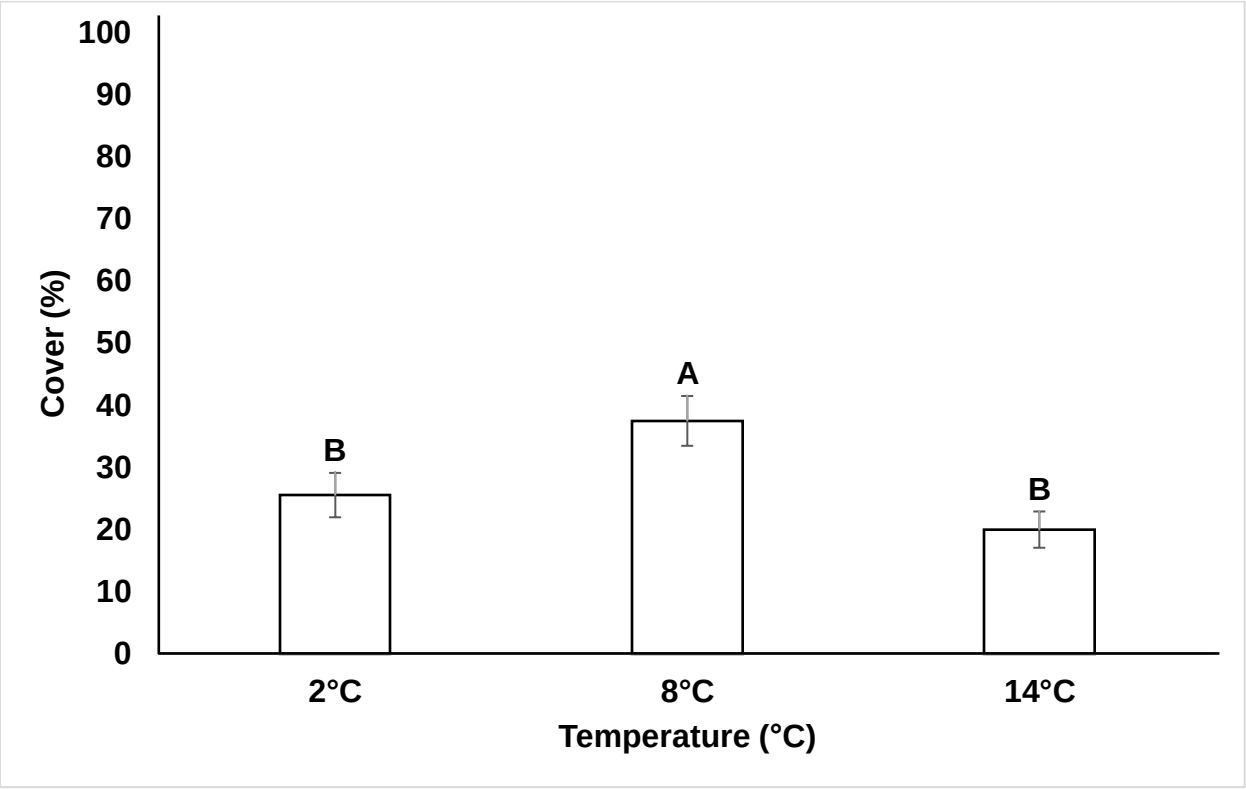

Figure 4. Comparison of percent test cover in green sea urchin corresponding to the three levels (2, 8, 14°C) within the temperature factor of model 1. There was a significant difference in sea urchin covering between 8°C and 2°C, but not between 2°C and 14°C (n = 216).

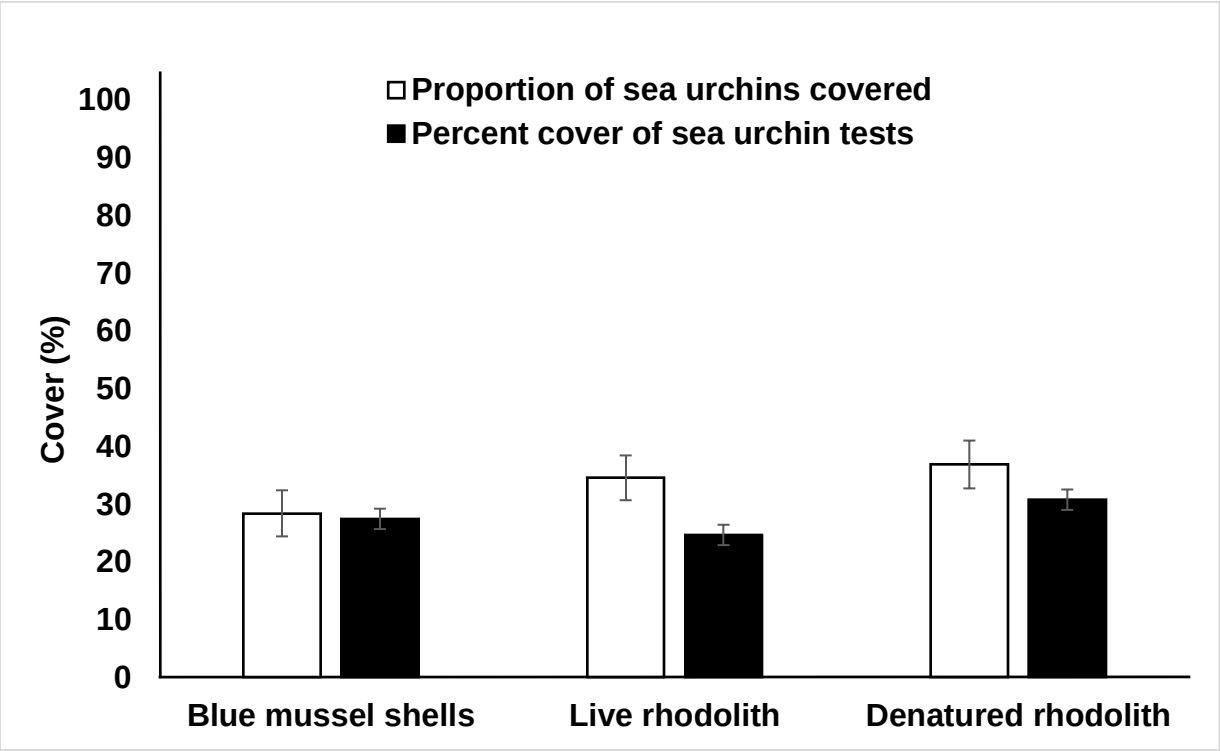

Figure 5. Proportion of sea urchins covered, and percent test cover exhibited by green sea urchin. Uppercase letters correspond to significance between proportion of sea urchin covered, whereas lowercase letters correspond to significance between percent test cover of sea urchins. The same letter means there is no significant difference between factor levels, whereas different letters mean there is a significant difference between factor levels (n = 216).

Table 2. Summary of model 2 t-test examining the effect of sea urchin size (S) on percent test cover of green sea urchin (*Strongylocentrotus droebachiensis*) between Experiment 1 and Experiment 2 (n = 48).

Source of variation	***df***	**SS**	**MS**	**F value**	***p***
S	1	3168.7	3168.7	5.0212	0.02991
Residuals	46	29029.2	631.1		

df degrees of freedom, SS sum of squares, MS mean square, *p* p-value

our size factor at our t = 60 min interval. In proportion to all covered sea urchins from our model 2 data, 59.3±4.3% were small sea urchins, while 40.7±4.1% were large sea urchins. Small sea urchins exhibited the highest percent test cover (29.6±2.9%), while large sea urchins exhibited the lowest percent test cover (13.3±1.6%).

4. DISCUSSION

Experiment 1 showed that covering in green sea urchin is affected by temperature, which supports our prediction that green sea urchin covers (1) less in cold (2°C) and warm (14°C) seawater, as it is outside of thermal optima. Experiment 1 also showed that covering in green sea urchin is not affected by covering material type, which contradicts our predication that green sea urchin covers (2) more with live rhodoliths fragments than with blue mussel shell fragments or denatured rhodoliths fragments. Experiment 1 and Experiment 2 showed that covering in green sea urchin is affected by sea urchin body size, which supports our prediction that green sea urchin covers (3) more when small (1 to 2 cm in test diameter, t.d.) than large (4 to 5 cm t.d.) in still water conditions.

4.1 Temperature

Sea urchins covered more at 8°C than at 2°C and 14°C. These findings are in accordance with green sea urchin grazing rates investigated by Frey and Gagnon (2015), whereby grazing rates of green sea urchin increase with temperature between 3°C and 12°C. There was a sharp drop in sea urchin grazing activity above 12°C, therefore the 3°C to 12°C temperature range could reflect green sea urchin thermal optima. Our findings indicate that both temperature extremes of 2°C and 14°C are outside of the proposed green sea urchin thermal optima for covering, as sea urchins covered less at 2°C and 14°C than at the midpoint of 8°C. The 6°C increase (or decrease) from our

8°C intermediate could have conferred a lower degree of covering in green sea urchin due to a slowing of neuromuscular-mediated behaviours. Brothers and McClintok (2015) found that increases of 4°C from a 28°C baseline can lead to lower covering rates in the common Floridian sea urchin *L. variegatus*, possibly caused by slowing of neuromuscular-mediated behaviours. Green sea urchin activity was highest from 3°C to 11°C (Rullens 2016) and 3°C to 12°C (Frey & Gagnon 2015). However, we observed lowered covering in green sea urchin at our 2°C to 14°C extremes, indicating that 3°C and 11°C or 12°C could become benchmarks outside which a sharp drop in neuromuscular-mediated behaviours (like covering) are observed.

4.2 Covering material type

Proportionally, sea urchins covered more with denatured rhodoliths than with live rhodoliths or blue mussel shells. Sea urchin percent test cover was also greatest with denatured rhodoliths over live rhodoliths and blue mussel shells. Neither the proportion nor percent test cover of sea urchins with any of the 3 material types was found to be statistically significant, indicating that covering material does not affect sea urchin covering. These findings contradict our prediction that green sea urchin would cover more with live rhodoliths than with denatured rhodoliths or blue mussel shells due to settlement/chemical cues. Steller and Caceres-Martinez (2009) concluded that invertebrate larvae settle on live rhodoliths, which act as nursery grounds, due to settlement cues that draw the larvae to the rhodoliths. Since small (1 to 2 cm t.d.) sea urchins were used to test for covering material preference, we predicted their younger age might aid in retaining larval settlement cues when compared to the older, large (4 to 5 cm t.d.) sea urchins. However, our findings suggest there is no larval settlement cue retained in small green sea urchin. It is possible that boiling the rhodoliths was not sufficient to completely denature the chemical cues.

Rullens' (2016) field data showed green sea urchin covered proportionately more with rhodoliths than with shells. However, sea urchin percent test cover with rhodoliths vs shells was not statistically significant ($p > 0.1$), which supports our findings since percent test cover was our response variable in our statistical models. Therefore, our findings, alongside those of Rullens (2016) suggest that green sea urchin may cover proportionately more with rhodoliths (living or dead), but sea urchin percent test cover does not vary significantly with covering material type.

4.3 Sea urchin body size

We found that small (1 to 2 cm t.d.) sea urchins exhibited greater covering than large (4 to 5 cm t.d.) sea urchins. This finding supports our prediction that small sea urchins cover more than large sea urchins under still water conditions. Dumont et al. (2007) reported that covering in green sea urchin exposed to strong wave surge was significantly greater than sea urchins exposed to still water conditions. Further, the extent to which sea urchins covered in their laboratory experiments increased with decreasing size. Dumont et al. (2007) suggested the smaller juveniles may cover more than larger adults due to increased susceptibility to predation and dislodgment. Though our findings align with the covering-size trend, we had no predators or wave action present in our containers. However, we cannot rule out either predation or wave action, as placement of the sea urchins into their containers may have elicited either a predation or dislodgment response. Sea urchins may have perceived our handling of them as a predation threat, and once placed into their containers, they may have initially covered to avoid further dislodgment. Any initial covering by sea urchins concerning predation or dislodgment cues could have settled by the $t = 60$ min interval used in our statistical models, potentially negating their influence on our results.

Rullens (2016) reported similar results to Dumont et al. (2007). Small (1 to 2 cm t.d.) green sea urchins covered more than large (4 to 5 cm t.d.) sea urchins under increasingly wavy

conditions. In large sea urchins, wave surge did not alter the amount of exhibited covering, further supporting the notion that small sea urchins cover more to avoid dislodgement due to their small size and lower overall weight compared to large sea urchins. Since wave action was not present in our study, our results could help determine a baseline covering degree in green sea urchin by size class.

4.4 Conclusion and future directions

Our 2-week laboratory experiments supported our prediction that green sea urchin covers (1) less in cold (2°C) and warm (14°C) seawater, as it is outside of thermal optima. Our experiments did not support our prediction that green sea urchin covers (2) more with live rhodoliths fragments than with blue mussel shell fragments or denatured rhodoliths fragments. Experiment 1 and Experiment 2 did support our prediction that green sea urchin covers (3) more when small (1 to 2 cm in test diameter, t.d.) than large (4 to 5 cm t.d.) in still water conditions. We showed that green sea urchin percent test cover significantly dropped 6°C above and below our 8°C treatments, a proposed optimal temperature for small sea urchin activity (Frey & Gagnon 2015, Rullens 2016). Small sea urchins exhibited a greater proportion and percent test cover than large sea urchins using rhodoliths fragments at 8°C. We also showed that both the proportion of sea urchins covered and sea urchin percent test cover with either live rhodoliths, denatured rhodoliths, or blue mussel shell fragments did not vary significantly.

Our results may help establish a baseline for temperature-induced covering values in green sea urchin under still water conditions, values which are not currently covered in the literature. Incorporating more temperatures within the 2°C to 14°C range to test for sea urchin covering could refine the scale for what temperature(s) green sea urchin covers most. Rullens (2016) found that sea urchins covered more with rhodoliths under increasingly wavy conditions. Future research

combining our experimental setup with a wave action component could determine if sea urchins cover more with certain types of covering material under increasingly wavy conditions with varying seawater temperatures. Destructive grazing habits in green sea urchin make it a bioengineering species in Newfoundland's coastal waters (Frey & Gagnon 2015, Schuster et al. 2022). Research indicates that sea urchin mobility decreases with increasing covering (Rullens 2016). Therefore, improving our understanding of factors responsible for eliciting covering in green sea urchin (which occurs during sea urchin grazing) may help us better understand sea urchin grazing dynamics.

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APPENDIX A

Latin square design, water bath and container assignment process

In the 3x3 Latin square grid design (Fig. A.1), each of the three covering material types was assigned a letter. The above process ensured no covering material appeared more than once in each row and column of containers, an occurrence that could cause pseudoreplication (Hurlbert 1984). To randomize water bath temperature assignments, an excel command producing a random number from 1 to 3 was used. Each of these numbers corresponded to a temperature value: 1 = 2°C, 2 = 8°C, 3 = 14°C. We assigned Latin squares to water baths by finding the 12 possible 3 x 3 Latin square combinations and arranging them in a 3 x 4 grid to form a haphazard number table. Rows were assigned a number from 1 to 3, and columns were assigned a number from 1 to 4 (Fig. A.2). A random number between 1 and 3 was generated for rows, then repeated for numbers 1 to 4 for columns. The output was 1 of the 12 pre-made Latin square designs, which was then assigned to the three water baths left, middle, then right in terms of their placement relative to the experimenter. This process was repeated for all trials of Experiment 1.

A	B	C
C	A	B
B	C	A

Figure A.1 General layout of a Latin square, where no letter is found more than once in each row or column. Each letter corresponds to a different type of covering material, while each of the 9 cells represents one experimental unit holding one sea urchin. Our assignment of Experiment 1 covering materials followed the Latin square design, with each of the three letters designating either live rhodoliths, denatured rhodoliths, or blue mussel shells.

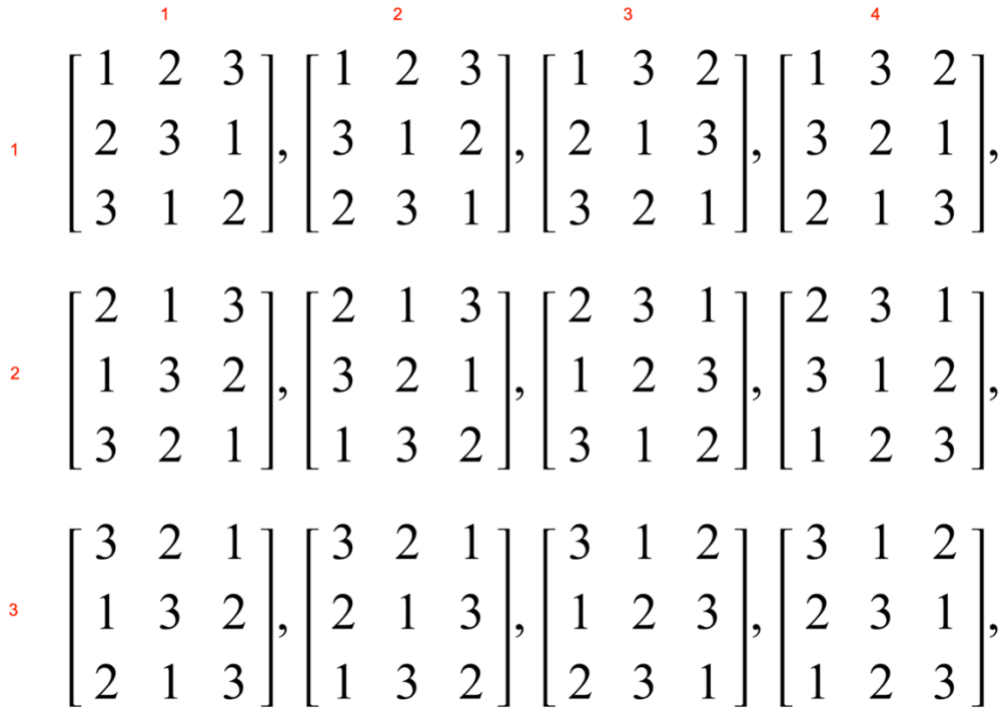

Figure A.2. Haphazard number table used for Latin square assignment. Numbers above the 3x3 Latin square grids correspond to column. Numbers to the left of the 3x3 Latin square grids correspond to row. The 9 numbers within each set of brackets corresponds to 1 of 12 possible Latin square arrangements assigned to a given water bath. Latin square arrangements were assigned to water baths through random number generation. First, a random number from 1 to 3 was generated for row, then a random number from 1 to 4 was generated for column. The resultant Latin square arrangement was then assigned to a water bath in a given trial. This process was repeated for all water baths in all trials, whereby the Latin square arrangements were assigned to the water baths from left to right (in terms of their placement relative to the experimenter).

APPENDIX B

Change in container seawater dissolved oxygen values during preliminary trials

From 27 October to 10 November, 2022, we conducted preliminary trials in the baths to gain a general understanding of sea urchin covering. The preliminary trials experiment setup was similar to the real trials experiment setup, except dissolved oxygen percent (DO%) was the focus instead of covering response. Our goal was to assess how DO% changed in containers of varying treatment types to test whether DO% dropped substantially from the start to the end of our real trials. Substantial drops in DO% from sea urchin metabolic consumption could have conferred lower activity, and subsequently lower covering values exhibited by green sea urchin. Further, a differential drop in DO% across varying treatment types would have been non-ideal, as we wanted to do our best to ensure parameter values/factors like these were as homogenous as possible since we were not testing for them in the real trials.

Covering material was provided to sea urchins to visually assess their covering in response to changing DO%. Large sea urchins (4 to 5 cm t.d.) covered far less than small sea urchins (1 to 2 cm t.d.) over 1 h increments. When sea urchins did not cover, the majority of them crawled to the top of the container walls and stayed at the water line. Small sea urchins covered far more with live rhodoliths than with blue mussel shells, both in containers with just live rhodoliths, and in containers with both materials available. Small sea urchins covered as quickly as 5 min with live rhodoliths in one trial, and within 10 min all sea urchins either adorned themselves with some type of covering material or moved up to the water line.

We used different bath temperatures to examine whether DO% varied with temperature throughout our trials. All three baths were randomly assigned temperatures 2, 8, or 14°C, where 8 containers were filled with seawater. The 8 containers each had a different treatment, as follows: a container with just seawater, a container with live rhodolith fragments in seawater, a container with a small 1 to 2 cm t.d. sea urchin in seawater, and a container with both the rhodolith fragments and a small sea urchin in seawater. The remaining four treatment types were a container with just seawater and an air stone, a container with live rhodolith fragments in seawater and an air stone, a container with a small (1 to 2 cm t.d.) sea urchin in seawater and an air stone, and a container with both the rhodolith fragments and a small sea urchin in seawater with an air stone. The standalone seawater containers were a control for DO% at each of the three temperatures. Containers with just rhodolith fragments in seawater and just a 1 to 2 cm t.d. sea urchin were to isolate how each organism independently affects DO% (i.e., their metabolic influence on DO%, since rhodoliths produce DO and sea urchins consume DO). The containers with both the rhodolith fragments and a 1 to 2 cm t.d. sea urchin depicted how DO% would change in real trials, mimicking a real container setup with a sea urchin and a covering material type. We duplicated these four treatment types with air stones, providing a standard to compare DO% levels between the non-aerated and aerated treatments. Through this comparison, we were able to discern if DO% levels in our containers dropped enough over time to necessitate aeration during our real trial acclimation period. DO% measurements were taken every 15 min during 3 h with a YSI multiparameter meter, and one more round of measurements again at 20 h. This DO% measurement process was one run, and in total three runs were completed to calculate an average value. There was no significant change in DO% over the 3 h period (Fig. B.1) in any of the treatments, but there was a significant change in the treatments after 24 h (Fig. B.2). These results indicate that air stones were not

necessary for the 3 h real trial durations, but were necessary for the 20-h temperature acclimation period since the DO% dropped both greatly and differentially for the varying treatment types.

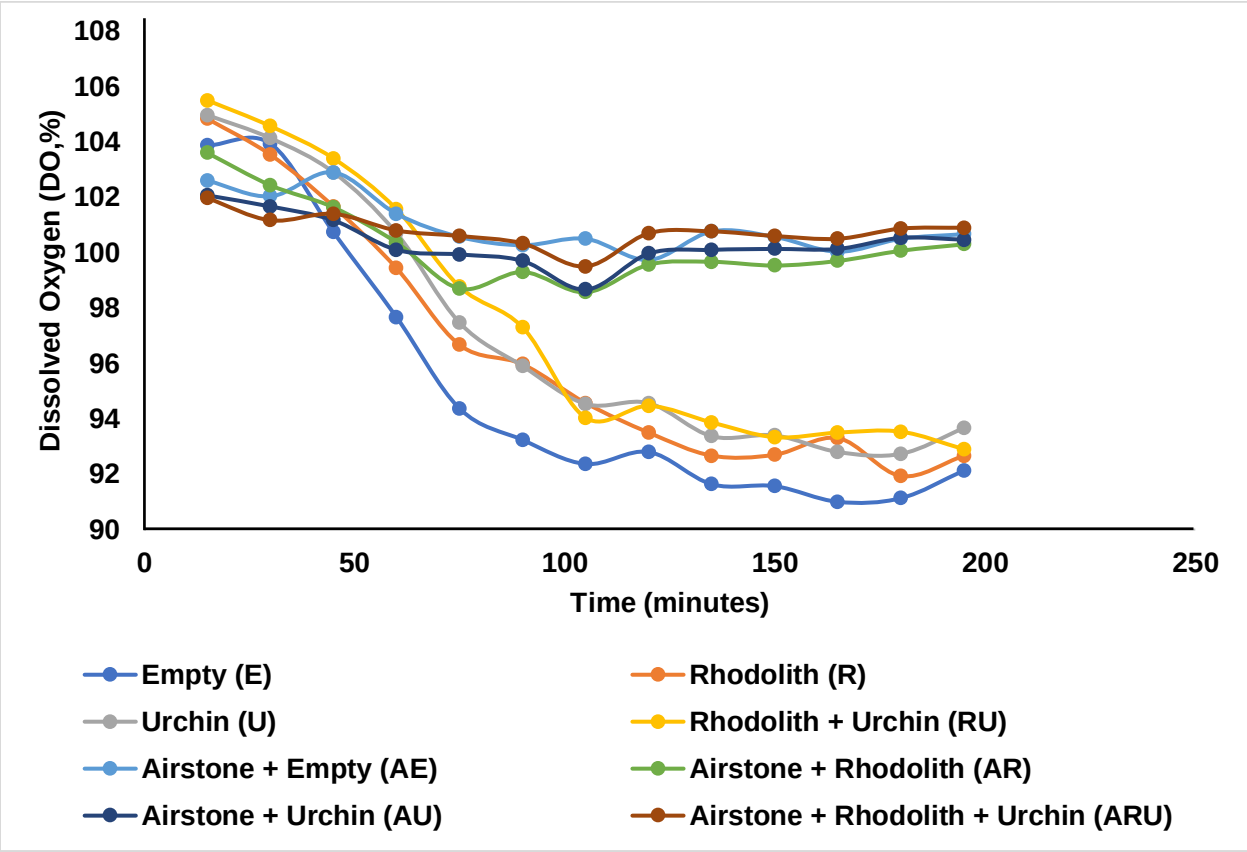

Figure B.1. Change in dissolved oxygen percent (DO%) over a 3 h period in sweater at 2°C. DO% in the non-aerated treatments dropped by as much as 12%, whereas DO% in the aerated treatments dropped by as much as 3% from start to end of trials. The 9% difference between DO% of the aerated and non-aerated treatments over 3 h was not enough to necessitate aeration during trials.

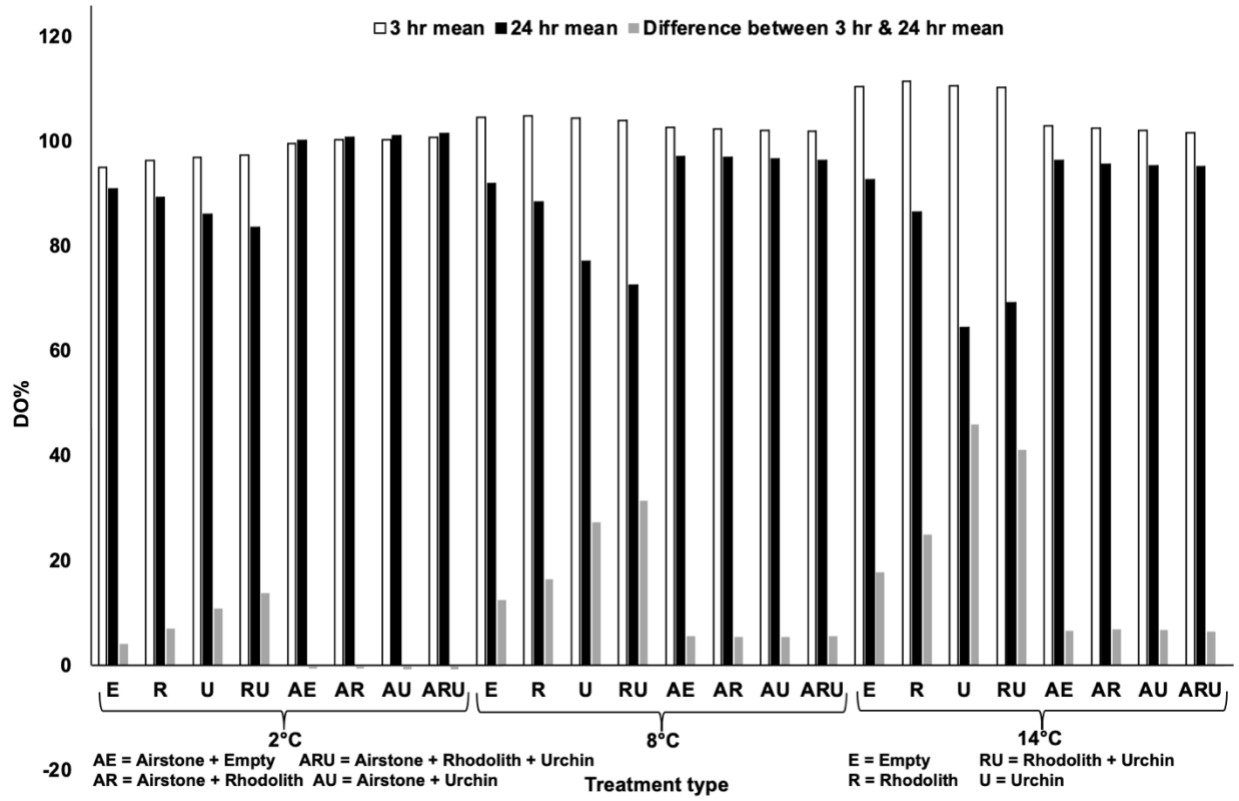

Figure B.2. Differences in dissolved oxygen percent (DO%) values of the 3 h mean and the 24 h mean. Each of the 8 treatment types are grouped under one of the 3 corresponding temperatures. Every treatment type was evaluated for DO% after 3 h and 24 h, whereafter the difference between 3 h and 24 h DO% was calculated (n = 48).

APPENDIX C

Image analysis error of sea urchin percent test cover assessments

To evaluate the accuracy of our visual percent cover assessments of green sea urchin tests, we compared our visual percent test cover values to our ImageJ-analyzed percent test cover values. The difference between our visual sea urchin percent test cover assessments and our ImageJ sea urchin percent test cover values was reported as a margin of error. We used 3 different time intervals (t = 40, 60, 180 min) to show the temporal trend of visual percent test cover accuracy in sea urchins. Due to the varying shapes of the fragmented covering materials, we calculated a mean value for the margin of error for both rhodoliths and blue mussel shells at each time interval. Ten (10) images were analyzed for percent test cover measurements for each covering material type (rhodoliths and blue mussel shells) at all 3 time intervals. In total, 60 images were analyzed and compared to visual percent test cover measurements for 6 mean margins of error, expressed as percent error (Fig. C.1). The largest mean margin of error was in the t = 180 blue mussel shell category (4.3%), followed by t = 180 rhodoliths (-1.0%), then t = 60 rhodoliths (0.7%), t = 40 blue mussel shell (0.62%), t = 40 rhodoliths (0.61%), and t = 60 blue mussel shell (-0.15%). A negative value indicates an underestimation of the degree of covering in sea urchins with a given covering material at a given time interval.

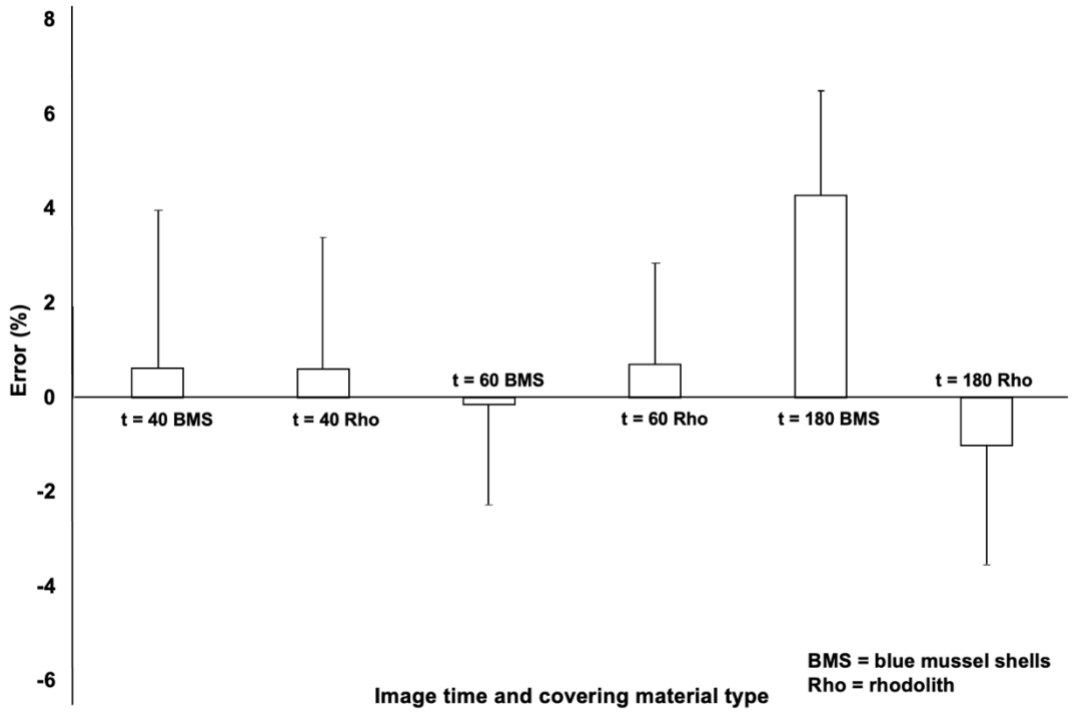

Figure C.1. Mean percent error values for image analysis. Positive y-values represent overestimation in visual sea urchin percent test cover measurements, whereas negative y-values represent underestimation in visual sea urchin percent test cover measurements. Overestimation of visual sea urchin percent test cover occurred more frequently than underestimation of visual sea urchin percent test cover (n = 60).

APPENDIX D

Field data

To support our lab-based findings, we analyzed field data gathered by Rullens (2016). Our ImageJ analysis of sea urchin covering from the provided 20 images yielded that 65.7% of sea urchins covered, while 34.3% did not cover. Of the 65.7% of covered sea urchins, we assessed what type of covering material the sea urchins covered with most: shells, rhodoliths, pebbles, red algae, or kelp. In proportion to all covered sea urchins, 59.8% of sea urchins covered with rhodoliths, 38.8% covered with shells, 13.8% covered with red algae, 12% covered with pebbles, and 11.9% covered with kelp. The percent cover of sea urchin tests was 35.4% with kelp, 17.5% with red algae, 16.7% with rhodoliths, 12.1% with pebbles, and 8.5% with shells (Fig. D.1).

There was a significant difference between the proportion of sea urchins that covered with rhodoliths or shells, and between sea urchins that covered with shells and red algae. There was no significant difference between the proportion of sea urchins covering with red algae, pebbles, or kelp. There was a significant difference between the percent cover of sea urchin tests with kelp or red algae. There was no significant difference between the percent cover of sea urchin tests with red algae, rhodoliths, pebbles, or shells.

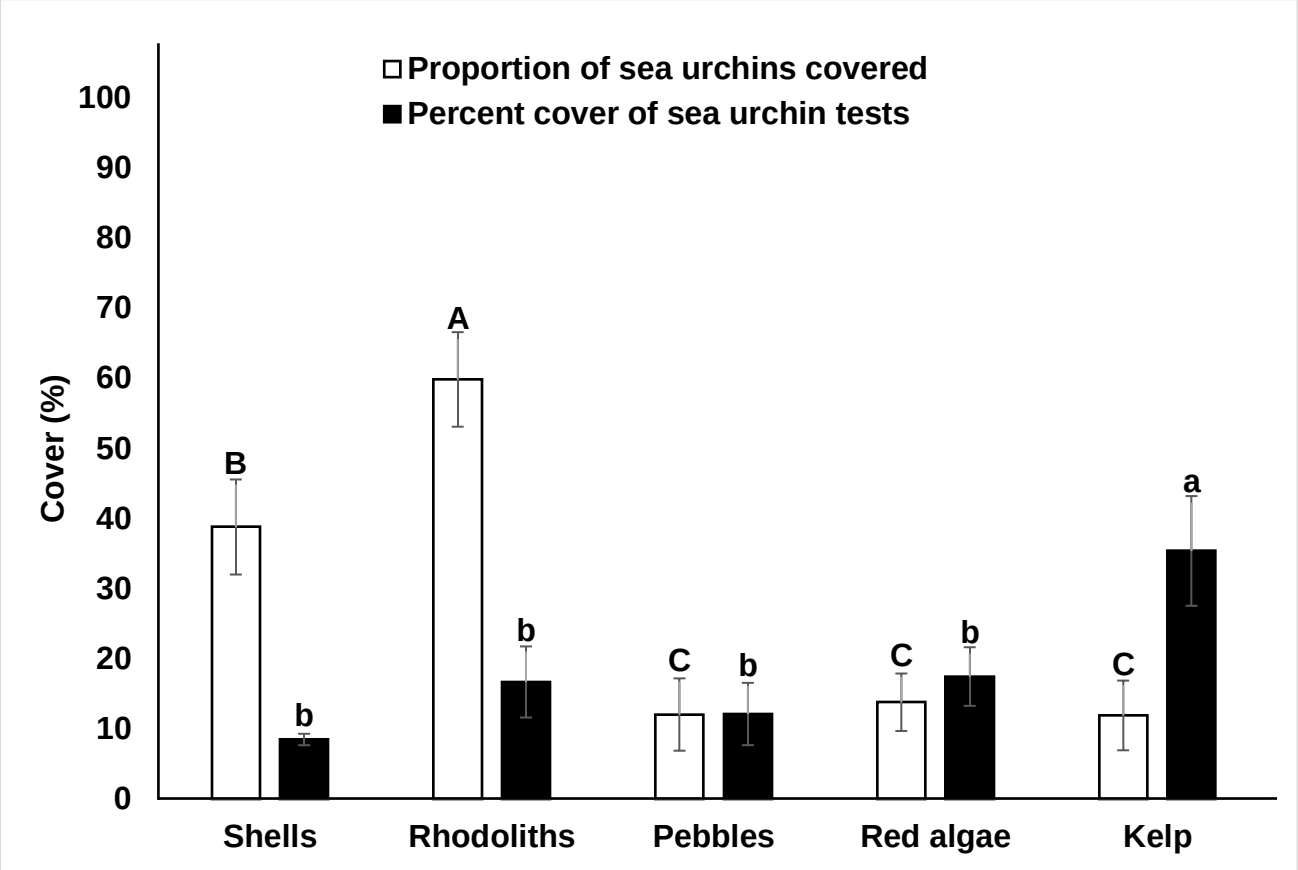

Figure D.1. Proportion of sea urchins covered and percent test cover of sea urchins with different covering materials from field data. Each of the 5 covering material types has a corresponding value for the proportion of sea urchins that covered with it and the percent test cover of sea urchins with that covering material. Uppercase letters correspond to significance between proportion of sea urchin covered, whereas lowercase letters correspond to significance between percent test cover of sea urchins. The same letter means there is no significant difference between factor levels, whereas different letters mean there is a significant difference between factor levels (n = 20).

APPENDIX E

Process of time interval selection for statistical analysis

Once we finished gathering values for our visual assessments of green sea urchin percent test cover, we had to determine which time interval values were most appropriate from Experiment 1 and Experient 2 for our statistical model inputs (i.e., model 1 & model 2). Out of 10 time intervals (t = 5 to t = 180 min) we obtained one average trendline, from which we chose the interval where percent cover of sea urchin tests plateaued. (Figs. E.1 & E.2).

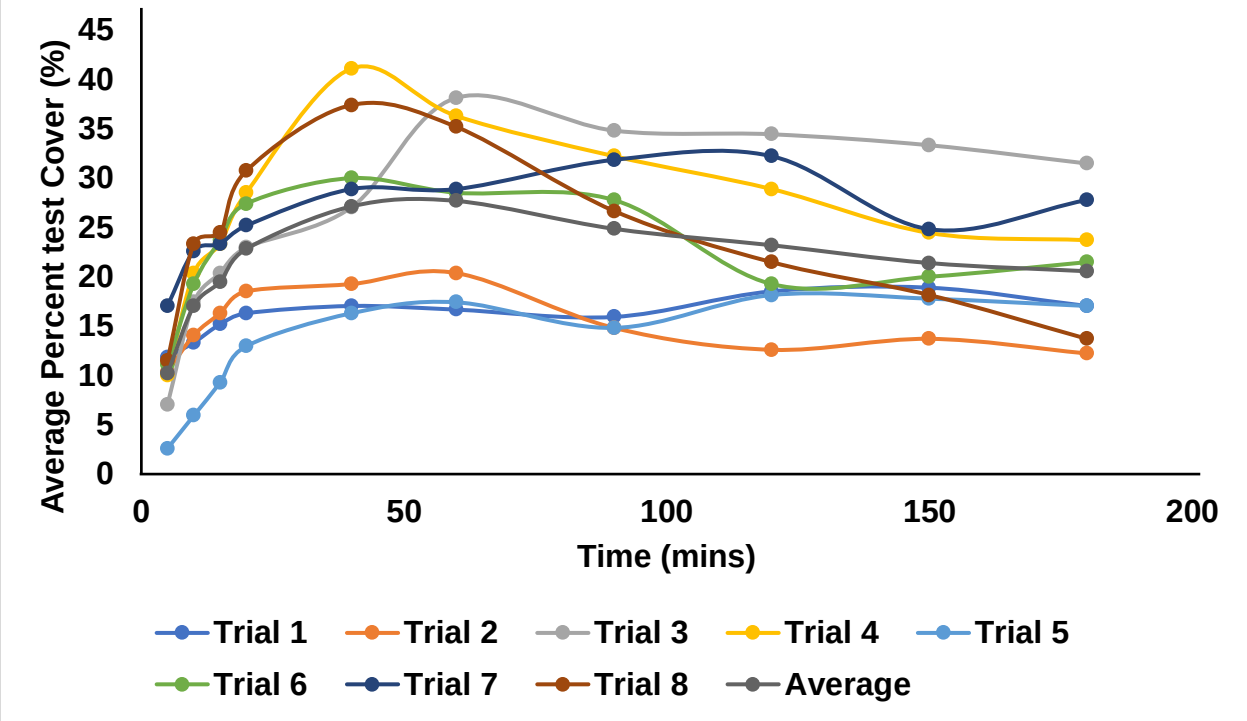

Figure E.1. Average sea urchin percent test cover for Experiment 1 at the 10 time intervals. On average, sea urchin percent test cover increased during the first 40 min, then plateaued at 60 min.

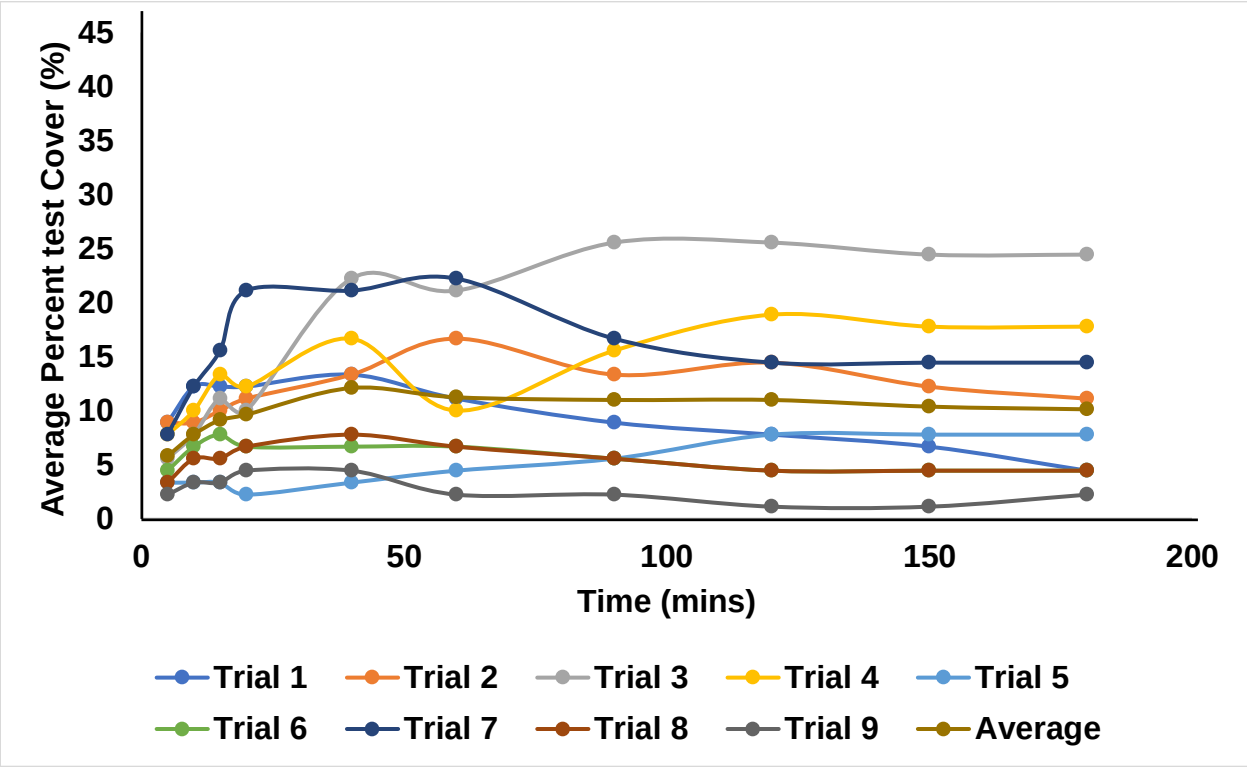

Figure E.2. Average sea urchin percent test cover for Experiment 2 at the 10 time intervals. On average, sea urchin percent test cover increased during the first 15 min, suffering a slight decrease at 20 min. Beyond 20 min, sea urchin percent test cover increased again until 60 min at which it plateaued.