Distribution and dynamics of shell-boring *Polydora* on *Crassostrea gigas*

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Abstract:

The proportion of live *Crassostrea gigas* infected with Polydorid spionid worms was surveyed at several locations around San Juan Island. Thirty infected *C. gigas* were collected from the Argyle Lagoon locations and worm galleries and holes on the inside and outside of shells were counted and assigned to a shell region. The proportion of borings that penetrate the inner layer of nacre was calculated. The area of each shell region was determined to find the density of worm populations and shells were cut to measure the thickness of different regions. Oyster populations with the highest proportion of infected individuals also had higher per-shell population densities. The proportion of nacre invasions remained constant as the population density of worms varied. Although the average thickness of the whole shell did not affect the proportion of inner nacre borings, the adductor scar and hinge regions showed a negative correlation between nacre bore holes and thickness.

Introduction:

The surfaces of large marine organisms offer a substrate for other benthic organisms. Several species of invertebrates and algae are known to etch or bore into the shells of bivalves such as clams, mussels, and oysters. This process is known as "fouling" and while it does not always lead to mortality for the host organism, in aquaculture farms, high levels of fouling organisms may decrease the marketability of product shellfish(Oakes & Fields, 1996). The study of such interactions could be of economic use.

The oyster, *Crassostrea gigas* is an imported species that has likely spread from the Wescott Bay Oyster Farms and other aquaculture facilities along Northern Salish Sea. *C. gigas* were introduced to the West coast of the US from Japan in the 1920s as a commercial species. Although it was originally believed that the oysters would not be able to survive in the introduced environments due to colder water temperatures in the introduced habitats, feral oyster communities can often be found in areas surrounding aquaculture facilities(Guy & Roberts, 2010). Wheras the polydorids will colonize other bivalves, they are common in the oysters living Argyle Lagoon and their range may depend in part on the distribution of *C. gigas*.

Some species of spionid polychaete worms in the genus *Polydora* bore into the shells of various bivalves and can be identified by two characteristic palps that they expose from boring holes and tubes(Kozloff & Price, 1996). Polydorids are characterized by hook shaped neuropodia after the sixth setiger and a modified fifth segment, which is enlarged and bears a pair of rows of giant setae(Light, 1978). As in all members of the family Spionidae, they have two long, grooved tentacles which they can use to either suspension feed or deposit feed. While the means by which Polydorids are able to burrow into calcium carbonate is still not fully understood, the worms use acid secretions in the boring process(Haigler, 1969). Scanning electron microscope images of the inside surfaces of bored holes have also suggest that the worms scrape the internal walls of the boreholes(Sato-Okoshi & Okoshi, 2000).

An uncertain aspect of the mollusc-polychaete interaction is whether it is detrimental to the host organism. A recent study testing the breaking strength of *Littorina* snail shells that had been inhabited by polydorids found that fouled shells were significantly more susceptible to breaking(Buschbaum, Buschbaum, et al., 2007). The calcium carbonate structure of the shells of *Littorina* does, however, vary from that of oysters. SEM photographs show several shell microstructures that varied between mollusk species and among different layers of individual shells(Sato-Okoshi & Okoshi, 2000). The shells of the Littorinidae have "crossed lamellar" microstructure in which the calcium carbonate forms elongate crystals organized into pairs of rows of sheets at a specific angle to the surface of the shell(Wilmot, Barber, Taylor, & Graham, 1992). Oyster shells differ by forming much weaker "foliate" microstructure that consists of a single layer of tilted crystals(Sato-Okoshi & Okoshi, 2000). It may be of interest to see if polydorid fouling similarly weakens this second type of shell microstructure.

In this study, oysters infected with polydorids were surveyed at several locations around San Juan Island to gauge the prevalence of the interaction. In the lab, I surveyed the density of worm populations in shells and the proportion of the population that enters the nacre of the shell to determine if dense infestations can compromise the strength of the shells.

Methods:

Distribution of Polydora-Crassostrea

Oyster populations were surveyed for the presence of *Polydora* borings by counting oysters with and without conspicuous signs of boring along the shore of four locations around San Juan Island including Friday Harbor Labs, Argyle Lagoon/North Bay, English Camp, and Wescott Bay Oyster Farms. Oysters were counted by tallying them in categories of either "bored" or "unbored" individuals. Oysters were tallied as "bored" if there were visible signs such as exposed galleries (burrows that had been eroded away, leaving grooves in the surface of the shell), small tubes, or boreholes. Due to high population densities at the oyster farm and in Argyle Lagoon, a survey of a haphazardly selected portion of the population was taken (115 individuals at the oyster farm and 82 individuals in Argyle Lagoon). At all other locations, all individuals were counted and tallied in the same way. Argyle Lagoon and Wescott Bay Oyster farms were also subdivided into four sites within each location. Locations were then ranked in order of highest to lowest general oyster population densities. All surveys were taken during low tide over the course of one week.

Polydora infestations into inner nacreous region of oyster shells

Thirty oysters showing conspicuous signs of boring (Fig.1) were collected from Argyle Lagoon/North Bay. They were brought back to the lab and placed in a sea table and watched for the tentacles of living worms emerging from bore holes. The oysters were then placed in a 0.79M solution of magnesium chloride mixed with equal parts seawater for several minutes to relax them as well as any attached organisms. Oysters were then boiled in tap water until they opened, whereupon the valves were separated and tissue removed. I marked the inside of each valve with a pen, dividing them into four regions (Fig.2):

1. Hinge: from one side of the hinge connection to the other, going around a deep point in the concave portion of the shell
2. Adductor scar: the kidney-shaped region at which the adductor muscle connects to each valve
3. Margin: the outer region running around the perimeter of the shell from the edge to where the peripheral ruffles smooth out
4. Other: the region surrounded by the margin and hinge regions, excluding the adductor scar

The inside divisions were traced onto a transparency that was used as a guide for marking the outer surface of the shells so that the markings on the inside and outside of the shell represented the same regions. The areas of the shell and its regions were calculated by taking a picture of the inner side of each valve behind a centimeter grid drawn on a transparency and counting the approximate number of squares contained in each region. The thickness of each region of the upper valve of each shell was also found by cutting across the width of the valve with a tile cutter, through the margin, other, and adductor scar regions and measuring them with calipers. The thickness of the hinge region could be measured with calipers without cutting.

Wormholes, galleries, and tubes were then counted within each region on both sides of each valve and recorded. Because very few worms actually penetrated the inside of the shell, galleries that could be seen through the nacre were also counted along with worm-shaped mud blisters (discolored bulges).

Results:

Distribution of Polydora-Crassostrea

Signs of *Polydora* boring were found in all four sites where *Crassostrea* were surveyed. The highest proportion of spionid infestations occurred in sites within Argyle Lagoon and Wescott Bay Oyster farm, whereas lowest were at Friday Harbor Labs and English Camp (Table 1). Ranking the subdivisions of the locations from highest to lowest observed oyster density and graphing the percent of the population infected by polydorids, I found that polydorid infections become more prevalent as population density increases (linear regression, R^2=0.568, p=0.012)(Fig. 4).

Polydora infestations into inner nacreous region of oyster shells

By comparing the percent of worm galleries that entered the inner nacre and the area of the valve, I found that the percent nacre invasions did not vary with the overall size of the oyster shell (Linear regression, R^2<0.001, p=0.886)(Fig.5). The percent nacre invasions also did not vary with density of borings on the outer surface of the shell, indicating that the proportion of nacre invasions does not vary with worm population density (Linear regression, $R^2 < 0.01$, p=0.459)(Fig.6).

Among shell regions, the most nacre invasions were observed on the adductor scars and the fewest on the hinge (Fig.8). Between the two regions, the number of invasions on the adductor scar was significantly higher than on the hinge (ANOVA, p <0.001)(Fig.9). The thickness of the shell at the adductor muscle was also significantly less than at the hinge (ANOVA, p <0.001)(Fig.9).

The overall average thickness of each shell correlated weakly with the percent of nacre invasions (Linear regression, R^2 = 0.163, p = 0.022)(Fig. 10). While the largest number of invasions was typically in the adductor scar and the percentage of worms in the nacre of the adductor scar was less in shells with thicker adductor scars (Linear regression, R^2 =0.293, p =0.01)(Fig. 11), the thickness of the scar was not significantly lower than in the margin (1-tailed t-test, p=0.239) or "other" (1-tailed t-test, p=0.091) regions.

Discussion:

My results show that the *Polydora-Crassostrea* interaction can be found in a variety of locations on San Juan Island, but is more prevalent where oyster populations are high. Although the determination of density at each site was preliminary, it shows that there is a positive relationship between oyster density and prevalence of boring. This means that oysters growing in lower densities will be less susceptible to *Polydora* infections. It would be interesting to look at rates population growth of polydorids in communities of oysters living in different densities to see if dense oyster populations are more susceptible to shell weakening by worm boring.

In some oysters, *Polydora* tubes reach all the way through the prismatic shell layer into the nacre, but the number of inner nacre invasions does not depend on the population density of Polydorids on the shell. Although there is a high variance in percent nacre invasions, the average infected shell has about 12.5% of its worm burrows enter the nacre. In addition, the number of nacre invasions did not correlate with the overall area of the shell, suggesting that the age and size of an infected oyster does not affect the number of borings that can make it deep into the shell.

Because the proportion of the worm population invading the nacre stays constant, the internal boring density will increase with increasing outer density (Fig.7). Assuming the worm burrows weaken the nacre sufficiently to decrease the breaking strength of the shell, the shell breaking strength will weaken and oysters' susceptibility to predation will increase with increasing *Polydora* population density. An oyster with a growing population of worms may be able to maintain its shell strength by growing fast enough to maintain the same external worm density.

If the worm population is increasing at a rate greater than the rate at which the oyster can add shell area, they pose a threat to the oyster.

The largest number of borings that could be seen from the inside of the shell occurred in the region of the adductor scar. While the thickness of the shell was much lower in this region for many of the shells, it was not significantly thinner than for the margin and "other" regions (Fig.8). It was, however, much thinner than the hinge region, which displayed the lowest percentage of borings that entered the inner nacre. This suggests that the thickness of the shell region may have some effect on the worms' ability to bore through the shell. This was contradicted by the comparison of the average width of whole shells and the percentage of nacre invasions, which showed no relation between a given oyster's average shell thickness over all regions and worm invasions. Within the adductor scar region, however, there was a significant decrease in percent invasions with increasing thickness. This suggests that the worms' boring dynamics varies between the different regions of the shell, possibly due to differences in the ability of the oyster to secrete nacre at different regions of its shell. Another possible explanation is that the oyster's age and its thickness may affect its susceptibility to higher rates of worm population growth.

A further area of research may be to look at what differences there are in the layers of nacre between regions of the shell as well as how the oyster might lay down nacre to cover up invading borers. It would also be interesting to look at rates of population growth of polydorids in communities of oysters living in different densities to see if dense oyster populations are more susceptible to worm boring. Another aspect to this interaction that I observed was that shell-boring algae were found around many of the worm galleries in the nacre. It would be of interest to study whether there is an ecological relationship between the worms and the algae.

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Tables and Graphs:

Table 1. Field sites listed in order of oyster density (high to low) and percent of population infection

Location	Oyster Density Rank	Population Infection (%)
Argyle 4 (Lagoon)	1	82
Wescott 1	2	66.67
Wescott 3	3	76
Wescott 2	4	63.33
Argyle 1	5	6.88
Wescott 4	6	86.67
Argyle 3	7	0
Argyle 2	8	9.20
FHL	9	16.28
English Camp	10	4

Figure 1. Worm galleries on outside of shell

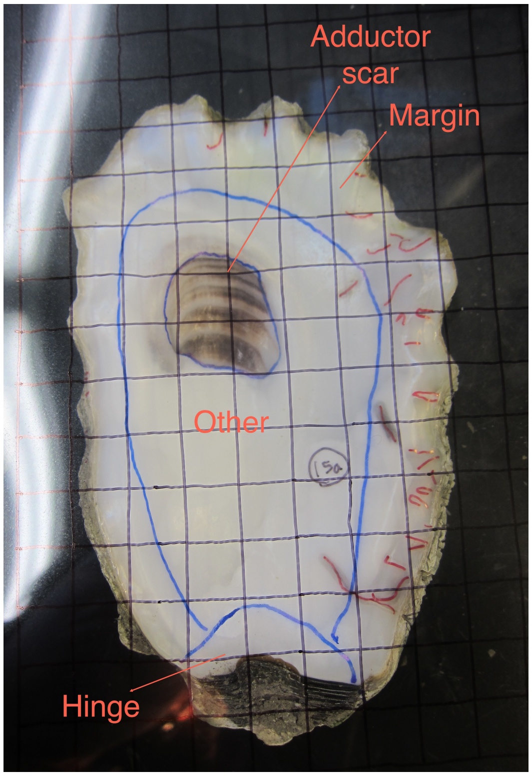


Figure 2. Regions of shell

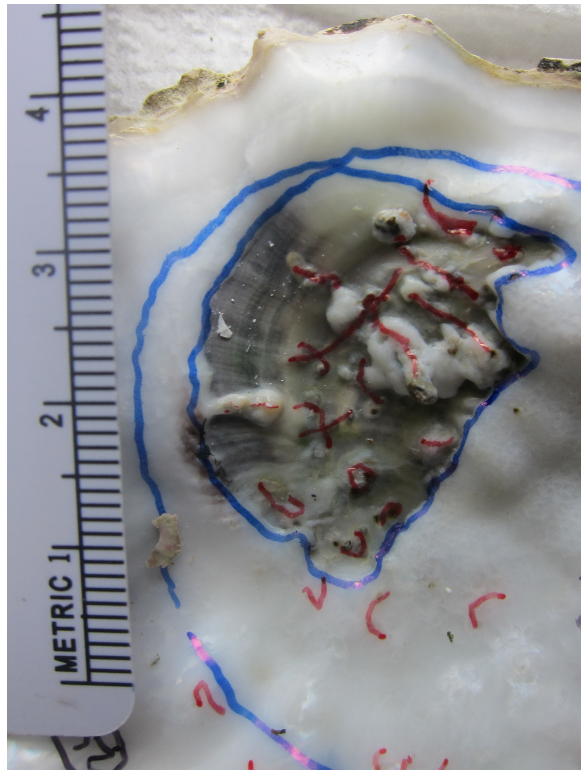


Figure 3. Worm borings invading the inner nacre of the shell

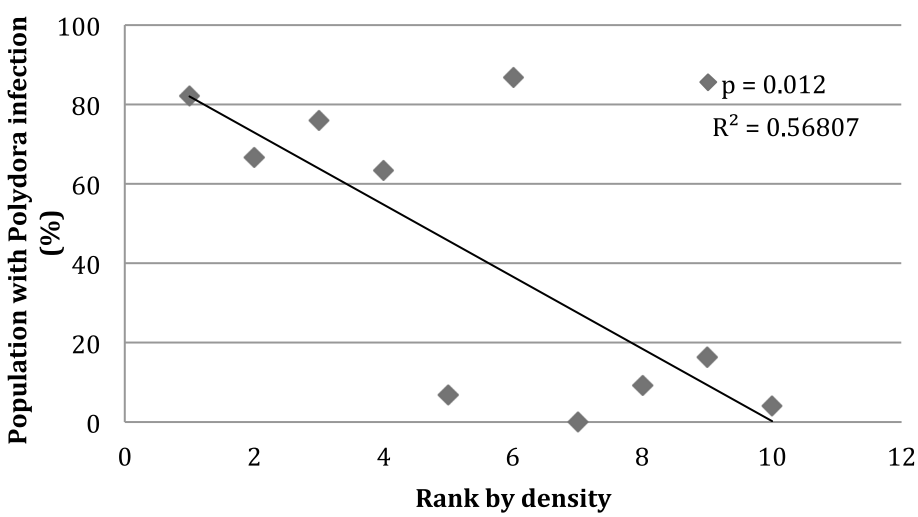


Figure 4. Showing percentage of populations infected with polydorids at locations as ranked by density (see Table 1).

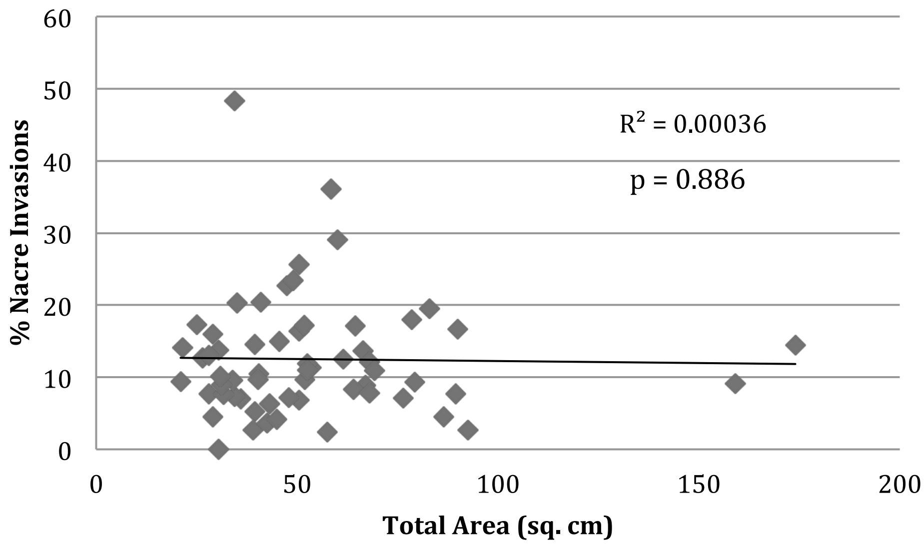


Figure 5. Relation of the percent of invasions that make it into the inner nacre of the shell to the total area of each shell.

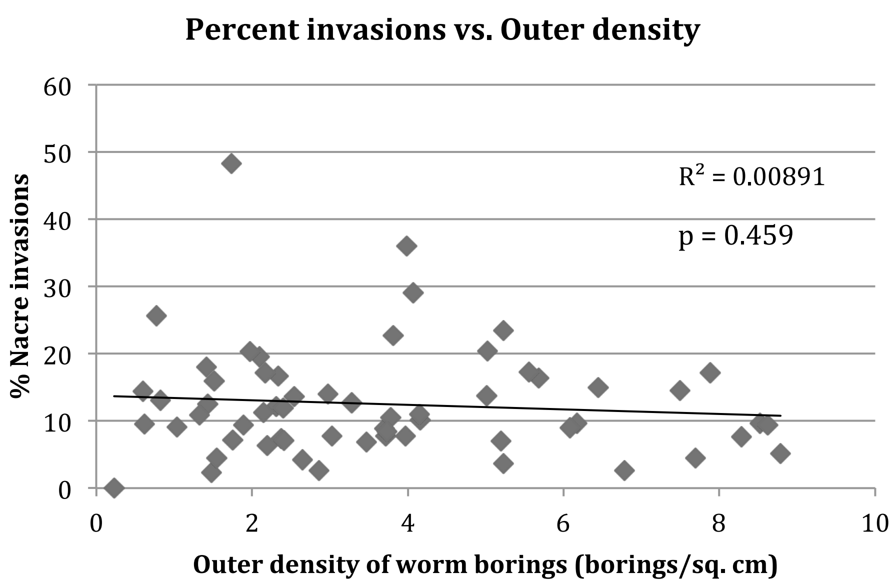


Figure 6. Relation of the percent of invasions that enter the inner nacre of the shell to density of borings on the outer surface of the shell.

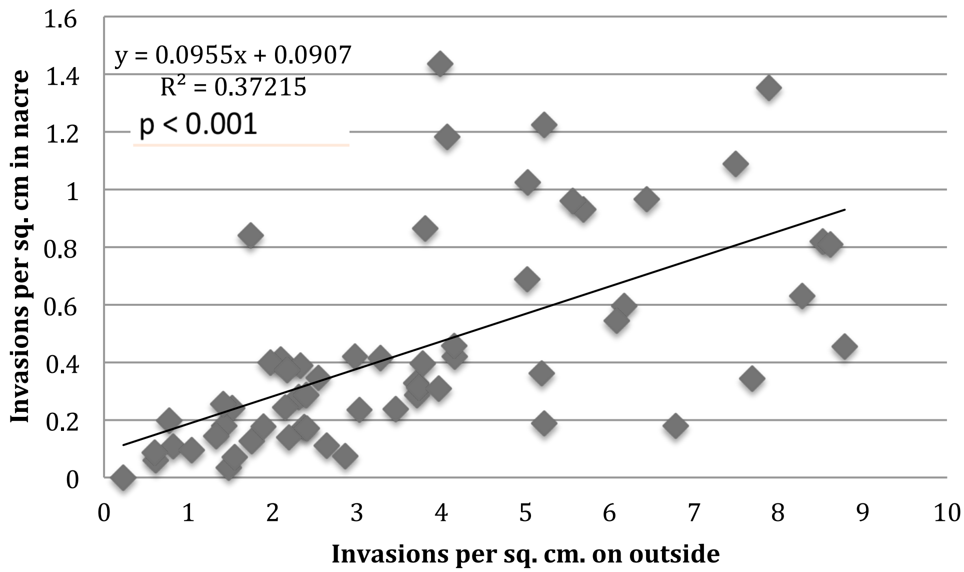


Figure 7. Relation of the density of borings on the outside of the shell to the density of borings in the nacre

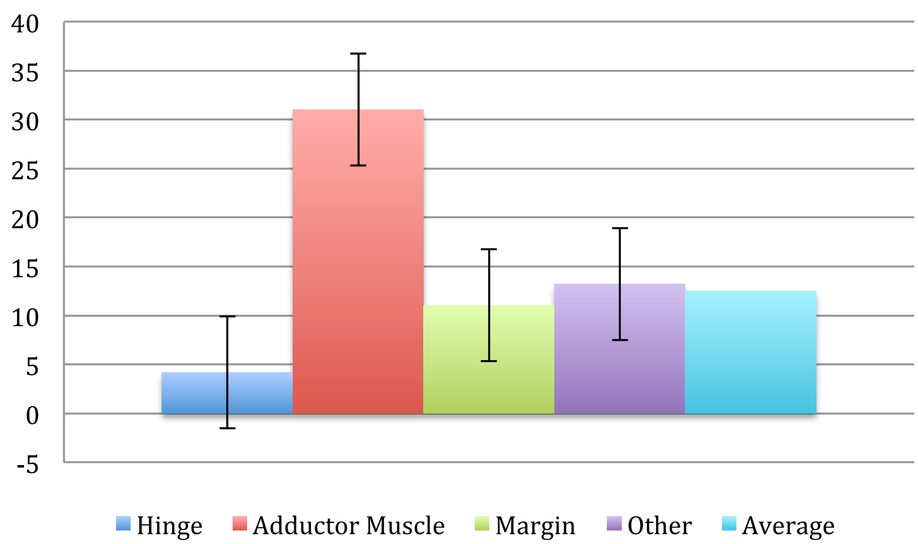
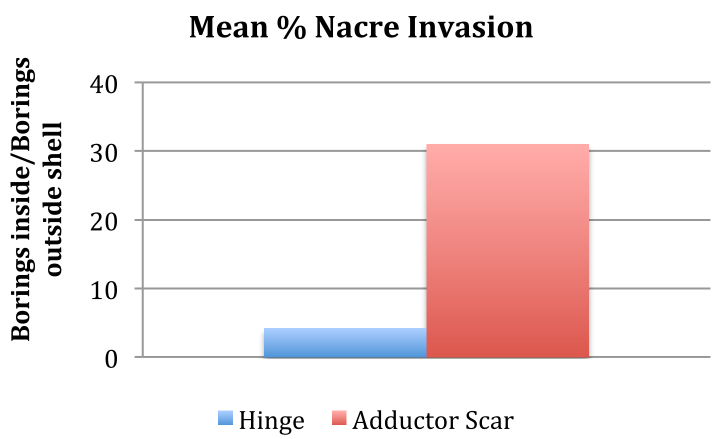


Figure 8. Mean percent of invasions by region of the shell

A.



B.

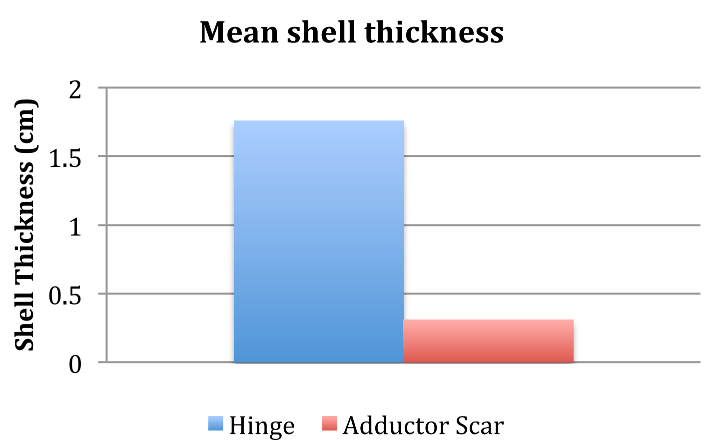
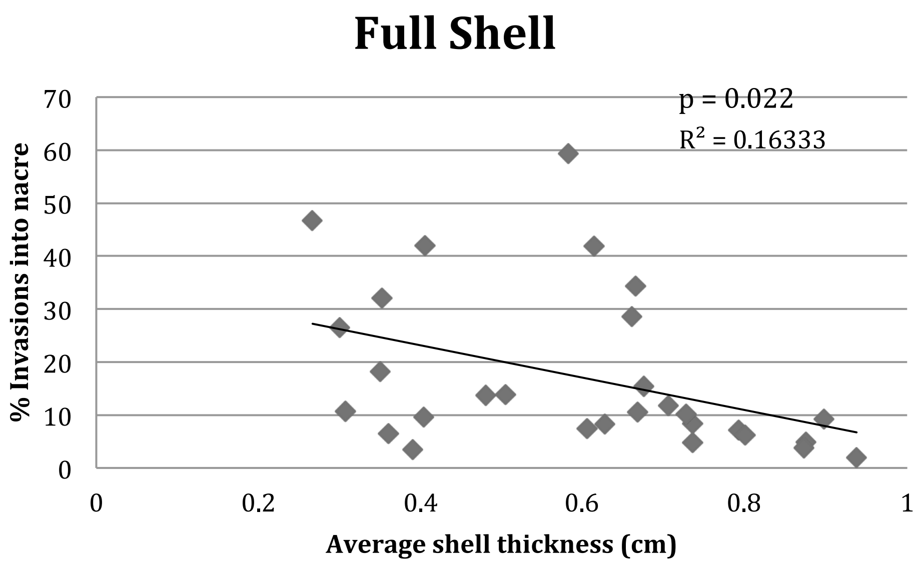


Figure 9. A. Comparisons of mean percent of invasions entering the inner nacre and B. shell thickness at the adductor scar and hinge regions.

A.



B.

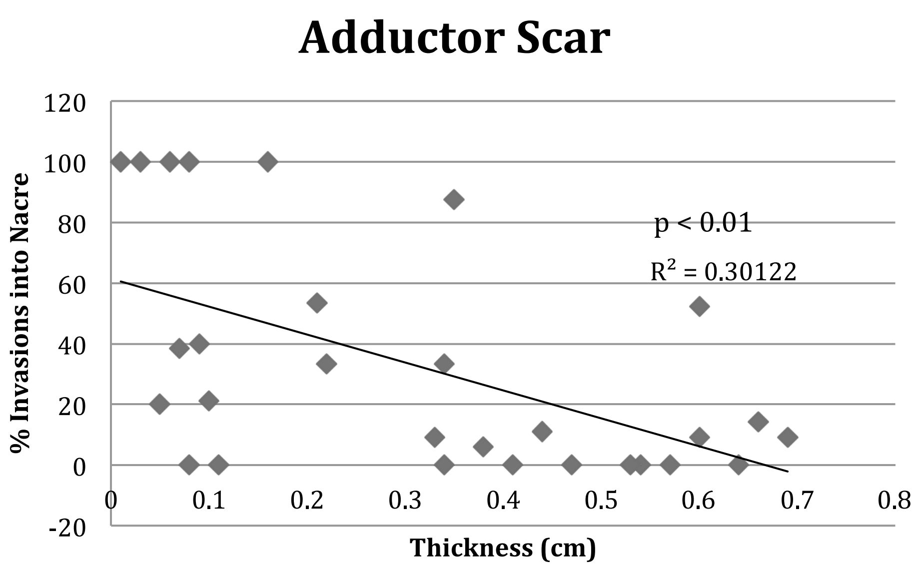


Figure 10. A. Relation of the percent of borings entering the inner nacre and the average whole shell thickness and B. within the adductor scar region.