

# Urchin Feces: a Possibly Vital Nutritional Link to Benthic Organisms

Jessica Kimber

Marine Environmental Research Experience 2012

Keywords: *Strongylocentrotus franciscanus*, urchins, *Nereocystis*, Agarum, food web, calorimetry, feces, diet variation

## **Abstract**

Detritus is a vital source of nutrients for many benthic organisms, but its composition and dynamics in the ocean are relatively unstudied. Sea urchin feces may provide an important link from the relatively shallow benthic areas to other benthic communities, such as those in the deep-sea, as urchin “mobs” are capable of mowing down entire forests of algae and, of that algae, their digestive systems absorb on average only 60% of the calories available. Through these relatively unprocessed feces, a large amount of material could be available to benthic communities, making the feces a potentially vitally important nutrient conveyer to benthic organisms. To gain insight into the composition of urchin feces, and more insight into their digestive system, I conducted a series of tests on the caloric content of algae and urchin feces. First, I tested two species of algae at various stages of decay for caloric content. Then, I tested aged and fresh feces of 12 urchins on diets of either *Nereocystis luetkeana* (*Nereocystis*), or *Agarum fimbriatum*, for caloric content using a modified version of a micro assay technique. I found that aging dramatically increases the caloric values of both *Nereocystis* feces and tissue samples, however in *agarum* feces and tissue samples aging has little effect. This could have significant implications in considering where benthic organisms acquire their nutrients, as well as the importance of sea urchins in providing a link to those organisms.

## **Introduction**

The ocean's food webs are vast and complex, and much about the dynamics between marine organisms and their food is unknown. One such unknown dynamic centers around the benthic community- a community largely separated from the easily accessible biomass in and around photic zone. Specifically, how this community obtains the nutrients and energy necessary to survive, especially the benthic community of the deep sea.

Much of this energy seems to come from detritus matter as it sinks from the productive photic zones and areas near them, to deeper parts of the ocean (Newell, 1965). However, the composition and nutritional content of this detritus is relatively unstudied. The nutrients from the photic zone must have some means of transferring from the upper water column to deeper areas. If detritus is the main driver of this transport, biotic links must be responsible for creating the vital detritus that sinks down.

It is possible that, in some areas, sea urchins provide such a biotic link. Sea urchins are infamous for their ability to dramatically change an ecosystem due to the huge amount of algae they consume. When urchins are removed from an environment, many algal species flourish, including species that may not have been found in that area with urchins present, and the diversity of algae in that ecosystem can dramatically increase (Paine and Vadas, 1969). An increase of urchins has the opposite affect; the urchins can consume entire forests of algae. An extreme but nevertheless exemplary instance of this is the classic case of sea urchin population rise and kelp forest decline with the removal of sea otters, a

major urchin predator. In most instances, as urchin population booms with sea otter decline, kelp forests decrease dramatically in biomass (Estes and Duggins, 1995). Moreover, a large quantity of kelp that urchins consume is brought down by the urchins from higher in the water column to lower. Urchins are capable of sinking large stalks of algae through their combined weight (Larson, et al., 1980) and are also capable of catching and weighing down drift algae, a resource that otherwise would not be as readily available to the benthic community. However, for all the algae that urchins consume, their relatively inefficient digestive system causes them to have a low caloric absorbance rate. This rate of absorption ranges from around 36-91% absorption, with an average rate of about 60% (Vadas, 1977).

The fact that urchins consume a large quantity of algae, an important energy resource, and also have a relatively poor rate of caloric absorption from that algae, leads to the assumption that their feces could be an important source of nutrients as they sink through the water column in the form of detritus. As mentioned previously, detritus is already an established form of sustenance for the benthic community. Some benthic filter feeders rely on detritus, a large portion of which is fecal material, for nutrients and energy (Newell, 1965). In addition to simply providing left over energy from urchin digestion, it is possible that the benefits of urchin feces and other detritus materials to the benthic community could be even greater than anticipated. Already, there have been studies on the increase of caloric value from bacterial colonization on plant detritus material (Mann, 1988), which found that microbes do indeed settle and colonize algal detritus, and provide a source of nitrogen and biomass as their colonies expand. Considering then, that a large portion of urchin feces is relatively unprocessed plant material, urchin feces may

gain caloric value as they age, providing an even greater energy source to the benthic community than simply through the caloric intake of digested algae.

With these implications possible, I first tested the absorption rate of urchins by comparing the caloric content of their feces to the caloric content of the original algae. Although this has already been partially tested by other researchers (Vadas 1977), I decided to elaborate on their findings, both for the sake of confirmation and for the sake of testing a new micro assay technique to measure caloric content, due to general concerns of inaccuracy with the common bomb calorimetry technique. Second, I wanted to compare the caloric content of two kinds of algae with known preferences among urchins, *Nereocystis luetkeana* (*Nereocystis*), and *Agarum fimbriatum* (*Agarum*). *Nereocystis* is known to be preferred by urchins over most other kelps when available, while *Agarum* is known to be very unfavorable. When presented with a large variety of algae, urchins consumed nearly 20x as much *Nereocystis* as *Agarum* (Vadas, 1977). *Agarum* is also well known for having high concentrations of polyphenolic compounds, which deter herbivores (Hammerstrom et al., 1998). It seems likely that these compounds account for the low preference towards *Agarum* in urchins. Lastly, I tested to see how aging affected the caloric content of both types- *Nereocystis*-fed feces (*Nereocystis* feces) and *Agarum*-fed urchin feces (*Agarum* feces)- to examine a possible increase of caloric content with aging due to bacterial/fungal colonization.

## **Methods**

### Experimental organisms

Three specific organisms were utilized in my experiment, the urchin *Strongylocentrotus franciscanus*, and two algae, *Nereocystis luetkeana* (*nereocystis*), and *Agarum fimbriatum* (*agarum*). The two algae were selected due to the known preferences urchins have for either species- urchins prefer *Nereocystis* and generally avoid *Agarum*- and due to the known chemical properties of the algae, namely the high concentrations of herbivore-detering polyphenolic compounds in *Agarum* (Hammerstrom et al., 1998). All organisms were collected from around the Friday Harbor Laboratory area on San Juan Island, Washington. Fresh algae were collected weekly for feeding the urchins. Fourteen urchins were collected for the experiment.

#### Experimental set up

I separated out the fourteen urchins out into two groups based on their assigned diets in two temporary tanks for the first week of the experiment. Seven urchins were fed *Agarum ad libidum*, and seven *Nereocystis ad libidum*. The urchins were kept in tanks circulated with seawater pumped directly from Friday Harbor on San Juan Island throughout the experiment.

After the first week in the temporary tank, I placed twelve of the urchins in their testing tank. I constructed 12 urchin testing containment units by drilling approximately fifteen 2.5cm diameter holes into 10-liter plastic buckets, spaced relatively evenly over the bucket's cylinder (figure 1). I wedged a plastic grate with approximately 1cm mesh onto the bottom of the buckets, keeping the grate between 2-8cm above the bottom of the buckets.

I placed each urchin into an individual bucket, and placed the buckets around the edges of a 2.5 meter diameter tank, alternating the buckets by diet around the edge of the circular tank. Throughout the experiment, urchins had near constant access to their assigned algae.

I kept the urchins in the testing buckets for 1 week before cleaning out their buckets by tipping the buckets over into an adjacent tank. After cleaning, I left the urchins for one day. That next day, I collected feces from beneath the grate by carefully pointing a turkey baster between the grates at the bottom of the bucket, blowing up urchin feces and water along the sides of the bucket, and then sucking up the feces with the baster as it floated upwards (see figure 2). I then placed the feces in 25ml test tubes, and carefully poured out as much water as possible without spilling any feces into the tank, leaving about 1ml of water and feces in each test tube. These pellets, the “fresh” samples, were immediately frozen, and kept for processing at a later period. I added new feces to the test tubes daily for 4 days and then refroze them, until I had collected approximately 10mL of feces. For the last 3 days of fresh feces collection, I altered my collection method from blowing up the feces with the turkey baster and then sucking it up, to removing the algae and grate (urchins generally kept to the side of the bucket, so removing them was unnecessary), and sucking up the feces directly at the bottom with the turkey baster. This method proved to be far more time efficient for collecting the feces, however there was possibly increased risk of contamination from algal cells such as diatoms settling at the bottom of the bucket.

One week later after the fresh feces collection, I cleaned the buckets once more, then one week after that I removed the urchins from their buckets and placed them in their initial holding tanks, still separated by diet. I left the feces in the buckets for one additional week, meaning the feces were aged between 1-2 weeks, depending on when they were deposited by the urchin. I then collected the feces by removing the grate and sucking up the feces with a turkey baster, then depositing it in test tubes. For each urchin, I collected approximately 10-15ml of feces in seawater.

I then scooped approximately 1 gram (wet weight) of feces out of each test tube with a metal scooping rod, and placed each sample into an aluminum weigh boat and dried them at 150 degrees Celsius for 90 minutes. Along with the feces samples, I also collected two approximately 4x4 square samples of fresh *Agarum* and *Nereocystis*, which I finely chopped with a razor and placed in weigh boats, and heated along with the other samples. The fresh *Nereocystis* samples I collected from a few centimeters off where the blade connects to the bulb, and the fresh *Agarum* samples a couple of centimeters off of the midrib on either side. The aged *Agarum* tissue sample (aged for 1 month) I was able to collect and prepare similarly to the fresh sample by cutting off a 4x4 section off of the midrib and then chopping it finely and drying it. However, the aged *nereocystis* tissue sample was broken down after the month of aging to the point of all anatomy of the algae being unrecognizable. As such, the aged *Nereocystis* tissue was collected randomly via an aquarium net, deposited directly into the aluminum weigh boats and dried.

After all weight was converted to dry weight, I pounded the samples to a powder using a mortar, then weighed this powder to ~25 mg for each sample, put the powder into 25ml test tubes, and began chemical analysis on each sample.

### Chemical Analysis

In order to test the samples, I followed a micro assay calorimetry technique. My basic methods are outlined in the Gosselin and Qian (1999).

I made a few changes to this technique. To optimize the amount of material used to increase the accuracy of my samples, I used approximately 30 mg of dry weight material, and as such changed the amount of the potassium dichromate oxidizing solution to 10mL added to the sample tubes. I then incubated as directed. After the two incubation periods, I took 0.5mL of solution from each sample tube and placed each in another test tube then, as directed in the original instructions, added 4 mL of the potassium iodide/starch solution, and waited for 20 minutes. From there, I went straight to the calorimetry measurements, without adding the RO water as directed. Wavelengths were measured at 575nm, using a DR 5000 spectrophotometer.

Due to the major changes I made to the original directions, many tests were conducted to ensure the accuracy of the test. The conclusive test that determined the effectiveness of the revised technique tested a series of dry weights of *Nereocystis*, ranging from 0mg to 80mg. A clear linear relationship was established (figure 3), confirming my technique was effective enough to proceed with using carbon amounts far larger than those specified in the original methods.

Standards were prepared in a similarly to the samples. I weighed out approximately 5mg, 10mg, 15mg, 20mg, 30mg and 50 mg of reagent grade glucose, and tested for caloric content using the same modified chemical technique as used for the samples. This produced the standard line (figure 4) which I would compare my samples to, in order to determine the amount of glucose present in each sample. The standard line used had a relatively high  $r^2$  value of .92, further confirming the effectiveness of my modified technique.

### Post Processing

To convert my samples' measurements from absorbance to caloric content, I calculated the amount of glucose in each sample by using the equation from my standard curve, converted to calculate for units of glucose (mg).

$$\text{Units of Glucose} = (\text{Absorbance of Sample} + 0.067) / 0.013$$

I then divided the calculated amount of glucose for each sample by the sample's dry weight, giving us units of glucose/milligram. With that, I used the conversion from units of glucose to calories outlined in Gosselin and Qian (1999), to calculate the calories of each sample.

$$= (15.7 * (\text{Units of Glucose}/\text{mg})) / 4.18$$

All data was processed in Microsoft Excel, which was also used to perform t-tests on samples. The means for each sample set from these t-tests were used for the direct sample comparisons.

## **Results**

Full mean caloric values for each test are displayed in figures 5 and 6. No significant difference ( $p$ -value=.699) was found between the caloric content of fresh *Agarum* tissue and fresh *Nereocystis* tissue. There was also no significant difference between fresh agarum tissue and fresh *Agarum* feces ( $p$ =.0721), fresh *Agarum* feces and aged *Agarum* feces ( $p$ =.78622), and fresh *Agarum* feces and fresh *Nereocystis* feces ( $p$ =.161).

However, aged *Nereocystis* feces had significantly higher caloric content than fresh *Nereocystis* feces ( $p$ = 0.0225), aged *Nereocystis* feces had a higher caloric content than aged *Agarum* feces ( $p$ = 0.016), aged *Nereocystis* tissue had a higher caloric content than aged *Agarum* tissue ( $p$ = 0.000), fresh *Nereocystis* tissue had a higher caloric content than aged *Nereocystis* tissue ( $p$ =0.000), and fresh *Agarum* had a higher caloric content than aged *Agarum* tissue ( $p$ =0.002)

## **Discussion**

My results show that several factors significantly affect the caloric value of various algal materials (tissue, or processed tissue in feces). The two most prominent of these factors are the species of algae from which the material originated, and the age (amount of decay) of the material.

My results clearly show that *Nereocystis* materials gain caloric value as they age. After the feces aged for just 1-2 weeks, they nearly doubled in caloric value from 5.7 calories per mg dry weight to 11.23 calories per mg dry weight. The uneaten tissue mirrors this pattern, more than doubling in caloric value 3.9 calories per mg dry weight to 9.6 calories per mg dry weight over a period of 1 month. These results could have significant

implications for the caloric value of detritus as it flows through the water column. If detritus gains caloric value as it ages, its value as it takes time to sink to deeper areas of the ocean could substantially increase. And in an area known to depend greatly on the input of detritus (Newell, 1965), *Nereocystis* materials and other algae with similar properties could be a vital source of nutrients to those deep-sea organisms. Early studies have also found that detritus has a relatively high concentration of nitrogen, which is inferred to be contributed by the fungi and bacteria that settle on it (Newell, 1965). In addition to being potentially vital in caloric value, the bacteria that settle on *Nereocystis* material could be vital to deep-sea benthic organisms in adding essential nutrients.

Also relevant to nutrient flow through various ocean zones is the caloric values I measured for *Agarum*. *Agarum* is one of the most common kelps in the San Juan Archipelago area (Britton-Simmons, 2004), and as such it could potentially provide energy for organisms throughout the ecosystem, especially aged *Agarum* if it were as calorically valuable as aged *Nereocystis*. However, I measured no such aging process in *Agarum* tissue or in urchin feces containing *Agarum*. It seems safe to infer that the chemical deterring properties agarum possesses in its fresh tissue also prevent the tissue from deteriorating and acquiring bacterial/fungal colonies as it ages (on the time scale of 4 weeks), or as it passes through an urchin gut. This is supported by past research, which found no significant increase in microbial colonies on *Agarum* blades after 5 weeks of aging (Sosik, A.S., and Simenstad, unpublished). Thus, while *Nereocystis* could be a vital nutritional source as it sinks through the water column, *Agarum* appears to be rather nutritionally inaccessible to those bacterial and/or fungal colonies that provide added nutritional benefits to *Nereocystis* material. Perhaps as *Agarum* ages further than the 4

week period my agarum was aged, the herbivore deterring compounds it contains could break down, making it more nutritionally accessible, however further research would need to be done to test this. It is notable that instead of gaining caloric value with age as *Nereocystis* tissue did, *Agarum* tissue decreased in caloric value with age. This could be error due to a relatively small pool of replicates (n=6), or could be caused by some unknown mechanism. Replication of this experiment, or further research could perhaps account for this difference.

The caloric difference measured between fresh *Nereocystis* and fresh *Agarum* materials was insignificant (see results for p-values). This could indicate that the main mechanism driving urchin preference away from *Agarum* is not based on the nutritional content, but rather on other factors, such as the chemical composition.

The amount of calories absorbed from either alga was also shown to be insignificant. This confirms the general idea that urchins absorb very little calories from their food. However, this goes against previous research on the absorption rates of urchins (Vadas, 1977) so the insignificant difference could also be attributed to the sample size of the experiment, possibly the stress the urchins experienced from experimental set up, or the availability of kelp in the experiment. Past research found that urchins nutrient absorption rate decreases with an increased availability of algae to the urchin (Minor and Scheibling, 1997). Due to the confined environments urchins experienced during my experiment, urchins were in almost constant contact with algae. This ease of access to food could cause them to have a lower absorbance rate than established previously by other research. A larger sample size, further research on how the set up affected the stress on the urchins (i.e. water flow, spatial constraints, amount of disturbance), or a replication of this

experiment with varying availability of algae would be necessary to determine the cause of this low/nonexistent rate of absorption.

There were various potential sources of error in this experiment. Urchin feces, particularly that of the *Nereocystis* diet, agitates easily. When I cleaned the buckets, or collected feces, there was a portion of feces that flowed from the holes in the buckets, and could have contaminated the other samples. There was also a film of diatoms that developed after a few days on the buckets, and it is possible some of that film contaminated the feces. The aged *Nereocystis* tissue used in the experiment was kept in clumps, and as such it was impossible to determine which part of the tissue I were sampling, so consistency between sections of fresh *Nereocystis* tissue and aged *Nereocystis* tissue could not be assured.

My findings, both in results and methods, could be expanded on in many ways by future researchers. First, I have created a new method for determining caloric content with a larger sample size than specified in the original micro assay procedure (Gosselin and Qian 1999). After replication to ensure accuracy, this method could be used for any number of research purposes, from research similar to mine such looking how the caloric values differs with a wider range of algal tissues used in experimentation, or essentially testing anything with the purpose of finding the caloric values of samples with ~10-50mg dry weights. It would also be useful to other research to compare the effectiveness of this method with other methods of calorimetry, such as bomb calorimetry.

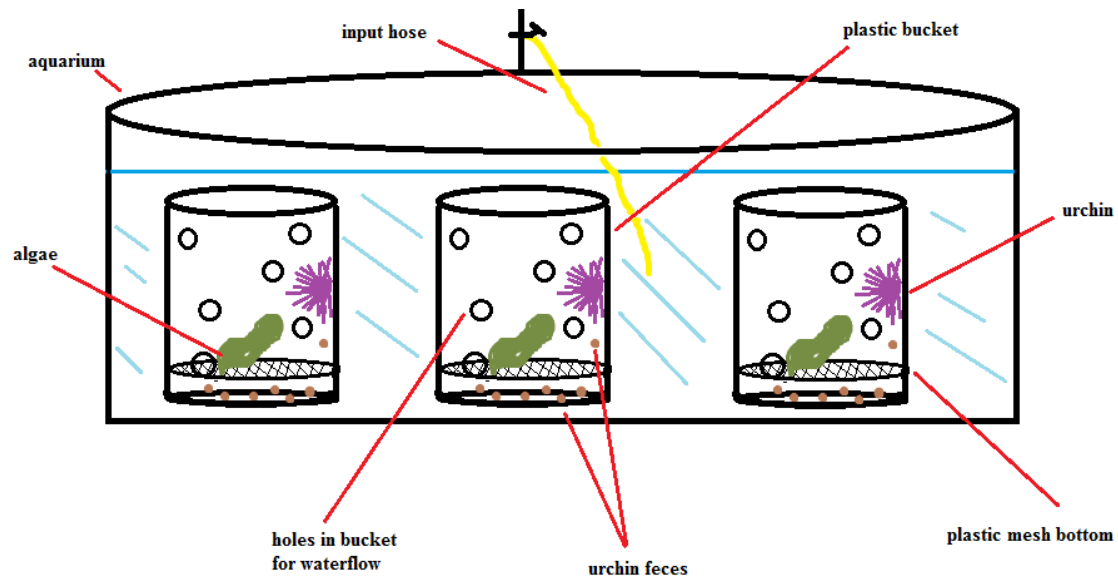
Further research could also be carried out based upon my findings concerning algal materials. Researchers could conduct an experiment similar to mine, but expand the

species of either algae or urchins used, to test how species might affect caloric value. Determining the composition of the fungi and/or bacteria that might accumulate on each type of algal material could also prove to be ecologically important.

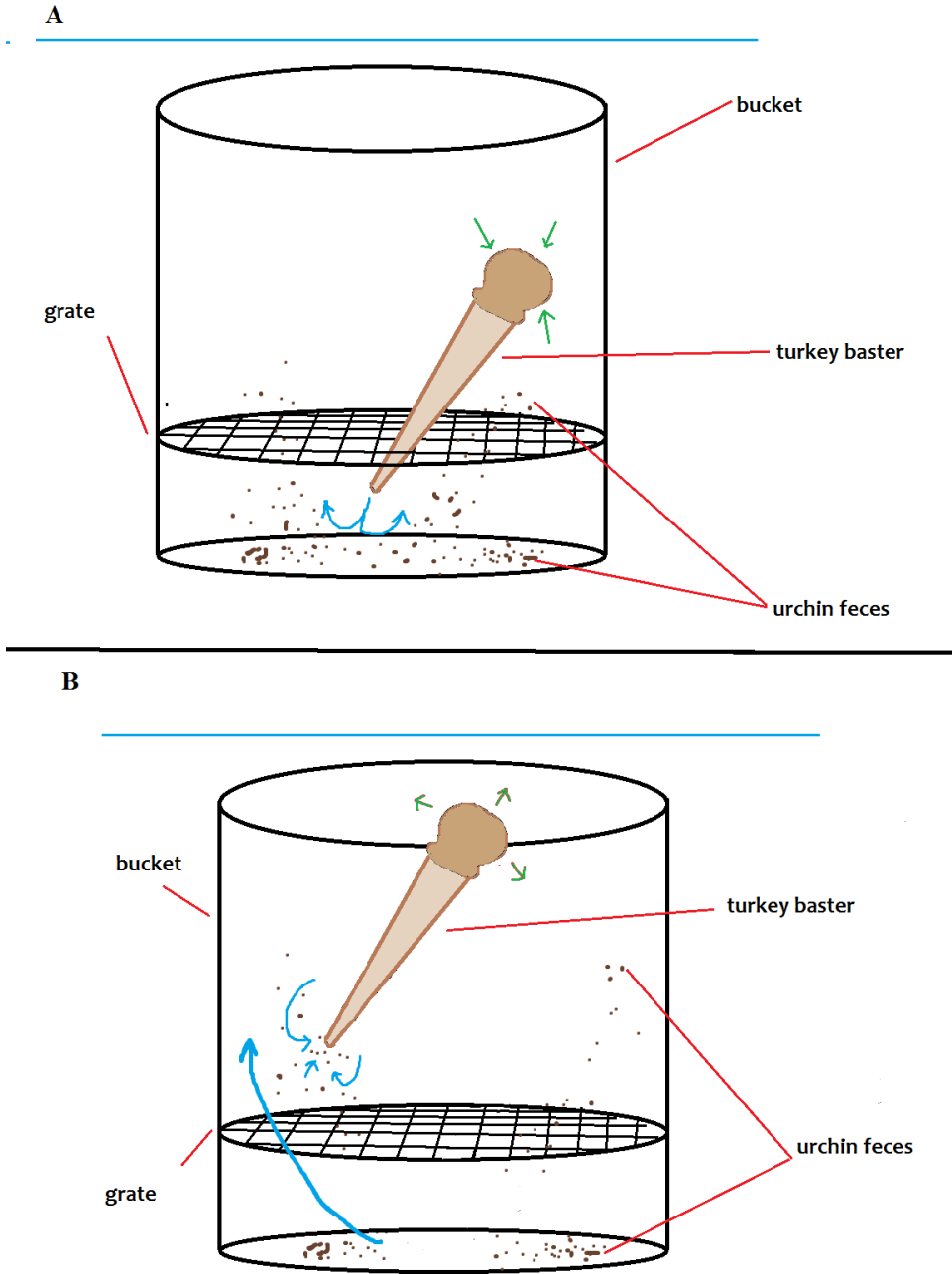
### **Acknowledgements**

I would like to thank my mentors in this project, David Duggins and Megan Dethier. Megan helped me set up the project, and was always willing to answer my questions as they came up. David helped me through the chemistry, coordinated much of the project, was responsible for creating the refined chemical analysis technique, and of course was always willing to help with the project. I would also like to thank Wendel Raymond and Alex Lowe, for collecting the urchins and algae for me on their dives. And I would like to thank Marianne Porter, for her assistance in putting together the information I gathered.

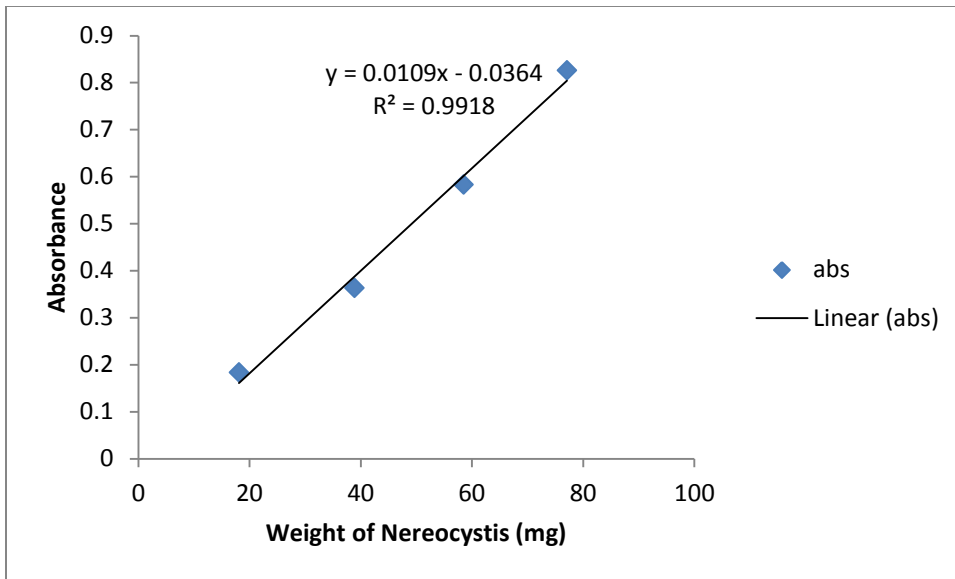
This work was funded in part by a National Science Foundation grant to Duggins et al.



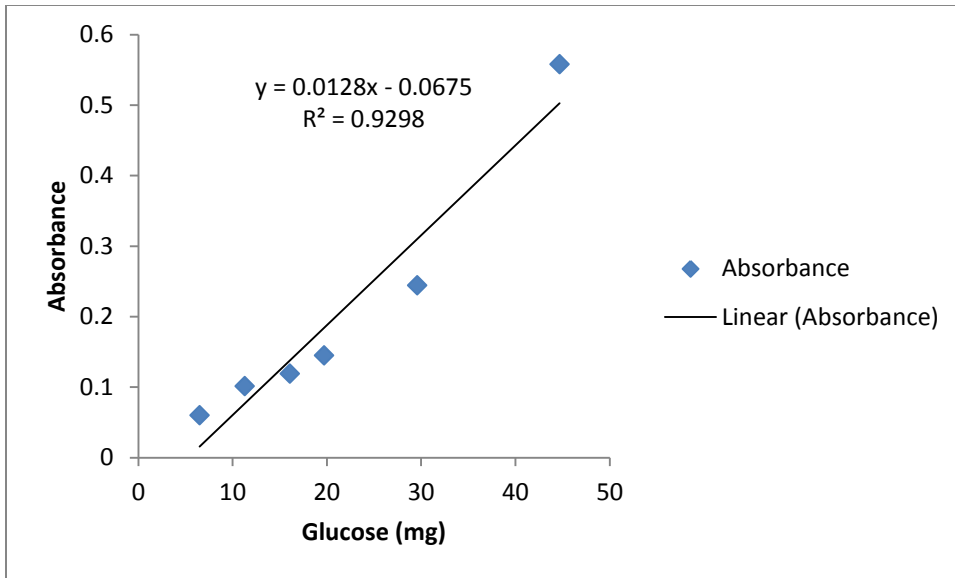
**Figure 1. A cross section of the urchins in their buckets. 12 urchins were kept in a large circular aquarium, in individual buckets. Holes were drilled into the buckets for water flow, and a plastic mesh was wedged over the bottom of the tank for a barrier between the urchin and their feces.**



**Figure 2.** Water was first flushed by pushing water at the bottom through the turkey baster, sending feces upwards through the grate (A). Fecal pellets were then collected as they floated upwards (B). Urchins were present in the buckets at the time of feces collection, but were omitted from the diagram for clarity.



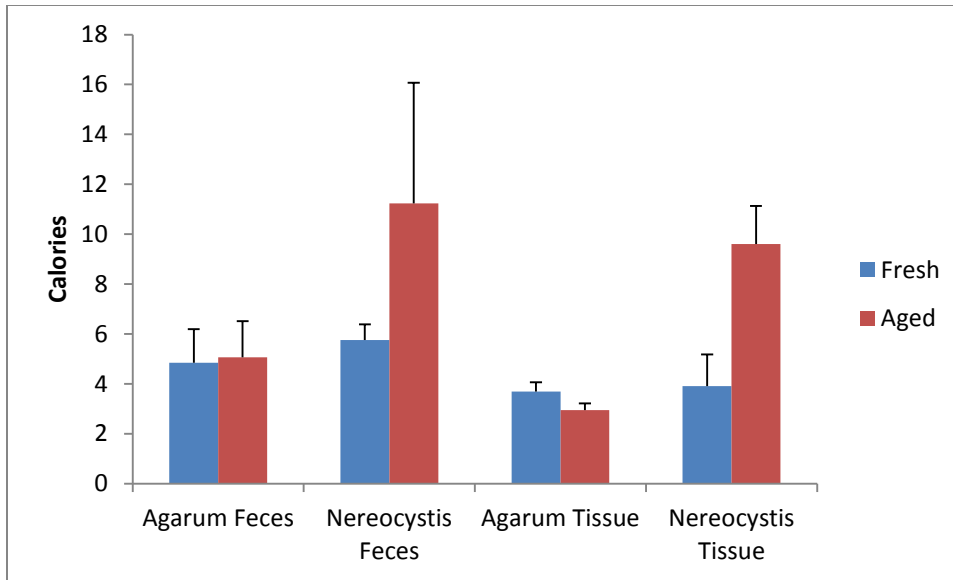
**Figure 3. The absorbance values of solution with standard weights of nereocystis. The clean linear relationship shows that the revised micro assay method is effective in measuring relative absorbance at different weights.**



**Figure 4.** The standard used for converting absorbance of each sample to amount of glucose. Set weights of glucose were measured out and then run through the chemical analysis used for the samples.

|       | <i>Agarum</i> Fed Feces | <i>Nereocystis</i> Fed Feces | <i>Agarum</i> Tissue | <i>Nereocystis</i> Tissue |
|-------|-------------------------|------------------------------|----------------------|---------------------------|
| Fresh | 4.842 ± 1.34            | 5.761 ± .626                 | 3.695 ± .364         | 3.909 ± 1.27              |
| Aged  | 5.067 ± 1.44            | 11.23 ± 4.84                 | 2.944 ± .274         | 9.603 ± 1.52              |

**Figure 5. Shows the mean caloric values of 8 treatments. All units are in calories. All treatments had 6 replicates (n=6), with the exception of aged *Nereocystis* feces, which had 4 replicates (n=4).**



**Figure 6. A graph showing the mean caloric values of each treatment. All treatments had 6 replicates (n=6), with the exception of aged *Nereocystis* feces, which had 4 replicates (n=4). All fresh/aged dynamics were found to be significant (with  $p < .02$ ) with the exception of fresh *Agarum*-fed feces vs. aged *Agarum*-fed feces ( $p = .78$ ).**

## Works Cited

- Britton-Simmons, K.H.** (2004). Direct and indirect effects of the introduced alga *Sargassum muticum* on benthic, subtidal communities of Washington State, USA. *Marine Ecology Progress Series*. **277**:61-78
- Durante, K.M., and Chia, F.** (1991). Epiphytism on *Agarum fimbriatum*: can herbivore preferences explain distributions of epiphytic bryozoans? *Marine Ecology Progress Series* **77**:279–287
- Estes, J., and Duggins D.O.** (1995). Sea Otters and Kelp Forests in Alaska: Generality and Variation in a Community Ecological Paradigm. *Marine Ecology Progress Series*. **165**:293-305
- Gosselin, L.A., and Qian, P.** (1999). Analysing energy content: a new micro-assay and an assessment of applicability of acid dichromate assays. *Hydrobiologia* **390**:141-151
- Larson, B.R., Vadas, R.L., and Keser, M.** (1980). Feeding and nutritional ecology of the sea urchin *strongylocentrus droebachiensis* in Maine, USA. *Marine Biology* **59**:49-62
- Mann, K.H.** (1988). Production and use of detritus in various freshwater, estuarine, and coastal marine ecosystems. *Limnol. Oceanography* **33**:910-930
- Minor, M.A., Scheibling, R.E.** (1997). Effects of food ration and feeding regime on growth and reproduction of the sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology* **129**:159-167
- Newell, R.C.** (1965). The role of detritus in the nutrition of two marine deposit feeders, the prosobranch *Hydrobia ulvae* and the bivalve *Macotna balthica*. *Proceedings of the Zoological Society of London* **144**:25-45
- Paine, R.T., and Vadas, R.L.** (1969). The effects of grazing by sea urchins *strongylocentrotus spp.*, on benthic algal populations. *Marine Biology* **4**:79-86
- Sosik, A.S., and Simenstad, A.S.** Microbial Trophic Levels: Isotopic Evidence and Consequences in Macroalgal Detritus-based Coastal Food Webs (unpublished)
- Vadas, R.L.** (1977). Preferential Feeding: An Optimization Strategy by Sea Urchins. *Ecological Monographs*. **47**:337-371