Responses of the mysid *Archaeomysis grebnitzkii* to phytoplankton and kelp diets

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ABSTRACT

The mysid *Archaeomysis grebnitzkii* is an important detritovore and prey in many food webs. In this experiment A. *grebnitzkii* was feed phytoplankton, blended fresh kelp, and blended aged kelp for 15 days while growth and diet preference were measured. Growth was measured as a molt rate and condition index. The preference was measured by the absorbance of chlorophyll a in solution. Unfortunately, growth was not observed in any of the treatments and chlorophyll a did not provide any indication of preference for a specific diet. Of all the treatments fresh kelp had the least molts and the lowest condition index. A strong negative response to fresh kelp particles was recorded.

INTRODUCTION

Spatially distinct food webs may be linked by the transfer of energy and nutrients, a process known as "spatial subsidy" (Polis et al. 1997). Spatial subsidies of detritus allow secondary productivity 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).

The bull kelp *Nereocystis luetkeana* grows rapidly, fixes large amounts of carbon, and is known to have low levels of polyphenols (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 derived from 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).

Algae are a food source for many mysid species (Mauchline, 1980). Mysids are crustaceans of the Order Mysidacea, found in both pelagic and benthic environments. They are omnivorous filter feeders eating 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, such as groupers (Eusebio et al. 2010). They thus present a unique opportunity to follow the transfer of energy in nutrients from primary producers and POM up the food web to large prey.

This investigation addresses two null hypotheses: (1) Mysids show no change in growth between diets of fresh and aged kelp; and (2) Mysids show no preference between fresh and aged kelp diets. We measured growth rates and mysid preference for aged or fresh detritus, predicting that aged kelp would positively affect growth rate.

MATERIALS AND METHODS

The experiment took place at the University of Washington's Friday Harbor Laboratories (FHL). Mysids were fed three diet treatments in two separate trials. The first trial (trial 1) ran for 9 days and the second (trial 2) for 15. During these trials the growth and feeding rates were measured.

Mysid collection

The mysid *Archaeomysis grebnitzkii* was used for feeding trials due to its abundance and ease of acquisition. Specimens were collected at Eagle Cove, a small, sandy, south-facing beach with a shallow slope on the south end of San Juan Island, WA. Mysids were collected by dragging large aquarium dip nets across the bottom in about a foot of water, kicking up sediment and allowing it to filter through the net. Some wild Mysids were frozen directly after being caught to be used for condition index analysis.

Kelp and diet preparation

Nereocystis luetkeana was chosen for the experimental diets due to its abundance and ease of acquisition in the area near FHL and due to its low levels of polyphenols. *N. luetkeana* was collected at Turn Rock, northeast of Turn Island, WA. Only non-reproductive areas of blades were collected. For each kelp diet, 150g wet weight of *N. luetkeana* was blended in 1500mL of filtered seawater until all material could fit through a 300μm 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 kelp 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 among treatments.

Because most species of mysids eat phytoplankton, using a phytoplankton control diet ensured that the food would be consumed and some growth would be observed (Mauchline 1980). The control diet was an industrial blend of phytoplankton paste 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. 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 averaging 10mm in body length were added to each tank. Large or obviously brooding mysids were avoided because measurable growth was unlikely to be observed in these individuals during the trial period. Very small individuals were also not

chosen because of the difficulty in finding and catching them in the tanks.

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 using a flurometer. Chlorophyll a is a commonly used measure of algal biomass. The flurometer measures the amount of red fluorescence released by chlorophyll a molecules when blue light was applied. Preference was measured in raw fluorescence units (RFU) to detect a relative change in chlorophyll a. 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. Two tanks were set aside in each treatment as settling tanks. These tanks were identical to the others in setup except they did not have mysids. Consumption was determined by the relative change of chlorophyll a measured over the course of each feeding cycle.

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. In trial 2, which ran longer, we compared the final dry weight of mysids to the telson length. This kind of weight to size ratio creates a condition index that is an indicator of

nutritional state, because a healthier animal weighs more per unit length than a starving animal. All mysids used for the condition index were collected when they died or at the end of the trial.

RESULTS

Absorbance

The absorbance of chlorophyll a throughout the feeding trials is seen in Figures 1 and 2. The amount of chlorophyll decreased as the feeding trials continued in all three treatments. This decrease in chlorophyll is due to both the mysids feeding and the natural decrease in absorbance of the solution as seen in the settling tanks (Fig 2). To obtain the relative rate of feeding we subtracted the average absorbance in the settling tanks from the average absorbance of the treatment tanks (Fig 3).

Growth

We counted the molts per treatment throughout the trial period (Table 1). The phytoplankton treatment had more total molts in both trials than fresh and aged kelp treatments (Fig. 4). The phytoplankton treatment was significantly greater than the fresh and aged kelp treatments in trial 2 only (ANOVA, trial 1 $p>.05$, trial 2 $p=.01$).The kelp diets had no difference in molts between fresh and aged (ANOVA, trial 1 $p>.05$, trial 2 $p>.05$).

We graphed the cumulative number of molts starting on day 3 (Fig 5). Any molts found on or before day 3 were not included because they are likely unrelated to growth on the diet treatments. A trend developed where individuals in the fresh kelp treatment

stopped molting after day 3 while those in other treatments continued. Animals in the aged kelp treatment did not start molting until day 9.

There were no significant changes in body length or weight during the feeding trials in any of the treatments (t-tests, $p>.05$). In the condition index (Fig. 6) a couple of trends are visible. The fresh from the field wild mysids had the greatest dry weight to telson length (W/L) ratio and the fresh treatment had the lowest W/L ratio of all diet treatments. The wild mysids had a greater W/L ratio than the kelp diet treatments (t-tests, wild & aged $p=.01$, wild & fresh $p=.001$, wild & phytoplankton $p>.05$).

Mortality

We counted the number of mysids alive at the end of each 3 day feeding cycle. The number of surviving mysids in all treatments dropped as the experiments continued (Fig 7, 8). In trial 1, after 9 days there was 56% mortality in the fresh kelp treatment while there was no more than 20% mortality in the other two treatments (Fig 7). In trial 2, mortality in the fresh treatment reached 61% on day 9 and 89% when the experiment ended on day 15 (Fig 8). In both trials the aged kelp treatment had the lowest mortality rate (Fig 7, 8).

DISCUSSION

We designed the experiment expecting to feed for 30 days but we discovered some interesting trends in molts and mortality. We repeated the experiment and happily saw the same trends.

Absorbance

While we found a change in chlorophyll a from the start to the end of the feeding cycle in all treatments, there were no consistent patterns. If the mysids were eating the food we would expect to see the adjusted absorbance of treatments increase as the differences between readings from settling and mysid tanks increased. None of the treatments produced this increase and only the fresh kelp treatment had any steady change in chlorophyll. The fresh kelp treatment had the opposite trend where the adjusted absorbance was decreasing over the feeding period. The observed decrease in overall chlorophyll a absorption over the feeding periods could have been due to the natural settling of the treatments. Thus this chlorophyll data does not provide any indication of preference for a specific diet. This method for measuring preference was not very accurate. We measured the absorbance of the particles suspended in solution assuming the mysids would be eating those particles but they may have been eating the debris that settled and this would be undetectable in our manner of measurement.

Growth

In order to grow, mysids have to molt so molt rate is an indirect measurement of growth. Mysid molts were observed in all three treatments, especially in the phytoplankton treatment. Based on the large number of molts present, the animals in the phytoplankton treatment were growing. The fresh treatment on the other hand, had few molts so likely the mysids were not growing or at least not as fast as the phytoplankton treatment. Those animals in the aged treatment continued to molt throughout the trial in consistent numbers. However, we found that the molts were very fragile, and hard to catch. It is likely that our molt count is underrepresentative of the actual number of molts that occurred in each treatment. Changing the water more frequently could remedy this

issue. Overall, the molt data suggest that phytoplankton might be providing the best food for growth.

Neither length nor weight measurements suggested that there was measureable growth observed from the start to the end of the trials in any treatment. However, our condition index using a weight to size ratio at the end of the trials did show differences among groups of animals. The wild mysids were in better condition than all of the treatment mysids. Animals in the fresh kelp treatment had the lowest condition index of all the treatments, corresponding with the pattern observed in the molting.

Any changes in weight or length that occurred were so small that our system of measuring was not precise enough to detect it. This is probably why we saw no change in growth. The beginning measurements were coarse, while the end measurements were taken when the animals were dead so better tools could be used to obtain a more precise measurement. Also many of the dead mysids were destroyed, torn to bits, and beheaded when we collected them leading to data that could skew the results.

Mortality

Our mortality data confirmed that some aspect of the fresh kelp treatment was very poor for the mysids. In both trials the mysids died faster in tanks with fresh kelp than with phytoplankton and aged kelp. The aged kelp treatment had the fewest deaths, suggesting it is better than the phytoplankton and the fresh kelp. This pattern in the mortality data correlates with the molts and condition index. A healthier animal will have a higher condition index and more molts while a dying animal will have a low condition index and fewer molts.

The strong negative response to fresh kelp particles in both trials was unexpected. *Nereocystis luetkeana* has few defensive compounds like polyphenols that should hinder herbivory or poison mysids. Mysids in all treatments were handled the same way, so handling cannot account for differences in mortality. Starving mysids have been shown to cannibalize (Mauchline 1980), but there were no missing bodies in the fresh treatment tanks. The particle sizes of fresh and aged kelp were the same, and the mysids were able to survive on the aged particles. Thus for unknown reasons the mysids were likely unable to utilize the fresh kelp. This result deserves further investigation.

In conclusion, our experiments did not allow us to determine if mysids show a change in growth between kelp diets and if mysids show a preference between fresh and aged kelp diets. It did however provide us with an interesting observation about fresh kelp and ideas for further research.

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LITERATURE CITED

Duggins, D. O. and J. E. Eckman. 1997. Is kelp detritus a good food for suspension feeders? Effects of kelp species, age and secondary metabolites. Marine Biology **128**:489-495.

Eusebio, P. S., R. M. Coloso, and R. S. J. Gapasin. 2010. Nutritional evaluation of mysids *Mesopodopsis orientalis* (Crustacea: Mysida) as live food for grouper *Epinephelus fuscoguttatus* larvae. Aquaculture **306**:289-294.

Findlay, S. and K. Tenore. 1982. Nitrogen-source for a detritivore - detritus substrate versus associated microbes. Science **218**:371-373.

Levinton, J. S. 1985. Complex interactions of a deposit feeder with its resources - roles of density, a competitor, and detrital addition in the growth and survival of the mudsnail *Hydrobia totteni*. Marine Ecology-Progress Series **22**:31-40.

Levinton, J. S., J. E. Ward, and S. E. Shumway. 2002. Feeding responses of the bivalves *Crassostrea gigas* and *Mytilus trossulus* to chemical composition of fresh and aged kelp detritus. Marine Biology **141**:367-376.

Mann, K. H. 1988. Production and use of detritus in various fresh-water, estuarine, and coastal marine ecosystems. Limnology and Oceanography **33**:910-930.

Mauchline, J. 1980. The biology of Mysids and Euphausiids. Advances in Marine Biology. **18**:1-681.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. Toward an integration of landscape and food web ecology: The dynamics of spatially subsidized food webs. Annual Review of Ecology and Systematics **28**:289-316.

Winter, F. C. and J. A. Estes. 1992. Experimental evidence for the effects of polyphenolic compounds from *Dictyoneurum californicum* Ruprecht (Phaeophyta: Laminariales) on feeding rate and growth in the red abalone *Haliotus rufescens* Swainson. Journal of Experimental Marine Biology and Ecology **155**:263-277.

FIGURES

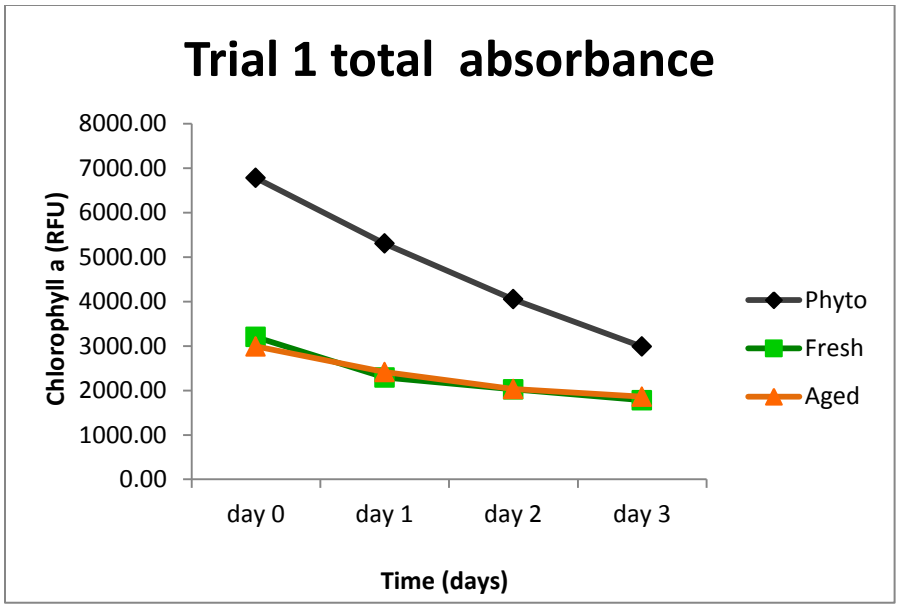

Figure 1 Absorbance of Chlorophyll a during the feeding period.

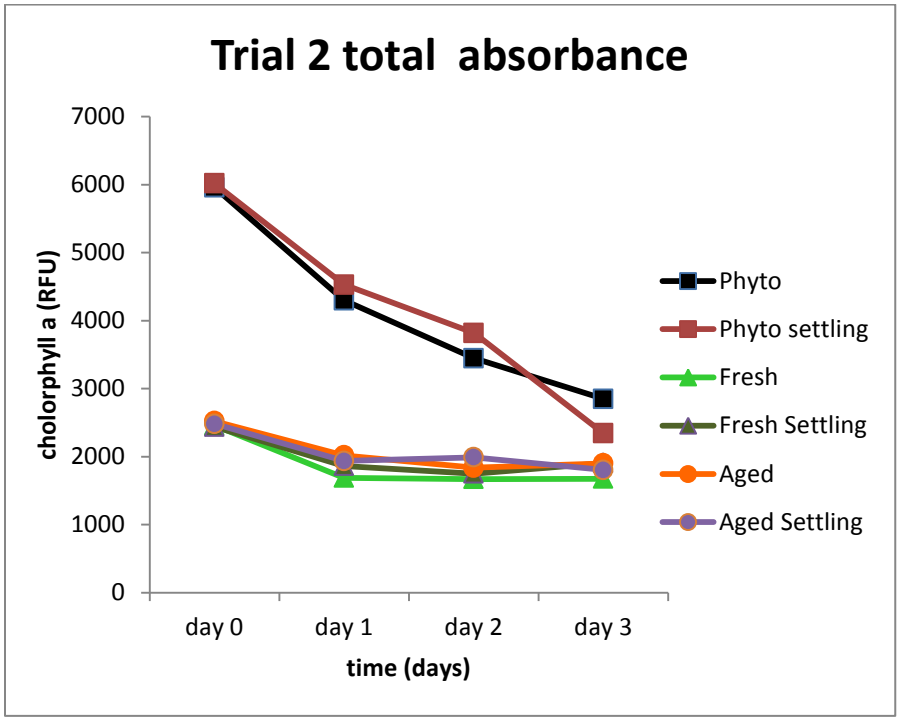

Figure 2 Absorbance of Chlorophyll a during the feeding period.

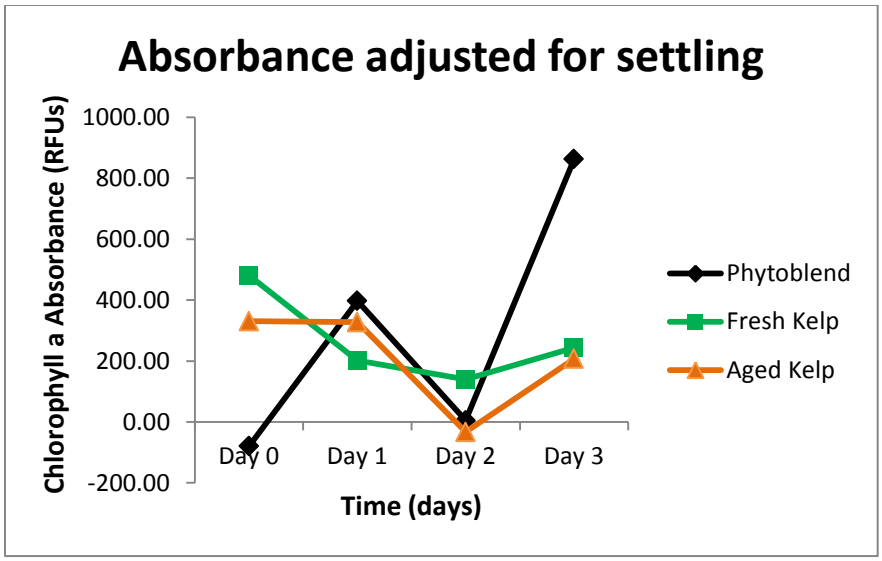

Figure 3 The average chlorophyll a absorbance in all tanks containing mysids from day 0 to day 3 adjusted for settling.

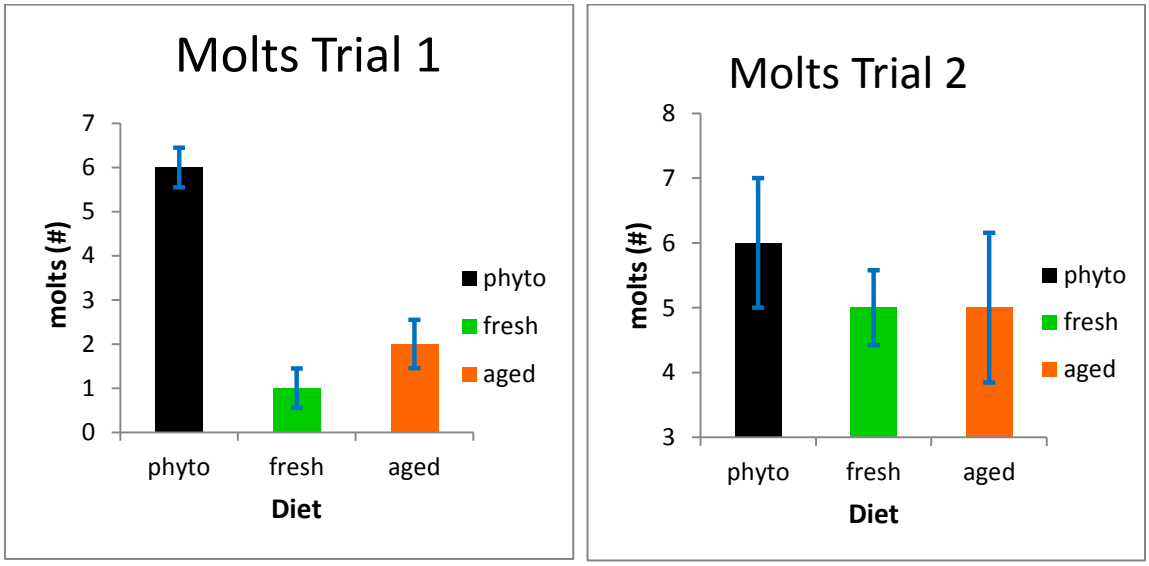

Figure 4 Total number of molts collected over both trial periods. The difference between fresh and aged in both trials was not significant. Error bars are calculated standard deviation of means.

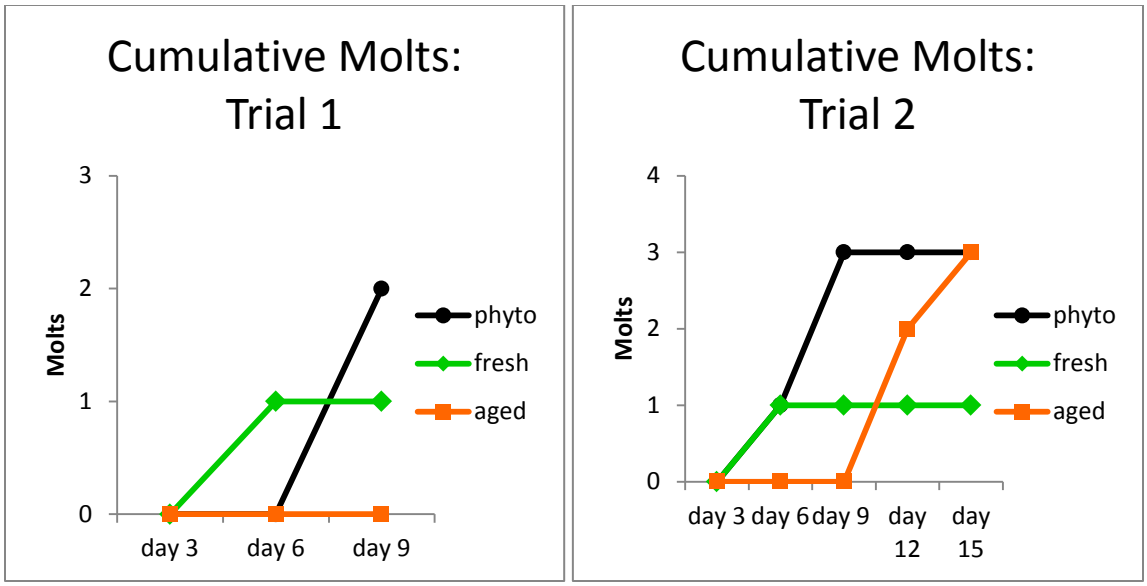

Figure 5 Cumulative molt counts for each trial starting on day 3.

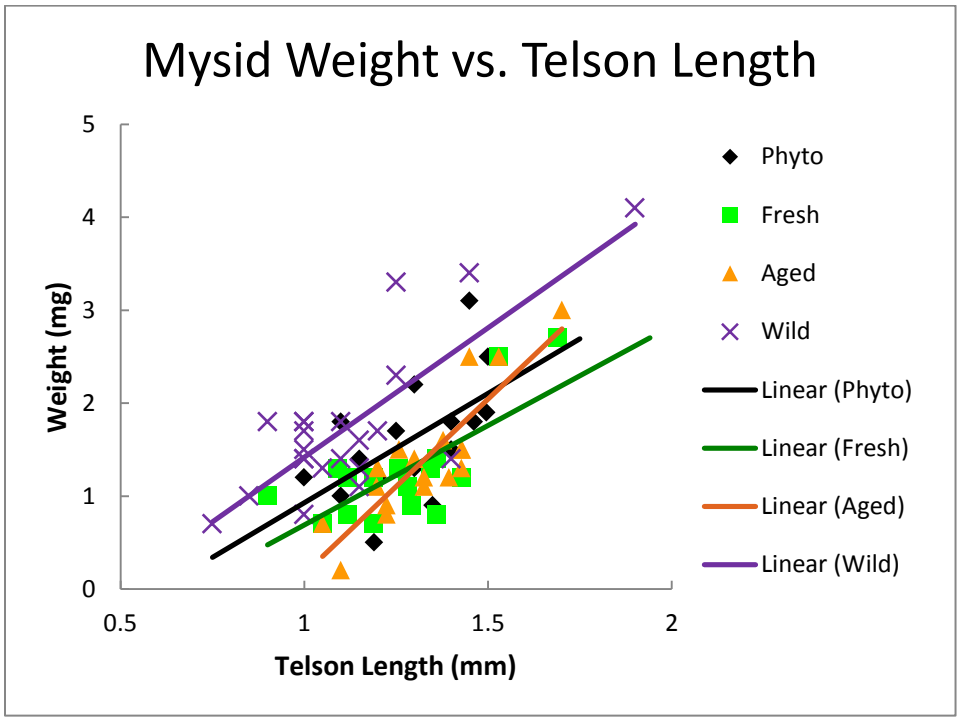

Figure 6 Ratio between dry weight of mysids and telson length of mysids. Trial 2 data only.

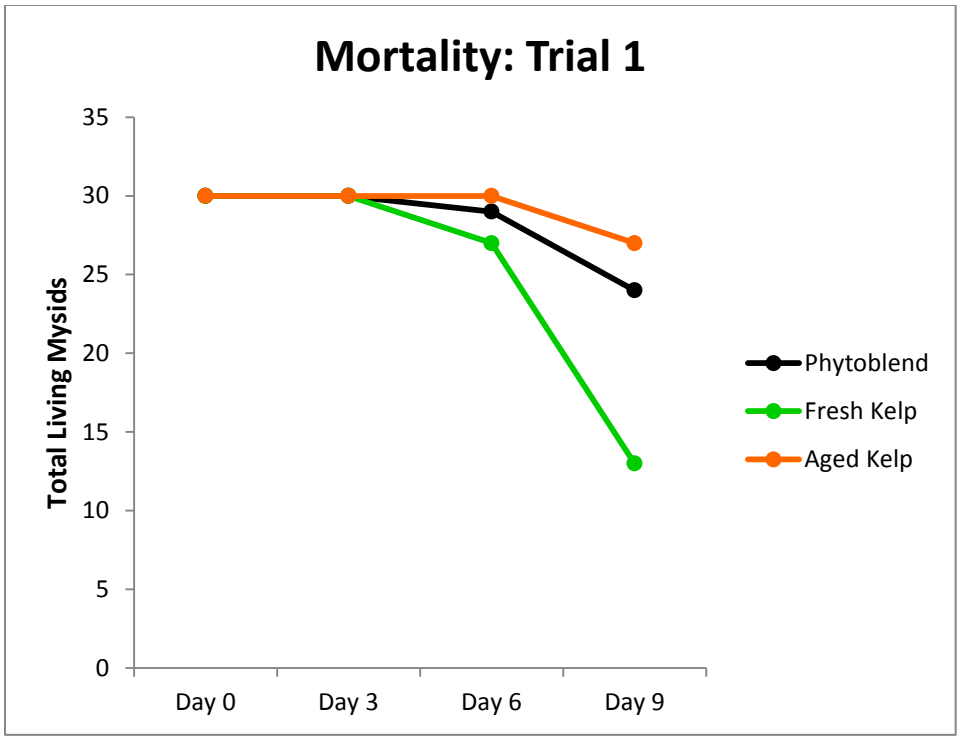

Figure 7 Mortality of mysids in each treatment of trial 1 over time. Each treatment began with 30 mysids.

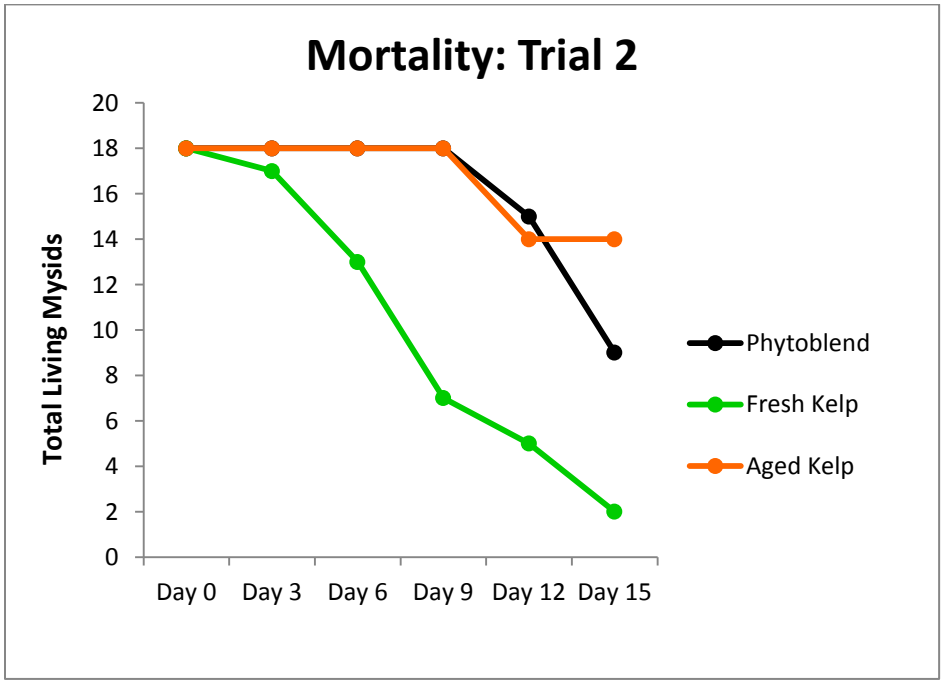

Figure 8 Mortality of mysids in each treatment of trial 2 over time. Each treatment began with 18 mysids.

TABLES

Table 1 Total molts by day

Trial 1 Molts	Treatment	day 0	day 3	day 6	day 9		
	phyto	0	4	0	2		
	fresh	0	0	1	0		
	aged	0	2	0	0		
Trial 2 Molts	**Treatment**	**day 0**	**day 3**	**day 6**	**day 9**	**day 12**	**day 15**
	phyto	0	3	1	2	0	0
	fresh	0	4	1	0	0	0
	aged	0	2	0	0	2	1