

**Feeding responses of the mysid *Archaeomysis grebnitzkii* to fresh and aged blended
*Nereocystis luetkeana***

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ABSTRACT

The mysid *Archaeomysis grebnitzkii* is a small detritivorous crustacean that is prey to many low level consumers. In vitro, we fed *A. grebnitzkii* fresh and aged blended *Nereocystis luetkeana* as well as a blend of phytoplankton and measured preference and differential growth between these diets. We were unable to detect feeding preference or changes in length or weight, however the fresh kelp treatment had a much higher mortality rate than the other treatments, a result we were able to reproduce in a second trial.

INTRODUCTION

Algae and other autotrophs serve as the foundation for most food webs. The bull kelp *Nereocystis luetkeana* grows rapidly, fixes large amounts of carbon, and is known to have low levels of polyphenolics (Winter and Estes 1992), making them a readily available and nutrient rich food source. As an individual alga ages, biomass is broken down and enters the ecosystem as particulate organic matter (POM), which comprises the remains of algae and other living organisms. Some of the larger organic material that is broken off of kelps is captured by herbivores, but much of it is broken down into smaller particles that are available to deposit feeders (Levinton 1985). Marine suspension feeders are thus exposed to a mixture of particles from phytoplankton to POM (Findlay and Tenore 1982, Mann 1988).

Spatially distinct food webs may be linked by the transfer of energy and nutrients, a process known as a “spatial subsidy,” feeding consumers in environments that have low primary productivity (Polis et al. 1997). Aged and detrital algae, common spatial subsidies, are thought to have greater nutritional value than fresh algal material.

Suspension feeding bivalves in nearshore ecosystems show preference for particles of aged kelp over particles of fresh kelp (Levinton et al. 2002). As kelp decays, it undergoes degradation of the protective polyphenolic compounds which are thought to deter herbivory and hinder bacterial growth (Duggins and Eckman 1997).

Algae are a food source for many mysid species (Mauchline, 1980). Mysids are crustaceans of the order Mysida found in both pelagic and benthic environments. They are omnivorous filter feeders who eat small planktonic organisms such as copepods and diatoms as well as organic detritus (Mauchline 1980). Mysids filter feed by collecting phytoplankton and other microscopic food on the setae of the endopodites of the thoracic appendages and mouthparts. In many species, the anterior thoracic endopods form a food basket where food is held until needed (Mauchline 1980). Mysids in turn are prey for many larval fishes (Eusebio et al. 2010) and as such present a unique opportunity to track the transfer of energy from primary producers up through the food web.

Here we manipulated the diet of captive mysids for varying lengths of time during which feeding rates, growth, and mortality were measured. This investigation addresses two null hypotheses: Mysids will show (1) no preference for one treatment or (2) change in growth between diets of fresh and aged kelp. We will be measuring growth rates and mysid preference for aged or fresh diets.

MATERIALS AND METHODS

Mysid Collection

The mysid *Archaeomysis grebnitskii* was chosen for feeding trials due to abundance and ease of acquisition. Specimens were collected on 25 April and 11 May, 2012, at Eagle Cove, a small, sandy, south-facing beach with a shallow decline on the

south end of San Juan Island, Wa. Mysids were collected by dragging large aquarium dip nets across the bottom in 0.25m to 0.5m of water, kicking up sediment and allowing it to filter through the net.

Kelp and Diet Preparation

Nereocystis luetkeana was selected for the experimental diets due to its abundance and ease of acquisition in the area near the University of Washington's Friday Harbor Labs and due to its low levels of polyphenolics (Winter and Estes 1992). Non-reproductive areas of *N. luetkeana* were collected at Turn Rock, northeast of Turn Island, Wa. For each kelp diet, 150g wet weight of *N. luetkeana* was blended with a Cuisinart blender (model CPB-300) in 1500mL of filtered seawater for approximately 10 minutes total and passed through a 300 μ m filter. The kelp for the fresh diet was then poured into four 16-cube ice cube trays, resulting in 64 cubes, and frozen to keep fresh. Since some kelp was lost in the filtering process, we estimate each cube contained approximately 2g wet weight of blended *N. luetkeana*. The kelp for the aged diet was blended and then allowed to decay in a dark room kept at 8° C for 10 days in filtered seawater on a stir plate before it was frozen in the same manner.

In previous studies on detrital kelp, the tissue was centrifuged after blending (Duggins and Eckman 1997). Our aged diet treatment was not centrifuged to prevent possible loss of micronutrients that could be important for bacterial colonization, and the fresh diet was not centrifuged to keep particle size consistent.

Because most species of mysids are known to eat phytoplankton, using this control diet ensured that the food would be consumed and some growth will be observed (Mauchline 1980). The control diet was an industrial blend of phytoplankton paste (Spat

Formula, Innovative Aquaculture Products, Ltd., British Columbia) consisting of the diatoms *Chaetoceros-B* and *Phaeodactylum tricornutum*, and the heterokont *Nannochloropsis oculata*. To get a similar food concentration as the two kelp diets, 2g wet weight of the phytoplankton blend was mixed with 1500mL of filtered seawater and frozen in ice cube trays.

Treatment and Replicate Setup

Each of the 3 diet treatments was used in 8 replicate tanks with mysids and 2 without, for a total of 30 tanks in a temperature controlled room set at 8° C. Tanks without mysids were used as controls to determine the settling rate of the food particles. Each 4L Mason jar “tank” was set up with an aquarium airstone and 1L of filtered seawater. Mysids survive better if the bottoms of the culture vessels were covered with sandy mud (Mauchline, 1980), so 60 mL of clean sand was added to every tank. A frozen diet cube of the appropriate treatment was blended frozen in 100 mL of filtered seawater to be resuspended and then diluted to 1L, and added to each tank. The tanks had a total of 2L of filtered seawater-food mixture per tank.

Six mysids were added to each tank. Large or obviously brooding mysids were avoided because measurable growth is unlikely to be observed in these individuals during the trial period. Very small individuals were also not chosen because of the difficulty in catching and finding them in the tanks. The mean body length was 11.9mm ±9mm.

Each feeding cycle lasted 3 days. At the end of the feeding cycle, the mysids were

collected, the tanks drained, sand replaced, tanks refilled, frozen diet cubes resuspended and added, and the mysids returned.

Measurements

Feeding preference was measured by absorbance of chlorophyll a in the tank water in raw fluorescence units (RFU). Water samples were taken from each tank every day during the first three feeding cycles. During the remaining six feeding cycles, samples were taken as the feeding cycle started and again 3 days later, directly before draining the tanks. Consumption was determined by the relative change of suspended particles recognized by a fluorometer on wavelengths for chlorophyll a. Suspended food was measured every day over the course of each feeding cycle. Replicate tanks of each treatment we set up without mysids to ascertain settling rate of food particles.

Mysid growth was determined by measuring length and weight of the mysids at the beginning and again upon their death or at the end of the trial. Mysid molts, if present, were collected when the water was changed every 3 days. Length of living mysids was measured in a thin, clear, rectangular container with millimeter markings on the back. Weight was determined by placing a mysid in a known quantity of water on a scale.

Mysids were collected and frozen on the final day of the experiment or as they died. Telson lengths were measured on dissecting microscopes with the aid of ocular micrometers; then the mysids were placed in pre-weighed microtubes in a warming oven at approximately 50° C for 5 hours.

One diet cube from each treatment was also thawed, dried, and ground to measure caloric content in a bomb calorimeter and to measure stable isotope and fatty acid biomarkers.

Data Analysis

For summary statistics, we report $\bar{x} \pm$ standard deviation. Fluorometry data was analyzed by comparison of standard deviation overlap. For comparison of molt rates we ran a single factor ANOVA. For comparison of length and weight we used pairwise t-tests. Condition index data was compared by ANOVA and post hoc pairwise comparison. Mortality was graphed as total number of surviving mysids over trial days in each treatment.

RESULTS

Preference

Fluorometry data measured in Raw Fluorescence Units (RFUs) was used to calculate change in suspended particulate in all tanks. There are no significant difference between feeding tanks and settling tanks (Fig. 1).

Growth

Total number of molts across each treatment was used as an indicator of growth. The phytoplankton treatment produced the most molts; there was no significant difference between fresh and aged treatments (Fig. 2, $F = 1.157$, $df = 2$, $P = 0.317$).

Viewing the molts cumulatively, we ignored any found on or before day 3 because they are likely unrelated to the diet treatment (Fig. 3). This method reveals a trend in which mysids in the fresh treatment stopped molting early while aged kelp and phytoplankton treatment mysids continued to molt.

At the start of the experiment, the average wet weight of an individual mysid was $0.2g \pm 0.05g$; the average length was $11.9mm \pm 9mm$. When measured again at the end of

trial 1, there were no significant changes in weight ($F = 1.92$, $df = 1$, $P = 0.196$) or length in any treatment ($F = 1.45$, $df = 1$, $P = 0.239$).

Condition

The dry weight and telson length of 73 individual mysids were compared as a condition factor (Fig. 4). Trends in the data were not well enough aligned for an analysis of covariance; but wild mysids measured after being frozen on the day of their capture showed much higher fitness than mysids in the experimental treatments (wild and aged: $p = 0.001$; wild and fresh: $p = 0.001$). Differences between experimental mysids were not significant.

Mortality

As the feeding trial progressed, a pattern of mysid deaths appeared. At 9 days, mortality was at less than 20% in both the phytoplankton and aged kelp treatments, but there was 56% mortality among mysids in the fresh treatment (Fig. 5). Due to this unanticipated trend, we started a second trial in which mortality in the fresh treatment reached 61% on day 9 and 89% on day 15 when the experiment ended (Fig. 6).

DISCUSSION

We failed to falsify the null hypothesis that mysids would show no preference between food treatments. The fluorometry data shows no trends when adjusted to account for the settling rates of the food particles (Fig 1). If mysids were eating the blended kelp, after subtracting the settling rates we would expect to see the lines of all treatments gently curving up on this graph as the differences between readings from settling and mysid tanks increase. It is unclear whether mysids were feeding on already-settled kelp

particles or those still in the water column. It is possible the change in number of particles due to mysid activity was below the level of detection on the machine we used. It seems a fluorometer using the chl a wavelengths is simply not a good tool for quantifying POM. This will be an important obstacle to overcome for any future investigations into mysid feeding preference.

We also failed to falsify the null hypothesis that mysids would show differential growth between food treatments. None of our measurement methods saw meaningful differences between mysids eating fresh and aged *Nereocystis*. The molt count charts which start on day 3 (Fig 3) hint at a trend of increased molting in the phyto and aged treatments and decreased molting in the fresh treatment starting after 6 to 9 days. We believe our molt counts significantly under-represent actual molting activity; a study designed for this data set may indicate greater growth from an aged kelp diet.

Our inability to measure change in length or weight of the mysids stems from the coarseness of our measuring methods. *A. grebnitzkii* are fragile and cannot withstand being removed from seawater for the amount of time required to weigh them or measure them with calipers, so we used gentler, and therefore less precise, methods of measurement. This issue, compounded by their miniscule mass, may mean weighing them at the start of an experiment is not feasible. Finding the length of a mysid might be accomplished harmlessly and accurately through the use of photo analysis software.

The comparison of dry weight and telson length as a condition factor did not produce clear distinctions between the fitness of the mysids in any of the treatments; the only meaningful differences are between the wild mysids and those from the two

Nereocystis treatments. The conditions of the experiment seem to have severely stressed all our animals regardless of treatment.

The general pattern in the data seems to be a tendency for mysids in the fresh *Nereocystis* treatment to underperform the other two treatments, but not to a statistically significant degree; the fascinating exception comes from the mortality rate in the fresh *Nereocystis* tanks (Fig. 5). It is interesting that trial 2 reproduced these results (Fig. 6) and implies there has been a significant change in the kelp during the aging process.

The trends we observed in mortality between the aged and fresh *Nereocystis* treatments are puzzling. The obvious difference between a fresh and an aged kelp would be polyphenols, but *Nereocystis luetkeana* was chosen specifically because of its low levels, so they aren't being poisoned, at least in this way (Winter and Estes, 1992). Starvation doesn't seem to be a likely cause either. Mysids are known to cannibalize each other when starved (Mauchline, 1980), and we witnessed *A. grebnitzkii* doing exactly this in the lab separate from our experiment, but we didn't lose a single body from the fresh treatment in trial 2. In fact, the only place we did lose a body in trial 2 was the phyto treatment, the treatment with the most evidence of growth and health. Further study of this would be aided by a starvation treatment for comparison. The primary observable difference between our fresh kelp and aged kelp was their consistency. We frequently witnessed blended kelp particles clinging together in the tanks to form gelatinous masses, probably due to colloids. It seems plausible that during the aging process, bacterial colonization broke down the kelp in some way that mysids are unable to on their own.

While this investigation was unable to show any differences in the feeding preference or growth of *A. grebnitzkii*, we did reveal an interesting correlation between

fresh *Nereocystis luetkeana* and mysid mortality. We believe the techniques we developed here are useful to further study of spatial subsidies and POM using mysids as a model organism.

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FIGURES & TABLES

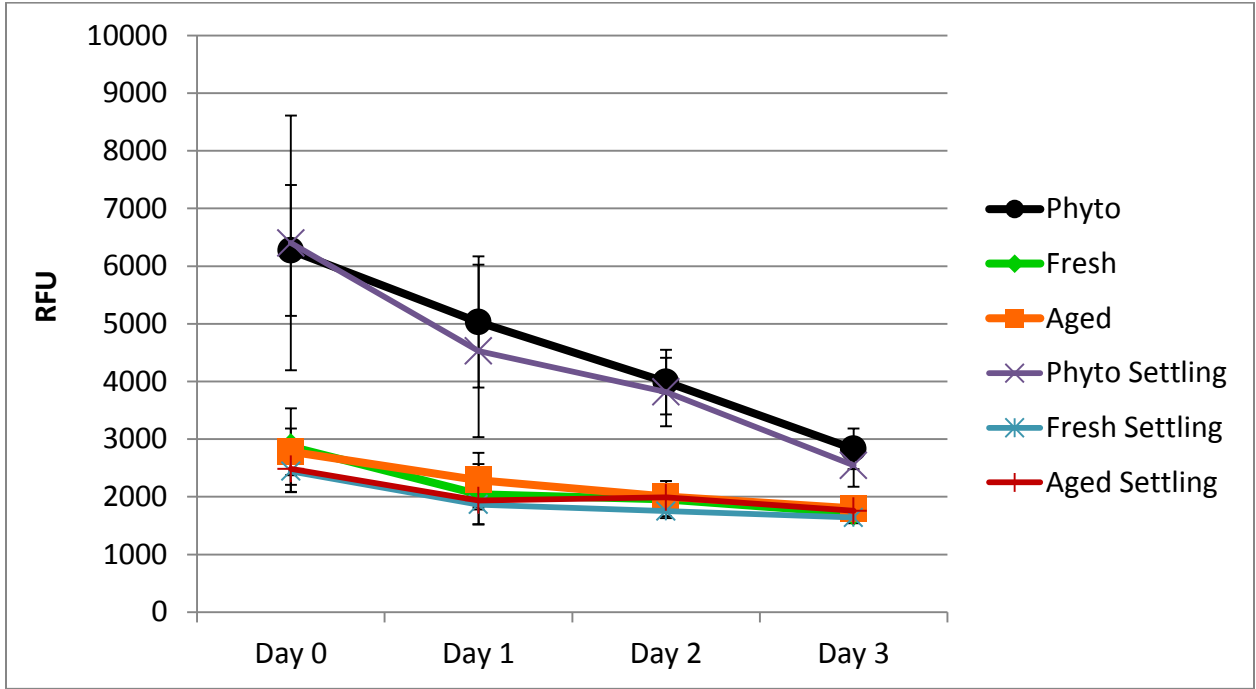


Fig. 1 Average Raw Fluorescence Units (RFUs) of experimental treatments and their settling tanks. There are no significant trends.

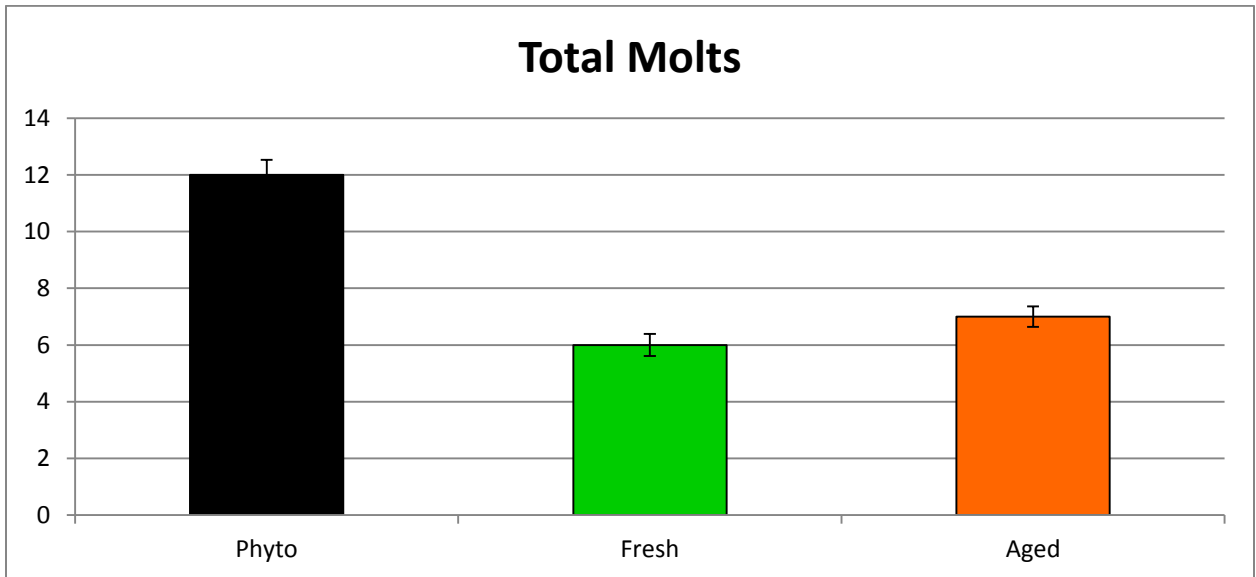


Fig. 2 Total number of *A. grebnitzkii* molts collected over both trial periods. The difference between the fresh and aged treatments is not significant.

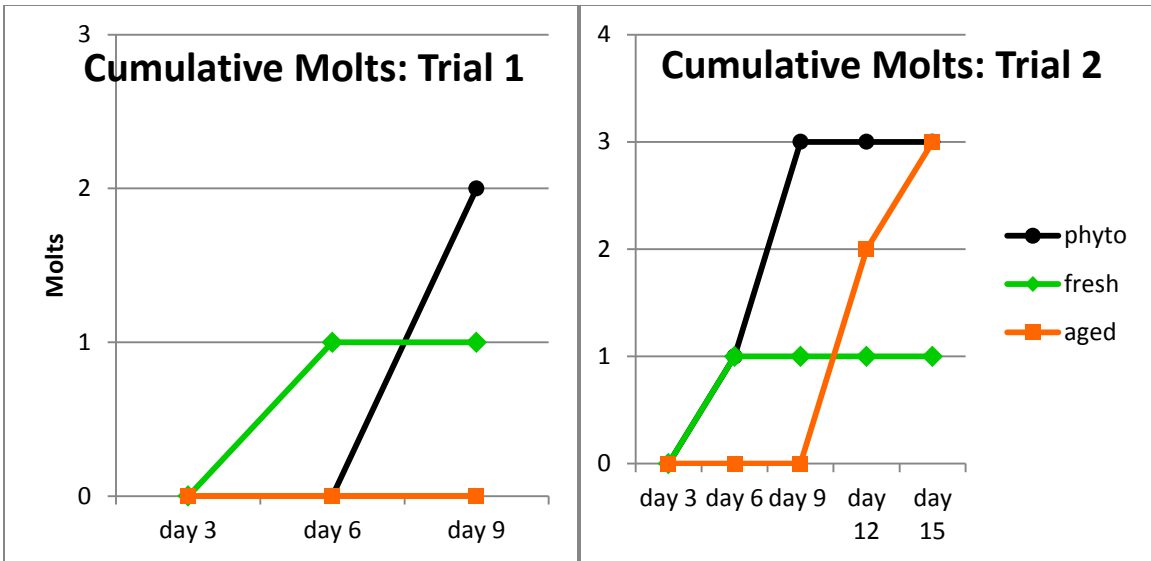


Fig. 3 Cumulative molt counts from all tanks in each trial. Molts before day 3 are ignored because it is unlikely that food treatments were influencing growth by this point.

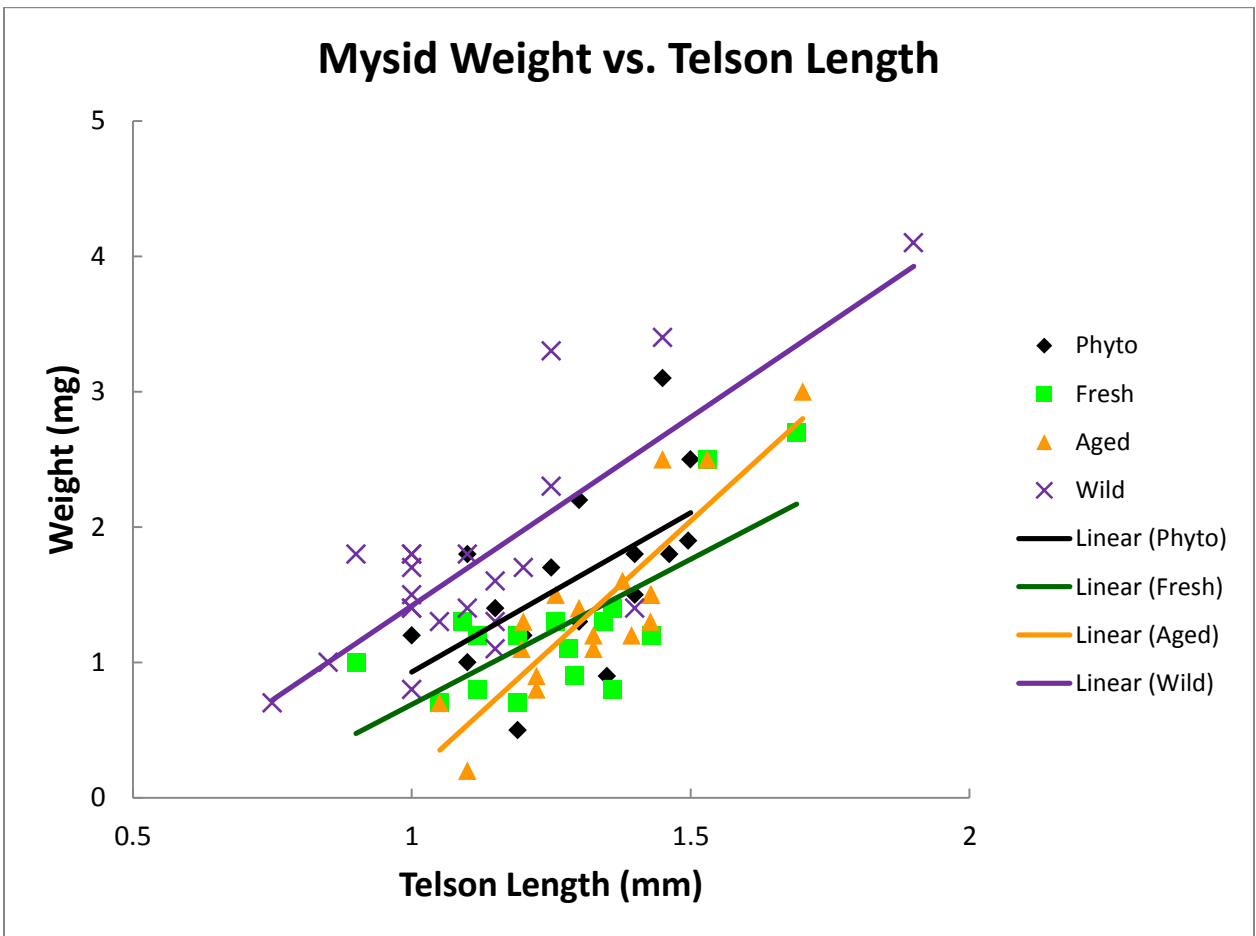


Fig. 4 Dry weight graphed against telson length in *A. grebnitzkii* individuals. Phyto, n = 16; Fresh, n = 17; Aged, n = 18, Wild, n = 22.

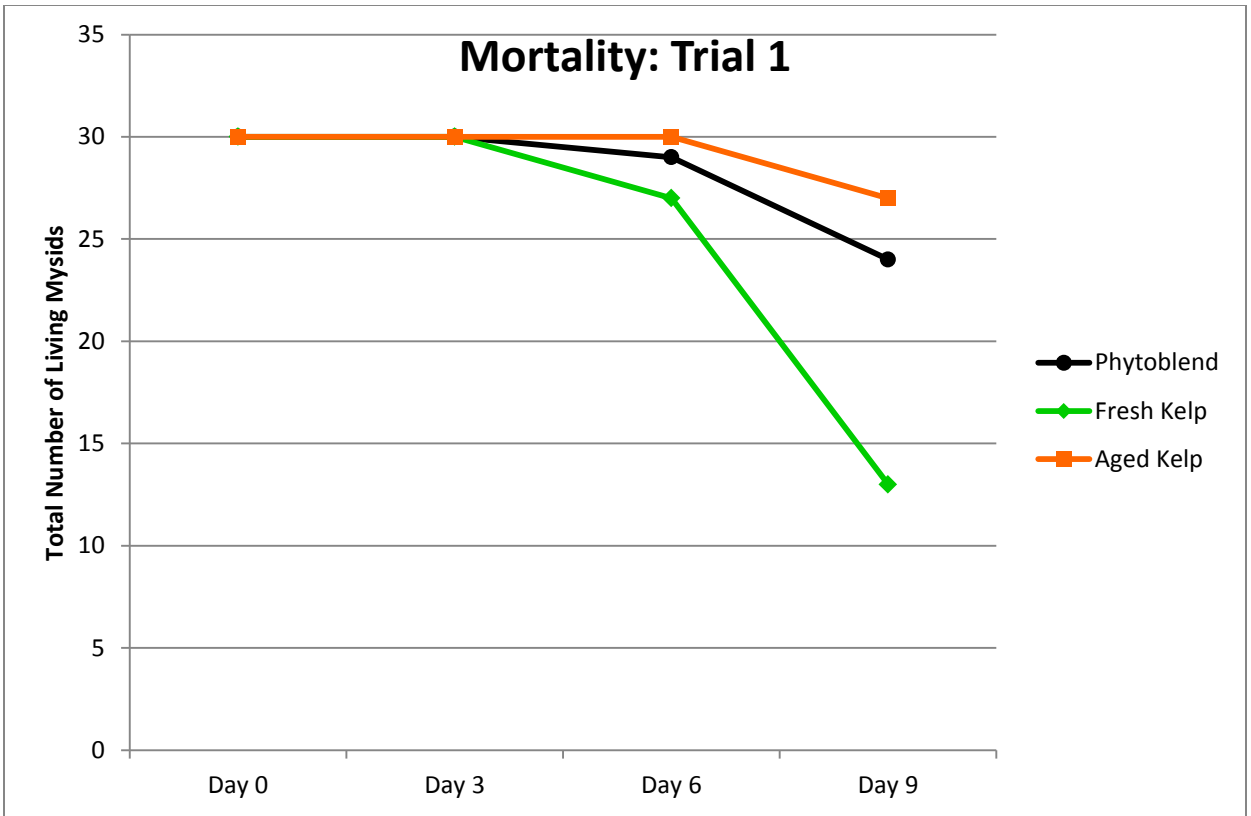


Fig. 5

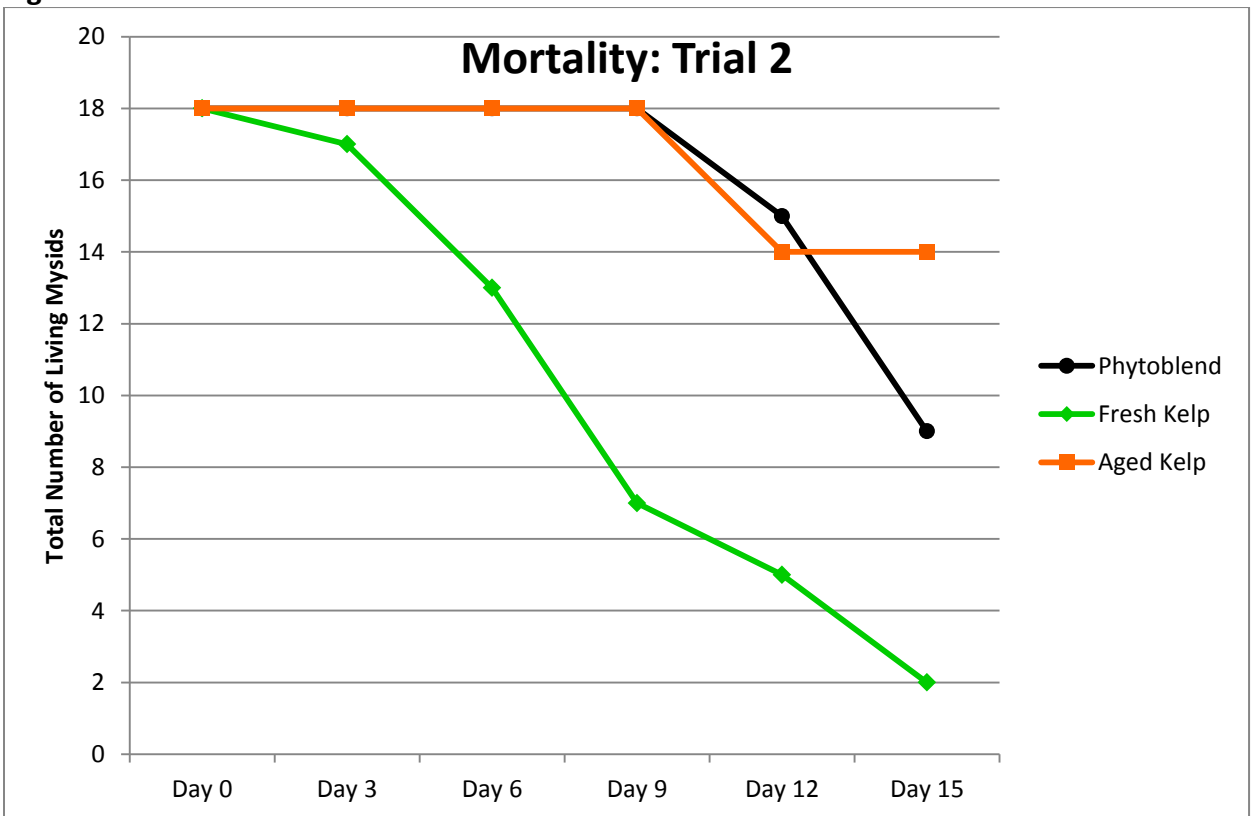


Fig. 6