

EXAMINING THE EFFECTS OF DIFFERENT DIETS AND SALINITIES ON COPEPOD POPULATION GROWTH

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*ABSTRACT: The coastal oceans are subject to climate impacts leading to sea level rise, increases in the frequency and intensity of storms, and increased precipitation. These events can lead to a rise in the amount of fresh water entering coastal ecosystems from runoff or rainfall, which cause decreases in ocean salinity. Understanding marine food web dynamics requires an understanding of how species interactions will respond to environmental changes of this kind. Sea urchins are key members of nearshore food webs and may help to link food availability between shallow and deep zones along coastal areas. Sea urchins possess a very inefficient digestive system, which means that their feces may possess large amounts of available nutrients which other organisms can use as a viable food source. This research studied the population growth of *T. californicus* copepods in both low salinity and normal seawater environments, and with diets of either fresh *Ulva* or urchin fecal *Ulva*. The calorie content for these different diets was also examined. Results show that both diet and salinity significantly affected population growth, low salinity is the better environment, and fresh *Ulva* is the better diet.*

Climate change is affecting coastal environments in many ways, but one of the major concerns is its effects on ecosystems, as deterioration of marine community structure is increasing (Doney et al., 2012). Coastal ecosystems are sensitive to sea level rise, changes in the frequency and intensity of storms, and increased amounts of precipitation (Harley et al., 2006). In the coming years we may see heavier rainfall, which in turn can lead to increased amounts of runoff into the ocean, and decreases in ocean salinity (Curry & Mauritzen, 2005). In this rapidly changing marine environment, population-level shifts are occurring, which decrease stability and recovery potential and can lead to altered species interactions in coastal systems (Hallegraef, 2010; Worm et al., 2006).

Within marine habitats, primary producers like benthic algae and phytoplankton are essential to the food web, as they constitute the base on

which all other species rely for energy. The next trophic level contains consumer organisms like zooplankton, including copepods, which eat phytoplankton. Further up this web are a variety of fish species that depend on zooplankton for their main food source (Richmond, Wethey, & Woodin, 2007). Food web dynamics are driven by the nature and abundance of primary food sources that are available to these higher trophic levels (Wallner-Han et al., 2015). Understanding these feeding dynamics requires an understanding of how species interactions will respond to environmental changes such as salinity (Norkko et al., 2007).

Coastal ecosystems have particularly complicated food webs. There are two distinct sources of primary production: phytoplankton and benthic macroalgae. Much of the organic matter entering the benthic food web derives from this material sinking to the deep subtidal

zone. Benthic communities are biologically diverse, so small shifts in their environment can create large changes in higher trophic levels (Van Oevelen et al., 2006). The importance and magnitude of this linkage between pelagic and benthic subsystems remains poorly studied (Sullivan et al., 1991). Because coastal systems are shallow, benthic fauna have direct access to primary producers, in the form of live or dead algae including detached pieces of macrophytes. In some systems, benthic consumers may get nutrition from the fecal matter of other species (Sauchyn & Scheibling, 2009). Although it is still unclear exactly how important this fecal detritus is as an adequate food source, my experiment investigates the possible importance of this connection.

Green sea urchins (*Strongylocentrotus droebachiensis*) are a common species whose fecal matter may provide a substantial amount of nutritious particulate matter following the consumption of macroalgae. Because of their very inefficient digestion (Mamelona & Pelletier, 2005), fresh urchin feces can contain high levels of nitrogen and phosphorus (Koike, 1987). This nutrient rich byproduct can act as a beneficial source of energy for some types of marine species (Sauchyn & Scheibling, 2009), such as copepods, and in a lab setting can lead to rapid population growth when it is the only food source (Kobelt & Dethier, 2015).

A strong linkage between copepods and nearshore fish species such as juvenile salmonids exists in many coastal ecosystems, including the Pacific Northwest (Naiman & Sibert, 1979). *Tigriopus californicus* is an abundant harpacticoid copepod that is commonly located within high tide pools in San Juan Archipelago. In this habitat, they predominantly consume algae and detritus (Morris, Abbott, & Haderlie, 1980, Dethier et al., 2014). This is an ideal species to use for experimentation because it can be easily kept in culture and has a short reproductive cycle (Dethier et al., 2014). In this experiment, *T. californicus* will be used to test how food sources and selected environmental

parameters interact to control population growth. By quantifying the population growth seen over at least one generation, and varying the physical environment, we can better predict how food web dynamics may be affected as greater climate change events occur along the coastal oceans.

Methods

For this study, copepod populations were raised under 4 treatments, with each treatment having four replicates, totaling 16 populations. *T. californicus* copepods were collected from a high tide pool at Cattle Point on San Juan Island. Egg-bearing females were identified and sorted under the microscope. Mason jar ‘aquaria’ were set up by adding 20 egg-bearing females per jar. Half of the jars were filled with normal salinity seawater directly from the Friday Harbor Labs (FHL) sea tables in lab 3. The other half were given a low salinity treatment by filling them with 50% RO water and 50% seawater. Jars were labeled and kept on the windowsill in lab 3, out of direct sunlight to minimize temperature variation, however, a gradual seasonal cooling over the course of the experiment occurred. The temperature range in the jars was between 9.5-15°C over the experimental period, with an overall average of 12 °C.

Two large-sized green sea urchins (*Strongylocentrotus droebachiensis*) were collected from FHL sea tables located in lab 3 and lab 1, while seven more were collected from an urchin trap located off the FHL Dock. These urchins were divided into groups of three, placed in buckets, and maintained with constant water flow in a sea table located in lab 3. They were fed a consistent diet of sea lettuce (*Ulva sp.*) collected from the FHL docks. Feces from the urchins were collected with a turkey baster on a weekly basis and fed directly to the populations of copepods. The remaining collected feces were frozen for chemical analysis in the lab 3 freezer.

Each of the jars containing copepods were fed

ad libidum with either diced fresh *Ulva* or urchin feces. Treatments were as follows: low salinity with fresh *Ulva*, low salinity with urchin feces, normal salinity with fresh *Ulva*, and normal salinity with urchin feces. Each population's food and water were refreshed regularly as needed every 7-10 days, by pouring water through a series of different size filter sieves, then washing the filtrate back into jars with clean seawater. Water changes were performed as infrequently as possible—only when the jars started to develop a surface film and bottom cloudiness—in order to minimize the number of individuals lost.

At the end of 6 weeks, each jar was treated with 95% ethanol to kill the *T. californicus* and allow for easy counting. Fluids were drained through a sieve and manual extraction of large algal remnants was performed before counting. Counting was completed under a dissecting microscope and included all egg-bearing females, adults, sub-adults and larval (nauplii) forms. Separation of adults and sub-adults depended on size and coloration factors, as adults were larger and possessed a darker segmented appearance throughout their body. Juveniles and larval forms were distinguished by their shape and number of legs, as larval forms contained three pairs of legs and bodies that were rounder than the juvenile forms. These counts were totaled and used for statistical analysis using Excel. Final population sizes were compared with a 2-factor ANOVA (with factors salinity and food type).

Caloric content of the food for the *T. californicus* populations was considered as an additional factor that might influence population growth. Samples of fresh *Ulva* and urchin feces, as well as their respective frozen versions, were used for calorimetric analysis with potassium dichromate oxidation from the methods outlined by Gosselin & Qian (1998) and modified by D. Duggins. These analyses were designed to test the caloric difference between fresh *Ulva* and fecal matter diets, as well as examining if there are any effects on caloric content caused

by freezing. Fresh blades of *Ulva* were tested and compared to 2 week old frozen blades. The second set of analyses examined the difference between fresh urchin feces and frozen urchin feces, as well as quantifying the calories from *Ulva* frozen for 6 weeks.

All samples were placed in the drying oven in foil trays to remove excess water. *Ulva* required drying time of between 14-18 hours whereas urchin feces only took 5-6 hours. Then 60 mg of dried sample was weighed and placed in a test tube with 10 mL of dichromate solution. The test tube was gently vortexed to mix and heated in an oven set to 115° C for 30 minutes, mixing halfway through. After the heating and mixing process, 0.5 mL of the mixture was then transferred to a new tube with 4 mL of potassium iodide solution, mixed, and left to sit for 20 minutes. Each sample was read at 575 nm in a spectrophotometer. The absorbance data was converted into calories with a regression equation generated from a glucose standard curve. Due to the expiration time for potassium and iodide reagents, analyses of *Ulva* samples were tested in sets 6 weeks apart, with each set run using a fresh batch of reagents and new standard glucose curve performed. We found that fresh reagents generated slightly different glucose curves, therefore, samples run with different batches were not directly compared.

Results

After 50 days, the total population size per jar for *T. californicus* had grown from the 20 original individuals to an average range of 91 individuals (in a population raised on feces from *S. droebachiensis* fed only *Ulva* (henceforth “fecal *Ulva*”) and 17 PSU), to 254 individuals (for population raised on fresh *Ulva* in low salinity of 17 PSU). Final average population sizes at 29 PSU were 28 fed fecal *Ulva* and 112 fed fresh *Ulva* diets (Figure 1). Thus a fresh *Ulva* diet resulted in more growth when compared with fecal *Ulva* diet, and low salinity consistently had greater growth than high

salinity. The effect of low salinity was even greater when the fresh *Ulva* food is present, as is seen in the statistical interaction (ANOVA diet p value <0.0001, salinity p value <0.0001, interaction p value =0.003).

The population composition in terms of abundances of the individual life stages differed among treatments in both the number of individuals (Figure 2), as well as how the proportions of each life stage (Figure 3) were represented. For egg-bearing females, low salinity showed a significant positive effect over seawater with 46% more individuals seen, while diet made only a small difference among treatments and its effect was not significant (Figure 4: ANOVA diet p value=0.064, salinity p value=0.016, interaction p value=0.100). In terms of the proportions of the populations, egg-bearing females differed among the treatments; the proportion of egg-bearing females increased 10% with the fecal *Ulva* diets over the fresh *Ulva* diets. Salinity did not make a difference but there is a significant interaction between the two factors seen (Figure 5: ANOVA diet p value=0.004, salinity p value=0.125, interaction p value=0.05).

For the non-egg-bearing adults, both population abundance and proportions were significantly affected by diets and salinity. Results for abundance indicate that low salinity is more ideal than seawater, as there were 66% more adults seen. The fresh *Ulva* diet also resulted in a 35% increase in adults over populations fed fecal *Ulva*, with no interaction (Figure 6: ANOVA diet p=0.003, salinity p <0.0001, interaction p=0.210). In terms of the proportion of adults within populations, only salinity showed an effect. The seawater treatment contained a 12% increase of adults over the low salinity treatment, though diet did not make a difference and there was no interaction (Figure 7: ANOVA diet p value=0.256, salinity p value=0.003, interaction p value=0.287).

For sub-adults' abundance there were effects of both salinity and diet, and a significant interaction of these factors. The low salinity

treatment resulted in 37% more sub-adults seen than in the seawater treatments, while fresh *Ulva* showed 30% more sub-adults than populations with fecal *Ulva* diets (Figure 8: ANOVA diet p value <0.001, salinity p value <0.001). When at low salinity and with a fresh *Ulva* diet, the maximum number of sub-adults were found among all of the treatments (interaction p value=0.008). The same pattern was not seen in proportion of sub-adults in the populations (Figure 9: ANOVA diet p value=0.109, salinity p value=0.09, interaction =0.280).

For the larval stage population abundance, there was a significant effect with salinity and diet, as well as an interaction. In the larval stage, low salinity treatments resulted in 33% more larvae growth over the seawater treatment, while fresh *Ulva* resulted in 28% more larvae than in fecal *Ulva* diets (Figure 10: ANOVA diet p value<0.0001, salinity p value <0.0001). When there was both low salinity and fresh *Ulva* diet, the larval stage reached maximum abundance, with a significant interaction (p value=0.02). The proportion of larval stages within the populations also showed effects of both diet and salinity, but with no interaction. The low salinity treatments resulted in a 9% greater proportion of larvae over the seawater treatments, while fresh *Ulva* diet showed with a 7% increase in the larval proportion over the fecal *Ulva* diet (Figure 11: ANOVA diet p value=0.013, salinity p value=0.003, interaction =0.062).

Chemical analysis results comparing the caloric content of fresh *Ulva* and fecal *Ulva* diets showed that the fresh *Ulva* was significantly higher in calories than fecal *Ulva* (Figure 12: t-Test p value=0.006). On average, fresh *Ulva* had a caloric content of 1.55 calories/mg while the fecal *Ulva* diet had a 1.29 calories/mg, a decrease in value of about 16%. Caloric values of *Ulva* also changed significantly after the freezing process. *Ulva* samples which had been frozen for a one week duration and for an extended 6 week duration were tested, and both showed a significant increase in caloric

content over the fresh *Ulva*. The one week frozen sample contained a caloric content of approximately 1.66 calories/mg whereas the fresh version had an average of 1.46 calories/mg, indicating an approximate 20% increase over the fresh *Ulva* (Figure 13: t-Test p value = 0.028). The 6-week extended frozen sample also showed similar results, with the frozen *Ulva* having a significantly higher value at 1.89 calories/mg over the fresh *Ulva* at 1.54 calories/mg (Figure 14: t-Test p value < 0.001), a difference of approximately 19%. The effects of freezing fecal *Ulva* diets did not show similar results; there was not a significant caloric difference between the frozen *Ulva* fecal diet and the fresh *Ulva* fecal diet (Figure 15: t-Test p value = 0.119).

Discussion

Both diet and salinity significantly affected population growth of *T. californicus* copepods. Feces produced by urchins that have been fed only *Ulva* do not appear to be an effective food for *T. californicus* population growth. The quality of fecal material produced on a particular algal diet depends on the absorption efficiency of urchins, and on the chemical composition of the food (Sauchyn & Scheibling, 2009). This study determined that fecal *Ulva* is a poor food source possibly due to its chemical composition. Based on caloric content analyses, fecal *Ulva* had 20% fewer calories than fresh *Ulva*. This contrasts with previous research on other algal species, where the caloric content of urchin feces fed *Nereocystis luetkeana* and *Saccharina latissima* was much higher than both fresh *N. luetkeana* and fresh *S. latissimi*, and the fecal diets resulted in increased copepod population growth (Kobelt & Dethier, 2015). Further study would be warranted to determine what the caloric content difference is between fresh *Ulva* and these kelp species, as well as their urchin fecal counterparts.

Surprisingly, the data showed that there were clear changes to the caloric content of *Ulva*

seen with freezing; frozen samples had a 20% increase of calories compared with the unfrozen samples. The caloric content in frozen algae has been insufficiently studied, so it is unclear exactly why it increased. Further investigation into this subject is needed.

The experiments also showed a higher rate of total copepod population growth in low salinity treatments. Both treatments testing salinity differences (17 PSU vs 29 PSU) resulted in the low salinity treatments having greater population growth than the normal seawater treatments. This could relate to the normal habitat of *T. californicus*. This copepod is found in the high intertidal pools along the Pacific coast, where it lives to avoid predation (Dethier, 1980). Tide pools are subject to physical variation on a daily basis, stemming from sunlight, waves, precipitation, and tides. Heavy rains or evaporation can change the salinity within them, and forces species there to quickly adapt to this drastic change. Species show more effective mechanisms to cope with stress if they frequently experience a more variable environment (Lewis, Brown, Edwards, Cooper, & Findlay, 2013). Although the experiment did not change the salinity of water over the course of the 50 days, it could be that this species of copepod is already better suited for a low salinity environment just due to its natural habitat. Future research could benefit from monitoring populations from different locations to see if these same trends are observed.

The proportions of life stages seen within the treatment populations suggest that different life stages are affected by different combinations of diet and salinity. Some, like the egg-bearing females, seem to be negatively affected by diet type, whereas non-egg-bearing adults were changed more by salinities. The larval stages are affected by both diet and salinities, but the sub-adults show no changes in population growth from either. These outcomes could be due to long term exposure to poor conditions, which often cause decreases in reproductive periods and delayed development (Emlen, 1966). Larval

development can be affected by differences in the chemistry of seawater, such as the absence or presence of particular dissolved compounds (Wilson & Armstrong, 1961). Running this experiment for a longer duration that spans multiple generations would clarify these trends.

Even though feces from urchins fed *Ulva* do not seem to provide the proper nutritional needs for *T. californicus* populations to thrive, this does not mean that *Ulva* is not itself a good food source. *Ulva* is an abundant species found through the entire Pacific Ocean, which could provide a link between intertidal zones along coastal marine communities, and many herbivore species living in the coastal subtidal zone effectively consume it as a food source. While *T. californicus* is not directly present in deep benthic communities, many harpacticoid copepod species are present, and are an essential component of deep subtidal food webs. Discovering that they showed a positive growth rate in low salinity environments gives hope that other coastal species could possess these same characteristics. As climate events are going to increase, withstanding such environmental changes is going to be imperative for marine species survival.

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Figures

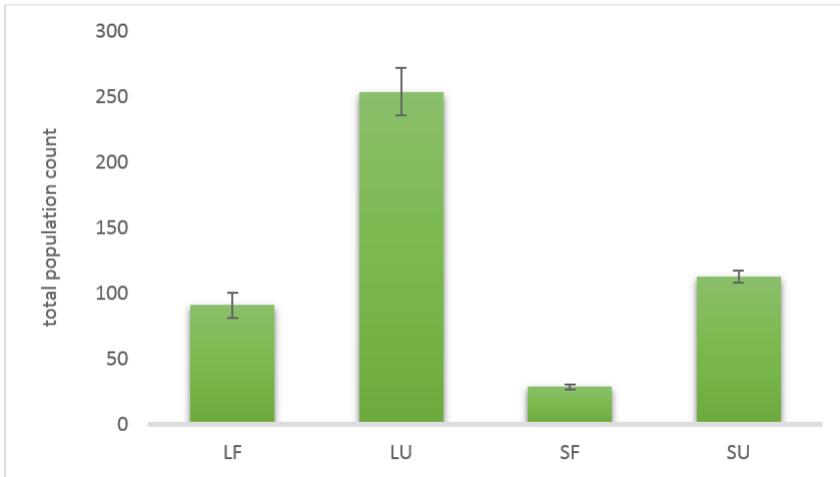


Figure 1. A comparison of total *T. californicus* population counts for each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin Feces (SF) and seawater/fresh Ulva (SU). Bars are average among the 4 jars per treatment with standard error.

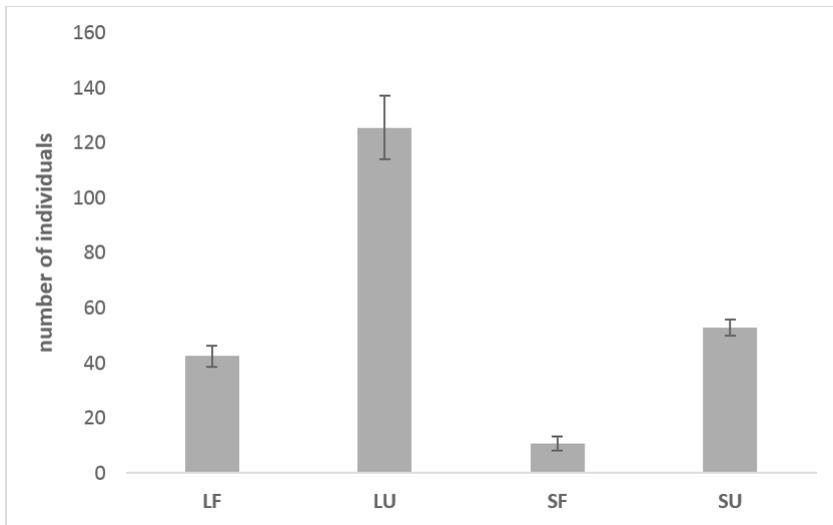


Figure 2. A comparison of total *T. californicus* population counts for each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Bars are the average among the 4 jars per treatment.

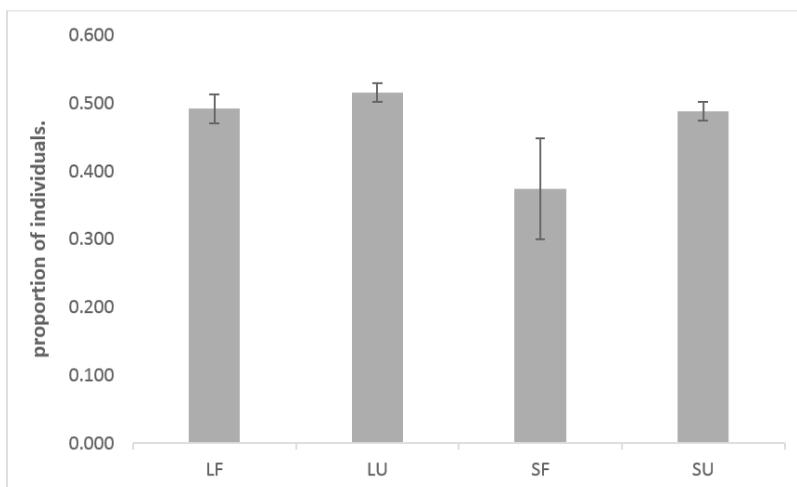


Figure 3. Comparison for the proportion of *T. californicus* populations made up each life-history state in each treatment including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Values are averages among the 4 jars per treatment.

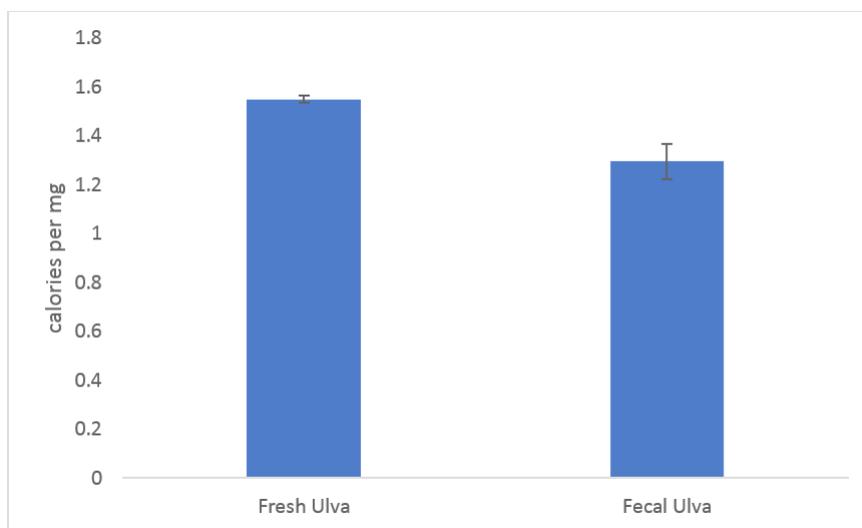


Figure 4. A comparison of total *T. californicus* population counts for egg-bearing females in each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin Feces (SF) and seawater/fresh Ulva (SU). Bars are the SE averages among the 4 jars per treatment with standard error bars.

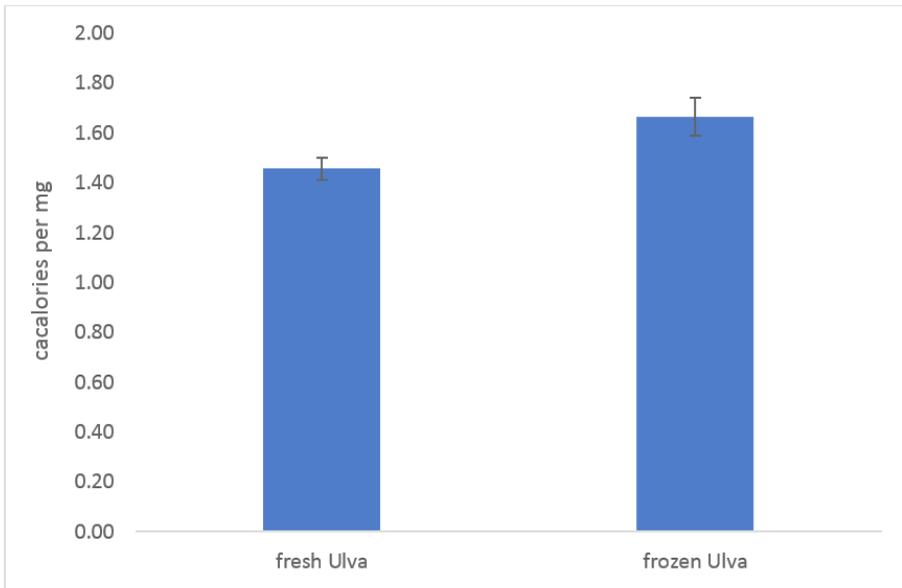


Figure 5. Comparison in proportion of *T. californicus* egg-bearing females in each treatment including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment with standard error.

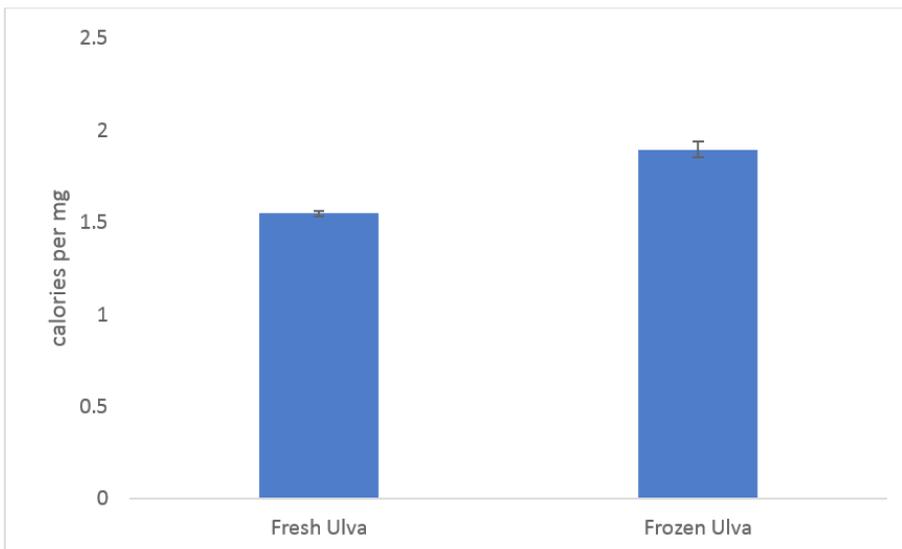


Figure 6. A comparison of total *T. californicus* population counts for non-egg-bearing adults in each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin Feces (SF) and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment with standard error.

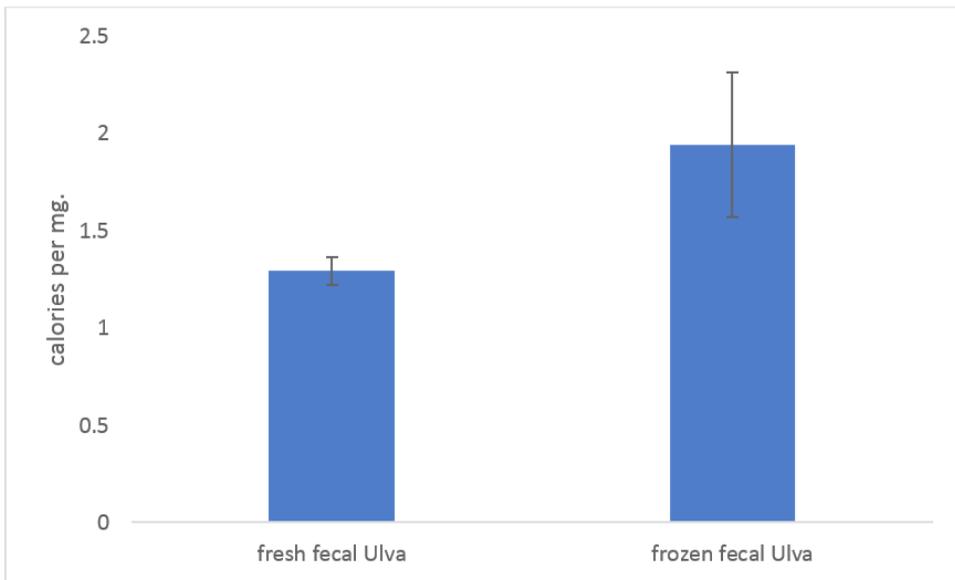


Figure 7. Comparison of the proportion of *T. californicus* non egg-bearing adults for each treatment including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment with standard error.

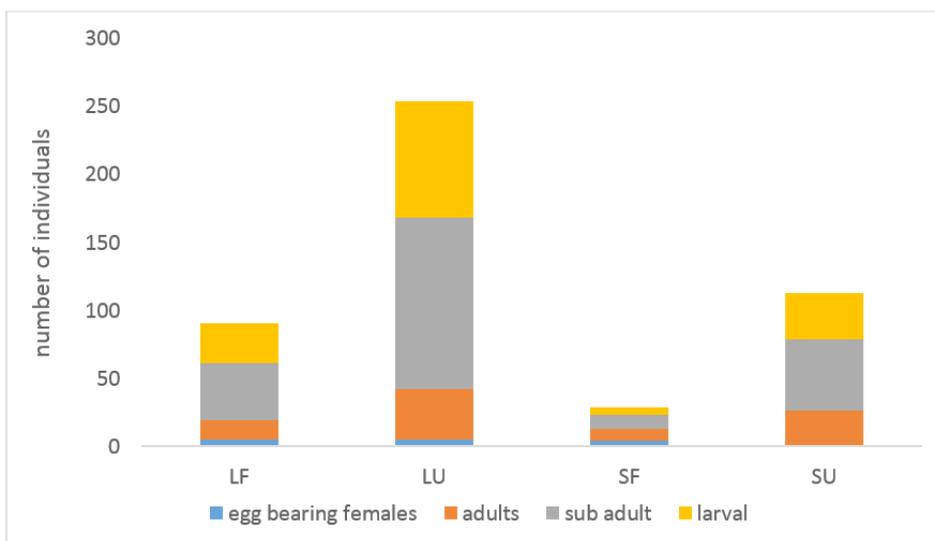


Figure 8. A comparison of total *T. californicus* population counts for sub-adult stages seen each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin Feces (SF) and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment with standard error.

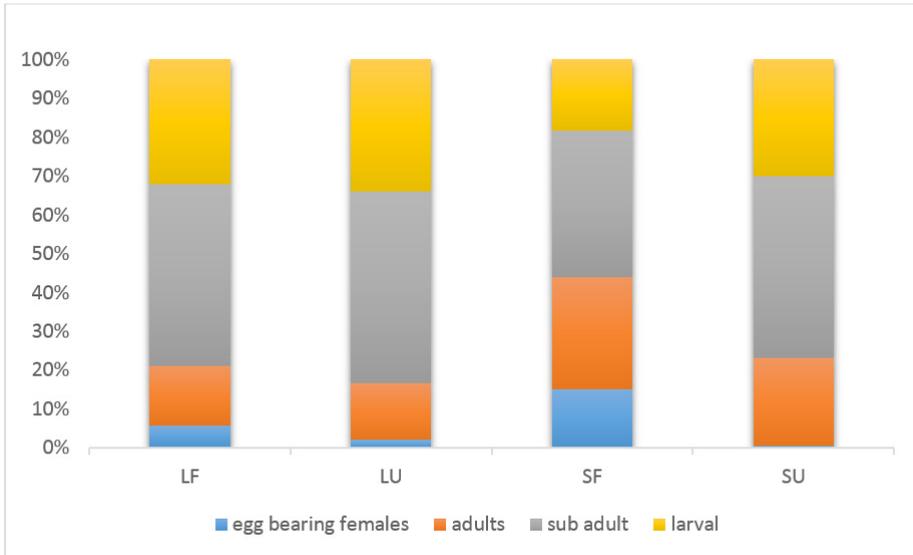


Figure 9. Comparison for the proportion of *T. californicus* sub-adults for each treatment including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment showing standard error.

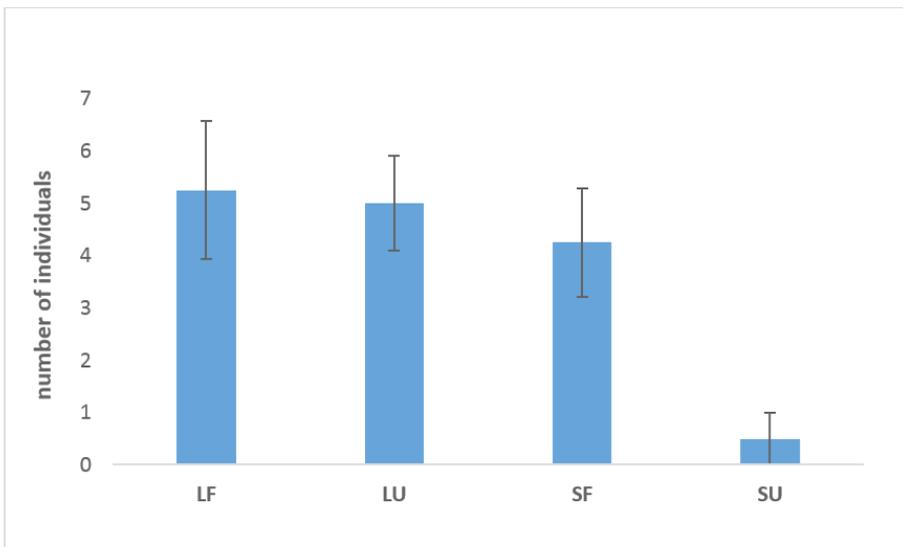


Figure 10. A comparison of total *T. californicus* population counts for larval stages seen each treatment, including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF) and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment showing standard error.

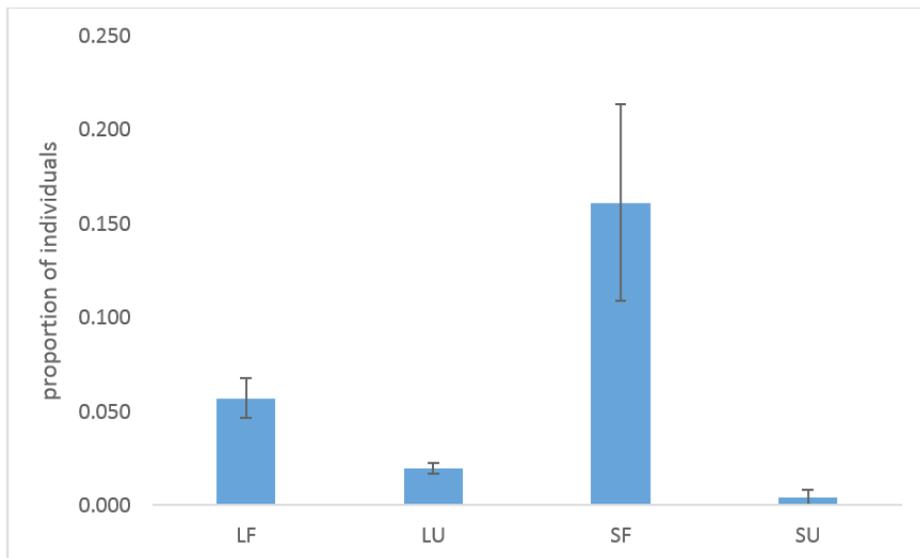


Figure 11. Comparison for the proportion of *T. californicus* larval for each treatment including low salinity/urchin feces (LF), low salinity/fresh Ulva (LU), seawater/urchin feces (SF), and seawater/fresh Ulva (SU). Bars are the averages among the 4 jars per treatment and show standard error.

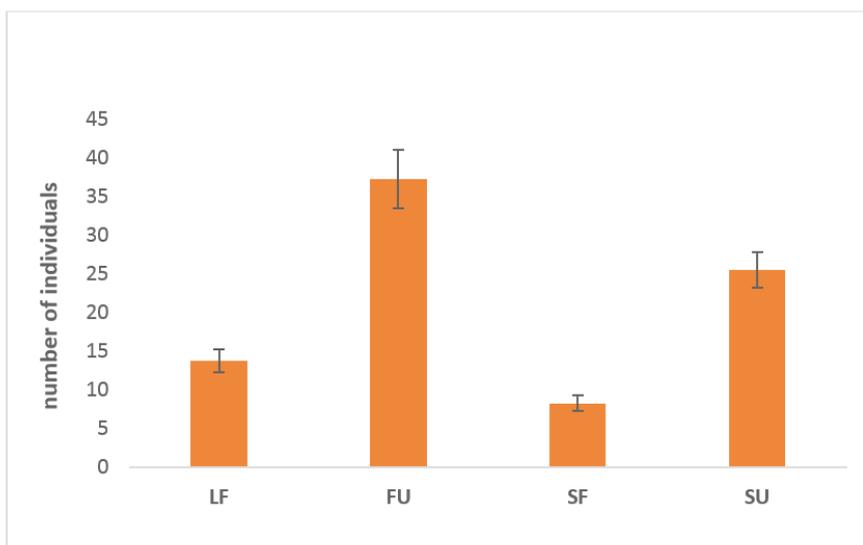


Figure 12. Comparison of the caloric content for fresh Ulva and fecal Ulva, showing fresh Ulva to be significantly higher than fecal Ulva by 16 % (t-Test p value=0.006) Bars are the averages among the 10 samples of each type and show standard error.

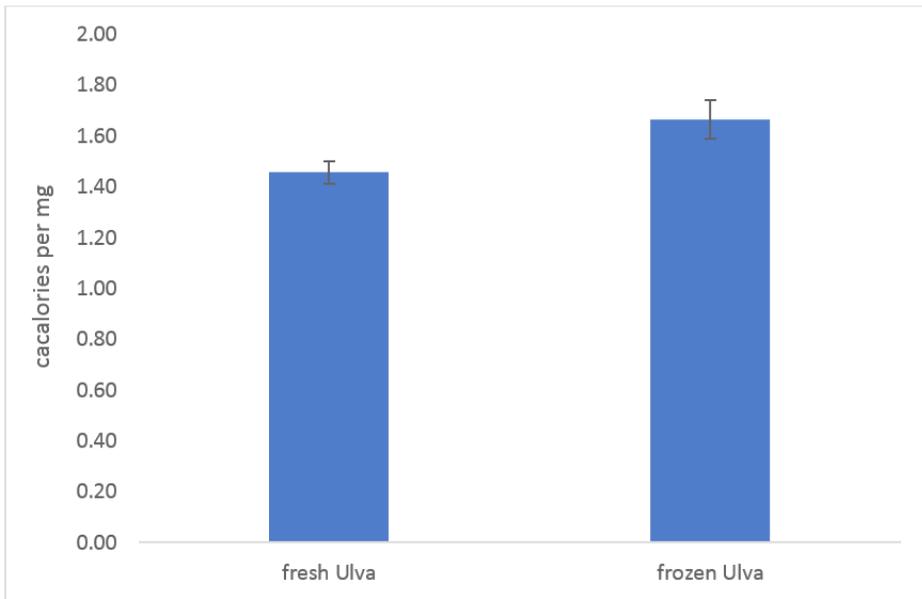


Figure 13. Comparison of the caloric content for fresh Ulva and 1 week old frozen Ulva, showing frozen Ulva to be significantly higher than fresh Ulva by 20%. Bars are the averages among the 15 samples of each type and contain standard error bars.

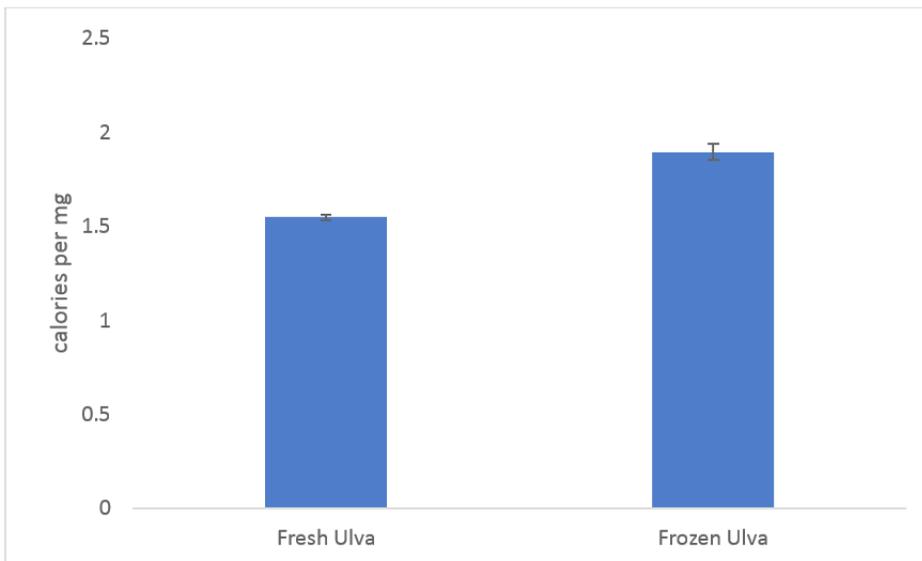


Figure 14. Comparison of the caloric content for fresh Ulva and 6 week old frozen Ulva, showing six week old frozen Ulva to be significantly higher than fresh Ulva by 19% . Bars are the averages among 10 samples per type and standard error.

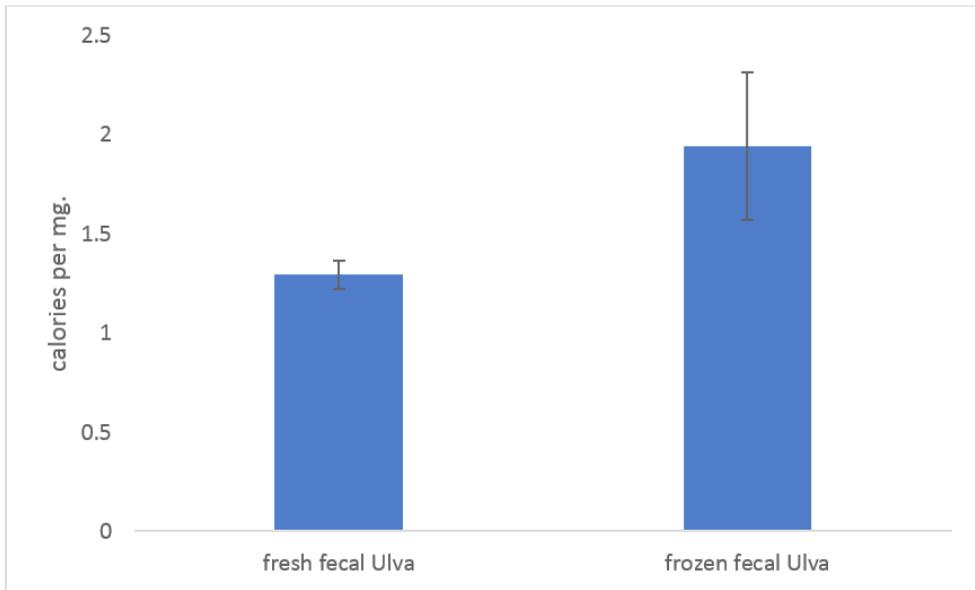


Figure 15. Comparison of the caloric content for fresh fecal Ulva and frozen fecal Ulva, showing no statistical significance in either treatments. Bars are the averages among 10 samples per type and show standard error.