Prey Preference of *Pisaster ochraceus* and *Leptasterias hexactis* Recruits of the Rocky Intertidal along the Central Oregon Coast

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Introduction

Sea stars are well known on the Pacific coast as being keystone species. Keystone species display a disproportionately large role in the prevalence and population levels of other species within the community (Paine 1969). They maintain diversity within the rocky intertidal by preventing monopolization by any one species. Paine (1969) found that *Pisaster ochraceus* played a major role in controlling the lower limits of the high zone mussel beds. With this knowledge, Menge (1972) found that *P. ochraceus* normally prey upon medium to large-sized species and *Leptasterias hexactis* is often found feeding on smaller prey. According to the competitive exclusion principle, complete competitors cannot coexist, meaning that both *P. ochraceus* and *L. hexactis* cannot dominate the rocky intertidal together (Hardin 1960). Consistent with this principle, when the competitively dominant *P. ochraceus* is absent, *L. hexactis* will expand its niche to include the larger prey normally consumed by *P. ochraceus* (Menge 1972). This would suggest that competition plays a role in the diets of these two species.

In addition to competitive exclusion, multiple other factors contribute to species diet. *P. ochraceus* and *L. hexactis* have generalized diets that depend on relative prey abundance, seasons, and areal variation (Mauzey 1968). For example, Mauzey (1968) found that *P. ochraceus* fed on whatever is most abundant. Often, *Mytilus californianus* was most abundant and was the preferred prey. *L. hexactis*, on the other hand, had a varied diet that correlated with depth. *Balanus cariosus* was preferred in shallow water, while a variety of holothurian species were preferred in deep water (deeper than 10 meters).

Despite this long-standing knowledge of adult sea star diets, particularly *P. ochraceus'* preference for mussels and *L. hexactis'* preference for barnacles (Mauzey 1968), we know little about the interactions and prey preferences in their recruit stages. The growing influence of climate change on today's ecosystems highlights the importance of baseline research. Understanding the dynamics of this large group of recruits will help predict interaction webs at younger life stages, effects on prey populations, and create a baseline in order to assess how climate change may affect these populations in years to come. Delving into the recruit stage of sea stars will complement other sea star diet knowledge and give dietary background for entire sea star life cycles.

We observed a strong year class for both *P. ochraceus* and *L. hexactis* along the rocky intertidal zones near Newport, Oregon in 2013. The strong year class and lack of general data on sea star recruits prompted us to investigate more. We found numerous studies on reproductive strategies and feeding habits of adult sea stars. A study done by Bruce A. Menge (1972) had looked at how *P. ochraceus* and *L. hexactis* compete for food. We decided to build off this study and focus on recruits.

The goal of our study was to determine if there is a difference in prey preferences between juvenile *L. hexactis* and *P. ochraceus* and if competitor presence affects these preferences. Following a predator-prey model, this project tests interactions and dietary preferences of sea star recruits. In particular, we addressed the following questions: (i) What is the preferred prey of *P. ochraceus* and *L. hexactis* recruits? (ii) What influence does a *L. hexactis* juvenile recruit have on the feeding of a *P. ochraceus* juvenile recruit and vice versa?

Based on previous studies on adult sea star diets, our null hypothesis was that *P. ochraceus* and *L. hexactis* would have the same prey preference. Alternatively, we predicted these recruits would prefer one prey species more than the others. Our other null hypothesis was that when *P. ochraceus* and *L. hexactis* are exposed to competitor cues they will prey on the same species. Our alternative hypothesis was that these two sea star species would prefer different prey when exposed to competitor cues. This paper considers these hypotheses in order to better understand the nature of recruits' diets and therefore better understand sea star life cycles, protection strategies for future year classes, and possibilities for further studies as baseline data expands.

Materials and Methods

Study Site

The animals used in this study were collected from three sites along the central Oregon coast near Newport, Oregon during the early summer months of 2013. The sites included Boiler Bay (44° 50' N, 124° 03'W), a site with both exposed and protected rocky intertidal zones and tidepools wrapping around a small point, Seal Rock (44° 50' N, 124° 08' W), a site protected by a wall of rocks with some protected rocky areas but mostly sand, and Strawberry Hill (44° 15' N, 124° 07' W), a site stretching linearly to the south with exposed and protected rocky intertidal zones and tidepools.

Sample Collection

During early low tides, we collected *P. ochraceus* and *L. hexactis* specimens using a paint scraper to allow for even removal off the rocks without tearing rays. A total of 32 *L. hexactis* and 27 *P. ochraceus* recruits were collected for the study. We collected more *L. hexactis* because five recruits died after the first day of feeding. We replaced them promptly with new recruits.

We collected prey species simultaneously at the three sites. *Semibalanus cariosus, Littorina* spp., and *Mytilus* spp. were scraped off of rocks using the same paint scrapping method. We immediately cleaned the prey in the lab to ensure epibionts would not influence the results of the study.

Prey Preference Experiments

We used three tanks, with circulating sea water and an oxygen aerator, at the Hatfield Marine Science Center in Newport, Oregon in the early summer months of 2013. We put 18 individuals into each tank in separate, screened containers. The first tank held only *P. ochraceus*. The second tank held only *L. hexactis*. The third tank held 9 *P. ochraceus* specimens and 9 *L. hexactis* specimens to involve competitor cues (see table 1). We assigned prey options consistently in each tank so even numbers of recruits were given each choice. The prey option treatments included combinations of *S. cariosus* with *Littorina* spp., *S. cariosus* and *Mytilus* spp., and *Littorina* spp. with *Mytilus* spp. All specimens were in a similar size range to eliminate variation in results due to body size. We weighed, measured, and assigned each recruit a number corresponding to prey options to be presented upon feeding.

After a one day acclimation period, we presented them with two prey choices based on the labels previously assigned. We counted, weighed, and recorded the prey before presenting them to the recruits. We standardized the weight of the prey presented to each recruit, giving them about 1.0g of *Littorina* spp., 1.7g of *Mytilus* spp., and 1.7g of *S. cariosus*. Each day we recorded the number of prey eaten, of what type, and the remaining weight of the prey in the containers. We then repeated the feeding steps, but this time replacing anything eaten with new prey. Water levels were checked daily for consistency and adjusted accordingly. After four days of feeding and recording eaten prey, we reweighed the recruits.

Statistics

From these data collected, we ran statistical analyses using Microsoft Excel 2007. To determine if the recruits had a preference toward particular prey, we ran Welch's t-tests to compare the average consumption of biomass and prey individuals per day for each prey species.

To correct for multiple comparison errors, we used the Bonferroni correction method to assess ps with a significance level of 0.05/3=0.017. To investigate the affects of competition on prey consumption, we ran Welch's t-tests comparing the average consumption of biomass and prey individuals per day within each sea star species with and without competitor cues. We applied the Bonferroni correction with a significance level of 0.05/6=0.0083.

Results

Preferred Prey

Mytilus spp.

When looking at the average weight of prey consumed per day between the two sea star species, *P. ochraceus* consumed *Mytilus* spp. with a mean of 0.07 ± 0.02 (SE) grams (g) per day, whereas *L. hexactis* consumed a mean of -0.01 ± 0.02 g per day (Figure 1). We found a strongly significant difference in biomass of *Mytilus* spp. consumed by these two species (t_{33} = -3.15, p = 0.003), where *P. ochraceus* consumed more.

When looking at the average prey consumed based on counts, *P. ochraceus* consumed *Mytilus* spp. with a mean of 0.74 ± 0.12 individuals per day (Figure 2). *L. hexactis* consumed *Mytilus* spp. with a mean of 0.22 ± 0.10 individuals per day (Figure 2). We found a significant difference in *Mytilus* spp. consumption among these species (t_{33} = -3.1, p = 0.004), again with *P. ochraceus* consuming more.

Littorina spp.

Based on average biomass consumed per day, the mean amount of *Littorina* spp. eaten by *P. ochraceus* was 0.03 ± 0.01 (SE) g per day and -0.02 ± 0.01 g per day for *L. hexactis* (Figure 1). We found the consumption of *Littorina* spp. by *L. hexactis* and *P. ochraceus* to have weak non-significance (t_{34}=-2.62, p=0.01).

Based on average individual consumption per day, *P. ochraceus* ate *Littorina* spp. at a rate of 0.74 ± 0.13 individuals per day. *L. hexactis* ate *Littorina* spp. at a rate of 0.22 ± 0.11 individuals per day (Figure 2). This pattern was weakly non-significant (t_{20} = -2.65, p = 0.02).

Semibalanus cariosus

P. ochraceus consumed a mean of 0.06 ± 0.01 (SE) g per day of *S. cariosus* when considering average biomass eaten. *L. hexactis* ate a mean of 0.08 ± 0.03 g per day of *S. cariosus* (Figure 1). We found no significance in this difference (t_{21}=0.73, p=0.48).

When considering average individuals consumed per day, *P. ochraceus* consumed a mean of 0.064 ± 0.15 individuals per day and *L. hexactis* consumed 0.26 ± 0.15 individuals per day (Figure 2). This difference also had no significance ($t_{19} = 1.3$, $p = 0.2$).

Summary

Overall, in the presence of *Littorina* spp. as a prey option, *L. hexactis* preferred *S. cariosus* significantly more than *Mytilus* spp. (p=0.005) (Figure1, Figure 2). In addition, *P. ochraceus* preferred *Littorina* spp. and Mytilus spp. significantly more than *L. hexactis* did (Figure 2).

Competitor Cue Influence

We found no significant difference in prey consumption when exposing both species to competitor cues (Figure 3, Figure 4).

When considering average biomass consumed daily with exposure to competitor cues, *P. ochraceus* did not consume any prey significantly more than another (*S. cariosus* t_{16}=1.26, p= 0.19; *Littorina* spp. t_{7}=-0.48, p=0.64; *Mytilus* spp. t_{7}=0.13, p=0.9). *L. hexactis* also did not consume one prey particularly more than another (*S. cariosus* t_{7}=-1.16, p= 0.28; *Littorina* spp. t_{6}=-0.26, p=0.8; *Mytilus* spp. t_{7}=-1.99, p=0.09).

When considering average individuals consumed daily, we found that *P. ochraceus* again did not consume one prey significantly more that the others when exposed to competitor cues (*S. cariosus* t_{11}=1.86, p=0.09; *Littorina* spp. t_{16}=0.14, p=0.89; *Mytilus* spp. t_{11}=0.79, p=0.45). *L. hexactis* also did not show a difference in prey consumption (*S. cariosus* t_{11}=-1.83, p=0.09; *Littorina* spp. t_{12}=0.3, p=0.77; *Mytilus* spp. t_{5}=-1.22, p=0.28).

Overall, these results indicate that no statistical significance can be established when comparing prey consumption of recruits exposed to competitor cues and those not exposed.

Discussion

Previous study into the diets of adult sea stars suggests that the diets of *L. hexactis* and *P. ochraceus* vary over several factors but by-and-large overlap (Mauzey 1968; Menge 1972; Menge 1974). The results of our study generally show this overlap, but they also give new insight into dietary choices of sea star recruits. In accordance with our hypothesis *P. ochraceus* and *L. hexactis* recruits did show preference towards particular prey.

The comparison of prey consumption within each sea star species showed *L. hexactis* having a preference for *S. cariosus* over *Mytilus* spp. and no preferential trend toward *Littorina* spp., reaffirming the results found by Mauzey (1968) in his study on *L. hexactis* adults. The adults showed a greater preference for barnacles overall. Together these studies suggest that *L. hexactis* maintains a palette for barnacles throughout its life history. *P. ochraceus* was shown to have no preference toward any of the prey species, which does not align with most studies done on adults. Mauzey's (1968) study shows a strong preference of *P. ochraceus* towards mussels, particularly *Mytilus californianus*. Further study could confirm these findings and suggest that *P. ochraceus* shifts its diet as it matures. This dietary ontogenetic shift was observed in *Heliaster helianthus* by Manzur et al. (2010). Although different prey was consumed over the size-classes, Manzur et al. attributed the shift to morphometric and mechanical restrictions as opposed to different prey preferences. *P. ochraceus* may experience a similar limitation at younger life stages, instead of actually preferring a different species.

P. ochraceus has previously been known to prefer mussels in adulthood (Mauzey 1968), which we see in our recruit data as well. The comparison of prey consumed between both species showed *P. ochraceus* having a greater preference for *Littorina* spp. and *Mytilus* spp. than *L. hexactis* did. This observation aligns with *L. hexactis*' preference for *S. cariosus*. While *P. ochraceus* is feeding on *Littorina* spp. and *Mytilus* spp., *L. hexactis* is feeding on *S. cariosus*. Menge (1972) describes how a competitive interaction dilutes the functional importance of *P. ochraceus* as a dominant community constructer and *L. hexactis* as a key predator of primary consumers. With *L. hexactis* feeding on the species least preferred by *P. ochraceus*, our study supports Menge's hypothesis that *L. hexactis* fills the niche left by the dominant *P. ochraceus*. In our study the species were kept in separate containers from each other with free reign on prey. No direct competition occurred. *L. hexactis* could be filling the vacant niche as a result of evolutionary development or out of true preference for *S. cariosus*. Knowing the basis of this niche partitioning will explain some aspects of the strength of *L. hexactis'* and *P. ochraceus'* interactions, which is currently unclear especially at the recruit level.

When larger starfish are absent, *L. hexactis* will widen its niche to include larger prey, suggesting that competition limits the diet of these adult starfish (Menge 1972). Our study found that recruits do not follow this pattern when exposed to competitor cues. This may be the result of not having enough data, as we only collected data for four day, or of not accurately testing competition. *L. hexactis* may not respond behaviorally to competitor cues. In this case, competition was not fully tested by this study, but future studies incorporating direct or indirect interactions may find more consistent results. Even if *L. hexactis* can respond to competitor cues, competition may not play a role at the recruitment stage. At this stage food may not be a limiting factor.

In correspondence with having a small collection of data, this study also did not see feeding from all of the recruits consistently, if at all. The low feeding rates could be the result of nutrient reserves within the specimens' pyloric caeca. *L. hexactis* and *P. ochraceus* have been found to survive as long as 48 weeks without feeding (Mauzey 1967). Chia (1969) found that after ten weeks of starvation nutrients were still highly detectable in the pyloric caeca of *L. hexactis*. Our specimens may have fed less than they would normally, under less stressful conditions, because they didn't need to eat to survive and they could have already had enough nutrients in their system to maintain adequate health.

Lab stress may have also played into our results. Taking the sea star recruits, which are already vulnerable in their natural rocky intertidal habitat, to our lab may have put them under extreme stress. There was no natural substrate in the containers we used, leaving the plastic and wire mesh as the only substrate available for the recruits to adhere to. The sea water circulation system in the tanks could not fully simulate the diurnal tides we see daily on the Oregon coast. Menge and Sutherland (1976) described in detail how predator exposure to stress causes sheltering during these periods, consequently reducing their feeding activity. A similar study by Petes, Mouchka, Milston-Clements, Momoda, and Menge (2008) showed how predatory sea stars exhibited higher mortality under stressful conditions. Their studies were conducted in the field; however those results could apply to the reactions we saw in our recruits.

This lab stress experienced by the sea star recruits could have also affected the prey in similar ways. These species would experience similar shock to the lack of natural substrate and abnormal water levels. As suggested by Petes et al. (2008) prey will use energy to defend themselves against stress which will result in greater susceptibility to predation. This means that the prey we used could have spent more energy adapting to the tanks than to protecting themselves from the recruits, which would give results deviant from natural dynamics.

As Mauzey (1968) warned, laboratory results alone are inconclusive. Sea stars have been proven to prefer different prey in the laboratory than what is preferred in the ocean; therefore observations from lab studies should be used in conjunction with field data to give greater meaning to the results (Mauzey 1968). Further study should be considered for this realm of marine science. Field studies should be pursued, focusing on solidifying recruit sea star diets and filling in the holes of sea star life stages. The data from this study can be used in conjunction with future field studies of similar data to appropriately establish necessary background records.

The findings of this study are applicable to a broader understanding of the dynamics of the rocky intertidal zone and potential system modeling for predictions of environmental effects. These results give a better understanding of the interspecific interactions between these two keystone species at younger life stages and how they choose prey. With longer more elaborate study, long-term predictions and can be made about populations for both the sea stars and their prey, and these results can be more strongly supported to create baselines for recruit sea star diets. This will help gauge the health of oceans and can be the starting off point to make predictions and models of how climate change could alter the rocky intertidal system.

Tank Number	**1**					
Species	*P. ochraceus*					
Treatment 1	B & M	B & M	B & M	B & M	B & M	B & M
Treatment 2	B & S	B & S	B & S	B & S	B & S	B & S
Treatment 3	S & M	S & M	S & M	S & M	S & M	S & M

Tank Number	**2**					
Species	*L. hexactis*					
Treatment 1	B & M	B & M	B & M	B & M	B & M	B & M
Treatment 2	B & S	B & S	B & S	B & S	B & S	B & S
Treatment 3	S & M	S & M	S & M	S & M	S & M	S & M

Tank Number	**3**					
Species	*P. ochraceus*			*L. hexactis*		
Treatment 1	B & M	B & M	B & M	B & M	B & M	B & M
Treatment 2	B & S	B & S	B & S	B & S	B & S	B & S
Treatment 3	S & M	S & M	S & M	S & M	S & M	S & M

Table 1. Three tanks were used in the study set up. Tank one contained 18 *P. ochraceus* recruits only. Tank two contained 18 *L. hexactis* recruits only. Tank three contained 9 *P. ochraceus* recruits and 9 *L. hexactis* recruits in order to simulate competition through chemosensory cues of one another. Within each tank there were three different prey treatments: 1-barnacles and mussels (B & M), 2-barnacles and snails (B & S), 3- snails and mussels (S & M). Tank one and two contained six replicates of each treatment. Tank three contained three replicates of each treatment for each species.

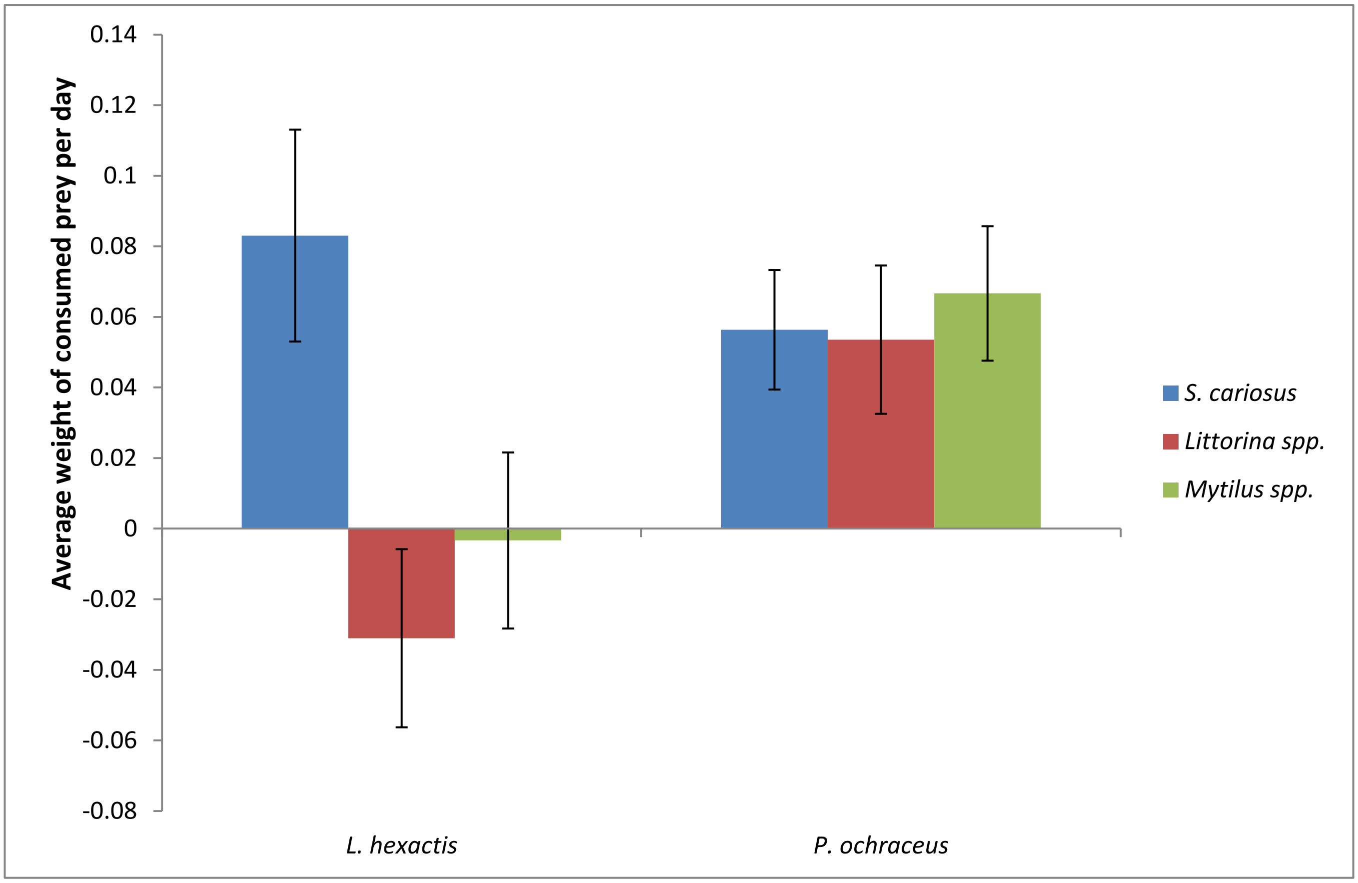

Figure 1. Average weight of prey consumed per day between *P. ochraceus* and *L. hexactis* recruits. Data are shown for L. hexactis and P. ochraceous both with and without exposure to competitor cues. Sample sizes were equal among both species. Bars are shown as means with standard error of biomass consumptions per day over four days for each prey species presented (*S. cariosus*, *Littorina* spp., *Mytilus* spp.).

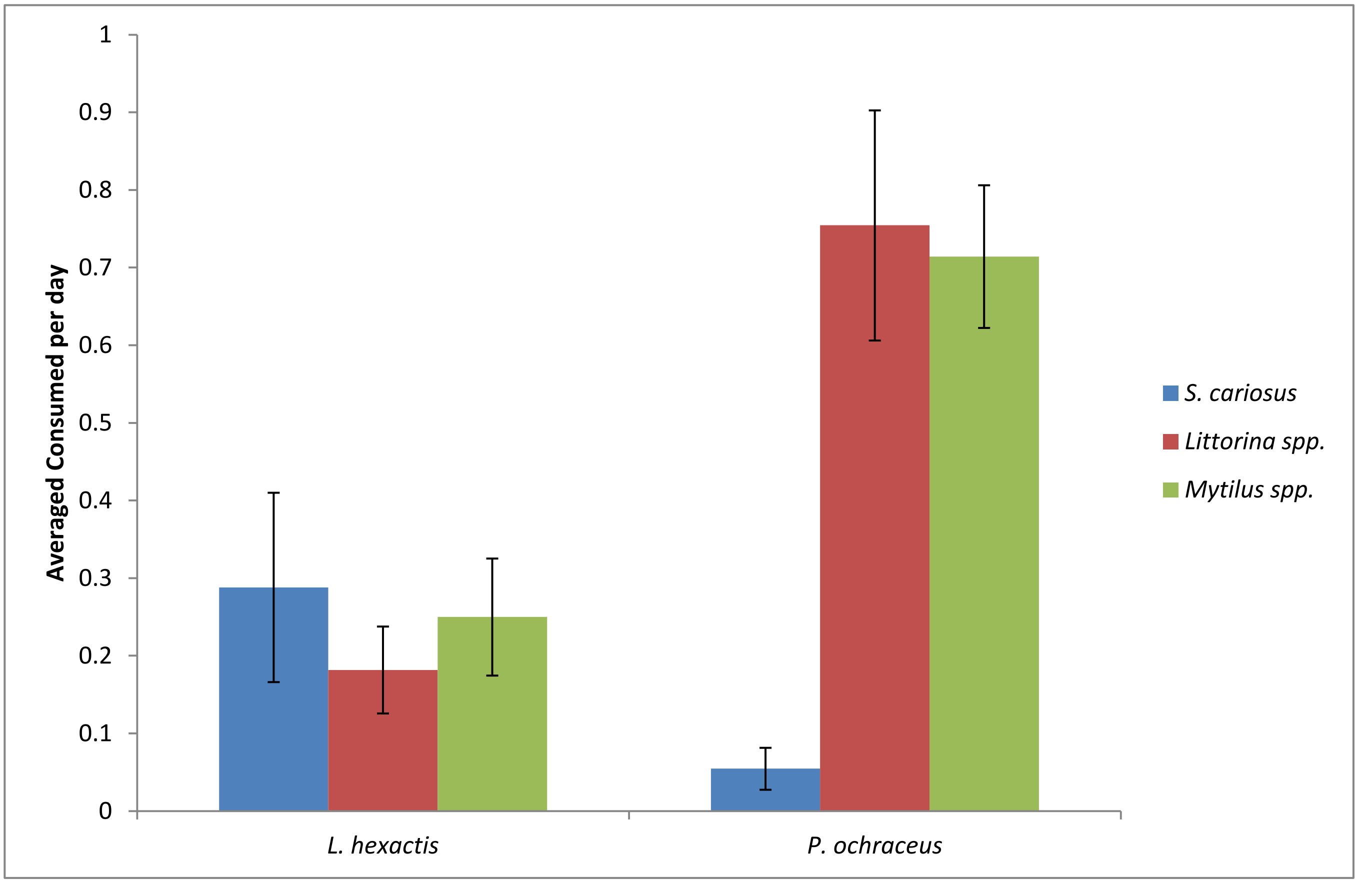

Figure 2. Average individual prey consumed per day between *P. ochraceus* and *L. hexactis* recruits. Data are shown for L. hexactis and P. ochraceous both with and without exposure to competitor cues. Sample sizes were equal among both species. Bars are shown as means with standard error of individual prey consumptions per day over four days for all prey options (*S. cariosus*, *Littorina* spp., *Mytilus* spp.).

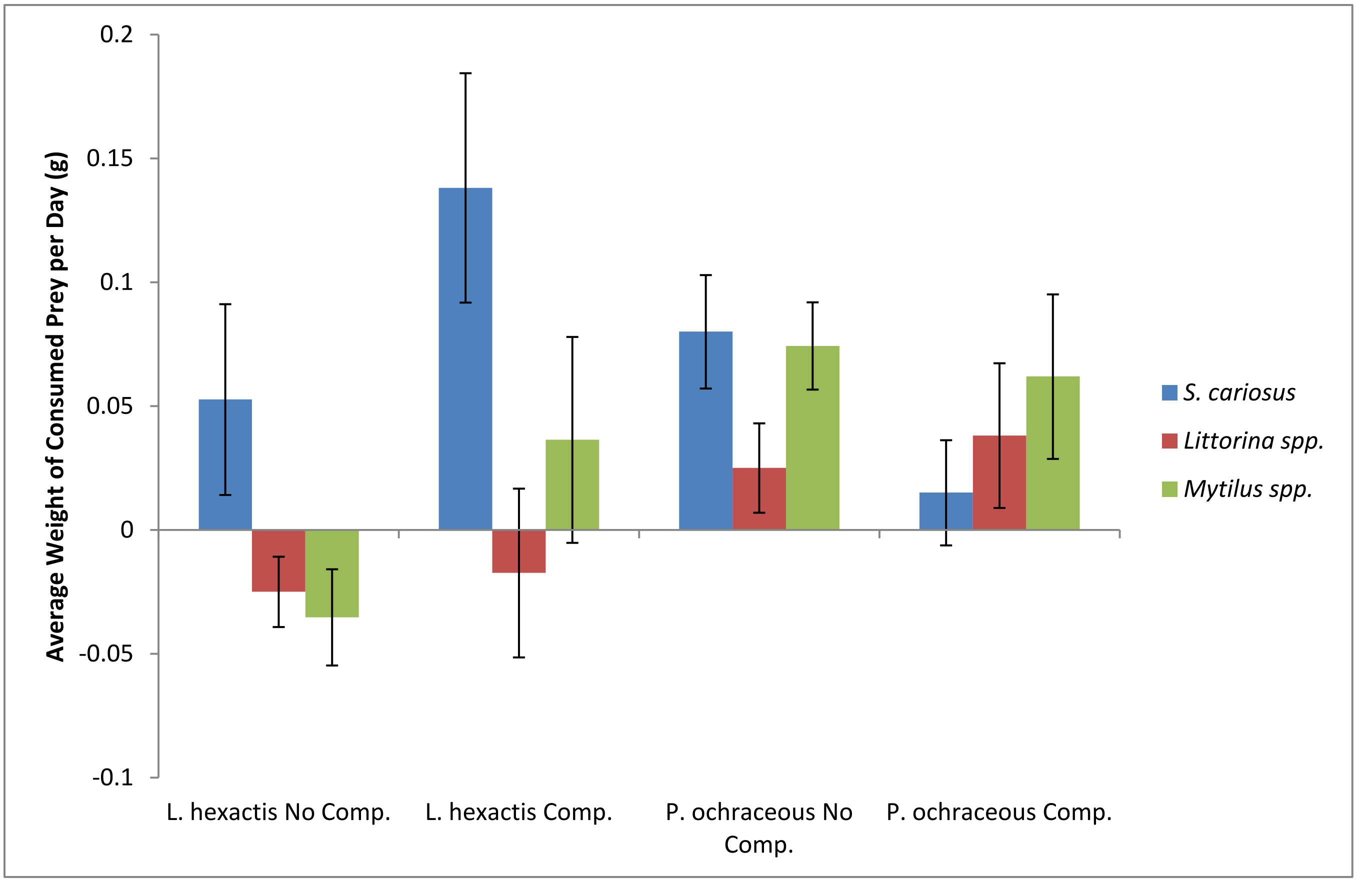

Figure 3. Average weight of prey consumed per day between *P. ochraceus* and *L. hexactis* recruits. Data are shown for L. hexactis and P. ochraceous when exposed and not exposed to competitor cues. Sample sizes were unequal between the two treatments (18 specimens for those not exposed and 9 for those exposed), but the difference was consistent between the two species. Bars are shown as means with standard error of biomass consumed per day over four days for all prey options (*S. cariosus*, *Littorina* spp., *Mytilus* spp.).

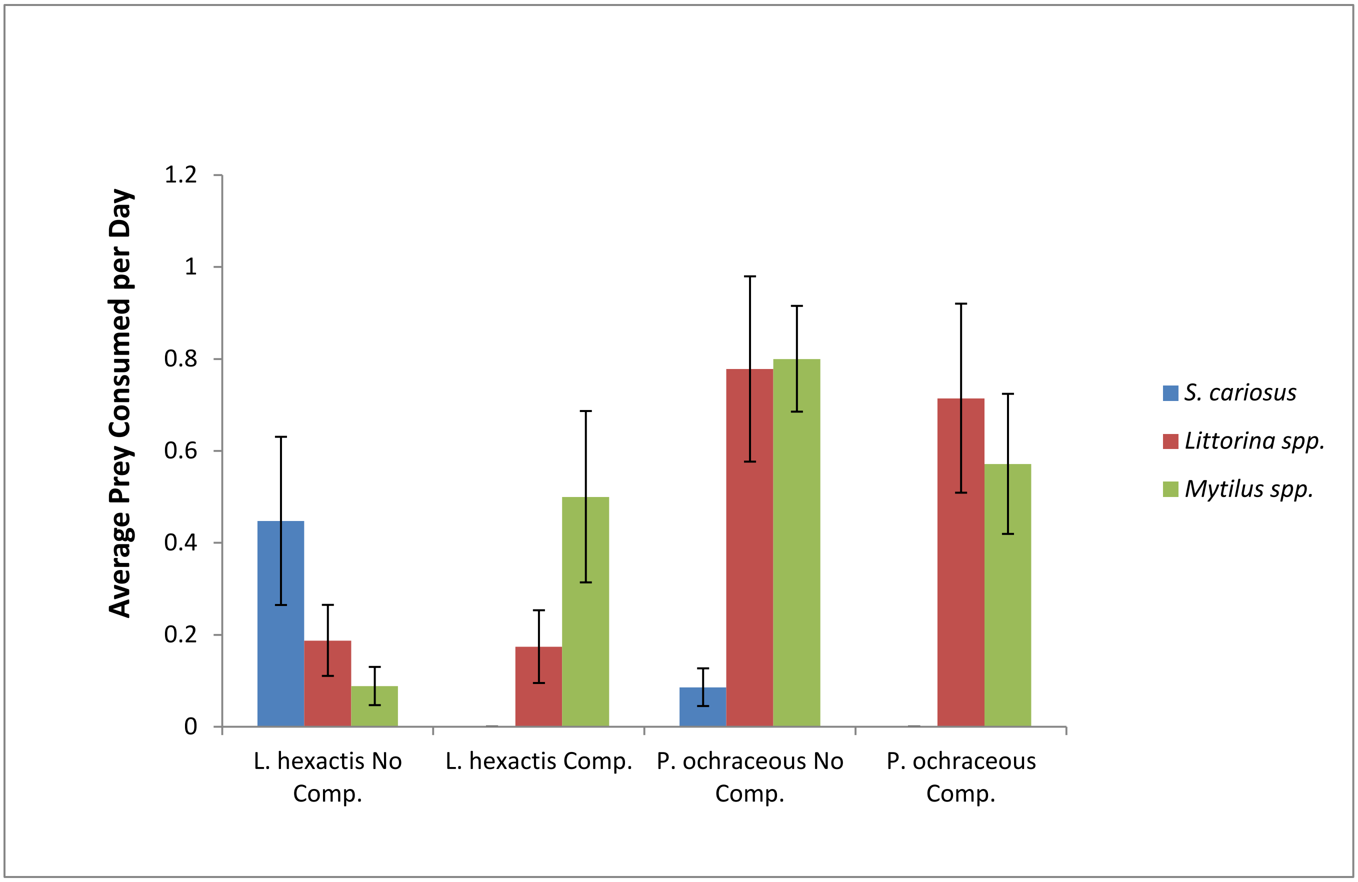

Figure 4. Average number of individual prey consumed per day between *P. ochraceus* and *L. hexactis* recruits. Data are shown for L. hexactis and P. ochraceous when exposed and not exposed to competitor cues. Sample sizes were unequal between the two treatments (18 specimens for those not exposed and 9 for those exposed), but the difference was consistent between the two species. Bars are shown as means with standard error of individual prey consumed per day over four days for all prey options (*S. cariosus*, *Littorina* spp., *Mytilus* spp.).

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