EFFECTS OF STOCK ORIGIN ON THE GROWTH AND SURVIVAL OF THE EASTERN OYSTER, CRASSOSTREA VIRGINICA, IN SOUTHEASTERN NORTH CAROLINA

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ABSTRACT

The use of live oysters (*Crassostrea virginica*) for water quality mitigation and for oyster reef restoration has received considerable attention in the past decade. Oysters for such management efforts are routinely purchased from hatcheries or relocated from natural reefs with little consideration for the possibility that the oysters may exhibit local adaptation to the environmental conditions of their natal site. Local adaptation might influence oyster growth and survival following transplant, thus potentially reducing the benefits of these management approaches. This study examined oysters from two tidal creeks (Bradley Creek and Pages Creek) in New Hanover County, North Carolina, for evidence of local adaptation using reciprocal transplant and common garden strategies. Reciprocal transplants were conducted with growth and survival of oysters monitored for 3 months in the late summer (Transplant 1) and 8 months covering the fall and winter (Transplant 2). Stock origin had a significant effect on growth. The Bradley Creek stock had better relative growth than the Pages Creek stock in Transplant 2, regardless of site (Transplant 1 data did not exhibit any clear trends in growth). The Bradley Creek stock also had better overall survival rates than the Pages Creek stock in both transplants (Bradley: 49%, 48%; Pages: 34%, 30%). In both transplants, the Bradley Creek stock had higher survival at all three sites, including the common garden. Environment also had a significant effect on growth and survival and effected stocks similarly. Both stocks performed best in the same site, but that site differed between transplants. Growth and survival were highest for both stocks in Bradley Creek in Transplant 1, and were highest for both stocks in the common garden in Transplant 2. Each stock's performance was site

dependent. However, the two stocks performed very differently from each other within each site, suggesting local adaptation (phenotypic differentiation that persists after common environmental conditions), likely a result of selective mortality. This study indicates that the source of brood stock for restoration or water quality mitigation may have significant impacts on project success.

ACKNOWLEDGEMENTS

I thank the Bay Shore community and the Bradley Creek Marina in Wilmington, North Carolina for allowing me access to field sites through their properties. In addition, I would like to greatly thank all the people who provided field and laboratory assistance, including Chris, Bonnie and Adam Broders, and especially Luther Smeilus.

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INTRODUCTION

Atlantic coastal estuaries have exhibited declining water quality in recent decades (Mallin et al., 2000). This decline in overall water quality can be traced to a variety of problems such as a rapidly growing coastal population leading to increased coastal development, extensive agriculture, and increased coverage of the watershed by impervious surfaces, all of which contribute to high coliform levels, high concentrations of heavy metals, increased point and non-point sources of pollution, eutrophication, toxic algal blooms and increased suspended solids (Mallin et al., 2000).

Runoff from terrestrial sources contributes significantly to the problem of eutrophication by increasing nutrient loads (Nybakken, 2000). These nutrient additions have resulted in a rise in eutrophication, which in turn has resulted in an increase in frequency, extent, and magnitude of hypoxia in coastal areas (Noxon, 1995; Vitousek et al., 1997; Diaz, 2001). Eutrophication is the process by which bodies of water become enriched in dissolved nutrients that stimulate the growth of photosynthetic organisms (Nybakken, 2001). When all these organisms die, the activity of decomposers can deplete dissolved oxygen leading to local hypoxia or anoxia (Hinrichsen, 1998). Tidal creeks in New Hanover County, North Carolina are especially susceptible to toxic algal blooms and eutrophication due to high nutrient inputs and poor flushing (Mallin et al., 2000), which leaves the nutrients to accumulate instead of being removed by increased tidal flux (Mallin et al., 2000). High nutrient inputs frequently are the result of extensive fertilizer use in adjacent developments and golf courses (which are known for high fertilizer use) (Mallin et al., 2000; Nybakken, 2000).

U.S. mid-Atlantic coast estuaries were once characterized by large complexes of oyster reefs (Luckenbach, 1999). The loss of oyster reefs from many of the Atlantic Coast estuaries where they were once abundant has been largely a consequence of disease (Allen et al., 1993), habitat degradation (Rothschild et al., 1994; Hargis and Haven, 1999), and over harvest (Alphin et al., 2004; Committee on Nonnative oysters in the Chesapeake Bay, 2004). Now that these reefs have disappeared, the effects are being felt in several different ways. Oyster reefs are considered key marine habitats (Jackson et al., 2001) and provide essential habitat, food and protection for many commercial and non-commercial species (Luckenbach, 1999; Posey et al., 1999). Oysters have also been shown to change the physical and biological parameters of estuarine systems (Harwell, 2004; Dame, 1999; Dame et al., 2000; Nelson et al., 2003; Mann, 2000), by increasing surface area, altering flow regimes, removing particulates, and reducing erosion (Cressman et al, 2003; Meyer et al., 1997). Several studies have shown that dense populations of suspension-feeding shellfish have the potential for having a significant impact on basin-wide water quality (Cloern, 1982; Cohen et al., 1984; Dame, 1996). Oysters have tremendous economical value as well. According to National Marine Fisheries data, even with diminished landings, oysters contributed $89,071,000 to the US seafood industry in 2002. In North Carolina, the oyster harvest has dropped significantly in recent years; the value of the catch in 2002 was 83% lower than in1987 (oysters brought in $2,875,306 in 1987, and only $991,004 in 2002) (National Marine Fisheries Services, 2002).

Recent interest in oysters and other filter feeders has increased because they have been shown to improve water quality (Nelson et al., 2003), which has been a long-standing problem for the coast over the past several decades. Oysters have a 50% efficiency rate at retaining particles of sizes as small as 2 micrometers and pump water through their gills at a rate of roughly 3-26 L/h (Shumway, 1996). In addition, oysters also trap non-ingestible particles (such as suspended solids) and expel them as pseudofeces, which settle to the bottom. This removal of suspended solids increases water clarity and may be a source of food for other organisms. This filtering activity has been shown to improve phytoplankton productivity (Luckenbach, 1999; Dame and Libes, 1993) by reducing the amount of suspended sediment in the water column, and therefore improving light penetration and clarity (Nelson et al., 2003; Mann, 2000). Interest in this technique has also increased because oyster reefs provide habitat for commercially important taxa (Posey et al., 1999), a separate but equally important reason for restoration.

Because of their filtering capabilities, ability to survive in somewhat eutrophic waters, and their beneficial habitat and fisheries value, many studies, such as Breitburg et al. (2000), have suggested oysters to be a possible management tool for improving poor water quality. Regardless of the reason for oyster restoration (water quality, fisheries value, habitat, etc), the type of oyster used could have a significant impact on the success of the restoration efforts.

Such restorations could rely on either hatchery-raised or wild oysters that are transplanted to a site, or by increasing local recruitment in areas targeted for mitigation. Local recruitment may be enhanced in several ways, including improving environmental conditions to a level conducive to the survival of juvenile oysters, or more commonly, providing hard substrate necessary for spat settlement (North Carolina Division of Marine Fisheries, 2000). With decreasing natural populations in some areas, conservationist groups, managers, and oyster biologists have had to look increasingly at hatchery seed each year to supply their restoration and research efforts (Allen et al., 1993).

Restoration efforts involving oysters have had variable success for several reasons, including inadequate reef construction for specific areas, reefs made of materials that don't attract and retain oyster spat, insufficient density of oysters planted, and placement in creeks with low coverage of live oysters or extreme conditions not satisfactory for oyster growth (Coen and Luckenbach, 2000). While all of these extrinsic factors influence the subsequent growth and survival of the oysters after planting, some of the observed variability may result from intrinsic variation among oysters used in the restorations (Shumway, 1996; Dittman et al., 1998).

It has recently been recognized that "physiological races" of oysters exist and that there is considerable individual variation in how oysters respond to environmental factors (Shumway, 1996; Dittman et al., 1998). This variation has been seen in reciprocal transplants, and common garden experiments, and differentiation among populations has been seen in gel electrophoresis studies

(Buroker, 1983). This variation often occurs across different geographic areas. Wide-ranging species often exhibit this type of variation, known as geographical variation. This has been observed in a range of organisms, including blue mussels (Hilbish and Hoehn, 1985), Atlantic silversides (Conover and Present, 1990; Lagomarsino and Conover, 1993; Billerbeck et at., 1997), and the Eastern oyster (Dittman et al., 1998), all of which are wide-ranging species. Dittman et al (1998) observed significant variability in response to the environment in oysters from different sites, and concluded that they experienced post-settlement selection based on local conditions. In fact, differences in sites of origin have been considered responsible for most differences that persist in populations of oysters, mussels and clams (and other wide-ranging species) upon transplantation, including growth and survival (Kautsky et al., 1990; Iglesias et al., 1996).

This variation among different geographic areas could be a result of genetically based differences, through either isolation among populations or post-settlement selection. Environmental selection pressures can result in geographic variation by altering the composition of local genotypes (Dittman et al., 1998; Mayr, 1963; Endler, 1977), ultimately resulting in local adaptation. This often occurs across clines in an environmental factor (Endler, 1977; Berven and Fill, 1983). Physiological and life history trait differences in these situations can be attributed to the survival of different genotypes in different locations, or post settlement selection. Genetic variation via post settlement selection has been observed in organisms such as wood frogs (Berven and Gill, 1983), American eels (Williams et al., 1973), mussels (Koehn et al., 1976; Iglesias et al., 1996), barnacles (Hedgecock, 1986), limpets (Johnson and Black, 1984), and Eastern oysters (Alphin, 2004).

This variation among different geographic areas could also be a result of environmentally based variation, which can cause genetically similar organisms to behave differently when confronted with different environmental conditions. Theoretically, an organism with the same genotype may exhibit different phenotypes depending on what environment the animal inhabits. It has been said that the phenotype is a reflection of the genotype that's modulated by the environment (Allen et al., 1993). Conversely, organisms displaying a particular phenotype in one environment may exhibit a very different phenotype in another. These genotype-environment interactions are physiological, a phenomenon known as acclimatization, and are one other source for the observed variation among local populations of species that have large geographic ranges, or occur across clines. Such variation has been observed in many different groups of organisms including plants, vertebrates, crustaceans, insects, and mollusks (Dehnel, 1955; Chapin and Chapin, 1981; Conover and Schultz, 1995).

Common garden experiments and reciprocal transplants are standard ways to determine which mechanism is functioning in a species' geographic variation in physiological and life history traits. The degree of persistence of physiological differences after transplantation has been assumed to provide insight on the nature of those differences (i.e. whether they were a result of genetic differences, or different environmental pressures) by other studies as well

(Iglesias et al., 1996; Kautsky et al., 1990). Differences among organisms from different locations that disappear once environmental conditions are equalized (i.e. in a common garden) can be deemed environmentally rather than genetically based. Differences in traits that persist once the environmental differences have been removed may be attributed to more genetic differences among the respective stocks, as opposed to acclimatization. Whether the geographic variation is a result of genetic differences or environmentally induced differences or a combination, this phenomenon has significance for oyster restoration efforts and especially for water quality mitigation as the impact of those transplanted oysters may be strongly influenced by their original locale.

Managers have limited options for stock selection and often rely on hatchery-produced seed (Allen et al., 1993; Kennedy et al., 1996), which is often the product of limited brood stock (Kennedy et al., 1996). Because hatchery-raised oysters are grown in relatively pristine conditions, it is inevitable that these oysters undergo some level of selection (whether deliberate or inadvertent) for superior performance (growth rate and survival) in these conditions (Allen et al., 1993). Such settings are generally some of the most pristine environments that can be achieved in a hatchery or are available in our coastal waters. The performance of such stocks in highly impacted waters is not generally known.

In New Hanover County, NC, a number of tidal creek systems exist with a relatively wild range of environmental conditions, oysters that recruit and survive in different tidal creeks experience vastly different environmental conditions and thus may have undergone adaptation to ambient conditions. Adaptation would have arisen by selection (post-settlement mortality), coupled with some degree of isolation (low immigration, restricted gene flow). In Wilmington, North Carolina (34°N latitude, 77°W longitude), tidal creeks range wildly in respect to water quality. If oysters have locally adapted to these different conditions then their performance following transplantation to areas with vastly different conditions may be suboptimal.

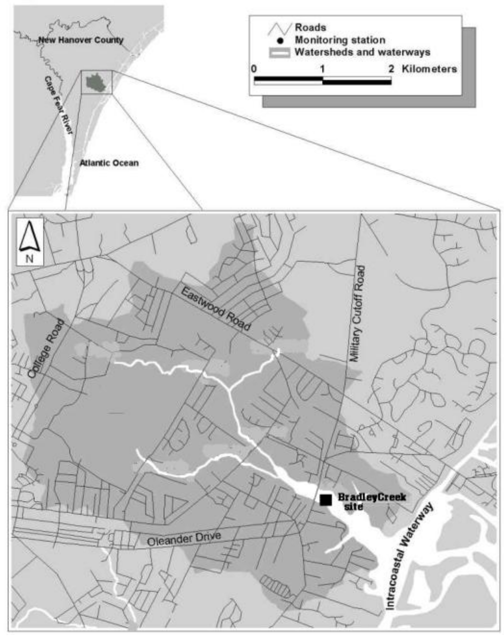
This study employed reciprocal transplants and a common garden approach to determine if oysters from two tidal creeks exhibited local adaptation (phenotypical differences that persist under common environmental conditions) with respect to performance (growth rate and survival). The null hypothesis tested, was population origin does not have an effect on growth and survival of the Eastern oyster, *Crassostrea virginica*.

STUDY SITES

Reciprocal Transplant Sites

Sites chosen for the reciprocal transplant for both Transplant 1 (Summer) and Transplant 2 (Fall-Winter) were the lower reaches of Bradley Creek (34°12'N latitude, 77°50'W longitude) and of Pages Creek (34°16'N latitude, 77°46'W longitude), both in New Hanover County, near the city of Wilmington, North Carolina (Figure 1). These two creeks were selected based on significant differences in their environmental conditions (Mallin et al., 2003; Mallin et al., 2005). Bradley Creek is the most polluted and impacted creeks in New Hanover

A.



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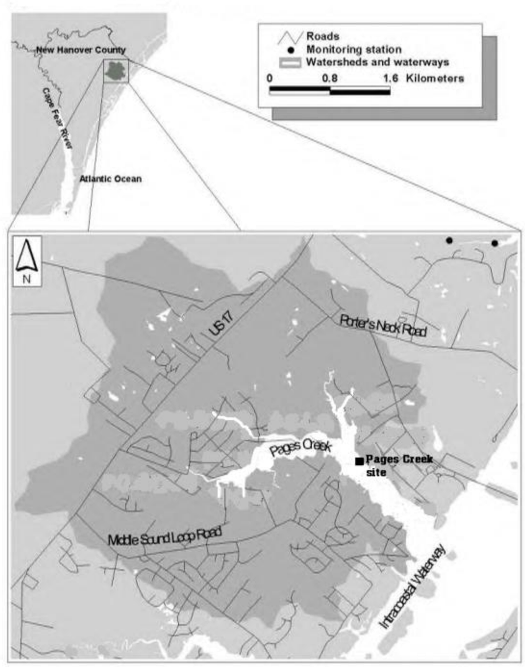


Figure 1: Reciprocal transplant sites (A=Bradley Creek watershed, B=Pages Creek watershed) for Transplant 1(Summer) and Transplant 2 (Fall-Winter). Squares represent reciprocal transplant sites.

County (although still only moderately affected), whereas Pages Creek is one of the most pristine (Mallin et al., 2000).

Routine water quality monitoring shows Bradley Creek to more frequently exhibit low dissolved oxygen (attributed to high nutrient inputs) than Pages Creek (Mallin et al., 2003). Results from Mallin et al. (2003), Mallin et al. (2004), and Mallin et al. (2005) have also indicated that Bradley Creek has also exhibited substantially higher levels of ammonium and Nitrate.

Although not statistically compared, there are several aspects in which Pages Creek and Bradley Creek differ that would have an effect on the oysters that live within those creeks. Bradley Creek was first closed to shellfishing in 1947, due to elevated *Escherichia coli* concentrations (Mallin et al., 2000b). In contrast, several large portions of Pages Creek are still open to shellfishing.

Federal regulations state that to remain open to shellfishing, the creek must have an average coliform unit count of 14 CFU/100mL or less, and the creek cannot exceed 43 CFU/100 mL more than 10% of the time (Mallin et al., 2000). *E. coli* levels determine whether or not shellfishing is allowed in that body of water, which has an effect on oysters in the form of fishing pressures. Bradley Creek's watershed covers more land than Pages Creek (2,448 hectares vs. 1,230), has a larger human population (13,657 people vs. 4,185), has a higher percentage of land developed (77.8% vs. 69.4%), and has a higher ratio of percentage impervious coverage to percentage developed land (33.3% vs. 12.5%) (Mallin et al., 2000).

Common Garden Site

The site chosen for the common garden for both transplants was along the Intracoastal Waterway (ICWW) behind the University North Carolina Wilmington's Center for Marine Science (34°08'N latitude, 77°51'W longitude), also in New Hanover County near the city of Wilmington, North Carolina (Figure 2). Residential homes, and a small handful of small businesses and a marina surround it. This location is closed to shellfishing.

MATERIALS AND METHODS

Experimental Design Overview

In the first transplant, stock success at each site was assessed by growth and survival. Growth was monitored by individually labeling and measuring height and width of each oyster by hand on a monthly basis. Soft tissue dry weight of a subset of each stock at each site was also examined at the beginning and end of the transplant. Survival was monitored monthly by visual inspection. Unfortunately there was very high mortality within the first month in the common garden site, so this aspect of the study is not discussed.

The second transplant's experimental design was modified in response to the mortality problems encountered in Transplant 1. Instead of measuring each individual oyster every month, a total cage yield was taken to get an average weight per oyster in an effort to reduce handling stress and time spent out of their environment. The number of oysters was also increased: instead of 150 oysters

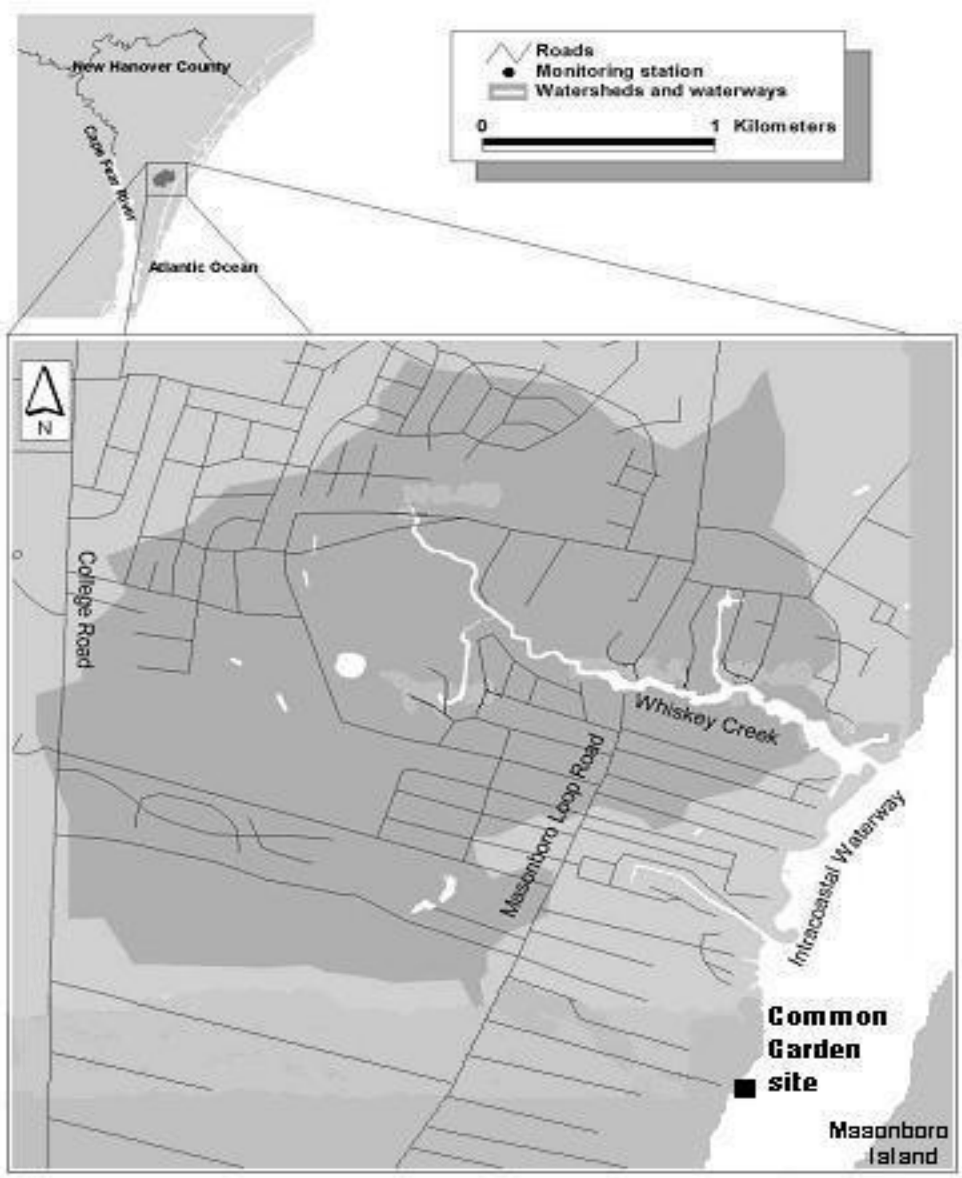


Figure 2: Common garden site, along the Intracoastal Waterway. The square represents the common garden site.

of each stock at each site and 50 oysters of each stock at the common garden, 300 oysters of each stock were deployed at each site and 300 oysters of each stock at the common garden.

In Transplant 2 stock success at each site was evaluated by monitoring survival and an indirect measure of growth. For transplant 2, growth was defined as changes in cage group over time. Change in the size of the oysters was monitored by individually measuring all oysters for height and total wet weight at the beginning and end of the transplant. Soft tissue dry weight for a small subset of each stock at each site was also measured at the beginning and end of the transplant. The status of the oysters (growth) was assessed monthly by taking each cage's total wet weight of all living oysters to get an average weight per oyster. Survival was monitored by visual inspection monthly.

In both transplants, each stock's success across sites was assessed using various statistical methods (see Methods section for Transplant 1 and Transplant 2) to see if the two stocks exhibited different or similar trend patterns across the different environmental conditions of each site.

Transplant 1 (Summer)

To obtain stock for this experiment, 350 oysters were collected from each of Bradley and Pages Creeks, breaking any groups into singles. All oysters were given individual specific labels using Bee-dots (from "The Bee Works" company, Canada), and placed randomly in 16 0.5m x 0.5m x 0.1m plastic mesh cages.

Cages were fastened to iron stakes that were driven into the substrate to keep them from moving or being disturbed. At the two reciprocal transplant sites and at the common garden site, the cages were installed ontop of living oyster reef (this kept cages approximately 7cm off the bottom) to minimize the effects of sedimentation. Six cages were deployed in Bradley Creek, 3 containing 50 oysters from Bradley Creek and 3 containing 50 oysters from Pages Creek. Another 6 cages of the same composition were deployed in Pages Creek, resulting in a complete reciprocal transplant. Cages were assigned to sites randomly. Growth and survival were monitored for 3 months (June 5, 2004 – September 8, 2004).

Oyster Growth

I assessed growth by measuring shell height (as defined as the longest axis of the oyster, from umbo to outer edge), shell width (as defined as the longest axis perpendicular to shell height), and soft tissue dry weight (STDW). Height and width measurements were taken monthly with calipers. A small exercise was performed to make sure the level of accuracy was acceptable when measuring oysters by hand, using calipers. After measuring 5 oysters 5 times at random (sizes: 35.78 – 65.3 mm in height) standard deviation between measures of the same oyster ranged from $\pm$ 0.158 mm to $\pm$ 0.638 mm. Soft tissue measurements were done once at the beginning of the experiment (using oysters not used in the deployments) and once at the end of the experiment (on randomly selected oysters from each stock after they were removed from each site). To do this, the soft tissue of 25 oysters from each stock was dissected, placed into pre-weighed aluminum pans, and weighed (wet weight). Tissues were then dried for 48 hours at 78 ºC and then reweighed (dry weight).

Prior to transplant, ANOVA was used to compare shell height and shell width across cages, 1-way ANOVA was used to look at stock height between sites, and Analysis of Covariance (ANCOVA) was used to look at condition (as estimated by STDW vs. Height relations) between the two stocks. At the end of the transplant, ANCOVA was used to compare initial and final condition of the transplanted oysters, with shell height as the covariate. Differences in growth (as defined as all treatments' individual oysters difference in shell height for that particular month) of each stock at each site were analyzed using Friedman's method for randomized blocks, a separate test done for each site. For this statistical test, the average growth for each cage for each time point in the site is recorded in that site's table (columns = time points; rows = cages). All growth averages within a column are then ranked (highest growth in that time point = 6, lowest = 1). The sum is found for each cage's row of ranks ($\Sigma^{b}R_{ij}$) and a χ^2 value is calculated (Sokal and Rohlf, 1995). This test determines whether the cages (and stock, indirectly) differed significantly in growth. For example, if stock A grew significantly faster than stock B, then the cages containing stock A would consistently have a higher ranking and result in a significant χ^2 estimate. Caging effects were thought to be minimal because cages were randomly placed following each monthly assessment.

At each measurement date, dead oysters were removed and shell height was measured. These data were used in a logistic regression test to determine if the stocks experienced size-dependant mortality. For this test, the mortality rates were based on the height of the oysters at the beginning of the transplant, and the height of the living and dead oysters at the end of the first month of the transplant, because this is when the most mortality took place. In addition to the logistic regression test, the height of the dead oysters was compared to the height of the surviving oysters each month for each stock at each site to determine significant differences (ANOVA).

Oyster Survival

Oyster survival was assessed monthly by visual inspection. Dead oysters were removed and shell height measured. Survival analysis was used to test for homogeneity of each stock's survival curves across sites. Survival probabilities were calculated based on the number of oysters surviving from each stock at each site for each time point. Survival curves were generated from these survival probabilities, and these curves were compared statistically using the Mantel-Haenzel log-rank test (M-H log-rank test). In this test, all oyster survival numbers of a stock are pooled together and each of that stock's site survival curves are separately compared to that overall stock survival curve to see if they are homogenous, yielding a χ^2 value. This is a nonparametric test (Marubini and Valsecchi, 1995). Relative risk calculations were also done to look at a stock's chances of surviving when transplanted to either of the reciprocal transplant sites. Relative risks after *t* time units were calculated as follows:

$$RR = F_2(t) / F_1(t)$$

Where $F_1(t)$ and $F_2(t)$ are the death risks in the expected and actual treatments, respectively (expected treatment was a stock being transplanted back to its creek of origin, and actual treatment was a stock being transplanted in the new creek). A risk of <1 suggests that the oyster has a better chance of survival being transplanted to that creek as opposed to being transplanted back into their creek of origin. A risk of >1 suggests that the oyster has a better chance of survival being transplanted back into their creek of origin as opposed to being transplanted to that creek. A risk of 1 suggests that the oyster has the same risk of mortality if transplanted to that creek as it does if it were transplanted to its creek of origin. Relative risks provide information, but are not a statistical test (Marubini and Valsecchi, 1995).

Water Quality

All samples were taken at the same position in the tidal cycle, during similar size tides, and at the same location at the grow out location each time. Each parameter was measured once for each creek at each time point to give a brief snapshot of the environmental conditions at monthly samplings. Total suspended solids were measured by filtering 450 mL of water into pre-weighed filters, weighed, dried, and reweighed. Temperature observations were made using a thermometer, held approximately 10 cm below the water surface during each sampling. Salinity was determined using a refractometer.

Transplant 2 (Fall-Winter)

To obtain stock for the second transplant, 900 oysters were collected from the Bradley Creek site and 900 oysters from the Pages Creek site, carefully breaking any groups into singles. Oysters were weighed, measured (shell height), and then placed in 0.5m x 0.5m x 0.1m plastic mesh cages, 100 oysters per cage. An effort was made to use only oysters between 40 mm and 70 mm due to methods used to monitor oyster growth. Oysters were assigned to cages at random. Cages were assigned to sites at random as well.

Cages were installed overtop of living reef in the same manner as they were in Transplant 1(Summer). Six cages were deployed in Bradley Creek, 3 containing 100 oysters from Bradley Creek and 3 containing 100 oysters from Pages Creek. Another 6 cages of the same composition were deployed in Pages Creek, resulting in a complete reciprocal transplant. Six additional cages were deployed at the ICWW site; 3 contained 100 oysters each from Bradley Creek, and 3 contained 100 each from Pages Creek. Growth and survival was monitored for 7 months (October 2, 2004 – April 26, 2005).

Oyster Growth

At the start of the transplant, all oysters were measured (shell height) and weighed (shell + tissue + water in the oyster = total wet weight (TWW)). An

ANOVA looked at shell height across cages, and a one-way ANOVA examined stock yield between sites. An ANCOVA of TWW, with shell height as the covariate was used to determine if there were significant differences between stocks and between sites for each stock at the start of the transplant. ANCOVA analysis was used because it accounts for stock differences in the function of how height affects TWW by looking at the TWW-shell height relationship as opposed to TWW by itself. An additional 25 oysters from each stock were sacrificed and soft tissue dry weight (STDW) determined using the same methods as in Transplant 1(Summer). ANCOVA was used to determine if oyster condition differed between the stocks prior to the transplant.

Monthly estimates of growth were obtained by determining yield for each cage. To measure yield, dead oysters were removed and all of the surviving oysters from each cage were weighed together. Average weight per oyster (AWO) was calculated by dividing cage yield by the number of surviving oysters and used as an estimate of overall stock growth. Differences in AWO attributable to stock, site and month were explored by a 3-way ANOVA. Significant effects were further examined using a Tukey's HSD test to determine if stocks differed between sites.

Additionally, final shell height measurements were made with calipers, and TWW measures were taken. TWW of each stock between sites was explored using an ANCOVA. STDW was also determined for 25 oysters from each stock by site combination at the end of the transplant. ANCOVA was used to evaluate changes in STDW condition between stocks, and also changes in STDW condition of each stock between sites.

Oysters found dead at monthly samplings were measured (shell height) and the size of the dead were compared to the estimated size of the survivors. Size dependent mortality was evaluated by plotting the size of the dead oysters at each time point relative to the size of the surviving oysters at the initial and the final time point. Statistical analysis were not possible due to lack of knowledge of death dates within each month, and due to no size of the living oysters each month (only dead were measured at monthly sampling).

Oyster survival

Oyster survival was monitored and recorded monthly by visual inspection. Dead oysters were removed from the cages prior to measuring cage yield. The M-H log-rank test, as described in the methods for oyster survival in Transplant 1, was used to compare survival between the two stocks, and among sites. Relative risk calculations, also described in the methods for oyster survival in Transplant 1, explored a stock's chances of survival when transplanted to the two reciprocal transplant sites, or the common garden site.

Water Quality

Temperature, salinity and total suspended solids were measured during monthly samplings in Bradley Creek, Pages Creek, and the ICWW. Analytical and statistical methods were the same as those for Transplant 1(Summer).

RESULTS

Results Overview

In transplant 1 (3 months), cage did not have an affect on growth at either site, suggesting no differences between stocks. There were no clear trends in growth for either stock at either site. On average, oysters from Bradley Creek increased in height at both sites, whereas oysters from Pages Creek increased in height in the Bradley Creek site, but decreased in the Pages Creek site, although none were significant. Survival of the Bradley Creek stock was 49% (50% in Bradley Creek; 48% in Pages Creek), and the Pages Creek stock was 34% (48% in Bradley Creek; 23% in Pages Creek). Both stocks suffered extensive mortality within the first month of being transplanted to the common garden site in the Intracoastal Waterway (26% survivorship of Bradley Creek stock; 31% survivorship of Pages Creek stock). For this reason, the data from the ICW site was not used in any analysis for Transplant 1. The absence of this treatment from the experimental design and the low survival of oysters in each cage at the other two (especially the Pages Creek stock transplanted back into Pages Creek), made a second transplant necessary.

At the end of Transplant 2's 7 months, stock and site had a significant effect on average weight per oyster. The Bradley Creek stock increased average weight per oyster at every site while Pages Creek stock decreased average weight per oyster at each site, and both stocks grew the best in the common garden (the ICW) and the worst in the Bradley Creek site. By the end of the 7 months, survival of the Bradley Creek stock was 48% (18% in Bradley Creek; 49% in Pages Creek; 77% in the ICW). Pages Creek survival was 30% (19% in Bradley Creek; 34% in Pages Creek; 38% in the ICW).

Transplant 1 (Summer) Results

Oyster Growth

Prior to transplant, the differences between stocks were not significant at each site (ANOVA $F_{(15)} = 0.07$, $p = 0.791$). However, there were significant differences among cages (ANOVA: shell height: ($F_{(11)} = 8.47$, $p<0.001$); width: ($F_{(11)} = 5.36$, $p< 0.001$) at the beginning of the transplant. The significant cage effect can largely be attributed to 2 cages containing Pages Creek oysters, based on pairwise comparisons (Tukey's HSD test) (Figure 3). A One-way ANOVA revealed a significant difference in Bradley Creek stock shell height between the two reciprocal transplant sites ($F_{(1,298)}= 13.92$, $p<0.001$). There was also a significant difference in Pages Creek stock shell height between sites (ANOVA $F_{(1, 298)}= 11.602$, $p<0.001$). In both cases, the oysters deployed to the Bradley Creek site were larger than those deployed to the Pages Creek site.

By the end of Transplant 1, Bradley Creek oysters increased in height at both sites. On average, oysters from Bradley Creek transplanted to Bradley Creek (B in B) grew 1.2 mm in height, and oysters from Bradley Creek transplanted to Pages Creek (B in P) grew 1.5 mm in height. Oysters from Pages Creek transplanted to Bradley Creek (P in B) increased 1.7 mm in height in 3 months, oysters from Pages Creek transplanted to Pages Creek (P in P)

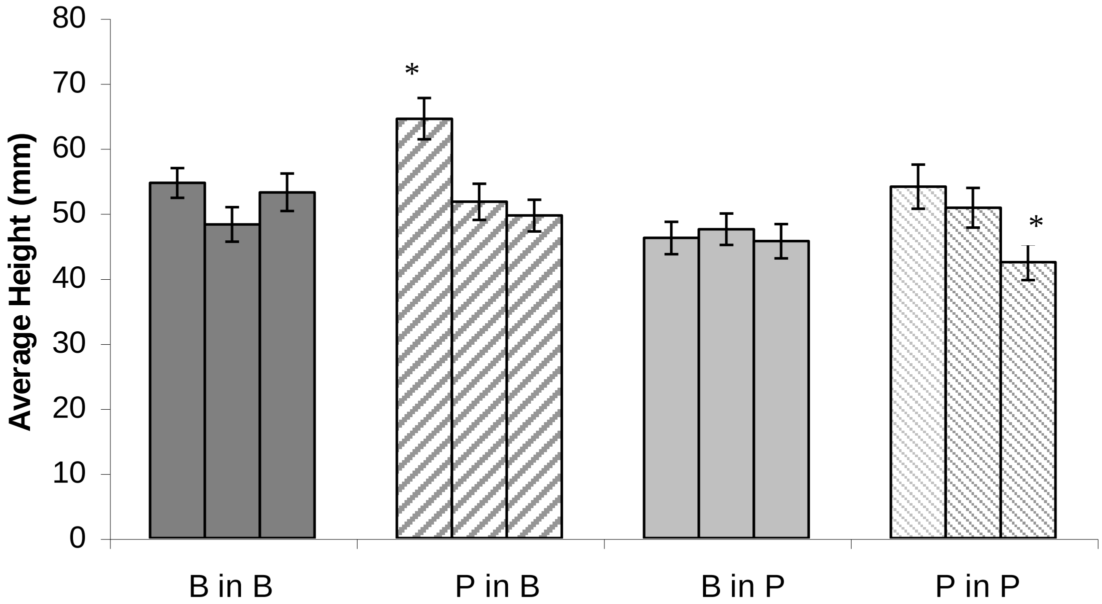


Figure 3. Transplant 1(Summer). Average shell height of all oysters in each cage for each stock at each site prior to deployment. Vertical lines represent standard error. Each bar is one of three replicate cages for that treatment. Cages differing significantly from the rest are marked with a *. For this figure as well as all following, "B in B" represents Bradley stock transplanted to Bradley Creek; "B in P" represents Bradley stock transplanted to Pages Creek; "P in B" represents Pages stock transplanted to Bradley Creek; "P in P" represents Pages stock transplanted to Pages Creek.

decreased of 0.7 mm in height (Figure 4). Decrease in average shell height was likely due to measurement error and damage to oyster shells during handling or possible predation. At both sites, Friedman's method for randomized blocks reveal that cage did not have a significant affect on average growth, an indirect indicator that stock did not have a significant effect on growth (Bradley Creek site $\chi^2 = 2.04$, $p > 0.75$; Pages Creek site $\chi^2 = 3.95$, $p > 0.50$). There were no clear patterns in growth observed between the two stocks.

Soft tissue dry weight (STDW) exhibited a non-linear relationship with shell height and thus both the STDW and shell height data was log transformed prior to ANCOVA analysis. ANCOVA analysis was used because it accounts for stock differences based on a relationship between height and STDW. Pages and Bradley Creek oysters exhibited significantly different condition at the beginning of the experiment (ANCOVA $F = 6.1$, $p = 0.017$). The homogeneity of slopes model (ANCOVA) indicated that neither stock ($F_{(1)} = 0.06$, $p = 0.812$), nor the interaction ($F_{(1)} = 0.06$, $p = 0.812$) had a significant effect on STDW. However, height did significantly affect STDW ($F_{(1)} = 83.55$, $p < 0.001$). ANCOVA same slopes model indicated that stock did have a significant effect on STDW, Pages Creek oysters were significantly lighter than Bradley Creek oysters ($F_{(1)} = 6.1$, $p = 0.017$) (Figure 5).

Condition of oysters for Bradley Creek stock, as estimated by STDW vs. height slopes, did not change over the course of the transplant. Analysis of covariance of STDW detected a significant effect of shell height on STDW ($F_{(1)} = 46.68$, $p < 0.001$), but neither site (Bradley versus Pages Creek $F_{(1)} = 0.91$, p=

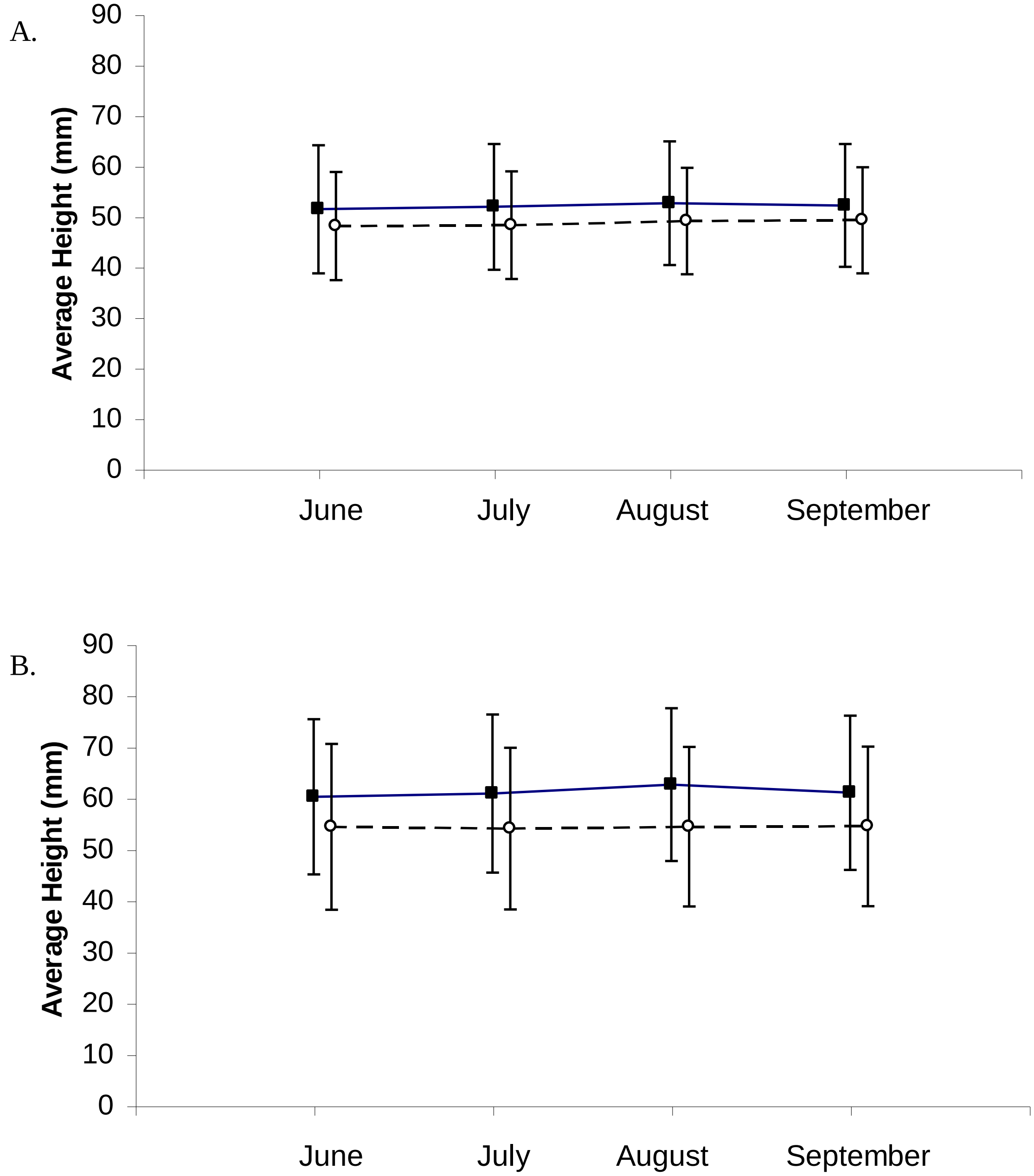


Figure 4. Transplant 1 (Summer). Average of all treatments' individual oysters height for each month for the two stocks of oysters (A = Bradley Creek oysters, B = Pages Creek oysters) at reciprocal transplant sites (Bradley Creek site = black squares; Pages Creek site = white circles). Data points are the average height of all oysters of that stock at that site, and vertical lines represent standard error.

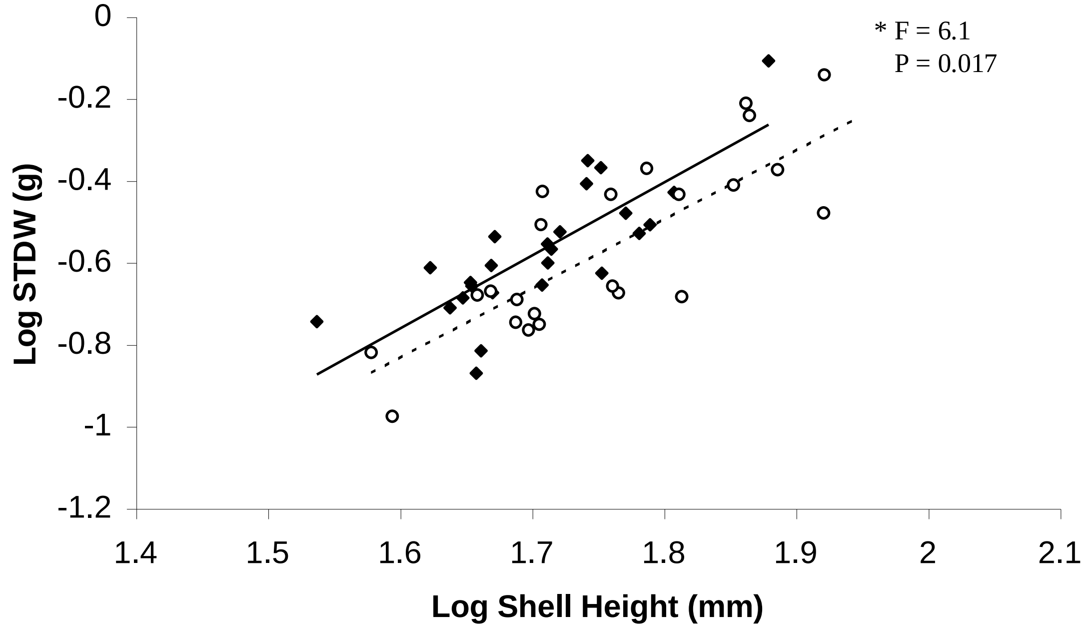


Figure 5. Transplant 1 (Summer). Log soft tissue dry weight (STDW) versus log shell height plots for all oysters prior to transplant. (Bradley Creek oysters = solid, black squares; Pages Creek dashed, white squares). Regression equations are as follows: Bradley: y = 1.7848x – 3.6187 (r^2 = 0.6253); Pages: y = 1.6939x – 3.545 (r^2 = 0.7164). There is a significant difference between the STDW condition of the two stocks, as marked by the *.

0.345), nor the interaction ($F_{(1)}$ =0.96, p=0.333) were significant. ANCOVA same slopes model also showed that there was no site effect ($F_{(1)}$ =0.21 p=0.652). However, the ANCOVA same slopes model revealed significant difference between initial and final STDW measurements ($F_{(1)}$ =25.28 p=<0.001) (Figure 6), showing Bradley Creek oysters to have decreased in condition by the end of the transplant. Condition did not differ between Pages oysters deployed in Pages Creek and those deployed in Bradley Creek 3 months after transplant ($F_{(1)}$ =1.23, p=0.274). ANCOVA same slopes model suggested the same, that there was not a site effect (site $F_{(1)}$ =1.71, p=0.198). There was no significant change between the initial and final STDW assessments in the Pages Creek stock, regardless of site ($F_{(1)}$ =0.00, p=0.966) (Figure 6).

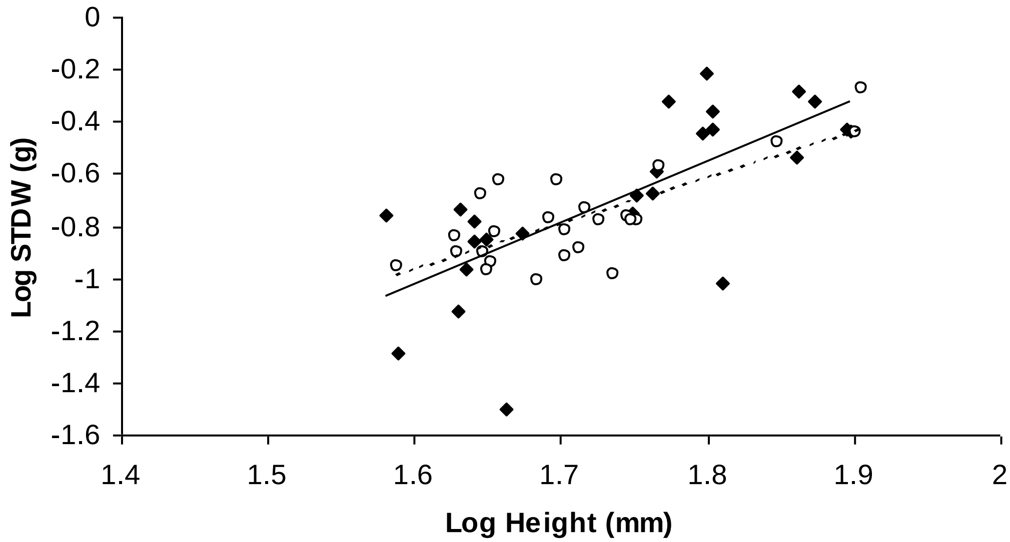
Oyster Survival

Overall survivorship of Bradley Creek oysters at all sites was 49%, and overall survivorship of Pages Creek oysters was 34%. Survival of each stock/site combination is shown in Figure 7.

Evaluation of survival curves suggests that all treatments (except P in P) show similar patterns regardless of site of deployment or stock (Figure 8). The exception to this generalization, P in P, exhibited 33% survivorship, as compared to other treatments, which exhibited between 60 and 50% survivorship.

Using the M-H log-rank test (Survival analysis), site-specific survival curves for each stock over the duration of the transplant were found to be homogeneous relative to their average stock performance for Bradley Creek oysters (B in B: $\chi 2$ = 0.170, p = 0.918; B in P $\chi 2$ = 0.488, p = 0.784), but not

A.



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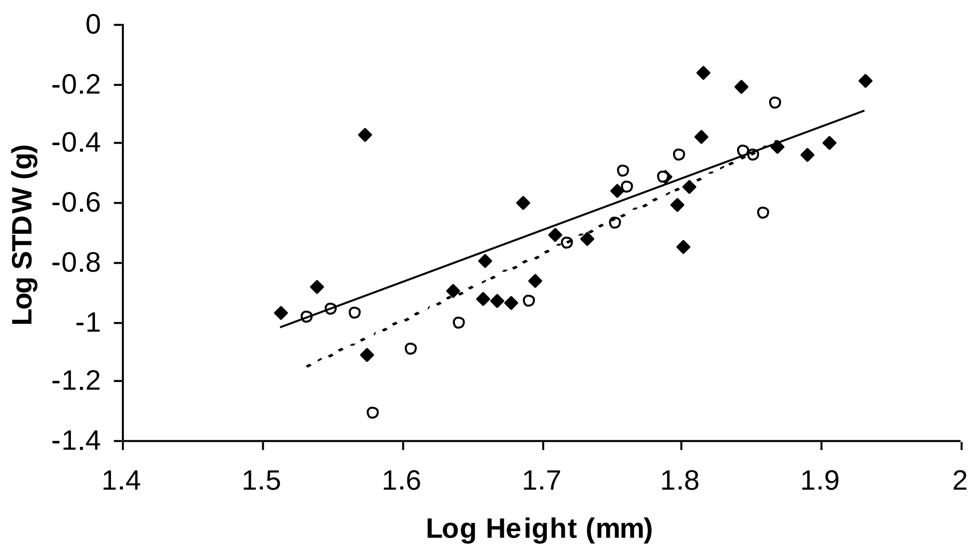


Figure 6. Transplant 1 (Summer). Final log shell height versus log soft tissue dry weight (STDW) regressions for the two stocks of oysters, (A = Bradley Creek oysters, B = Pages Creek oysters) at reciprocal transplant sites (Bradley = solid, black squares; Pages = dashed, white circles). Regression equations are as follows: B in B $y = 2.3539x – 4.7855$ ($r^2 = 0.5211$); B in P $y = 1.7642x – 3.793$ ($r^2 = 0.5851$); P in B $y = 1.7431x – 3.6552$ ($r^2 = 0.5617$); P in P $y = 2.2555x – 4.6069$ ($r^2 = 0.7951$).

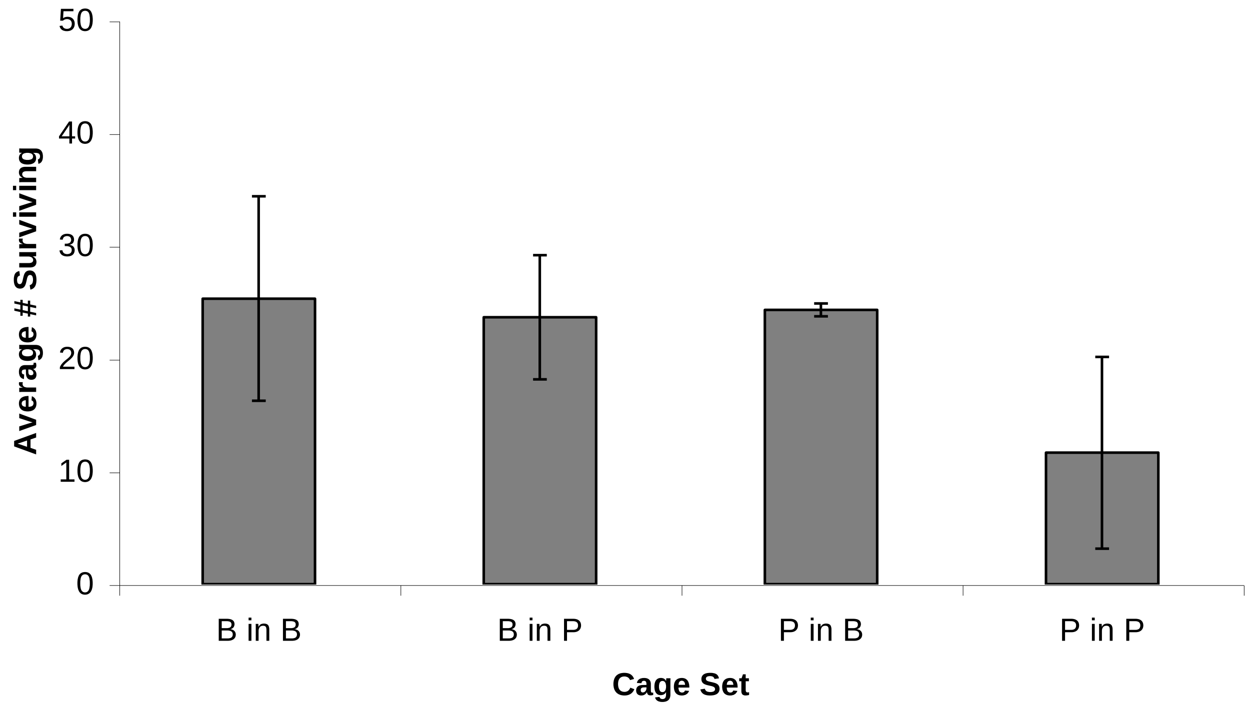
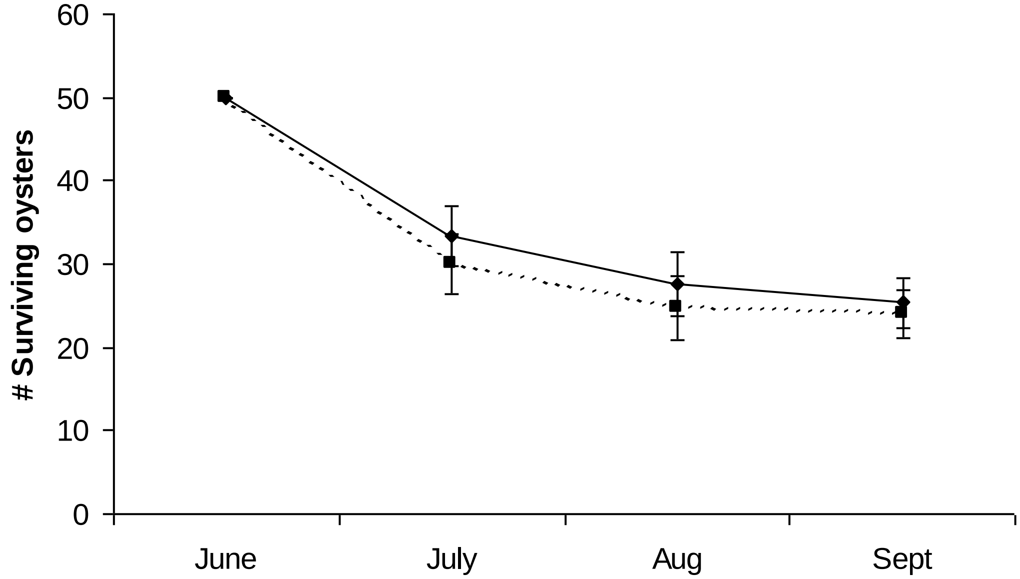


Figure 7. Transplant 1 (Summer). Average number of surviving oysters (out of the original 50 oysters per cage) among each group of 3 replicate cages. Vertical lines represent standard error.

A.



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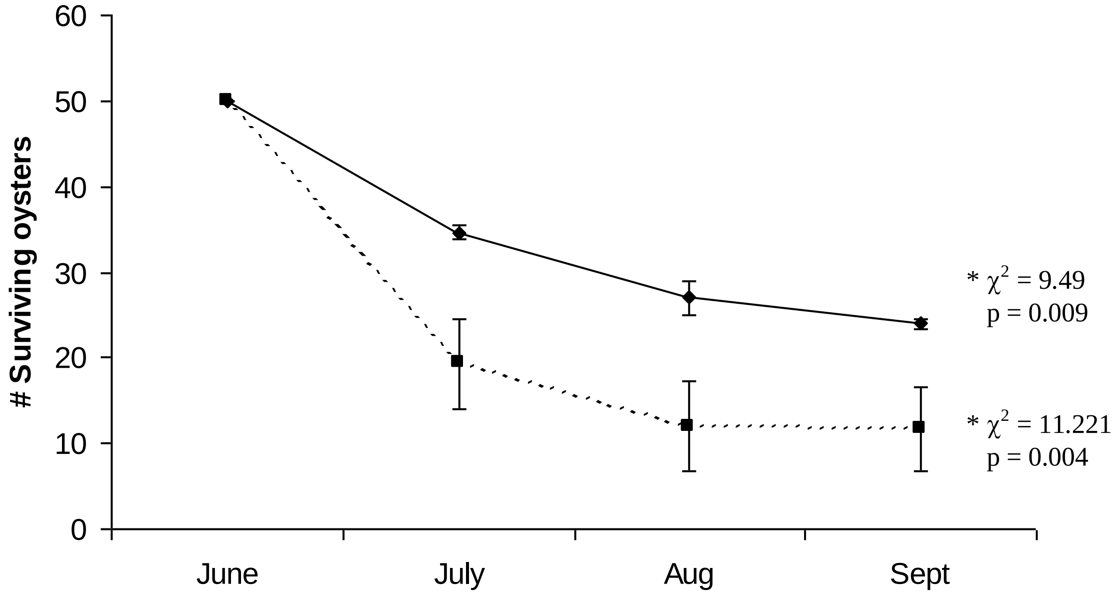


Figure 8. Transplant 1 (Summer). Survival curves for oysters of the two stocks (A = Bradley Creek oysters, B = Pages Creek oysters) at reciprocal transplant sites (Bradley Creek site = solid, Pages = dashed). Data points are the average of the three replicate cages. Vertical lines represent standard error. Significant differences between the treatments' survival curve and the survival of the stock overall, exhibited by both P in B and P in P, are marked by a *.

for Pages Creek oysters (P in P: $\chi 2 = 11.221$, $p = 0.004$; P in B: $\chi 2 = 9.49$, $p = 0.009$).

Relative risks were calculated to see what the oyster's risk of mortality was if transplanted to another creek as opposed to being transplanted back to its creek of origin. The calculations reveal that overall, on a month-by-month basis, Bradley Creek oysters have a much better chance of survival if transplanted back into Bradley Creek, where Pages Creek oysters surprisingly have a much better chance of survival if transplanted to Bradley Creek. These relative risks suggest a site effect. However, the site-specific survival curves shown in Figure 8 show the two stocks have different magnitudes of differences between sites, suggesting an origin effect as well. Monthly individual risk calculations are shown in Table 1.

Size-dependent Mortality

ANOVA reveals that the average shell height of the dead oysters was not significantly different from the oysters that survived (B in B $F = 0.06$, $p = 0.803$; P in B $F = 0.00$ $p = 0.945$; B in P $F = 2.55$, $p = 0.122$). The only exception to this trend was for Pages oysters transplanted to Pages Creek, where significant differences in shell height between the living and dead oysters were observed (ANOVA $F = 8.23$, $p < 0.05$). The average size of the oysters that died was less than that of the oysters that survived in July and in August. September data for this treatment was not applicable for a test due to only 1 oyster death (Figure 9).

Table 1. Transplant 1 (Summer). Relative Risk Calculations (Survival Analysis) for Bradley Creek stock and Pages Creek stock for each month. A risk of <1 suggests the oyster has a better chance of survival being transplanted to that creek as opposed to being transplanted back into their creek of origin. A risk of >1 suggests that the oyster has a better chance of survival being transplanted back into their creek of origin as opposed to being transplanted to that creek.

Origin	Month	Relative Risk of being transplanted to new creek as opposed to being transplanted to creek of origin
Bradley	June	1.200
	July	1.255
	August	1.408
Pages	June	0.500
	July	0.292
	August	1.166

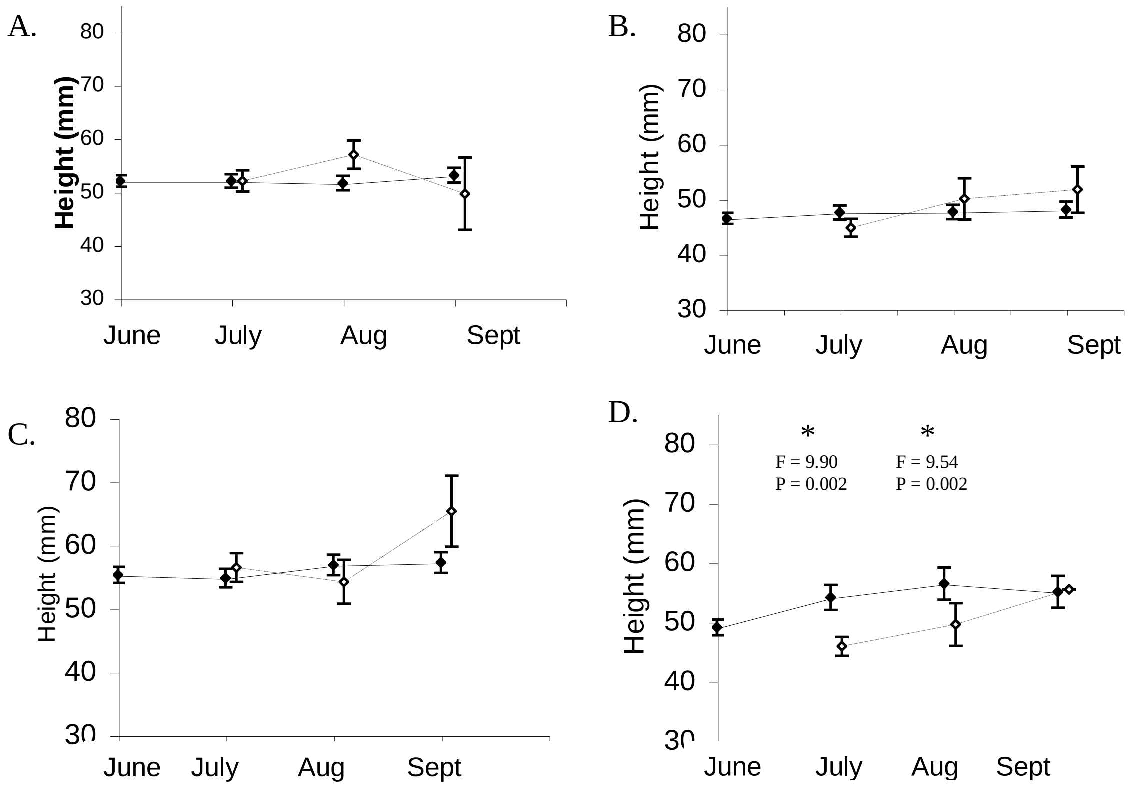


Figure 9. Transplant 1(Summer). Average shell height of oysters, both living and dead (living = black, dead = white), over time for each stock at each site (A = B in B, B = P in B, C = B in P, D = P in P). Vertical lines represent standard error. Significant differences between average height of living and average height of dead only occurred in P in P; they are marked by a * (ANOVA).

A logistic regression test suggests the same. There was no significant size-dependent mortality (Figure 10) except for Pages oysters transplanted to Pages Creek. For all treatments except P in B, a trend was seen in that the mortality rate decreases as oyster height increases. For P in B, the mortality rate increases as size increases (although not significant). However, as shown in the previous test, only one treatment (P in P) had a significant difference between its slope and zero, indicating a significant relationship between height and mortality (P in P: χ^2=7.9, p = 0.0049). All treatments' slopes and p-values are reported in Table 2.

Transplant 2 (Fall-Winter) Results

Oyster Growth

Despite efforts to control for size across cages, there were significant differences in shell height among cages (ANCOVA Bradley: $F_{(8)}$ = 4.0, p = <0.001; Pages: $F_{(8)}$ = 4.0, P = 0.014) prior to deployment in the field (Figure 11). However, pairwise comparisons reveal that for both stocks, log height did not differ significantly between sites (Bradley oysters: $F_{(2)}$ = 2.0, p = 0.126; Pages oysters: $F_{(2)}$ = 2.0, p = 0.252).

There were minimal differences in each stock's cage yield (total mass of living oysters) between sites prior to transplant. A one-way ANOVA indicated significant difference in Bradley Creek stock cage yield between sites (F=0.21, p=0.012). A Tukey's HSD test revealed that the yield of those transplanted to Bradley Creek were not significantly different from those transplanted to Pages Creek (p=0.093), or from those transplanted to the Intracoastal Waterway

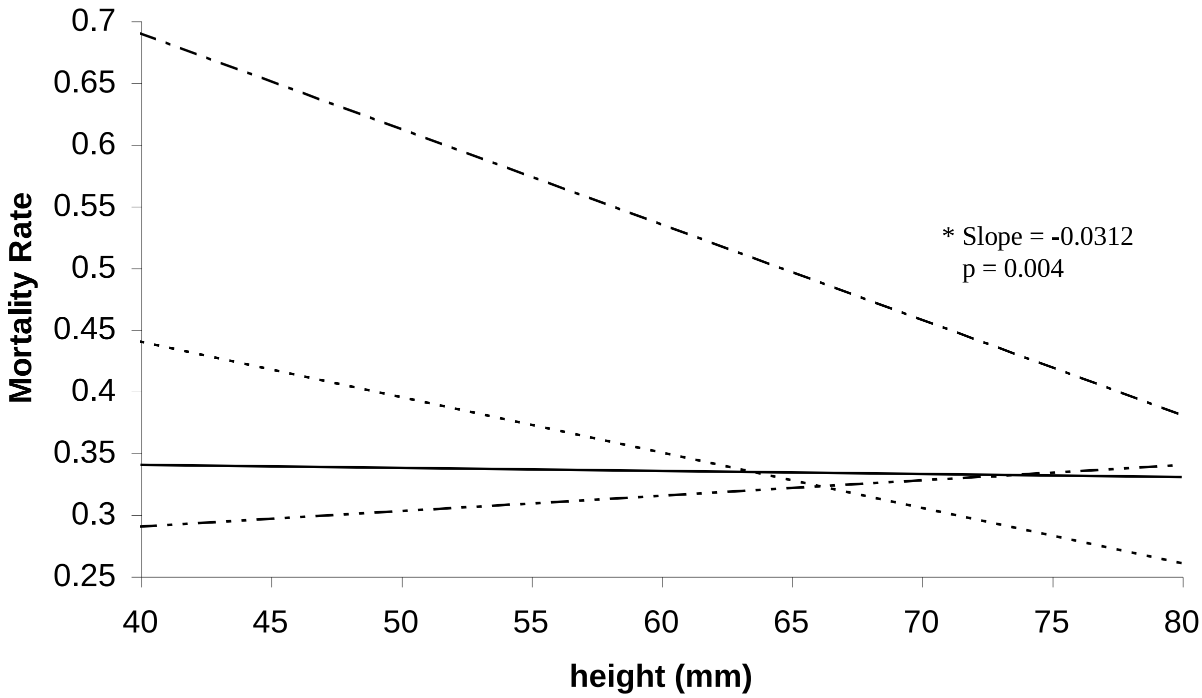


Figure 10. Transplant 1(Summer). Mortality rate of oysters of two stocks at two sites (B in B = solid line; B in P = dashed line; P in B = lines with 2 dots; P in P = lines with 1 dot), estimated by a logistic regression test. Significant differences between a treatment's mortality rate : height slope and a slope of zero, exhibited only by P in P, are marked by a *. Mortality rates are based on the size of all living oysters at the beginning, and the size of all the dead oysters at the end of the first month after transplant.

Table 2. Transplant 1 (Summer). Results of Logistic Regression tests on size-dependent mortality among oysters. Significant effects are marked by a *.

Site	Origin	Intercept	Slope	χ^2 value	P-value
Bradley	Bradley	-0.6437	-0.0009	0.01	0.942
Bradley	Pages	-1.0786	0.0047	0.16	0.6868
Pages	Bradley	0.5043	-0.0196	1.92	0.1654
Pages	Pages	2.0140	-0.0312	7.90	0.0049 *

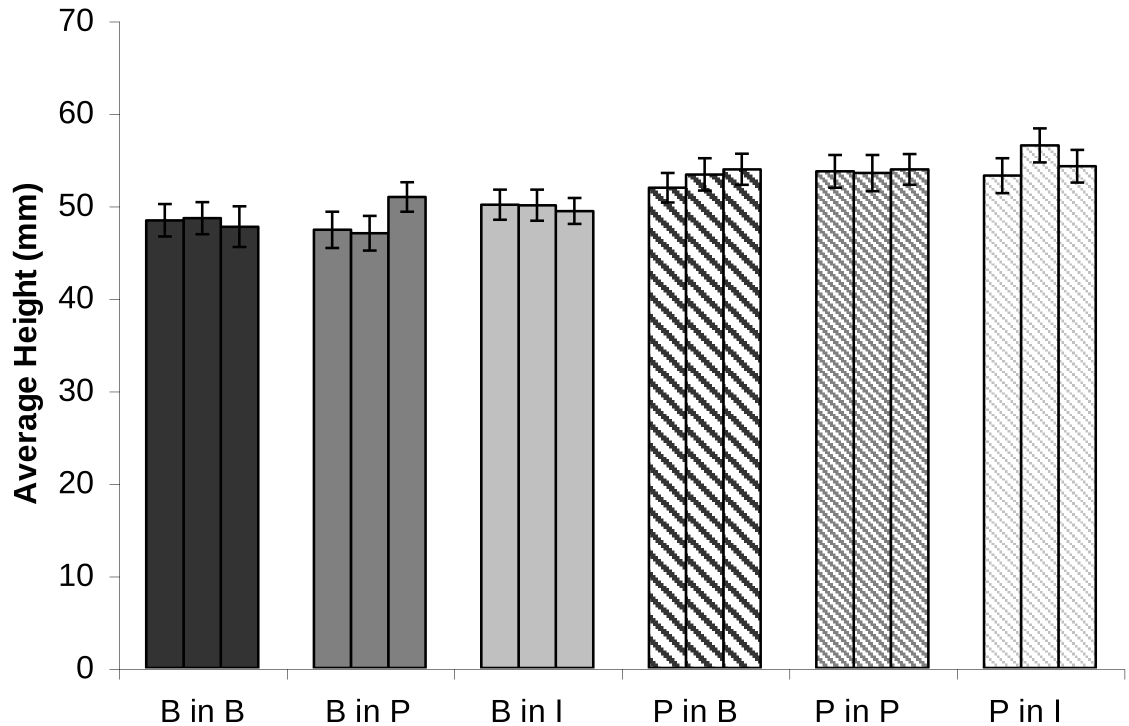


Figure 11. Transplant 2 (Fall-Winter). Average shell height of all oysters in all cages previous to transplant. Vertical lines represent standard error. Each bar represents one of three replicate cages for that treatment. “B in I” represents Bradley stock transplanted to the Intracoastal Waterway’ “P in I” represents Pages stock transplanted to the Intracoastal Waterway; all other treatments are the same as previously described (Figure 3). There were no significant differences between cages at each site.

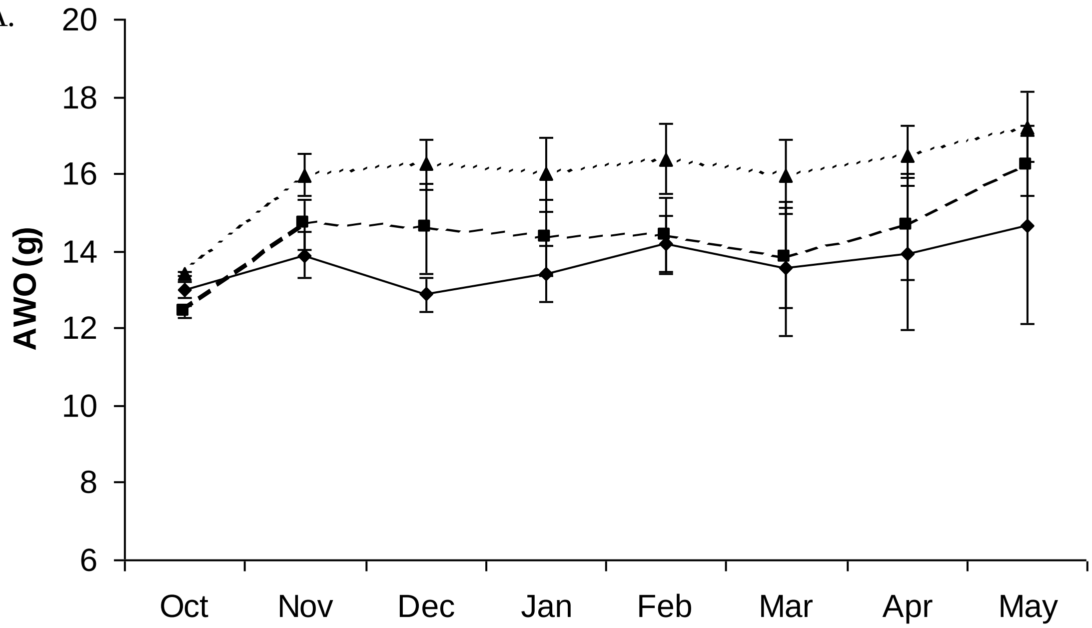
(p=0.211). However, the yield of those transplanted to Pages Creek was significantly greater than the yield of those transplanted to the Intracoastal Waterway (p=0.010). The one-way ANOVA indicated that there were no significant differences among the Pages Creek cage yields between sites (F=4.781, p=0.057).

Analysis of final average weight per oyster (AWO) measurements for all oysters at all sites (3-way ANOVA with interaction) indicated that there was significant stock by site interaction for AWO ($F_{(2)}$ = 4.32; p= 0.016), as well as a time by stock interaction ($F_{(6)}$ =8.66, p<0.001). Stock also had a significant effect on growth ($F_{(1)}$ =87.4, p<0.001), as did site ($F_{(2)}$ =41.66 p<0.001) (Table 3). Bradley Creek stock increased AWO at every site. By the end of the 7 month transplant, on average, B in B increased AWO 1.509 g, B in P increased 2.213 g, and B in I increased 3.083 g. Tukey's HSD test shows that B in B and B in P were significantly different from B in I (B in B vs B in I: p<0.001, B in P vs B in P: p=0.029). B in B and B in P were not significantly different (p=0.758) (Figure 12). As with the Bradley Creek oysters observations, there was a significant interaction between stock and site effecting the measurements for the Pages Creek oysters (Table 3). By the end of the 7 months, Pages Creek oysters decreased AWO at every site. On average, P in B decreased AWO by 6.059 g, P in P decreased 3.352 g, and P in I decreased 2.309 g. The Tukey's HSD indicated that P in P (p=0.001) and P in B (p<0.001) were both significantly different from P in I. P in B were also significantly different from P in P (p<0.001) (Figure 12).

Table 3. Transplant 2 (Fall-Winter). Results of a 3-way ANOVA (time, stock, site) with interaction to see if there were effects on Average Weight per Oyster. Significant effects are marked by a *.

Effect	SS	Df	Ms	F	P
Time	36.3	6	6.0	2.18	0.05 *
Stock	242.9	1	242.9	87.4	<0.001*
Site	231.6	2	115.8	41.66	<0.001*
Time*Stock	144.3	6	24.1	8.66	<0.001*
Time*Site	20.4	12	1.7	0.61	0.826
Stock*Site	24.0	2	12.0	4.32	0.016*
Time*Stock*Site	4.1	12	0.3	0.12	1.000

A.



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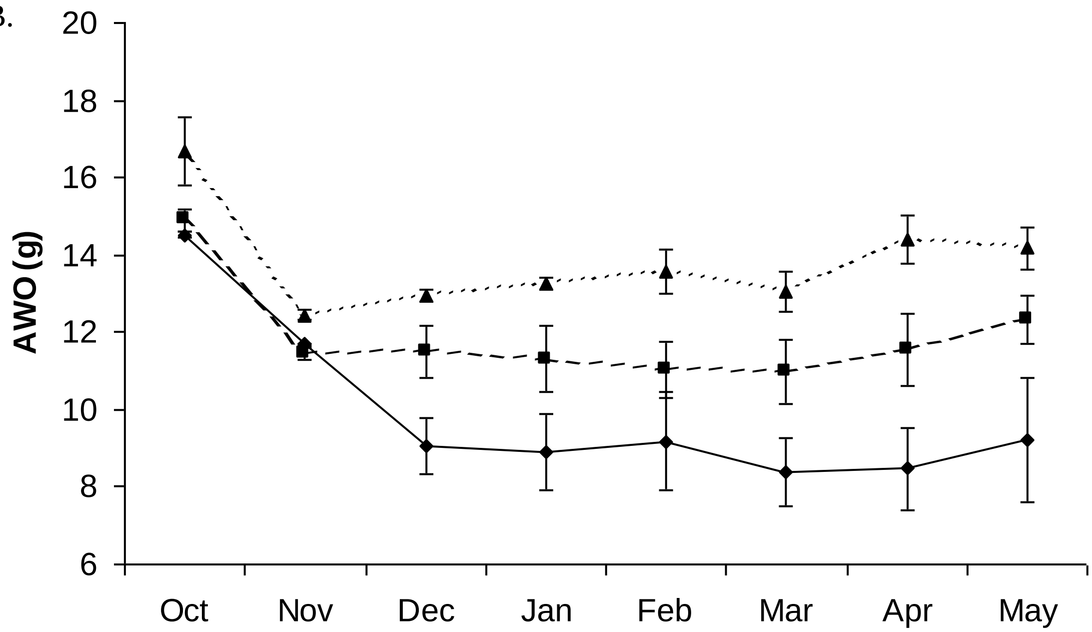


Figure 12. Transplant 2 (Fall-Winter). Change in Average weight per oyster (AWO) for the two stocks of oysters (A = Bradley Creek oysters, B = Pages Creek oysters) at reciprocal transplant and common garden sites (Bradley Creek site = solid, diamonds; Pages Creek site = squares, large dashed; ICWW site = triangles, dashed). Data points are average of three replicates, with standard error bars. Significant effects on AWO are presented in Table 3.

Prior to transplant, soft tissue dry weight (STDW) exhibited a non-linear relationship with shell height and thus both the STDW and shell height data were log transformed prior to ANCOVA analysis. Homogeneity of slopes test reveals that there was no stock*height interaction ($F_{(1)}$ =0.84, p= 0.364). An ANCOVA same slopes model also showed that there was no stock effect at the beginning of the transplant ($F_{(1)}$ =0.50 p=0.482) (Figure 13).

An ANCOVA showed the final STDW versus height slopes of B in B, B in P, and B in I to be significantly different ($F_{(2)}$ =3.24, p=0.045), suggesting that height has a different effect on STDW depending on transplant destination. However, a separate slopes model indicated no site effect on STDW condition ($F_{(2)}$ =2.89, p=0.062) suggesting that site only has an effect on condition in the largest oysters (Figure 14). ANCOVA same slopes model also suggest that condition did not change over time for Bradley Creek oysters (F=3.78, p=0.055). At the end of the transplant, a homogeneity of slopes model indicated that there was no site*height interaction affecting STDW ($F_{(2)}$ =2.16, p=0.123). ANCOVA same slopes model also suggested that site did not have an effect on STDW for Pages Creek oysters ($F_{(2)}$ =2.27 p=0.111) (Figure 14). ANCOVA same slopes model suggest that condition did not change over time for Pages Creek oysters (F=0.10, p=0.757).

Like STDW, total wet weight (TWW) and shell height data were log transformed prior to ANCOVA analysis. The test for homogeneity of slopes revealed significant differences between the stocks (stock*log height interaction F=20.00, p<0.001), necessitating the use of a separate slopes model. A

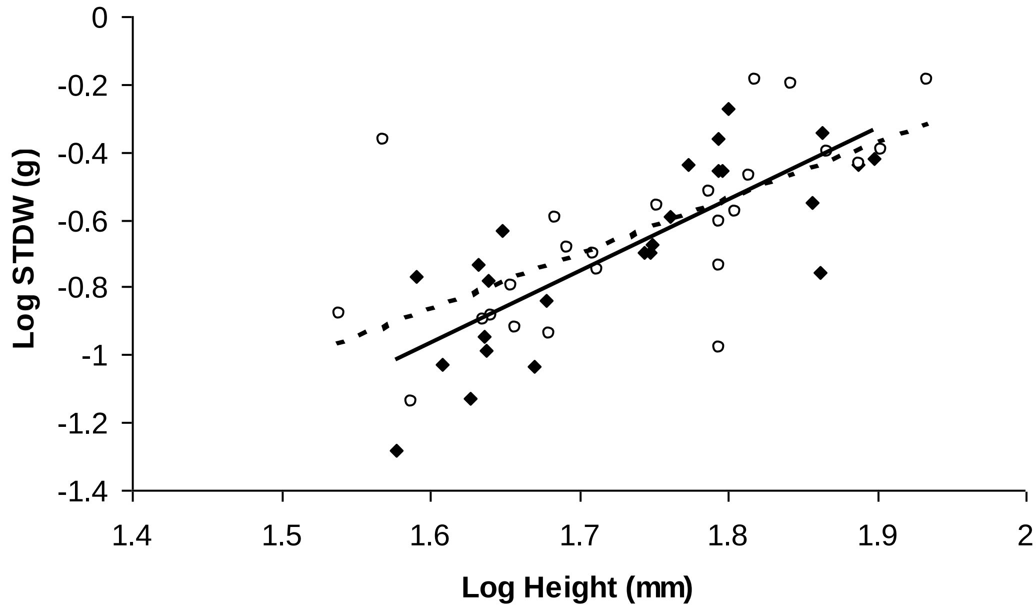
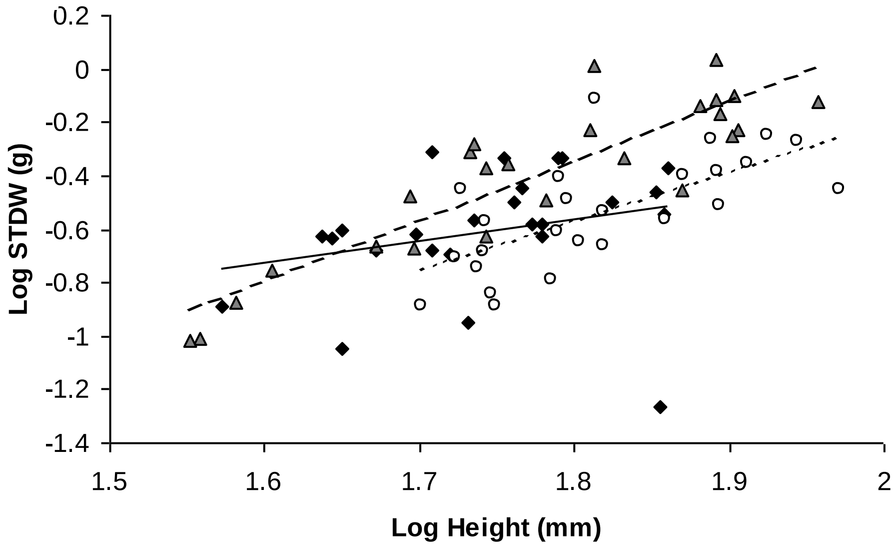


Figure 13. Transplant 2 (Fall-Winter). Log soft tissue dry weight (STDW) versus log shell height regressions for the two stocks of oysters (Bradley Creek oysters = solid, black; Pages Creek oysters = white, dashed) previous to the start of the transplant. Regression equations are as follows: Bradley y = 2.1139x – 4.3512 (r^2 = 0.6279); Pages y = 1.6464x – 3.5027(r^2 = 0.4538).

A.



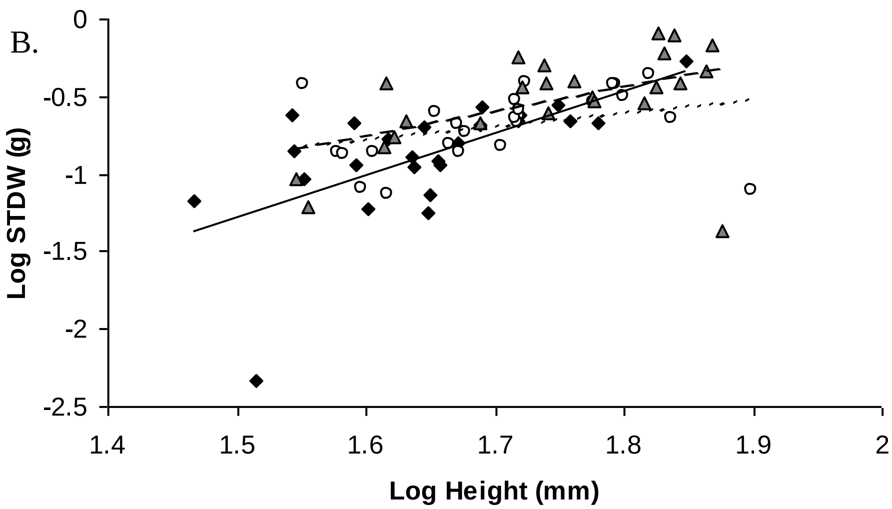


Figure 14. Transplant 2 (Fall-Winter). Log shell height versus log soft tissue dry weight (STDW) for the two stocks of oysters (A = Bradley Creek oysters B = Pages Creek oysters) at reciprocal transplant and common garden sites (Bradley Creek site = black diamonds, solid line; Pages Creek site = white circles, small dashed line; ICWW site = gray triangles, large dashed line) at the end of the transplant. Regression lines are as follows: B in B y = 0.8162x – 2.0293 (r^2 = 0.0754); B in P y = 1.8552x – 3.9133 (r^2 = 0.4684); B in I y = 2.2374x – 4.3737 (r^2 = 0.7903); P in B y = 2.6936x – 5.3108 (r^2 = 0.3798); P in P y = 0.8395x – 2.1212 (r^2 = 0.1102); P in I y = 1.5922x – 3.3028 (r^2 = 0.2439).

significant effect of stock was observed (stock $F_{(1, 1786)}$ =21.00, p<0.001), and Pages oysters were, generally, lighter than Bradley Creek oysters of the same shell height at all sites. Analysis of the STDW did not reveal any differences between stocks (same slope model $F_{(1,47)}$ =0.50, p=0.482)(Figure 13), suggesting that the differences in wet weight may be due to differences in shell mass or in amount of water in the tissue. ANCOVA revealed that TWW by height slopes did not differ significantly between sites prior to the start of the transplant study ($F_{(2)}$ =2.0, p=0.196) (Figure 15).

At the end of the transplant, however, the analysis of TWW resulted in a significant interaction between site and height (site*height F=7.1, p=0.001). A separate slopes model found site to significantly impact TWW (site $F_{(2)}$ =6.8, p=0.001). The Tukey's test revealed no significant difference between B in B and B in P (p=0.844), but there was a significant difference between B in B and B in I (p<0.001) and between B in P and B in I (p<0.001), showing B in I to be significantly heavier. There is an evident change in TWW condition suggested by the significant change in slope of B in I oysters relative to the initial estimate (Figure 16). ANCOVA revealed that the regression of TWW on shell height was not significantly different between sites for the Pages Creek oysters prior to transplanting ($F_{(2)}$ = 1, p= 0.252). After the transplant, there was still no significant difference between P in B, P in P, and P in I (ANCOVA $F_{(1)}$ = 0.94, p= 0.391). A common slope model to test for effect of site indicated the same (Site $F_{(2)}$ =2.5 p=0.083) (Figure 16).

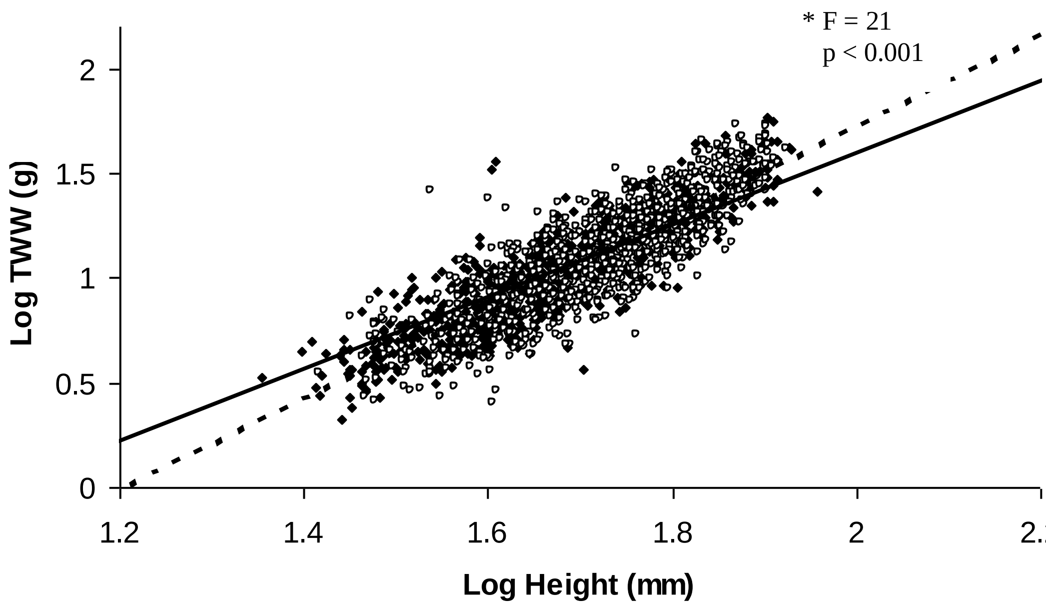
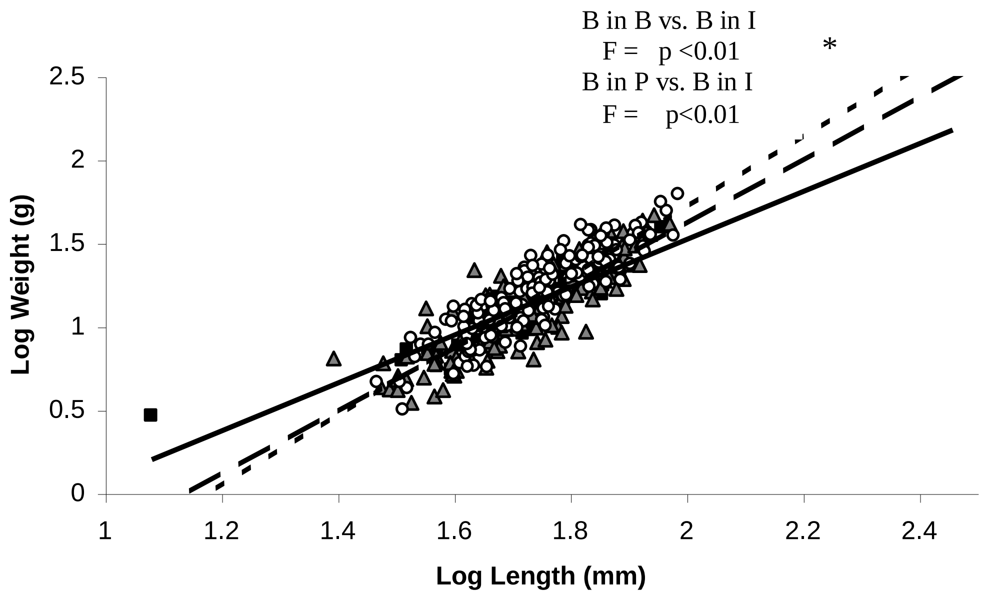


Figure 15. Transplant 2 (Fall-Winter). Log total wet weight (TWW) versus log shell height regressions for the two stocks of oysters (Bradley = black diamonds, solid line; Pages = white circles, dashed line) prior to transplanting. Regression lines are as follows: Bradley y = 1.7199x – 1.8461 (r^2 = 0.665); Pages y = 2.1708x – 2.6284 (r^2 = 0.6788). There was a significant difference in TWW condition between the two stocks, as marked by the *.



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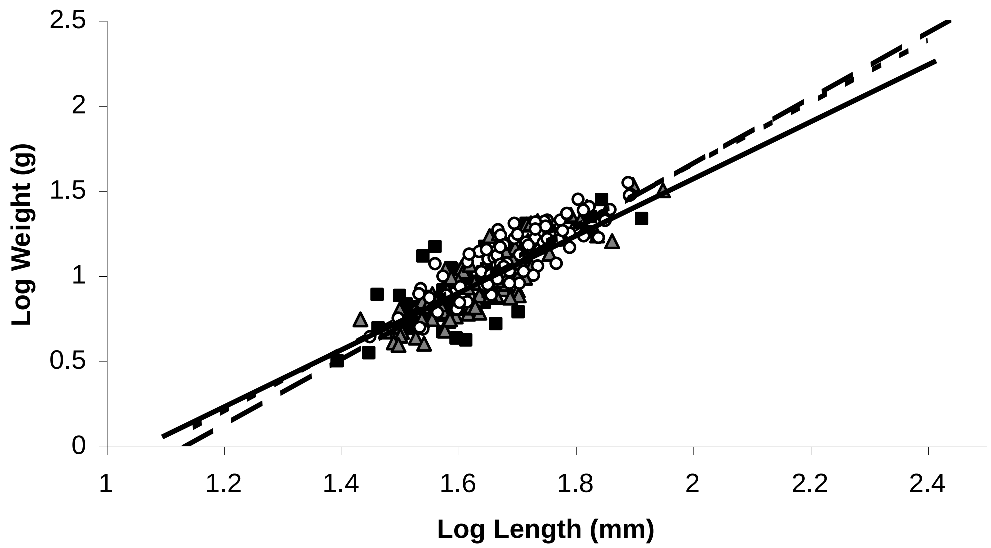


Figure 16. Transplant 2 (Fall-Winter). Final log shell height versus log total wet weight (TWW) regressions for the two stocks of oysters (A = Bradley Creek oysters, B = Pages Creek oysters) at the three sites (Bradley = black squares, solid line; Pages = gray triangles, large dashed line; ICWW = white circles, small dashed line). Regression equations are as follows: B in B Log y = 1.4367Log x – 1.352(r^2 = 0.7909); B in P Log y = 1.8922Log x – 2.1586 (r^2 = 0.7361); B in I Log y = 2.1001Log x – 2.476 (r^2 = 0.687); P in B Log y = 1.8261Log x – 1.9964 (r^2 = 0.7647); P in P Log y = 1.6746Log x – 1.7856 (r^2 = 0.6211); P in I Log y = 1.929Log x – 2.2004 (r^2 = 0.7458). Significant differences between a stock's treatments were only seen by B in I, which was significantly different from B in B and B in P, as marked by the *

Oyster Survival

Overall survivorship at all sites was 48% for the Bradley Creek stock, and 30% for the Pages Creek stock. Average survival among each set of replicate cages for each stock at each site is illustrated in Figure 17. Survival curves show Bradley Creek oysters survival inconsistent between sites, doing very well in the ICCW and poorly in Bradley Creek. Pages Creek exhibited a more consistent pattern, and did not do well in any sites (Figure 18).

An overall test for homogeneity (M-H log-rank test) of survival curves revealed that B in B and B in I exhibited distinct survival curves relative to those observed for all Bradley Creek oysters (B in B: $\chi 2= 147.17$, $p<0.001$)(B in I: $\chi 2= 68.43$, $p<0.001$); but that the B in P curve was not different ($\chi 2=11.15$, $p= 0.084$). For the Pages Creek oysters, only the P in B survival curve differed from the average of all Pages Creek oysters at all sites ($\chi 2= 13.85$. $p= 0.003$).

Relative risk calculations revealed that on a month-by-month basis, oysters from Bradley Creek had an overall lower risk of mortality if transplanted into Pages than if put back into Bradley Creek. The risk was reduced for Bradley Creek oysters transplanted to ICWW. Oysters from Pages Creek had a greater risk of mortality if they were transplanted into Bradley Creek than if they were to be put back into Pages Creek, but had approximately the same risk if they are transplanted to the ICWW. As in Transplant 1, relative risk suggested a site effect, however, site-specific survival curves in figure 18 show the two stocks had different magnitudes of difference between sites, suggesting an origin effect as

A.

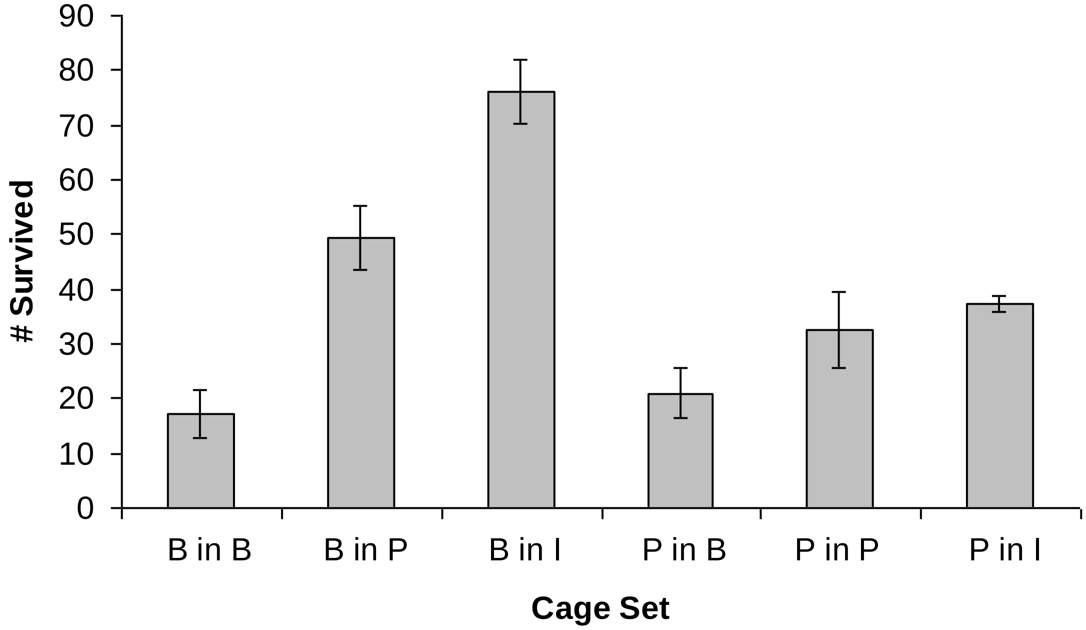
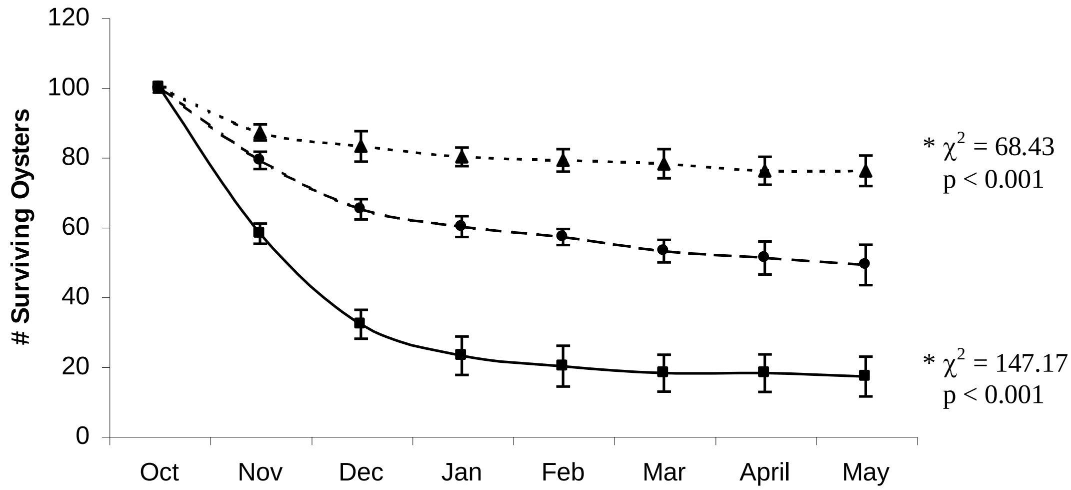


Figure 17. Transplant 2 (Fall-Winter). Average survival among each set of three replicate cages (out of the original 100 oysters per cage) for each stock at each site. Vertical lines represent standard error.

A.



B.

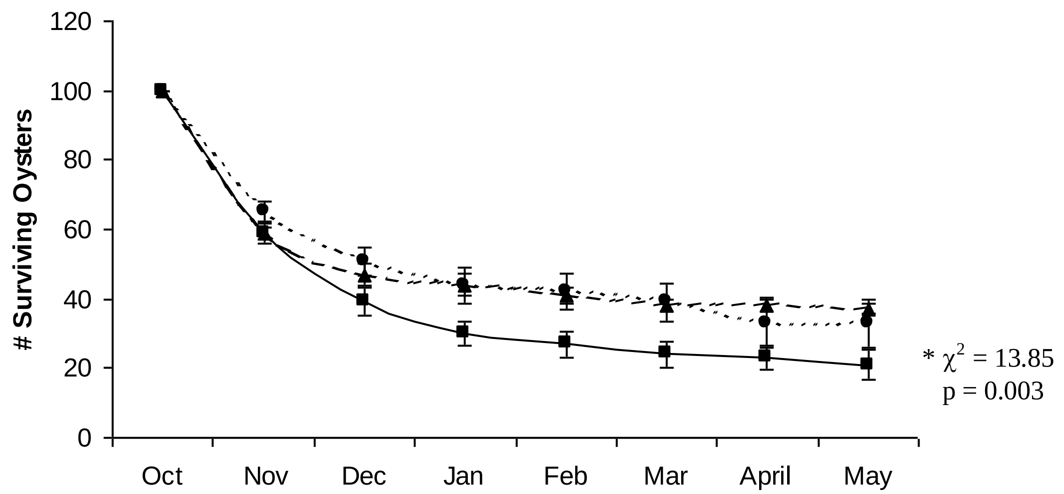


Figure 18. Transplant 2 (Fall-Winter). Survival curves for the two stocks of oysters (A = Bradley Creek oysters, B = Pages Creek oysters) at reciprocal transplant and common garden sites (Bradley Creek = squares, solid; Pages Creek = circles, small dashed; ICWW = triangles, dashed). Data points are average of three replicate cages. Vertical lines represent standard error. Significant differences between treatments' survival curve and the survival curve of the stock overall, exhibited by B in B, B in I, and P in B, are marked by a *.

well. Relative Risk calculations broken down by month, site and origin are illustrated for both stocks in Table 4.

Size-dependent Mortality

The hypothesis of size dependent mortality was explored by looking at the size of the dead oysters at each month relative to the size of the surviving oysters at the initial and final time points. There were no consistent trends found among treatments (Figure 19). Because there were no sizes of living oysters during monthly samplings, this was exploratory rather than evaluative.

Water Quality

Transplant 1 (Summer)

Over the 3-month study, the Bradley Creek site exhibited a range in total suspended solids of 0.028-0.105 g/L, a range in temperature of 25-28 ºC, and a range in salinity of 8-26 parts per thousand (ppt). Over the same time period, the Pages Creek site exhibited a range in total suspended solids of 0.096-0.126 g/L, a range in temperature of 25-28 ºC, and a range in salinity of 24.5-32 ppt (Figure 20). Water quality data was not analyzed statistically due to lack of replication.

Transplant 2 (Fall-Winter)

Over the 7-month study, Bradley Creek exhibited a range in temperature of 9-30 ºC, in salinity of 5-36 ppt, and in total suspended solids of 0.024-0.073 g/L. Over the same 7 months, Pages Creek exhibited a range in temperature of

Table 4. Transplant 2 (Fall-Winter). Relative Risk Calculations (Survival Analysis) for Bradley Creek Oysters and Pages Creek Oysters for each month. A risk of <1 suggests the oyster has a better chance of survival being transplanted to that creek as opposed to being transplanted back into their creek of origin. A risk of >1 suggests that the oyster has a better chance of survival being transplanted back into their creek of origin as opposed to being transplanted to that creek.

Origin	Month	Relative Risks	
		Relative risk of being Transplanted to the ICWW as opposed to Bradley	Relative risk of being transplanted to Pages as opposed to Bradley
Bradley	October	0.309	0.500
	November	0.109	0.390
	December	0.103	0.262
	January	0.127	0.381
	February	0.289	0.947
	March	-	-
	April	-	-
		Relative risk of being Transplanted to Bradley as opposed to Pages	Relative risk of being transplanted to ICWW as opposed to Pages
Pages	October	1.169	1.160
	November	1.586	0.936
	December	1.605	0.489
	January	2.183	1.323
	February	1.286	0.840
	March	-	-
	April	9.826	15.000

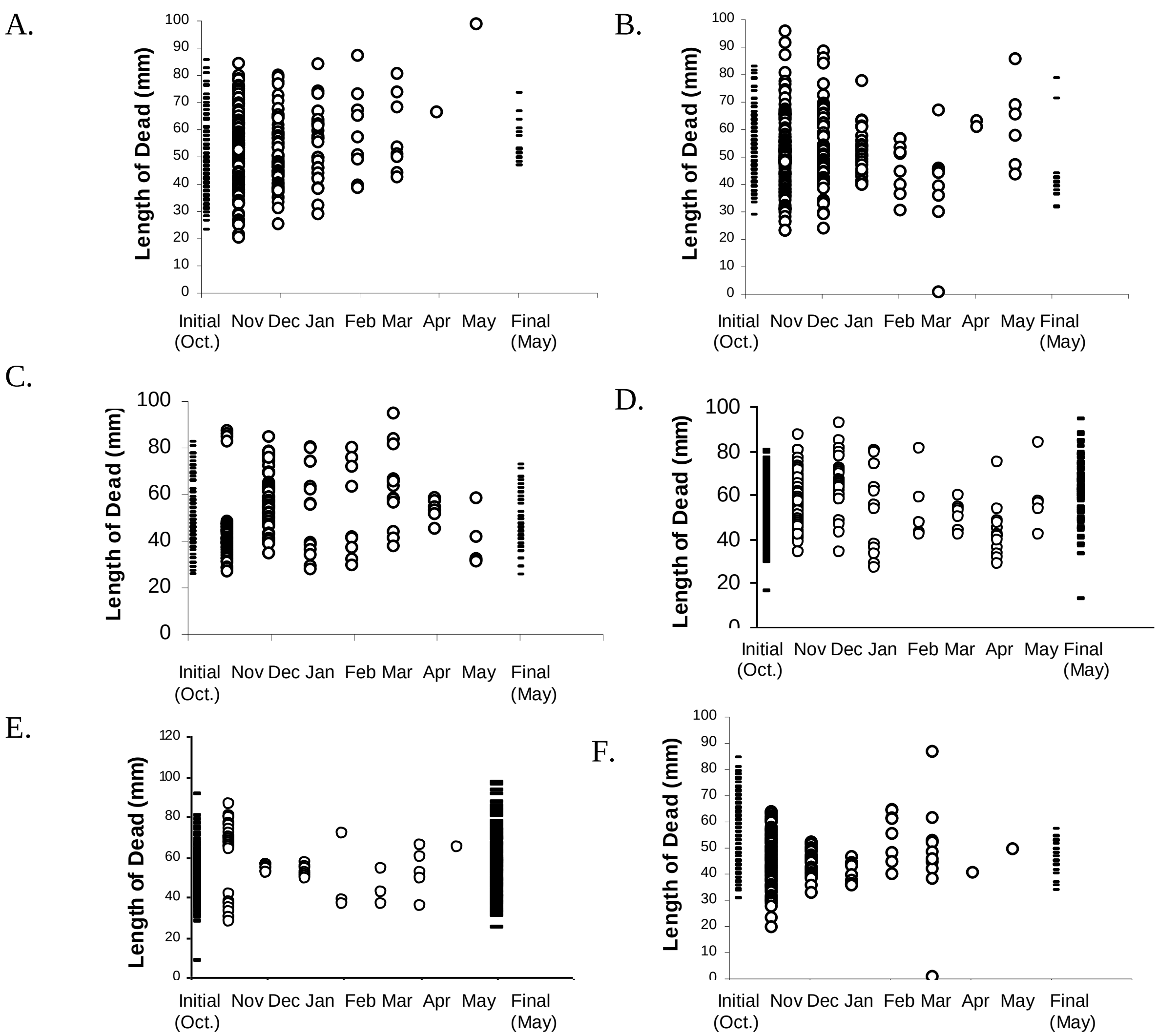


Figure 19. Transplant 2 (Fall-Winter). Initial and final shell heights of all oysters, both living and dead (live oysters = black squares, dead oysters = white circles) for each month in between, by site and stock (A = B in B, B = P in B, C = B in P, D = P in P, E = B in I, F = P in I). The horizontal lines represent the median of all initial oysters for each set. Horizontal line connects the initial and final median.

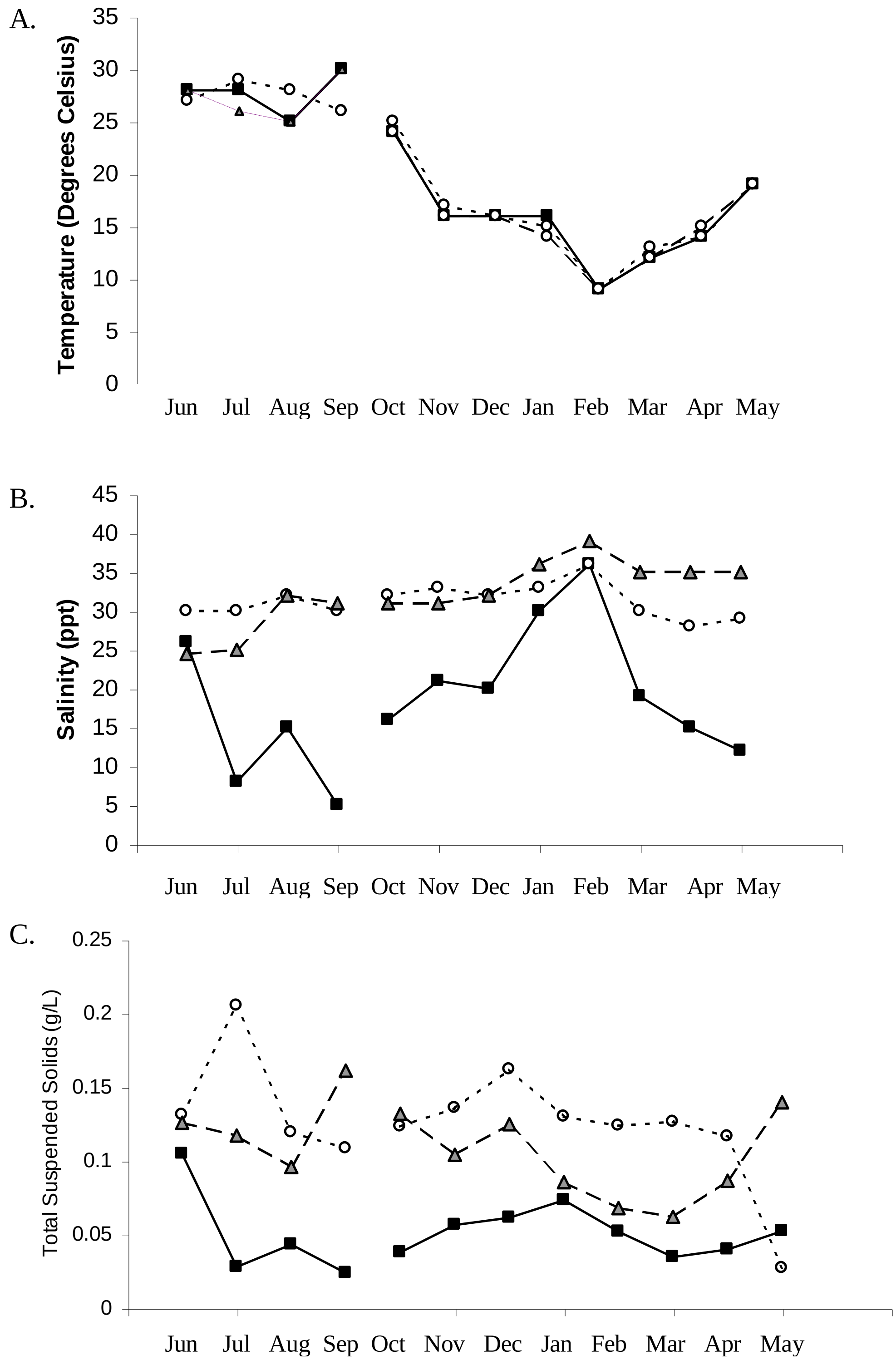


Figure 20. Three sets of environmental conditions (A = Temperature, B = Salinity, C = Total Suspended Solids) for reciprocal transplant and common garden sites (Bradley Creek = black squares, solid; Pages Creek = white circles, small dashes; ICWW = gray triangles, large dashes) during the two transplants.

9-30 ºC, in salinity of 31-39 ppt, and in total suspended solids of 0.062-0.162g/L. The ICWW's temperature ranged from 9-26 ºC, salinity ranged from 28-36 ppt, and total suspended solids ranged from 0.027-0.162 g/L (Figure 20).

DISCUSSION

This study provides evidence for local adaptation between two stocks of the Eastern oyster, *Crassostrea virginica*, from two local tidal creeks. Local adaptation (phenotypical differences that persist under common environmental conditions) was evident from differences in growth observed between the two stocks. Transplant 1 did not show a clear trend in oyster growth between stocks (as shown in Friedman's method for randomized blocks), possibly due to short transplant duration and high mortality of transplanted oysters (3 months). However, in Transplant 2, oysters originating from Bradley Creek exhibited higher relative growth than oysters originating from Pages Creek. One possibility that could account for the finding is size-dependent mortality. For size-dependent mortality to be the key factor driving the growth variation between the two stocks, the average size of the living oysters would have to be larger than the average size of the dead oysters each month for oysters originating from Bradley Creek, and vice versa for oysters originating from Pages Creek. There was, however, only minimal difference between the average size of the dead oysters and the average size of the living oysters each month for both oysters from Bradley Creek and oysters from Pages Creek, indicating that the major determinant of the growth differences between the two stocks to be growth rate, not size-dependent mortality. Furthermore, a logistic regression test found that mortality trends in the two stocks were similar. There would have to be opposite trends exhibited between the stocks (all Bradley stock mortality rates decrease as size increases; all Pages stock mortality rates increase as size increases) to explain the observed patterns of growth. Transplant 2 (Fall-Winter) exhibited similar trends with respect to the average size of the living oysters and the average size of the dead oysters, which were similar, suggesting no size-dependent mortality.

There is a possibility that because the actual death dates of oysters within each month are not known there is potential for growth artifacts, which could result in me overestimating or underestimating the differences between the living and the dead oysters. In order for size-dependent mortality to have been overlooked in this study, time of the month death occurred and size of the dead could not be random, there would have to be some relationship between the two factors.

Growth variation between oyster stocks was previously seen by Dittman et al. (1998) who found that even after several transplants, one stock of oyster was consistently larger than other stocks, which they concluded to be a consequence of genetic differences between the stocks. Several studies also found positive correlations between genetic make up (individual heterozygosity, specifically) and growth rate in *C. virginica* (Koehn and Shumway, 1982; Walne, 1958; Singh and Zouros, 1978; Zouros et al., 1980; Brown et al., 1994). Differences in growth rate have been evaluated more closely in some studies, which have revealed that individual differences in physiological rates like clearance rate (a measure of filtering capacity) and absorption efficiencies, which could account for differences in growth observed between animals of different origins (Iglesias et al., 1996). Similar differences, although not likely in this case, could potentially account for growth differences observed between oysters in Bradley Creek and Pages Creek.

Survival patterns also support the hypothesis of local adaptation of *C. virginica* populations. The two stocks used in this study exhibited very different survival patterns. The Bradley Creek stock had higher overall survival than the Pages Creek stock in both transplants and exhibited higher overall survival rates in all three sites, including the common garden site (ICWW in Transplant 2). This was of particular interest because the environmental conditions in the ICWW appear to be more similar to Pages Creek's conditions than Bradley Creek's, with respect to temperature, salinity and total suspended solids, as seen in Figure 20. Other studies also found that different populations of marine invertebrates survive differently when transplanted into sites with different environmental conditions. This was observed in populations of mussels (Kautsky et al., 1990) and acorn barnacles (Bertness and Gaines, 1993), and oysters (Alphin, 2004; Dickie et al., 1984). In each case, differences in survival were attributed to genetic differences among stocks. In this study, the survival patterns observed, suggest that the differences are stock related rather than environmental.

Relative growth and survival data indicate that the Bradley Creek stock outperforms the Pages Creek stock. There was, however, evidence for an environmental effect on performance. Bradley Creek stock had consistently greater growth relative to the Pages Creek stock, but the magnitude of the difference was site-dependent. This supports the conclusion that while there is a genetic component to the differential performance among these populations, there was also an environmental influence on growth. Similar results were also found by Dittman et al. (1998), who concluded that environmental conditions at transplant sites had a very strong affect on growth parameters such as shell size. Experience in Oyster aquaculture supports this, observing that oysters planted in certain environmental conditions (such as optimal food levels) get heavier quicker than if they were planted in other areas (Wallace, 1951).

These findings are intriguing because the oyster's long-lived pelagic larval stage provides a potential for high gene flow, limiting genetic differentiation between geographically proximal locations (Burton 1983, 1986; Hedgecock, 1986). However, in general, genetic differentiation is still possible through strong local differences in selection pressures (Bertness and Gaines, 1992; Mayr, 1963; Endler, 1977; Hochachka and Somero, 1984). If individuals representing different portions of a species gene pool survive differently in different locations, then genetically based differences in various traits may occur in adults in different populations. This has been shown to occur in several populations of marine species with broad dispersal, including eels (Williams et al., 1973), mussels (Koehn et al., 1976, 1984), barnacles (Hedgecock, 1986) and limpets (Johnson and Black, 1984), as well as oysters (Alphin, 2004). The two creeks used in this study differ in a number of characteristics (population, watershed area, percent of land developed, and percent of land covered by impervious surfaces). Although there is no direct evidence that there is selective mortality, data suggest that environmental differences could cause selective mortality, resulting in the observed variation between the two stocks of oysters.

In general, alternative local adaptation can be a result from geographic isolation. Geographic isolation occurs when adjacent populations cannot or do not exchange individuals. Due to the close proximity of Bradley and Pages Creek (approximately 7.5 kM), a stronger argument can be made for post settlement selective mortality as the cause of any potential differentiation between the two stocks. However, realized dispersal may often be less than potential dispersal due to patchy habitat conditions (Hare and Avise, 1996), and limited flushing can cause creeks to retain larvae generated by local populations. Limited dispersal in estuaries that suffer from low flushing rates may lead to restricted gene flow and subsequent adaptation to local conditions (Bertness and Gaines, 1993). Due to factors similar to these, Dittman et al. (1998) concluded that the effective population size for reproduction of some *C. virginica* populations is a factor of 1×10^5 smaller than the apparent population size, despite the long-lived pelagic larvae. Smaller effective population sizes for reproduction would make geographic isolation a more plausible explanation for differentiation between oysters from different locations, even if they are close together such as the Bradley and Pages Creeks.

Previous studies in other systems have also suggested that oyster populations are locally adapted and may differ genetically despite the potential dispersal of their larvae (Stauber, 1950; Hillman, 1964; Loosanoff and Nomejka, 1951). Dittman et al. (1998) concluded that persistent effect of origin, even after many generations of propagation in a common environment, supports the hypothesis that there is genetic differentiation among certain *Crassostrea virginica* populations.

CONCLUSIONS

This study provides evidence for local adaptation (phenotypical differences that persist under common environmental conditions) in two stocks of *C. virginica* from two local tidal creeks, Bradley Creek and Pages Creek. Relative growth data indicates that the Bradley Creek stock had greater growth relative to the Pages Creek stock. Survival data revealed that the Bradley Creek stock had consistently higher survival rates relative to the Pages Creek stock. The stocks' survival curves were site dependent, but were very different from each other at each site. Growth data coupled with survival data support the hypothesis that certain local populations of *Crassostrea virginica* exhibit local adaptation in growth and survival. Future studies looking at F_1 generations of both stocks would be wise to determine whether the differences are a result of long-lasting environmental effects, or genetic factors.

For restoration purposes, this study suggests that some stocks may handle the transplant process better. This study also indicates that a stock's performance is dependent on the transplant site, as well as the stock's origin. These findings are significant because they illustrate that the level of success achieved in a restoration effort may be strongly affected by source of the oyster used. It may be beneficial to target particular stocks for restoration purposes. This study indicates that specific stocks could be more effective than others when considering specific restoration projects. Therefore, we could potentially select stocks that provide consistent survivorship across a variety of sites, a conclusion also suggested by Alphin (2004). Alphin's 2004 study, like this one, suggested that while constant survivorship can be achieved by use of certain stocks, growth is still variable. These findings may also have implications for the strategy of oyster relaying for aquaculture, and as well as hatchery procedures.

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