UNIVERSITY OF OREGON

Braudrick

Colonial Ascidian Dispersal: What are the effects of adult population density and isolation on recruitment?

Nate Braudrick
Oregon Institute of Marine Biology
63466 Boat Basin Road
Charleston, OR

Abstract

Invasive species are thought to be one of the largest ecological threats to healthy ecosystems today. This paper is an attempt to show how anthropogenic transport of species with short dispersal distances is the most important factor in their colonization of new areas. In the Charleston, Oregon marina three study sites were set up to attempt to show the significance of isolation and adult population density on the recruitment of colonial ascidians, an encrusting member of the fouling community. Significant results were found supporting the hypothesis that greater adult population density results in increased recruitment. The second hypothesis of isolation decreasing the recruitment of colonial ascidians was not supported with significant results.

Introduction

Colonial ascidians are invertebrates of the phylum Chordata, subphylum Urochordata, and class Ascidiacea (Lambert, 2005). The species covered in this paper are encrusting sessile members of the fouling community. Colonial ascidians are found in low intertidal and subtidal habitats. This is largely due to the fact that they are unable to tolerate desiccation (Lambert and Lambert, 1998). The habitats they prefer include man-made objects that float, such as boat hulls and floating docks that remain constantly underwater and near the surface. These surfaces allow colonial ascidians to be easily studied. Among the colonial ascidians present around the Coos Bay, Oregon estuary in the marina at Charleston are the relatively recent invader *Botrylloides violaceus* from Japan and the long present Atlantic species *Botryllus schlosseri*. Much is known about the life history of *B. schlosseri*, but the recent invader species *B. violaceus* has been studied less. Their life histories are similar.

Botryllus schlosseri and *Botrylloides violaceus* colonies are started by a sexually produced lecithotrophic tadpole larva which metamorphoses into the initial zooid, the oozooid of the colony (Grosberg, 1988; Lambert, 1998). These oozooids then asexually reproduce forming morphologically and genetically identical zooids that form a colony. The process of forming functional adult blastozooids is deemed "blastogenesis" and takes approximately six days (Grosberg, 1988). The process of asexual reproduction is continuous in a colony and there are always three stages present: adult feeding zooids, primary developing buds on the adult zooids, and secondary developing buds on the primary buds (Grosberg, 1988). Approximately every five to seven days the adult feeding zooids are reabsorbed, primary buds complete their development, secondary buds become primary buds, and new secondary buds form. The duration of the asexual cycle has been inversely correlated to water temperature (Grosberg, 1988). *B. schlosseri* has had two life history patterns identified, both semelparous and iteroparous (Grosberg, 1988; Ayre et al., 1997). According to Grosberg 1988 recruitment stops at the beginning of winter, an effect of temporally varying selection.

Colonial ascidians have a short duration planktonic larval period lasting from a few minutes to less than a day (Ayre et al., 1997). The duration of the larval period is too short to allow cross-oceanic dispersal. Because of this, colonial ascidians such as *B. violaceus* are good indicator species of invasion. The invasive species most likely used trans-Pacific freighters as vectors for their dispersal. This could have occurred through three methods, encrusting on the hull of the ship, as a metamorphosed adult attached to debris in the ballast water, or as unmetamorphosed larvae in the ballast water. The first two of the three options are most likely, although unmetamorphosed larvae have been collected from ballast water after transoceanic shipping (Lambert and Lambert, 1998). Other vectors of invasion for the region studied include transport of introduced oysters that are farmed, a common practice in the South Slough of Coos Bay (Hewitt, 1993). Species of colonial ascidians that are known to encrust oysters include *Botryllus schlosseri* on Atlantic oysters and *Botrylloides violaceus* on Japanese oysters (Hewitt 1993). Imported mussels have been found to be a vector for invasion in other areas as well. Anthropogenic transport of ascidians began centuries ago by the fouling of ship hulls (Lambert 2005).

Botryllus schlosseri was first described in 1766 in Europe but was found in New Zealand and Australia by 1928 and is believed to have reached the San Juan Islands by the 1960s or 1970s (Lambert and Lambert, 1998). The species of colonial ascidians, *B. schlosseri* and *B. violaceus*, have a very short larval dispersal distance, usually only a few meters (Ayre et al., 1997). Once established in major ports colonial ascidians are able to travel the shorter distances between harbors by fouling the hulls of pleasure craft (Cohen and Carlton, 1995). In Bodega Bay, California colonies of *Botrylloides violaceus* have dispersed over 200 times the distance of the tadpole larvae through the detachment of ascidian fouled eel grass blades (*Zostera marina*) from their bases and passive transport by currents (Worcester, 1994). The limiting factor of dispersal for the tadpole larvae may be circulation because of their limited mobility (Lambert, 2005).

This study examines the relationship of increased adult population density and recruitment. Because these invasive species have a short dispersal range I hypothesized recruitment would decrease as distance from adult populations increases. The three hypotheses tested were: plate depth will not have a significant effect on recruitment, recruitment rate will be greater where population density of adults is higher and recruitment rate will be greater in either boat basin than in the channel.

Methods

To measure the effects of adult density and isolation on colonial ascidian dispersal, three locations were used in the marina in Charleston, OR (43°320.730' N 124°19.610' W). These three locations varied in distance from both the ocean and Coos Bay. The furthest removed from the ocean was the inner boat basin, followed by the channel, and finally the outer boat basin. All three locations are protected from the bay by a rocky breakwater. In each location I set up three sites, in as linear a fashion as was possible, varying in distance from 35 to 88 meters from one another (Figure 1). The recruitment plates consisted of 8" square ¼" thick black plexiglass whose surfaces were roughed using a wire brush. I hung the plates horizontally from a string at depths of ½ and 1 meter with an 8oz lead weight attached 6" from the bottom plate to ensure stability. I chose the depth of 1 meter both because it ensured the plates in the channel would remain submerged at low tide and not hit the bottom and also this was an effective depth in previous experiments (Grosberg, 1988). I hung the plates from floating docks for in the inner and outer boat basin locations (sites 1-3, 7-9). On the sites in the channel (sites

4-6) a more complicated setup was necessary because were no docks from which to hang the plates. Plates were attached to rope in the same manner as in the inner and outer boat basin sites. I secured the rope to a crab pot buoy, and the buoy was attached to shore by a 30ft of rope. Another rope attached the buoy to a concrete block with both 15ft of rope and 7ft of small diameter surgical tubing. The surgical tubing acted to control the length of the anchor line to ensure that the plates would not wash up on shore at low tide. I found this to be the case at the first observation on October 16, 2009. I added the surgical tubing and the plates were placed further out into the channel. This solved the problem for the remainder of the experiment.

The study occurred during the end of the recruitment period during the fall of 2009. The plates for the inner boat basin (sites 1-3) were in place between 10/14/2009 and 11/6/2009. The plates for the channel (sites 4-6) were in place between 10/14/2009 and 11/6/2009, but because they washed up on shore on 10/15/2009 the data range is from 10/16/2009 to 11/6/2009. The plates for the outer boat basin (sites 7-9) were put in later than the other two sites and were in place from 10/22/2009 to 11/6/2009. Data was gathered every two to three days during the study period for all sites.

I measured relative adult density for the inner boat basin (site 1-3) and outer boat basins (site 7-9) by random sampling presence and absence over 102 meters of dock. I chose this value because it was the length of the docks surrounding site 7, a boat launch which had less dock than any other site (Figure 1). I generated 30 random numbers for each of the six sites (1-3, 7-9).

At each random number a 1 meter stick was lowered vertically and if at least one adult colonial ascidian crossed the line of the stick I marked its presence, if an adult colony was not present across the stick I marked its absence. There was a total of 90 data points for each the inner and outer boat basins.

Data Analysis

I gathered data by photographing the downward facing side of each plate 2-3 times depending on picture quality. The pictures were taken using a 10 megapixel digital camera. Later, I chose five dates as the data for the experiment at as close to five day intervals as possible. I chose the best pictures for each plate and loaded each into Photoshop CS4 for analysis. I divided each photograph into 16 squares and counted the number of colonial ascidians in each square, on each photograph, for each day. Colonies on the top or edge of the plate were not counted. Because each plate was not cleaned after each photograph the data is in the form of cumulative recruitment at each time point. To determine the number of recruits per day the number of individuals at time t-1 was subtracted from the number of individuals at time t. This value was divided by the number of days in the interval between t and t-1 to give recruitment per day. This is used as a measure of recruitment but it must be noted that individual colonies were not tracked so the data is in the form of average number of recruits per day where recruitment = settlement – mortality.

I tested the hypothesis that plate depth would have no effect on recruitment to ensure there was no statistical difference between the two depths of plates. I tested this hypothesis with a paired two tailed student's t-test comparing the recruitment per day of plates at depth 0.5m and 1.0m. I ran this test for two time periods (10/21 - 10/26 and 10/30 - 11/4). A paired two tailed student's t-test was chosen because it was deemed important to directly compare plates at the same location but different depths. This is because all other factors were deemed to be controlled except for the depth of the plates.

I used an unpaired two tailed student's t-test to discern if there were statistically significant results for the hypothesis of recruitment being greater where adult population density was greater. I compared two specific time periods (10/21 - 10/26 and 10/30 - 11/4). The inner boat basin and the outer boat basin were compared using a chi squared analysis to tell if there was a significant difference between the adult population densities at the two locations. I then ranked each site by adult population density and graphed recruitment per day on the y-axis. This allowed me to run a Spearman's rank model to determine if there was statistical dependence between average recruitment per day and adult population density.

I tested the third hypothesis with an ANOVA comparing the recruitment per day for three locations (inner boat basin, channel, and outer boat basin) over two time periods (10/21 - 10/26 and 10/30 - 11/4). For this hypothesis it was assumed that the sites in the channel (4-6) were isolated because no adult colonies were found on the near shore (pers. observation), although adult colonies have been seen on the rip-rap on the far side of the channel by Richard Emlet (pers. communication, see Figure 1). The null hypothesis was tested using an ANOVA followed by a multiple comparisons test in separate comparisons of recruitment over two time periods (periods 10/21 – 10/26 and 10/30 – 11/4) for all three locations (sites 1-9).

Results

The average daily recruitment in the inner boat basin declined over the study period showing a net loss on the plates at site 3 over the period between 10/26 and 10/30 (Figure 2). There was less recruitment in the channel than in the inner boat basin throughout the study, but a decreasing trend in average daily recruitment occurred between 10/26 – 10/30 (Figure 3). The recruitment at the outer boat basin was similar to that in the channel, and less than in the inner boat basin. Recruitment in the outer boat basin declined dramatically over the period 10/26 – 10/30 but recovered during the remainder of the study (Figure 4).

The first hypothesis that there would be a significant difference in recruitment between the plates at depth 0.5m and 1.0 m was tested using the null hypothesis that there would be no significant difference in recruitment between plates at different depths. The null hypothesis was tested during two time periods, 10/21 – 10/26 and 10/30 – 11/4 (Figures 5 and 6). The results of the t-tests were non-significant with $P = 0.79$ and 0.30 for the two study periods respectively (Table 1). A nonsignificant difference in settlement with depth allows the use of plates as replicate measures of recruitment effectively doubling the sample size and increasing statistical power in the tests of subsequent hypotheses.

The second hypothesis of greater relative adult population density yielding increased recruitment was tested against the null hypothesis: there will be no significant difference in recruitment with greater adult population density. The relative density of adult populations was significantly greater in the inner boat basin than that of the outer boat basin (Figures 7 and 8, P=.001). Recruitment was significantly greater in the inner boat basin than the outer boat basin for both intervals (P=0.009 and P=0.014 for respective intervals, see also Figures 9, 10 and Table 2). Adult density and recruitment were significantly correlated for the first study period (P=0.01) but not for the second study period (P=0.08) (Figures 11 and 12).

The third hypothesis that isolation would have the effect of decreased recruitment was tested against the null hypothesis that there would be no significant difference in recruitment regardless of isolation. Recruitment in the inner boat basin (sites 1-3) was significantly different from that at the other two locations (sites 4-9) (P<0.05). The channel (sites 4-6) and outer boat basin (sites 7-9) were not significantly different (P>0.05, Figures 13 and 14; Table 3).

Discussion

The insignificant effect of depth on recruitment rate was expected (Figures 5 and 6; Table 1). This showed that there were no artifacts in the data from plate depth. This also allowed me to double the number of data points in the Spearman's rank model giving twelve data points rather than six.

The significance of adult population density on recruitment of a limited dispersal sessile invertebrate has been shown in the past, but has been supported in this experiment (Ayre et al., 1997) (Figures 7-10; Table 2).

The hypothesis that isolation would negatively effect recruitment is consistent with the theory of island biogeography (TIB). TIB predicts that the number of species present on an island is positively correlated to its size and inversely correlated to its isolation (distance from continent). The number of species is inversely correlated to isolation because many species are dispersal limited; preventing them from colonizing isolated islands. The settlement plates can be considered "islands" of habitable surface. The very limited dispersal capacity of colonial ascidians could create isolation by distance.

Current also plays a large role in the dispersal distance of planktonic larvae with limited mobility. This is because mobility is largely used to enter and exit currents (Mchenry and Patek, 2004). The sites used for isolation were in the channel between the boat basins, and while exact current flow is unknown, through the tidal action of the area a large amount of flushing of the inner boat basin happens twice a day (personal observation). This would greatly increase the flow rate in the narrow channel where the experimental plates were placed which would carry more larvae past the settlement plates as they are whisked by. Because of the high adult density in the outer boat basin it was expected that there would be a significant difference between the recruitment rates seen there and in the channel. The reason for the insignificant difference in recruitment may be that the larvae are whisked out of the outer boat basin and into Coos Bay. The reason for the exceptionally high recruitment found in the inner boat basin may be due to the retention of larvae, where weak currents allow the larvae to settle near the adult colonies. I have no evidence to substantiate this hypothesis or its effects on the study of the connection between isolation and recruitment (Figures 12, 13; Table 3).

The data in this experiment, rate of recruitment per day, is unusual for a settlement experiment. It is in this form because of the varying length of sample intervals (4 or 5 days). As a measure of recruitment, I deem this to be valid because a previous study reported that mortality between recruitment and the first asexual reproduction is less than 8% (Grosberg, 1988). Data was collected over a period of 24 days, but was only analyzed for two five day periods of time (10/21 – 10/26 and 10/30 – 11/04). This is because of an unusual trend in the data over the four day period of 10/26 – 10/30 (Figures 2-4) where on three plates a negative population growth occurred. The period prior to 10/21 could not be used because data for all three locations was not available. After 10/21, summer recruitment may have been coming to a close because of the reduced rates of recruitment throughout the experiment (Grosberg, 1988).

The results of the experiment examining the effects of isolation on recruitment assumed that the channel was isolated because very few adult colonies have been observed (Richard Emlet, pers. communication) while the boat basins were assumed not to be isolated because of the relatively high adult colony density.

More research is needed on the topic of isolation and its effects on recruitment. If an experiment could be designed so that isolation was shown in a way where it was not surrounded by source populations on either side significant results may be found. Using a slough, like South Slough upriver from the Charleston marina may show that larval dispersal is limiting the spread of invasive species such as *Botryllus schlosseri* and *Botrylloides violaceus*.

Acknowledgements:

I would like to thank Richard Emlet for helping me design and carryout this experiment, as well as allowing me to use many of his supplies to complete it. I would also like to thank all who invested time in helping me photograph the plates, especially Zachary Calef who was there day or night, rain or shine.

Table 1: Data analysis for two periods of study, 10/21 – 10/26 and 10/30 – 11/4, comparing two depths of plates at all study sites, 0.5 meters and 1.0 meters. Probability value is given that the two means come from the same dataset.

Study Period	0.5 m (mean±SE) n=9	1.0 m (mean±SE) n=9	P-value
10/21 – 10/26	1.01±0.58	0.94±0.35	0.79
10/30 – 11/4	0.91±0.29	0.68±0.19	0.30

Table 2: Data analysis for two periods of study, 10/21 – 10/26 and 10/30 – 11/4, comparing two locations: the inner boat basin (sites 1-3) and outer boat basin (sites 7-9). Probability values are given that the mean population changes per day from both datasets are from a single dataset.

Study Period	Inner (mean±SE) n=6	Outer (mean±SE) n=6	P-value
10/21 – 10/26	2.47±0.65	0.33±0.15	0.009
10/30 – 11/4	1.53±0.321	0.50±0.134	0.014

Table 3: Data analysis for two periods of study, 10/21 – 10/26 and 10/30 – 11/4, comparing three locations: the inner boat basin (sites 1-3), the channel (sites 4-6), and the outer boat basin (sites 7-9). Groups from each study period labeled with different letters are significantly different (P<0.05).

Study Period	Inner (mean±SE) n=6 (Group)	Outer (mean±SE) n=6 (Group)	Channel (mean±SE) n=6 (Group)
10/21 – 10/26	2.47±0.65 (A)	0.33±0.15 (B)	0.14±0.06 (B)
10/30 – 11/4	1.53±0.321 (A)	0.50±0.134 (B)	0.37±0.11 (B)

Figure 1: This satellite image of the marina in Charleston, Oregon. The red triangles denote locations where settlement plates were deployed and are labeled above in white writing. Sites 1-3 are in the inner boat basin, sites 4-6 are in the channel, and sites 7-9 are in the outer boat basin. The scale bar given is 100 meters long.

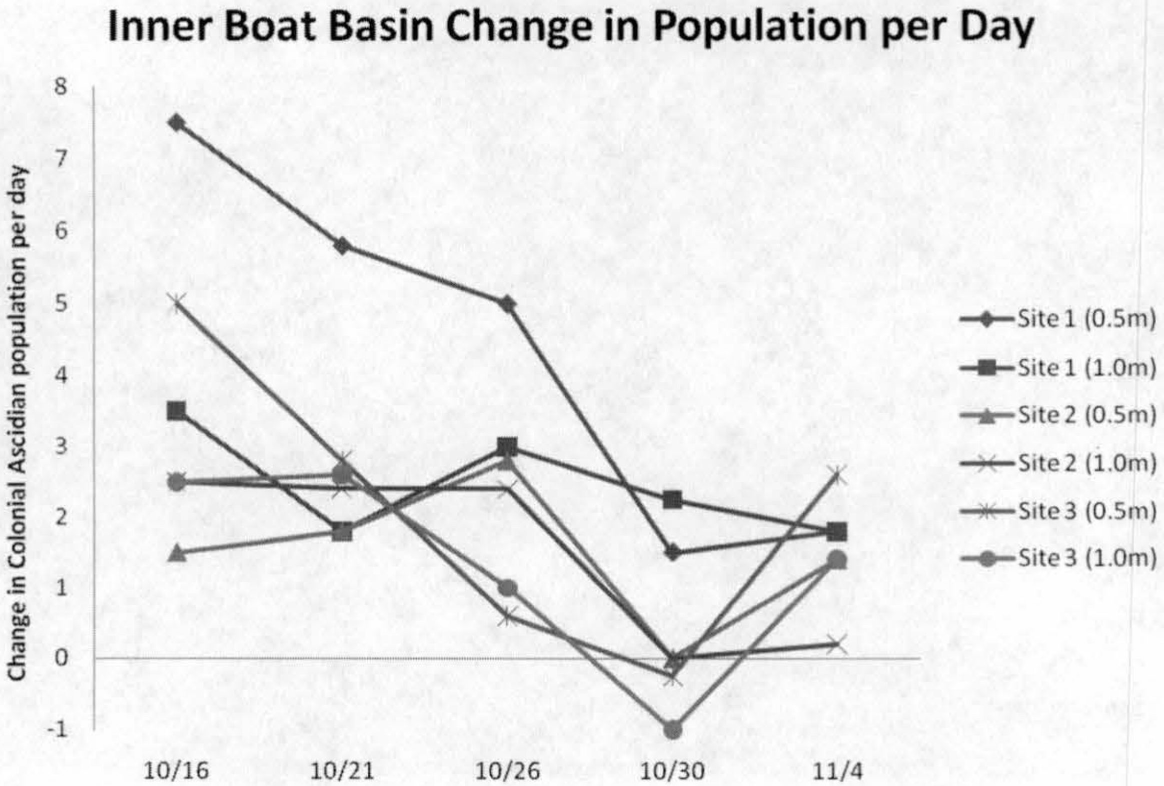

Figure 2: This chart shows the change in population size per day for the inner boat basin (sites 1-3) over the study period. The y-axis shows the average change in colonial ascidian population per day between each marked point. Each study period is four or five days. The x-axis shows a progression of time, from the first data collection to the last. Each series is labeled with the site number and depth of the plate.

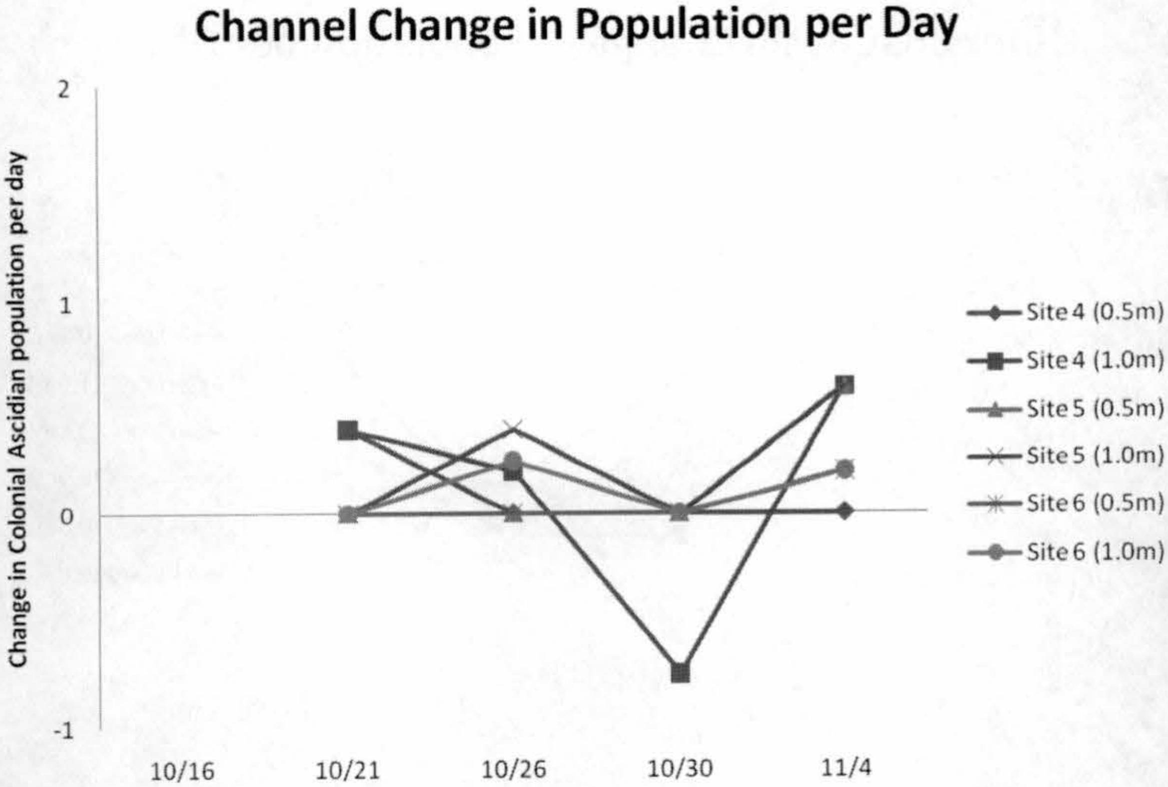

Figure 3: This chart shows the change in population size per day for channel (sites 4-6) over the study period. The y-axis shows the average change in colonial ascidian population per day between each marked point. Each study period is four or five days. The x-axis shows a progression of time, from the first data collection to the last. Each series is labeled with the site number and depth of the plate.

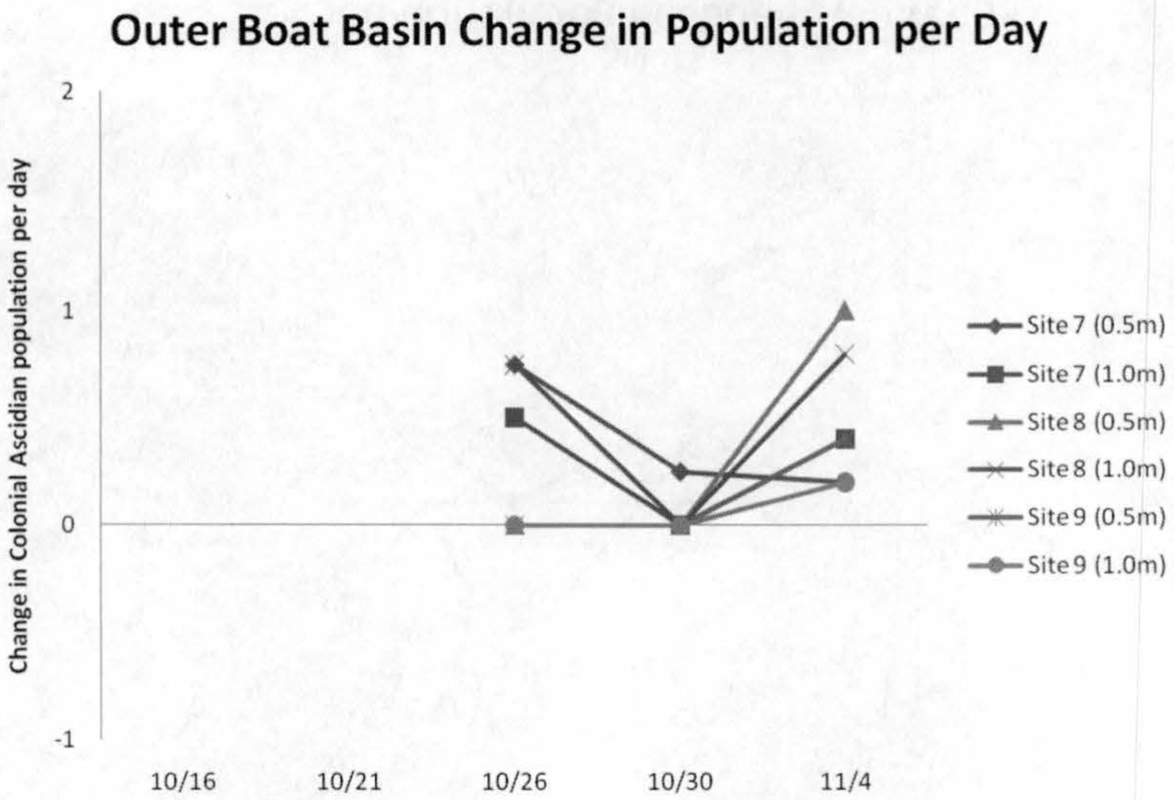

Figure 4: This chart shows the change in population size per day for outer boat basin (sites 7-9) over the study period. The y-axis shows the average change in colonial ascidian population per day between each marked point. Each study period is four or five days. The x-axis shows a progression of time, from the first data collection to the last. Each series is labeled with the site number and depth of the plate.

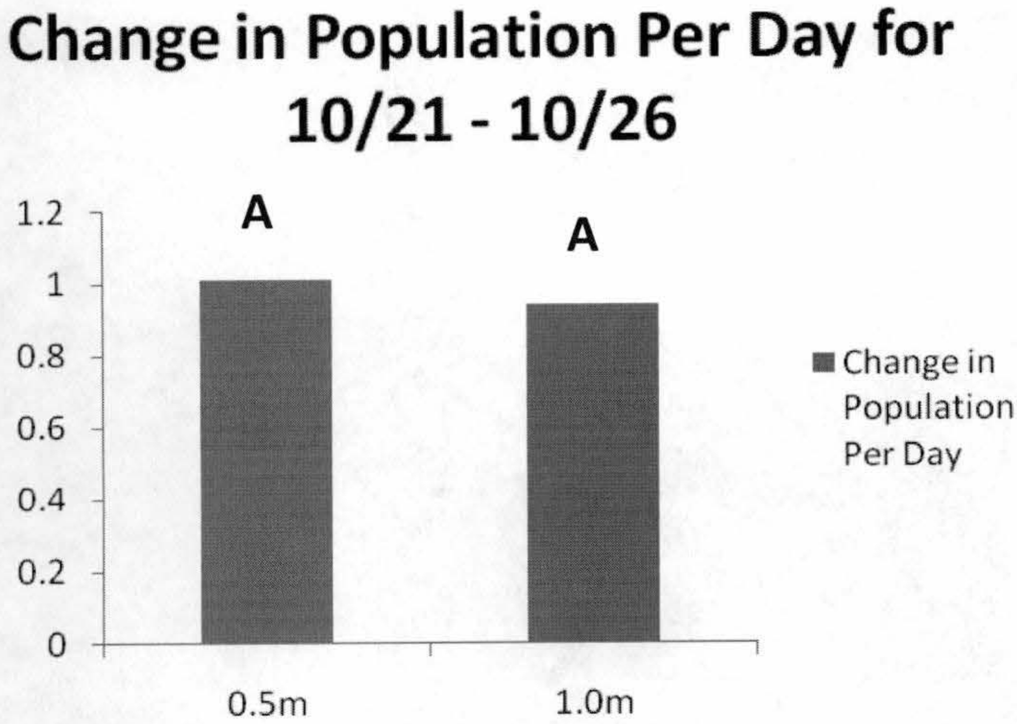

Figure 5: This chart shows the average change in population per day comparing two plate depths at all locations in the study over the period 10/21 to 10/26. The depths compared are 0.5 meters and 1.0 meters. The y-axis shows the average change in population per day over the study period. The x-axis labels the two different depths, 0.5m and 1.0m. Bars with different letters over them are significantly different ($P<0.05$).

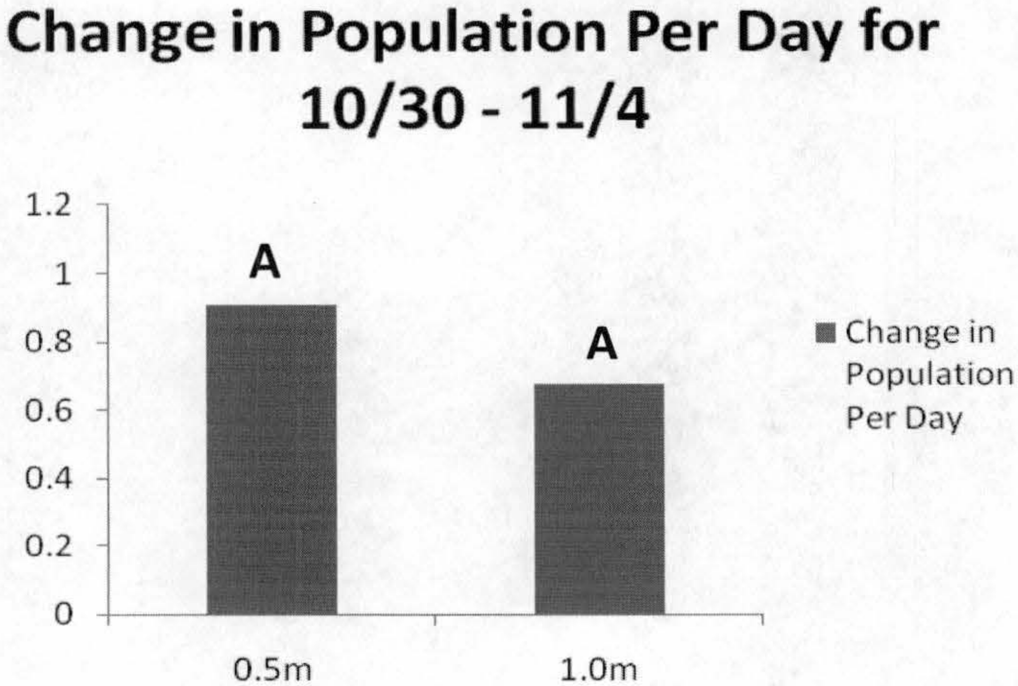

Figure 6: This chart shows the average change in population per day comparing two plate depths at all locations in the study over the period 10/30 to 11/04. The depths compared are 0.5 meters and 1.0 meters. The y-axis shows the average change in population per day over the study period. The x-axis labels the two different depths, 0.5m and 1.0m. Bars with different letters over them are significantly different (P<0.05).

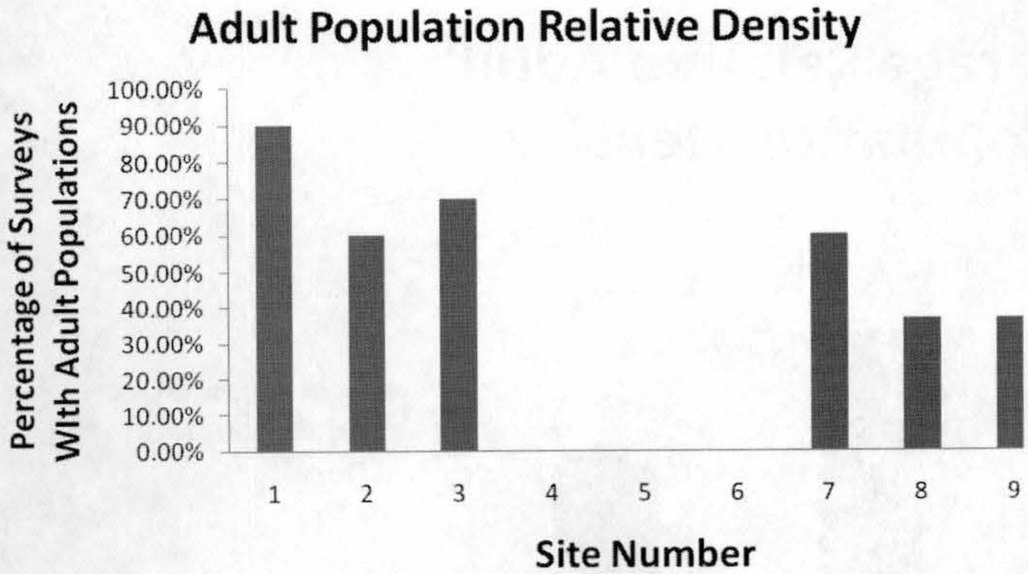

Figure 7: The y-axis shows percentage of sight studies that yielded an adult colonial ascidian for nine study sites. The x-axis shows the site number, sites 1-3 are the inner boat basin, sites 4-6 are in the channel (no measurements possible), and sites 7-9 were the outer boat basin. Thirty sightings were taken at random over 102 yards of floating dock at each site.

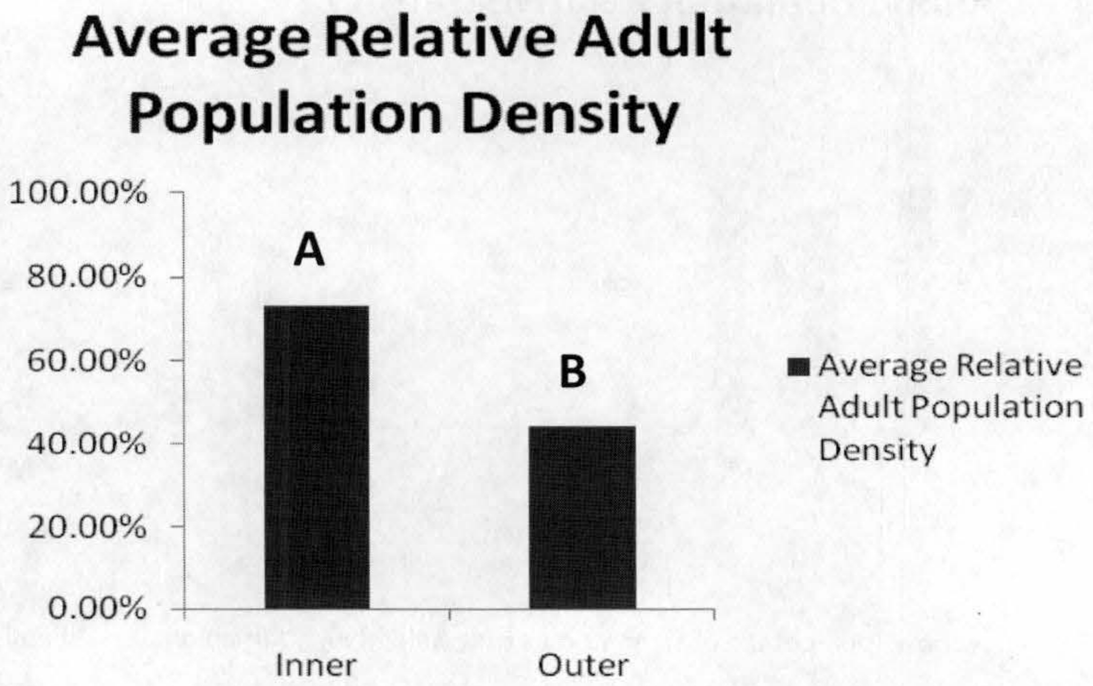

Figure 8: This chart compares the average relative density of adult colonial ascidians of two locations of study, the inner (sites 1-3) and outer (sites 7-9) boat basins. The y-axis shows the percentage of the 90 sightings that had an adult colonial ascidian. The x-axis labels the two locations of study, the inner and outer boat basins. Bars with different letters over them are significantly different ($P<0.05$).

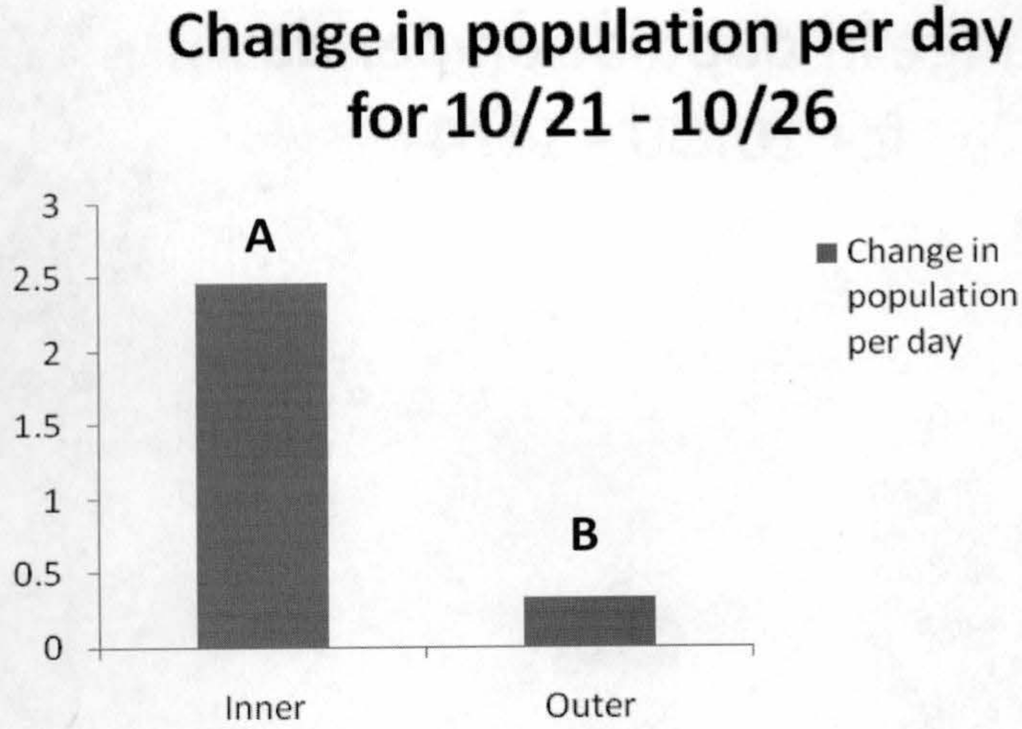

Figure 9: This chart shows the average change in population per day comparing two locations of study over the period 10/21 to 10/26. The locations are the inner (sites 1-3) and outer (sites 7-9) boat basins. The y-axis shows the average change in population per day over the study period. The x-axis labels the two different locations, inner and outer boat basins. Bars with different letters over them are significantly different ($P<0.05$).

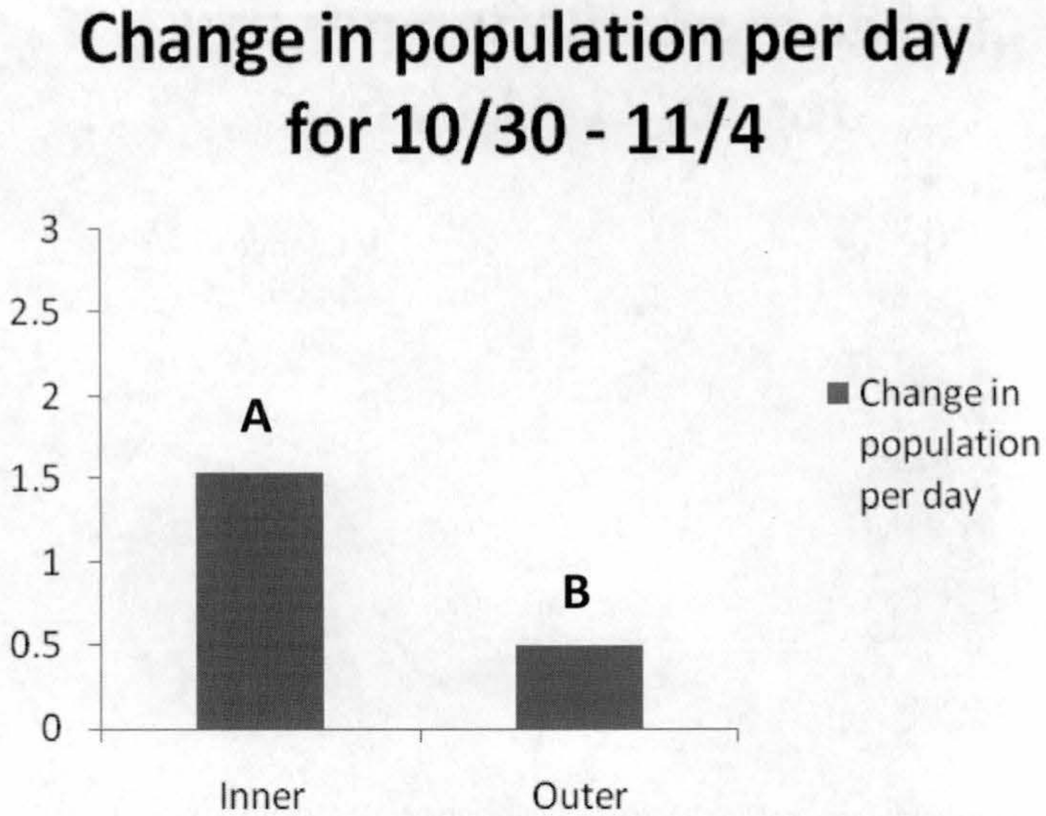

Figure 10: This chart shows the average change in population per day comparing two locations of study over the period 10/30 to 11/04. The locations are the inner (sites 1-3) and outer (sites 7-9) boat basins. The y-axis shows the average change in population per day over the study period. The x-axis labels the two different locations, inner and outer boat basins. Bars with different letters over them are significantly different ($P<0.05$).

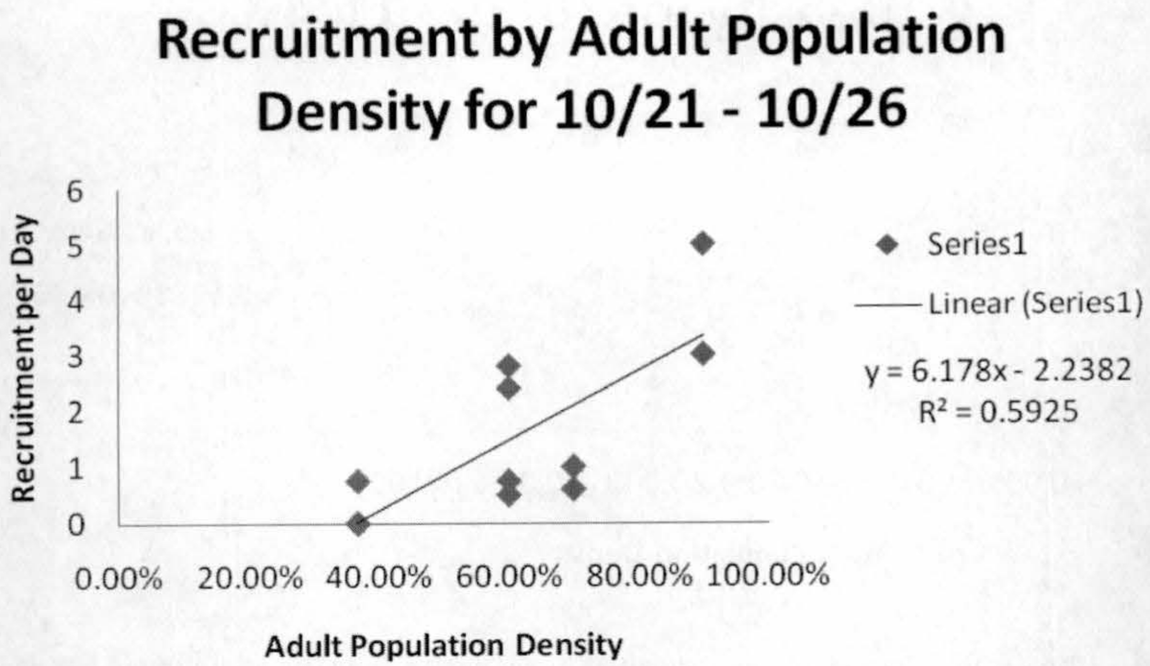

Figure 11: This graph plots adult population density on the x-axis against recruitment per day on the y-axis over the study period between 10/21 and 10/26. The sites measured are from both the inner and outer boat basins (1-3, 7-9). There are a total of 12 data points. A linear regression was added in the form Y = mX + b. The equation and R^2 value are listed on the graph.

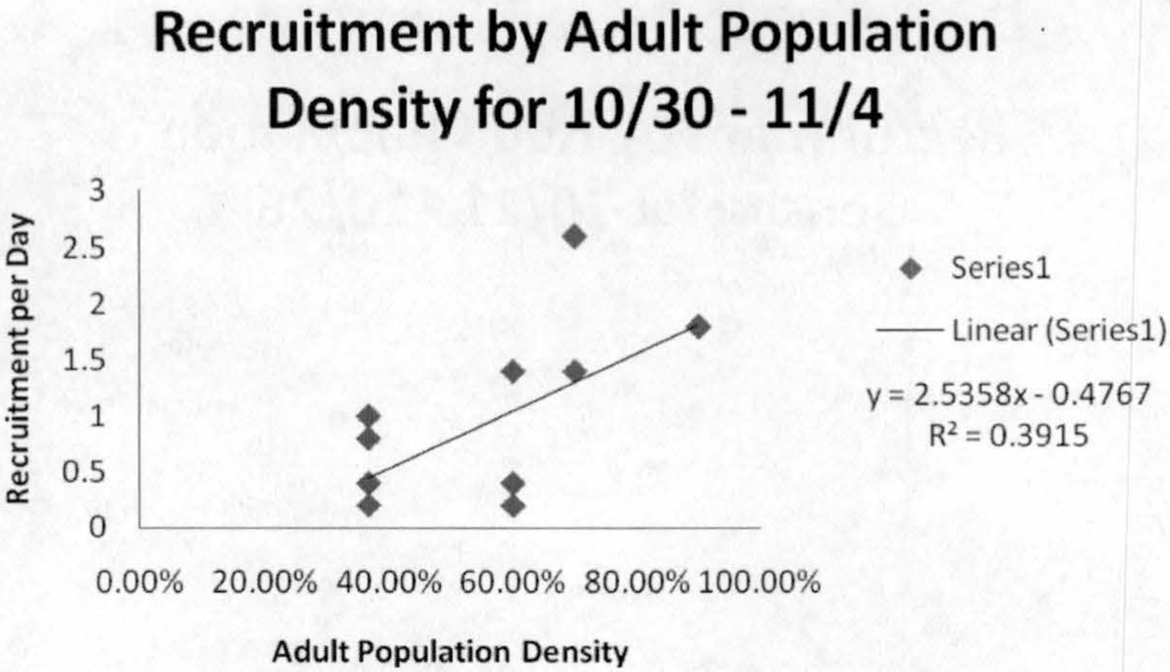

Figure 12: This graph plots adult population density on the x-axis against recruitment per day on the y-axis over the study period between 10/30 and 11/4. The sites measured are from both the inner and outer boat basins (1-3, 7-9). There are a total of 12 data points. A linear regression was added in the form Y = mX + b. The equation and R^2 value are listed on the graph.

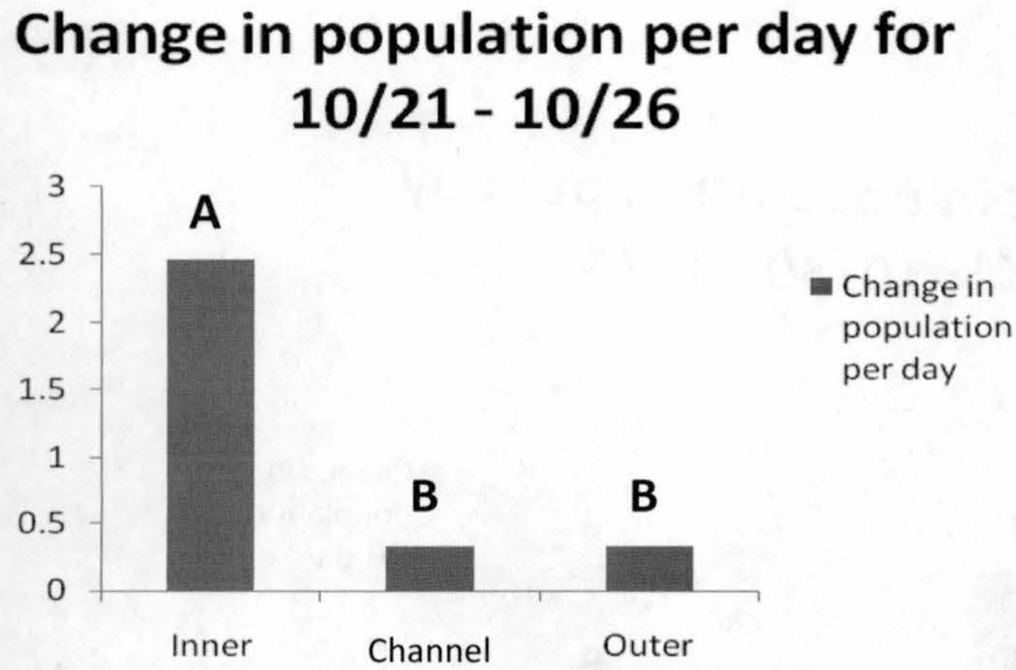

Figure 13: This chart shows the average change in population per day comparing three locations of study over the period 10/21 to 10/26. The locations are the inner boat basin (sites 1-3), the channel between the boat basins (sites 4-6), and the outer boat basin (sites 7-9), a. The y-axis shows the average change in population per day over the study period. The x-axis labels the three different locations: inner boat basin, channel between boat basins, and outer boat basin. Bars with different letters over them are significantly different (P<0.05).

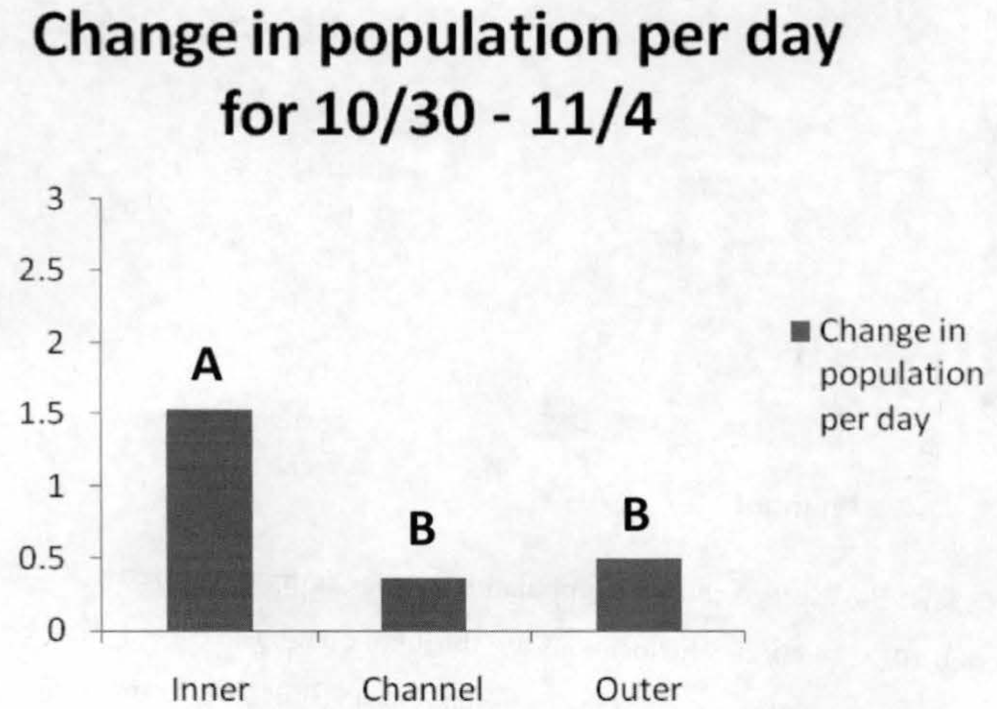

Figure 14: This chart shows the average change in population per day comparing three locations of study over the period 10/30 to 11/04. The locations are the inner boat basin (sites 1-3), the channel between the boat basins (sites 4-6), and the outer boat basin (sites 7-9). The y-axis shows the average change in population per day over the study period. The x-axis labels the three different locations: inner boat basin, channel between boat basins, and outer boat basin. Bars with different letters over them are significantly different ($P<0.05$).

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