The effect of fresh and aged *Agarum fimbriatum* and *Nereocystis luetkeana* diets on the population growth of *Tigriopus californicus*

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Abstract

The study explores the effect of diets of fresh and aged *Agarum fimbriatum* and *Nereocystis luetkeana* on the population growth of *Tigriopus californicus*. Fresh diets were expected to increase abundance, and the two algal species were expected to produce different sized populations. Founding populations of 30 gravid females were fed weekly a piece of one of 4 diets (fresh / aged x *A. fimbriatum* / *N. luetkeana*) for 32 days. Fresh *N. luetkeana* resulted in the largest population growth (1845 +/-633.8) and fresh *A. fimbriatum* the smallest (521 +/-32.5). This trend was consistent for the nauplius, copepodite and adult life stages, as well as the gravid female populations. Hatching rates were studied and significant differences were found among the treatments. Gravid females fed fresh *N. luetkeana* diets hatched eggs the fastest (15.7 hrs +/-12.4) and fresh *A. fimbriatum* the slowest (35.1 +/-21.6). Polyphenolic anti-herbivory compounds in *A. fimbriatum* are suggested as the primary cause for the findings.

Introduction

Deep subtidal environments (DSE) support an abundant and diverse population of secondary producers including sea urchins and copepods (Vetter 1995; Britton-Simmons et al. 2009). In the San Juan Archipelago (SJA), the DSE starts below 23 m where little light reaches the seafloor (Britton-Simmons et al. 2009). Consumers can survive in the DSE despite the lack of vegetative growth because of subsidies of significant amounts of drift macrophytes that are transported there by hydrodynamic forces (Britton-Simmons et al. 2009). Ninety-seven percent of observations within a 60-km^2 section of sea floor in the SJA noted drift macrophytes, with the majority of biomass contributed by kelps (Britton-Simmons et al. 2012). This drift provides both habitat and food for the secondary producers.

Drift macrophytes are often aged by the time they settle in the DSE. Most species of algae show physical and microbial decay throughout the aging process (Mews et al. 2006). As aging occurs, algae are thought to increase in food quality for grazers due to bacterial colonization (Chenelot and Konar 2007). Aged kelp may also be more palatable due to the breakdown of polyphenolic secondary metabolites which usually deter grazing (Duggins and Eckman 1997). Two experiments were undertaken on DSE species to test these assumptions. Contrary to expectations, isopods grew faster when fed fresh versus aged *Nereocystis luetkeana* diets over a 10-week experiment (Burgess unpubl.), and red urchins showed an increase in gonad mass when fed fresh versus aged algae (Raymond unpubl.). More studies are required on DSE grazers to understand the nutritional value of drift macrophytes.

Harpacticoid copepods are an abundant and integral secondary producer of the DSE (Vetter 1995), where they provide a trophic linkage between drift macrophytes and commercially important species like Pacific salmon (Webb 1991) and shrimp (Jensen and Jensen 1985). *Tigriopus californicus* is a harpacticoid copepod commonly found in splash pools in the SJA. Powlik (1996) found the total generation time to be 21 days under higher temperature “summer” conditions. Morris et al. (1980) state that *T. californicus* is primarily a browser of algae and detritus, which in addition to the ease of keeping this species in culture and the short generation time makes it ideal for population growth experiments.

This study looked at the effects of a fresh versus aged algal diet on the population growth of *T. californicus* over a 4-week period. Specifically, two of the dominant algae in the SJA, *Nereocystis luetkeana* and *Agarum fimbriatum*, were used for the diet. *N. luetkeana* is often preferred by herbivores because of its low phlorotannin content (Duggins and Eckman 1997), whereas *A. fimbriatum* is often avoided by herbivores because it contains among the highest known percentages of total polyphenolics among northeast Pacific brown algae (Steinberg 1985). Based on the previous results which showed an increase in isopod growth and sea urchin gonad mass with fresh algal diets (Burgess unpubl.; Raymond unpubl.), I hypothesized that a diet of fresh algae would increase the population growth rate and fecundity of *T. californicus*. I also expect to see unequal growth rates between the two algal diets due to the differences in phlorotannin content.

Methods

Algae collection -- Fresh *Nereocystis luetkeana* blades were harvested weekly for 4 weeks from April 19 to May 17 from open water near Turn Island (N 48.535, W -122.964). Pieces of the blade used in the experiment were cut from within 1 meter of the meristem and did not contain any visible sori. Fresh *Agarum fimbriatum* blades were harvested at the same time from under the FHL pier (N 48.545, W -123.012) at a depth of ~5 m.

Algae aging -- A mesh bag (30 cm x 30 cm, constructed from window screen) was filled with fresh blades from *N. luetkeana* and placed in a 5-gallon bucket with holes to allow water flow. The bucket was submerged in a darkened tank of circulating sea water for 1 week. This same method was applied to *A. fimbriatum* blades in a separate tank. Aged algal diets were cut from these blades.

Test species -- *Tigriopus californicus* were collected from two splash pools on the FHL property and stored in glass jars with no additional food at room temperature. After 2 days, 12 groups of 30 gravid females were collected and put into 12 jars filled with 600 mL of filtered sea water.

Population growth experiment -- Three 2 cm x 2 cm pieces of fresh unwashed *N. luetkeana* were added to jars 1-3. Three similar sized pieces of 1-week old *N. luetkeana* were added to jars 4-6. Identical steps were taken for fresh and 1-week old *A. fimbriatum* for jars 7-9 and 10-12. The jars were stored at 15°C in a 16:8 light:dark cycle. After 7 days, each piece of algae was removed and rinsed over its jar to remove any feeding or attached copepods. A new piece of fresh or aged algae was put into each jar to replicate initial conditions. This process was repeated weekly for 4 weeks. After 32 days the total population, as well as adult, copepodite, nauplii and gravid female abundances within that population were counted and recorded.

In situ hatching rate experiment – At the end of the population growth experiment, ten gravid females from each replicate were individually placed into 7 mL scintillation vials filled halfway with filtered sea water. The egg sac of each female was monitored every 8 hours over the span of 96 hours to determine when the eggs hatched.

Natural population hatching rate experiment – 20 gravid females were collected from Reuben Tarte State Park and South Beach on San Juan Island, WA. Each female was placed into a 7 mL scintillation vial filled halfway with filtered sea water. The egg sac of each female was monitored every 8 hours over the span of 180 hours to determine when the eggs hatched.

Results

Total population of *Tigriopus californicus* increased across all four diets from a founding population of 30 gravid females. Both fresh and aged *Nereocystis luetkeana* diets resulted in significantly higher abundances of total individuals (Figure 1; ANOVA, $p = 0.004$), adults (Figure 2; ANOVA, $p = 0.003$) and gravid females (Figure 3; ANOVA, $p = 0.007$) than *Agarum fimbriatum* diets. Algal age was not a factor in determining copepod abundance (t-test, $p > 0.05$ for all comparisons).

Hatching rate in the in situ experiment was significantly shortened for females fed fresh *N. luetkeana* compared to either fresh *A. fimbriatum* or aged *N. luetkeana* (Figure 4; ANOVA, $p = 0.0003$). Reuben Tarte females hatched eggs at a faster rate than South Beach females in the natural populations experiment.

Discussion

Tigriopus californicus populations grew faster on a diet of *Nereocystis luetkeana* than on *Agarum fimbriatum*. The adult life stage of the populations provided the most accurate count due to the larger body size, and these results showed an even greater effect of diet than the population count as a whole. The polyphenolic secondary metabolites found in relatively high concentrations in *A. fimbriatum* are the most likely cause for the low population counts in those treatments. Literature indicates that the polyphenolics reduce food quality by reducing assimilation efficiency in grazers (Duggins and Eckman 1997). Polyphenolic compounds are thought also to prevent bacteria and fouling agents, which increase food quality by enhancing nitrogen that accompanies detrital microflora development, from colonizing on the alga

(Duggins and Eckman 1997). This colonization likely occurs sooner on *N. luetkeana* and increases its food quality, further leading to the difference in population abundances seen between the two algae.

The anti-herbivory polyphenolics found in *A. fimbriatum* break down over time (Duggins and Eckman 1997). This helps explain the slightly higher population growth in aged versus fresh *A. fimbriatum* treatments. Though Duggins and Eckman (1997) found that polyphenolic concentrations in *A. fimbriatum* decreased by over 80% in one week, their samples were ground up, which probably helped to increase decomposition rate. Since my *A. fimbriatum* samples were much larger (2 cm x 2 cm), the difference in population growth would likely have been clearer if the *A. fimbriatum* was ground up or aged longer than one week.

Other factors may play a role in the treatment differences seen. *N. luetkeana* blades are thicker and were found to have roughly 5 times the mass of *A. fimbriatum* per unit area. The size of algal pieces used for feeding was based on area, thereby providing *N. luetkeana* treatments with more algal mass for consumption. The *N. luetkeana* pieces also decomposed faster in the jars, which may have allowed more effortless consumption when compared to the tougher *A. fimbriatum*. A consequence of this decomposition was that used *N. luetkeana* pieces could not be removed from the jars after a week without the risk of removing *T. californicus* individuals living within the detritus. Therefore, *N. luetkeana* treatments only had fresh or aged pieces added weekly, while *A. fimbriatum* pieces were replaced weekly. This resulted in providing *T. californicus* a choice of fresh or aged pieces in the fresh *N. luetkeana* treatments. This choice diet could be beneficial to the consumer and account for part of the difference in population growth stated above.

The in situ hatching rate studies showed that a diet of fresh *N. luetkeana* resulted in gravid females hatching their eggs significantly faster than when fed fresh *A. fimbriatum* and aged *N. luetkeana*. This accelerated life cycle is likely one cause of the large population found in the fresh *N. luetkeana* treatment of the population growth experiment. Differences in hatching rate could be caused by at least two factors. The first is that the eggs are taking longer to develop on the mother, likely a result of poor nutrition. The second is that gravid females are capable of delaying deposition of their eggs in stressful conditions, as seen by Kahan et al. (1988) in high density (> 20 ind ml^{-1}) populations. Though the densities in the treatments were far below this threshold, other stressful conditions, such as poor nutrition, likely trigger this reaction as well. High density conditions weren't observed in the natural populations either, so it's possible that the South Beach females were under stressful conditions induced by malnutrition as well.

T. californicus populations grow faster on a diet of *N. luetkeana* than *A. fimbriatum.* This illustrates the importance of *N. luetkeana* drift in the DSE, because abundant populations of harpacticoid copepods in the lower levels of the food web strengthen all consumers above them, up to apex predators like *Orcinus orca*. Conservation of *N. luetkeana* kelp forests is therefore essential for the health of any food web that includes harpacticoid copepods at the primary and secondary levels.

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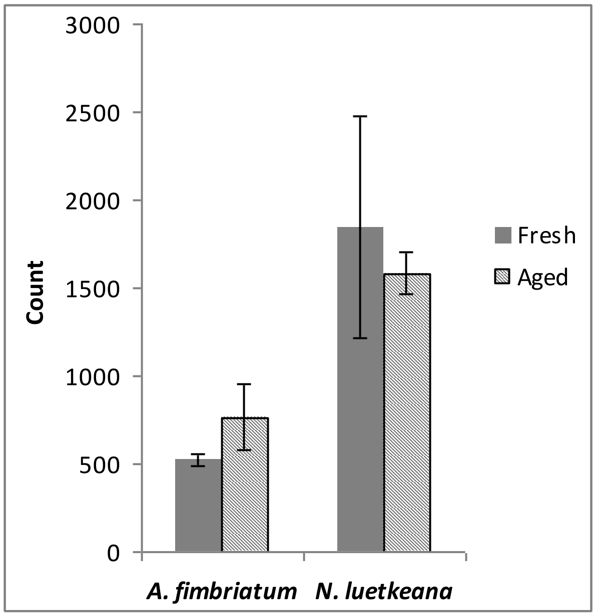

Figure 1. Total population count of *Tigriopus californicus* on 4 algal diets

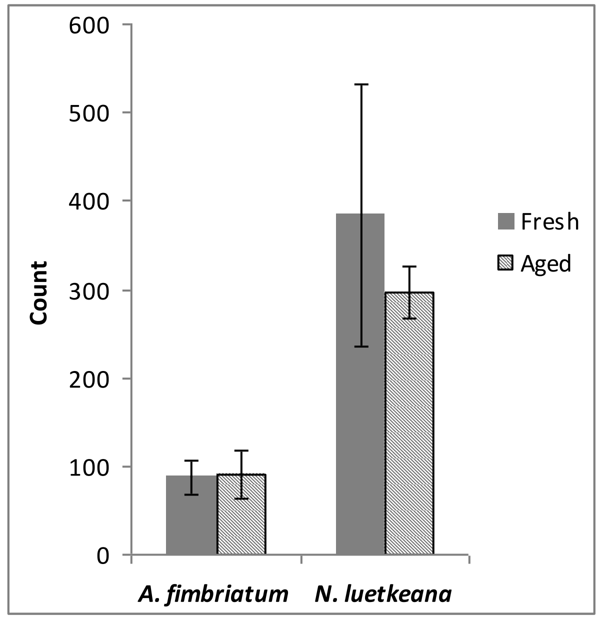

Figure 2. Adult population count of *Tigriopus californicus* on 4 algal diets

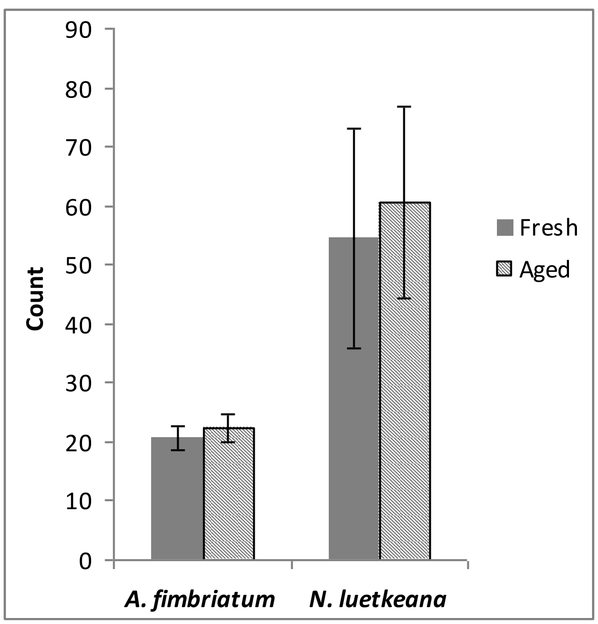

Figure 3. Gravid female population count of *Tigriopus californicus* on 4 algal diets

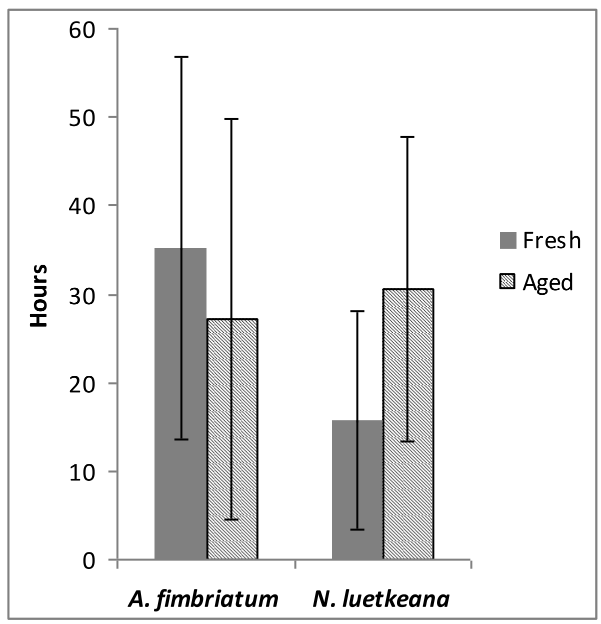

Figure 4. Time until hatching of eggs from gravid *Tigriopus californicus* females on 4 algal diets

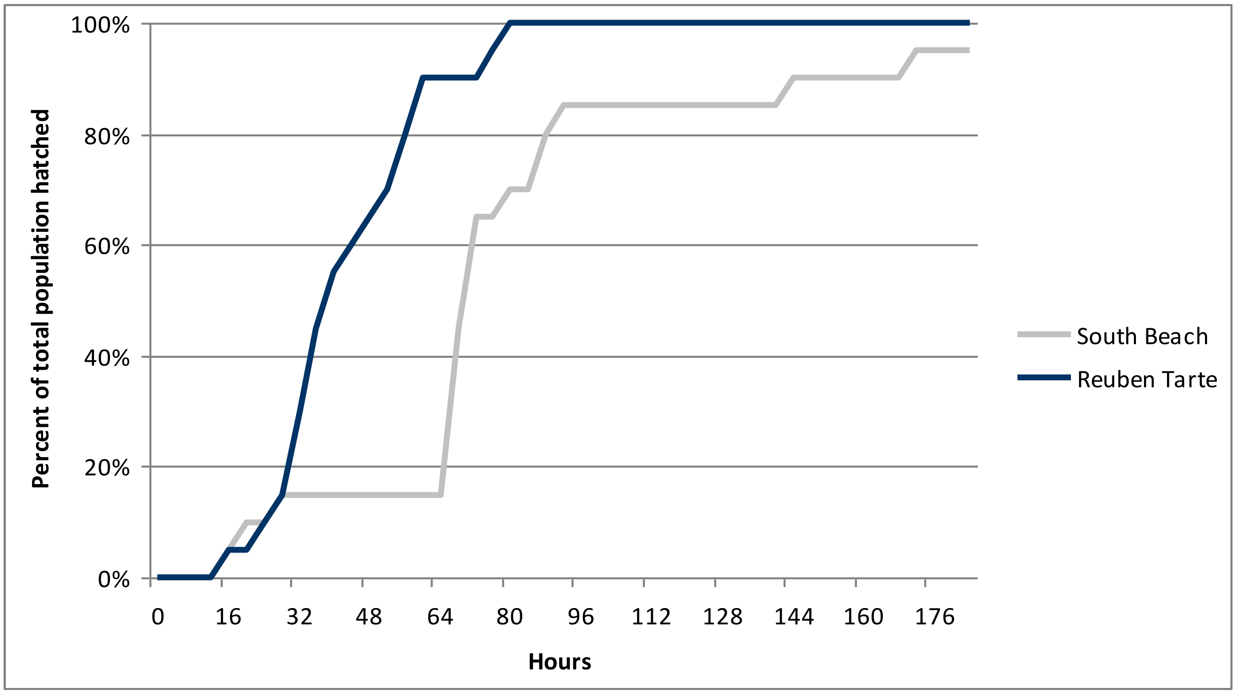

Figure 5. Hatching rate of two natural *Tigriopus californicus* populations on San Juan Island, WA

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