Shellfish growers have expressed interest in developing high-yielding oyster strains through selective breeding. This dissertation has three objectives to help determine the effects of genetic and environmental variation on production traits (body weight, survival, and yield) of Pacific oysters grown in the Pacific Northwest.

Objective 1: Determine if relative family performance at harvest is influenced by the nursery conditions in which the families are raised. Algal feeding rate during juvenile development was found to affect adult body weight and survival ($P<0.01$) among outbred families and adult body weight and yield ($P<0.029$) among inbred families. No significant genotype x nursery environment interactions were found among outcrossed families ($P>0.339$). Adult body weight and yield were significantly affected by genotype x nursery environment interactions ($P<0.019$) among inbred families, with rank changes occurring in the most stressful nursery environments. Results suggest differences in nursery feeding regime should not significantly alter relative field performance of outbred oyster families and should not alter relative field performance of inbred families under all but the most stressful juvenile growing conditions.
Objective 2: Determine effects of genotype x environment (GxE) interactions on field performance traits of oysters grown in the Pacific Northwest. Average family adult body weight, survival, and yield were all significantly affected by GxE interactions when raised in four dissimilar environments (P<0.01), however, correlations among performance characters across sites were greater than 0 (P<0.05). Indirect selection in a single environment targeting improved yield in all other environments was 48% to 91% as effective as direct selection within each environment separately. Results suggest development of broadly-adapted oyster strains should be possible using a small number of well selected evaluation environments.

Objective 3: Determine heritability of adult oyster body weight. Parent-offspring analysis estimated heritability of adult body weight to range from −0.01 (± 0.17) when parents and offspring were raised in dissimilar environments, to 0.33 (± 0.12) when parents and offspring were raised in similar environments. A strong negative correlation was found between parental body weight and offspring survival (P<0.001). Although offspring were only evaluated in a single growing environment, caution should be exercised when selection is performed on adult oyster body weight to indirectly improve yield.
Improving Pacific Oyster (*Crassostrea gigas*) Production Through Selective Breeding.

by
Sanford Evans, III

A DISSERTATION
submitted to
Oregon State University

in partial fulfillment of
the requirement for the
degree of

Doctor of Philosophy

Presented December 8, 2004
Commencement June 2005
Doctor of Philosophy dissertation of Sanford Evans, III presented on December 8, 2004.

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ACKNOWLEDGEMENTS

Generous support was provided by the Molluscan Broodstock Program, located at the Hatfield Marine Science Center (HMSC), Newport, OR. Additional funding provided from the 2001 and 2002 Anja Robinson Shellfish Fellowships, and the 2003 Walter G. Jones Fisheries Development Award.

Raising oysters from larvae to market-size requires both manpower and time. I am indebted to the staff of the Molluscan Broodstock Program for providing both. Many people have participated in my research since the first crosses were made in 2000. In particular, assistance provided by Dave Jacobson, Sean Matson, John Brake and Drew Mosher in the nursery and in the field. Ebru Onal and Salina Gaskill provided algae on demand to keep the growing oysters happy while housed at HMSC. I am also thankful for the assistance provided by Paul Lang, David Stick, and Jennifer Ferschweiler. Alan Barton, Dan Troop and Chris Emerson were critical in allowing me to keep on schedule during the flurry of oyster counting and weighing, up and down the coast, during 2004.

This research could not have been completed without the support of the commercial oyster farms where the experiments were conducted. I would like to thank to Liu Xin and everyone at Oregon Oyster Farm, Yaquina Bay, OR, and Joth Davis, Lizzie Nelson, and everyone at Taylor Resources, Dabob Bay, WA, for their assistance and use of growing grounds and facilities.
I would also like to thank Anja Robinson for instructing me on the essentials of the culture of oyster larvae. In addition, Chris Brooks generously provided raw data from which I was able to select sires and dams for Chapter 4. Thanks also to Mike Blouin and Eric Hoffman for confirming parentage of all crosses used in this research. Committee members (Chris Langdon, Mike Blouin, Mark Camara, and Howard Meyer) were very helpful assisting in both the design of the experiments as well as in the editing of this document.

Last, but certainly not least, I would like to acknowledge the love and support from my parents, Sandy and Sally, my lovely wife Kathy and my beautiful daughter Mia, whose arrival into this world gave extra incentive to finish this degree on time and get a "real" job.
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IMPROVING PACIFIC OYSTER (*Crassostrea gigas*) PRODUCTION THROUGH SELECTIVE BREEDING.

CHAPTER 1

INTRODUCTION

Shellfish farming along the west coast of the United States is forecasted to produce over 40,000 metric tons (live weight) of oysters in 2004 (Chew and Toba, 2001). Although oysters are farmed in all West coast states, most production occurs in the protected waters of Puget Sound, Willapa Bay, and Grays Harbor, Washington. The most commonly cultured oyster is the Pacific oyster (*Crassostrea gigas*) which reaches market size in two to three years under typical growing conditions. Although husbandry of aquatic animals can be traced back to 1100 B.C. in China (Shell, 1991), significant genetic improvement of fish and shellfish has only occurred recently, primarily due to the development of techniques that allow for breeder-controlled mating (Refstie, 1990; Gall, 1991). Similarly, the Pacific oyster industry has existed on the West coast of the United States since the early 1900’s, however it wasn’t until the 1970’s that commercial growers began to shift from wild-collected spat to hatchery-produced spat (Conte et al., 1996), and began to consider the benefits of genetic improvement (Lannan, 1972; Hershberger et al., 1984).
The Pacific oyster industry may benefit from a selective breeding program for two main reasons. The first reason is economic. Domestic demand for Pacific oysters is projected to remain stable (Chew and Toba, 2001) while demand for exportable product is expected to increase (Hedgecock et al., 1997). At the same time Chew and Toba (2001) forecast pollution and human encroachment will reduce productive oyster growing grounds along the West coast. Further, declines in Eastern oyster (*Crassostrea virginica*) production has maintained market demand for oysters grown in the Pacific Northwest (Conte et al., 1996). Therefore, in order for the Pacific oyster industry to maintain a constant or increasing level of production, oyster yield (i.e. meat production per number of oysters planted; Quayle 1988) must increase. The Pacific oyster industry may also benefit from a selective breeding program because research suggests that genetic improvement of growth traits in Pacific oysters is possible, either through additive (Lannan, 1972; Hedgecock, 1991; Langdon et al., 2003) or non-additive (Hedgecock et al., 1995) gene action. Evidence from other shellfish species affirm growth traits can be improved through genetic selection (e.g. Stromgrem and Nielsen, 1989; Rawson and Hilbish, 1990; Hadley et al., 1991; Sheridan, 1997; Ibarra et al., 1999; Nell et al., 1999; Perez and Alfonsi, 1999).

In 1995 a selective breeding program was initiated at the Hatfield Marine Science Center, Newport, Oregon, to develop high yielding strains of Pacific oysters suitable for production throughout the Pacific Northwest (Hedgecock et al., 1997). Family-based phenotypic selection was employed to directly select on average family yield. Results
after one generation of selection indicated that yield was heritable and that gains in yield could be made through selective breeding (Langdon et al., 2003). However, questions remain about the possible impacts of genotype x environment interactions on oyster performance as well as the heritability of yield’s causal components (i.e. body weight and survival).

*Genotype x environment (GxE) interactions*

Identification of superior broodstock in the Pacific Northwest is complicated by the broad range of environments in which oysters are commercially grown. Growout occurs in protected bodies of water from which oysters filter-feed on naturally occurring phytoplankton. Within these protected waters, specific culture sites may be intertidal or subtidal and experience salinity concentrations from brackish to marine. Farmers have no control over the quality or quantity of food the oysters receive, nor can farmers control temperature or quality of the water in which the oysters are grown. With such a broad range of growing environments, there is a risk that a strong genotype x environment (GxE) interaction may prevent the development of ubiquitously superior oyster strains.

The interaction between genotype and environment is an important force in the artificial or natural selection of a character (Falconer and Mackay, 1996). Falconer (1952) suggested that the same trait measured in two different environments could be treated statistically as two separate characters. The genetic correlation between these two
characters reflects the extent to which the same genes are involved in their expression in both environments (pleiotropy). If the genetic correlation between these characters is positive, improvement in one environment is expected to produce a favorable correlated response at the second environment. If the genetic correlation between these characters is negative (i.e. a significant GxE), improvement in one environment will result in an unfavorable correlated response in the second environment, and a ubiquitously superior genotype may not exist (e.g. Rawson and Hilbish, 1991). In such a case, development of environment-specific strains may be required.

GxE interactions acting on composite characters are expected to increase in frequency as the number non-interacting causal component traits increase (Baker, 1987). Yield is a life-history trait that is the result of many causal components, the most basic ones mentioned above being body weight and survival. Body weight and survival are, in turn, affected by other causal traits such as tolerance to salinity (Innes and Haley, 1977), temperature (Beattie et al., 1980), disease (Haskin and Ford, 1988) and aerial exposure (Littlewood, 1988). At present no structured experiment has been performed to measure the strength of GxE interactions on oyster performance in Pacific Northwest commercial growing environments.

Similarly, it is unclear if a genotype x nursery environment interaction affects relative family performance later in life. When a genotype x nursery environment interaction is strong enough, the relative performance of a genotype after two years in the field may
depend upon the nursery environment in which it was raised during the spat stage. If nursery environments have a large impact on relative field performance then commercial hatcheries would need to standardize their husbandry practices in order to allow the development of industry-wide strains of high-yielding oysters.

**Heritability of oyster body weight**

Additive genetic variation must exist for the trait of interest if a breeding program is to be based on recurrent selection. Fortunately, evidence suggests that most oyster growth traits are heritable (e.g. see review by Sheridan, 1997; Nell et al., 1999). Two studies have been performed to estimate the heritability of individual body weight in Pacific oysters. The first (Lannan, 1972), used full-sib analysis to estimate the broad-sense heritability for total weight \( H^2 = 0.33 \pm 0.19 \) and meat weight \( H^2 = 0.37 \pm 0.20 \). These estimates are possibly biased upwards due to the inclusion of non-additive genetic effects. More recently (Hedgecock, 1991), the narrow-sense heritability of meat weight at harvest size was reported to be approximately 0.20, however, no variance estimate was given. Studies based on other oyster species (including the genera *Crassostrea*, *Saccostrea*, and *Ostrea*) also indicate a moderate level of heritability for growth traits measured at harvest size (e.g. see review by Sheridan, 1997; Nell et al., 1999).

Although farmers have expressed interest in selecting for high yielding oyster strains (Hedgecock et al., 1997), only one study has directly measured the heritability of oyster
yield (Langdon et al., 2003). Yield is a product of individual body weight and survival. It appears to be assumed by most researchers that by selecting for individual growth traits (such as body weight) there will be a correlated increase in yield. This assumption has yet to be tested for Pacific oysters in the Pacific Northwest. Survival cannot be discounted as an important component of yield. In some growing environments, survival accounted for over 90% of the observed variation in average family yield (Evans et al., 2002). In addition, significant response to selection for high survival has been reported in C. gigas exposed to elevated water temperature (Beattie et al., 1980) and summer mortality conditions (Degremont et al., 2003) and in C. virginica exposed to MSX (Haplosporidium nelsoni; Haskins and Ford, 1988), suggesting that these survival traits are at least partly under additive genetic control.

Family selection has been used to directly select for improved yield (Langdon et al., 2003). However, indirectly improving yield through individual selection on body weight also has advantages. The primary advantage is in the simplicity of the breeding design, reduced resources required, and increased selection differential (Gall, 1991; Falconer and Mackay, 1996). Indirectly improving yield through selection on individual body weight would be possible if 1) oyster body weight is heritable and 2) improvement in body weight results in a favorable correlated response in offspring yield.

This dissertation has three objectives. The first objective was to determine if relative family performance at harvest (i.e. rank) is influenced by the nursery conditions in which
the families were raised. The second objective was to determine the magnitude and significance of genotype x environment interactions on performance traits of oysters planted in typical commercial growing environments in the Pacific Northwest. The third objective was to determine if adult oyster body weight is heritable and if selection on body weight results in a correlated response in survival or yield. These objectives will help determine how genetic and environmental variation affects performance characters of Pacific oysters grown in the Pacific Northwest.
CHAPTER 2

EFFECT OF DIETARY RESTRICTION DURING JUVENILE DEVELOPMENT ON ADULT PERFORMANCE OF PACIFIC OYSTERS (*Crassostrea gigas*).

ABSTRACT

An experiment was performed to determine if dietary restriction in the nursery significantly affects the relative field performance of Pacific oysters (*Crassostrea gigas*) at harvest. Five outbred full-sib families (*F*=0) and three inbred full-sib families (*F*=0.203) were created in August 2000, and reared under each of three feeding levels during juvenile development in the nursery. In June 2001, 40 individuals from each family-nursery treatment combination were stocked into each of ten replicate lantern net tiers and deployed subtidally in Yaquina Bay, OR, USA. Measurements of yield (kg tier⁻¹), individual body weight (g), and survival (%) were recorded after one and two growing seasons in the field. ANOVA was used to determine if genotype, nursery environment, or genotype x nursery environment interactions significantly affected performance traits after one and two growing seasons in the field. Average outbred family yield after two growing seasons was significantly affected by genotype (*P*<0.01), but not by nursery environment (*P*=0.052) or genotype x nursery environment interaction (*P*=0.87). Components of yield (i.e. individual body weight and survival) were affected by both genotype and nursery environment (*P*<0.01), but not genotype x nursery environment interaction (*P*>0.34). Average inbred family yield and average body weight after two growing seasons were significantly affected by genotype (*P*<0.01), nursery environment (*P*<0.029) and genotype x nursery environment interaction (*P*<0.019). Average inbred
family survival was affected by genotype (P<0.01) but not nursery environment (P=0.929) or genotype x nursery environment interaction (P=0.197). Significant rank changes among inbred families for both individual body weight and yield occurred only under the most stressful nursery feeding regime. Results suggest differences in nursery feeding regime should not significantly alter relative field performance of outbred oyster families and should not alter relative field performance of inbred families under all but the most stressful juvenile growing conditions.

1. INTRODUCTION

The phenotype (P) of an individual for a quantitative trait is a function of both genotype (G) and environment (E), such that \( P = G + E \). In a similar manner, the phenotypic variation (\( V_P \)) for a quantitative trait in a population is due to genetic variation (\( V_G \)) and environmental variation (\( V_E \)) within that population. Variation due to environment can be partitioned further to include both temporary and permanent effects. Temporary environmental effects are reversible, while permanent environmental effects persist even after environmental conditions have changed. For example, the effect of water temperature on bivalve growth rate is considered a temporary environmental effect; growth rate will tend to decrease as water temperature moves away from the optimal range, but growth rate will increase again if water temperatures return to within the optimal range (e.g. Shumway, 1996). Conversely, high-energy diets fed to pre-pubertal (3 to 10 months of age) dairy heifers may affect milk yield throughout maturity by
impeding mammary development (Silva et al., 2002). Reduced milk yield in adulthood (due to impaired mammary development) can therefore be considered a permanent effect of juvenile dietary environment.

Animal and plant breeders are aware of the need to account for permanent environmental effects in order to maximize genetic gain (e.g. Falconer and Mackay, 1996; Bourdon, 2000; Lynch and Walsh, 2000). Permanent environmental effects have been found to account for nearly 50% of observed phenotypic variation in dairy cattle milk production (Keown, 1989) and Angus cattle fat thickness (Hassen et al., 2001). Maternal effects are perhaps the most commonly sited source of permanent environmental variation among agronomically important species (Houpt and Hintz, 1982/83; Falconer and Mackay, 1996, and references therein; Lay et al, 1998), however, many other stressors experienced during development have also been shown to permanently affect adult phenotypic variation (Moberg and Wood, 1982; Creel and Albright, 1988; Goater, 1994; Desai and Hale, 1997; O’Steen, 1998; Brooks, 2000; Lay, 2000; Madsen and Shine, 2000; Metcalfe and Monaghan, 2001; Pahkala et al., 2001; Lee and Petersen, 2003).

Oyster aquaculture often involves two distinct management phases: 1) the nursery phase, and 2) the grow-out phase. The nursery phase typically begins with spawning of the parents, through the larval and spat stages, and ends when the oysters are planted in the field. The nursery phase can last for several months and frequently occurs in land-based facilities with strict control over environmental conditions and algal diet quantity and
quality. The growout phase begins when the animals are planted in the field and ends when the animals reach market size and are harvested. The duration of the growout phase depends upon the quality of the growout site, culture method used, and targeted market size. There is concern, however, that effects of the nursery environment may carry-over and alter the expression of adult oyster phenotypes. When viewed in this way, we can consider the effects of nursery environment as a source of permanent environmental variation in field performance.

From a statistical standpoint, two sources of nursery environmental variation are of concern when selecting on adult performance traits. The first is simply the main effect of nursery environment (Does environmental variation in the nursery affect adult phenotype?) and the second is the interaction between variation in nursery environment and genotype (Does the relative performance of genotypes at harvest depend upon the environment experienced in the nursery?). Both carry-over effects can significantly impact a breeding program by inflating environmental variation acting on performance characters, thereby reducing heritability and genetic gain (Falconer and Mackay, 1996; Bourdon, 2000).

Dietary restriction in the nursery has been shown to affect juvenile shellfish growth and survival (His and Seaman, 1992; Rheault and Rice, 1996; Wikfors et al., 2001; Pechenik et al., 2002) and could be one stressor that permanently affects adult performance. Although the effects of temporary starvation during development on subsequent larval
and juvenile growth and survival have been studied (His and Seaman, 1992; Pechenik et
al., 2002), little attention has been given to the effect of nursery feeding restriction on
growth and survival of adult shellfish. Further, few studies account for genotype and the
possibility of a genotype x nursery environment interactions affecting adult phenotype.
This experiment was performed to determine if dietary restriction in the nursery
significantly affects performance traits of Pacific oysters (Crassostrea gigas) at harvest
and, if so, does this source of variation result in a change in the relative performance
among genotypes.

2. METHODS

2.1 Broodstock selection and spawning

Five pair-wise crosses were made among putatively unrelated C. gigas parents. These
crosses were performed using full or half-sibs of parents used to create previously
evaluated families from a selective breeding program underway at the Hatfield Marine
Science Center, Newport, Oregon, U.S. (Langdon et al., 2003). Families that were
recreated were chosen so that they would likely be high, mid, and low-yielding families
at harvest and therefore increase the likelihood of observing a significant family effect on
offspring field performance. Three pair-wise crosses were also made among full or half-
sibs to create inbred families. Due to uncertain pedigrees, the average inbreeding
coefficient (F) for all three inbred crosses was estimated as per Evans et al. (2004) to be
0.203. Inbred families to be recreated were again chosen such that they would likely be high, mid, and low-yielding families at harvest. The use of non-randomly selected families limits the scope of interpretation of family effects, but was considered necessary due to the limited number of crosses possible within the nursery system.

Fertilization and larval rearing followed methods outlined by Langdon et al. (2003). Briefly, selected parents were conditioned for approximately 6 weeks in 18° C sand-filtered seawater and fed a continuous ration of *Cheatoceros calcitrans* and *Isochrysis galbana* delivered at 50,000 to 80,000 cells ml⁻¹. Once conditioned, gametes were stripped from the parents and fertilized. Fertilized eggs from each family (at a concentration of approximately 100 eggs ml⁻¹) were allowed to develop at 25° C for 24 h in separate 20 l containers filled with 0.2 μm-filtered seawater. Straight-hinge larvae were then stocked at a concentration of 10 ml⁻¹ into family-specific 100 l tanks filled with 0.2 μm-filtered seawater at 25° C. Water was changed in larval tanks twice per week. After the first week, larval concentrations were reduced to 1 larva ml⁻¹. Larvae subsequently retained on a 243 μm sieve (approximately two weeks post spawn) were induced to metamorphose using 2x10⁻⁴ M epinephrine (Coon et al., 1986). Successfully metamorphosed spat were transferred to family-specific 15-cm diameter upwellers. Larvae from each family were sieved and induced to metamorphose twice per week until nearly all of the larvae were transferred to the 15-cm upweller system (approximately 4 weeks post spawn). Water temperature in the 15-cm upweller system was maintained at 18° C and a mixed algal diet (*C. calcitrans* and *I. galbana*) supplied ad libitum. Spat
were sieved from each upweller weekly and those retained on a 1.4 mm sieve were
transferred to family-specific 28-cm diameter upwellers, discussed below.

2.2 Nursery feeding treatments.

Spat from each family were exposed to different nursery feeding regimes intended to
produce markedly different growing environments. Nursery treatments began when all
oyster spat in the 15-cm upwellers could be retained on a 1.4 mm sieve. Approximately
1,500 1.4-mm spat from each family were stocked into each of three family-specific 28-
ctm upwellers. Each of the three upwellers per family were then assigned to one of three
nursery feeding treatments (high, medium, and low feeding regimes, described below).
Flow rate through each 28-cm upweller was maintained at approximately 3 l min\(^{-1}\) and
water exchange rate through each of the two upwelling systems (each holding 16
upwellers) was approximately 6 water volume exchanges d\(^{-1}\). Water temperature in the
upwellers was maintained at 14° C. Details of the three nursery treatments are given
below.

Oyster spat in the “high” algal ration treatment received a continuous supply of \(C.\)
calcitrans and \(I.\) galbana at a targeted concentration of 50,000 to 80,000 cells ml\(^{-1}\) in the
28-cm upwellers. Once spat were retained on an 8 mm sieve, they were transferred to 2
mm mesh spat bags (0.90 m x 0.23 m, LxW) and held in a storage tank that received
sand-filtered seawater. Water temperature in the storage tank was not controlled and
followed ambient seawater fluctuations (average approximately 10.2°C; max/min 6.9°C / 15.9°C). Again a mixed algal diet was delivered continuously to the storage tank at a targeted concentration of 50,000 to 80,000 cells ml⁻¹. Animals remained in the storage tank until being planted out in the field.

Animals in the “medium” algal ration treatment received the same feeding regime in the 28-cm upwellers as the high ration treatment. However, once spat were retained on an 8 mm sieve, they were moved to 2 mm mesh spat bags in a storage tank that received only 1 feeding day week⁻¹, effectively stunting oyster growth. Algal concentrations during feeding days were maintained at 50,000 to 80,000 cells ml⁻¹. Storage tank water temperatures were similar to those in the high algal ration treatment. Animals remained in the storage tank until being planted out in the field.

Oyster spat in the “low” algal ration treatment received *C. calcitrans* and *I. galbana* at a targeted concentration of 50,000 to 80,000 cells ml⁻¹, 2 to 4 days week⁻¹ while in the 28-cm upwellers. Once spat were retained on a 4.75 mm sieve, they were transferred to a storage tank and fed only 1 day week⁻¹. Algal concentrations during feeding days in the storage tank were maintained at 50,000 to 80,000 cells ml⁻¹. Storage tank water temperatures were similar to those in the high algal ration treatment. Animals remained in the storage tank until being planted out in the field.

2.3 Field production.
From May 25 to June 5, 2001, 35 spat from each family were randomly selected, weighed, and stocked into each of 10 replicate mesh sleeves (2 mm mesh; 0.30 m x 0.30 m, LxW). Two of the 10 replicate sleeves from each family were then randomly assigned to each of five vertical blocks of a ten-tier lantern net (5 mm mesh), with blocking intended to account for variation in field performance traits due to water depth. Each lantern net compartment had a diameter of 0.51 m and a height of 0.17 m. Lantern nets were deployed approximately six miles up the Yaquina River, Oregon, U.S. (44.6° N, 124.1° W), at a commercial oyster farm. After one growing season in the field (153 days), oysters from each replicate were cleaned of biotic and abiotic fouling, counted and the collective weight of all live animals measured to the nearest gram. Average individual oyster weight per replicate was calculated by dividing the total bag weight by the number of live oysters. At this point, oysters were transferred from the small mesh sleeves directly into lantern nets. These data were collected again after the following winter (264 days in the field) and after the second growing season (490 days in the field) when the experiment was terminated.

2.4 Data analysis.

Unless otherwise stated, all analyses were performed using SAS statistical software (SAS, V.8, 2002, SAS Institute, Cary, NC, USA). The statistical significance of family and nursery feeding treatment on average plant-out body weight was determined using a
two-factor fixed-effect ANOVA. Due to lack of space, family cultures within each nursery feeding treatment were not replicated, therefore the effect of a family x nursery treatment interaction on average plant-out body weight could not be determined.

Replicate measures of yield (kg replicate\(^{-1}\)), average body weight (g) and survival (%) were taken once the animals were planted out in the field. Following a completely randomized block design, a mixed-model ANOVA was used to determine if block (i.e. water depth), family, nursery treatment or a family x nursery treatment interaction significantly affected field performance traits (P<0.05). Block was considered a random effect, while family and nursery treatment were considered fixed effects. From these ANOVA tables, sum of squares were used to estimate the effect-size (\(\eta^2\)) of each independent variable and interpreted as the fraction of the total variation which could be explained by each of the main effects and interaction effects in the model (Tabachnick and Fidell, 1989; DeLacy et al., 1990). Decomposition of estimated mean squares to compute variance components was not possible due to the fixed-effects included in this study. Least square means were determined for all family-nursery treatment combinations and used to produce response curves to illustrate the sensitivity of each family’s performance at harvest to changes in nursery dietary regime, using mean treatment plant-out body weight, averaged across all families, as a proxy for the overall effect of each nursery feeding treatment (Iwamoto et al., 1986; Falconer, 1990).
Variation in average family plant-out body weight within nursery treatments could bias results measured in the field. Regression analysis was used to determine if variation in initial plant-out weight among families within each nursery treatment significantly affected interim and harvest performance traits. If the regression was significantly different from 0, field performance measures were adjusted along the slope to a common (i.e. treatment average) initial plant-out weight. This adjustment was performed separately within each nursery feeding treatment. Natural log (ln) transformations were performed as needed to ensure linearity of the covariate (Sokal and Rohlf, 1995).

3. RESULTS

3.1 Effect of nursery feeding regime on initial plant-out body weight.

Nursery feeding regime significantly affected body weight at plant-out of outbred spat (P<0.001, ANOVA; Figure 2.1a). Average whole wet body weights, across all outbred families, in the high, medium and low nursery feeding treatments were 0.530 g, 0.367 g, and 0.079 g, respectively. No significant family effect was detected on average spat weight at plant-out (P=0.1446). Survival through the nursery phase among outbred families was high and not significantly affected by treatment (P=0.1856) or family (P=0.1318). Similarly, average spat whole wet weight at plant-out among inbred crosses was also significantly affected by nursery treatment (P<0.0001) and not by family (P=0.067; Figure 2.1b). Average whole wet body weights at plant-out among inbred
crosses for the high, medium, and low nursery feeding regimes were 0.446g, 0.324, and 0.067 g, respectively. Survival of inbred crosses through the nursery phase was not affected by nursery treatment (P=0.2981) or family (P=0.1683).
Figure 2.1. The effect of nursery feeding regime (low, medium, and high) on average individual plant-out live weight (g) of (A) five outbred families and (B) three inbred families.
3.2 Effect of plant-out body weight on field performance within nursery treatments.

Although not significant, there was some variation among family plant-out body weights within all nursery treatments which could bias field results (Section 3.1 and Figure 2.1). Variation in plant-out weight among outbred families within each nursery treatment had little effect on field performance (Table 2.1). An exception is the positive correlation between plant-out weight and survival in the low treatment at days 153 and 264. These correlations were lost after the second growing season (day 490).

Field performance of inbred families was more sensitive to variation in plant-out body weight within nursery treatment than outbred families (Table 2.1). In the medium feeding regime treatment, significant positive correlations were found between plant-out weight and body weight as well as yield at days 153 and 264. By day 490 plant-out weight was negatively correlated with all performance traits within nearly all nursery treatments.
Table 2.1. Within-treatment correlation coefficients ($r$) between the natural log of initial replicate plant-out weight and field performance traits after 153, 264, and 490 days in the field. All reported correlations are significant ($P < 0.05$). Statistically insignificant correlations are represented with a dash (-).

<table>
<thead>
<tr>
<th>Trait</th>
<th>Nursery Feeding Treat.</th>
<th>Correlation coefficient ($r$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 153</td>
</tr>
<tr>
<td><strong>Body Weight (g)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbred</td>
<td>High</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-</td>
</tr>
<tr>
<td>Inbred</td>
<td>High</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>0.493</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-</td>
</tr>
<tr>
<td><strong>Survival</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbred</td>
<td>High</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>0.298</td>
</tr>
<tr>
<td>Inbred</td>
<td>High</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-</td>
</tr>
<tr>
<td><strong>Yield (kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outbred</td>
<td>High</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-</td>
</tr>
<tr>
<td>Inbred</td>
<td>High</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Med</td>
<td>0.522</td>
</tr>
<tr>
<td></td>
<td>Low</td>
<td>-</td>
</tr>
</tbody>
</table>
3.3 Effect of nursery feeding regime on individual oyster body weight in the field.

Body weight gain occurred primarily during the summer months (the growing season for shellfish in temperate regions; Quayle, 1988), with little or no growth during the winter months (Figures 2.2a and 2.3a). The parallel growth trajectories for all treatments during the second growing season suggest little compensatory weight gain; treatments that produced larger animals at plant-out are the treatments with the largest individuals at harvest. Body weight of outbred families was significantly affected by block (i.e. water depth), nursery treatment, and family through the growout phase (P<0.0044; Table 2.2). Family x nursery treatment interactions were never found to be significant (P>0.308). Similar trends were seen among inbred families with the notable exception of a significant family x nursery environment interaction present at harvest (P=0.019; Table 2.2). The significance of the block effect was typically due to increased body weight in the shallowest block for both inbred and outbred families.
Outbred families

A. Individual body weight.

B. Survival.

C. Yield.

Figure 2.2. The effect of nursery feeding treatment on (A) average individual body weight, (B) survival, and (C) yield, over the entire experimental growout phase for outbred families. Treatment effects are represented by the mean of all five families. Significance of treatment effects within each sampling period is indicated by “ns” (P>0.05), “*” (P>0.01), and “**” (P<0.01) as determined by analysis of variance (see Table 3).
Inbred families

A. Individual body weight.

![Graph showing the effect of nursery feeding treatment on individual body weight.]

B. Survival.

![Graph showing the effect of nursery feeding treatment on survival percentage.]

C. Yield.

![Graph showing the effect of nursery feeding treatment on yield.]

Days in the field

Figure 2.3. The effect of nursery feeding treatment on (A) average individual body weight, (B) survival, and (C) yield, over the entire experimental growout phase for inbred families. Treatment effects are represented by the mean of all three families. Significance of treatment effects within each sampling period is indicted by “ns” (P>0.05), “*” (P>0.01), and “**” (P<0.01) as determined by analysis of variance (see Table 3).
Table 2.2. Analysis of variance output (mean squares “MS” and P-values “P”) for performance measures at all time periods for outbred and inbred families. “Treatment” refers to nursery dietary treatment, “TxF” refers to nursery treatment x family interaction. P-values < 0.05 printed in bold.

<table>
<thead>
<tr>
<th>Source</th>
<th>Outbred</th>
<th>Day 153</th>
<th>Day 264</th>
<th>Day 490</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 153</td>
<td>Day 264</td>
<td>Day 490</td>
<td></td>
</tr>
<tr>
<td>Body Weight</td>
<td>MS</td>
<td>P</td>
<td>MS</td>
<td>P</td>
</tr>
<tr>
<td>Block</td>
<td>108.95</td>
<td>&lt;0.001</td>
<td>82.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
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<td>&lt;0.001</td>
<td>803.71</td>
<td>&lt;0.001</td>
</tr>
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<td>0.004</td>
<td>24.76</td>
<td>0.008</td>
</tr>
<tr>
<td>TxF</td>
<td>7.23</td>
<td>0.308</td>
<td>6.03</td>
<td>0.538</td>
</tr>
<tr>
<td>Error</td>
<td>6.06</td>
<td></td>
<td>6.89</td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td>Block</td>
<td>0.0012</td>
<td>0.780</td>
<td>0.0039</td>
</tr>
<tr>
<td>Treatment</td>
<td>0.0299</td>
<td>&lt;0.001</td>
<td>0.0076</td>
<td>0.075</td>
</tr>
<tr>
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<td>0.0080</td>
<td>0.022</td>
<td>0.0105</td>
<td>0.007</td>
</tr>
<tr>
<td>TxF</td>
<td>0.0023</td>
<td>0.564</td>
<td>0.0014</td>
<td>0.870</td>
</tr>
<tr>
<td>Error</td>
<td>0.0027</td>
<td></td>
<td>0.0029</td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td>Block</td>
<td>0.1713</td>
<td>&lt;0.001</td>
<td>0.0807</td>
</tr>
<tr>
<td>Treatment</td>
<td>1.2765</td>
<td>&lt;0.001</td>
<td>1.1578</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Family</td>
<td>0.0481</td>
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</tr>
<tr>
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<td>0.498</td>
<td>0.0106</td>
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</tr>
<tr>
<td>Error</td>
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<td>0.0152</td>
<td></td>
</tr>
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<table>
<thead>
<tr>
<th>Source</th>
<th>Inbred</th>
<th>Day 153</th>
<th>Day 264</th>
<th>Day 490</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 153</td>
<td>Day 264</td>
<td>Day 490</td>
<td></td>
</tr>
<tr>
<td>Body Weight</td>
<td>MS</td>
<td>P</td>
<td>MS</td>
<td>P</td>
</tr>
<tr>
<td>Block</td>
<td>25.82</td>
<td>&lt;0.001</td>
<td>25.98</td>
<td>&lt;0.001</td>
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<tr>
<td>Treatment</td>
<td>238.54</td>
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<td>246.36</td>
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</tr>
<tr>
<td>Family</td>
<td>15.48</td>
<td>0.003</td>
<td>8.84</td>
<td>0.276</td>
</tr>
<tr>
<td>TxF</td>
<td>2.83</td>
<td>0.353</td>
<td>3.74</td>
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<tr>
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<td>2.52</td>
<td></td>
<td>2.35</td>
<td></td>
</tr>
<tr>
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<td>Block</td>
<td>0.0035</td>
<td>0.454</td>
<td>0.0030</td>
</tr>
<tr>
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<td>0.012</td>
<td>0.0275</td>
<td>0.006</td>
</tr>
<tr>
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<td>0.531</td>
<td>0.0088</td>
<td>0.177</td>
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<tr>
<td>TxF</td>
<td>0.0049</td>
<td>0.271</td>
<td>0.0097</td>
<td>0.111</td>
</tr>
<tr>
<td>Error</td>
<td>0.0037</td>
<td></td>
<td>0.0050</td>
<td></td>
</tr>
<tr>
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<td>Block</td>
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<td>&lt;0.001</td>
<td>0.0282</td>
</tr>
<tr>
<td>Treatment</td>
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<td>0.3450</td>
<td>&lt;0.001</td>
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<td>0.0140</td>
<td>0.010</td>
</tr>
<tr>
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<td>0.0060</td>
<td>0.1144</td>
<td>0.0074</td>
<td>0.046</td>
</tr>
<tr>
<td>Error</td>
<td>0.0031</td>
<td></td>
<td>0.0029</td>
<td></td>
</tr>
</tbody>
</table>
Figures 2.4a and 2.5a illustrate the magnitude of the main effects and interaction effects on individual body weight over time (153, 264 and 490 days in the field) for outbred and inbred families. These figures show two important trends. First, the fraction of phenotypic variation in individual body weight explained by variation in nursery dietary treatment decreases over time from over 54% at days 153 and 264, to 9.8% at day 490 for outbred families and from approximately 60% at days 153 and 264 to 23.7% at day 490 for inbred families. Second, the fraction of phenotypic variation in individual body weight explained by variation among families increases over time from approximately 3.3% at the interim measurements to 10.2% at harvest for outbred families and from approximately 3.0% at the interim measurements to 12.9% at harvest for inbred families.

3.4 Effect of nursery feeding regime on average family survival in the field.

Average survival was high after the first growing season (outbred: >90%; inbred: >88%) and after the first winter (outbred: >90%; inbred: >86%) for all treatments, followed by a severe mortality event during the second growing season (Figures 2.2b and 2.3b). Survival of outbred families was affected by nursery treatment and family at the first measurement period (P<0.022), and only affected by family at the second measurement period (P=0.0071; Table 2.2). At harvest, after the mortality event, all main effects were significant (P<0.0023). The family x nursery treatment interaction, however, remained non-significant (P=0.9464).
Figure 2.4. The percent of total phenotypic variation among outbred families that can be explained by block, family, nursery environment, family x nursery environment, and experimental error for (A) individual body weight, (B) survival, and (C) yield. Sum of squares were used to estimate eta² values for three measurement periods (153, 264 and 490 days in the field). Significance of each factor is indicated by “ns” (P>0.05), “*” (0.05>P>0.01), and “***” (P<0.01) as determined by analysis of variance (see Table 3).
Figure 2.5. The percent of total phenotypic variation among inbred families that can be explained by block, family, nursery environment, family x nursery environment, and experimental error for (A) individual body weight, (B) survival, and (C) yield. Sum of squares were used to estimate \( \eta^2 \) values for three measurement periods (153, 264 and 490 days in the field). Significance of each factor is indicted by "ns" (\( P > 0.05 \)), "*" (\( 0.05 > P > 0.01 \)), and "**" (\( P < 0.01 \)) as determined by analysis of variance (see Table 3).
Inbred families differed in that only nursery feeding treatment significantly affected survival during the first two measurement periods. At harvest, survival of inbred families was only affected by block (i.e. water depth; P=0.0001) and family (P<0.0001). Block effects were again primarily due to increased survival in the shallowest block for both inbred and outbred families. Trends in $\eta^2$ for both outbred and inbred families were similar to those described for individual body weight with a general decrease in the effect of nursery dietary treatment over time and an increase in the effect of family over time (Figure 2.4b and Figure 2.5b).

3.5 Effect of nursery feeding regime on average family yield in the field.

Yield is a function of both individual body weight and survival. Prior to the second growing season, average treatment yield was primarily determined by body weight, and consequently, those treatments that produced the largest individuals were the highest yielding. This pattern can be seen at day 153 and day 264 (Figures 2.2c and 2.3c). However, after the mortality event during the second growing season, average treatment yield of outbred families became a function of both individual body weight and survival. Average treatment yield of inbred families remained largely a function of body weight, as there was little differential mortality by day 490 between nursery feeding treatments.

Yields of outbred families were significantly affected by block (P<0.001) and nursery treatment (P<0.001) at the two interim measurements (Table 1). Yield of outbred
families at harvest was affected by block (P<0.001) and family (P<0.001) but not family x nursery feeding environment interactions (P=0.874). Yields of inbred families were affected by all main effects at all time periods. The effect of a genotype x nursery environment interaction on yield of inbred families was not significant at day 153 (P=0.114), but was significant at days 264 (P=0.045) and 490 (P=0.009). The shallowest blocks tended to display the highest yields for both inbred and outbred families.

Trends in $\eta^2$ for both inbred and outbred families were similar to those described for individual body weight and survival with a decrease in the effect of nursery dietary treatment over time and an increase in the effect of family over time (Figure 2.4c and Figure 2.5c). The amount of total phenotypic variation in yield explained by nursery dietary treatment decreased from approximately 54% during the interim measurements to 2.7% at harvest in outbred families and from over 60% at the interim measurements to 3.7% at harvest in inbred families. The amount of total phenotype variation in yield explained by family increased from approximately 3% during the interim measurements to 26.3% at harvest in outbred families and from approximately 3% at the interim measurements to 30.6% at harvest in inbred families.

3.6 Family sensitivity of adult performance traits to variation in the nursery environment.

Response curves in Figure 2.6 measure the sensitivity of each family's adult performance traits at harvest to changes in nursery feeding environment (using average initial
treatment plant-out weight as a proxy for the overall effect of nursery feeding treatment). Figure 2.6 supports conclusions drawn from ANOVA in Table 2.1, that family x nursery environment interactions do not significantly affect adult phenotype of outbred families. Among outbred families, rank stability is maintained across all nursery environments, for all performance traits. For example family 4 is the highest yielding family at harvest across all nursery feeding regimes, while family 2 is the lowest yielding family across all nursery feeding regimes. All rank changes that do appear are among similarly performing families (i.e. rank changes among the best performers, or rank changes among the worst performers).

Response curves also support ANOVA results indicating that a significant genotype x nursery environment interaction affects harvest body weight and yield of inbred families. Figures 2.6d-f illustrate rank changes in the most extreme nursery treatment (i.e. “low” nursery feeding regime).
Figure 2.6. The sensitivity of harvest performance measures for both outbred (A-C) and inbred (D-E) families to changes in nursery feeding regime. Average treatment plant-out weight is used as a scale of the overall effect of each of the three nursery feeding regimes.
4. Discussion

This study demonstrates environmental variation experienced by hatchery-raised Pacific oysters during juvenile development can permanently affect adult phenotype, even after 490 days in the field. Significant effects of nursery dietary regime on plant-out body weight found in this study are consistent with the findings of other researchers who have shown bivalve growth rate to be plastic in response to varying levels of dietary restriction (His and Seaman, 1992; Rheault and Rice, 1996; Wikfors et al., 2001; Pechenik et al., 2002). Commercial shellfish hatcheries attempt to maximize oyster growth rate by providing a continuous supply of algal feed while the animals are in the nursery, similar to the “high” feeding regime in the present study. Conditions similar to the “medium” feeding regime may be encountered occasionally during periods of algae shortages. Conditions as extreme as the “low” feeding regime in this study are unlikely to occur in commercially viable shellfish hatcheries.

4.1 Individual body weight.

Parallel growth trajectories among all treatments after day 153, for both inbred and outbred families (Figures 2.2a and 2.3a), and the significant effect of nursery feeding regime on body weight even after 490 days in the field (Table 2.2), suggest little or no compensatory weight gain in response to reduced rations in the nursery. Similar results were reported by Brooks (2000), who found oyster spat stunted in the nursery by
exposure to episodic freshwater pulses (simulating heavy rainfall events in estuaries) remained, on average, smaller, after two growing seasons in the field, than oysters which experienced constant salinity seawater in the nursery. Although successful use of dietary restriction to elicit compensatory growth has been reported (e.g. Hayward et al., 1997), it is generally agreed to be a complex phenomenon, dependant upon the age at which food restriction is applied, the duration of food restriction and the severity of restriction (e.g. Lay, 1998). With these variables in mind, the dietary restrictions applied in this study may have resulted in conditions for which the oysters were physiologically unable to compensate.

4.2 Survival.

Family significantly affected survival at harvest for both inbred and outbred families (Table 2.2; Figure 2.6). Other researchers have shown oyster survival to be under genetic control. Haskins and Ford (1988) found Eastern oyster (C. virginica) lines selected for improved tolerance to MSX had significantly higher survival than unselected lines. Beattie et al. (1980) found Pacific oysters selected to tolerate elevated water temperature had higher survival in the field than unselected oysters. More recently, Degremont et al. (2003) found tolerance of Pacific oysters to summer mortality in France to be highly heritable.
Nursery treatments also affected field survival. However, after 490 days in the field the effect of nursery treatment on survival was only seen among outbred families, and not among inbred families (Table 2.2). It is interesting to note the inverse relationship between average treatment plant-out weight and average treatment survival at harvest among the outbred families (Figures 2.6). Two possible explanations for this trend are offered below.

First, it is possible that large animals at plant-out remained larger in the field (see Figure 2.6a) reducing their ability to tolerate environmental stress during the second growing season. The trade-off between growth rate and/or body size and survival has been reported in a variety of taxa (Li et al., 1996; Bradford et al., 1999; Miller et al., 2000; Norry and Loeschcke, 2002; Olsson and Shine, 2002). Although this correlation has not been observed directly in oysters (Beattie et al., 1980; Ernande et al., 2002), some researchers have suggested a positive correlation between size and mortality may explain patterns seen in summer mortality syndrome in C. gigas (Glude, 1975). Reproductive effort (i.e. proportion of energy resources dedicated to reproduction) in shellfish tends to be positively correlated with both mortality rate (Beattie et al., 1980; Ernande et al., 2002) and body size (Bayne et al., 1983; Roff, 1992), which could result in size-specific mortality during periods of elevated water temperature and rapid gonad development. This hypothesis is consistent with observations in the present study, where greater mortality occurred in nursery treatments with large individuals versus nursery treatments with smaller individuals. The parallel growth trajectories found in this study (Figures
2.2a and 2.3a), suggest that it is not differential growth rate, but differential body weight that accounts for variation in mortality among the treatments. Interestingly the inverse correlation between body weight and survival in outbred families only exists when considered across nursery treatments. No obvious correlation exists within each nursery treatment (e.g. family 4 consists of both high survivors and heavy individuals in any given treatment, Figure 2.6a and 2.6b). This suggests that body weight alone does not account for the observed variation in harvest survival. The inverse relationship between average nursery treatment plant-out weight and average treatment survival at harvest was only observed in outbred families and not in inbred families, possibly due to the overall smaller size of inbred oysters (approximately 50g) versus the larger outbred individuals (approximately 83 g), small number of inbred families (n=3), or interactions between gametogenesis and inbreeding.

A second possible explanation is that oysters exposed to restricted algal rations in the nursery were conditioned to handle stress more effectively than nutritionally non-stressed animals, and consequently enjoyed higher survival in the field. Stress during early development has been shown to permanently affect phenotypic expression later in life (see review by Lay, 2000 and review by Metcalfe and Monaghan, 2001) across a wide variety of species (e.g. Hales and Barker, 1992; Desai and Hales, 1997; O’Steen, 1998; Lay, 2000; Pahkala et al., 2001; Lee and Peterson, 2003), including bivalves (Brooks, 2000). Researchers have examined many early-life stressors that include nutrient restriction (Hales and Barker, 1992; Lay et al., 1998), handling (e.g. Lay, 2000), physical
environment (O’Steen, 1998; Brooks, 2000; Pahkala et al., 2001; Lee and Petersen, 2003) and social isolation/grouping (Moberg and Wood, 1982; Houpt and Hintz, 1982/83; Creel and Albright, 1988). Lay (2000) reported stressed neonatal mice were able to better tolerate stress later in life than non-stressed neonatal mice. The opposite trend was found among livestock species such as lambs, calves and foals. Although the exact mechanisms for these phenomena are largely unknown, Lay (2000) notes that “if exposing livestock to stress during development can increase their ability to cope with stressful situations, then manipulation of this phenomenon in a controlled manner will allow producers to ‘programme’ stock for specific management systems.”

4.3 Yield.

Yield is a commercially important character in shellfish aquaculture (Hedgecock et al., 1997; Langdon et al., 2003) and is defined as “meat production in relation to amount (number) of seed planted” (Quayle, 1988). As such, yield is a function of two primary causal components: body weight and survival. During periods of high survival (days 158 and 264), little variation in yield could be attributed to variation in survival, therefore yield was largely determined by variation in body weight. This trend, however, reversed during the second growing season, when survival became the dominant component of yield. As a result, the treatment with the smallest animals but the highest survival (the “low” nursery feeding treatment) became the highest yielding treatment (although not significantly different from the other treatments). In addition, as mentioned above, an
increase in average treatment body weight at plant-out resulted, on average, in an
increase in average treatment body weight at harvest and a decrease in average treatment
survival at harvest among outbred families (Table 2.2; Figures 2.6a and 2.6b). This
tradeoff resulted in no net nursery treatment effect on harvest yield. It is also important
to note that this experiment was performed in a single growing environment, and that the
observed trends may be environment-specific. For example, if the severe mortality event
during the second growing season had not occurred, the role of body weight may have
remained the dominant component of yield, resulting in treatments with the largest
individuals at plant-out being the highest yielding at harvest.

4.4 Effect of inbreeding.

Inbred families behaved differently from outbred families in several ways. First, field
performance among inbred families was significantly affected by genotype x nursery
environment interactions (Table 2.2). Ranking of inbred family performance was stable
between the high and medium nursery feeding treatments for all traits at harvest (Figure
2.6). Significant rank changes among families for both individual body weight and yield
occurred only under the most stressful ("low") nursery feeding regime. Further, the rank
changes can be traced to a single crossover event between families 1 and 3. Inbred
families are considered to be more sensitive to environmental stress than more
heterozygous genotypes (Reich and Atkins, 1970; Schnell and Becker, 1986; Leon, 1994;
Haussmann et al., 2000; Myrand et al., 2002), with the latter displaying greater
performance stability across variable environments. Results from the present study suggest that inbred families may also be more sensitive to the effects of permanent environmental stress.

Inbred and outbred families also differed in that all harvest performance measures of inbred families within each nursery treatment were significantly negatively correlated with initial plant-out body weight (r<-0.416; P<0.05; Table 2). These correlations will act to bias results measured at harvest, primarily by contributing to variation among families within nursery treatments. It is unclear why the negative correlation between initial plant-out body weight and harvest performance traits exists only among inbred families. Again, this may be a result of heightened environmental sensitivity with increased genomic homozygosity. The fact that the correlations occur only at harvest suggests that inbred and outbred families differed in their response to the mortality event during the second growing season.

Lastly, Figure 2.6 shows inbreeding depression primarily affected body weight (Outbred = 85g; Inbred = 55g), with little affect on survival (outbred = 40%; inbred = 44%). Evans et al. (2004) attributed a similar pattern to the purging of individuals homozygous for early acting lethal recessive genes from the population during the nursery phase, resulting in fewer than expected individuals homozygous for lethal recessive alleles evaluated in the field. Due to limited culling by size in the nursery, as practiced in this experiment, individuals homozygous for deleterious alleles affecting growth would not have been
purged in the nursery and therefore would have remained in the population and been evaluated as poor growers in the field.

4.5 Relative effects of independent variables.

It is important for breeders to consider the relative effects of block, family, nursery environment, and family x nursery environment interactions on the characters of interest over time. P-values generated from ANOVA are useful for assigning statistical significance but not as useful for determining the practical significance of specific independent variables. The effect-size ($\eta^2$) is a statistic used to describe the amount of total phenotypic variation explained by each main and/or interaction effect, when these effects are considered fixed (Tabachnick and Fidell, 1989). The more traditional approach of using mean squares to estimate variance components is, strictly speaking, only applicable to random effects. Due to the small number of families used in this study and non-random selection of families, the absolute levels of $\eta^2$ have limited scope of inference, however, they do allow the relative changes in effect-size over time to be examined within this experiment. The effect of nursery treatment decreased over time and the effect of family increased over time among both outbred and inbred families for all performance characters (Figures 2.4 and 2.5). All else being equal, this would indicate that the accuracy of selecting superior performing families should increase as the length of the growout phase increases. The partial eta$^2$ ($\eta^2_p; SS_{Family}/(SS_{Family} + SS_{Error})$) suggests that this is true even as the relative contribution of unexplained error increases.
over time for body weight and yield (Figure 4 and 5). Other researchers have reported similar effects of time on the increase in family divergence (Refstie and Steine, 1978; Gjedrem, 1983; Mckay et al., 1986; Toro and Newkirk, 1990).

4.6 Implications for breeding programs.

Management strategies to account for permanent nursery environmental effects on adult phenotypes can be adopted relatively easily by selecting superior families from within cohorts (or contemporary groups) made up of families of the same age and raised in the same nursery environment. This approach would reduce or even eliminate variation due to nursery environment ($E_N$), and is analogous to accounting for variation due to herd effects in terrestrial animals (where all members of a herd experience the same juvenile and adult growing environments). The absence of a significant genotype x nursery environment effect on harvest performance measures among outbred families suggests that relative family performance should be stable across a wide range of nursery feeding environments. Managing around a significant genotype x nursery environment interaction would be more difficult and in extreme cases could require the development of nursery specific lines of selection. The significant genotype x nursery environment interactions affecting inbred family field performance only occurred in the most stressful nursery environment and therefore may be unlikely to affect families raised under more typical commercial feeding regimes.
The results of this study indicate that the nursery environment can significantly affect performance characters measured after two growing seasons (490 days) in the field. Genotype x nursery environment interactions did not affect outbred family field performance, but did affect inbred adult body weight and adult yield. Rank changes in field performance occurred among inbred families raised in the most stressful feeding environment. These results also indicate that selection efficiency for all performance traits measured in this study should increase as the number of days in the field increase due to a reduction in the effect of nursery environment and an increase in the effect of family over time. Further work is needed to determine if the inverse correlation between average treatment plant-out weight and survival at harvest is due to differential adult body weight or conditioning to nutrient stress as juveniles.
5. REFERENCES


CHAPTER 3

EFFECTS OF GENOTYPE X ENVIRONMENT INTERACTIONS ON PACIFIC OYSTER (*Crassostrea gigas*) PERFORMANCE IN THE PACIFIC NORTHWEST.

ABSTRACT

Broadly adapted genotypes (i.e. "generalists") are required if a single line of selection is to improve Pacific oyster (*Crassostrea gigas*) production throughout the heterogeneous oyster growing environments found in the Pacific Northwest. An experiment was conducted to determine if the relative rankings of average family performance (i.e. body weight, survival and yield) remain stable across a wide range of growing environments. Twenty-four to 27 full-sib oyster families were each planted at an intertidal and subtidal suspended culture site in both Yaquina Bay, Oregon, and Dabob Bay, Washington. Each family was represented by up to 12 replicate growout bags (stocked with 60 spat each) or 12 replicate lantern nets compartments (stocked with 40 spat each). Oysters were planted at all four sites in Fall 2002 and yield (kg live weight replicate⁻¹), body weight (g) and survival (%) measured in Spring and Fall 2003 and at harvest in Summer 2004. Harvest body weight, survival, and yield were all significantly affect by genotype, environment, and genotype x environment interactions (P<0.01). Genotype, environment, and genotype x environment interaction, accounted for 5%, 80%, and 3% of the total phenotypic variation in harvest body weight, respectively. Genotype, environment, and genotype x environment interaction, accounted for 42%, 14%, and 6% of the total phenotypic variation in harvest survival, respectively. Genotype, environment, and genotype x environment interaction, accounted for 14%, 60%, and 5% of the total
phenotypic variation in harvest yield, respectively. Although the effects of the genotype x environment interactions were statistically significant, phenotypic correlations among average family yields between all sites were significantly greater than 0 ($r_p > 0.432; P < 0.001$) indicating high-yielding families in one environment tended to be high-yielding in other environments. These results suggest that selection for broadly-adapted generalists families at a limited number of sites should be able to indirectly improve mean oyster yield at dissimilar sites throughout the Pacific Northwest.

1. INTRODUCTION

Agronomically important traits of domestic plant and animal species are commonly affected by genotype x environment (GxE) interactions (e.g. Haldane, 1947; Baker, 1987; DeLacy, 1990), which occur when the relative performance of genotypes depends upon the environment in which they are raised. These interactions can impact breeding programs by reducing the association between phenotype and genotype, thereby reducing heritability ($h^2$). In addition, and perhaps more importantly (Haldane, 1947), GxE interactions can result in significant rank changes among genotypes across environments. When strong enough, GxE interactions may require breeders to develop different lines of selection for each unique growing environment.

Shellfish growers have expressed interest in developing broadly-adapted, high-yielding strains of Pacific oysters (*Crassostrea gigas*) to improve oyster production along the west
coast of the United States (Hedgecock et al., 1997). Two aspects of this selection goal increase the probability of encountering a GxE interaction: 1) the heterogeneous target environment and 2) the complexity of the character of interest. The highly heterogeneous target environment includes commercial oyster farms along the west coast from California to Alaska. In addition to geographic variation, culture environments vary dramatically as a function of tidal exposure, salinity fluctuation, type of substrate, food quality and quantity, culture method and countless other biotic and abiotic factors (Quayle, 1988; Conte, 1996). Variation in the growing environments of many terrestrial and some aquatic species can be minimized through the application of inputs such as irrigation, fertilizers, complete artificial diets, etc (e.g. Ceccarrelli, 1989; Landau, 1992). Unfortunately, with few exceptions (e.g. farm relocation or changing culture methods), farmers are largely unable to alter shellfish growing environments (Conte et al., 1996).

The probability of encountering a GxE interaction is also a function of the complexity of the character of interest (Baker, 1987). Yield, defined as oyster “production in relation to amount (number) of seed planted” (Quayle, 1988), is a composite character made up of two primary causal components: oyster body weight and survival. In general, the probability of experiencing a GxE interaction for a composite trait is expected to increase as the number of non-interacting causal components increase (Baker, 1987). It should be noted that body weight and survival are only the most basic components of yield; body weight and survival are themselves made up of many secondary causal components (e.g. feed consumption rate, feeding efficiency, protein assimilation efficiency, temperature...
tolerance, salinity tolerance, etc.).

Although Pacific oyster yield (Langdon et al., 2003), body weight (Lannan, 1972; Hedgecock, 1991), and survival (Beattie et al., 1980; Degremont et al., 2003) have all been shown to be heritable, the degree to which GxE interactions may slow progress towards developing high-performing strains broadly-adapted to the Pacific Northwest has not been directly tested. In this study, full-sib Pacific oyster (Crassostrea gigas) families were evaluated at four typical, but very different, oyster growing environments to address two main questions: 1. Do GxE interactions affect oyster yield, individual body weight, and survival in the Pacific Northwest?; and 2. Can oyster performance be indirectly improved throughout the Pacific Northwest based on performance at a limited number of evaluation sites?

2. METHODS

2.1 Spawning and nursery protocol

A total of 300 adult Crassostrea gigas were collected from Dabob Bay, Washington, USA (47.8° N, 122.87° W), and transported to the Hatfield Marine Science Center (HMSC), Newport, Oregon, US (44.6° N, 124.1° W), in January 2002. Animals were held in 18° C sand-filtered seawater and fed a mixture of Isochrysis galbana (Iso) and Cheatoceros calcitrans (Cc) at a concentration of approximately 50,000 to 80,000 cells
ml$^{-1}$ until ready to spawn. In April 2002, 68 individuals were stripped-spawned as per Langdon et al. (2003) to create 34 full-sib families. Fertilized eggs were allowed to develop into veliger larvae (D-larvae) for 24 hours in cross-specific 20-l containers filled with 25$^\circ$ C, 0.2 $\mu$m-filtered seawater.

D-larvae from each cross were then stocked into each of two 60-l larval culture containers at a concentration of 10 larvae ml$^{-1}$. Each larval tank is referred to here as a culture and each pair-wise mating is referred to as a genotype, therefore there were 2 cultures for each of 34 genotypes resulting in 68 larval tanks. Larvae were fed daily with a mixture of Iso and Cc at concentrations ranging from 30,000 to 80,000 cells ml$^{-1}$, depending on age (Breese and Malouf, 1975). Larval tanks were drained and re-filled with 25$^\circ$ C 0.2 $\mu$m-filtered seawater twice per week. During water changes, larvae were retained on 37 $\mu$m sieves for the first week and 80 $\mu$m sieves for the second week. During the third week larval cultures were drained through a 243 $\mu$m sieve onto an 80 $\mu$m sieve. All larvae retained on the 243 $\mu$m sieve were exposed to $2 \times 10^4$ M epinephrine in order to induce metamorphosis (Coon, 1986).

Successfully metamorphosed spat were transferred to culture-specific 15-cm diameter upwellers. Upwellers were held in a semi-recirculating system which received approximately 6 exchanges d$^{-1}$ of 25$^\circ$ C UV-irradiated 1 $\mu$m filtered seawater. Once all larvae had metamorphosed, the number of spat per upweller was randomly thinned to 10,000. Spat were allowed to grow until retained on a 1.4 mm sieve, then transferred to
culture-specific 28-cm diameter upwellers in a larger upweller system. These larger upwellers were supplied with 18° C 1 μm filtered seawater and fed an Iso/Cc mixture at a final concentration of approximately 50,000 to 80,000 cells ml\(^{-1}\). Once all animals were transferred from the 15-cm upwellers, the number of oysters per 28-cm upweller was randomly thinned to 5,000. Oysters were then allowed to grow until retained on a 6.4 mm sieve, before being transferred to culture-specific spat bags (2 mm mesh) held in storage tanks receiving ambient 1 μm-filtered seawater (mean 12.4° C; range 9.9° -18.4° C) and batch-fed to a final concentration of approximately 80,000 to 100,000 cell ml\(^{-1}\) of a Cc/Iso mixture twice per week. The reduced temperature and limited feeding was intended to slow oyster growth, minimizing the variation in spat weight within and between cultures prior to planting in the field (Langdon et al., 2003).

After approximately 80% of the oysters were sieved from the 28-cm upwellers, spat in the storage tank were counted and weighed for subsequent planting in the field. Six replicate bags of 40 oysters were weighed for each of two subtidal sites (utilizing 10-tier, 0.51-m diameter lantern nets; 5 mm mesh) and six replicate bags of 60 oysters weighed for each of two intertidal sites (utilizing rectangular 0.53 m x 0.81 m; 7 mm mesh) from each culture. Regardless of culture method, all oyster were first stocked into 0.3 m x 0.3 m sleeves (2 mm mesh). These sleeves were then inserted into either lantern nets compartments or growout bags.

2.2 Field trials
2.2.1 Experimental environments

Four growing environments were examined in this study: 1. intertidal on-bottom culture site in Yaquina Bay, Oregon; 2. subtidal suspended culture site in Yaquina Bay, Oregon; 3. intertidal on-bottom culture site in Dabob Bay, Washington; 4. subtidal suspended culture site in Dabob Bay, Washington. These four environments were intended to represent four very dissimilar oyster-growing environments encountered in the Pacific Northwest (Quayle, 1988). Yaquina Bay is characterized as an estuarine environment subject to both tidally and seasonally induced fluctuations in salinity. Salinity in Yaquina Bay can range from 0 % due to winter runoff to 35 % during dry-season high tides. Conversely, Dabob Bay is a relatively deep embayment off Hood Canal in Puget Sound. This environment is characterized by constant and high salinity, dropping only slightly during winter rain-events. Data loggers (developed by F. Smith, Northwest Research Associates, Seattle, WA) were deployed at all four environments to record temperature and salinity at 30 minute intervals.

2.2.2 Measurement of average genotype yield, body weight, survival

Oysters were planted out at the four test environments in the Fall of 2002. Each test environment was partitioned into three blocks, accounting for either intertidal aerial exposure or subtidal depth in the lantern nets. Each genotype was represented by up to four replicates per block (two replicates from each of the two cultures within genotype). Due to varying survival in the nursery, some genotypes had fewer than four bags per
block and some crosses were not planted at all four evaluation environments. In the Spring of 2003 (day 192 in the field) oysters were removed from the 2-mm mesh sleeve and all live oysters from each replicate were cleaned of biotic and abiotic fouling, counted, and the collective weight of all live animals measured to the nearest gram. Animals were restocked directly into either the intertidal growout bags (7 mm mesh) or the lantern nets (5 mm mesh) after this initial measurement period. These measurements were recorded again in the Fall of 2003 (day 370) and in the Summer of 2004 (day 664 at Dabob Bay and day 697 in Yaquina Bay). The collected data allowed for replicated estimates of average genotype bag weight (kg replicate\(^{-1}\)) and survival (%) in the field. Average individual oyster weight per replicate was calculated by dividing the total bag weight by the number of live oysters. Finally, yield was determined by standardizing each replicate bag weight to a uniform number of seed planted (i.e. 100), therefore intertidal bag weight was multiplied by the quantity (100/60) and subtidal bag weight multiplied by the quantity (100/40).

2.3 Statistical analysis

Unless otherwise stated, all analyses were performed using SAS statistical software (SAS, V.8, 2002, SAS Institute, Cary, NC, USA). Normal probability plots and residual plots were used to assess normality and equality of variance for all performance measures.

2.3.1 Adjustment for variation in initial weight
Due to variable spat performance in the nursery, average individual plant-out weight per family ranged from 0.221 to 0.385 g. To account for possible bias due to variation in plant-out body weight, field performance measures were regressed against initial body weight separately for each of the four evaluation environments. If a significant relationship was present between initial plant-out weight and field performance, field performance values were adjusted along a common slope to the mean plant-out weight for that environment (e.g. Rawson and Hilbish, 1990; Langdon et al., 2003). These adjusted values were used in subsequent analyses.

2.3.2 Four-environment and single-environment analysis of variance

A random-effects, nested two-factor analysis of variance (ANOVA) was used to test the significance of environment, block within environment, genotype, culture within genotype, and genotype x environment interaction (Romagosa and Fox, 1993) across all four growing environments on adjusted oyster body weight, survival and yield in the Spring and Fall of 2003 and the Summer of 2004. Variance components were determined using restricted maximum likelihood (REML) estimation methods. Environment was considered a random variable (rather than fixed) so variance due to environment, and therefore fraction of total phenotypic variation, could be estimated. Because environments were not randomly selected, variation due to environment may not accurately reflect the true variance among all environments in the Pacific Northwest. All survival values were arc-sine transformed prior to analysis (Sokal and Rohlf, 1995). Live body weight at day 192 was natural-log transformed to ensure homogeneity of variance.
across the four environments.

Similar analyses were performed to determine if block, genotype, culture within genotype, and block x genotype interaction significantly affected performance within each growing environment. Variance components were again estimated for all main and interaction effects using REML.

2.3.3 Phenotypic correlation ($r_p$).

Pair-wise correlations of mean genotype performance across environment is another way to test for the significance of genotype x environment interactions (Lynch and Walsh, 2000). When no GxE interaction is present, relative performance of genotypes should not change across environments (i.e. high rank stability) and $r_p$ should not differ significantly from 1. However, if a significant GxE interaction exists, the relative performance of genotypes will change across environment (i.e. low rank stability), and $r_p$ will be significantly less than 1. For the purposes of correlating performance among environments, all data were first standardized as the deviation (in standard deviation units) from the appropriate block mean within each environment. This set each block mean at all environments equal to 0 with a standard deviation of 1. Expressing performance in this manner ensured equality of variance across environments as well as reduced bias due to missing cells in above or below average blocks (Newkirk and Haley, 1983). Bootstrap re-sampling techniques (Phillips, 2001; 10,000 re-samples) were used to calculate the phenotypic correlation between genotype means for all pair-wise
combinations of environments. These techniques were also used to test if \( r_p < 1 \) and \( > 0 \) (t-test, \( P < 0.05 \)). Correlation of performance traits across environment were only made between cultures within each cross that did not share a common nursery environment (e.g. culture A versus culture B, see Section 2.1). As a result, similarity between genotypes at different growout sites due to shared nursery environment was removed, leaving only the correlation among genotypes (both additive and non-additive; Lynch and Walsh, 2000).

Phenotypic correlations were also determined in this manner among different characters within each environment. This measure provided an indication of how influential each of the two causal components (individual live body weight and survival) were in determining yield. Correlations between individual live body weight and survival were also determined. Again, to avoid bias due to shared nursery environment, correlations were never made between traits measured on the same culture within a genotype (Lynch and Walsh, 2000).

2.3.4 *Intraclass correlation* (\( t \)) and *broad-sense heritability* (\( H^2 \))

The intraclass correlation (\( t \)) represents the fraction of total variance that can be explained by between group variance (\( \sigma_b^2 \)) relative to within group variance (\( \sigma_w^2 \); Falconer and Mackay, 1996): \( t = \sigma_b^2 / (\sigma_b^2 + \sigma_w^2) \). In this study, within a given test environment, the "between group" source of variance was due to genotype (\( \sigma_G^2 \)) and "within group" variance was due to all other sources of uncontrollable variation (i.e. culture within
genotype, \( \sigma^2_{G(G)} \); genotype x block, \( \sigma^2_{GB} \); and experimental [within block] error, \( \sigma^2_e \).

Estimation of \( t \) was therefore calculated as:

\[
 t = \frac{\sigma^2_b}{\sigma^2_b + \sigma^2_w} = \frac{\sigma^2_G}{\sigma^2_G + [\sigma^2_{G(G)} + \sigma^2_{GB} + \sigma^2_e]} .
\]  

(1)

Block was considered a controllable nuisance variable and excluded from the estimation of \( t \). Variation around \( t \) was calculated as per Becker (1992).

Sibling analysis uses the intraclass correlation (\( t \)) among groups of relatives to estimate \( h^2 \) of a character such that \( h^2 = t / r \), where \( r \) is the coefficient of relationship (Falconer and Mackay, 1996). Full-sib matings conducted in this study prevents estimation of narrow sense heritability (\( h^2 \)), but does allow for the estimation of broad sense heritability (\( H^2 \)), which includes variation due to non-additive sources of genetic variation. Typically, when full-sib families are considered, data is collected on individuals resulting in an \( r \) among relatives of 1/2. \( H^2 \) then becomes 2(\( t \)). However, in the present study \( r \) represents the coefficient of relationship between replicate bags, each of which are composed of up to 40 to 60 individuals. As a result, the performance of each replicate bag is an estimate of the average offspring performance of a particular set of parents. As the number of individuals within each replicate increases, the coefficient of relationship (\( r \)) between groups of siblings approaches 1, and the formula to estimate broad sense heritability reduces to \( H^2 = t \). Note because the true value of \( r \) may be less than 1, \( t \) represents the
lower limit of $H^2$. Therefore, $t$ was used as a conservative estimate of $H^2$ and $\sigma$, as an estimate of $\sigma_H^2$ (Langdon et al., 2003).

2.3.5 Expected efficiency of indirect selection across environments

Although ANOVA and correlation coefficients allow specific hypotheses to be tested [e.g. $\Pr(\sigma_{GxE}^2 > 0)$ and $\Pr(r_p < 1)$], the effect of GxE interactions on progress towards genetic gain is best illustrated by considering variance components (i.e. heritability) and correlation across environments simultaneously (Falconer, 1952). The expected efficiency of indirect selection on a trait in environment B for gains in environment A (CR$_A$) was compared to direct selection in environment A (RA) as per Falconer (1952):

$$\frac{CR_A}{RA} = \frac{r_a h_B}{h_A}$$

(2)

where $r_a$ is the additive genetic correlation between the same character in environment A and B, and $h$ is the square root of the narrow sense heritability of that character in environments A and B, respectively. The value of this approach is in the ease of interpretation. For example a $CR_A/RA$ value of 0.8 indicates that indirect selection in site B for gains in site A will be 80% as effective as direct selection in site A.

The use of full-sib families in this experiment requires two assumptions to be made in order for a valid comparison of direct and correlated responses across environment to be
made (Roff, 1995). The first is that the phenotypic correlation between mean genotypic performance calculated in the present study must approximate the additive genetic correlation as required by Falconer (1952). Although it is understood that inclusion of non-additive genetic effects may inflate $r_P$ over $r_a$, some researchers suggest that, for animals in general, phenotypic correlations tend to closely approximate additive genetic correlations, and can often be determined with a much higher degree of precision (Mousseau and Roff, 1987; Cheverud, 1988; Roff, 1995; Roff, 1996; but see Willis et al, 1991). Also, as mentioned earlier, there is no environmental correlation between performance measures at any two pair of environments, therefore $r_P$, as measured here, can be considered the “broad sense” genetic correlation, including both additive and non-additive genetic correlations. For the purposes of this study it is assumed that $r_P$ is an acceptable approximation of $r_a$.

The present study requires a second assumption to be made because with full-sib matings it is impossible to estimate the narrow-sense heritability ($h^2$), only the broad-sense heritability ($H^2$) which can, again, be inflated due to the inclusion of non-additive sources of genetic variation. Mousseau and Roff (1987) found a positive correlation between estimates of heritability based on parent-offspring regression ($h^2$) and full-sib analysis ($H^2$) using 33 characters in 11 independent studies (9 species) and found no significant bias in heritability based on estimation method. Further, because we only require that the ratio of $h_A/h_B$ approximates $H_A/H_B$, we need only assume that if $h$ changes across environment, $H$ changes proportionally. It is unclear how the ratio of additive and non-
additive genetic variation could change, if at all, across environments (Hoffman and Parsons, 1991). Therefore, in the absence of data specific to oysters, it is assumed in this study that \((H_A/H_B)\) is approximately equal to \((h_A/h_B)\).

3. RESULTS

3.1 Average environment yield, individual body weight and survival over time

Table 3.1 summarizes average temperature and salinity for the three measurements periods at subtidal environments in both Dabob and Yaquina Bays. Average water temperature over the entire experimental period was slightly higher in Yaquina Bay (mean temperature 13.4° C; range 6.2° C to 23.6° C) than in Dabob Bay (mean temperature 11.8° C; range 6.1° C to 23.0° C). Due to complications in retrieving data, salinity was only recorded over 84-87% of the growth trial at the Yaquina Bay sites and 39 to 54% of the growth trial at the Dabob Bay sites. The two environments differed dramatically in terms of average annual salinity and in seasonal salinity fluctuations. Yaquina Bay had an average salinity of 20.6‰ over the experimental period, ranging from short periods of 0‰ in the Winter to 35.6‰ during the Summer and Fall. Dabob Bay had an average salinity concentration of 28.3‰, ranging from 16.9‰ during the Winter and Spring to 32.7‰ during the summer. The total salinity fluctuation was much more dramatic in Yaquina Bay, including daily fluctuations due to tidal cycles and annual fluctuations between wet and dry seasons. Conversely, Dabob Bay showed little
tidal influence and much smaller fluctuations due to season. Water quality at Dabob Bay and Yaquina Bay likely differed in many other ways, however, a complete biotic and abiotic characterization of these two environments was beyond the scope of this study.

The primary difference between intertidal and subtidal culture environments within each body of water was stress from desiccation and extreme temperature fluctuations due to aerial exposure. In Yaquina Bay, oysters planted intertidally were exposed to air for approximately 11% of the 697 day growth trial, including short periods of freezing air temperatures in the winter of 2003/2004 and spikes in air temperatures up to 35°C in the summers of 2003 and 2004. Likewise, oysters planted intertidally in Dabob Bay were exposed to air for approximately 9% of the 664 day growth trial, including short periods of freezing air temperature in the winters of both 2002/2003 and 2003/2004 and spikes to 38° C in the summer of 2003 and 34° C in the summer of 2004.
Table 3.1. Average water temperature at the subtidal (depth ≈ 1 m) and intertidal (+ 0.3 m MLLW) within Dabob Bay, WA, and Yaquina Bay, OR, over the three measurement intervals. Salinity recorded subtidally in both embayments. Values in parentheses represent min/max range. Data recorded every 30 minutes during 84 to 87% of the field growth trial in Yaquina Bay and 39 to 54% of the field growth trial in Dabob Bay.

<table>
<thead>
<tr>
<th>Embayment</th>
<th>Parameter</th>
<th>0 to 192</th>
<th>193 to 370</th>
<th>370 to 664/697</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabob Bay, WA</td>
<td>Intertidal</td>
<td>9.80</td>
<td>14.72</td>
<td>10.61</td>
</tr>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>(-1.68/25.23)</td>
<td>(1.38/38.10)</td>
<td>(-4.73/28.24)</td>
</tr>
<tr>
<td></td>
<td>Subtidal</td>
<td>10.82</td>
<td>14.45</td>
<td>10.63</td>
</tr>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>(6.1/21.5)</td>
<td>(7.80-23.03)</td>
<td>(6.07-17.61)</td>
</tr>
<tr>
<td></td>
<td>Salinity (%)</td>
<td>30.0</td>
<td>25.7</td>
<td>27.2</td>
</tr>
<tr>
<td></td>
<td>(27.1/30.9)</td>
<td></td>
<td>(16.9-32.7)</td>
<td>(21.8-31.5)</td>
</tr>
<tr>
<td>Yaquina Bay, OR</td>
<td>Intertidal</td>
<td>10.22</td>
<td>13.87</td>
<td>11.70</td>
</tr>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>(3.08/16.74)</td>
<td>(6.35/35.92)</td>
<td>(-0.41/34.48)</td>
</tr>
<tr>
<td></td>
<td>Subtidal</td>
<td>10.93</td>
<td>16.29</td>
<td>13.14</td>
</tr>
<tr>
<td></td>
<td>Temperature (°C)</td>
<td>(7.44/18.35)</td>
<td>(8.98-23.61)</td>
<td>(6.21-23.10)</td>
</tr>
<tr>
<td></td>
<td>Salinity (%)</td>
<td>14.1</td>
<td>23.5</td>
<td>20.9</td>
</tr>
<tr>
<td></td>
<td>(0/28.9)</td>
<td></td>
<td>(0/34.2)</td>
<td>(0/35.6)</td>
</tr>
</tbody>
</table>
Figure 3.1 shows trends in average live oyster body weight, survival and yield over the entire experimental period at all four environments. Live body weight (g; Figure 3.1a) was primarily affected by degree of exposure (i.e. intertidal v. subtidal). Interestingly, average live body weight at harvest was almost identical between subtidal Yaquina Bay and subtidal Dabob Bay despite dissimilar salinity profiles. Survival (Figure 3.1b) at harvest was similar between environments with no clear pattern in the effects of exposure and/or watershed. Trends in survival over time were similar across all environments, as well, with a steep decline in survival during the first winter, followed by a leveling in the rate of mortality until harvest. The Dabob Bay intertidal site showed the highest mortality after the first measurement period, and although the rate in mortality declined at subsequent measurements, survival in this environment remained the lowest until harvest. Yield, by definition, is a combination of average live body weight and survival. With no substantial differences in average site survival, the general pattern in yield over time (Figure 3.1c) was more similar to that of individual body weight, with subtidal environments supporting higher yields than intertidal environments.
Figure 3.1. Average individual live body weight (g; A), survival (%; B), and yield (kg 100 seed\(^{-1}\); C) at all four experimental environments over the entire growout phase.
3.2 Analysis of variance of performance traits

3.2.1 P-values and variance components

Table 3.2 displays performance measures which required adjustment due to variation in initial plantout weight. This table also indicates the strength of association between the initial plantout weight and the performance trait in the field. For most traits the influence of initial weight diminished over time, having little or no affect by harvest. The only environment that required adjustment at harvest was subtidal Dabob Bay, where initial weight was positively correlated with body weight, survival and yield.

Due to variable survival in the nursery, not all families were planted at all four environments, resulting in empty cells and an unbalanced experimental design. Analyses performed with general linear models using Type III and Type IV sum of squares, as well as analyses performed with a balanced subset of the data, all showed similar results, suggesting the imbalance was not too severe. Consequently, results from analyses performed with Type III sum of squares, using all the data, are presented here. When all four environments were considered simultaneously, significant (P<0.016) effects of environment, block within environment, genotype, culture within genotype and genotype x environment were found for nearly all performance measures (Table 3.3). Block within environment did not affect survival at day 192 (P=0.279) and culture within genotype did not significantly affect individual body weight at day 370 (P=0.225).
Table 3.2. Correlation coefficients significantly greater than zero (P<0.05) between field performance traits and initial oyster body weight for all measurement periods at all sites. Dashes indicate no significant correlation (P>0.05).

<table>
<thead>
<tr>
<th>Environment</th>
<th>Day</th>
<th>Correlation coefficient (r)</th>
<th>Body weight (g)</th>
<th>Survival (%)</th>
<th>Yield (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabob Bay, intertidal</td>
<td>192</td>
<td>0.137</td>
<td>-</td>
<td>0.238</td>
<td>0.338</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>-</td>
<td>-</td>
<td>0.214</td>
<td>0.249</td>
</tr>
<tr>
<td></td>
<td>664</td>
<td>-</td>
<td>-</td>
<td>0.168</td>
<td>0.325</td>
</tr>
<tr>
<td>Dabob Bay, subtidal</td>
<td>192</td>
<td>0.252</td>
<td>0.238</td>
<td>0.352</td>
<td></td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>-</td>
<td>0.214</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>664</td>
<td>0.168</td>
<td>0.205</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yaquina Bay, intertidal</td>
<td>192</td>
<td>0.191</td>
<td>-</td>
<td>0.232</td>
<td></td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>697</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yaquina Bay, subtidal</td>
<td>192</td>
<td>0.310</td>
<td>-</td>
<td>0.352</td>
<td></td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>0.134</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>697</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.3. Significance of environment, block within environment, genotype, culture within genotype and genotype x environment interaction on performance traits measured at three time periods. Fraction of the total phenotypic variation explained by each variable ($\sigma^2/\sigma^2_P$) based on variance components computed using REML.

<table>
<thead>
<tr>
<th></th>
<th>Day 192</th>
<th></th>
<th>Day 370</th>
<th></th>
<th>Day 664/697</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body Weight</strong></td>
<td></td>
<td>$P$-value</td>
<td>$\sigma^2/\sigma^2_P$</td>
<td></td>
<td>$P$-value</td>
<td>$\sigma^2/\sigma^2_P$</td>
</tr>
<tr>
<td>Environment</td>
<td>3</td>
<td>&lt;0.01</td>
<td>0.82</td>
<td>&lt;0.01</td>
<td>0.882</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Block(Environment)</td>
<td>8</td>
<td>&lt;0.01</td>
<td>0.02</td>
<td>&lt;0.01</td>
<td>0.015</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Genotype</td>
<td>27</td>
<td>&lt;0.01</td>
<td>0.03</td>
<td>&lt;0.01</td>
<td>0.023</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Culture(Genotype)</td>
<td>24</td>
<td>0.016</td>
<td>0.01</td>
<td>0.225</td>
<td>0.001</td>
<td>0.015</td>
</tr>
<tr>
<td>Genotype x Env.</td>
<td>74</td>
<td>&lt;0.01</td>
<td>0.02</td>
<td>&lt;0.01</td>
<td>0.022</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Error</td>
<td>787</td>
<td>0.11</td>
<td></td>
<td>0.057</td>
<td></td>
<td>0.077</td>
</tr>
<tr>
<td><strong>Survival</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environment</td>
<td>3</td>
<td>&lt;0.01</td>
<td>0.177</td>
<td>&lt;0.01</td>
<td>0.188</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Block(Environment)</td>
<td>8</td>
<td>0.279</td>
<td>0.001</td>
<td>&lt;0.01</td>
<td>0.010</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Genotype</td>
<td>27</td>
<td>&lt;0.01</td>
<td>0.515</td>
<td>&lt;0.01</td>
<td>0.433</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Culture(Genotype)</td>
<td>24</td>
<td>&lt;0.01</td>
<td>0.065</td>
<td>&lt;0.01</td>
<td>0.044</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Genotype x Env.</td>
<td>74</td>
<td>&lt;0.01</td>
<td>0.039</td>
<td>&lt;0.01</td>
<td>0.045</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Error</td>
<td>786</td>
<td>0.203</td>
<td>0.279</td>
<td>0.320</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Yield</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Environment</td>
<td>3</td>
<td>&lt;0.01</td>
<td>0.707</td>
<td>&lt;0.01</td>
<td>0.687</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Block(Environment)</td>
<td>8</td>
<td>&lt;0.01</td>
<td>0.039</td>
<td>&lt;0.01</td>
<td>0.023</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Genotype</td>
<td>27</td>
<td>&lt;0.01</td>
<td>0.063</td>
<td>&lt;0.01</td>
<td>0.097</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Culture(Genotype)</td>
<td>24</td>
<td>&lt;0.01</td>
<td>0.013</td>
<td>&lt;0.01</td>
<td>0.014</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Genotype x Env.</td>
<td>74</td>
<td>&lt;0.01</td>
<td>0.047</td>
<td>&lt;0.01</td>
<td>0.040</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Error</td>
<td>787</td>
<td>0.131</td>
<td>0.139</td>
<td>0.174</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Variance components were estimated using restricted maximum likelihood (REML) and therefore resistant to biases due to unbalanced data (Sokal and Rohlf, 1995). Figure 3.2 shows that the fraction of total phenotypic variation each effect accounted for depended greatly on the character under consideration. Total variation in body weight was accounted for primarily by variation due to environment (79-88%) with smaller fractions accounted for by variation among crosses (2.3-5%) and genotype x environment interaction (1.5-3.4%). Culture within genotype accounted for the smallest fraction of variation in body weight (0.1-0.5%). No consistent effect of time was observed in the variance components of individual body weight. Total variation in survival, however, was accounted for largely by genotype (41-51%). Variation due to experimental error was much higher for this trait (20-32%), with a reduced effect of environment (14-19%). There was a weak trend in the effect of time on the magnitude of variance components, with a reduction in the amount of variation accounted for by genotype from 51.5% at day 192 to 41.7% at harvest, and an increase in the amount of variation attributable to experimental (random) error from 20.3% at day 192 to 32.0% at harvest. Total variation in yield was accounted for primarily by environment, which decreased from 71% at day 192 to 60% at harvest. Variation in yield attributable to genotype, however, increased from 6.3% at day 192 to 13.6% at harvest. Variation attributable to random error also increased over time from 13.1% at day 192 to 17.1% at harvest.
Figure 3.2. Fraction of total phenotypic variation in body weight (A), survival (B), and yield (C) explained by site, block within site, cross, culture within cross, site x cross interaction, and unexplained error. Variance components estimated using restricted maximum likelihood.
3.2.2 **Broad sense heritability of performance traits.**

Variance components for each trait at each site were used to estimate the broad sense heritability ($H^2$) for all measurement periods (Table 3.4). The $H^2$ of live body weight at harvest ranged from 0.456 to 0.554, with estimates at environments within Dabob Bay being slightly higher than those in Yaquina Bay. The $H^2$ of survival ranged from 0.357 (intertidal Dabob Bay) to 0.699 (intertidal Yaquina Bay); the $H^2$ estimates for the two subtidal sites were similar (0.523 v. 0.577). The $H^2$ of yield followed a similar trend as the $H^2$ of survival, with the highest $H^2$ at intertidal Yaquina Bay and the lowest at intertidal Dabob Bay, the subtidal sites were intermediate.

For all sites, the $H^2$ of body weight increased from day 192 ($H^2$=0.088-0.380) to harvest ($H^2$=0.456-0.554). With the exception of the subtidal Dabob Bay site, $H^2$ of survival deceased over time. The $H^2$ of yield remained unchanged or decreased slightly at the Dabob Bay sites and increased at the Yaquina Bay sites from day 192 to harvest.
Table 3.4. Broad-sense heritabilities ($H^2 \pm SE$) of performance traits for all measurement periods at all four environments. $H^2$ calculated as the fraction of genetic variation divided by the total phenotypic variation ($\sigma_g^2 / \sigma_p^2$). Standard errors estimated as per Becker (1996).

<table>
<thead>
<tr>
<th>Environment</th>
<th>Day</th>
<th>Body weight</th>
<th>Survival</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabob Intertidal</td>
<td>192</td>
<td>0.088 (0.052)</td>
<td>0.479 (0.084)</td>
<td>0.436 (0.085)</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>0.518 (0.083)</td>
<td>0.384 (0.084)</td>
<td>0.359 (0.083)</td>
</tr>
<tr>
<td></td>
<td>664</td>
<td>0.528 (0.083)</td>
<td>0.357 (0.083)</td>
<td>0.352 (0.083)</td>
</tr>
<tr>
<td>Dabob Subtidal</td>
<td>192</td>
<td>0.380 (0.087)</td>
<td>0.574 (0.083)</td>
<td>0.427 (0.088)</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>0.404 (0.088)</td>
<td>0.526 (0.086)</td>
<td>0.459 (0.088)</td>
</tr>
<tr>
<td></td>
<td>664</td>
<td>0.554 (0.085)</td>
<td>0.577 (0.083)</td>
<td>0.456 (0.088)</td>
</tr>
<tr>
<td>Yaquina Intertidal</td>
<td>192</td>
<td>0.287 (0.076)</td>
<td>0.784 (0.051)</td>
<td>0.527 (0.080)</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>0.476 (0.081)</td>
<td>0.748 (0.057)</td>
<td>0.636 (0.071)</td>
</tr>
<tr>
<td></td>
<td>697</td>
<td>0.456 (0.082)</td>
<td>0.699 (0.064)</td>
<td>0.644 (0.071)</td>
</tr>
<tr>
<td>Yaquina Subtidal</td>
<td>192</td>
<td>0.222 (0.070)</td>
<td>0.641 (0.072)</td>
<td>0.351 (0.081)</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>0.450 (0.082)</td>
<td>0.548 (0.079)</td>
<td>0.395 (0.082)</td>
</tr>
<tr>
<td></td>
<td>697</td>
<td>0.466 (0.083)</td>
<td>0.523 (0.081)</td>
<td>0.404 (0.082)</td>
</tr>
</tbody>
</table>
3.3 Phenotypic correlation.

Pair-wise phenotypic correlations between environments for all characters at harvest are shown in Table 3.5. All correlations were significantly larger than 0 (P<0.03) and significantly less than 1 (P<0.01). The highest correlations among yields (\( r_p = 0.812 \)) as well as survival (\( r_p = 0.814 \)) were found between the two environments located in Yaquina Bay. In addition, the lowest correlations for both yield (\( r_p = 0.430 \)) and survival (\( r_p = 0.471 \)) were found between the subtidal Yaquina Bay site and the intertidal Dabob site. The highest correlation in body weight was found between the two environments within Dabob Bay (\( r_p = 0.681 \)). The lowest correlation in body weight was found between the intertidal Yaquina site and the subtidal Dabob site (\( r_p = 0.464 \)).

Phenotypic correlations among individual body weight, survival and yield at harvest within each environment are shown in Table 3.6. In all cases there was a significant positive correlation between survival and yield (\( r_p = 0.595 \) to 0.835). Body weight, however, was only significantly correlated with yield at the intertidal Yaquina site (\( r_p = -0.442; P = 0.047 \)). There was a tendency for the correlations between harvest body weight and harvest survival within each environment to be negatively correlated, although only significant in one environment (intertidal Yaquina Bay, \( r_p = -0.592, P = 0.006 \)).
Table 3.5. Correlation coefficients ($r_p$) of average genotype performance between environments for harvest yield, live body weight and survival. Correlation coefficients and standard errors (in parentheses) were determined using bootstrap sampling (10,000 samples, Phillips, 2001). All correlation coefficients were significantly greater than 0 ($P<0.05$) and significantly less than 1 ($P<0.05$).

<table>
<thead>
<tr>
<th>Environment</th>
<th>Dabob Subtidal</th>
<th>Yaquina Intertidal</th>
<th>Yaquina Subtidal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live body weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dabob Intertidal</td>
<td>0.681</td>
<td>0.581</td>
<td>0.595</td>
</tr>
<tr>
<td></td>
<td>(0.106)</td>
<td>(0.098)</td>
<td>(0.123)</td>
</tr>
<tr>
<td>Dabob Subtidal</td>
<td>0.464</td>
<td>0.494</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.131)</td>
<td>(0.150)</td>
<td></td>
</tr>
<tr>
<td>Yaquina Intertidal</td>
<td>0.527</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.136)</td>
<td></td>
</tr>
<tr>
<td>Survival</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dabob Intertidal</td>
<td>0.667</td>
<td>0.620</td>
<td>0.471</td>
</tr>
<tr>
<td></td>
<td>(0.140)</td>
<td>(0.161)</td>
<td>(0.155)</td>
</tr>
<tr>
<td>Dabob Subtidal</td>
<td>0.793</td>
<td>0.718</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.089)</td>
<td>(0.097)</td>
<td></td>
</tr>
<tr>
<td>Yaquina Intertidal</td>
<td>0.814</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.058)</td>
<td></td>
</tr>
<tr>
<td>Yield</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dabob Intertidal</td>
<td>0.657</td>
<td>0.641</td>
<td>0.432</td>
</tr>
<tr>
<td></td>
<td>(0.108)</td>
<td>(0.143)</td>
<td>(0.162)</td>
</tr>
<tr>
<td>Dabob Subtidal</td>
<td>0.693</td>
<td>0.541</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(0.115)</td>
<td>(0.166)</td>
<td></td>
</tr>
<tr>
<td>Yaquina Intertidal</td>
<td>0.812</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.071)</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.6. Correlation coefficients ($r_p$) of average genotype performance between harvest yield, live body weight and survival within all environments. Correlation coefficient determined using bootstrap sampling (10,000 samples, Phillips, 2001). Standard error estimates in parentheses. Asterisks indicate correlation coefficients differ from zero at the $P<0.05$ (*) and $P<0.01$ (**) levels of significance.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Yield (kg bag$^{-1}$)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dabob Intertidal</td>
<td>Live body weight (g)</td>
<td>-0.039 (0.168)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Survival (%)</td>
</tr>
<tr>
<td>Dabob Subtidal</td>
<td>Live body weight (g)</td>
<td>0.157 (0.156)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Survival (%)</td>
</tr>
<tr>
<td>Yaquina Intertidal</td>
<td>Live body weight (g)</td>
<td>-0.442 (0.176)*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Survival (%)</td>
</tr>
<tr>
<td>Yaquina Subtidal</td>
<td>Live body weight (g)</td>
<td>-0.091 (0.156)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Survival (%)</td>
</tr>
</tbody>
</table>
3.4 Expected efficiency of correlated response.

Table 3.7 shows both expected pair-specific efficiencies as well as the expected average efficiency of indirect selection at one environment compared to the response of direct selection at all other environments. For yield, the most efficient environment for selection is expected to be intertidal Yaquina Bay, where, on average, indirect selection for improved yield at the remaining three environments considered in this study was 90.5% as efficient compared to direct selection at all environments separately (range CR/R: 0.825-1.025). Based on these results, the intertidal Yaquina Bay site is also expected to be the most efficient environment for the selection of survival which was 89.4% as efficient compared to direct selection at all other environments separately (range CR/R: 0.869-0.941). Expected efficiencies of indirect selection for body weight were lower than for yield or survival, with the most efficient environment of selection being the intertidal Dabob Bay site which was 64.1% as efficient compared to direct selection at all other environments separately (range CR/R: 0.625-0.665). Although, intertidal Dabob Bay site was expected to be the most efficient environment for indirectly selecting for individual body weight, it was the worst environment for indirectly selecting both survival (average CR/R = 0.453) and yield (average CR/R = 0.484).
Table 3.7. Efficiency of indirect selection versus direct selection based on pair-wise comparisons of environments for live body weight, survival, and yield at harvest. Selection site means represent the average efficiency of indirect selection compared to direct selection all other environments.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Target Environment</th>
<th>Dabob Intertidal (DI)</th>
<th>Dabob Subtidal (DS)</th>
<th>Yaquina Intertidal (YI)</th>
<th>Yaquina Subtidal (YS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live body weight</td>
<td>DI</td>
<td>0.698</td>
<td>0.540</td>
<td>0.559</td>
<td></td>
</tr>
<tr>
<td></td>
<td>DS</td>
<td>0.665</td>
<td>0.421</td>
<td>0.453</td>
<td></td>
</tr>
<tr>
<td></td>
<td>YI</td>
<td>0.625</td>
<td>0.511</td>
<td>0.533</td>
<td></td>
</tr>
<tr>
<td></td>
<td>YS</td>
<td>0.633</td>
<td>0.539</td>
<td>0.521</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>0.641</td>
<td>0.583</td>
<td>0.494</td>
<td>0.515</td>
</tr>
<tr>
<td>Survival</td>
<td>DI</td>
<td></td>
<td>0.848</td>
<td>0.869</td>
<td>0.570</td>
</tr>
<tr>
<td></td>
<td>DS</td>
<td></td>
<td>0.525</td>
<td>0.872</td>
<td>0.684</td>
</tr>
<tr>
<td></td>
<td>YI</td>
<td></td>
<td>0.444</td>
<td>0.720</td>
<td>0.704</td>
</tr>
<tr>
<td></td>
<td>YS</td>
<td></td>
<td>0.389</td>
<td>0.754</td>
<td>0.941</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>0.453</td>
<td>0.774</td>
<td>0.894</td>
</tr>
<tr>
<td>Yield</td>
<td>DI</td>
<td></td>
<td>0.749</td>
<td>0.867</td>
<td>0.461</td>
</tr>
<tr>
<td></td>
<td>DS</td>
<td></td>
<td>0.578</td>
<td>0.824</td>
<td>0.509</td>
</tr>
<tr>
<td></td>
<td>YI</td>
<td></td>
<td>0.474</td>
<td>0.583</td>
<td>0.643</td>
</tr>
<tr>
<td></td>
<td>YS</td>
<td></td>
<td>0.401</td>
<td>0.575</td>
<td>1.025</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td></td>
<td>0.484</td>
<td>0.636</td>
<td>0.905</td>
</tr>
</tbody>
</table>
4. DISCUSSION

4.1 Experimental environments

Environments were chosen for this experiment to include four very different, but not uncommon, oyster growing environments in the Pacific Northwest. Not surprisingly, oysters sibling groups cultured at subtidal environments had heavier average individual weights and were higher yielding than sibling cultured in intertidal environments. In addition, blocks lower in the intertidal zone tended to have heavier individuals and higher yielding replicates. Similar effects of exposure on the growth of intertidal *C. gigas* have been reported by other authors (Quayle, 1988; Langdon et al., 2003; Evans et al., 2004). Salinity has also been shown to affect oyster growth. Brown and Hartwick (1988), reported slower growth of *C. gigas* in areas with low salinity (<20 %o) compared to areas with higher salinity. Controlled studies have shown reduced growth when salinity is too low (<20 %o) or too high (>40 %o) for both larval and adult bivalves (Newkirk, 1978; review by Shumway, 1996; Taylor et al., 2004). In the present study the effect of salinity is confounded with water temperature and watershed (which may include food quality and abundance), such that the differences (or similarities) in the oyster performance between Dabob Bay and Yaquina Bay can not be attributed to a single factor (such as salinity).
Survival followed a similar pattern over time at all environments, with high mortality occurring during the first measurement interval (days 0 to 192), followed by little additional mortality. High mortality rates among small or young oysters have been reported by several authors (Agius et al., 1978; Brown and Hartwick, 1988), especially when the oysters are planted out late in the year (Mallet and Haley, 1983). The effect of aerial exposure on oyster survival was not as clear as the effect of exposure on body weight. In Yaquina Bay, survival was highest at the intertidal environment, while in Dabob Bay survival was highest at the subtidal environment. Differences between intertidal and subtidal environments in Yaquina Bay first occurred between days 192 and 370. It is unclear why the Yaquina subtidal site experienced higher mortality than the intertidal site but may be, in part, mortality associated with increased productivity of the subtidal environment (Glude, 1975). Beattie et al. (1980) observed reduced morality of C. gigas planted higher in the intertidal zone compared to siblings planted lower in the intertidal zone. Littlewood (1988) was unable to explain increased mortality observed in subtidally versus intertidally cultured C. rhizophorae. Differences between intertidal and subtidal environment in Dabob Bay first occurred between days 0 and 192. The initial drop in survival at the intertidal Dabob Bay site may have been due to the fact that the animals were still quite small when they experienced freezing air temperatures during Winter 2002/2003. The effects of freezing air temperature during Winter 2003/2004, may have been reduced due to the larger size of the oysters at this time (Askew, 1972; Brown and Hartwick, 1988). The thermal buffering of seawater resulted in oysters cultured at the subtidal Dabob site never experiencing temperatures below 6° C.
Again, inferences regarding the effect of salinity on survival based on comparisons between Yaquina and Dabob Bay suffer the same problems with confounding variables as discussed with body weight (above). However, Taylor et al. (2004) reported a similar trend for the pearl oyster *Pinctada maxima* with high (>40%) and low (<20%) salinity significantly affecting growth but not survival. It is worth noting that the trends in survival observed in the present study suggest that a “summer mortality” event did not occur. These events are characterized by catastrophic mortality of animals during periods of high productivity and elevated water temperatures (Glude, 1975; Beattie et al., 1980).

Trends in average yield at each environment were, by definition, a combination of body weight and survival. The similarity in average survival between all four environments (50-65%), resulted in the trends of yield over time closely following the trends observed in body weight.

4.2 Components of total phenotypic variation across all environments

The importance of environment, block within environment, genotype, culture within genotype, genotype x environment interaction, and unexplained error differed greatly depending on the character. The majority of variation in live body weight was explained by variation among environments (79-88%). Conversely, the majority of the variation in survival across all four environments was explained primarily by genotype (41-51%).
Mallet and Haley (1983) found growth rate in *C. virginica* was largely determined by environment and survival largely determined by genotype. These results underscore the importance of both locating farms in environments which allow for maximal growth and utilizing high-surviving oyster genotypes. This does not discount the need for selective breeding for growth, as there is still considerable genetic variation for body weight at harvest within each environment.

Trait heritability was determined within each environment, excluding variation due to environment and block within environment (considered controllable sources of variation and easily accounted for in most experimental designs used by breeders). Estimates of $H^2$ were high and similar to published estimates for Pacific oyster adult body weight at 18 months ($H^2=0.33 \pm 0.19$; Lannan, 1972) and yield after two growing seasons in the field ($H^2=0.36$ to 0.61; Langdon, 2003). The generally high $H^2$ estimates for all traits indicate that these performance characters are under a high degree of genetic control. Based on a literature review, Hoffman and Merila (1999) concluded that there is no consistent effect of environmental quality on the heritability of traits. Indeed, in the present study, only survival showed a positive relationship between $H^2$ and phenotypic value (i.e. as average site survival increase, so did the heritability of survival). The $H^2$ of body remained fairly stable across environment (from 0.46 to 0.55), even as average site harvest body weight rose from 46 g to 105 g.

Culture within genotype represents the amount of variation attributable to nursery
environment (i.e. variation among larval tanks and upwellers). Although often statistically significant, the total amount of variation explained by this term was small for all characters (0.1-6.5%) and was always less than the variation due to genotype (approximately 1/5). These results suggest that, under the protocol used here, there is little need to replicate crosses within the nursery in order to reduce error in field performance estimates owing to variation in nursery growing environment, especially when resources in the nursery culture are limited.

The effect of genotype x environment interaction was significant in all cases. However, variation due to GxE interactions (2.0-6.2%) was always less than variation due to genotype (2.3-51.5%). Further, $r_p$ of performance traits for all pair-wise combination of environments were both significantly greater than 0 and significantly less than 1, suggesting that some, but not all, of the genes responsible for determining phenotypic expression of performance traits were shared between environments.

GxE interactions are common in many plant and animals species (e.g. Haldane, 1947; Falconer, 1952; Baker, 1987; Hallauer, 1987; Romagosa and Fox, 1993), and, according to Price and Schluter (1991), “should be expected to be present virtually whenever selection pressures vary with the environment”. DeLacy et al. (1990) summarized data from over 100 multi-location terrestrial crop variety trials and reported variability (expressed as fraction of total sum of squares) due to GxE was almost always greater than the variability due to genotype alone. Research on several finfish species such as *Salmo*
solar (Refstie, 1990), Salmo alpinus (Nilsson et al., 1990), and Onchyrynchis mykiss (=Salmo gairdneri, Iwamoto et al., 1986; Fishback et al., 2002), suggest that GxE interactions are not a concern and account for a small fraction of the total phenotypic variation. The absence of GxE interactions for these species may be in part, due to relatively high degree of control over the growing environment, thereby reducing the overall environmental variation. GxE interactions appear to be more common in cultured shellfish, which may reflect the poor degree of control over shellfish growing environments. Newkirk (1978) found significant GxE interaction among Crassostrea virginica larvae from four populations and their hybrids cultured at four different salinities. Mallet and Haley (1983) reported a significant GxE interaction on the growth rate among five genetically distinct groups of C. virginica planted out at two environments in New Brunswick. Similarly, Mallet et al. (1986) found the correlation among Mytilus edulis family growth rates across two environments to be significantly greater than 0 (r=0.58, P=0.05), however, the correlation among average family survival across environments was not different from 0. Rawson and Hilbish (1991) found significant rank changes in growth rate of the hard clam, Mercenaria mercenaria, among five locationas along the Atlantic Coast of the United States, with no genotype being ubiquitously superior at all environments. More recently, Langdon et al. (2003) found that yields of Pacific oyster (Crassostrea gigas) families were significantly affected by GxE interactions (P<0.001). The positive correlation of mean family yield across environments [r from 0.30 (ns) to 0.35 (P=0.04)] allowed the identification of several broadly adapted families ("generalists").
4.3 Effects of GxE interactions on oyster breeding in the Pacific Northwest

The ability to develop broadly-adapted strains will depend, in part, on the strength of the GxE interaction acting on the character of interest. Results presented here suggest that the effects GxE interactions on body weight, survival, and yield are not large enough to prevent selection in a limited number of well chosen environments from resulting in favorable correlated gains in other environments in the Pacific Northwest. However, it is essential that the selection environment 1. show a positive genetic correlation with all production environments, and 2. allow genotypes to be easily discriminated (i.e. high $h^2$ or $H^2$; Falconer, 1952; Weber and Wrick, 1990).

The average expected efficiency of a particular environment in which to conduct indirect selection for improvement of yield at the other three environments in this study (Table 7) ranged from 48% (selection occurring at intertidal Dabob Bay) to 91% (selection occurring at intertidal Yaquina Bay). The low efficiency of intertidal Dabob Bay was, in part, due to the relatively low $H^2$ for yield at that environment (0.35) and the poor $r_p$ with other environments (0.432 to 0.657). The most efficient environment for indirect selection for yield was intertidal Yaquina Bay, which had the highest $H^2$ for yield (0.64) and relatively high $r_p$ with the other environments (0.641 to 0.812). Interestingly, it was found that indirect selection at the intertidal Yaquina environment targeting improved harvest yield at the subtidal Yaquina site is expected be more effective than direct
selection at the subtidal Yaquina site (i.e. $r_p H^2_{Y1} > H^2_{Ys}$). Indirect selection is generally found to be less effective than direct selection (Falconer and Mackay, 1996), but occurred in this case due to the high $r_p$ and the large disparity in $H^2$ of yield between the two environments.

On average, the correlation of average genotype body weight across environments was lower than for survival and yield. Consequently, indirect selection of body weight across environments was also less efficient. The most efficient environment for indirectly selecting for body weight was the intertidal Dabob Bay site (average CR/R = 0.641), which was the least efficient environment to indirectly select for yield (average CR/R = 0.484). From an applied standpoint, the low efficiency of indirect selection targeting body weight suggests that GxE interactions affect body weight to a larger degree than survival or yield. Environments with high average efficiency to indirectly select for yield also tended to be efficient for indirectly selecting for survival, due to the high phenotypic correlation between the two traits. Although it is tempting to conclude that intertidal Yaquina Bay is the single best environment in which to conduct selection to improve oyster yield throughout the Pacific Northwest, consideration should be given to how correlations between sites change over time. Marine and estuarine environments are known to fluctuate due to oceanic and local perturbations on an annual or decadal basis (e.g. Cloern and Jassby, 1994). Although terrestrial plant and animal breeders use genotype x location (as measured here) as an approximation for the effects of genotype x time (Romagosa and Fox, 1993), it is unclear how sporadic events, such as “summer
mortality" (Glude, 1975; Beattie, 1980) will effect the correlation between performance traits across sites.

Statistically significant genotype x environment interactions were found for all Pacific oyster performance characters, however, the amount of total phenotypic variation that they accounted for was relatively small. Results from this study suggest oyster genotypes can be identified that display high performance across a wide range of growing environments typical of the Pacific Northwest. In addition, these results suggest that genetic improvement of broadly adapted Pacific oyster strains is possible using a limited number of well selected evaluation environments. The breeder must determine if a reduction in selection efficiency due to indirect selection can be offset by the reduction of resources (e.g. time, labor, money, etc.) needed to evaluate genotypes in a limited number of environments. If the reduction in selection efficiency is unacceptable, locally adapted strains may have to be developed via direct selection in each unique environment.
5. REFERENCES


CHAPTER 4

DIRECT AND CORRELATED RESPONSES TO SELECTION FOR
INDIVIDUAL BODY WEIGHT IN THE PACIFIC OYSTER (Crassostrea gigas).

ABSTRACT

Three experiments were performed to examine the heritability of body weight among adult Pacific oysters evaluated in Yaquina Bay, OR, USA, and to determine if selection for individual body weight will result in correlated responses in survival and yield. The first two experiments utilized midparent-offspring regressions to estimate the heritability of adult oyster body weight and the significance and sign of correlated responses. In Experiment 1 both parents and offspring were evaluated in an “upriver” environment in Yaquina Bay. In Experiment 2 parents were evaluated in a “downriver” environment, while offspring were evaluated in an “upriver” environment. Experiment 3 was designed to contrast body weight, survival, and yield of offspring (evaluated upriver) derived from three large sires and three small sires mated to the same five females (all parents evaluated downriver). Twelve full-sib families were evaluated in Experiment 1, 19 families evaluated in Experiment 2, and 26 families evaluated in Experiment 3. In Spring 2002, 50 offspring from each family were stocked into each of ten replicate lantern net tiers and deployed subtidally in Yaquina Bay. Measurements of yield (kg tier⁻¹), individual body weight (g), and survival (%) were recorded after one and two growing seasons in the field. Heritability estimates for adult body weight ranged from 0.33 (± 0.12) in Experiment 1 to –0.01 (± 0.17) in Experiment 2. Contrasts in Experiment 3 found adult offspring derived from large sires were not significantly larger than offspring
from small sires (P=0.56). These results suggest that adult oyster body weight may be sufficiently affected by GxE interactions to render selection in the downriver Yaquina environment ineffective at improving body weight in the upriver environment. Significant correlated responses to selection were observed. A strong negative correlation was found between parental body weight and offspring survival (r<-0.43, P<0.01) and between parental body weight and offspring yield (r<-0.29, P<0.01). Contrasts in Experiment 3 found offspring derived from large sires to have significantly lower survival (P<0.01) and yield (P<0.01) than offspring derived from small sires. Although offspring were only measured in a single growing environment, caution should be exercised when selection is performed on adult oyster body weight to indirectly improve yield.

1. INTRODUCTION

Shellfish growers along the west coast of the United States have expressed interest in developing high-yielding strains of oysters through selective breeding (Hedgecock et al., 1997). Yield is defined as “meat production in relation to amount (number) of seed planted” (Quayle, 1988), and therefore a function of individual body weight and survival. One strategy to increase shellfish production is to increase body weight. Consequently, considerable research has been performed to determine if adult shellfish body weight can be improved through selective breeding. The heritability of growth traits (including body weight and growth rate) has been estimated for a wide variety of shellfish species such as
clams (Rawson and Hilbish, 1990; Hadley et al., 1991), mussels (Mallet et al., 1986; Stromgren and Nielsen, 1989), and scallops (Ibarra, 1999; Perez and Alfonsi, 1999). Heritability of growth traits has also been reported for oysters such as *Ostrea edulis* (Newkirk and Haley, 1983; Toro and Newkirk, 1990), *O. chilensis* (Toro et al., 1994), *Saccostrea commercialis* (Nell, 1996; Nell et al., 1999), *S. cucullata* (Jarayabhand and Thavornyutikarn, 1995), and *Crassostrea virginica* (Haley and Newkirk, 1982; Paynter and Dimichele, 1990).

Pacific oysters (*Crassostrea gigas*) account for over 90% of the oyster production in the Pacific Northwest of the United States (Chew and Toba, 2001). Only two estimates of heritability for body/meat weight exist for this species. Lannan (1972) reported the broadsense heritability of Pacific oyster whole body weight at 18 months of age to be 0.33 ± 0.19 (± 1 SE). This estimate, however, may be biased upward due to the inclusion of non-additive genetic effects. Hedgecock et al. (1991) estimated the narrow-sense heritability of meat weight of Pacific oysters at harvest size to be approximately 0.20, unfortunately, variance around this estimate was not reported.

Breeders also need to be aware of changes in characters that are genetically correlated to the character under selection (Falconer and Mackay, 1996; Bourdon, 2000). Selection for increased body weight will result in increased yield as long as body weight and survival are not negatively correlated. Few authors have considered the correlated response in survival when selection is applied to improve shellfish body weight or growth rate (but
In this study, three sets of experimental crosses were produced concurrently to address two primary questions: 1. What is the heritability of body weight among adult Pacific oysters evaluated in Yaquina Bay, OR?; and 2. Will selection for individual body weight result in a correlated response in average offspring survival or yield. The first two experiments utilized midparent-offspring regressions to estimate the heritability of individual oyster body weight and the significance and sign of correlated responses. These two experiments differed in the source population of the parents. The third experiment was designed to contrast body weight, survival, and yield of offspring derived from three large and three small sires mated to the same five females.

2. METHODS

2.1 Selection of parents

Individually tagged adult oysters from unselected pedigreed families were collected from lantern nets suspended at two locations within Yaquina Bay, OR (44.6° N, 124.1° W) during Summer 2001. The first site was located approximately 3 km from the mouth of Yaquina Bay ("downriver"), the second site was located approximately 15 km up the Yaquina River at a commercial shellfish lease-site ("upriver"). Body weight of all tagged
oysters were periodically measured from plant-out in Spring 1998 until harvest in Summer 2001 as a continuation of research initiated by Brooks (2000). No significant family x site interaction effect on average parental family body weight was found between the upriver and downriver sites after two growing seasons in the field (Spring 1998 –Winter 1999), however, a significant site effect was found (unpublished data). Assortative, pair-wise crosses were made based on live adult body weight after 700 days in the field within upriver broodstock (“Experiment 1”) and within downriver broodstock (“Experiment 2”). Crosses among full-siblings were avoided. An additional set of families were created by crossing the three largest males (147g mean live body weight after 700 days in the field) and the three smallest males (68 g mean live body weight after 700 days in the field) from the downriver population with five females from the downriver population (i.e. 6 male x 5 female factorial cross; “Experiment 3”). Dams in Experiment 3 were randomly selected with the only restriction being that they possessed a sufficient number of eggs to contribute to six crosses. Crosses conducted in Experiment 3 are shown in Table 4.1.
Table 4.1. Mating design for Experiment 3. Three large sires and three small sires were crossed with the same five dams, resulting in 30 full-sib families.

<table>
<thead>
<tr>
<th>Dam</th>
<th>Live body weight (g)</th>
<th>Small Sires</th>
<th>Large Sires</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sire 1</td>
<td>Sire 2</td>
</tr>
<tr>
<td>1</td>
<td>144.01</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>2</td>
<td>147.58</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>3</td>
<td>148.57</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>4</td>
<td>155.16</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>5</td>
<td>172.46</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>
2.2 Hatchery and nursery protocol

Broodstock were held in 18° C sand-filtered seawater and fed a mixture of *Isocrysis galbana* (Iso) and *Cheatoceros calcitrans* (Cc) at a concentration of approximately 50,000 to 80,000 cells ml⁻¹ until ready to spawn. Animals were stripped-spawned as per Langdon et al. (2003) in Fall 2001. Fertilized eggs were allowed to develop into veliger larvae (D-larvae) for 24 hours in cross-specific 20-l containers filled with 25° C, 0.2 μm filtered seawater. D-larvae from each cross were then stocked into family-specific 100-l larval culture containers at a concentration of 10 larvae ml⁻¹. Larvae were fed daily with a mixture of Iso and Cc at concentrations ranging from 30,000 to 80,000 cells ml⁻¹, depending on age (Breese and Malouf, 1975). Larval tanks were drained and re-filled with 25° C 0.2 μm filtered seawater twice per week. During water changes, larvae were retained on 37 μm sieves for the first week. During the second week larvae were retained on 80 μm sieves and stocking densities reduced to 1 larva ml⁻¹. During the third week, larval cultures were drained through 243 μm and 80 μm sieves. All larvae retained on 243 μm sieves were exposed to 2×10⁻⁴ M epinephrine in order to induce metamorphosis (Coon, 1986). In total, 12 crosses were made for Experiment 1, 23 crosses made for Experiment 2 and 30 crosses made for Experiment 3.

Successfully metamorphosed spat were transferred to family-specific 15-cm diameter upwellers. Upwellers were held in a semi-recirculating system which received
approximately 6 exchanges d\(^{-1}\) of 25°C UV-irradiated 1 μm filtered seawater. Once all larvae had metamorphosed (approximately 4 weeks post harvest), the number of spat per upweller was randomly thinned to 10,000. Spat were allowed to grow until retained on a 1.4 mm sieve, then transferred to family-specific 28-cm diameter upwellers in a larger upwelling system. These larger upwellers were supplied with 18°C 1 μm filtered seawater delivered at approximately 2.8 l min\(^{-1}\) and fed an Iso/Cc mixture at a final concentration of approximately 50,000 to 80,000 cells ml\(^{-1}\). Once all animals were transferred from the 15-cm upwellers, the number of oysters per 28-cm upweller was randomly thinned to 5,000. Oysters were allowed to grow until retained on a 6.4 mm sieve, before being transferred to family-specific spat bags (2 mm mesh) held in storage tanks. Storage tanks received ambient 1 μm filtered seawater (mean 9.5°C; range 7.0°C - 13.68°C) and batch-fed to a final concentration of approximately 100,000 cell ml\(^{-1}\) of a Cc/Iso mixture for 8 h, twice per week. The reduced temperature and limited feeding was intended to slow oyster growth, minimizing variation in spat weight within (due in part to variable setting dates) and between cultures prior to planting in the field (Langdon et al., 2003). After approximately 80% of the oysters were sieved from the 28-cm upwellers, spat in the storage tank were counted and weighed for subsequent planting in the field.

Due to variable performance in the nursery, some crosses did not produce enough spat to plant-out and evaluate in the field. All crosses made for Experiment 1 (12), 19 of 23
crosses made for Experiment 2 and 26 of 30 crosses made for Experiment 3 were evaluated in the field.

2.3 Field trials

In Spring 2002, 50 randomly selected oysters from each family were weighed and stocked into each of two replicate compartments in each of five vertical blocks in ten-tier lantern nets (0.3-m diameter, 2 mm mesh) for a total of 10 replicates per family. Blocking was conducted to account for variation in performance traits due to water depth (e.g. Langdon et al., 2003). Variable survival in the nursery resulted in some families having fewer than desired individuals, and therefore less than ten replicates. Stocked lantern nets were suspended from a raft at the upriver field site. In January of 2003 (317 days in the field) all live juvenile oysters from each replicate were cleaned of biotic and abiotic fouling, counted, and the collective weight of all live animals measured to the nearest gram. After measurements were taken, oysters were stocked into 0.5-m diameter lantern nets (5 mm mesh), and re-suspended at the upriver site. These measurements were recorded again in Fall 2003 on adult oysters (after 640 days in the field). The collected data allowed for replicated estimates of average family bag weight (kg replicate\(^1\)) and survival (%) in the field. Average individual oyster weight per replicate was calculated by dividing the total bag weight by the number of live oysters. StowAway data loggers (Onset Corp, MA, USA) recorded temperature every 2 h at a depth of approximately 1 m for the duration of the 640-day field trial.
2.4 Data analysis

Unless otherwise stated all statistical analyses were performed using SAS statistical software (SAS Institute, 2000, Cary, NC, USA). Normal probability plots and residual plots were used to assess normality and equality of variance for all performance measures in Experiments 1, 2, and 3. Log or arcsine transformations were applied to non-normal data sets.

2.4.1 Adjustment for variation in initial weight

Due to variable spat performance in the nursery, average individual plant-out weight per family from all three experiments ranged from 0.403 to 0.664 g. To account for possible bias due to variation in plant-out body weight, field performance measures from all three experiments were regressed against initial body weight. If a significant relationship was present, field performance values for all replicate units were adjusted along the slope to a common plant-out weight (e.g. Rawson and Hilbish, 1990; Langdon et al., 2003). These adjusted values were used in all subsequent analyses.

2.4.2 Standardized Selection Differential (SSD)

Body weights of all parents after 700 days in the field were standardized within either upriver (Experiment 1) or downriver (Experiment 2 and 3) populations. The standardized
selection differential (SSD) within each population was calculated as per Newkirk and Haley (1983):

$$SSD = \frac{(W - \bar{W}_P)}{SD_P}$$

where $W$ is the weight of a given sire or dam, $\bar{W}_P$ is the average body weight of both males and females in either the upriver or downriver parental population (including individuals not selected as parents) and $SD_P$ is the standard deviation of either the upriver or downriver parental population. Midparent SSD was calculated as the average SSD of the appropriate sire and dam. No attempt was made to standardize sires and dams separately, using sex-specific average weights and standard deviations, due to uncertainty of each animal’s sex after 700 days in the field (the age at which selection was applied; also see Toro and Newkirk, 1990). No block affect was detected in parental body weight after 700 days in the field, and therefore was not corrected for in the estimate of SSD.

An alternative method of computing SSD uses selection differentials standardized not only within population (upriver versus downriver) but also within sex. Results from the two methods were similar, and for ease of interpretation, only the method that standardizes parental performance without regard to sex is presented here.

2.4.3 Standardized Response Differential (SRD)
All offspring performance measures (i.e. body weight, survival and yield) were also standardized and referred to as the standardized response differential (SRD). SRD's were computed as (Newkirk and Haley, 1983):

$$SRD = \frac{(X_i - \bar{X}_i)}{SD_i}$$

where $X_i$ is the performance measure of a replicate lantern net compartment in the $i^{th}$ block, $\bar{X}_i$ is the mean performance measure of the $i^{th}$ block, and $SD_i$ is the standard deviation of that performance measure in the $i^{th}$ block. As a result, the mean SRD within each block was zero. All means and standard deviations were computed using data pooled from all three experimental crosses. The use of SSD and SRD ensured the variances in performance measures are consistent across generation (Falconer and Mackay, 1996) as well as allowing for a unit-less measure of all performance traits.

2.4.4 Heritability of individual body weight

Midparent-offspring regressions were used to estimate the narrow sense heritability of oyster body weight after two growing seasons in the field (Falconer and Mackay, 1996; Lynch and Walsh, 2000). Due to variable numbers of replicates per family in the field, the slope and standard error of the midparent-offspring regression of SRD on SSD for average individual body weight were computed as per Falconer (1963) for Experiment 1 and Experiment 2. The slope of the midparent-offspring regression was interpreted as the
narrow sense heritability of adult oyster body weight (Falconer and Mackay, 1996). T-tests were performed to determine if the slopes were significantly greater or less than 0 (Sokal and Rohlf, 1995). In addition, the sign and significance of correlations between parent and offspring body weight was determined.

In Experiment 3, analysis of variance and linear contrasts determined if SRDs of adult body weight differed significantly between offspring of large sires and offspring of small sires. The fixed-effects model included sire, dam and sire x dam interaction. Block effects were not included in the model because the mean SRD was zero for all blocks. Some sire-dam combinations failed to produce enough spat to plant out into the field, resulting in an unbalanced statistical design. Balance was achieved by excluding crosses from one female and one large sire (a total of 6 families) from the analysis. Contrasts were then performed between offspring of three small sires and two large sires, with each sire being crossed to the same four dams (i.e. 5 males x 4 females factorial).

2.4.5 Correlated response to selection

The sign and significance of the correlation (r) between adult parental body weight after 700 days in the field and juvenile offspring body weight after 317 days in the field were determined separately for Experiments 1 and 2. Correlations were also performed between adult parental body weight and offspring survival and yield as both juveniles (317 days in the field) and adults (640 days in the field).
The regression slope \((b)\) of adult midparent body weight SSD on juvenile offspring body weight SRD was also computed. The regression slopes \((b)\) of adult midparent body weight SSD on offspring SRD for survival and yield, as both juveniles and adults, were also computed for Experiments 1 and 2. All slopes were again weighted as per Falconer (1963) to account for unequal number of replicates per family. T-tests were performed to determine if the slopes were significantly greater or less than 0 (Sokal and Rohlf, 1995). The slope computed here is interpreted as the change in offspring performance in standard deviation units (as either juveniles or adults) per unit change in adult midparent body weight.

The correlated responses in Experiment 3 were determined by contrasting the SRD of offspring derived from large sires with the SRD of offspring derived from small sires. Specifically, contrasts between these two groups were performed for juvenile body weight, survival and yield as well as adult survival and yield. Data were again analyzed as a balanced 5 male x 4 female factorial mating design, as described in Section 2.4.4.

2.4.6 Impact of density dependent oyster performance

The field-testing environment in the present study was designed to emulate actual commercial growing conditions. As a result, stocking density within each replicate lantern net compartment was allowed to vary as function of replicate individual growth rate and survival. However, the effect of variable stocking densities should be considered when interpreting these results. Two correlations were performed to assess the impact of
variable stocking density due to field mortality on the expression of body weight and survival. First, the correlation between offspring adult body weight and offspring adult survival was measured to determine if an increase in average family survival (and therefore higher stocking densities) was associated with a decrease in average individual body weight. Second, juvenile offspring survival (measured at day 317) was correlated against offspring growth rate (g d\(^{-1}\)) during the second growing season, to determine if families with high stocking densities entering the second growing season exhibited slower than average second season growth rate.

3. RESULTS

Based on pooled data from all three experiments, initial plant-out weight was significantly positively correlated with juvenile body weight (r=0.235, P<0.001) and juvenile yield (r=0.130, P=0.004) as well as adult body weight (r=0.152, P<0.001), and adjusted as outlined in Section 2.4.1. Survival data in all experiments were arcsine transformed and adult offspring yield in Experiment 3 was log-transformed to meet assumptions of normality (Sokal and Rohlf, 1995).

3.1 Average individual body weight, survival and yield

Individual live body weight, averaged across all three experimental crosses, increased from 46.3 g at day 317 to 128.6 g at day 640 (Table 4.2). Average survival decreased
linearly with time, from 58.6% after the first growing season to 26.7% at harvest. Yield increased rapidly during the first growing season to 1.36 kg rep\(^{-1}\). Yield increased slower during the second growing season and at harvest averaged 1.72 kg rep\(^{-1}\).

The water temperature profile for the entire field trial is shown in Figure 4.1. Average water temperature was 13.9\(^{\circ}\) C, ranging from a winter low of 7.2\(^{\circ}\) C to a summer high of 23.2\(^{\circ}\) C. Water temperatures exceeded 20\(^{\circ}\) C for extended periods during the summers of 2002 and 2003.
Table 4.2. Mean and standard deviation for body weight (g), survival (%), and yield (kg) in each of the three experimental crosses. Adult values for parental population body weight measured after the 1997-1998 growing seasons (700 days in the field). Juvenile offspring performance measured after the 2002 growing season (317 days in the field) and adult offspring performance measured after the 2002 and 2003 growing seasons (640 days in the field). Pooled values represent average performance measures across all three experiments.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Test site</th>
<th>Juvenile Mean</th>
<th>Juvenile SD</th>
<th>Adult Mean</th>
<th>Adult SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parents body wt (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Upriver</td>
<td>-</td>
<td>-</td>
<td>112.32</td>
<td>20.25</td>
</tr>
<tr>
<td>2 &amp; 3</td>
<td>Downriver</td>
<td>-</td>
<td>-</td>
<td>139.69</td>
<td>38.01</td>
</tr>
<tr>
<td>Offspring body wt (g)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
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<td>45.89</td>
<td>7.12</td>
<td>127.10</td>
<td>20.09</td>
</tr>
<tr>
<td>2</td>
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<td>131.06</td>
<td>19.67</td>
</tr>
<tr>
<td>3</td>
<td>Upriver</td>
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<td>7.74</td>
<td>127.55</td>
<td>19.04</td>
</tr>
<tr>
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<td>46.29</td>
<td>7.49</td>
<td>128.56</td>
<td>19.52</td>
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<tr>
<td>Survival (%)</td>
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<td></td>
<td></td>
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<td></td>
</tr>
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<td>17.1</td>
<td>29.31</td>
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<td>18.0</td>
<td>27.49</td>
<td>15.51</td>
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<tr>
<td>Pooled</td>
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<td>58.64</td>
<td>17.20</td>
<td>26.75</td>
<td>14.63</td>
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<td>Yield (kg)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Upriver</td>
<td>1.46</td>
<td>0.48</td>
<td>1.83</td>
<td>0.97</td>
</tr>
<tr>
<td>2</td>
<td>Upriver</td>
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<td>0.49</td>
<td>1.57</td>
<td>0.83</td>
</tr>
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<td>3</td>
<td>Upriver</td>
<td>1.33</td>
<td>0.47</td>
<td>1.77</td>
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<td>Pooled</td>
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<td>1.36</td>
<td>0.47</td>
<td>1.72</td>
<td>0.96</td>
</tr>
</tbody>
</table>
Figure 4.1. Water temperature in Yaquina Bay, Oregon, from Spring 2002 to Winter 2003. StowAway (Onset Corp, Mass., U.S.A.) data logger recorded temperature (C) every 2 hours at a depth of approximately 1 m.
3.2 Heritability of individual body weight

Heritability ($h^2$) of body weight after two growing seasons in the field in Experiment 1 (both parents and offspring raised upriver) was $0.33 \pm 0.121$ (± 1 SE; bold values in Table 4.3). In Experiment 2, offspring of parents raised downriver showed no response to selection for body weight at harvest ($h^2=-0.013 \pm 0.172$). Results from Experiment 3 show that there were significant sire and dam effects (P<0.001) on adult offspring body weight (Table 4.4A). Although there was variation among sires, the difference between adult body weight of offspring derived from the large sires was not significantly different from the adult body weight of offspring derived from small sires (P=0.557; Table 4.4B).
Table 4.3. Correlated responses in individual body weight, survival and yield to selection on adult body weight for Experiments 1 and 2. *N* represents the number of families used in each experiment, *N̅* represents the average number of replicate compartments measured per family and *N̅N̅* is the total number of replicates measured per experimental cross. Correlation coefficients (*r*) were measured between adult parental body weight and performance of juvenile (day 317) and adult (day 640) offspring. The slope of the midparent-offspring regression (*b*), and standard errors (SE), were weighed for variable number of replicates as per Falconer (1963). Values in bold represent direct responses to selection and the slope (*b*) interpreted as the narrow sense heritability of adult oyster body weight. Correlation coefficients and regression slopes that differed from 0 at the *P*<0.05 and *P*<0.01 levels of significance are indicated with *, or **, respectively.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Parental test site</th>
<th>Offspring test site</th>
<th>Offspring trait</th>
<th><em>N</em></th>
<th><em>N̅</em></th>
<th><em>N̅N̅</em></th>
<th><em>r</em></th>
<th><em>b</em> (SE)</th>
<th><em>r</em></th>
<th><em>b</em> (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Upriver</td>
<td>Upriver</td>
<td>Individual Body Wt (g)</td>
<td>12</td>
<td>9.83</td>
<td>117</td>
<td>0.075</td>
<td>0.055 (0.072)</td>
<td>0.379**</td>
<td>0.330* (0.121)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Survival (%)</td>
<td>12</td>
<td>9.83</td>
<td>117</td>
<td>-0.321**</td>
<td>-0.262* (0.115)</td>
<td>-0.430**</td>
<td>-0.373* (0.152)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Yield (kg)</td>
<td>12</td>
<td>9.83</td>
<td>117</td>
<td>-0.202*</td>
<td>-0.166 (0.100)</td>
<td>-0.285**</td>
<td>-0.240 (0.151)</td>
</tr>
<tr>
<td>2</td>
<td>Downriver</td>
<td>Upriver</td>
<td>Individual Body Wt (g)</td>
<td>19</td>
<td>8.53</td>
<td>158</td>
<td>-0.022</td>
<td>-0.070 (0.150)</td>
<td>0.030</td>
<td>0.013 (0.172)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Survival (%)</td>
<td>19</td>
<td>8.53</td>
<td>158</td>
<td>-0.399**</td>
<td>-0.480** (0.133)</td>
<td>-0.464**</td>
<td>-0.515** (0.143)</td>
</tr>
<tr>
<td></td>
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<td>Yield (kg)</td>
<td>19</td>
<td>8.53</td>
<td>158</td>
<td>-0.311**</td>
<td>-0.408* (0.148)</td>
<td>-0.382**</td>
<td>-0.418* (0.147)</td>
</tr>
</tbody>
</table>
Table 4.4 A and B. Direct and correlated responses to selection in Experiment 3, where parents were evaluated at the downriver site and offspring evaluated at the upriver site. A. Analysis of variance of the effects of sire, dam, and sire x dam interaction on juvenile and adult offspring body weight, survival and yield. B. Linear contrasts between offspring derived from large and small sires for juvenile and adult body weight, survival, and yield. Values in bold represent direct responses to selection.

A.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Source</th>
<th>Juvenile (day 317)</th>
<th></th>
<th>Adult (day 640)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>d.f.</td>
<td>MS</td>
<td>P</td>
<td>d.f.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BW</td>
<td>Sire</td>
<td>4</td>
<td>2.093</td>
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<tr>
<td></td>
<td>Dam</td>
<td>3</td>
<td>15.128</td>
<td>&lt;0.001</td>
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<tr>
<td></td>
<td>Sire x Dam</td>
<td>12</td>
<td>0.268</td>
<td>0.938</td>
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</tr>
<tr>
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<td>Error</td>
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<tr>
<td>Survival</td>
<td>Sire</td>
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<td>12.256</td>
<td>&lt;0.001</td>
<td>4</td>
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<tr>
<td></td>
<td>Dam</td>
<td>3</td>
<td>1.466</td>
<td>0.053</td>
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<tr>
<td></td>
<td>Sire x Dam</td>
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<td>0.016</td>
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<td>Error</td>
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<tr>
<td>Yield</td>
<td>Sire</td>
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<td>7.765</td>
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B.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Juvenile (day 317)</th>
<th></th>
<th>Adult (day 640)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ave offspring body wt (g)</td>
<td></td>
<td>Ave offspring body wt (g)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Large</td>
<td>Small</td>
<td>P</td>
<td>Large</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>46.157</td>
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<td>128.42</td>
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<td>Survival (%)</td>
<td>47.547</td>
<td>54.125</td>
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<td>28.510</td>
</tr>
<tr>
<td>Yield (kg)</td>
<td>1.253</td>
<td>1.466</td>
<td>&lt;0.0001</td>
<td>1.525</td>
</tr>
</tbody>
</table>
3.3 Correlated response to selection for individual body weight

3.3.1 Juvenile offspring body weight

Selection on adult midparent body weight resulted in no significant correlated responses in juvenile offspring body weight in Experiment 1 \((r = 0.075, P=0.420)\) or in Experiment 2 \((r=-0.022, P=0.787; \text{Table 4.3})\). The slope of the regression between adult parental body weight and juvenile offspring body weight, in standard deviation units, did not differ significantly from zero in both Experiment 1 and 2 (Table 4.3). In Experiment 3, analysis of variance indicated that juvenile offspring body weight was significantly affected by both sire and dam adult body weight (Table 4.4A), but not by an interaction between sire and dam. Contrasts performed in Experiment 3 showed body weight of offspring from large sires did not differ significantly from body weight of offspring from small sires \((P=0.347; \text{Table 4.4B})\).

3.3.2 Juvenile and adult offspring survival

Significant negative correlations \((P<0.001)\) were found between adult parental body weight and both juvenile offspring survival (Experiment 1, \(r=-0.321, P <0.001\); Experiment 2, \(r=-0.399, P<0.001\)) and adult offspring survival (Experiment 1, \(r=-0.430, P<0.001\); Experiment 2, \(r=-0.464, P<0.001\); Table 4.3). In addition, all slopes between midparent body weight and offspring survival were significantly less than zero (Table 4.3). In Experiment 3, analysis of variance indicated that juvenile offspring survival was significantly affected by sire \((P<0.01)\) but not by dam \((P=0.053; \text{Table}\)
Adult offspring survival was significantly affected by both sire and dam (P<0.01). The interaction between sire and dam significantly affected both juvenile and adult offspring survival (P<0.02). Contrasts performed in Experiment 3 also indicated that offspring from large sires had significantly poorer survival than offspring from small sires as both juveniles (P<0.001) and adults (P<0.001; Table 4.4B).

3.3.3 Juvenile and adult offspring yield

Significant negative correlations were seen between adult parental body weight and both juvenile offspring yield (Experiment 1, r_p = -0.202, P=0.029; Experiment 2, r_p = -0.311, P=<0.001) and adult offspring yield (Experiment 1, r_p = -0.285, P=0.002; Experiment 2, r_p = -0.382, P=<0.001; Table 4.3). The slopes between adult midparent body weight and juvenile and adult offspring yield were significantly less than zero in Experiment 2, but not in Experiment 1, although the slopes were also negative. In Experiment 3, analysis of variance indicated that juvenile and adult offspring survival were significantly affected by sire (P<0.001) and dam (P<0.001; Table 4.4A). The interaction between sire and dam significantly affected juvenile offspring survival (P=0.011) but not adult offspring survival (P=0.077). Contrasts performed in Experiment 3 indicated that offspring from large sires had significantly lower yields as juveniles (P<0.01) and as adults (P<0.01) compared with offspring from small sires (Table 4.4B).

3.4 Effect of variable stocking density at interim and harvest
No significant correlations were found between average family body weight at harvest and average family survival at harvest for Experiment 1 ($r = 0.031, P=0.737$), Experiment 2 ($r = -0.061, P=0.444$) or Experiment 3 ($r = -0.001, P=0.991$). In addition, correlation between interim survival (i.e. stocking density) and growth rate ($g d^{-1}$) during the second growing season was not significant for Experiment 1 ($r = 0.011, P=0.902$) and Experiment 2 ($r = 0.031, P=0.695$), but was significant, and positive, for Experiment 3 ($r = 0.184, P=0.006$). As a result, no attempt was made to correct performance for variable stocking densities.

4. DISCUSSION

4.1 Heritability of body weight after two growing seasons

Heritability of adult body weight differed dramatically between Experiment 1 (0.33 ± 0.121) and Experiment 2 (-0.013 ± 0.172). The primary difference between the two experimental crosses was the environment in which the parents were evaluated. In Experiment 1 both parents and offspring were evaluated at the upriver site, while in Experiment 2 parents were evaluated at the downriver site and offspring evaluated at the upriver site. These results suggest site-specific adaptations, with the resemblance between parent and offspring dependant upon environment (i.e. a genotype x environment interaction). Because Yaquina Bay is an estuarine environment, tidal influence and seasonal rainfall can result in large differences in temperature and salinity.
between the upriver (15 km from the mouth of the bay) and downriver (3 km from the mouth of the bay) sites. Tolerance to elevated temperature (Beattie et al., 1980) and variable salinity (e.g. Castagna and Chanley, 1973) could explain site-specific adaptation, resulting in a poor correlation between parental performance downriver and offspring performance upriver. An alternative explanation is that there was simply no additive genetic variation present in the downriver population. Although the author believes this to be unlikely (as both upriver and downriver populations were derived from the same base population), this theory cannot be directly tested in this study because offspring were not evaluated in the downriver environment.

The narrow sense heritability estimated in Experiment 1 (where parents and offspring were both evaluated in the upriver environment) is consistent with published heritability estimates for adult shellfish body weight and growth rate. The broadsense heritability ($H^2$) of adult Pacific oysters body was reported by Lannan (1972) to be $0.33 \pm 0.19$. The narrow sense heritability of adult Pacific oyster meat weight was reported by Hedgecock et al. (1991) to be approximately 0.2. The heritability of adult body weight and/or growth rate has also been reported in Ostrea edulis ($0.24 \pm 0.20$; Toro and Newkirk, 1990), Saccostrea cucullata ($0.28 \pm 0.06$, Jarayabhand and Thavornyutikarn, 1995) and Mercenaria mercenaria ($0.43 \pm 0.06$; Hadley et al., 1991). Several other studies also report significant response to selection for improved body weight, although estimates of heritability were not reported (see review by Sheridan, 1997).
Stocking density has been shown to affect shellfish growth rate (Jarayabhand and Newkirk, 1989; Holiday et al., 1991; Taylor et al., 1997; Southgate and Beer, 2000). The design of the experiments presented here allowed stocking density to vary as function of survival and body weight, which should be addressed when considering bias of heritability estimates. Increased average body weight could lead to elevated stocking densities (biomass) per replicate and consequently decreased growth rate. Under these conditions the observed heritability of body weight will likely underestimate the true population heritability, and could serve as an explanation for the absence of a measured direct response to selection for body weight in Experiments 2 and 3. However, the significant and relatively high narrow-sense heritability measured in Experiment 1, in which density was also allowed to vary, suggests that variable stocking density was not entirely responsible for the results seen in Experiments 2 and 3. In addition, no correlations were found between harvest body weight and harvest survival nor were correlations found between the number of oyster present at the interim measurement and growth rate during the second growing season. Nonetheless, it is possible, based on research in other shellfish, that variable stocking density may have biased the reported heritability estimates downward.

4.2 Correlated responses to selection

No correlated response in juvenile body weight was observed as the result of selection on adult body weight. These results suggest that either the two characters are not genetically
correlated (but see Toro and Newkirk, 1990), average body weight in offspring had not
differentiated sufficiently to statistically detect a response (e.g. Refstie and Steine, 1978;
Gjedrem, 1983; Mckay et al., 1986; Toro and Newkirk, 1990), or that, as suggested in
Section 4.1, genotype x environment interactions mask the genetic similarities between
adult parents and juvenile offspring. In the latter case, environmental variation may be
due to location (i.e. upriver versus downriver) or time (1997-1998 field trials for parents
versus 2002-2003 field trials for offspring).

In all three experiments significant inverse relationships existed between adult midparent
body weight and both offspring survival and offspring yield (P<0.01), regardless of
parental source population (upriver or downriver). Although stocking density may again
bias these results, the literature suggests that survival is much less sensitive to variation in
stocking density compared to growth rate (Holiday et al., 1991; Taylor et al., 1997). In
addition, the decrease in offspring survival with increased parent body weight was seen
even after the first growing season, when the animals were still quite small (average
individual body weight of 46 g), suggesting that stocking density was not the cause of the
observed trend.

The fact that large parents produced poorer surviving offspring than small parents in the
present study, suggest that there may be a negative genetic correlation between body
weight and survival under certain environmental conditions. The biological mechanisms
for these inverse correlations are unknown, but there is evidence in the literature that
suggests smaller oysters may enjoy higher survival for a variety of reasons. Reproductive
effort (i.e. proportion of energy resources dedicated to reproduction) tends to be
positively correlated with both mortality rate and body size and (Bayne et al., 1983; Roff,
1992; Moal et al., 2003), resulting in size-specific mortality during periods of elevated
water temperature and rapid gonad development (e.g. Askew, 1972; Glude, 1975). These
results are consistent with findings of Beattie et al. (1980), who were successful in
breeding high-surviving oyster strains by increasing their glycogen content during warm
summer months, effectively reducing their reproductive effort. These studies suggest
larger animals, through their increased reproductive effort, could be more susceptible to
physical and biological stressors compared to smaller oysters. It is also worth noting that
the growing environment in Yaquina Bay during this experiment was stressful for the
oysters (as evidenced in the low average survival), possibly due to high water
temperatures in the summers of 2002 and 2003 (Figure 4.2) as well as fluctuating salinity
in winter (unpublished data). Some research suggests that the sign of the correlation
between growth rate and survival may be environment-specific (Norry and Loeschcke,
2002). It is therefore possible that in more benign environments midparent size may be
uncorrelated or even positively correlated with offspring survival and yield.

The inverse relationships between parental body weight and offspring survival found in
the present study differs from the three studies reported in the literature that have
considered the effects of selecting for increased oyster body weight on offspring survival.
In Ostrea edulis Newkirk and Haley (1983) found no difference in body weight between
offspring of parents selected for increased body weight and unselected controls. They
did, however, find that offspring of selected parents had significantly higher survival than
offspring of unselected control parents. In a study utilizing midparent-offspring
regression to estimate heritability of live weight in *O. edulis*, Toro and Newkirk (1990)
found no correlated response in offspring survival. Similarly, in *Saccostrea cucullata*,
Jarayabhand and Thavornyutikarn (1995) found survival of offspring derived from
parents selected for increased growth rate tended to be higher, but not significantly
different from, survival of offspring derived from unselected control parents. The only
comment on the cause of the observed mortality was by Newkirk and Haley (1983), who
suggested handling mortality may have made differences in survival between genotypes
more apparent.

4.3 Effects of direct and indirect responses to selection on breeding programs

Although body weight is an important commercial trait, improvement of oyster
production will be dependant upon increasing yield. Therefore survival must also be
considered in any breeding program with the goal of improving yield. Family selection
has been used to allow average body weight, survival and yield to be treated as
continuous traits (versus threshold traits; Bourdon, 2000; Langdon et al., 2003).
Unfortunately, family selection has several disadvantages compared with individual (or
mass) selection. Selection intensity (*i*) is typically lower when family selection is applied
versus individual selection due to the ability to evaluate more individuals than families.
In addition, phenotypic variation among family means is typically smaller than among individuals. Together, this results in a reduced selection differential \( (S = i\sigma_p) \) when family selection is applied compared to individual selection (Gall, 1991; Falconer and Mackay, 1996). Further, resources and labor demands associated with the production, maintenance, and tracking of the many family groups required to conduct family selection, may prevent the adoption of such breeding schemes by commercial farmers (Tave, 1995). Indirect selection is a common strategy used to make genetic gains in characters that are too difficult or too costly to measure directly (Falconer and Mackay, 1996; Bourdon, 2000). An attractive candidate for an indirect measure of oyster yield is individual body weight, which can be measured on an individual-basis. Unfortunately, results from this study suggest that selection for increased body weight could result in decreased survival and yield. Even in Experiment 1, where parents and offspring were evaluated at the upriver site, gains made in average individual body weight were outweighed by the correlated reduction in survival, resulting in a net decrease in yield.

Results from this study confirm findings by other authors that suggest adult oyster body weight is heritable (e.g. Sheridan, 1997). However, these results also suggest that adult oyster body may be sufficiently affected by genotype x environment interactions occurring within Yaquina Bay to render selection in the downriver environment ineffective at improving body weight at the upriver environment. Lastly, the strong negative correlation between parental body weight and offspring survival suggests that selection for improved body weight may actually decrease offspring yield. Although
offspring were only measured in a single growing environment, it is recommended that caution be exercised when selection is performed on adult oyster body weight in an attempt to indirectly improve yield.
5. REFERENCES.


CHAPTER 5

GENERAL CONCLUSIONS

Husbandry of aquatic animals can be traced back to 1100 B.C. in China (Shell, 1991), however, significant genetic improvement of fish and shellfish has occurred only recently, primarily due to the development of techniques that allow for breeder-controlled matings (Refstie, 1990; Gall, 1991). Similarly, it wasn’t until the 1970’s that commercial oyster growers along the West coast of the United States began to shift from wild-collected spat to hatchery-produced spat (Conte et al., 1996) and began considering the benefits of genetic improvement.

In 1995 a selective breeding program was initiated at the Hatfield Marine Science Center, Newport, Oregon, to develop high yielding strains of Pacific oysters suitable for production throughout the Pacific Northwest (Hedgecock et al., 1997). Family-based phenotypic selection was employed to directly select on average family yield. Results after one generation of selection indicated that yield was heritable and that gains in yield could be made through selective breeding (Langdon et al., 2003). However, questions remain about the possible impacts of genotype x environment interactions on oyster performance as well as the heritability of yield’s causal components (i.e. body weight and survival).
Oyster breeding programs in the Pacific Northwest will benefit from a better understanding of the genetic and environmental factors affecting oyster performance. This dissertation had three objectives. The first objective (Chapter 2) was to determine if relative family performance at harvest is influenced by the nursery conditions in which the families were raised. The second objective (Chapter 3) was to determine the magnitude and significance of genotype x environment interactions on performance traits of oysters planted at typical, but dissimilar, commercial sites in the Pacific Northwest. The third objective (Chapter 4) was to determine the heritability of adult oyster body weight and if selection on body weight resulted in a correlated response in survival or yield. The implications of this research on oyster breeding programs are summarized below.

Chapter 2. Effects of dietary restriction during juvenile development on performance of adult Pacific oysters (Crassostrea gigas).

Nursery environment was found to significantly affect performance characters (body weight, survival and yield) even after two growing seasons (490 days) in the field. However, no significant genotype x nursery environment interaction was detected in the field performance of outbred crosses, suggesting relative field performance remains stable across a wide range of nursery feeding regimes. The significant genotype x nursery environment interactions affecting inbred family field performance only occurred in the most stressful nursery environment and therefore may be unlikely to affect families raised under more typical commercial feeding regimes. Consequently, it is advised that
selection occur only among families that have experienced the same or similar nursery
environments (e.g. conduct selection within “cohorts” made up of 50 to 60 families; Langdon et al., 2003). These results also indicated that selection efficiency for all performance traits measured in this study should increase as the number of days in the field increase due to a reduction in the effect of nursery environment and an increase in the effect of family over time. Lastly, an inverse correlation between average treatment plant-out weight and average treatment survival at harvest was observed among outbred families. Further work is needed to determine if this relationship is due to differential adult body weight or conditioning to nutrient stress as juveniles.

Chapter 3. Effects of genotype x environment interaction on Pacific oyster (Crassostrea gigas) performance in the Pacific Northwest.

Although statistically significant genotype x environment interactions were found for all Pacific oyster performance characters (body weight, survival, yield) across dissimilar sites (P<0.001), the amount of total phenotypic variation that the interactions accounted for was relatively small. In addition correlations between performance traits across environments were significantly greater than 0 (r_p>0.432; P<0.05). These results suggest that genetic improvement of broadly-adapted Pacific oyster strains is possible using a limited number of well selected evaluation environments. Indirect selection in a single environment targeting improved yield in all other environments was 48% to 91% as effective as direct selection within each environment separately. It is essential that oyster performance within the environment where selection occurs be positively genetically
correlated with performance at all production environments, and must allow genotypes to be easily discriminated (i.e. high $h^2$ or $H^2$; Falconer, 1952; Weber and Wrick, 1990). The intertidal Yaquina environment appears to be the best environment in which to perform selection to indirectly improve yield at all other experimental environments. Further work is needed to determine how stable the observed correlations between sites remain over time. In addition, the breeder must determine if a reduction in selection efficiency due to indirect selection can be offset by the benefits associated with avoiding the development of site-specific lines and also by minimizing the number of field test sites. If the reduction in selection efficiency is unacceptable, locally adapted strains may have to be developed via direct selection in each unique environment.

Chapter 4. Direct and correlated responses to selection for individual body weight in Pacific oysters (Crassostrea gigas).

Chapter 4 confirms findings by other authors that suggest adult oyster body weight is heritable (e.g. Sheridan, 1997). However, these results also suggest that adult oyster body weight may be sufficiently affected by genotype x environment interactions as to render selection in the downriver Yaquina Bay environment ineffective at improving body weight in the upriver Yaquina Bay environments. At first, these results appear inconsistent with the findings reported in Chapter 3, which suggested GxE interactions should not prevent the development of broadly-adapted high-yielding strains of oysters. However, in Chapter 3, body weight was the least efficient character in which to apply indirect selection (e.g. 49% to 64% as efficient as direct selection within each site),
suggesting that GxE interactions impact this trait more than survival and yield. Further, a
suitable environment for indirectly improving performance at other sites requires the trait
have a relatively high heritability at the site of selection and show a high positive genetic
correlation will all production sites. It is not clear that the downriver Yaquina Bay
environment met these criteria.

Lastly, the strong negative correlation between parental body weight and offspring
survival suggests that selection for improved body weight may actually decrease
offspring yield. Family selection has been successful at improving average family yield
in Pacific oysters (Langdon et al., 2003). The disadvantage of family selection is
primarily the decreased selection differential (S), due to decreased phenotypic standard
deviation and/or decreased selection intensity (i), compared to individual selection (Gall,
1991; Falconer and Mackay, 1996). In addition, family selection requires resources be
devoted to the development, evaluation and storage of numerous family groups.
Alternatively, selection based on individual performance could capitalize increased S and
be accomplished with fewer nursery culture tanks and smaller field test plots. Body
weight would be an ideal indicator trait to indirectly select for yield if selection for body
weight resulted in a positive correlated response in yield. Unfortunately, selection for
increase adult body weight in Chapter 4 resulted in a correlated decrease in survival and
consequently a decrease in average family yield. Although these offspring were only
evaluated in a single growing environment, it is recommended that caution be exercised
when selection is performed on adult oyster body weight in an attempt to indirectly improve yield.
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