The Effects of Competition with Invasive Fouling Species on the Size and Abundance of the Native Olympia Oyster in the Elkhorn Slough

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Abstract The Olympia oyster, *Ostrea lurida*, the only oyster species native to western North America, may be threatened by competition for space and food with invasive fouling species. Although the intensity of competition could vary by tidal height, studies in different estuaries have found contradictory results. Therefore, an individualized study for a specific estuary is necessary to make sound oyster restoration decisions. In this study, I investigated whether resource competition with invasive fouling species limits Olympia oyster size and abundance in the Elkhorn Slough, an estuary in central California. I also examined how invasive fouler abundance and competition intensity varies with tidal height. I hypothesized that the Olympia oysters would be negatively affected by this competition and these effects would be greatest below mean lower low water, since this is where invasive fouling species would be most abundant. I employed two manipulative experiments, however, these studies yielded limited results due to a relative lack of oyster recruitment. I also conducted a mensurative experiment of the existing fouling community, which indicated that the oysters were negatively affected by interspecific competition, as oyster abundance was negatively proportional to that of invasive fouling species. Contrary to my predictions, oyster size was not affected by competition or tidal height. However, other significant tidal height effects were observed: in general, invasive species were most abundant in subtidal elevations, while oysters, though physiologically better suited for the subtidal, were most abundant at the intertidal.

Introduction

Over the last few decades, the rate of introduction of invasive species and the consequences of these invasions have greatly increased in the United States partly due to the growth of the human population, rapid transport of people, and various changes in the environment (Pimentel 2000). Introduced species not only have important economic impacts, but can also affect the abundance and diversity of native species in many areas (Cohen and Carlton 1998). Specifically, coastal habitats have become among the most invaded systems on the planet (Grosholz 2002). This is due in part to the introduction of invasive species for aquaculture, both intentionally and accidentally, and through international shipping between harbors (Wasson *et al.* 2001).

Since boat traffic and aquaculture can introduce species that have attached to ship hulls and to mollusk shells, these vectors frequently transport species adapted to hard substrates. In some California estuaries, where these are the principal vectors for invasive species introduction, studies have determined that hard substrates are more invaded than soft substrates (Wasson *et al.* 2005). This difference in the intensity of invasion could also be explained by the fact that many bays and estuaries do not have much natural hard substrate, and what hard substrate they do have has been added relatively recently by human activities, such as the construction of bridges and harbors (Heiman et al. 2008). The majority of native species in estuaries with these conditions are adapted to soft sediments, therefore, even when invasive species associated with these soft substrates are introduced into an area, they face greater competition and predation from native species and may not become established (Wasson *et al.* 2005).

However, there are some native species associated with hard substrate that may now be threatened by various invasive species, including the Olympia oyster, *Ostrea lurida* (=*Ostreola conchaphila*, Carpenter 1864), the only oyster native to western North America. The historical distribution of this oyster species extends from Alaska to Panama (Grosholz 2007), but is typically restricted to isolated and widely spaced bays and estuaries (Baker 1995). Several abiotic factors influence the distribution of Olympia oysters. The lack of hard substrate on which oysters can attach limits their abundance and growth in several bays (McGowan and Harris 2007). High sedimentation rates, caused by erosion from activities such as agriculture and dredging, can bury the oysters, and thereby reduce their growth and limit their recruitment (Barrett 1963). Olympia oysters are also not able to tolerate salinities below 15-25 parts per thousand for very long, and thus are affected by regular inputs of freshwater (Baker 1995).

The Olympia oyster's population has been reduced or nearly depleted in most estuaries over the last 150 years, possibly due to overharvesting (Baker 1995), greater tidal restriction, and increased erosion (Wasson 2008, pers. comm.), among other factors. The significant reduction in the Olympia oyster population may represent a significant loss to various estuarine environments because of the important ecological role of the Olympia oyster. Like many bivalve mollusks, Olympia oysters form clumps and beds by permanently attaching one of their valves on natural or artificial hard surfaces (Kirby 2004). Thus, they serve as "ecosystem engineers" by providing hard, complex structures that many species use as habitat (Coen et al. 2007). The presence of these oyster beds increases local species richness (Kimbro and Grosholz 2006) and may improve water quality by removing phytoplankton (Coen et al. 2007). In addition, Olympia oysters are a food source for various animals popular among humans for recreation, such as ducks, crabs, and rays (Baker 1995).

Because of their vital role, restoration plans are currently being considered or in effect for Olympia oysters in various estuaries (White *et al.* 2009, Wasson 2009, pers. comm.). In order to implement restoration plans for this species successfully, it is also necessary to understand the biotic factors that limit the distribution and abundance of the Olympia oyster (Young et. al 2005). Particularly, an important factor may be its competition with other fouling species, species that attach to hard substrates, for example, barnacles and mussels. A large percentage of the fouling species that may compete with these oysters are non-native. This competition takes several forms. One is interspecific competition for space in estuaries that have limited amounts of hard substrate (Osman 1977). If the non-native competitor attaches first to the hard substrate, a rock for example, this may prevent the oyster larvae from attaching and surviving on this location (Connell 1961). In addition, if both competitors are established, the species that grows fastest, in this case the non-native competitor will overgrow the one that grows slowest and usually cause the latter to die (Osman 1977). Another possible form of competition is for food. Both the Olympia oyster and its fouling competitors are filter feeders. As observed with assemblages of filter-feeding bivalves, it is possible that, at least on a localized level, there exists food depletion that affects the species' survivorship and growth (Peterson and Black 1987).

Previous studies have looked into the effects of interspecific competition with non-native fouling species. For example, Trimble (2007) found that invasive fouling species limited oyster survival by 50% and growth of the organisms by 20% in Willapa Bay (Washington, USA).

However, the intensity of competition may vary by tidal height. Trimble (2007) also found that oyster survival was higher at lower depths, and thus that the intensity of competition was likely greatest at higher tidal elevations. On the other hand, Bishop and Peterson (2006), in a study with another oyster species, found that competition intensity was greatest at lower tidal heights. In many estuaries, the non-native fouling species are present predominantly below mean lower low water (MLLW), the average of the lower of two low tides, determined over a 19 year period (Bishop and Peterson 2006). This is because above this area, they species experience physiological stress from being exposed to the air, which is especially damaging during times of the year when these fouling species are exposed the longest and when there are higher temperatures. Typically, oysters are thought to be less successful in the intertidal than below MLLW, since the oysters are covered by water and feed for a shorter period in the intertidal region. However, since the other fouling species are less tolerant to prolonged aerial exposure, the reduction in competition with fouling species in the intertidal would compensate for the costs of a reduced feeding time, and therefore the intertidal would be a suitable refuge from competition in which the oysters would be most successful.

These opposing conclusions may be due to certain differences between the two study locations, for example, the timing of the tides that may expose the invasive fouling species and the oysters to temperature extremes. Thus, an individualized study for a specific bay or estuary may be necessary to make restoration decisions. I conducted this study in the Elkhorn Slough, a coastal wetland located in central California which the Olympia oysters have occupied for thousands of years, and which maintains connectivity between the northern and southern California populations of these oysters (Wasson 2009, pers. comm.). Like many estuaries, it has been affected by a decline in the Olympia oyster population (Baker 1995), as well as invasions by fouling species (Wasson et al. 2005). While this estuary has native species that could compete with the Olympia oysters, these native fouling species have only been found near the mouth of the slough, where other factors prevent the establishment of Olympia oysters. Additionally, the Elkhorn Slough has strong tidal flushing along its main channel, which reduces the effects of sedimentation and freshwater input (Wasson et al. 2001), potential confounding factors for this study.

Wasson *et al.* (2005) found that 84% of sessile, or permanently attached, species cover on hard substrates in the Elkhorn slough was non-native. In particular, the growth and survival of

Olympia oysters appears to be limited by a non-native sponge, *Hymeniacidon sinapium,* and an invasive reef-forming worm, *Ficopomatus enigmaticus* (Fig. 1, Wasson and Castaneda 2008, elect. comm.). A recent study conducted at the Elkhorn Slough found that there was little evidence for competition negatively affecting oysters (Wasson, unpublished data). However, this study only looked at the high and low intertidal. Since non-native species, particularly the non-native sponge, have been observed to occur predominantly below MLLW, it is important to assess the effects of competition in this area.

Figure 1: Invasive Fouling Species on the Rocks of the Elkhorn Slough. Pictured right: invasive yellow sponge, *Hymeniacidon sinapium*, and on the upper right, an Olympia oyster close to being overgrown. Pictured left (image by K. Wasson): invasive tubeworm, *Ficopomatus enigmaticus*, and dead Olympia oyster at the center of the picture (upper valve missing)

Therefore, this study addresses the following research questions: How is the size and abundance of the Olympia oyster affected by resource competition with invasive fouling species? And what is the effect of tidal height on the abundance of invasive fouling species and the intensity of competition. I hypothesized that the native oysters would be negatively affected by this competition and would thus be smaller and less abundant. Additionally, I predicted that the invasive fouling species would be most abundant below MLLW, and therefore the intensity of competition would also be greatest in the subtidal. In answering these questions, I employed two manipulative experiments at three different tidal heights, one in which I manipulated the density of the non-native competitors and another which looked at the success of the Olympia oyster in attaching and surviving on surfaces already extensively covered with fouling species. In addition, I conducted a mensurative experiment (a study in which the system of study is not manipulated, but instead is observed with a rigorous design and statistical hypothesis) of the oysters and fouling species already present in the rocks of the Elkhorn Slough.

Methods

To assess the effects of interspecific competition on the Olympia oyster, I conducted my study at two sites within the Elkhorn Slough State Marine Reserve (Monterey County, CA, USA). Specifically, these two sites, Kirby Park and the South Marsh Footbridge (Fig. 2) are located in the mid-upper slough, where there is the greatest number and highest density of Olympia oysters in this estuary.

Figure 2: Partial Map of Elkhorn Slough, Monterey CA. Kirby Park is roughly located at 36º50'24N, 121º44'37W, and the South Marsh Footbridge at 36º49'16N, 121º44'14W. Image by Google Earth

To examine the effects of tidal elevation, I divided the settlement plates and survey quadrats into three groups. One of these groups corresponded to the mean lower low water (defined as 0 in the remainder of this study). The other groups are 1.5 feet above and 1.5 feet below the MLLW (called +1.5 and -1.5, respectively). Although this may appear to cover a small range, in the Elkhorn Slough, Olympia oysters are most abundant around MLLW and have an upper limit of about two feet above MLLW, and a lower limit that remains unknown (Wasson 2008, pers. comm.).

Manipulative Experiment A: Footbridge Experiment To determine if the presence of invasive fouling species affects Olympia oysters, I installed settlement plates on the footbridge in the South Marsh. Thirty standard split bricks (settlement plates) were labeled with a ceramic paint, drilled with a small hole in the middle fit a screw with an eyebolt, and suspended from the footbridge. Fifteen settlement plates were placed on each side of the footbridge and spaced about 50 centimeters apart, thus preventing the plates from colliding as frequently and breaking or falling. They were suspended with 3/8 inch rope and cable ties, which allowed the experiment to be easily removed once the study was concluded while being able to withstand the strong currents at this site. In addition, by suspending these settlement plates from a bridge, I was able to monitor and modify these plates at any time, rather than having to do this on the limited occasions where the tide was low enough to do these activities effectively.

To test the effects of the presence of fouling species, half of the settlement plates were cleaned by removing anything that was not an oyster with a standard scouring pad. To avoid removing oyster larvae in the process, only fouling organisms large enough to be conclusively identified as something other than an Olympia oyster were selectively removed, rather than cleaning the entire surface. The determination of which plates to clean was made randomly (of 10 selections made by a random number generator, the 5 highest numbers were placed in the clean group) so that five of the settlement plates at each tidal height were cleaned. This cleaning took place every two weeks, allowing enough time for new organisms to settle without overwhelming the cleaning process.

At the conclusion of this experimental component, I visually estimated the percent cover of various fouling species on the settlement plates, as well as the amount of bare space. In addition, I had planned to note the number of oysters and their size, measured along their longest axis. Only the underside of the bricks was analyzed, as this is where the oysters overwhelmingly settle as larvae (Hopkins 1935). However, all the surfaces of the plates, as well as the small area of the rope also covered with fouling species, were cleaned if in the clean group, to prevent the underside from being rapidly covered again. Although I had planned to run this experiment for eight months (July 2008-March 2009), I concluded this experimental component after five months due to a lack of oyster recruitment.

Manipulative Experiment B: Bare vs. Colonized Surface I had set up the same experiment on a floating dock located in Kirby Park, but due to an unforeseen removal of the dock, I instead deployed a similar experiment at both Kirby Park and the South Marsh. Fifteen additional bricks were deployed at each site in mid-August 2008, five at each tidal height. Instead of being suspended from a bridge or dock, the settlement plates were attached with cable ties to a 1-inch diameter PVC pipe that was then inserted into the ground to correspond with the right depth. This design allows the bricks to be suspended off the bottom, preventing the oysters from experiencing the possible negative effects of being covered in mud.

These settlement plates were assessed in March 2009, again visually estimating the relative abundance of invasive fouling species and the number and size of the Olympia oysters across the three tidal heights. I was also able to compare these plates to another set that had been deployed three months earlier (May 2008) at the same locations for a related experiment conducted by another researcher. These existing settlement plates were already covered extensively with non-native species by August, predominantly by the invasive tubeworm. By comparing these plates with the new ones with more bare space, I was able to assess whether the preemption of space by these invasive fouling species hinders the ability of Olympia oysters to survive and grow on a surface. However, my analysis was limited by the fact that the bricks deployed in May were not at all three tidal heights at both sites (Table 1). In addition, this design, unlike the first described, did not allow for regular cleaning and monitoring, as a tide of about 1 foot below MLLW would be necessary to access the settlement plates placed at -1.5.

Table 1: Settlement Plates Deployed in Kirby Park and South Marsh Footbridge in May and August 2008. # deployed indicates the number of settlement plates that were deployed at the specified site, tidal height, and date. Where # deployed in May does not match the # deployed in August, comparisons between bare and colonized plates could not be made.

		# deployed in May	**# deployed in August**
Kirby Park	+1.5	5	5
	0	5	5
	-1.5	0	5

	+1.5	0	5
South Marsh Footbridge	0	5	5
	-1.5	0	5

Mensurative Experiment Since the level of oyster recruitment varies drastically from year to year (Wasson 2008, pers. comm.), an observational component was instructive in addressing this research question in the event that no oysters attached to the settlement plates. I surveyed the existing fouling community in order to determine if there were correlations between the amount of non-native fouling cover with the size and abundance of the Olympia oysters at the three tidal heights. Using a stratified random sample, six quadrats were placed at each of the three tidal heights I was assessing. These quadrats were 50 centimeters on each side and had six intersecting strings, dividing the quadrat equally into sixteen smaller squares. I recorded the percent cover of all the fouling species present, as well as the number and size of the Olympia oysters. For quadrats with a very large number of oysters, where it would be excessively time consuming to measure all the oysters present, I only measured the oysters closest to the intersections of the strings of the quadrat, for a total of nine measurements. I also noted the number of dead oysters, and if possible, the cause of death, to determine whether overgrowth by invasive fouling species contributes significantly to oyster mortality. This mensurative experiment was completed in the South Marsh Footbridge rocks in early June 2008 and in the Kirby Park rocks in early July 2008.

Statistical Analysis I analyzed all the data collected from the experimental and observational study with a two-way analysis of variance (ANOVA) test, to account for interactions between tidal height and the treatment in the two experiments and between site and tidal height in the observational component. I used a Tukey's Honest Significant Difference (HSD) test to examine which tidal heights were significantly different from each other in the various measures I collected. I also used regression analysis to determine the strength of the correlation between the level of cover by invasive fouling species and the number and size of the Olympia oysters at the three tidal elevations. In addition, I employed a chi-squared test to analyze data from manipulative experiment B. All these statistical tests were performed with the statistical program JMP (v7.0.2/SAS Institute Inc./Cary, NC, USA).

Results

Manipulative Experiment A: Footbridge Experiment Oyster recruitment was not observed on any of the settlement plates. However, I was able to address the second research question concerning the effect of tidal height on the abundance of invasive fouling species. As expected, the percent cover of the invasive yellow sponge was greater on settlement plates that were not cleaned versus those that were cleaned periodically (2.54%±0.47 versus 0%, respectively). The abundance yellow sponge also varied significantly with tidal height (Table 2). It was most abundant at 0 (mean=2.00%±0.60) and absent at +1.5. The group of settlement plates that were not cleaned showed this same pattern, while those that were cleaned showed no significant difference in percent cover among the three tidal heights (Table 3). In contrast, invasive tubeworm cover was not affected by tidal height, but was slightly greater on plates that were cleaned (0.25%±0.07, Table 2).

Table 2: ANOVA results for percent cover of a yellow sponge, invasive tubeworm, unidentified bryozoan, and bare space in Manipulative Experiment A (Elkhorn Slough, July 2008-March 2009). Treatment refers to the response to the removal of the fouling species. n= 5

	Yellow sponge			Tubeworm			Unidentified Bryozoan			Bare Space		
	df	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**	**df**	**F**	**p**
Treatment	1	16.05	<0.01	1	6.38	0.02	1	360.87	<0.01	1	881.25	<0.01
Tidal Height	2	4.27	0.03	2	0.61	0.55	2	1.86	0.18	2	0.08	0.92
Treatment* Tidal Height	2	4.27	0.03	2	0.61	0.55	2	6.22	0.01	2	1.87	0.18

Table 3: Tukey's HSD results for the percent cover of yellow sponge and unidentified bryozoan in Manipulative Experiment A. Pairs within each organism that do not have the same letters are significantly different. n=5

	Cleaned			Not Cleaned		
	-1.5	**0**	**+1.5**	**-1.5**	**0**	**+1.5**
Yellow Sponge (Mean % cover)	<0.01[a]	<0.01[a]	<0.01[a]	3.75[ab]	4.50[b]	<0.01[a]
Unidentified Bryozoan (Mean % cover)	7.80[a]	1.60[a]	1.60[a]	57.50[b]	80.25[c]	75.60[bc]

Another fouling species, an unidentified colonial bryozoan (Fig. 3), also did not demonstrate a significant difference among the three tidal heights, but was much greater on plates that were not cleaned than those that were (Table 2). The only significant difference between tidal heights was within the settlement plates that were not cleaned (Table 3), in which the percent cover of this species was greater at 0 (mean=80.25%±4.67) than at -1.5 (mean=57.50%±4.67). The amount of bare space was also significantly different between the plates that were cleaned and those that were not (Table 2). The plates that were cleaned had an average percent cover of 94.47%, while those that were not cleaned only had on average 10.23% of bare space. However, the percent cover of bare space was not significantly different between the three tidal heights (Table 2).

Figure 3: Unidentified Bryozoan on Underside of Settlement Plate in Control Group, in the South Marsh Footbridge. Photo taken on 8/25/08, after four weeks of deployment.

Manipulative Experiment B: Bare vs. Colonized Surface In contrast to manipulative experiment A, the percent cover of yellow sponge was not significantly different between the newer and already colonized settlement plates in Kirby Park or between tidal heights (Table 4), and was completely absent from the plates in the South Marsh. Even within the group of the plates deployed in August, yellow sponge was not significantly different between the three tidal elevations (F=2.25, df=2, p=0.13). The invasive tubeworm was more abundant in this experiment than in manipulative experiment A. In Kirby Park, the percent cover of this invasive species was significantly higher at +1.5 than at 0, and also more abundant in plates deployed in May than those deployed months later (Table 4, 5). In the South Marsh, however, the invasive tubeworm was largely absent and was not significantly different between the colonized and initially bare settlement plates (F=1, df=1, p=0.35).

Table 4: ANOVA results for percent cover of yellow sponge, invasive tubeworm, bare space, and Olympia oysters in Kirby Park at 0 and +1.5. n=5

	Yellow sponge			Tubeworm			Bare Space			Olympia oysters		
	df	**F**	**p**	**df**	**F**	**P**	**df**	**F**	**p**	**df**	**F**	**p**
Bare/Colonized	1	1.00	0.33	1	18.94	<0.01	1	32.94	<0.01	1	3.28	0.09
Tidal Height	1	1.00	0.33	1	15.37	<0.01	1	52.16	<0.01	1	3.28	0.09
Bare/Colonized* Tidal Height	1	1.00	0.33	1	15.37	<0.01	1	51.61	<0.01	1	6.43	0.02

Table 5: Tukey's HSD test for percent cover of invasive tubeworm, bare space, and Olympia oysters in Kirby Park at 0 and +1.5. Pairs within each organism/factor that do not have the same letters are significantly different. n=5

	Bare		Colonized	
	0	**+1.5**	**0**	**+1.5**
Tubeworm (Mean % cover)	0.20^{a}	0.20^{a}	3.00^{a}	54.00^{b}
Bare Space (Mean % cover)	11.40^{a}	87.20^{b}	19.00^{a}	19.20^{a}
Olympia oysters (Mean % cover)	0.00^{a}	2.40^{b}	0.40^{ab}	$<0.01^{a}$

The percent cover of bare space was significantly greater in newer plates than those deployed in May in the South Marsh at MLLW (F=30.18, df=1, p<0.01, mean=43.60%±3.86 vs. 13.60%). In Kirby Park, the percent cover of bare space varied significantly with both tidal height and the date of deployment of the settlement plates (Table 4). The bare plates at +1.5 in Kirby Park had significantly more bare space than any of the other pairs (Table 5). Within the group of settlement plates deployed in August in both sites, the percent cover of bare space decreased with increasing depth (F=4.55, df=2, p=0.02).

Oyster recruitment was observed in this experimental study, but only in Kirby Park. The percent cover of live oysters was greater in the newer bricks than those that were already colonized by other fouling species at +1.5, but were not significantly different at 0 (Table 5).

Within the settlement plates in Kirby Park at +1.5, the proportion of "bare" plates with oysters was significantly higher than the proportion of colonized plates with oysters(3/5 vs. 0/5, respectively, χ^2=4.29, df=1, p=0.04). Among all the plates deployed in August in Kirby Park, the percent cover of the live oysters varied significantly with tidal height: oysters were most abundant at +1.5 (mean=2.40%±0.42), than at 0 and -1.5 (mean<0.01%, F=5.43, df=2, p=0.01). However, the average size of the oysters was not significantly different between the colonized and bare settlement plates, or between tidal heights (F=1.50, df=1, p=0.24).

Mensurative Experiment Most of the oyster measures I took for this mensurative experiment were significantly different between the three tidal heights. For example, the average size of the oysters was significantly greater at -1.5 than the other two tidal heights (Fig. 4). The percent cover of live oysters was greater by 6.25% at 0 than +1.5 and -1.5 (Fig. 5a). The number of live oysters demonstrated a similar pattern (F=35.34, df=2, p<0.01). The number of dead oysters was also significantly different between tidal heights, with +1.5 containing the largest number dead oysters (mean=8.92±1.16) and -1.5 the least (mean=3.92, F=4.80, df=2, p=0.02). The percent of dead oysters that were overgrown by invasive fouling species did not show a significant difference between tidal heights (F=0.98, df=2, p=0.40), but had inadequate power (0.183).

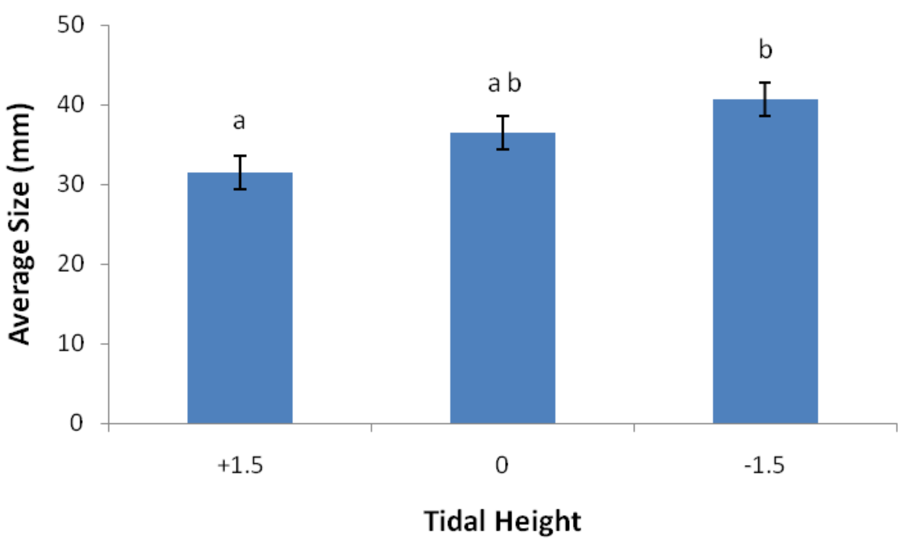

Figure 4: Average Oyster Size at Three Tidal Heights at Kirby Park and the South Marsh, June-July 2008. Bars indicate ± 1 standard error, SE(+1.5,0)=2.11, SE(-1.5)=2.46. Tidal heights with different letters above the bars are significantly different based on a Tukey's HSD test. ANOVA: n=6, df=2, F=4.35, p=0.02.

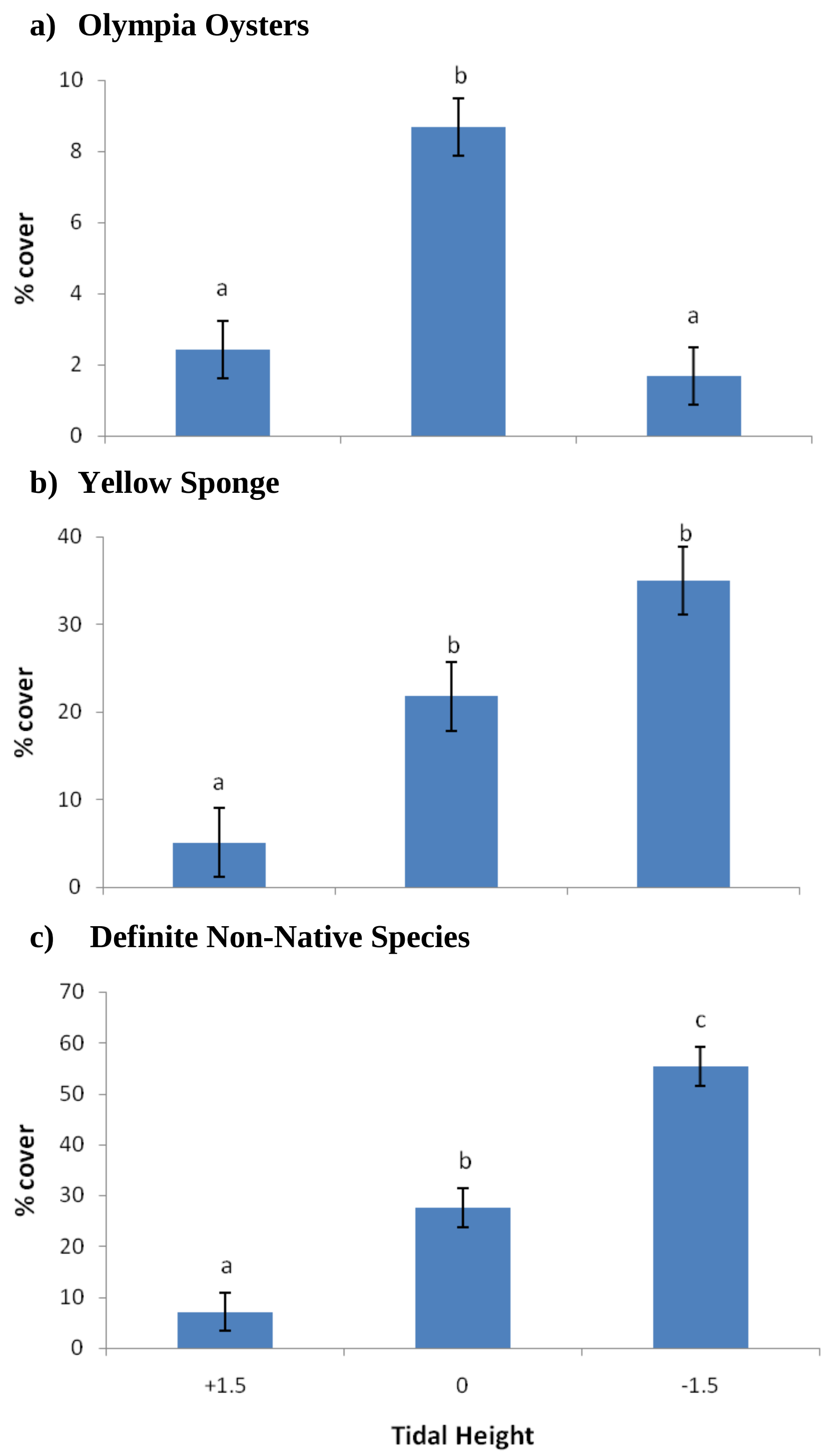

Figure 5: Percent Cover of Olympia Oysters, Yellow Sponge, and Definite Non-Native Species at Three Tidal Heights in Kirby Park and South Marsh, June-July 2008. Bars indicate ± 1SE. The tidal heights with different letters above the bars are significantly different based on a Tukey's HSD test. a) ANOVA: n=6, df=2, F=22.26, p<0.01, SE = 0.81, b) ANOVA: n=6, df=2, F=14.69, p<0.01). SE=3.91, c) ANOVA: n=6, df=2, F=40.84, p<0.01). SE=3.79

The percent cover of yellow sponge was also significantly different between the three tidal heights. Yellow sponge was most abundant at 0 and -1.5 (Fig. 5b). Invasive tubeworm was relatively prevalent in Kirby Park, but absent from the rocks by the South Marsh footbridge, and was not significantly different between tidal heights (n=6, df=2, F=0.15, p=0.86). Although not all of the fouling species found within a given quadrat could realistically be conclusively determined as native or non-native, the percent cover of definite non-native sessile animals showed a significant trend in tidal height, with the greatest amount of cover at -1.5 (Fig. 5c). Bare space was also significantly different between the tidal heights (n=6, df=2, F=12.59, p<0.01). The percent of bare space was greatest at +1.5 (mean=51.25%±3.34), and decreased with increasing depth (mean(0)=26.67%, mean(-1.5)=12.75%).

Olympia oyster size and abundance may be influenced by the percent cover of invasive fouling species, in general and specifically yellow sponge and invasive tubeworm, and the percent cover of bare space. However, only the percent cover of yellow sponge (Fig. 6) and definite non-native species (Fig. 7) demonstrated a significant negative correlation with the percent cover of live oysters.

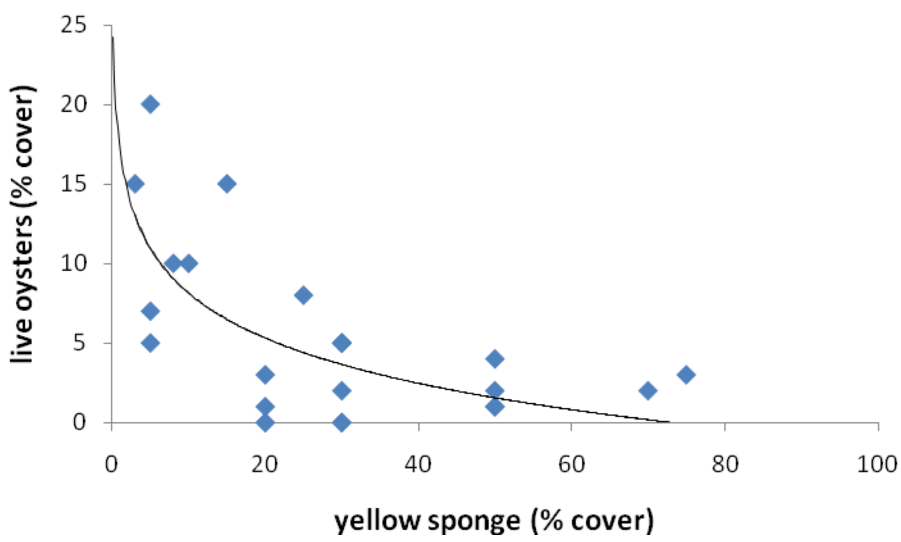

Figure 6: Percent Cover of Yellow Sponge vs. Percent Cover of Live Oysters at Tidal Heights 0 and -1.5. log(y)=2.25-0.03x, r^2=0.42, p<0.01

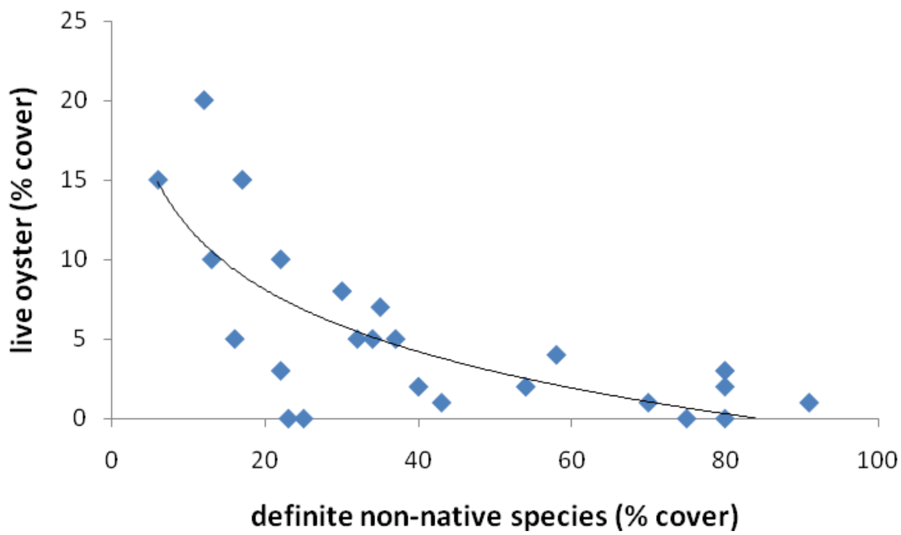

Figure 7: Percent Cover of Definite Non-native species vs. Percent Cover of Live Oysters at Tidal Heights 0 and -1.5. $\log(y)=2.59-0.03x$, $r^2=0.61$, $p<0.01$

Discussion

The three components of this study found significant results that addressed part or all of my research questions. Manipulative experiment A indicated that tidal height had an effect on the abundance of invasive fouling species and the bare space available for oysters to settle in the absence of these invasive foulers, but did not address the effects on Olympia oysters due to a lack of oyster recruitment. In the second manipulative experiment, both the oysters and some invasive fouling species were also found to vary with tidal height. Additionally, the relative success of the oysters on the bare versus colonized bricks indicated that the preemption of space affected oyster abundance, but not size. The mensurative experiment yielded some of the most significant results, indicating that the abundance both the Olympia oysters and the non-native fouling species varied with tidal height. Furthermore, as suggested by their negative correlation, Olympia oysters are less abundant in the presence of these invasive species, particularly below MLLW, though oyster size was not affected in this study.

The results the manipulative and mensurative experiments supported some of the study's hypotheses, but not all. I hypothesized that Olympia oysters would be less abundant as a result of resource competition with invasive fouling species. Since no oysters settled on the bricks on the South Marsh Footbridge, I was not able to test this hypothesis with manipulative experiment A.

However, the large difference between the amount of bare space on the settlement plates that were not cleaned versus those that were, a difference of nearly 85%, indicated that the oysters, had they been present, would have faced intense competition for space on settlement plates where the invasive fouling species were not removed. This result may have been confounded by the dominance of the unidentified colonial bryozoan on the settlement plates that were not cleaned. I have observed that this fouling species is abundant around bridges and other man-made structures in the Elkhorn Slough, but not on the rocks, even the ones in close proximity to these structures. Therefore, it is unlikely to compete with Olympia oysters under natural conditions and is instead a consequence of the experimental design.

Manipulative experiment B provided stronger support for this hypothesis, as oysters did recruit on the settlement plates for this component. The Olympia oysters present on these plates were all relatively small, indicating that the period of recruitment took place much later than in previous seasons in the Elkhorn Slough (Wasson 2009, pers. comm.). Still, the difference between the abundance of oysters between the bare and colonized plates indicated that competition for space between Olympia oysters and invasive fouling species could occur through the preemption of space by the fouling competitors. The settlement plates deployed later had a greater percent cover of Olympia oysters where oyster recruitment occurred than the plates that were already colonized by invasive fouling species, particularly the invasive tubeworm (Table 4, 5). This was also indicated by the amount of bare space, which was greater in the more recently deployed settlement plates. The percent cover of the Olympia oysters in the mensurative experiment, as it was negatively correlated with the percent cover of invasive fouling species (Fig. 7), including the invasive yellow sponge (Fig. 6), also supported the hypothesis that oyster abundance would be negatively affected by competition with invasive fouling species.

However, the second hypothesis to this research question, that oyster size would also be affected by this competition, was not supported by either of the components where oysters were present. In manipulative experiment B, average oyster size was not significantly different between the bare and colonized settlement plates. Contrary to my prediction, the mensurative experiment revealed that average oyster size was greatest in tidal heights where invasive foulers are most abundant, and was not correlated with the percent cover of definite non-native fouling species.

For this study's second research question, I hypothesized that invasive fouling species would be most abundant below MLLW, and thus this is where interspecific competition between the oysters and the invasive foulers would be most intense. In general, all three studies supported this hypothesis, although this did not hold true for all invasive fouling species at both Kirby Park and the South Marsh Footbridge. In manipulative experiment A, located only at the South Marsh Footbridge, yellow sponge was found to be present almost exclusively below MLLW (Table 3). While I was not able to test whether Olympia oysters are negatively affected by this competition, the subtidal distribution of the yellow sponge found in this experiment supports the hypothesis that if competition occurs, the oysters would face the greatest competition from yellow sponge at lower tidal heights. However, the invasive reef-forming worm did not show this strong tidal influence (Table 2). The amount of bare space also did not significantly vary with tidal height, but again, this may have been confounded by the presence of the unidentified bryozoan.

Results from manipulative experiment B largely did not support this hypothesis. In this component, yellow sponge did not vary by tidal height in this experimental study. While the invasive tubeworm was found to vary by tidal height (Table 4), this invasive fouling species was more abundant 1.5 feet above MLLW than at MLLW (Table 5). Similarly, the percent cover of live oysters was also greatest above MLLW in this manipulative experiment (Table 5), which was contrary to the prediction that the oysters would be physiologically restricted from higher tidal elevations. One possible reason for this distribution is that the tidal height was estimated, since tidal height data was only available at the mouth of the slough, not at the two sites where I conducted this study, and therefore these settlement plates may have been placed at an incorrect tidal height. In addition, within the settlement plates at 1.5 feet above MLLW, the cover of live oysters was greater in “bare” plates than the ones that were colonized, indicating that my hypothesis for that the preemption of space by invasive fouling species limited oyster abundance was correct at this tidal height. However, this same pattern was not observed at MLLW (Table 5). This difference, and the unexpected distribution of the Olympia oysters above MLLW, may be explained by the fact that the settlement plates at +1.5 were placed next to large cement pipes extensively covered by the invasive tubeworm, and to a lesser extent by Olympia oysters, while the ones at MLLW were not. Therefore, a future area of research could be to examine whether Olympia oysters prefer to settle in areas where there are oysters already present within a short distance.

The study of the existing oyster populations revealed various trends in relation to tidal elevation that supported this hypothesis. The Olympia oysters were most abundant at MLLW than at the other two tidal heights (Fig. 5a), since their abundance is likely limited above MLLW by the shorter immersion time, and thus shorter feeding time, and limited below MLLW by the presence of invasive fouling species. The number of dead oysters, greater above MLLW than below, suggested that physical factors had a greater effect on oyster mortality than the presence of invasive fouling species. The subset of those that were overgrown also did not show the pattern I predicted in my hypothesis, which could be an indication that competition occurs mostly through preemption of space, rather than after the oysters are established.

The largely subtidal distribution of invasive fouling species also supported this hypothesis. Yellow sponge was again most abundant below MLLW (Fig. 5b), and was negatively proportional to the cover of live oysters. The invasive tubeworm, on the other hand, did not show a strong response to different tidal elevations and was limited to Kirby Park. Still, the overall distribution of definite invasive fouling species was strongly dependent on tidal height and was most abundant below MLLW (Fig. 5c), and showed a negative correlation with the cover of live oysters (Fig. 7). This general distribution of invasive fouling species in the Elkhorn Slough can therefore explain why Olympia oysters were not more abundant below MLLW, despite the fact that at this tidal height, they would be protected from aerial exposure and be able to feed for a longer period of time.

My hypothesis the size of the Olympia oysters would also be affected by tidal height and competition with invasive fouling species found less support. In mensurative experiment B, the size of these oysters did not vary between tidal elevations or between the bare and colonized plates. The mensurative experiment found that oyster size did vary with tidal height, but not as I had predicted, since average oyster size was not significantly different between MLLW and 1.5 feet below MLLW (Fig. 4). The emersion time of the oysters may be a confounding factor in this instance. Although amount of bare space was much lower below MLLW than at the other two tidal heights, presumably the oysters would have still had room to grow in this tidal elevation, where they were immersed in water for the longest period of time. Nevertheless, in order to know with greater certainty whether competition with invasive fouling species reduces oyster size, it would be necessary to conduct a study similar to manipulative experiment A.

The study I conducted did concur with the Bishop and Peterson (2006) study, which also found that oysters were most successful in the intertidal, since they are largely protected from competition with invasive fouling species at this tidal height. Like the Trimble (2007) study, my investigation also indicated that interspecific competition limited oyster abundance (Fig. 6, 7), but his study found that oyster size was also reduced, while mine did not. In the question of the effects of tidal height, our results are contradictory, as Trimble determined that oysters were most negatively affected at higher tidal elevations. These opposing conclusions are most likely due to differences in the timing of the tides between our study sites, and the intertidal freezes in Willapa Bay that could limit oysters from higher tidal levels at that location, but not in Elkhorn Slough (Wasson 2009, pers. comm.). Finally, the results from my study also concurred with a previous study in the Elkhorn Slough (Wasson and Castaneda 2008, elect. comm.), both suggesting that the yellow sponge and invasive tubeworm limits oyster abundance in the Elkhorn Slough.

More broadly, the conclusions from this study also largely agree with those of studies examining space and food competition with other species. Connell (1961), in his study of the distribution of two barnacle species, also selectively removed a competing species at different tidal heights. His results suggested that both physical conditions and biotic interactions affected the distribution and mortality of the barnacle *Chthamalus stellatus*. In addition, this species shifted to the intertidal because it was able to tolerate aerial exposure and higher temperatures than its competitor was. Buss (1979) also examined competition, using two bryozoan species as his study organisms. Since these bryozoans, like the Olympia oysters, are sessile species, the space they occupy cannot be separated from the food they consume. Therefore, he suggested that competition for food between suspension feeders is a mechanism through which they compete for space; as one organism overgrows a slower growing one, it may be interfering with the overgrown species' access to food. While overgrowth was not shown to be a significant factor liming Olympia oyster abundance in this study, it is important to note that while I did not test the effects of food competition directly, as Buss (1979) indicates, this is linked to competition for space for sessile organisms.

This study helped to fill the gap in knowledge in expanding on the recent study conducted at the Elkhorn Slough (Wasson, unpublished) by examining tidal heights below MLLW, thus including the depth at which invasive fouling species were found to be most abundant. While this study did not find evidence for competition, my study did find evidence for competition across the three experimental components. In addition, this study detected a large interannual variability in oyster recruitment in the Elkhorn Slough, and low oyster recruitment previously only found in only two out of six sites examined in the Elkhorn Slough (Wasson, unpublished).

For future research examining these questions, I would suggest avoiding bridges and similar structures to prevent the establishment of the unidentified bryozoan that was a confounding factor in manipulative experiment A. Instead, a design similar to experiment B would be more effective, though with larger PVC pipes and longer rope to still allow for regular cleaning regardless of the tide. Studies with this design, however, should be done further away from cement pipes or other structures, especially if these are only present at some tidal heights, which have significant coverage by oysters or invasive fouling species, to avoid the possible confounding factor in manipulative experiment B.

Another future area of research is to examine these research questions at different times of the year. Seasonality was a factor in Bishop and Peterson's (2006) study with Simonue oysters because the changes in temperature between seasons affected the distribution of the fouling species. In addition, I noticed about three months after conduction the observational study that the yellow sponge near the South Marsh footbridge covered a greater area further up in tidal elevation, including well above MLLW. Therefore, it may be instructive to conduct this study across the seasons using these same procedures. Another possibility is to monitor a few small plots at different tidal heights, tracking changes in cover of invasive species and in Olympia oyster number and size over a prolonged period of time. Finally, future studies should take place at additional sites in the slough. The differences in the importance of the yellow sponge and tubeworm, as well as the level of oyster recruitment between Kirby Park and the South Marsh Footbridge indicate that there are significant site differences within the Elkhorn Slough. Site differences in this slough were also noted in the study by Wasson (unpublished), and thus future studies should examine more sites in order to better assess the threat invasive species may pose to the Olympia oyster.

The results from this study indicated that there are significant trends in the distribution of various species and provided evidence for the competition with invasive fouling species negatively affecting Olympia oysters, especially at lower tidal elevations. This built on the existing knowledge about the effects of tidal height on the interaction between these fouling species. In addition, it provided a trial run for different methods of addressing this question that could be repeated in future seasons with greater oyster recruitment. While it is not possible to generalize these results to all other estuaries, by detecting the high interannual variability in oyster recruitment and finding evidence for the preemption of space by invasive competitors limiting oyster abundance, this study will influence plans to restore the Olympia oyster population in the Elkhorn Slough.

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