Efficiency of *Strongylocentrotus franciscanus* in extracting calories from algal foods: implications for benthic communities.

Griffin Hoins[1, 2], Megan Dethier[1], David Duggins[1]

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[1] Friday Harbor Laboratories, University of Washington, Friday Harbor, WA 98250

[2] Department of Aquatic and Fishery Sciences, University of Washington, Seattle, WA 98105

Contact information:

Griffin Hoins

PO Box 217

Nordland, WA 98358

GWHoins@gmail.com

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Abstract

Algal subsidies are extremely important to the success of the deep benthos where there is little to no primary productivity. Little research has been done on the nutritional value of detritus, such as pieces of kelp, sinking into deep habitats. Sea urchin feces, in the form of detritus, may provide an energetic link to benthic communities with no primary productivity. Urchins are known to have an inefficient digestive system which leaves the potential for high food value in feces. I conducted a series of tests on the relative caloric contents of algae and urchin feces. First, I measured the biomass consumed and egested by urchins. Secondly, I quantified the caloric content of aged and fresh feces of 10 urchins fed diets of either *Nereocystis luetkeana* or *Saccharina latissima*. For both kelp diets, the caloric content of algal material increased after being consumed by urchins, and the longer the urchin feces aged, the higher the caloric value became. This could be significant in considering the importance of urchins as a link to benthic communities that rely heavily on detritus for their success.

Introduction

The transfer of nutrients between environments can be vital to the success of an ecosystem. For example, in temperate deep subtidal environments there is little to no light, limiting primary productivity to shallow nearshore habitats (Britton-Simmons et al. 2009). For herbivores in all habitats, energy and nutrients must come from such photic habitats. Macrophytes from productive nearshore environments may thus provide an important trophic subsidy to deep subtidal food webs, in the form of particulate organic

matter (POM) or larger pieces of sinking detritus (Polis et al. 1997). Consumers survive in the deep subtidal despite the lack of vegetative growth because of the abundance of detritus that is transported there by hydrodynamic forces (Britton-Simmons et al. 2009). Britton-Simmons et al. (2012) found that 97% of observations within a 60-km^2 section of sea floor in the San Juan Archipelago had drift macrophytes, and most of the biomass came from kelps. However, little research has been done on the nutritional value of this sinking detritus. What is the energetic link between algal subsidies and the benthos? An important link may involve the fecal material from sea urchins.

The sea urchin *Strongylocentrotus franciscanus* is an herbivore common in the subtidal zone, ranging from the shallow subtidal to deeper than 100m. When present in deep subtidal environments it consumes mostly drift algae (Britton-Simmons et al. 2009). Sea urchins are voracious herbivores and consume large amounts of kelp biomass daily, transforming it into fecal matter (Sauchyn and Sheibling 2009). As a sea urchin's digestive system is relatively inefficient, the fecal matter could be an important source of calories (Vadas 1977). The increase of caloric value from bacterial colonization on plant detritus material provides a source of calories as their colonies expand (Mann, 1988). A substantial portion of urchin feces is relatively unprocessed plant material, which means urchin feces may gain caloric value as it ages. This may provide an even greater energy source to the benthic community, since many benthic suspension or deposit feeders rely on detritus, a large portion of which is fecal material, for nutrients and energy (Newell, 1965).

In my experiment I focused on how efficiently sea urchins extract calories from their algal foods to determine how much of an energetic impact their fecal matter could

potentially have on benthic ecosystems. I compared the caloric content of the feces to the original algae consumed as well as differences in caloric value in feces of urchins fed N*ereocystis luetkeana* and *Saccharina latissima*, two kelp species often consumed in nature. I also tested how aging affected the caloric content of aged feces vs. fresh feces. Lastly, I measured assimilation efficiency of the urchins between the two kelp species.

Methods

Experimental Organisms

I compared the caloric content of two different algal species, *Nereocystis luetkeana* and *Saccharina latissima,* before consumption by *Strongylocentrotus franciscanus,* with the caloric content of fresh and aged urchin feces after consumption of these kelps. All organisms were collected from around the Friday Harbor Laboratory area on San Juan Island, Washington. Ten sea urchins were collected near Brown Island, *N. luetkeana* was collected from Reid Rock and *S. latissima* came from the lab docks. Fresh algae were collected weekly for feeding the urchins.

Experimental Set-up

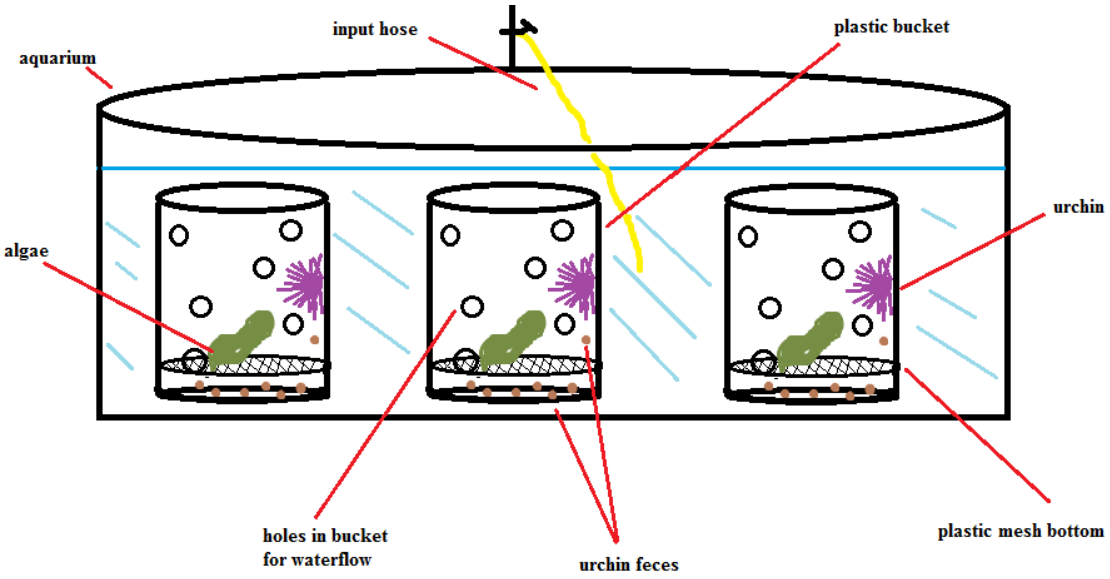

Figure 1. A cross section of the urchins in their buckets. 10 urchins were kept in individual buckets (Kimber 2012).

All of the sea urchins were placed in a temporary basket suspended off of the Friday Harbor Laboratories dock for a week without any added algae to ensure a similar state of starvation in all individuals. The ten sea urchins were then each placed in an urchin containment unit held within a large (2.5m diameter) circular tank. The containment units are 10-liter plastic buckets with approximately fifteen 2.5cm diameter holes drilled evenly around the walls to allow water flow. There is a plastic grate with approximately 1cm mesh wedged 2-8cm above the bottom of the buckets (Fig. 1). Each treatment had five replicate buckets. The urchins stayed in the testing buckets for one week while being fed their respective algae diet (*N. luetkeana* or *S. latissima*) ad libitum. After this first week each urchin bucket was fully cleaned by removing the urchin, the grate and scrubbing down the entire bucket. The next day each urchin was fed an excess amount of

their algal diet (pre-weighed) to ensure that they had constant access to their assigned algae throughout the day. Throughout the experiment this amount was approximately ~45 g for *N. luetkeana* and ~25 g for *S. latissima.* The following day I collected the extra algae, the fresh feces, weighed, recorded and also replenished each bucket with fresh algae. To collect the feces I siphoned all of the water in the bottom of the bucket through a fine filter, where all of the feces would collect. Afterwards I transferred the fecal matter from the filter into a scintillation vial and weighed the wet mass. These fresh samples were immediately frozen, and kept for processing at a later period. I repeated this process for four days with each of the five urchins in the *S. latissima* and *N. luetkeana* treatments.

Aged vs. Fresh Feces

On the first day after measuring, I took half of the *N. luetkeana* feces from each sample and put it in containers with mesh holes for flow that were submerged in a darkened tank of circulating seawater for four days. This acted as the aged-feces treatment. At the end of the four days, aged fecal matter, fresh fecal matter and fresh algal tissue were dried. For each, enough material to get ~20mg of dried sample including finely chopped fresh algal tissue was dried at 120 degrees Celsius for 90 minutes. The dried samples were pounded to a powder using a mortar and pestle and weighed out to ~20mg of each sample to begin chemical analysis.

Chemical Analysis

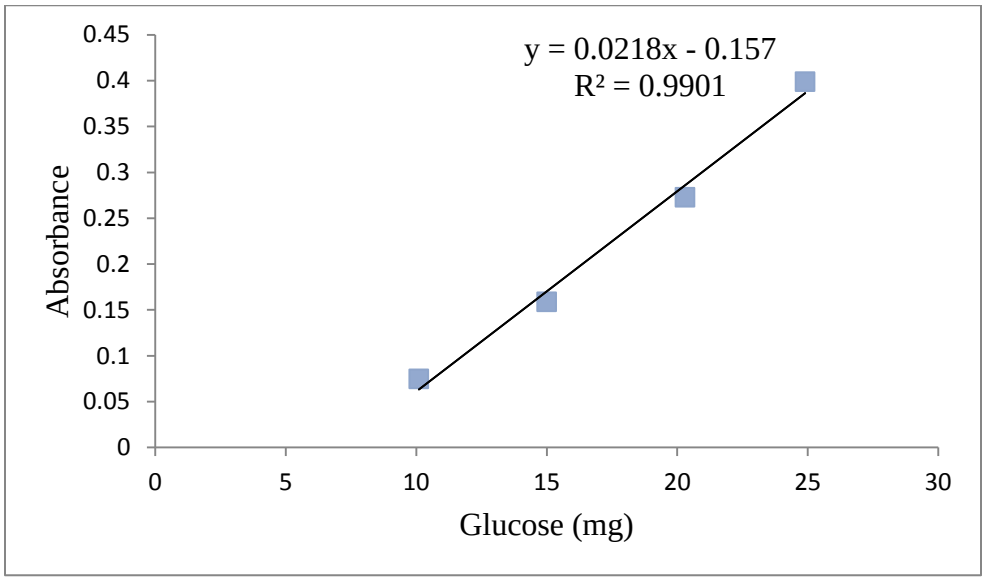

Figure 2. Glucose standard used for converting absorbance of samples to amount of glucose.

I used the general micro assay calorimetry technique that is outlined in Gosselin and Qian (1999) while applying the revisions made by Jessica Kimber (2012). To increase the accuracy of the samples, I used approximately 20mg of dry weight material, and as such changed the amount of the potassium dichromate oxidizing solution to 10ml added to the sample tubes. Samples were then incubated as directed. After the two incubation periods, 0.5ml of solution was taken from each sample tube and 4ml of the potassium iodide/starch solution was added and incubated for 20 minutes. Calorimetric measurements were made without adding the RO water as directed. Absorbance was measured at 575nm, using a DR 5000 spectrophotometer. Standards were prepared similarly to the samples. I weighed out approximately 5mg, 10mg, 15mg, 20mg, 25mg

and 30mg of reagent grade glucose, and tested for caloric content using the same modified chemical technique. This produced the standard line (Fig. 2) which I would compare my samples to, in order to determine the equivalent amount of glucose for each sample. The standard line had an $r^2 = 0.99$, further confirming the effectiveness of the modified technique.

Post Processing

To convert the sample 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).

$$\text{Units of Glucose} = (\text{Absorbance of Sample} + 0.157)/0.0218$$

I then divided the calculated amount of glucose for each sample by the sample's dry weight to give, 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.

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

Results

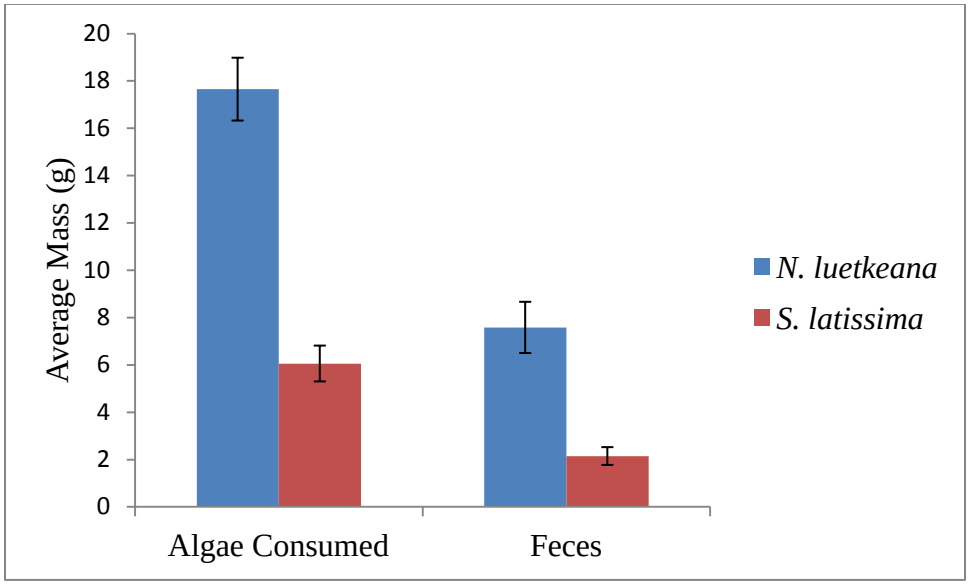

Figure 3. Average wet mass of algae that was consumed for two different algal species vs. average wet mass of feces produced. All treatments had 20 replicates (n=20). Standard error bars present.

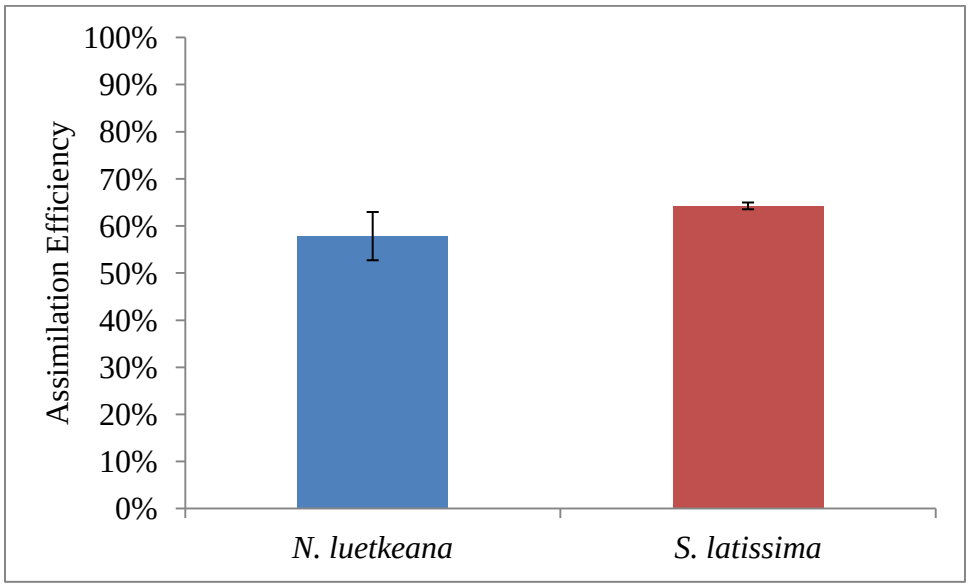

Figure 4. Assimilation efficiency of urchins fed two different algal species. All treatments had 20 replicates (n=20). Standard error bars present.

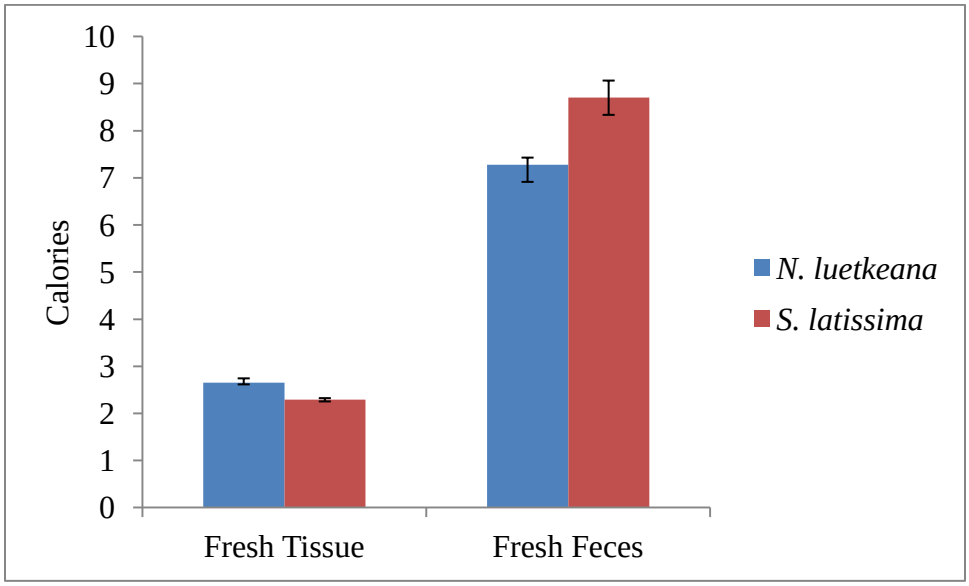

Figure 5. Mean caloric values from four treatments. Fresh tissue treatments had 5 replicates (n=5), and fresh feces treatments had 20 replicates (n=20). Standard error bars present.

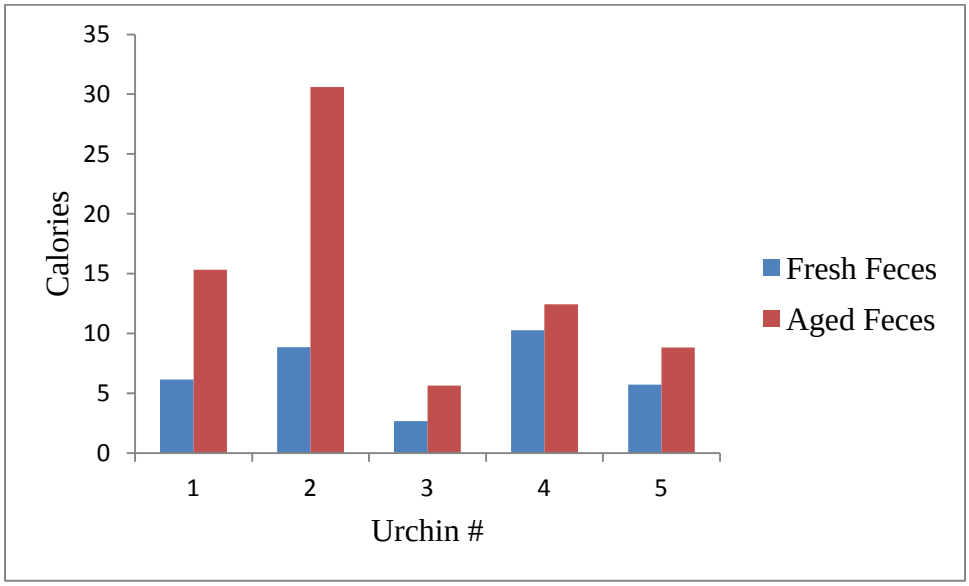

Figure 6. Caloric content of fresh feces vs. aged feces from 5 urchins in the *N. luetkeana* sample. Treatments had 5 replicates (n=5).

Urchins consumed significantly more *N. luetkeana* than *S. latissima* per day (Fig. 3; Welch's t-test, $p<0.0001$). The same result was found with weight of feces produced daily (Fig. 3; Welch's t-test, $p<0.0001$). The assimilation efficiency of algal material based on the ratio of mass of consumed versus egested daily was not significantly different between species of algae (Fig. 4; Welch's t-test, $p=0.2965$).

Comparison of the caloric value of fresh *N. luetkeana* and *S. latissima* tissues showed these to not be significantly different (1-way ANOVA, p= >.05); Fig. 5). There was also no significant difference between the caloric value of fresh feces from urchins fed *N. luetkeana* versus *S. latissima* (1-way ANOVA, $p=0.4269$; Fig. 5). However, feces from urchins had a higher caloric content than the fresh algal tissue; this result was significant for both *N. luetkeana* (Welch's t-test, $p=0.0108$) and *S. latissima* (Fig. 5; Welch's t-test, $p=0.0200$).

While there was high variation in the caloric content of urchin feces on a diet of *N. luetkeana*, the feces from all five urchins showed a significant increase in caloric content after being aged for four days; this consistency of direction of change is significant (probability of it happening by chance is 0.031; Fig. 6).

Discussion

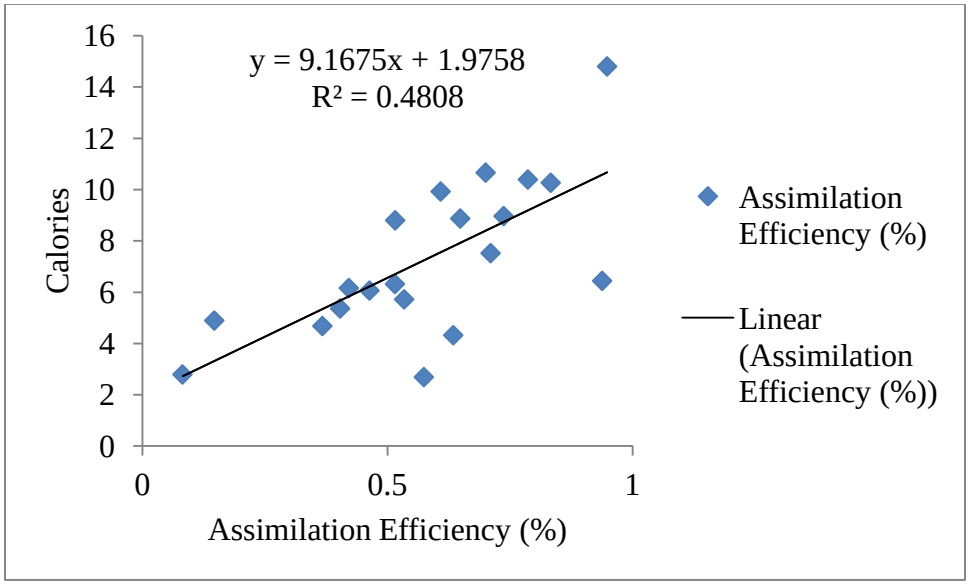

Figure 7. Caloric value of feces based on assimilation efficiency by urchins fed *N. luetkeana.* Treatment had 20 replicates (n=20).

My results show that there was no significant difference between caloric value of these two kelp species in either the form of urchin feces or fresh tissue. In both species, the caloric value increased after being consumed. The amount of time the ingested kelp stays in the gut before being egested appears to be the most important factor affecting the caloric value. The age of the material once it has been egested also affected caloric content (Fig. 6). This suggests that bacteria in the gut of the sea urchin are colonizing the plant material and creating an additional source of calories (Mann, 1988; Sauchyn and Sheibling 2009**)**. This colonization by microbes also continues after the feces have been egested as we can see by the aged-feces *N. luetkeana* treatment, which shows significant

increases in calories compared to the fresh feces treatment. While the two kelp species showed no significant difference between caloric value, Paine and Vadas (1969) show that there is variation in algal caloric value among other species. This suggests that algae with low caloric value would actually benefit detritus feeders more if passed through an urchin gut where it could be colonized by microbes and increase its caloric value. There was no significant difference in assimilation efficiency of algal biomass between the two kelp species. I found on average an assimilation efficiency of 60% for both algal species, Vadas (1977) found upwards of 90% assimilation efficiency for urchins eating *N. luetkeana*. When looking at caloric value of feces based on assimilation efficiency by urchins for *N. luetkeana,* there was a higher caloric value in the feces produced when algal material was being assimilated better and less feces was produced per day (Fig. 7). On the other hand urchins that egested almost as much as they took in had lower caloric content in their feces. This seems to demonstrate again that the longer the algal material stays in the gut of the sea urchin, the more calories it gains from urchin gut flora. This change was not statistically significant for *S. latissima.*

In all, these results have important implications for the caloric value of detritus as it is transported to benthic habitats. Lowe et al. (2014) saw an increase in abundance of grazers and detritivores found under sessile communities of red sea urchins in the San Juan Archipelago, particularly at increasing depth. This suggests that urchins play an important role in connecting the drift algal subsidy to the benthic zone as herbivores that can transform a large amount of drift algae into fecal material. Fawcett (2014) found that when given the choice between fresh *N. luetkeana* and *S. latissima* tissue, or the same tissue in the form of urchin feces, *Tigriopus californicus* showed a significant preference

for the fecal material. Also, if detritus gains calories as it ages, then falling to deep subtidal environments may substantially increase its caloric value. If algal species begin this increase in caloric value while still in the gut of urchins, urchin fecal matter may prove to be a vital source of nutrients in benthic communities that depend on the input of detritus for their success.

Acknowledgments

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