Feeding Preferences of Red Urchins, *Strongylocentrotus franciscanus,* to Drift Kelps

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Abstract:

Kelps and major contributors to primary production in temperate marine ecosystems. Kelps also make up a high amount of biomass and provide structure and habitat to many organisms. However, little kelp biomass is directly consumed. However, its biomass is transported via a spatial subsidy. This spatial subsidy links near-shore primary production with comparatively deep water primary consumers. One such consumer is the red sea urchin, *Strongylocentrotus franciscanus*. Urchins are known to feed on kelps transported by the spatial subsidy. These drift kelps may spend weeks drifting and degrading before they are encountered by an urchin. There has been little research on how the degradation or aging of kelps has on palatability to urchins. This study tests the feeding preference of red urchins to three species of kelp (*Nereocystis luetkeana*, *Agarum fimbriatum*, *Saccharina subsimplex*) that were both fresh and aged with the hope of gaining insight to how kelps move through the spatial subsidy. We also investigated how phlorotannin content changes as kelp age. Some research suggests that phlorotannins act as herbivory deterrent and that as kelp age they lose their phlorotannins. We found that *Nereocystis luetkeana* was the most preferred kelp species and that fresh kelps were preferred over aged ones. Analysis of phlorotannin content showed that there is a difference between species in phlorotannin content but the data suggests that there is little to no change through time. Further analysis indicated that urchins can detect the differences between kelps. This may mean that urchins act as a biological filter to detrital kelps as they are moved through the spatial subsidy and integrated into the food web.

Introduction:

Brown macroalgae of the order Laminariales (kelps) are vital to temperate coastal ecosystems. In some areas kelps can be a very abundant in the sublittoral zone. There, they are a food source for many organisms and create important three dimensional structure (Sano et al. 2001). While there is little direct grazing on kelps, their primary production may constitute an important spatial subsidy to other habitats (Mann 1988, Britton-Simmons et al. 2009). Spatial subsidies are nutrient and energy fluxes that link near-shore primary production to deep water habitats. The first step in integrating this subsidy is consumption of kelp biomass by primary consumers such as sea urchins. Urchins are known for their low assimilation efficiency (McBride et. al. 1998) which allows for their feeding to act as a distributer of kelp biomass. Urchins feed on drift algae, (Harrold and Reed 1985, Britton-Simmons et. al. 2009) breaking them down into smaller pieces which then can be consumed by smaller organisms. Urchin fecal matter is also added to benthic detritus, becoming a food source for even more organisms. An important but little-studied process of the spatial-subsidy food webs is the effect of gradual aging of kelp detritus as it is transported from near-shore habitat into deeper waters has on palatability to consumers such as urchins.

Vadas (1977) demonstrated that urchins (*Strongylocentrotus* spp.) show clear preference for certain species of kelp. However, little research has been done to address the effect of kelp aging on herbivore preference. Several factors may cause aged kelp to be treated differently that fresh kelp via herbivores. First, certain species of kelp, such as *Agarum fimbriatum*, have high levels of polyphenolic secondary metabolites, such as phlorotannins, which act as an herbivore deterrent (Duggins and Eckman 1997, Amsler et al. 1999). In at least one case, as kelps decompose there is a rapid loss of their chemical compounds; Duggins and Eckman (1997) found that particulate *A. fimbriatum* polyphenolic concentrations declined 60% in 24 hours in black-out conditions. This rapid reduction in polyphenolics suggests that decomposed kelps may be more palatable to herbivores. Second, since polyphenolic compounds may have antibacterial properties (Steinberg 1992, Zimmer and Butman 2000) decomposing kelps may be more prone to microbial colonization. Such microbes extract nitrogen from the surrounding water to synthesize proteins (Mann 1988), therefore increasing the nitrogen levels present "in" decomposed kelp. These higher nitrogen levels could mean that aged, kelp may be a better food source than fresh kelp, since marine herbivores are often limited by nitrogen (Mann 1988).

By definition drift kelp is not fresh, and can be many weeks old before it is encountered by a consumer. Britton-Simmons and colleagues (2009) observed that near-shore urchins capture many species of drift kelps, but it is unknown if these urchins select particular species or levels of decomposition. Urchins have also been observed feeding on kelp blades in water up to130m (David Duggins *pers. comm.*). Thus urchins probably encounter drift kelp of varying freshness. By understanding the feeding preference of *Strongylocentrotus franciscanus* to aged vs. fresh kelp across kelp species we will gain insight into how the kelp subsidy moves through the food web, and how aging affects palatability. A pilot study (Stephens 2008 unpublished data) suggested that aged kelps are preferred to fresh when compared within a species of kelp. To further investigate urchins' preference towards fresh and aged algae I conducted a series of pair-wise experiments using three kelp species (*Nereocystis luetkeana*, *Agarum fimbriatum,* and *Saccharina subsimplex*) each with fresh and aged samples. Based off of Stephens's (2008 unpublished data) project I predict that *Nereocystis* will be the most preferred species and that aged kelp samples will be preferred over fresh ones. I also investigated changes in polyphenolic concentrations via phlorotannin analysis as the kelps aged. Considering Duggins and Eckman (1997) polyphenol data I expect to see a decrease in phlorotannin content as kelps age. However I analyzed pholorotannin content on intact blades rather that particulate kelps. This data will provide a quantitative measure of the decrease in concentration through time and the concentration in aged samples that were presented to urchins.

Methods:

Kelp Collection and Preparation

The three kelp species, *Nereocystis luetkeana*, *Agarum fimbriatum,* and *Saccharina subsimplex* were collected from Point Caution, San Juan Island (48°33'43"N 123°00'56"W). *N. luetkeana* was collected from the surface. *A. fimbriatum,* and *S. subsimplex* were collected using SCUBA at a depth of 4-8m. Once collected the kelps were brought back to Friday Harbor Laboratories where they were kept in flow-through tanks until they were processed.

Samples were cut from the kelp blades for use in the preference experiment. All samples measured 10cm by 20cm and were taken from the same general location for each species.

No samples were taken closer than 20 cm to the end of the stipe, to avoid meristematic tissue. Also, great care was taken to avoid sori and midribs.

Kelp Aging

Three large flow-through tanks (one per species) were wrapped in black plastic and covered to block out all light. This environment was created to simulate deep-water conditions where large amounts of detrital kelp comes to rest. The kelps were left to age for 19 days.

Phlorotannin Analysis

In a separate blacked-out tank three samples of each species were kept for phlorotannin content analysis. Approximately every third day a ~2g (damp weight) sample was taken. These samples were placed in a -80°C freezer for a minimum of 24 hours. Phlorotannin content was measured though phlorotannin extractions following a modified Folin-Ciocaleau method (Van Alstyne 1995).

Urchin Collections

All urchins (*S. franciscanus*) were collected using SCUBA from Andrews Bay, San Juan Island (48°32’55”N 123°09’48”W) from a depth of 4-8m.

Feeding Preference Trails

Kelp preference was tested by offering paired pieces of kelp to individual urchins and observing what kelp was bitten first. For each trial a single urchin in a flow-though sea water table was offered two pieces of kelp simultaneously on opposite sides of its test. Before kelps were offered to the urchins they were given 5 min to acclimate to the sea water table environment. Urchins were given 15 minutes to make a choice once the kelp samples were offered. If there was no clear choice after that time then the urchin was said to have chosen neither kelp. This was done across the three kelp species and with fresh and aged samples, creating 12 treatments (Table 1). I did not conduct any treatment where the two kelp samples were the same species, as these were already tested in a pilot study (Stephens 2008 unpublished data).

Table 1

	AGFI-F	**NELU-F**	**SASU-F**	**AGFI-A**	**NELU-A**	**SASU-A**
AGFI-F						
NELU-F	**A**					
SASU-F	**B**	**E**				
AGFI-A	**S-2008**	**F**	**H**			
NELU-A	**C**	**S-2008**	**I**	**J**		
SASU-A	**D**	**G**	**S-2008**	**K**	**L**	

Pair-wise treatment combinations for fresh (xxxx-F) and aged (xxxx-A) for *Agarum fimbriatum* (AGFI), *Nereocystis luetkeana* (NELU) and *Saccharina subsimplex* (SASU). Combinations of "S-2008" were done in a pilot study by Stephens's pilot study in 2008.

Results:

Feeding Preference

Figure 1 shows the percentage of time that an algae was chosen if it was presented to a urchin. Table 2 summarizes the percentage a kelp was chosen if it was offered. Fresh *N. luetkeana* was chosen the most often of all other kelps. Chi-squared analysis showed that urchin choice was dependent on what kelps were offered (X^2=34.57, df=5, p-value=0.000002). To further investigate urchin preference I ran chi-squared analyses of fresh kelp vs. fresh kelp treatments (treatments A, B and E). The analyses showed that in treatments A and B urchin choice was non random (X^2=20.3, df=2, p-value=0.000039, X^2=10.27, df=2, p-value=0.005) (Table 3). The non randomness can be attributed to choices for *N. luetkeana* in treatment A (observed choices=15) and to choices for *S. subsimplex* in treatment B (observed choices=7).

Figure 1

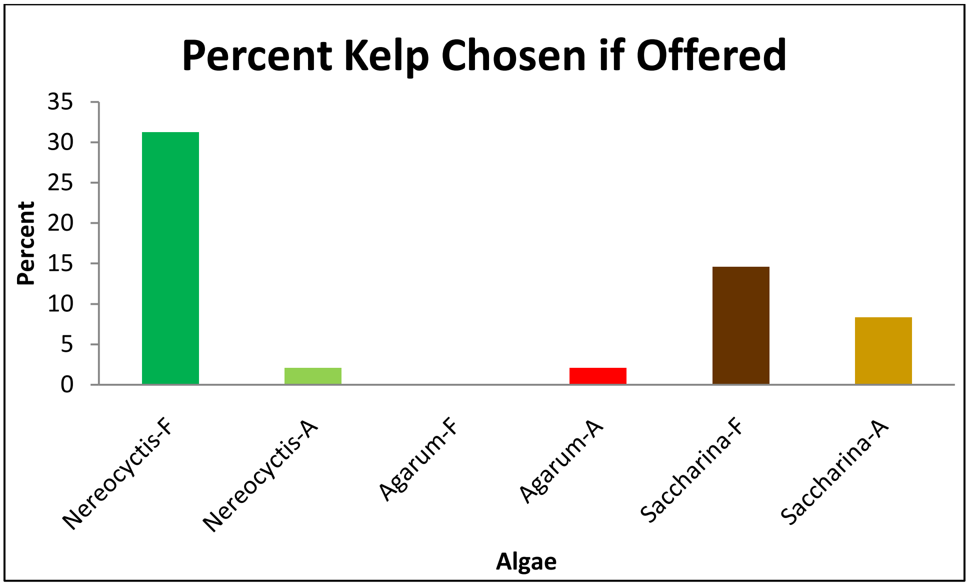


Table 2

Kelp	Percent chosen if offered	Percent other kelp chosen if offered	Percent neither chosen if offered
NELU-F	31.25	6.25	62.50
NELU-A	2.08	2.08	95.83
AGFI-F	0	22.92	77.08
AGFI-A	2.08	10.42	87.50
SASU-F	14.58	8.33	77.08
SASU-A	8.33	8.33	83.33

Table 3

Treatment	X^2 within	df	p-value
A	20.3	2	0.000039
B	10.27	2	0.005
E	3.78	2	0.15

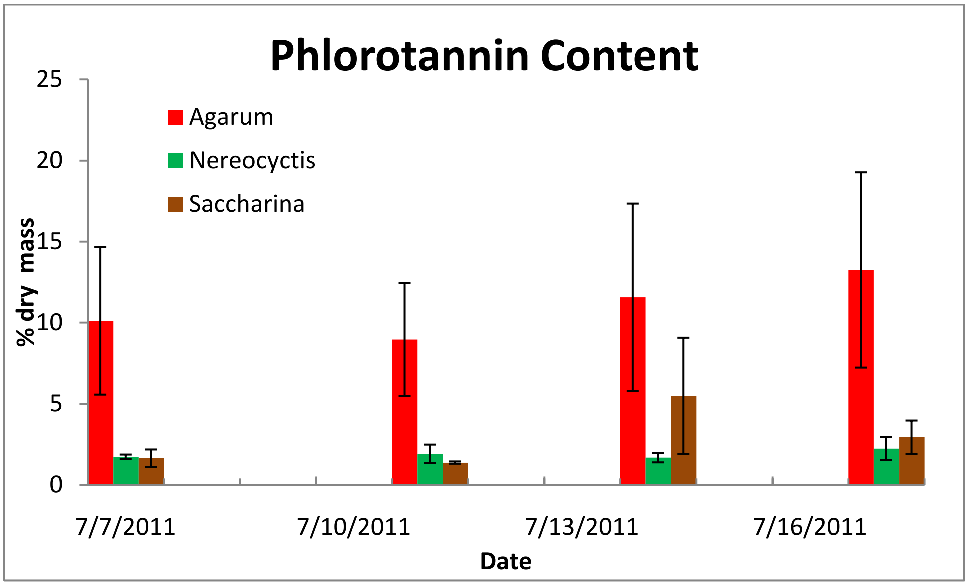
Phlorotannin Analysis

Phlorotannin concentrations differed significantly between *A. fimbriatum*, *N.luetkeana* and *S. subsimplex* for the first 4 samples (Table 4). From the graph of phlorotannin content (percent dry mass) through time (Figure 2) it is clear that *A. fimbriatum* is greater than both *N. luetkeana* and *S. subsimplex*.

Table 4

Sample Date	df	F	ANOVA p-value
7/7/2011	2	10.16	0.012
7/11/2011	2	12.96	0.007
7/14/2011	2	5.14	0.056
7/17/2011	2	9.05	0.015

Figure 2



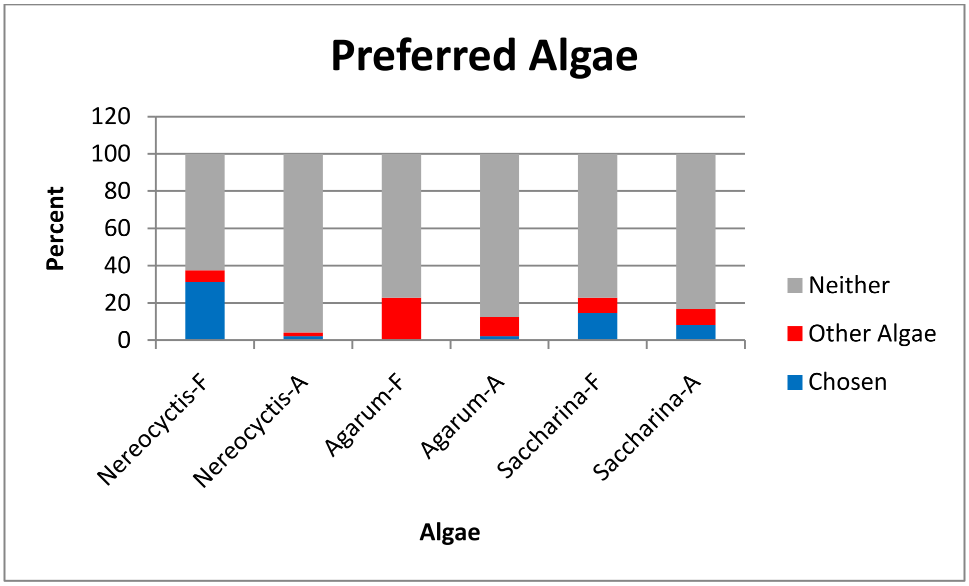
Discussion:

Feeding preference

Analysis of Figure 1 clearly shows that *N. luetkeana* was chosen the most often out of all kelps. This finding is consistent with Stephens's (2008 unpublished data) and Vadas's (1968, 1977) data that *N. luetkeana* is the preferred food of *S. franciscanus*. Figure 1 also shows that fresh kelps were chosen more often than aged kelps. This is in direct conflict with Stephens's (2008 unpublished data) findings where aged kelps were chosen significantly more than fresh kelps. When considering all the chi-squared analyses of urchin choice given a certain kelp was offered (Table 2, 3) it appears that urchins can detect differences between kelps. This has interesting implications for how certain species of kelp are integrated into the food web. It is plausible that urchins may act as a biological filter for how quickly kelps are processed by consumers. For example, *N. luetkeana* may we consumed by urchins before *A. fimbriatum* therefore delaying the entry of *A. fimbriatum* biomass into the food web.

Across all my treatments urchins chose neither kelp the majority of the time (Figure 3). This may be due to a variety of factors. First, 15 minutes may not have been enough time foe urchins to make a choice. Stephens (2008 unpublished data) protocol called for more time for urchins to make a choice. Second, I conducted my experiments in a lighted room. Vadas (1968) observed that algae consumption by urchins was the highest and most consistent in shaded or dark environments. Finally, urchins were moved in and out of tanks fairly frequently over the course of my trials. Arguably this introduced significant stress to the urchins which may have affected choice or desire to eat. While I allowed some time for the urchins to acclimate before I offered them kelps this may been insufficient.

Figure 3



Phlorotannin Analysis

The ANOVA analyses (Table 4), show a clear difference in phlorotannin content between species. Analysis on Figure 2 clearly indicates that *A. fimbriatum* was had higher phlorotannin content than both *N. luetkeana* and *S. subsimplex*. It is also interesting that this trend was consistent through time. While the high variability of the data makes statistical analysis of the change in phlorotannin content through time, Figure 2 suggests that there is little to no change. This finding is inconsistent with Duggins and Eckman's (1997) findings with *Agarum* and *Laminaria groenlandica* (now *Saccharina subsimplex*). This could be due to differential changes in kelp chemistry as they age in particulate matter (Duggins and Eckman) and in intact blades. These differences in how phlorotannin content changes though time has interesting implications for kelp consumers. Assuming that phlorotannins do play a role in herbivore palatability then particulate kelp matter may be more palatable than intact blades. This suggests that high phlorotannin kelps have to be physically broken down before their biomass can be assimilated into the food web.

The high variability in the phlorotannin data could be caused by multiple factors. First, there is natural interspecific variation in phlorotannin content (Duggins and Eckman 1997). Low sample size is probably amplifying these differences as well. Second, after the first four data points I changed my sampling methods which may explain the increased variability in the last three data points (not presented in Figure 2). Finally, I had unexpected issued in my extractions. In a number of samples and standard concentration runs some solutions formed precipitates and/or had comparatively cloudy solutions. It is impossible to say how much of an effect, if any, any of these issues affected my data. It is also plausible that a combination of these inconsistencies contributed to the variation in my data.

Overall Conclusions

Urchins preference to fresh kelps over aged ones calls into questions what weather microbial communities play a role in palatability of aged kelps. Mann (1988) demonstrated that associated microbial communities increase as kelp degrade and therefore increase the nitrogen in/around the kelps. I did not see much of a change in phlorotannin content as the kelps aged. Since phlorotannins are thought to have antibacterial properties (Steinberg 1992, Zimmer and Butman 2000) microbial communities may have been deterred from forming, therefore not increasing the palatability of aged kelps. While I did not directly measure nitrogen my findings suggest that there was little nitrogen associated with my aged samples, possibly due to an absence of microbial communities. Alternatively, urchins choice may be driven more by the physical degradation of a blade rather than the microbial biofilm. Further research is needed to conclusively describe the feeding preference of urchins to drift kelps and what effect of aging has on phlorotannin content.

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