Light and Temperature on Reef Herbivory: Effects of Algal Growth Conditions on Grazing Intensity by Juvenile and Adult Sea Hares

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Herbivore-algae interactions play a dominant role in reef health and stability. As climate change progresses, light exposure and sea surface temperature are predicted to increase. This study examines how herbivores respond to climate change-induced impacts on prey algae using the spotted sea hare (*Aplysia dactylomela*) and the red alga *Laurencia intricata*. Algae was cultured under increased light and temperature treatments then presented to adult and juvenile sea hares. The mass of algae consumed from each treatment by each age group was determined. It was found that algae grown under high temperature was eaten significantly less than algae grown of any other treatment. Adult sea hares ate significantly more per capita than juveniles and treatment preference was not significantly different between the two age groups. These results indicate that increasing sea surface temperature will likely have an impact on reef herbivory and macroalgal abundance and merits further study.

Introduction

The oceans and climate are currently experiencing change at an alarming rate, and their health and stability are projected to deteriorate for many decades to come[1]. It is essential to study the effects of these changes as they concern the welfare and future of the planet as well as its inhabitants[2].

Coral reefs are crucial, as they are locations of immense biodiversity and high ecological value[3]. They support intricate food webs and provide habitat for many organisms, including charismatic flagship species such as sea turtles and whales. Reefs also provide ecosystem services through jobs, food, tourism, recreation, cultural value, and storm protection totaling over AU$6.6 billion annually[4]. Thus, it is in the planet's and humanity's best interest to prioritize reef preservation during this pivotal time.

It has been well documented that coral reefs are under threat from climate change, as rapid ocean acidification coupled with increasingly frequent bleaching events is steadily eroding these calcifiers[5]. The complexity of physical, chemical, and biological variables involved in reef structure make it difficult to predict how these climatic changes will manifest. One topic that has spurred much controversy is the way climate change is predicted to impact algae[6]. It is well known that one of the dominant factors in reef health and productivity is the coral/macroalgae relationship. Herbivorous reef fishes and invertebrates graze heavily on macroalgae and control its growth in the process. In their absence, or in the presence of excess nutrients, algae will overgrow the coral, blocking incoming light and impeding coral photosynthesis and growth. Additionally, as macroalgae overtakes hard substrates, coral larvae are unable to settle and recruit, initiating a positive feedback loop eventually causing a phase shift from coral reef to macroalgae dominated benthos[7]. Phase shifts are often triggered by coral bleaching and mass mortality events (when the reef is already at a weakened state) and are exceptionally difficult to recover from. Algal phase shifts not only reduce the reef's aesthetic beauty and tourism value, but also lead to biodiversity loss, fishery collapse, and diminished shoreline protection. Thus, developing an understanding of the processes and precursors to phase shifts has substantial implications in multiple sectors[8]. Herbivory maintains reef health by preventing phase shifts from occurring[9], so it is worthwhile to explore how climate change may impact it. This paper seeks to address this issue by testing how herbivory is altered when presented with macroalgae grown under future climate conditions.

The herbivore selected for this study was *Aplysia dactylomela* (Rang, 1828), the spotted sea hare. Sea hares, a clade of gastropod mollusk, fill an important niche by feeding off algae containing chemical toxins not grazed on by other herbivores[10]. A. dactylomela is a cosmopolitan species, found across the world in tropical waters and reefs[11]. This species' broad distribution and key role in controlling toxin-laden algae makes A. dactylomela globally relevant and impactful, and thus an ideal choice for this experiment.

A. dactylomela feeds primarily on the red macroalga *Laurencia intricata*, sequestering the secondary metabolites to become unpalatable themselves[12]. *A. dactylomela* also uses *L. intricata*'s pigments to produce a purple ink believed to confuse the sensory organs of would-be predators[13]. The health of the alga determines the amount of chemical defenses it can produce as well as its nutritive value. Thus, as survival of *A. dactylomela* is affected by ability to grow large enough to reproduce while avoiding predation, there is an evolutionary advantage to feeding on algae that is healthier and more nutritious. In fact, several studies [10, 14] have shown that *Aplysia* sea hares are indeed able to detect and selectively feed on algae that is more nutritious. Given this, it follows that *A. dactylomela* will, when all else is constant, preferentially graze on the healthiest, most nutritious algae available.

Future climate models predict the Great Barrier Reef will experience elevated sea surface temperature, as well as increased light exposure during El Niño events: periods of weakened trade winds and altered circulation[15]. These stressors may impact the health and nutritional content of reef macroalgae, which would be apparent in the feeding preferences of herbivores, in this case *A. dactylomela*. By examining how herbivory changes when sea hares are presented with food sources similar to what they will encounter in the future, we can gather information on what reefs may begin to look like as climate change progresses.

It has been shown that light and temperature are two of the most important factors in determining growth rates of algae in the genus *Laurencia*[16] and thus, certain elements of the algae's physiology will likely be altered along with rate of growth and may influence sea hare feeding preference. A study [17] on growth rates of macroalgae under different conditions found that both temperature and light will negatively alter growth rate as well as pigmentation and nutritive value when altered from the natural optimal value. While this does not indicate how this algae will be grazed by herbivores, it would suggest that as a result of elevated light and temperature it is less healthy overall. However, a study [18] in 2000 suggests the opposite, finding that red abalone exhibited higher growth rates when fed algae cultured under higher light conditions, likely indicating an enhanced nutritional content. One study [21] in the Bahamas used a naturally occurring Nitrogen gradient to study the relative impact of N and herbivory (by *A. dactylomela* and others) on macroalgae. They found that *A. dactylomela* grazed more on *L. intricata* grown in high N conditions, presumably due to this elevated nutritional value, and that herbivory was the dominant factor dictating macroalgal species composition by consuming certain algaes to their preference. However, they did not significantly alter total macroalgae biomass and subsequent coral cover, as that was attributed largely to N content.

One study [19] found that dietary preference in limpets and chitons is largely determined by the ability of the animal's mouth parts to accommodate a given algae's structure and texture. For example, the periwinkle snail *Littorina Iittorea* possesses a large number of small sharp teeth with high surface area and primarily grazes on soft filamentous algae and microalgal films. Conversely, another periwinkle, *Littorina obtusata*, has fewer, stronger, and blunter teeth, and is able to prey on tough macrophytes [19]. A more general study on herbivory [20] looking at isopods and amphipods concluded that dietary preference is a result of either 'attractiveness', here loosely meaning health and nutritional value, or 'edibility'. The paper went on to elaborate that food choice in a given herbivore is dominated by food quality or ease of eating (rarely both), and is influenced by a variety of factors including habitat, physiology, predation pressure, size, and food source availability. To illustrate, the *Idotea baltica* isopod chooses hard, branched algae which, although more difficult to consume, is highly nutritive [20]. Meanwhile *Ampithoe valida*, an amphipod, favors soft, supple bladed algae because of this morphology and the fact that it is a more prevalent food source [20]. Additional elements can also come into play, such as a rigid food alga doubling as wave protection and predator defense for *I. baltica* [20]. While a sea hare is quite different from either an isopod or amphipod, this relationship has been shown to persist: the type of algae a sea hare species predates upon is often linked to the complexity of its radula, with highly specific radulae indicating a specialist, whereas more modest, uncomplicated radulae typically belong to generalists [22]. In a study regarding the related *Dolabella* sea hares, herbivory was determined largely by the softness of the algae, for in every case in which they were presented with two food choices, they chose the softer [23].

Still other studies [24] have deemed these oversimplifications, and emphasized the notion that food choices cannot always be reduced to simply energy, texture, or protein content. Instead, herbivores moderate their diet to predate on a variety of primary producers to achieve maximum nutritional complementarity [24]. In other words, they prefer a well balanced diet.

The 6cm long *Stylocheilus striatus* sea hare is frequently associated with cyanobacterial blooms, predating on the microalgae. It in turn is targeted by a carnivorous nudibranch (another variety of sea slug), with each nudibranch consuming more than 2 sea hares per day [25]. Predation of sea hares, and the resultant reduction in herbivory, lead to a 50% increase in cyanobacteria biomass [25]. In this case, smaller sea hares were 22 times more likely to be eaten than large individuals [25] so it is worth noting that juveniles may behave differently from adults due to predator avoidance behavior which could lead to differences in food preference. While *Aplysia* sea hares reach sexual

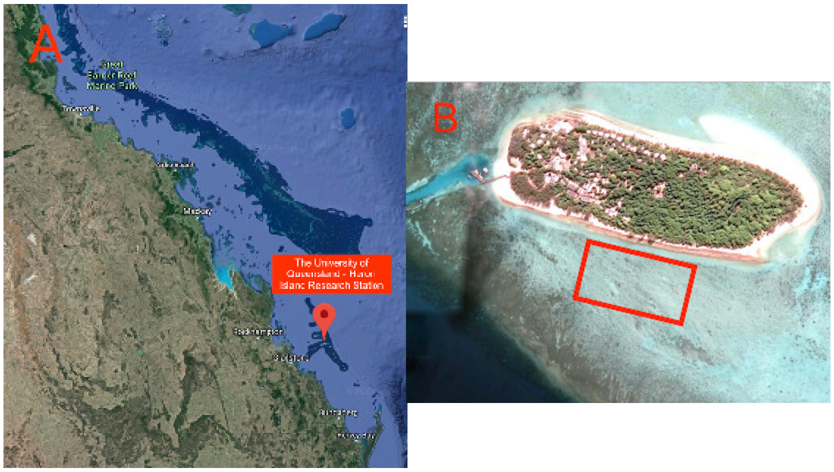

Figure 1. A) Satellite image showing location of Heron Island Research Station (HIRS). HIRS is located on Heron Island (23.442°S, 151.914°E) in the southern Great Barrier Reef. It is operated by the University of Queensland and is the reef's oldest and largest marine station. The Great Barrier Reef Marine Park is shown in dark blue with a red icon designating Heron Island; **B)** Aerial view of Heron Island. Experiments were conducted in HIRS facilities, with organisms and seawater collected on the southern reef flat within the Scientific Research Zone (rectangle outlined in red).
Image source: Google Earth, 2018

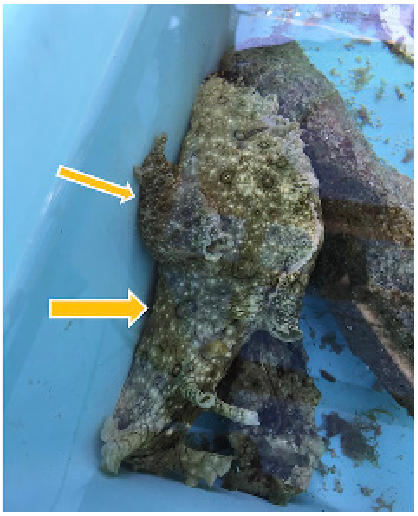

Figure 2. *Aplysia dactylomela*, the spotted sea hare, was the herbivore chosen for this study. Arrows show size difference of adult (bottom arrow) and 15 cm juvenile (top arrow) *A. dactylomela*. During a 3d collection period, two adults and four juveniles were found and moved to a large flow-through tank containing rocks from the reef flat and allowed to acclimate for 7d before participating in feeding trials. All were fed a diet of fresh *Laurencia intricata* macroalgae each day.

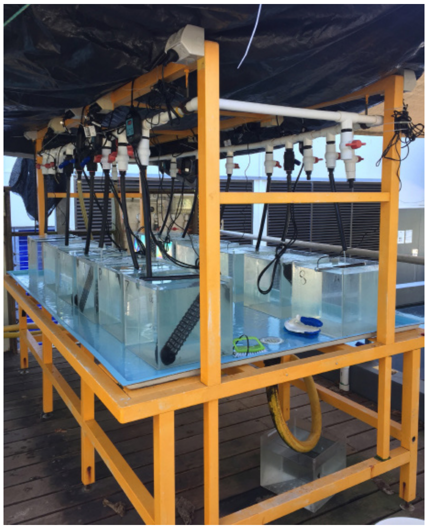

Figure 3. Experimental setup for algae treatment tanks. Twelve 125L flow-through tanks arranged beneath a shade screen to equalize natural light exposure. Tanks were randomly assigned one of four treatments: Control (C), increased light (L), increased temperature (T), and both increased light and temperature (B). There were three replicate tanks for each treatment. Tanks were filled with *Laurencia intricata* macroalgae and cultured for 5d under treatment conditions. Water for tanks was pumped directly off the reef flat at Heron Island Research Station where the algae was collected. Tanks and equipment were monitored daily to ensure proper functioning.

maturity within several months and spend half their life as adults, the juvenile phase is also significant [26] and thus should be included in analysis. There is evidence that some *Aplysia* species change their preferred algae as they mature[27], but understanding the evolution of dietary preference across life stages is largely unknown[28]. In this experiment, *Laurencia intricata* was exposed to increased temperature and light and the impact on herbivory by the sea hare *Aplysia dactylomela* was measured. Additionally, the impact on herbivory of adult and juvenile sea hares was compared. This study aims to address the following three questions:

1. Will *Aplysia dactylomela* consume *Laurencia intricata* grown in high temperature and/or light differently than that grown in control (present day) conditions?
2. Will adult and juvenile *Aplysia dactylomela* consume different amounts of *Laurencia intricata*?
3. Will adults and juveniles differ in preference between *Laurencia intricata* treatments?

As shifts in herbivory can have widespread impacts[29], answering these questions may shed light on the future of reef community structure, and thus improve management techniques and preparation for climate change.

Methods

Overview

This experiment was conducted at Heron Island Research Station on the Great Barrier Reef (Fig. 1A), and sea hares and algae were collected on the southern reef flat (Fig. 1B). Tides are semidiurnal, and a reef crest separates the flat from the ocean during low tide. Being shallow and partially isolated, the reef flat experiences high variation in temperature throughout the day (3–4°C) and season (6.5°C), with an average water temperature of 20°C in the winter and 28°C in the summer[30]. This experiment took place during Spring (October 7-16) with mean water temperatures of 24°C.

Over the course of 3d, a total of six Aplysia dactylomela were found: two Adults and four juveniles (half of which were 2cm long, the other half were 15 cm long) (Fig. 2). All were acclimated for 7d in a large, controlled flow-through tank using water pumped from the reef flat and fed a diet of fresh *Laurencia intricata*.

Algae Treatments

The culturing setup entailed growing *L. intricata* in four different treatments: increased light (L), increased temperature (T), both increased light and temperature (B), and control (C). Twelve 125L shaded flow-through tanks were randomly assigned to treatments, providing three replicates each (Fig. 3). Tanks in the L treatment were illuminated by external LED hood lights from 07:00 to 18:00 each day (Fig. 4A). The T treatment included submersible heaters programmed to raise the temperature of incoming water by 3°C at all times to mimic projected rise of sea surface temperature on the reef flat (Fig. 4B). B tanks were fitted with both hood lights and heaters (Fig. 4C). C tanks were left bare aside from the water inflow tube (Fig. 4D). A shade screen was hung above the tanks to equalize natural light exposure. Two five-gallon buckets of *L. intricata* were collected from the reef flat then divided evenly amongst the 12 treatment tanks and cultured for 5d.

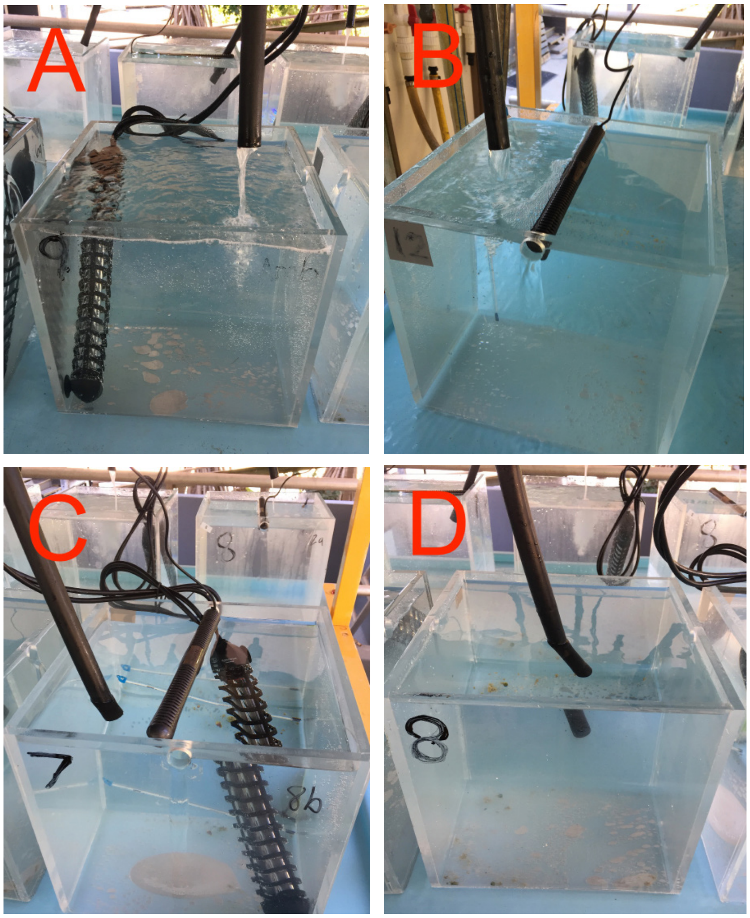

Figure 4. Setup of treatments in which *Laurencia intricata* algae was cultured. All are flow-through tanks using water pumped from the reef flat: **A)** Increased temperature treatment tank with submersible heater. These tanks were kept 3°C above ambient sea surface temperature on the reef flat and were tested daily with a thermometer to verify heater accuracy; **B)** Increased light treatment tank with external LED hood. Lights were positioned directly across the tank at the water surface and were turned on from 07:00 to 18:00 each day. They emitted white light; **C)** Combined light and temperature treatment tank containing both LED hood and submersible heater; **D)** Control treatment tank.

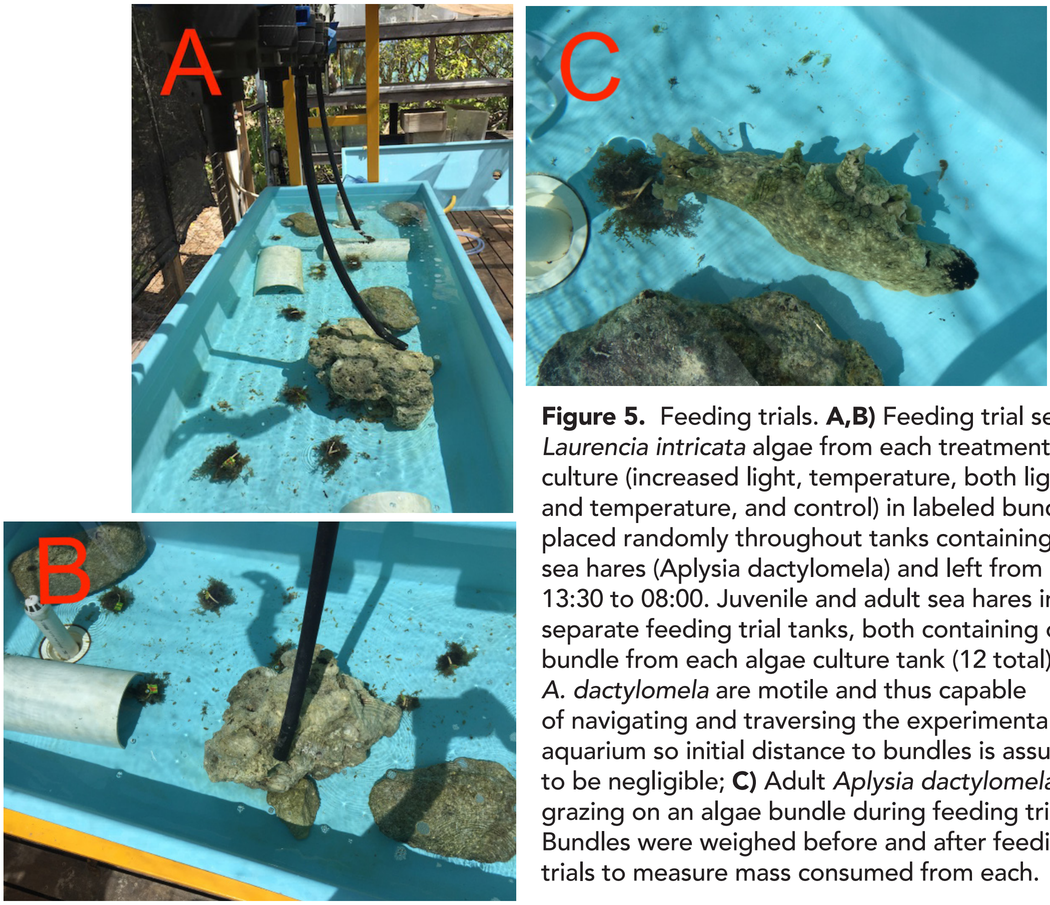

Figure 5. Feeding trials. **A,B)** Feeding trial setup. *Laurencia intricata* algae from each treatment culture (increased light, temperature, both light and temperature, and control) in labeled bundles placed randomly throughout tanks containing sea hares (Aplysia dactylomela) and left from 13:30 to 08:00. Juvenile and adult sea hares in separate feeding trial tanks, both containing one bundle from each algae culture tank (12 total). *A. dactylomela* are motile and thus capable of navigating and traversing the experimental aquarium so initial distance to bundles is assumed to be negligible; **C)** Adult *Aplysia dactylomela* grazing on an algae bundle during feeding trials. Bundles were weighed before and after feeding trials to measure mass consumed from each.

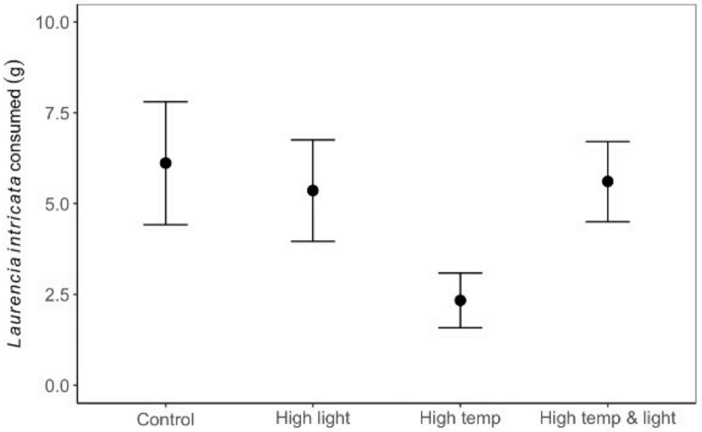

Figure 6. Mean grams of the red algae *Laurencia intricata* consumed per bundle per sea hare from each treatment culture over 18.5h. For control n=6; high light n=6; high temperature n=6, high light and temperature n=4. Bars represent standard error.

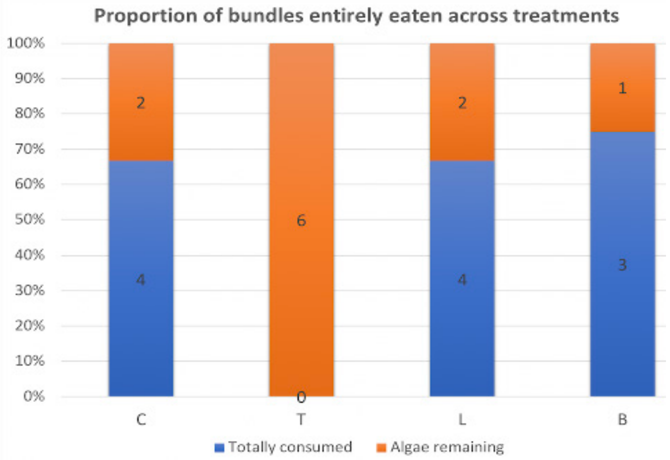

Figure 7. Proportion of red algae *L. intricata* bundles completely consumed by sea hares after 18.5 hours across treatments: Control (C), high temperature (T), high light (L), both high light and temperature (B). Number of bundles in each treatment and category is represented on each bar.

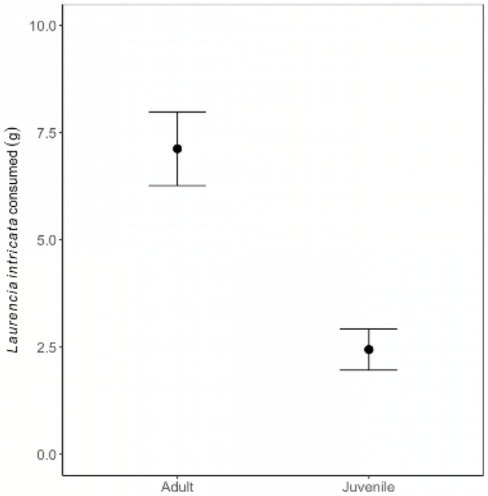

Figure 8. Mean grams of red algae *Laurencia intricata* consumed per bundle per individual by each juvenile (n=4) and adult (n=2) sea hares *Aplysia dactylomela* over a 18.5h period.

Feeding Trials

Algae in each tank was tied into two egg-sized bundles using rubber bands and labeled with treatment and tank number: a total of six replicate bundles per treatment. Bundles were blotted dry with a cloth for 5s then weighed and the mass recorded.

Sea hares were fasted for 12h before the feeding trial. Adults and juveniles were placed into separate tanks with one bundle each from every tank and treatment dispersed randomly throughout to control for differences in initial proximity to sea hares (Fig. 5). Trials began at 13:30 and continued until 08:00 the next morning (18.5h duration) at which time what remained of the bundles was removed, blotted, and weighed again. The difference in mass for each bundle was calculated and associated with the given treatment, tank number, and *A. dactylomela* age. In cases where all algae was consumed, the rubber band and label were weighed. To standardize results between adults and juveniles and achieve per capita values, difference in mass for each bundle was divided by number of sea hares in the tank. The timing of the experiment gave sea hares equal opportunity to feed during different times of both day and night.

Analysis

All data analysis was conducted using R version 3.4.2. and all figures were made through ggplot2. A preliminary QQplot and histogram were used to visually confirm normality of distribution. Data appeared slightly right skewed and were adjusted using a square root transformation (see Appendix). A Levene's test was then run to check the assumption of homogeneity of variance, and a Shapiro test to mathematically verify normality of distribution; both tests passed.

A two-way Analysis of Variance (ANOVA) was used to compare mass of *L. intricata* consumed between algal treatments as well as age groups of *A. dactylomela.* Age and algae treatment were orthogonal fixed factors, while tank number was a random effect of algae treatment. A post-hoc Tukey's test was then used to isolate significant differences between specific treatments.

Results

A. dactylomela ate significantly different quantities of algae across the different treatments($F_{(3,13)}=15.618$, $p=1.34\text{x}10^{-4}$). *A. dactylomela* ate significantly less algae from treatment T than the control or any other treatment ($p<0.05$), while there was not a significant difference in amount consumed between *L. intricata* treated with C, L, or B (Fig. 6).

Across C, L, and B treatments, approximately 70% of bundles had been completely consumed leaving only the rubber band. However not a single T bundle (0%) was entirely consumed (Fig. 7).

Adults and juveniles ate significantly different amounts: per capita, adult *A. dactylomela* ate significantly more *L. intricata* than juveniles ($F_{(1,13)}= 99.865$, $p=1.81\text{x}10^{-7}$), with each adult consuming on average 78.37g over the 18.5h period, and juveniles consuming 33.41g each (Fig. 8).

There was no significant difference in the age/treatment interaction term, meaning adults and juveniles ate the different treatments in similar ways. For full ANOVA and Tukey results see Appendix.

Discussion

Findings

Significant results for age likely indicate greater nutrient and metabolic needs for adults than juveniles. Juveniles require energy to grow, and as evidenced by these results, do consume a large amount of algae despite their small size. However, adults were demonstrated to eat over double that amount, as they are larger and likely require more energy to move and produce egg masses. That adults consume higher quantities of algae indicates they have a greater impact individually on algae control than juveniles, which play a smaller but still significant role. However, depending on the mortality rate of *A. dactylomela,* it is possible that there are enough juveniles to together account for the majority of algae consumed. Obtaining measurements for total algae consumed for adults and juveniles also enables future researchers to compare sea hare feeding habits with that of other herbivores to better understand the dominant species in reef macroalgae control.

A non-significant treatment age interaction term reveals that adults and juveniles did not exhibit significantly different preferences between treatments. This knowledge is critical, as it suggests that the changes in sea surface temperature and light exposure predicted to arise will affect the herbivory of all *A. dactylomela* similarly, regardless of age. This is unexpected, as the literature on other species in the *Aplysia* genus suggests juveniles and adults are known to have different preferences among algal species, and consequently, the physical and chemical characteristics of food sources[27]. *A. dactylomela* is unusual in that it specializes on *L. intricata* for its entire life. Thus, in order to gain a more thorough understanding of herbivory preferences across life stages, future experiments would benefit from studying additional sea hare species both within and outside the *Aplysia* genus to determine if this is a trend that holds or a singular characteristic of *A. dactylomela*.

> “Rising sea surface temperature could result in less herbivory, as animals unable to meet their energy needs are forced to eat more and travel farther.”

A. dactylomela were shown to eat significantly less *L. intricata* if it had been treated with high temperature. Observations during the culturing process noticed algae in T tanks appeared less pigmented than that of other treatments, and was more fragile to bundle. This could indicate a loss of chlorophyll and nutritional value, making it less appealing to *A. dactylomela*. As algae in T treatments would be equally easy (if not more so) to eat, this aversion is likely caused by a reduction in quality rather than an increase in difficulty [20]. This is consistent with the literature, in which thermal stress on algae has been shown to result in cellular damage and decreased protein, nitrogen, and caloric value[17]. Additionally, algae often responds to this by producing large quantities of heat shock proteins (such as the HSP60 and HSP70 classes) to repair damage[31]. It is possible that these chemical changes could also have an effect on the algae's taste and thus herbivore response.

Interpretations

One interesting result of this study was that despite a highly significant difference for the temperature treatment, there was no significant difference whatsoever for the B treatment of both temperature and light. This could imply that for *L. intricata*, light and temperature are antagonistic with respect to quality as a food source, meaning some feature of increased light is able to mitigate the negative effects of temperature on herbivory preference. This could be a result of an additional 3rd factor such as loading of microbial epiphytes. Low light and higher temperatures in water along with an organic substrate (algae) is known to facilitate bacterial growth[32], which could also make the algae less appetizing. Two 2011 studies [33, 34] on the Australian red alga *Delisea pulchra* found that loss of pigmentation occurs when the it is thermally stressed because warmer temperatures inhibit the alga's ability to produce chemical defenses, leaving it susceptible to bacterial loading and infection that results in loss of chlorophyll and other pigments. This is consistent with our observational evidence of *L. intricata* (also a chemically defended red alga) lacking coloration and beginning to decay in tanks with increased temperature. Furthermore, the fact that algae in B tanks did not appear any less pigmented, and was shown to be preferred equally to control algae by sea hares, could suggest that increasing light may allow the alga to combat (perhaps through increased photosynthesis and energy production) whatever degradation the increased temperature caused.

Another interpretation is that B and perhaps also L treated *L. intricata* were in fact less nutritious (though still preferable to T), and as a result, *A. dactylomela* compensated by consuming more of it to meet their needs. This has been shown to occur in several species of amphipods, but as a phenomenon is still largely understudied[35]. If this experiment were repeated, it would be possible to explore this by re-weighing the bundles multiple times during the experiment at shorter intervals to detect a ranking in preference and a succession from the most favored treatments to less desirable ones and gain a better understanding of what is actually occurring. As this experiment is relatively small-scale, and since so little has been studied on the topic, it is difficult to predict how increased temperature and light might impact future reefs, and further research is necessary to develop a more comprehensive understanding of the factors at play. However, the following discussion will outline several possible outcomes supported by these results.

Future Implications

If rising sea surface temperature causes *L. intricata* to lose nutritional value yet continue growing as usual, it could result in less herbivory, as animals unable to meet their energy needs or forced to eat more and travel farther (energetically expensive) to gain sufficient food will likely have reduced growth rate and reproductive capacity. This would result in decreased herbivory and thus allow *L. intricata* to spread and, if other algae and herbivores react similarly, could contribute to a phase shift. Alternatively, if higher sea surface temperatures damage the algae enough to impede its growth and reproduction, as before, this will reduce nutrition available to herbivores, but if unable to grow well even in the absence of predation, algae cover may remain at a low levels. While possibly less harmful to reefs than a phase shift, loss of a key food source can still result in bottom-up effects that reverberate through the food web through changes in trophic interactions. However, one potential positive is that the results for increased light might suggest that herbivory interactions could return to normal during high-light El Niño years. This could be beneficial given that most coral bleaching events occur during El Niño events and thus, when corals are most vulnerable. However, if the constant impact of increased temperature causes enough damage, it may be difficult to recover and re-establish depleted populations over the time scale of an El Niño.

Each of these scenarios have the possibility of contributing to far-reaching changes across the trophic pyramid and pose a threat to reef biodiversity. For this reason, it would be valuable for expand this study to include other species of algae that are dominant and widespread, as well as the most impactful herbivorous reef fish and invertebrates. Additionally, collecting long-term data on the health and fecundity of grazers fed different treatment algae would be beneficial.

Limitations and Considerations

This study was largely exploratory and small-scale in design, thus it is susceptible to all the usual limitations associated with this type of experiment, including small sample size and lack of site-level replication. Additionally, one tank experienced a heater malfunction and was discarded from the experiment so there were only two replicate bundles for B treatment in each age (four total, as opposed to six). Furthermore, only two adult *Aplysia* could be found over the entire three day span in which they were collected. According to Dr. Selina Ward of University of Queensland, who has been studying A. dactylomela at Heron Island for over a decade, they are typically highly abundant and finding so few is extremely unusual. This could point to additional factors at play, as whatever is causing such an uncharacteristically low abundance of *A. dactylomela* may also have an impact on the dietary preferences of those found. Some gastropods, such as the sea slug *Placida dendritica* have been shown to exhibit different taste preferences even among individuals of the same population, which is believed to have a genetic component[36]. It is not known if this variability of preference applies to *A. dactylomela* as well, but as only two adults and four juveniles were found, if this were the case the dietary choices of the six individuals studied may not be representative of the species as a whole.

Another consideration worth noting is that while some algae from each bundle was passively lost during the feeding trial (as it is delicate and small pieces likely broke off), this may have occurred disproportionately more for certain treatments. That is, the less healthy and thus more fragile algae may have fallen apart more and incorrectly been counted as eaten.

Acknowledgments

I would like to thank the University of Queensland and Heron Island Research Station for providing me with the resources and facilities to conduct this research. Additionally, I would like to thank Kristen Brown and Dr. Selina Ward of the University of Queensland for their guidance, knowledge, and support in designing, conducting, and analyzing this experiment. Additionally, I would like to acknowledge the University of Queensland's Jasmine Lee for assistance with statistical tests and Cole Shepherd of Stanford University for help in caring for the sea hares. Lastly, I am thankful for the Bing Overseas Studies Program for enabling me to visit Australia and funding my stay in Heron Island.

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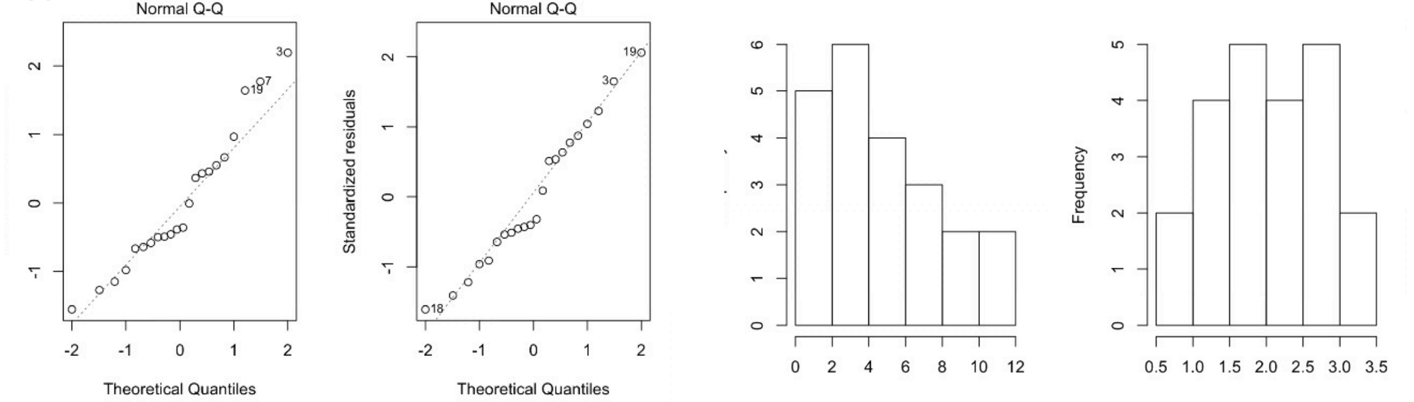
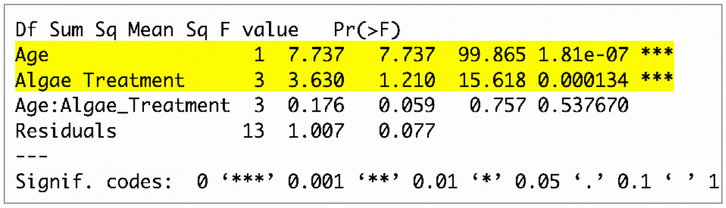
Appendix

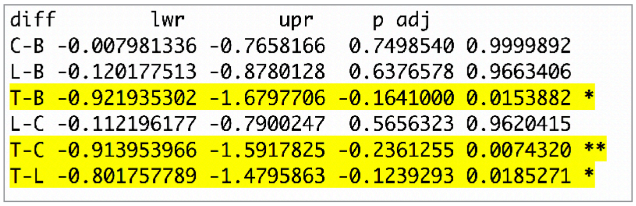


Appendix I. **A)** Histograms and **B)** QQplots of the data before (left) and after (right) implementing the square root transformation.

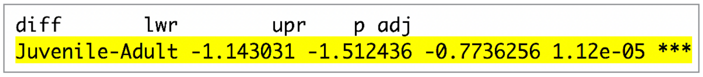


```
Df Sum Sq Mean Sq F value    Pr(>F)
Age                    1  7.737    7.737  99.865 1.81e-07 ***
Algae Treatment        3  3.630    1.210  15.618 0.000134 ***
Age:Algae_Treatment  3  0.176    0.059    0.757 0.537670
Residuals             13  1.007    0.077
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

Appendix II. Full results from the 2-way ANOVA with significant values highlighted in yellow.



```
diff        lwr         upr     p adj
C-B -0.007981336 -0.7658166  0.7498540 0.9999892
L-B -0.120177513 -0.8780128  0.6376578 0.9663406
T-B -0.921935302 -1.6797706 -0.1641000 0.0153882 *
L-C -0.112196177 -0.7900247  0.5656323 0.9620415
T-C -0.913953966 -1.5917825 -0.2361255 0.0074320 **
T-L -0.801757789 -1.4795863 -0.1239293 0.0185271 *
```



```
diff       lwr        upr    p adj
Juvenile-Adult -1.143031 -1.512436 -0.7736256 1.12e-05 ***
```

Appendix III. Results from TukeyHSD test performed on **A)** treatment (top), and **B)** age (bottom). Significant results highlighted in yellow.