

Assimilation efficiency of *Idotea wosnesenskii* on different algal diets

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Abstract:

Drift algae are an important resource in marine food webs and undergo a vital change in chemical composition during the degrading (or aging) process. I investigated the effects of aging algae and different species of algae on the Assimilation Efficiency (AE) of *Idotea wosnesenskii*, a grazing isopod found on San Juan Island. One week feeding (3 days) and starving (4 days) treatments were run with daily fecal matter collection. Weights of algae fed to isopods over 3 days and fecal matter subsequently produced were used to determine AE. The data show that in an alga with high levels of secondary metabolites, AE increased with algal age. This was not the case with another alga with few defensive chemicals, suggesting that aging causes a change in chemical composition, which increases AEs. Food preference was correlated with AE, as the preferred fresh algal species also had a higher AE. This study provides further support for why drift algae is important in food webs.

Introduction:

Photosynthetic primary producers provide most of the energy source for marine food webs (Polis et al. 1997). However, many of these ecosystems lack actively photosynthesizing plants, and the consumers must instead rely on detritus. Algae that break loose from where they grew and drift in the water (“drift algae”) can support detrital food webs and thereby increase production in otherwise low-food areas (Polis et al. 1997). Drift algae degrade with time, releasing nutrients that can be utilized by other plants (sea grass, epiphytes, etc.), and are a direct food source for animals (Hyndes et al. 2012). For example, at one site, a macroalgal species was able to coexist with purple sea

urchins because the urchins instead ate predominantly algal fragments from distant reef ecosystems (Vanderklift et al. 2008). Additionally, it has been estimated that only ten percent of kelp biomass is directly eaten, and the rest is transported as drift (or particulate organic matter) to various other habitats (Mann 1988). Therefore, drift algae may be a very important food source for a variety of consumers in a range of habitats (Vanderklift et al. 2008).

Animal food preference can be influenced by chemical composition of algae, specifically the amount of secondary metabolites present. In brown algae, chemical compounds called polyphenolics (phlorotannins) have been found to deter herbivores (Targett and Arnold 2001). In laboratory settings, the sea urchins *Strongylocentrotus drobachiensis* and *S. franciscanus* show strong preference for *Nereocystis* (low phenolic content) over *Agarum* species (high phenolic content) (Vadas 1977). Other organisms, like the isopod *Idotea wosnesenskii*, also prefer *Nereocystis* over *Agarum* (Burgess et al. 2012). Preference was positively correlated with assimilation efficiency (AE) in experiments on several sea urchin species, which chose food that gave them a higher AE (Vadas 1977). AE data can therefore help predict preference and may provide more understanding of the effects of phlorotannins on food choice. However, it is difficult to quantify and identify these effects because each animal's gut environment has unique interactions with phlorotannins. Acidic stomach conditions can oxidize polyphenolics and induce binding to carbohydrates or proteins, preventing absorption or inactivating herbivore digestive enzymes (Targett and Arnold 2001). Thus, animals with low pH gut environments may process phlorotannins less effectively. Nonetheless, there are other examples of invertebrates with acidic guts, like the crab *Mithrax sculptus*, that process

phlorotannin-rich food effectively (Targett and Arnold 2001) as well as instances where chemical composition of algae does not affect isopod food choice, and instead availability and habitat adaptation are the major influences (Jormalainen et al. 2001, Arrontes 1990).

The process of aging, or decomposition, may be vital to food webs. In *Laminaria hyperborea* kelp forests, primary production can be an important food source for consumers; however, bacterial decomposition of kelp is critical for making algae digestible for certain consumers (Norderhaug et al. 2003). Comparing consumption of both aged and fresh algal samples, researchers found decreased content of phlorotannins, a secondary metabolite, due to bacterial colonization in the aged samples. For species like the gastropod *Rissoa parva*, survival wasn't dependent on the aging of the kelp, but amphipods (*Jassa falcata*, *Lembos websteri*, *Ampithoe rubricata*, and *Gammarus locusta*) were unable to survive on diets of fresh *Laminaria hyperborean*. However, evidence of phlorotannin degradation by bacteria is inconclusive. Phlorotannin content did not decrease in the aging of the kelp *Agarum fimbriatum*, suggesting that the species may contribute less to detrital food webs than previously thought (Sosik and Simenstad in review). Large changes in fatty acid content were also observed in the "less defended" *Saccharina subsimplex* as well as increasing bacterial marker fatty acids (Galloway et al. in press). Hence algae with high phlorotannin content may be less susceptible to aging effects and a less viable food source for many consumers.

My experiment investigated the nutrient value of "fresh" and "aged" algae and its palatability for organisms living in detritus-supported environments. I was interested in seeing whether aging and chemical composition of algae influence AE (and ultimately

preference). I focused on the nutrient extraction of aged and fresh algae by *Idotea wosnesenskii*. Because aging reduces the presence of phlorotannins and these secondary metabolites deter digestion, I expected isopods fed aged *Nereocystis* to have a higher assimilation efficiency. Also, because animals adapt to their environmental conditions and drift algae are often a vital food source, AE should be higher with aged *Nereocystis* than fresh *Nereocystis*. In past experiments, fresh *Nereocystis* has been consistently preferred over aged (Burgess et al. 2012), so I am interested to see whether the AE will reflect that. Isopods also preferred *Nereocystis* over *Agarum* (Burgess et al. 2012), probably due to the higher concentrations of phlorotannins in *Agarum* (Targett and Arnold 2001) so I expect the AE to be higher for all *Nereocystis*. Both aged *Agarum* and fresh *Agarum* should have similar AE values since aging has been found to have little effect on degradation of phlorotannins in that alga (Sosik and Simenstad in review) and there was no difference in preference between aged/fresh *Agarum* (Burgess et al. 2012).

Methods:

This study was conducted in April and May 2013 at Friday Harbor Laboratories on San Juan Island, Washington, with *I. wosnesenskii*, an abundant grazing isopod. Twenty-two male subjects were gathered at Eagle Cove. Ten isopods were used for the first trial and then switched out with the other ten for the next trial. By alternating groups in/out into larger containers with water flow, the effects of stress from unnatural habitat conditions were reduced. Some preliminary trials were run to determine a suitable environment and feeding schedule. There were ten replicates per each trial with four treatments: Fresh *Nereocystis*, Aged *Nereocystis*, Fresh *Agarum*, and Aged *Agarum*.

Algae were obtained nearby Friday Harbor Labs. Aged algae was created by keeping blades for one week in dark, flow through tanks simulating subtidal conditions. This time farm was chosen because effects of aging were quicker than in previous studies (algae turned to “mush” faster) possibly due to unusually warm weather (Lowe and Eisenlord, pers.comm.).

Isopods were placed individually in clear plastic beakers with airstones and kept in a temperature controlled room at ~14°C. Aluminum foil lids kept the animals from climbing out. Removable mesh screens were inserted in each beaker to allow the isopod feces to fall to the bottom of the beaker and avoid being further broken up by the animal and to separate algal pieces from feces.

For each treatment, isopods were each given a ~3g piece of algae and fed for 3 days, after which I removed the algal remains, measured blotted algal wet weights, and froze them. Fecal matter was collected daily and frozen for a total 7 days after food was introduced. The dry weight of the leftover algae was obtained by placing sample vials (without caps) in the freeze drier overnight. Thus, wet and dry weights of algal pieces and feces could be compared. I dried incremental increasing weights of both wet *Nereocystis* and wet *Agarum* in an oven to establish a relationship between wet and dry algal weights. This was used to determine the starting dry weights of the algal samples. To control for changes in algal weight over the course of the treatment period without isopod consumption, ~3 g pieces of algae were kept in the cold room in plastic containers under similar conditions.

Fecal matter was filtered using pre-weighed 0.22 μm filters. The samples were then weighed (wet), freeze dried, and weighed again (dry). In all of the trials 8 replicates were used (4 of the original 22 animals died). In total, the experiment lasted four weeks.

To determine assimilation efficiency I used the equation $[(I-F)/I * 100]$ where I is the weight of ingested food, and F is the weight of fecal matter produced (Catalan et al. 2008, Romero et al. 2006). 6 out of 32 replicates were discarded because they were unrealistic: extremely high AE ($>110\%$), extremely negative AE ($<-50\%$), the animals died midway through the trial, or no fecal matter was produced.

Results:

The relationship between algal wet weights and their corresponding dry weights were determined with linear regressions. Fresh algal samples were dried overnight in an oven and then measured three times in 2-hour time intervals to determine a final dry weight. The equation for *Agarum* was $y = 0.1849x - 0.0053$ (Figure 1, $R^2 = 0.988$) where $y = \text{dry weight}$ and $x = \text{wet weight}$; *Nereocystis* was $y = 0.09x + 0.0088$ (Figure 2, $R^2 = 0.993$). Using these equations, dry weights were estimated from the algal wet weights originally given to the isopods in the feeding trials.

Controls were run to determine weight change of algae under the experimental conditions (Figure 3). Each sample's weight change was calculated then the proportion of the change to the original weight was calculated. This number was averaged (5 replicates for each) and used as a correction factor for the weight change of the algal samples in each replicate of the treatments. The average proportion of the

five samples was either added (if positive) to 1 or subtracted (if negative) from 1, and then multiplied by the weight of the pre-consumed algal sample. Control algal samples showed small but variable weight changes over the 3 days (Figure 3). The largest change was in the aged *Nereocystis*, which lost substantial mass, while aged *Agarum* actually grew. There was a significant difference between Aged *Agarum* and Fresh *Agarum* (pvalue~0.008).

Figures 4 and 5 depict the calculated Assimilation Efficiency (AE) for both the Wet and Dry weights. Both graphs show the same trends; in order of increasing assimilation efficiencies the treatments were: Fresh *Agarum*, Aged *Agarum*, Fresh *Nereocystis*, and Aged *Nereocystis*. The wet and dry weight-calculated assimilation efficiencies showed the same general pattern. However, the wet weight data was much more variable probably due to inconsistent blotting/differential water retention by algal pieces. Only FN and AA treatments in dry vs. wet weights had marginal or significant statistical difference ($p = 0.083$ and 0.00035 respectively) showing that the dry and wet number sets are not reporting similar values. Thus, only the dry weight data were used for statistical analysis, as these were less variable (fewer replicates with unrealistic values).

Both a two-way and one-way ANOVA test were performed on the data. Isopods consuming aging *Agarum* had significantly higher AE, but aging *Nereocystis* does not change its value (Table 2). Comparing species shows AE is very different for the two fresh algae, but not different for the two aged algae (Table 2).

Figure 6 shows the different algal amounts (average dry mass) consumed by the isopods in each treatment. Aged *Agarum* had the highest amount consumed followed by fresh *Agarum*, aged *Nereocystis*, and fresh *Nereocystis*.

Discussion:

Different algal species can affect the assimilation efficiencies of *I. vosnesenskii*. Isopods were found to assimilate fresh *Nereocystis* more efficiently than fresh *Agarum* (Figure 5, Table 2, $p = 0.018$), suggesting that there is a favorable element in *Nereocystis*, or something in *Agarum* that makes digestion more difficult. A likely explanation is the high concentration of phlorotannins (secondary metabolites) present in *Agarum* (Vadas 1977). These can interfere with digestion and therefore may decrease assimilation efficiency. These AEs correlate with some studies that found isopods consistently prefer *Nereocystis* over *Agarum* (Burgess et al. 2012). Thus, assimilation efficiency could be a good indication of food preference (also shown in Vadas 1977).

The process of aging can affect the AE as well. Aged *Agarum* treatments were assimilated more efficiently than fresh *Agarum* treatments (Figure 5, Table 2, $p = 0.462$). Therefore, during this aging process, there must be a change occurring in the chemical composition of the algae that makes it a more valuable (higher AE) food source for isopods. Though the chemical change occurring in the algae cannot be quantified from my data, the degradation of algae has been found to decrease phlorotannin concentration (Norderhaug et al. 2003) and, in turn, may influence AE. Thus, drift (aged) algae create a vital and necessary source of nutrients for certain consumers.

One unexpected result was that aged *Agarum* and aged *Nereocystis* AEs were not statistically different (Figure 5, Table 2, $p = 0.314$). Previous studies have suggested that *Agarum* may not contribute readily to detrital food webs due to its lack of degradation (Sosik and Simenstad in review). However, my results indicate that aged *Nereocystis* and aged *Agarum* have equally high AE for isopods, so drift *Agarum* may be just as useful to food webs as drift *Nereocystis*. Therefore, the aging process may affect *Agarum* and *Nereocystis* equally.

Surprisingly, the *Nereocystis* treatment group of isopods ate less and therefore produced less excrement than the *Agarum* treatment replicates (Figure 6). This group may have been exposed to stressful conditions and was unable to recover. When less algal mass was consumed and less fecal material produced, weight measurement errors increased. Thus, there were some extreme outliers that were discarded in the final analysis because they were unrealistic (a negative or 100% AE). To avoid encountering this problem again, different individuals would be used for each treatment and with longer feeding trials. In addition, a preliminary feeding trial using dyed/colored food could determine a more adequate starving period (for gut clearance) for an isopod (Vadas 1977). Studies on *Idotea baltica* have successfully utilized a different method of AE calculation that compares the amount of soluble sugars (assimilated fraction) to structural polysaccharides (insoluble and relatively indigestible – nonassimilated tracer) of both fecal matter and algal samples (Arrontes 1997, Jormalainen et al. 2001). This sort of additional data would be useful to support the AE values.

Future experimental directions include more analysis to quantify why aging or different algal species affect AE. For instance, the degradation of phlorotannins in aged

algae has been found to influence animal survival rates, and therefore may also impact AE (Arrontes 1997, Norderhaug et al. 2003). However, AE could have been influenced by factors other than the chemical composition of the algae, which were not examined in this study. For example, one study found that *Idotea baltica* accustomed to eating algae with chemical defenses had higher AEs on diets of algae with high phlorotannin concentrations (Jormalainen et al. 2001). Therefore, researchers propose that these isopods adapted to whatever food source is abundant, so that AE are influenced more strongly by the amount of an algal species present in an environment than by the chemical composition of the algae (Jormalainen et al. 2001). Thus, my group of isopods consuming *Nereocystis* may have eaten less algae and had higher assimilation efficiency on that algal diet because they were accustomed to consuming chemically defended species.

It would be useful to perform caloric content analysis of aged/fresh samples of both *Nereocystis* and *Agarum*. Caloric content of the algae and the fecal matter could indicate why the food is assimilated more efficiently. For example, *Agarum* has been found to have a higher caloric content than the more preferred *Nereocystis*, suggesting that its high concentration of secondary metabolites deter herbivores from a valuable, high-caloric food source. *Nereocystis* may have other distinct features that are beneficial and increase AE (Vadas 1977). However, contradictory data indicate high caloric content in aged *Nereocystis* and lower content in fresh/aged *Agarum* (Kimber 2012). Thus, it would be constructive to discern between these contrasting ideas and to compare caloric content with AE. If caloric content is positively correlated with assimilation efficiency,

then this may help explain why isopods process aged *Nereocystis* more effectively (Figures 4 and 5) and why they might prefer it.

Overall, this pilot experiment suggests trends in assimilation efficiencies are likely due to differential phlorotannin concentrations in algal species. Substantial increases in AE of animals fed aged *Agarum* indicate that the phlorotannin-rich algae could play a large role in detrital food webs, but perhaps not as significant as *Nereocystis*. Continuation of the project by analyzing the caloric content could enhance understanding of its correlation (or lack there of) with assimilation efficiency or food preference.

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Figures:

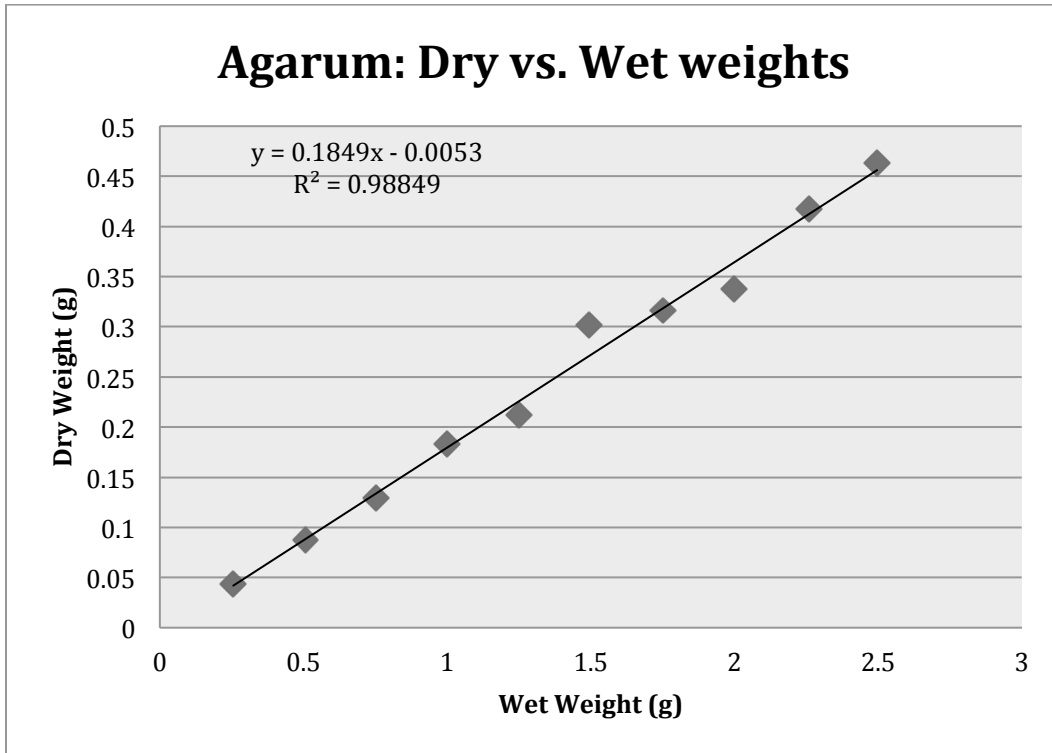


Figure 1: *Agarum* dry and wet weight relationship.

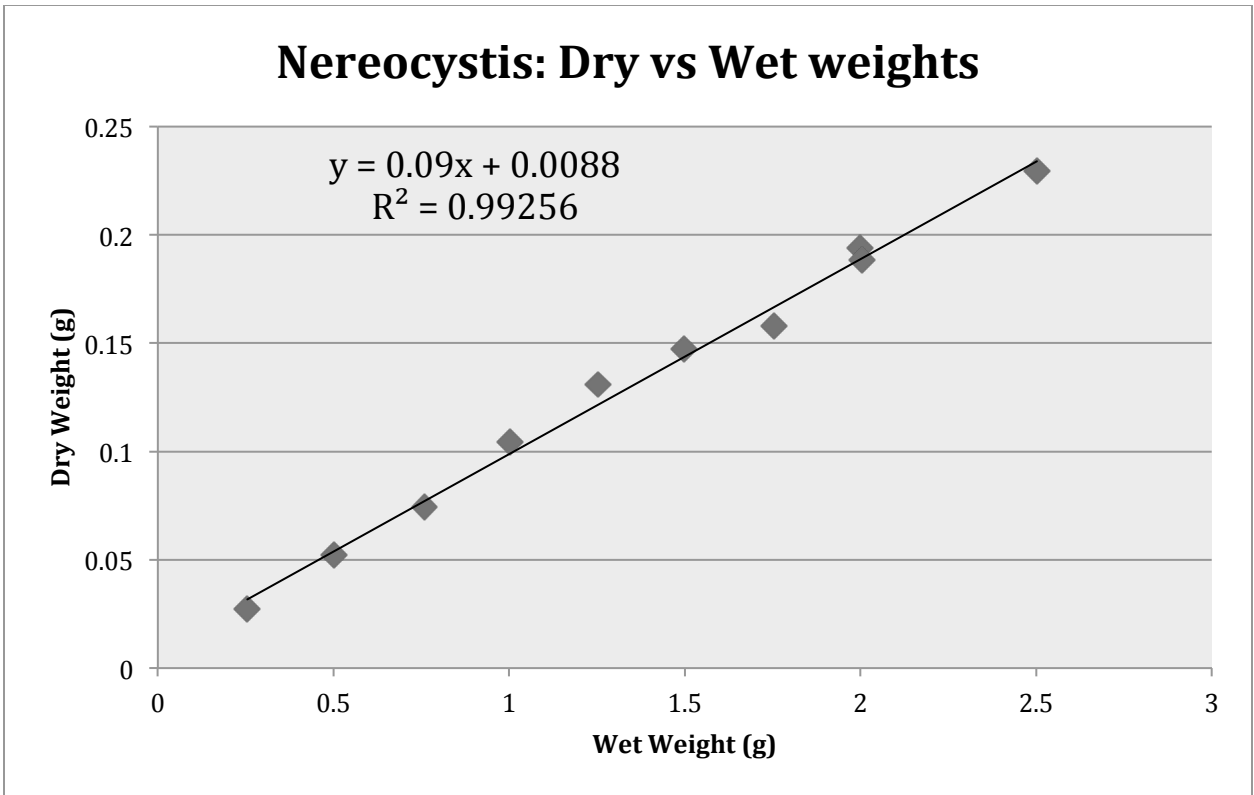


Figure 2: *Nereocystis* dry and wet weight relationship.

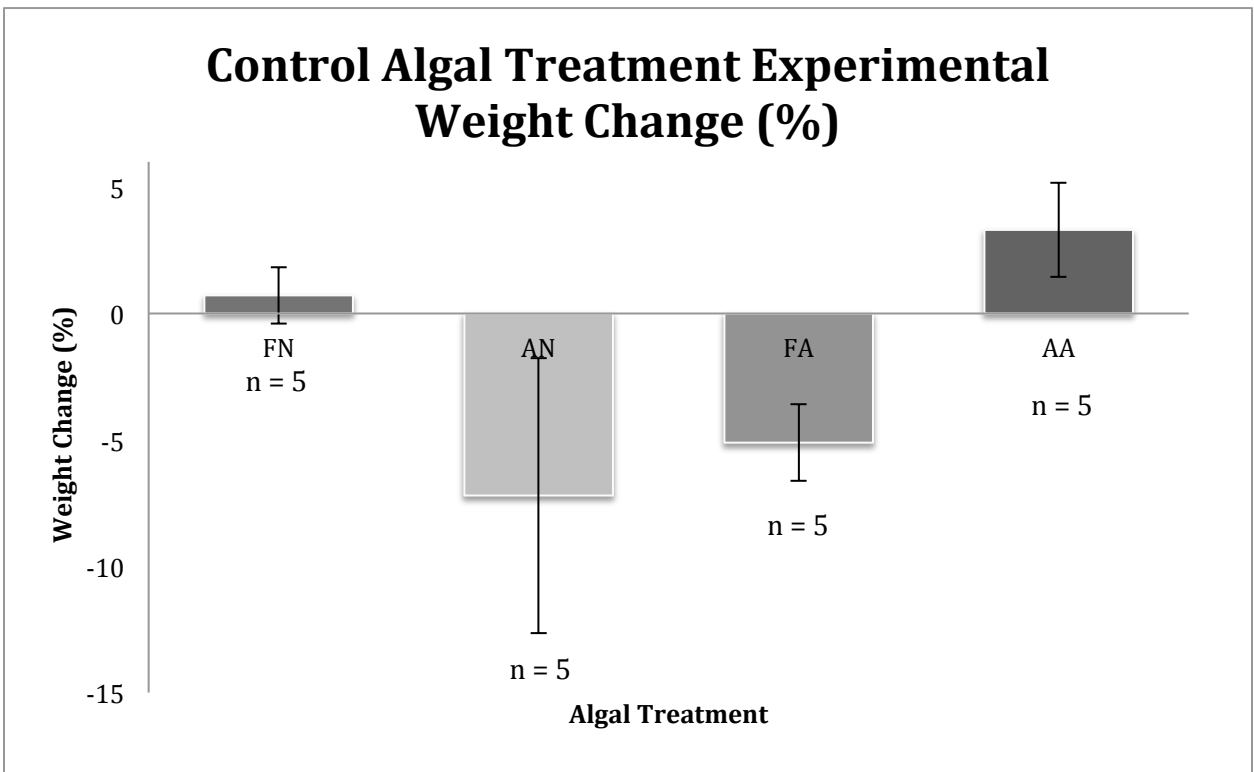


Figure 3: Average algal weight change in the control treatments (FN = fresh *Nereocystis*, AN = aged *Nereocystis*, FA = fresh *Agarum*, AA = aged *Agarum*). Standard error bars: 1.12, 5.45, 1.51, 1.86. Example calculation of fresh *Nereocystis* replicate 1: $(1+0.00717)*2.7179$, with 0.00717 being the weight gain proportion and 2.7179 being the pre-consumed algal wet weight.

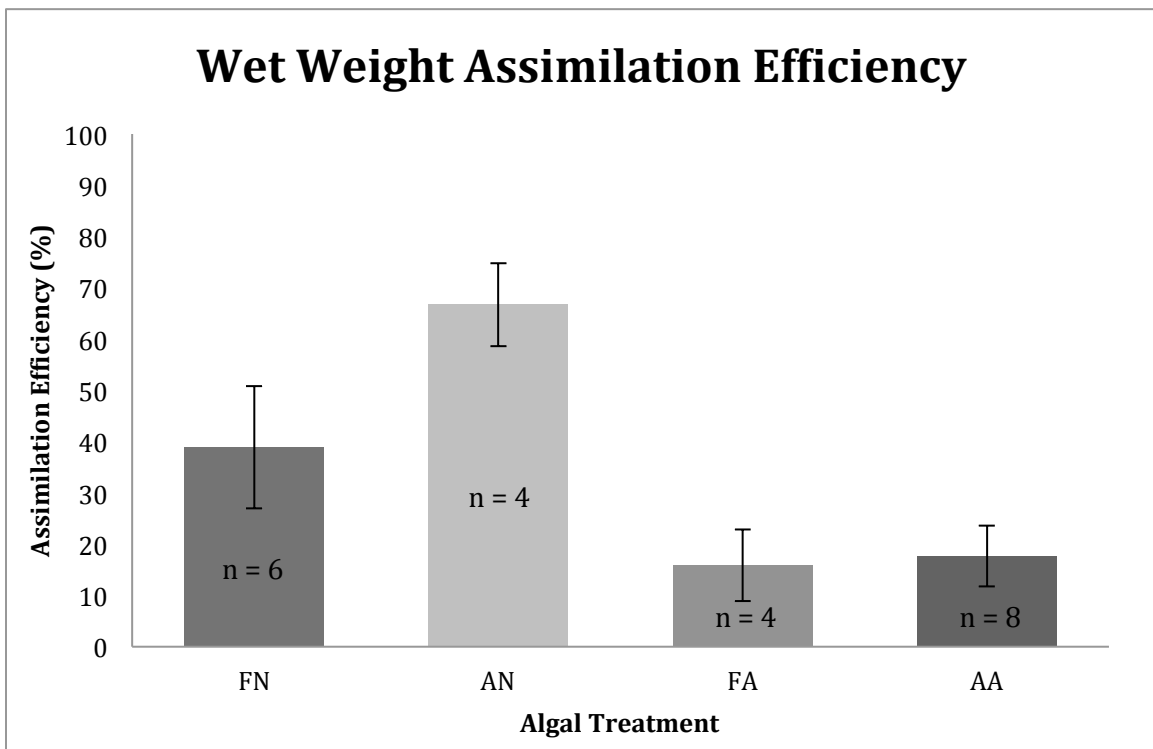


Figure 4: Standard errors listed from left to right: 11.95, 8.11, 6.95, and 5.92. (FN = fresh *Nereocystis*, AN = aged *Nereocystis*, FA = fresh *Agarum*, AA = aged *Agarum*). Outliers that have unrealistic values were discarded, which is why there are different n values.

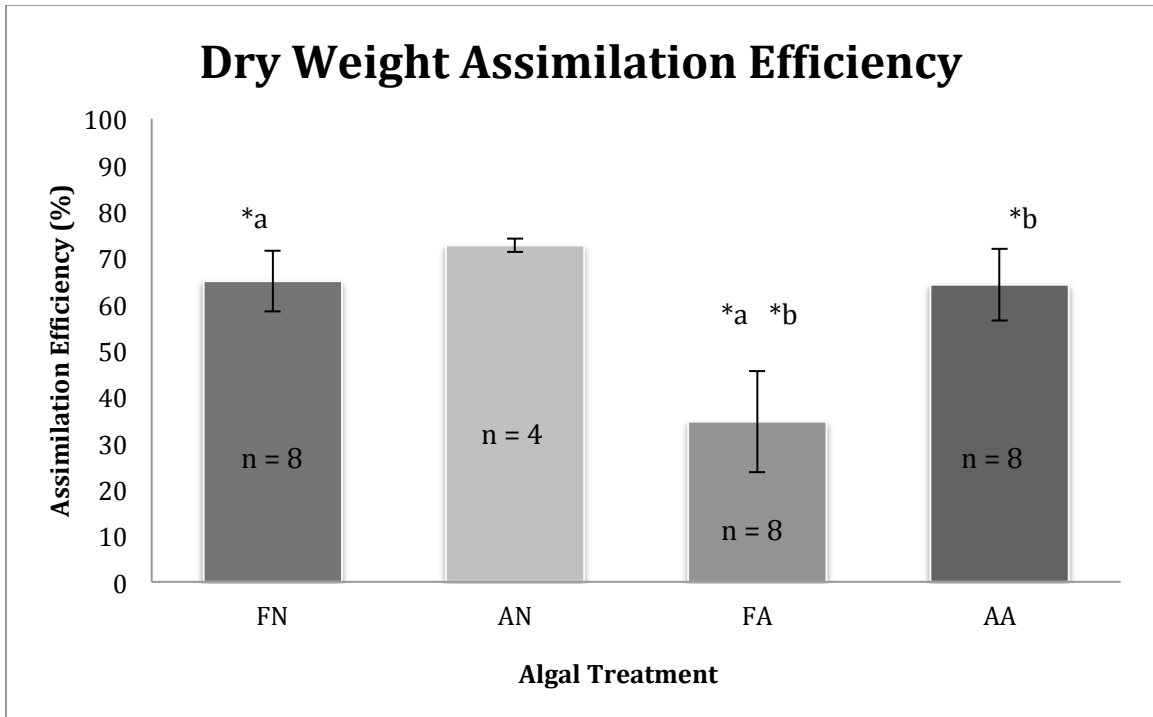


Figure 5: Standard deviations listed from left to right: 6.53, 1.42, 10.91, and 7.73; and *a, *b show data sets with statistically significant difference. (FN = fresh *Nereocystis*, AN = aged *Nereocystis*, FA = fresh *Agarum*, AA = aged *Agarum*).

Source	Prob > F
Species of Algae	0.0234
Fresh/Aged	0.0832
Species of Algae*Fresh/Aged	0.1523

Table 1: A two-way ANOVA test was performed on the Dry weight data (excluding the unrealistic values). There were statistically significant differences in AE between species of algae ($p = 0.0234$), marginal difference between Fresh/Aged treatments ($p = 0.0832$), and no interaction effect between the two variables ($p = 0.1523$).

Variable 1	Variable 2	Variable 2
	<i>Nereocystis</i>	<i>Agarum</i>
Fresh/Aged	0.6699	0.0462
	Fresh	Aged
<i>Nereocystis/Agarum</i>	0.018	0.314

Table 2: P values from a one-way ANOVA tests comparing AE between species of algae and fresh/aged samples. Fresh *Nereocystis* and Fresh *Agarum* ($p = 0.018$) had statistically significant differences as well as Aged and Fresh *Agarum* ($p = 0.0462$) were statistically different. Neither Aged *Agarum* and Aged *Nereocystis* ($p = 0.314$) were supported by statistical difference or Fresh and Aged *Nereocystis* ($p = 0.6699$).

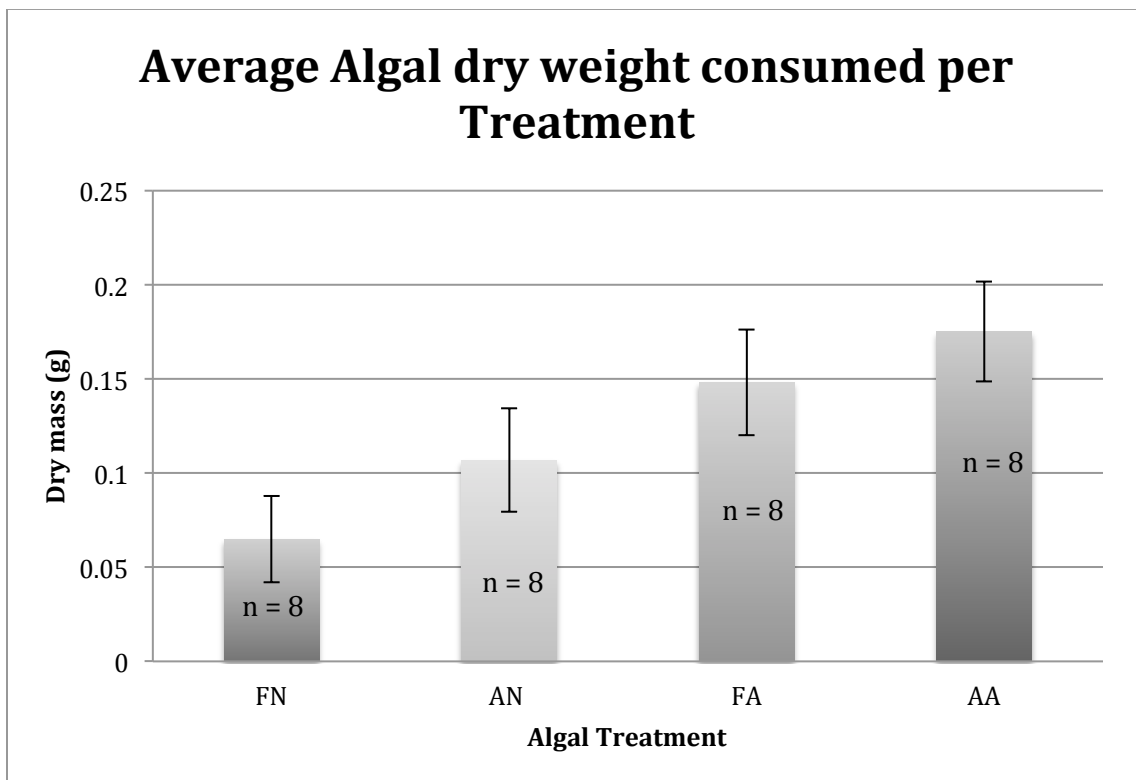


Figure 6: Average dry mass (g) of algae consumed for each treatment (FN = fresh *Nereocystis*, AN = aged *Nereocystis*, FA = fresh *Agarum*, AA = aged *Agarum*). AA has

the highest mass consumed while FN has the lowest amount consumed. Standard errors from left to right: 0.023, 0.027, 0.028, 0.027.