Analysis of Kelp Quality and Starvation on Grazing Behavior of *T. tridentata*

By

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Abstract:

The purpose of this experiment was to determine if *Tegula tridentata* exhibit awareness of the quality of kelp (*Lessonia trabeculata*) they consume, and, if so, what quality of kelp is preferred. A two-and-a-half-month study that consisted of three separate trials took place over Chile's winter (June - August 2016). Arenas were set up to introduce individuals of *T. tridentata* that had previously been starved at three different levels to kelp fronds from the species *L. trabeculata* with three levels of damage (damage types: physically damaged, naturally damaged, and undamaged). Behavior was monitored over a continuous four-hour period. In all trials, snails did not show a preference for fronds that had experienced natural damage over undamaged and artificially damaged fronds, and there was no affect of starvation. This indicates that *T. tridentata* may not exhibit choice in the quality of food that they consume and that starvation levels do not effect choice.

Introduction:

There are many examples of cases of chemical defense shared among terrestrial and marine plants in response to herbivory (Karban *et al.* 2014). Evidence suggests that this may prevent future herbivory in grazed plants and surrounding plants as well as possibly aid in the wound-healing process of previously grazed plants (Hammerstom *et al.* 1998; Karban *et al.* 2014). Some evidence suggests that such phenomenon also occurs in marine algae (Cronin & Hay 1996; Rohde *et al.* 2004). Induced, chemical defense consists of the release of secondary metabolites in response to injury from grazing (Daniel 2006; Dethier *et al.* 2014). Daniel (2006) defines secondary metabolites to be a group of organic compounds that do not get involved or regulated into the primary metabolism of plants. Secondary metabolites associated with bitter taste that may deter herbivores include both alkaloidal compounds as well as phenolics (tannins) (Daniel 2006; Dethier *et al.* 2014). The bitter taste associated with secondary metabolites affects how palatable a plant is to an herbivore (Dethier *et al.* 2014). In one study, brown seaweeds, *Ascophyllum nodosum,* that had been naturally grazed upon by small gastropods or crustaceans had a significant decrease of further attempts of herbivory than those that had not experienced grazing (Toth *et al.* 2007); this suggests that previously grazed seaweeds are able to respond by means of induced defense to prevent further grazing. *A. nodosum* responded to grazing by producing phlorotannins (secondary metabolites) that decrease palatability (Toth *et al.* 2007). Similarly, Cronin & Hay (1996) found that the brown algae *Dictyota menstrualis* responded to amphipod grazing by producing defensive secondary metabolites, reducing palatability. Though a decrease in palatability has been noted to be induced by grazing (Toth *et al.* 2007; Cronin & Hay 1996), it has not been completely determined if grazing intensity affects the overall strength of the inducible defense. There is also evidence that some seaweeds can detect chemical responses coming from neighbors that are experiencing grazing. One study found that there is a significantly stronger negative effect on herbivore consumption on brown seaweeds that were exposed to chemical cues from grazed seaweeds for approximately 11- 20 days than seaweeds exposed to more or less duration of grazing than that time period (Toth & Pavia 2007).

Some studies suggest that palatability in relation to grazing is herbivore specific (Dethier *et al.* 2014; Kappes *et al.* 2012; Thornber *et al.* 2008). Furthermore, as suggested in Kappes *et al.* (2012), some herbivores (gastropods) may have general nutrition preferences. In this study, Kappes *et al.* (2012) assessed the feeding and field behavior of native (German) gastropods and the invasive slug, *Arion lusitanicus.* This study used traps lined with baits of different nutrient type concentrations (acids/alcohols) to see what both the invasive and native gastropods preferred to consume. (In this case, the choice consumption was Pilsner beer.) If palatability in relation to grazing is herbivore specific, particular herbivores may show a preference toward different qualities of plants, e. g. palatability of kelp fronds in relation to aging (degradation) (Dethier *et al.* 2014). For example, Dethier *et al.* (2014) tested the value of aged kelp to isopods, urchins, and copepods in the species *Nereocystis luetkeana* (low polyphenolics; low bitterness) and *Agarum fimbriatum* (high polyphenolics; high bitterness) to see if kelp detritus had more value to those species than did fresh kelp; this study suggests that preference tended to be both species- and time- dependent, e. g. some species of herbivores preferred different species and degradation levels of kelp than did other species of herbivores. Moreover, as has been suggested by Dethier *et al.* (2014), there are few studies that observe aged versus fresh kelp-derived diets (Duggins & Eckman 1997; Levinton *et al.* 2002; Norderhaug *et al.* 2003). Thornber *et al.* (2008) suggests that some herbivores overlook certain secondary metabolites and execute preference toward morphological features (kelp textures). Although there are no studies to demonstrate this, it is possible that damaged/degraded kelp have weaker chemical defenses, thus explaining preferences that are shown by some herbivores.

The purpose of this study was to see if the Chilean turban snail, *Tegula tridentata*, displays a preference for the quality of kelp (*Lessonia trabeculata*) that those snails consume. Quality for the purposes of this study is defined to be the morphological state (damage) of the kelp. *Tegula tridentata* is a common species of snail in central Chilean subtidal ecosystems, and those snails are commonly known to show a grazing preference for *L. trabeculata* (Pereira *et al.* 2015; Qu *et al.* 2014) However, it has yet to be determined if this species of snail (*Tegula tridentata*) exhibit a preference for the quality of kelp that species choose to consume. This study addresses three questions: 1) do *T. tridentata* express a preference in the quality of kelp consumed, 2) what quality of kelp do *T. tridentata* prefer to consume, 3) does starvation of *T. tridentata* play a role in preference?

Methodology:

Collective Methods and Materials

This project was designed to determine if *Tegula tridentata* (snails) exhibited preference for the quality of *Lessonia trabeculata* (kelp) that they consume. This project took place during central Chile's winter during the months of June through August in 2016. The project consisted of three trials that differed by level of starvation. Each trial utilized choice arenas that contained the following treatment types: undamaged kelp vs. naturally damaged kelp, undamaged kelp vs. physically damaged kelp (Fig. 1), and naturally damaged kelp vs. physically damaged kelp.

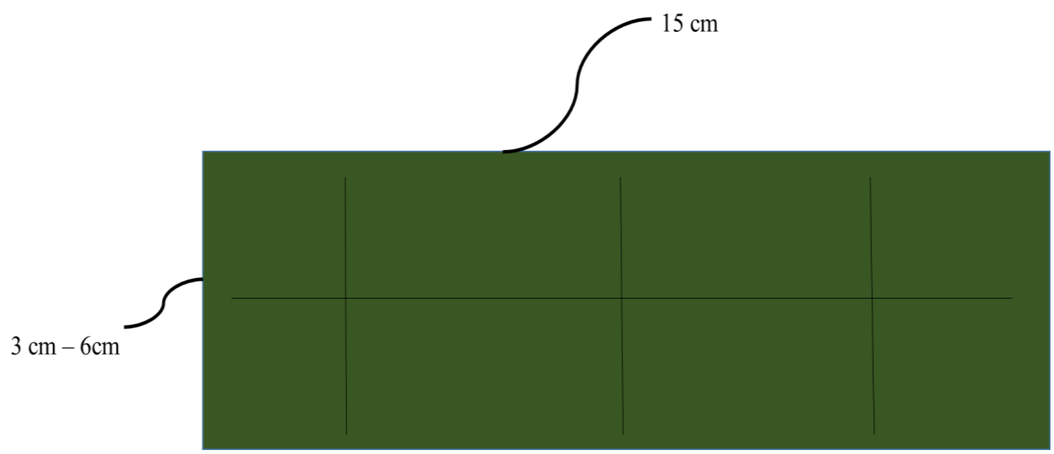


Fig. 1 Representation of what one slice of physically damaged kelp type looked like. A razor blade was utilized to make shallow slices onto each piece of this kelp type.

Each arena contained a grid to indicate location of each snail with the kelp frond choices at each end of the arenas (Fig. 2 & Fig. 3). Total kelp coverage in each arena was 28% algal cover on the bottom. Snails were initially placed in the center of each tank, and observations spanned a continuous four-hour period.

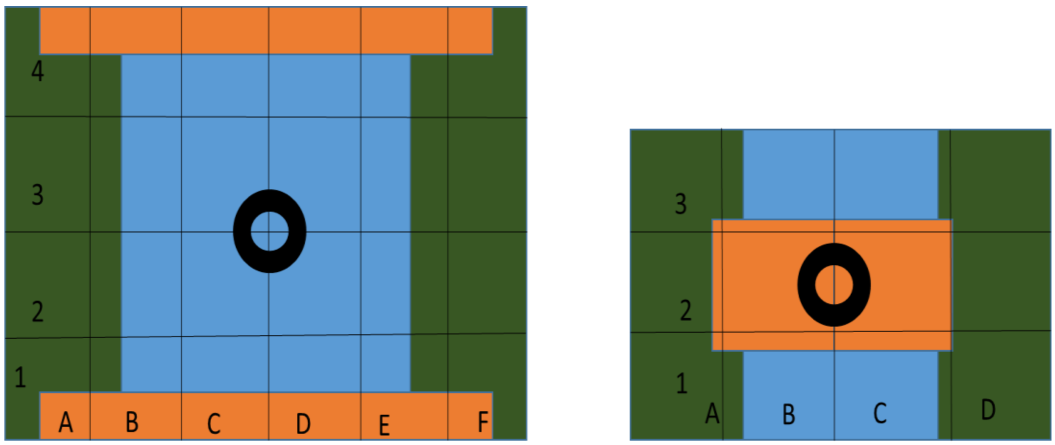


Fig. 2 Examples of the tank grids that were used on both tanks. The picture on the left shows a 2D version of the 30 cm x 18 cm tank (V = 4 L). Each block in the grid is 5 cm x 5 cm except for the 4th row, which is 3 cm x 5 cm. The picture on the right shows a 2D version of the 20 cm x 12cm tank (V = 1.4 L). Each block in the grid is 5 cm x 4 cm. Black circles represent the starting point for snails on each grid. Colors represent the following: green (kelp type), orange (tile), blue (bottom of tank), and black (snail).

All of the trials' snail behavioral observations took place over a continuous four hour rotation. All trials had a total of these numbers of observation per individual: 18 (Trial 1), 17 (Trial 2), and 17 (Trial 3). Preliminary studies indicated that

Tegula Tridentata were more active at night, so all trials were conducted at night. A red light was used while observing night time activity so as to not disturb the snails. Trial 1 used individuals who were 15 days starved, Trial 2 used individuals who were 17 days starved, and Trial 3 used individuals who were 20 days starved. The ranges of snail sizes for all trials were 9 mm – 17 mm (base diameter) and 6 mm – 17 mm (height). Tanks contained either 4 L or 1.4 L of sea water.

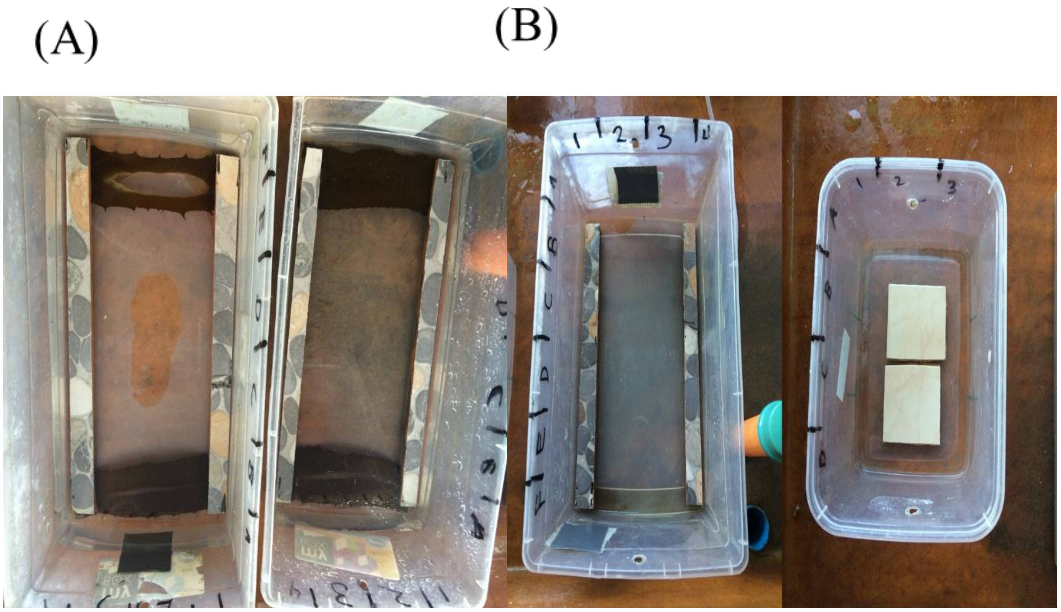


Fig. 3 Aerial views of the tanks utilized. (A) shows aerial views of the bigger tanks. The tank on the left shows an example of naturally damaged kelp vs. physically damaged kelp. The tank on the right shows an example of naturally damaged kelp vs. physically damaged kelp. (B) shows a side-by-side comparison of the tanks that were utilized. Right: bigger tank. Left: smaller tank.

Trial Data Analysis

There were two types of behavioral activity observed for each of the trials. The first type consisted of what *T. tridentata* were doing at a particular point in time. These categories consisted of grazing, crawling, or other movement (twisting of shell; antennae movement). The second type of behavioral activity consisted of where (substrate) each *T. tridentata* was at a particular point in time. These categories consisted of either kelp type 1, kelp type 2, or other substrate (not kelp). Kelp types for each arena set consisted of either naturally damaged kelp, undamaged kelp, or artificially damaged kelp. Kruskal-Wallis tests and post-hoc Nemenyi tests were used to analyze the two types of behavioral activities' categories for each of the trials.

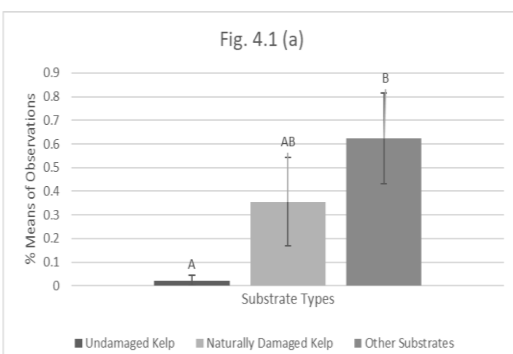
Results:

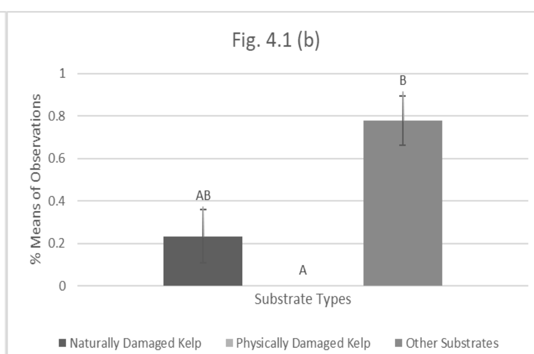
Guide to Figures

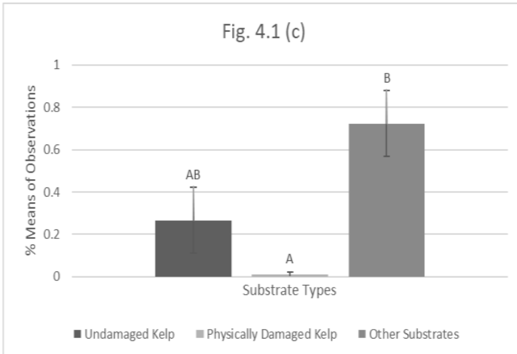
Each trial with .1 (a-c) represent where (substrate) each of the *Tegula tridentata* are in their respective tanks with treatment types at a particular point in time. Each trial with .2 (a-c) represent what behavioral activity *T. tridentata* are displaying at a particular point in time. Whole numbers before the decimal represent what trial each of the data from the figures respectively belong to, i.e. Fig. 4.1 represents on what substrate that individuals from Trial 1 are at a particular point in time. Figure types .1 and .2 correspond to each other, i.e. individuals in 4.1 (a) are the same individuals as 4.2 (a), only aspects such as choice and behavior are different. Figure types .1 (d) show a sample of another way of representing an individual’s placement per observation. To make figure locations more accessible, section headers have been used to indicate a difference in figures at a glance, i.e. Fig. 4.1 is listed under section header *Trial 1*. The following figure types for each trial with both .1 and .2 (a-c) were subjected to a Kruskal- Wallis ranked test as well as Nemenyi post-hoc tests calculated using the statistical program R.

Trial 1

For this portion of Trial 1, *T. tridentata* show a preference for Other Substrates (not kelp). Though there were no significant differences for Fig. 4.1 (a) substrate categories (P = 0.0778), Fig. 4.1 (b) and Fig. 4.1 (c) demonstrate significant p-values of P = 0.004609 and P = 0.005084. In those figures, *T. tridentata* demonstrate a preference for Other Substrates more than either kelp type 1 or kelp type 2. Fig. 4.1 (b) demonstrates a significant difference between the substrate categories for Physically Damaged Kelp and Other Substrates; however, there were no significant differences between those two categories and Naturally Damaged Kelp. Fig. 4.1 (c) demonstrates a significant difference between the substrate categories for Physically Damaged Kelp and Other Substrates; however, there were no significant differences between those two categories and Undamaged Kelp. Fig. 4.1 (d) shows an individuals' preference for the Other Substrate category at an observed time instance.







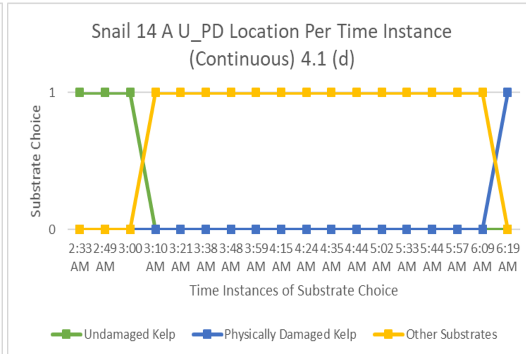


Fig. 4.1 (a-c) show collective percentages of means of *T. tridentata* substrate choice in a continuous, approximately 4-hour time period (n= 5; (a) P = 0.0778; (b) P = 0.004609; (c) P = 0.005084). Fig. 4.1 (d) shows a visual illustration of an individual snail's substrate choice at a particular time instance. The individual in Fig. 4.1 (d) was chosen at random from one of the treatment types.

Behaviorally, *T. tridenata* show a preference for the Other Behavior category. Fig. 4.2 (a) demonstrates no significant difference between the behavior categories (P = 0.1963). Fig. 4.2 (b) and Fig. 4.2 (c) demonstrate significant differences between categories (P = 0.01045; P = 0.02713). In both Fig. 4.2 (b) and Fig. 4.2 (c), there were significant differences between the behavior categories for Crawling and Other Behaviors; however, there were no significant differences between those two categories and Grazing.

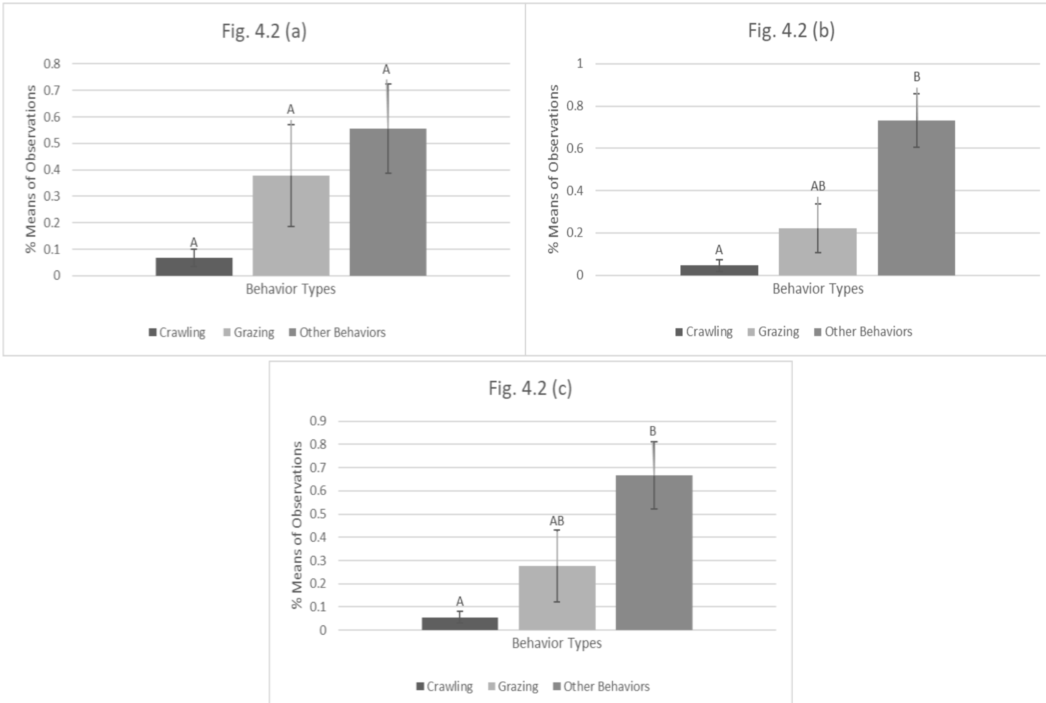


Fig. 4.2 (a-c) show collective percentages of means of *T. tridentata* behavior in a continuous, approximately 4-hour time period (n=5; (a) P = 0.1963; (b) P = 0.01045; (c) P = 0.02713). These graphs correspond with the respective Fig. 4.1 (a-c) graphs, i.e. individuals are the same in Fig. 4.2 (a) as are in Fig. 4.1 (a).

Trial 2

For this portion of Trial 2, *T. tridentata* show a preference for Other Substrates (not kelp). Though there were no overall significant differences for Fig. 5.1 (a) substrate categories (P = 0.0743), Fig. 5.1 (b) and Fig. 5.1 (c) demonstrate significant p-values of P = 0.002058 and P = 0.01078. In those figures, *T. tridentata* demonstrate a preference for Other Substrate more than either kelp type 1 or kelp type 2. Fig. 5.1 (b) and Fig. 5.1 (c) demonstrate a significant difference between Other Substrates and the other two kelp categories. Fig. 5.1 (d) shows an individuals' preference for the Other Substrate category at an observed time instance.

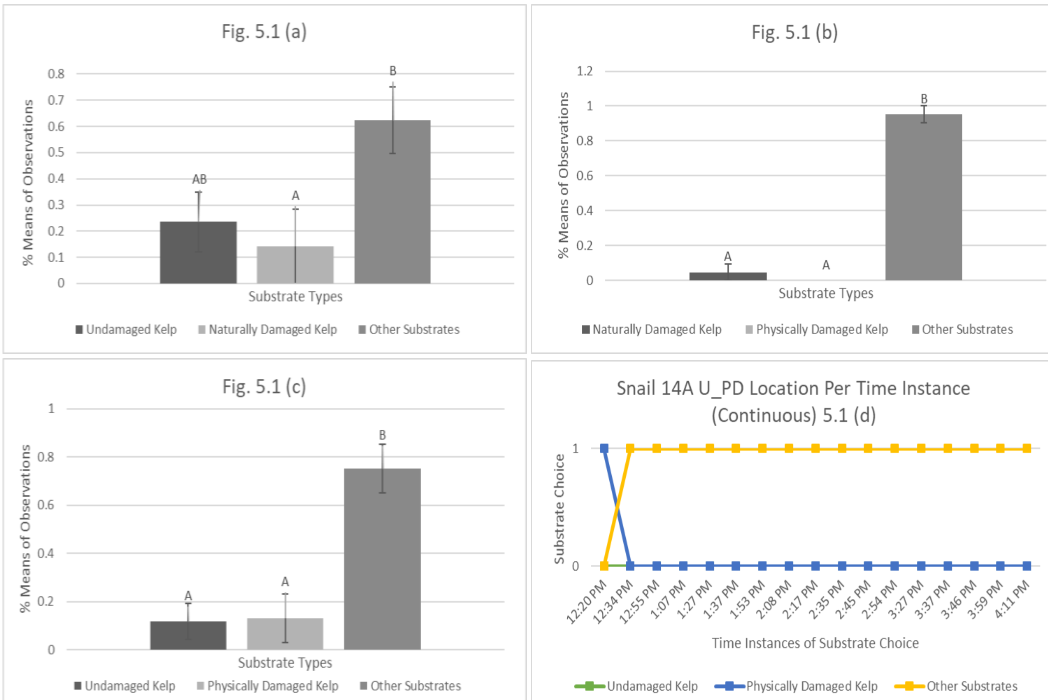


Fig. 5.1 (a-c) show collective percentages of means of *T. tridentata* substrate choice in a continuous, approximately 4-hour time period (n= 5; (a) P = 0.0743; (b) P = 0.002058; (c) P = 0.01078). Fig. 5.1 (d) shows a visual illustration of an individual snail's substrate choice at a particular time instance. The individual in Fig. 5.1 (d) was chosen at random from one of the treatment types.

Behaviorally, *T. tridenata* show a preference for the Other Behavior category. Fig. 5.2 (a), Fig. 5.2 (b), and Fig. 5.2 (c) demonstrate significant differences between categories (P = 0.0336 P = 0.01045; P = 0.02713). In both

Fig. 5.2 (a) and Fig. 5.2 (c), there were significant differences between the behavior categories for Crawling and Other Behaviors; however, there were no significant differences between those two categories and Grazing. Fig. 5.2 (b) demonstrates a significant difference between Other Behaviors and the other two categories.

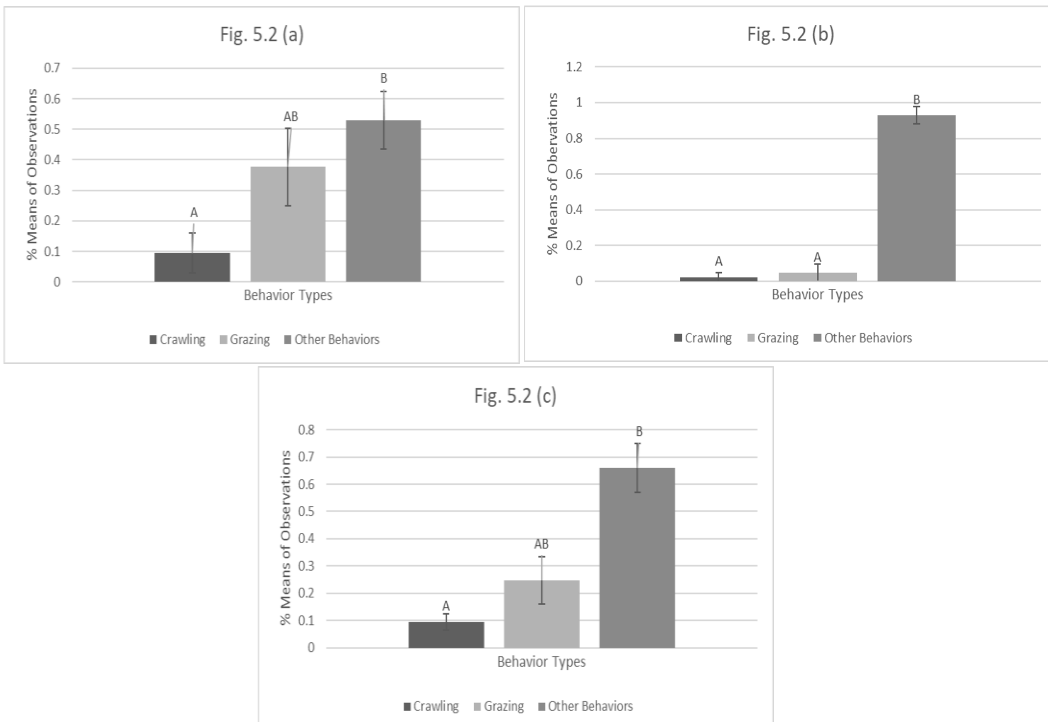


Fig. 5.2 (a-c) show collective percentages of means of *T. tridentata* behavior in a continuous, approximately 4-hour time period (n=5; (a) P = 0.0336; (b) P = 0.003832; (c) P = 0.009507). These graphs correspond with the respective Fig. 5.1 (a-c) graphs, i.e. individuals are the same in Fig. 5.2 (a) as are in Fig. 5.1 (a).

Trial 3

For this portion of Trial 3, *T. tridentata* do not show much of an overall preference for any of the categories. However, Fig. 6.1 (c) demonstrates a significant difference between Other Substrates and Physically Damaged Kelp, but no significant differences between those two categories and Undamaged Kelp (P = 0.011). Fig. 6.1 (a) and Fig. 6.1 (b) do not show any significant differences between any of the categories (P = 0.1187; P = 0.1927). Fig. 6.1 (d) shows an individuals' preference for the Other Substrate category at an observed time instance.

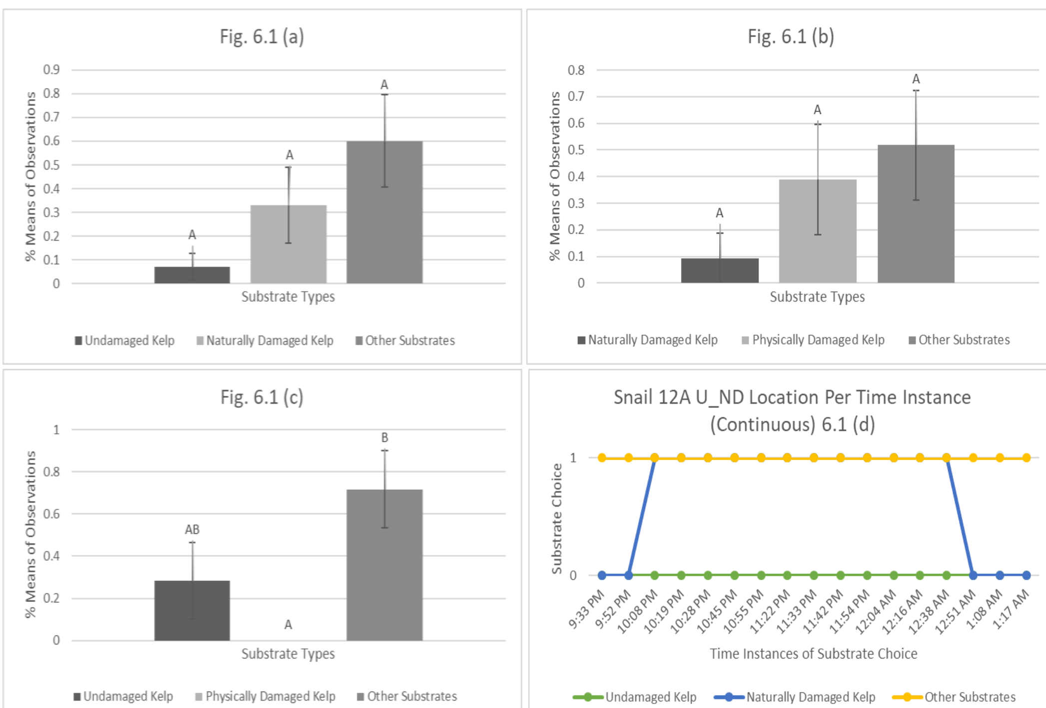


Fig. 6.1 (a-c) show collective percentages of means of *T. tridentata* substrate choice in a continuous, approximately 4-hour time period (n= 5; (a) P = 0.1187; (b) P = 0.1927; (c) P = 0.011). Fig. 6.1 (d) shows a visual illustration of an

individual snail's substrate choice at a particular time instance. The individual in 6.1 (d) was chosen at random from one of the treatment types.

Behaviorally, *T. tridenata* do not show preferences for any of the categories. Fig. 6.2 (a), Fig. 6.2 (b), and Fig. 6.2 (c) demonstrate no significant differences between any of the categories (P = 0.2029; P = 0.4143; P = 0.1753). The Nemenyi post-hoc test confirmed that there were no significant differences between the behavior categories.

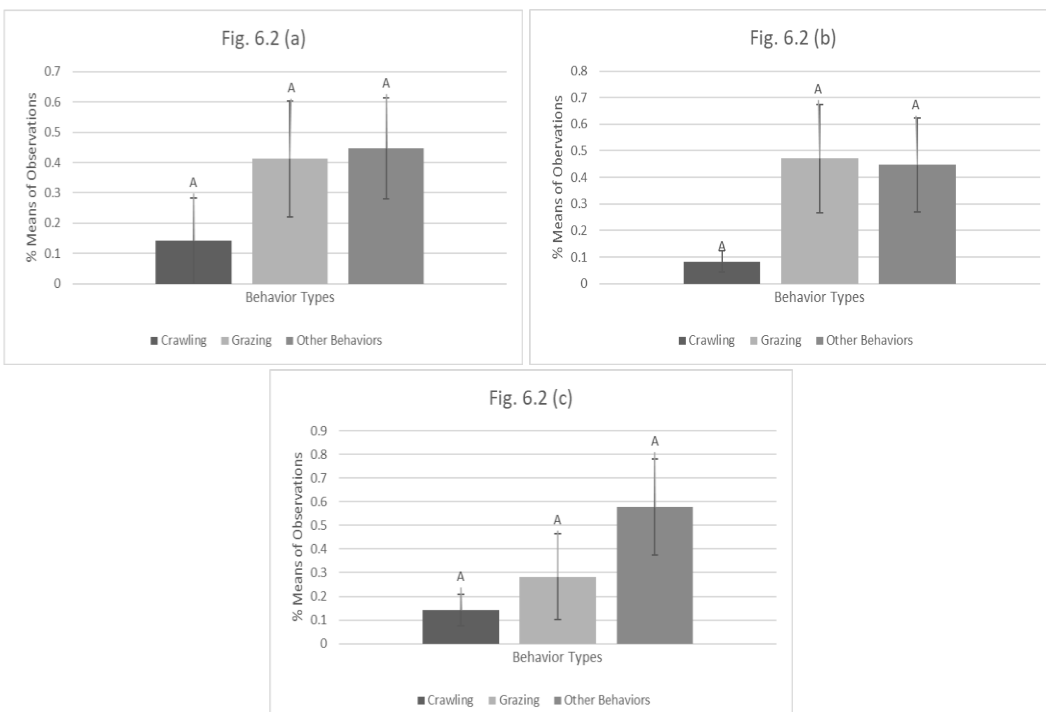


Fig. 6.2 (a-c) show collective percentages of means of *T. tridentata* behavior in a continuous, approximately 4-hour time period (n=5; (a) P = 0.2029; (b) P = 0.4143; (c) P = 0.1753). These graphs correspond with the respective Fig. 6.1 (a-c) graphs, i.e. individuals are the same in Fig. 6.2 (a) as are in Fig. 6.1 (a).

Discussion:

In most cases, *Tegula tridentata* do not seem to show much of a preference for either substrate or what behavior category was being displayed at a particular point in time (Fig. 4-6); there were no significant differences in the two types of behavioral activity categories. One example can be seen in Fig. 4.1 (a). There were significant differences between Undamaged Kelp and Other Substrates; however, there were no significant differences between those two categories and Naturally Damaged Kelp (n= 5; P = 0.0778). Another example can be seen in Fig. 4.1 (b). Fig. 4.1 (b) demonstrates differences between the categories of Physically Damaged Kelp and Other Substrate, while the third category in this figure of Naturally Damaged Kelp shows similarities to the other two categories (n= 5; P = 0.004609). The same can be said for Fig. 4.2 (b); there are significant differences between Crawling and Other Behaviors, while Grazing is similar to both of those categories (n = 5; P = 0.01045). These are a few examples of patterns that manifest in all the Trials. When differences were present in the trials, most of those individuals seemed to show a preference for the "other" categories; *T. tridentata* did not choose to be on any of the main categories. For example, Fig. 5.1 (b) shows a greater percentage of mean observations on Other Substrate than it does either Naturally Damaged or Physically Damaged Kelp (n = 5; P = 0.002058).

As the starvation level increased, there seemed to be less preference of behavior activity as to where or to what *T. tridentata* were taking part of; there were no significant differences between any of those categories. This can be seen in all figures of Trial 3 (Fig. 6). For example, Fig. 6.1 (a) shows no differences between Undamaged Kelp, Naturally Damaged Kelp, and Other Substrates (n= 5; P = 0.1187). Further testing and trials will be needed to determine if starvation played a role in the small differences that were displayed among all trials.

An aspect of the original design of this study was to measure the level of chemical defenses induced from aged/fresh kelp as well as the herbivore response. Certain circumstances, such as equipment unavailability. Future suggestions are featured with the original intent in mind. Thornber *et al.* (2008) suggests that this genus (*Tegula*) does not respond to secondary metabolite release; however, this genus may respond to morphological changes in kelp. However, Pereira *et al.* (2015) suggests that more grazing occurs on previously harvested kelp forests by *T. tridentata* by approximately 30% more than non-previously harvested kelp forests. Perhaps significant differences were not apparent in *Tegula tridentata* preference because the morphological changes (damage) or spatial variations made to *Lessonia trabeculata* (kelp) were either not great enough or too great. As performed in Kappes *et al.* (2012), gut analysis would have been another way to assess what preference of the snails would have been most prevalent. In the future, gut analysis would be a necessary step to compare Carbon:Nitrogen ratios to more accurately assess what each individual snail consumed (Kappes *et al.* 2012). Furthermore, because studies that involve aged kelp preference are sparse (Dethier *et al.* 2014), it may be beneficial to conduct more of those studies on different species of herbivores and plants in the future.

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