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ScienceDirect
Aquaculture 259 (2006) 124–137
Aquaculture
www.elsevier.com/locate/aqua-online

# Effect of dietary restriction during juvenile development on adult performance of Pacific oysters (*Crassostrea gigas*)

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Received 9 December 2005; received in revised form 19 May 2006; accepted 22 May 2006

**Abstract**

An experiment was performed to determine if dietary restriction in the nursery significantly affects the performance of Pacific oysters (*Crassostrea gigas*) after two years in the field. Five outbred full-sib families and three inbred full-sib families were created in August 2000, and reared under each of the 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 suspended in Yaquina Bay, OR, USA. ANOVA was used to determine if genotype, nursery environment, or genotype × nursery environment interactions significantly affected yield (kg tier$^{-1}$), individual body weight (g), and survival (%) after one and two growing seasons in the field. Average outbred family yield after two growing seasons in the field (490 days) was significantly affected by genotype ($P<0.01$), but not by nursery environment ($P=0.052$) or genotype × 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 × 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 × 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 × nursery environment interaction ($P=0.197$). Significant rank changes among inbred families for both individual body weight and yield occurred only among families reared under the most stressful nursery feeding regime. Although the effect of nursery feeding environment was detected even after two growing seasons in the field, the lack of a genotype × nursery environment interaction suggests differences in nursery feeding regime should not significantly alter relative field performance of outbred oyster families. Significant rank changes were seen among inbred families; however, relative field performance should not be affected under all but the most stressful juvenile growing conditions.


*Keywords:* Oyster; Breeding; *Crassostrea gigas*; Nursery; Permanent environment effect

## 1. Introduction

Phenotypic variation of polygenic traits is typically expressed as a function of both genetic and environmental sources of variation (Falconer and Mackay, 1996). When environmentally mediated effects persist over time, the variation due to environment can be partitioned further to include both temporary and permanent effects. Animal and plant breeders have long been aware of the need to account for permanent environmental effects in order to maximize genetic gain (e.g. Falconer and Mackay, 1996; Bourdon, 2000). Indeed, stress experienced during

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doi:10.1016/j.aquaculture.2006.05.035

juvenile development has been shown to permanently affect adult phenotypic variation in aquatic (Brooks, 2000; Lee and Petersen, 2003), amphibious (Goater, 1994; O'Steen, 1998; Pahkala et al., 2001) and terrestrial species (Desai and Hale, 1997; Lay, 2000; Madsen and Shine, 2000; Metcalfe and Monaghan, 2001), including commercially important species (Moberg and Wood, 1982; Creel and Albright, 1988; Keown, 1989; Hassen et al., 2001) and humans (Hales and Barker, 1992; Desai and Hale, 1997).

Considerable evidence exists suggesting nursery culture environment can significantly affect juvenile shellfish growth and survival. For example, feeding ration (e.g. His and Seaman, 1992; Rheault and Rice, 1996; Wikfors et al., 2001; Pechenik et al., 2002; Southgate and Beer, 2000), salinity (e.g. Brooks, 2000; Dudas et al., 2004; Lemos et al., 1994), water temperature (e.g. Lemos et al., 1994; Dudas et al., 2004) and stocking density (e.g. Holiday et al., 1991; Taylor et al., 1997) have all been shown to affect larval or juvenile growth and survival. However, it is unclear to what degree the effects of nursery environment can carry-over and affect market-sized adult phenotype (typically after two growing seasons in the field). If carry-over effects do occur, nursery environment can be said to have a permanent effect on adult shellfish phenotype, that is, the effect of nursery environment persists even after the nursery phase has ended.

Although the effects of temporary starvation 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 market-size adult shellfish. Further, few studies account for genotype and the possibility of genotype × nursery environment interactions affecting adult phenotype. This experiment was performed to determine if dietary restriction in the nursery significantly affects performance traits of adult Pacific oysters (*Crassostrea gigas*) after one and two growing seasons in the field and, if so, does this source of variation change relative adult performance among genotypes.

## 2. Methods

### 2.1. Broodstock selection and spawning

Pair-wise crosses were made among unrelated *C. gigas* parents to create five outbred families and among full or half-sibs to create three inbred families. Parents were chosen from previously evaluated families (Langdon et al., 2003) such that they would likely be high, mid, and low-yielding offspring at harvest and therefore increase the likelihood of observing a significant family effect on offspring field performance. Although the use of non-randomly selected families limits the scope of interpretation of family effects, it was considered necessary due to the limited number of crosses that could be raised in 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 *Isocrysis galbana* delivered at 50,000 to 80,000 cells $ml^{-1}$. In August 2000, gametes were stripped from the parents and fertilized. Fertilized eggs from each family were held at a concentration of approximately 100 eggs $ml^{-1}$ and 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 $2 \times 10^{-4}$ M epinephrine (Coon et al., 1986). Successfully metamorphosed spat were transferred into 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 from the larval tank into the 15-cm upweller system (approximately 4 weeks post spawn). Water temperature in the 15-cm upweller system was maintained at 23 °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 into 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 juvenile growing environments. Nursery treatments began in October 2000, when all oyster spat in the 15-cm upwellers could be retained on a 1.4 mm sieve. Approximately 1500 1.4-mm spat from each family were stocked into each of the three family-specific 28-cm upwellers. Each of the three family-specific upwellers were then assigned to one of the three nursery feeding treatments (high, medium, and low feeding regimes, described below). Flow rates through the 28-cm upwellers were maintained at approximately 3 l $min^{-1}$ and water

exchange rates through each of the two upwelling systems (each holding 16 upwellers) were 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 × 0.23 m, L × W) 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 15.9 °C/6.9 °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 planted out in the field in June 2001.

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 planted out in the field in June 2001.

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. The number of feeding days per week was increased from 2 to 4 in order for the spat to be retained on a 4.75 mm sieve by June, 2001. 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 planted out in the field in June, 2001.

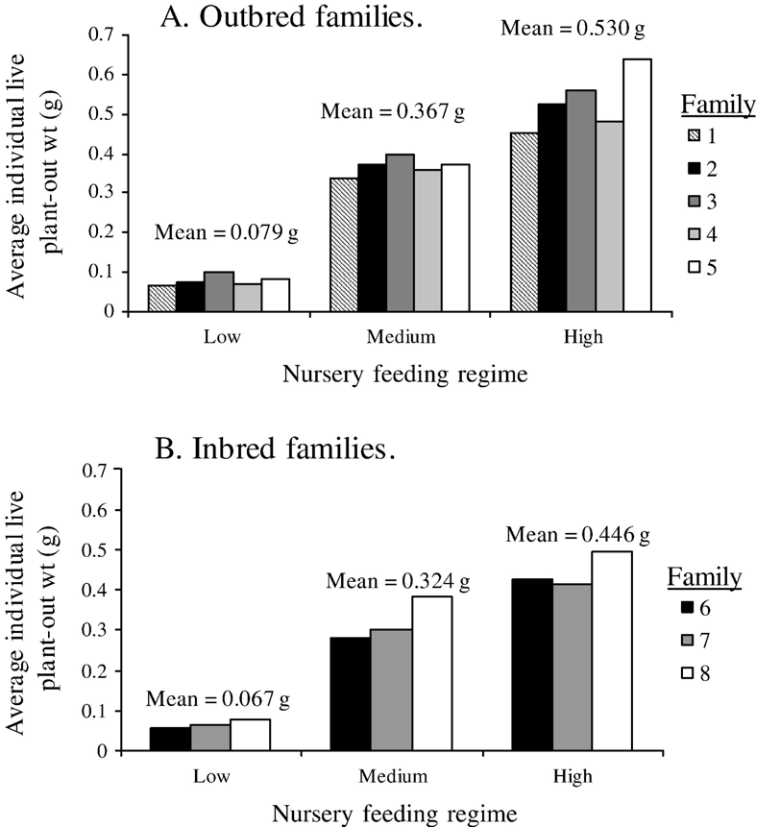


Fig. 1. Effect of nursery feeding regime (low, medium, and high) on average individual plant-out live weight (g) of (A) five outbred families and (B) three inbred families.

### 2.3. Field production

In June, 2001, 40 spat from each family–nursery treatment combination were randomly selected, weighed, and stocked into each of 10 replicate mesh sleeves (2 mm mesh; 0.30 m × 0.30 m, L × W). The 10 replicate sleeves from each family were then randomly assigned to each of the five vertical blocks of a ten-tier lantern net (5 mm mesh; compartment diameter of 0.51 m and a height of 0.17 m), with blocking intended to account for variation in field performance traits due to water depth. Lantern nets were deployed at a commercial oyster farm approximately 13 km from the mouth of the Yaquina River, Oregon, U.S. (44.6° N, 124.1° W). 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 oyster weight per bag by the number of live oysters. At this point, oysters were transferred from the small mesh sleeves directly into the 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) and the results were considered significant when $P<0.05$. The statistical significance of family and nursery feeding treatment on average plant-out body weight was determined using a two-factor fixed-effect ANOVA. Space limitation prevented the replication of family cultures within each nursery feeding treatment, therefore the effect of a family × 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 × nursery treatment interaction significantly affected oyster field performance traits. Block was considered a random effect. Family and nursery treatment were considered fixed effects as these were not randomly selected from a larger reference population. 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, 1996; 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. The small number of families used in this study and non-random selection of families limits the inferences that can be made regarding the absolute levels of $\eta^2$. However, we can examine the relative changes in the effect-size over time within this experiment.

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 coefficient 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 a linear relationship between plant-out weight and the measured performance traits (Sokal and Rohlf, 1995).

Table 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

| Trait | Nursery feeding treat. | Correlation coefficient ($r$) | | |
|---|---|---|---|---|
| | | Day 153 | Day 264 | Day 490 |
| *Body weight (g)* | | | | |
| Outbred | High | – | – | – |
| | Med | – | – | – |
| | Low | – | – | – |
| Inbred | High | – | – | −0.416 |
| | Med | 0.493 | 0.483 | – |
| | Low | – | – | −0.558 |
| *Survival* | | | | |
| Outbred | High | – | – | – |
| | Med | – | – | – |
| | Low | 0.298 | 0.355 | – |
| Inbred | High | – | – | −0.505 |
| | Med | – | – | −0.539 |
| | Low | – | – | −0.596 |
| *Yield (kg)* | | | | |
| Outbred | High | – | – | – |
| | Med | – | – | – |
| | Low | – | – | – |
| Inbred | High | – | – | −0.549 |
| | Med | 0.522 | 0.481 | −0.533 |
| | Low | – | – | −0.749 |

All reported correlations are significant ($P<0.05$). Statistically insignificant correlations are represented with a dash (–).

## 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; Fig. 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

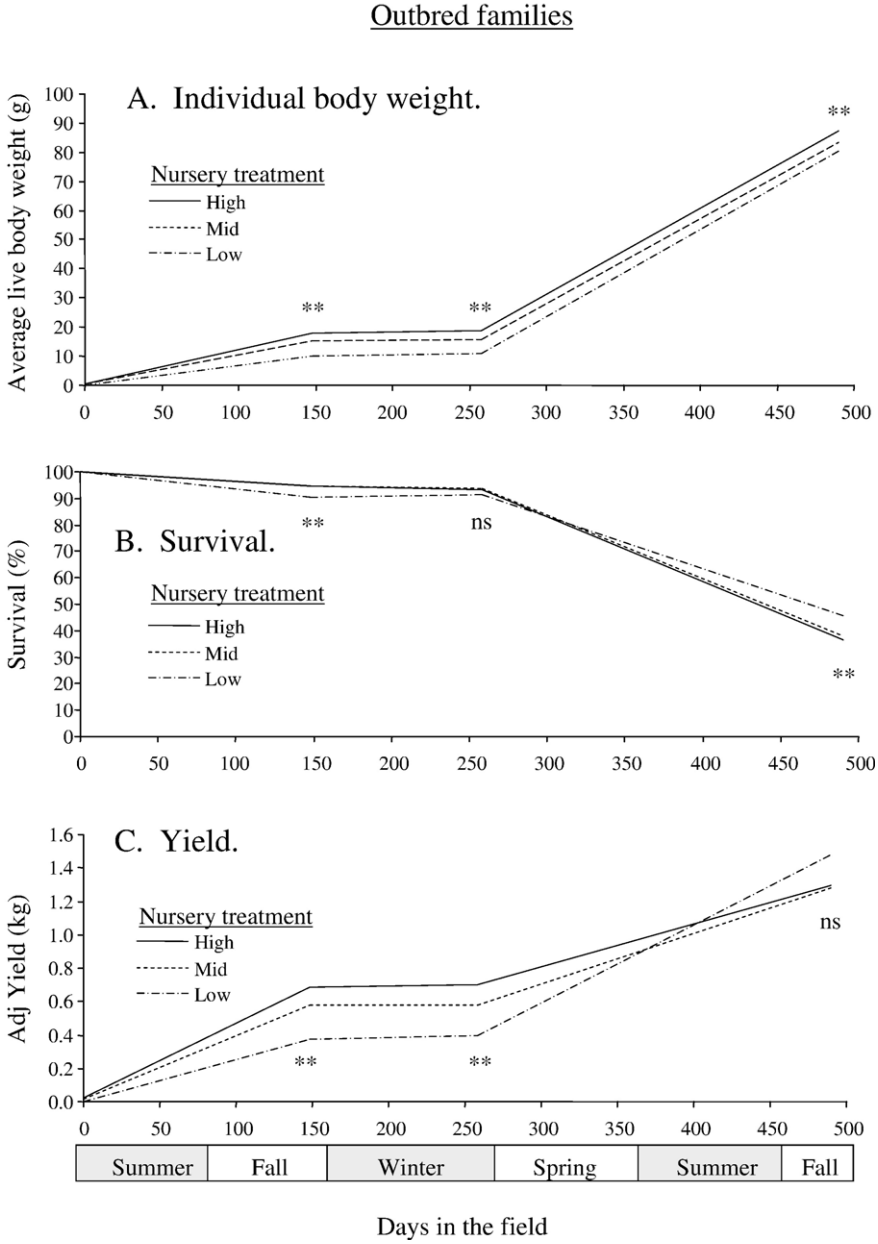


Fig. 2. Effect of nursery feeding treatment on (A) average individual body weight, (B) survival, and (C) yield, over the entire experimental growout phase for outbred families. Treatment effects are represented by the mean of all five families. Significance of treatment effects within each sampling period is indicated by "ns" ($P>0.05$), "*" ($0.05>P>0.01$), and "**" ($P<0.01$) as determined by analysis of variance (see Table 2).

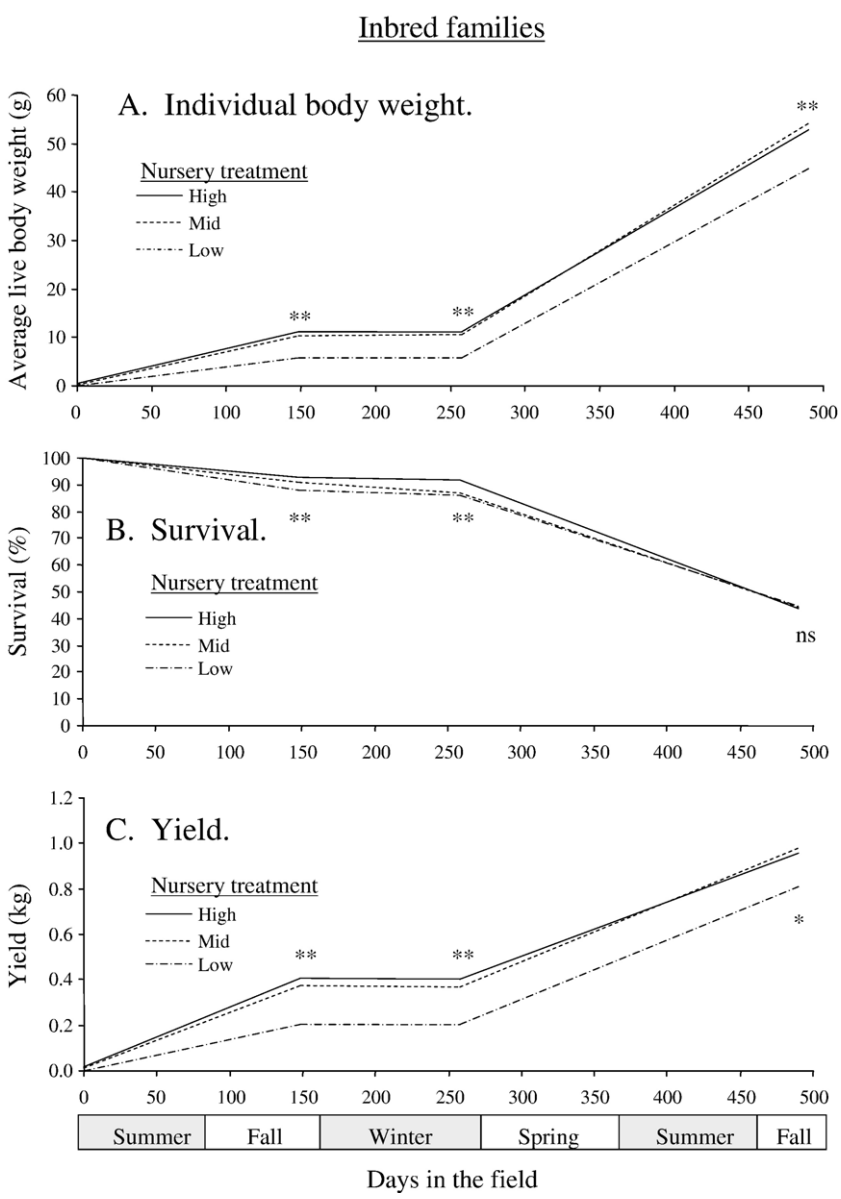


Fig. 3. Effect of nursery feeding treatment on (A) average individual body weight, (B) survival, and (C) yield, over the entire experimental growout phase for inbred families. Treatment effects are represented by the mean of all three families. Significance of treatment effects within each sampling period is indicated by "ns" ($P>0.05$), "*" ($0.05>P>0.01$), and "**" ($P<0.01$) as determined by analysis of variance (see Table 2).

was also significantly affected by nursery treatment ($P<0.0001$) and not by family using a $P=0.05$ level of significance ($P=0.067$; Fig. 1B). Average whole wet body weights at plant-out among inbred crosses for the high, medium, and low nursery feeding regimes were 0.446 g, 0.324, and 0.067 g, respectively. As with the outbred crosses, survival of inbred crosses through the nursery phase was high and not affected by nursery treatment ($P=0.2981$) or family ($P=0.1683$). Although body weight at plant-out of outbred crosses was generally heavier than plant-out body weight of inbred crosses, there was no significant difference ($P=0.1068$) between the two groups after accounting for the effects of nursery feeding regime and family within group (i.e. inbred or outbred).

### *3.2. Effect of plant-out body weight on field performance within nursery treatments*

Although not significant, there was some variation among average family plant-out body weights within

Table 2
Analysis of variance output (mean squares "MS" and *P*-values "*P*") for performance measures at all time periods for outbred and inbred families

| | Source | Day 153 | | Day 264 | | Day 490 | |
|---|---|---|---|---|---|---|---|
| | | MS | *P* | MS | *P* | MS | *P* |
| *Outbred* | | | | | | | |
| Body weight | Block | 108.95 | <**0.001** | 82.22 | <**0.001** | 523.40 | <**0.001** |
| | Treatment | 793.41 | <**0.001** | 803.71 | <**0.001** | 666.16 | <**0.001** |
| | Family | 24.17 | **0.004** | 24.76 | **0.008** | 348.78 | <**0.001** |
| | T × F | 7.23 | 0.308 | 6.03 | 0.538 | 73.37 | 0.339 |
| | Error | 6.06 | | 6.89 | | 64.16 | |
| Survival | Block | 0.0012 | 0.780 | 0.0039 | 0.255 | 0.0687 | **0.002** |
| | Treatment | 0.0299 | <**0.001** | 0.0076 | 0.075 | 0.1189 | <**0.001** |
| | Family | 0.0080 | **0.022** | 0.0105 | **0.007** | 0.2264 | <**0.001** |
| | T × F | 0.0023 | 0.564 | 0.0014 | 0.870 | 0.0054 | 0.946 |
| | Error | 0.0027 | | 0.0029 | | 0.0156 | |
| Yield | Block | 0.1713 | <**0.001** | 0.0807 | <**0.001** | 1.4351 | <**0.001** |
| | Treatment | 1.2765 | <**0.001** | 1.1578 | <**0.001** | 0.6101 | 0.052 |
| | Family | 0.0481 | <**0.001** | 0.0285 | 0.119 | 2.9855 | <**0.001** |
| | T × F | 0.0087 | 0.498 | 0.0106 | 0.692 | 0.0950 | 0.874 |
| | Error | 0.0094 | | 0.0152 | | 0.2009 | |
| *Inbred* | | | | | | | |
| Body weight | Block | 25.82 | <**0.001** | 25.98 | <**0.001** | 157.50 | **0.006** |
| | Treatment | 238.54 | <**0.001** | 246.36 | <**0.001** | 760.44 | <**0.001** |
| | Family | 15.48 | **0.003** | 8.84 | 0.276 | 415.42 | <**0.001** |
| | T × F | 2.83 | 0.353 | 3.74 | 0.184 | 125.63 | **0.019** |
| | Error | 2.52 | | 2.35 | | 39.83 | |
| Survival | Block | 0.0035 | 0.454 | 0.0030 | 0.661 | 0.0870 | <**0.001** |
| | Treatment | 0.0176 | **0.012** | 0.0275 | **0.006** | 0.0010 | 0.929 |
| | Family | 0.0024 | 0.531 | 0.0088 | 0.177 | 0.2843 | <**0.001** |
| | T × F | 0.0049 | 0.271 | 0.0097 | 0.111 | 0.0204 | 0.197 |
| | Error | 0.0037 | | 0.0050 | | 0.0132 | |
| Yield | Block | 0.0371 | <**0.001** | 0.0282 | <**0.001** | 0.6924 | <**0.001** |
| | Treatment | 0.3546 | <**0.001** | 0.3450 | <**0.001** | 0.2438 | **0.029** |
| | Family | 0.0258 | <**0.001** | 0.0140 | **0.010** | 2.0043 | <**0.001** |
| | T × F | 0.0060 | 0.1144 | 0.0074 | **0.046** | 0.2407 | **0.009** |
| | Error | 0.0031 | | 0.0029 | | 0.0656 | |

"Treatment" refers to nursery dietary treatment, "T × F" refers to nursery dietary treatment × family interaction. *P*-values < 0.05 printed in bold.

each nursery treatment, which could bias field results (Section 3.1 and Fig. 1). Variation in plant-out weight within each nursery treatment had little effect on field performance among outbred families (Table 1). An exception was the positive correlation between plant-out weight and survival in the low treatment at days 153 and 264. These correlations were not evident 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 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 the performance traits within nearly all nursery treatments.

### 3.3. Effect of nursery feeding regime on individual oyster body weight in the field

Body weight gain in the field occurred primarily during the summer months, with little or no growth during the winter months (Figs. 2A and 3A). The parallel growth trajectories for all treatments during the second growing season suggest little compensatory weight gain, that is, treatments that produced larger animals at plant-out were 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). Family × 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 × nursery

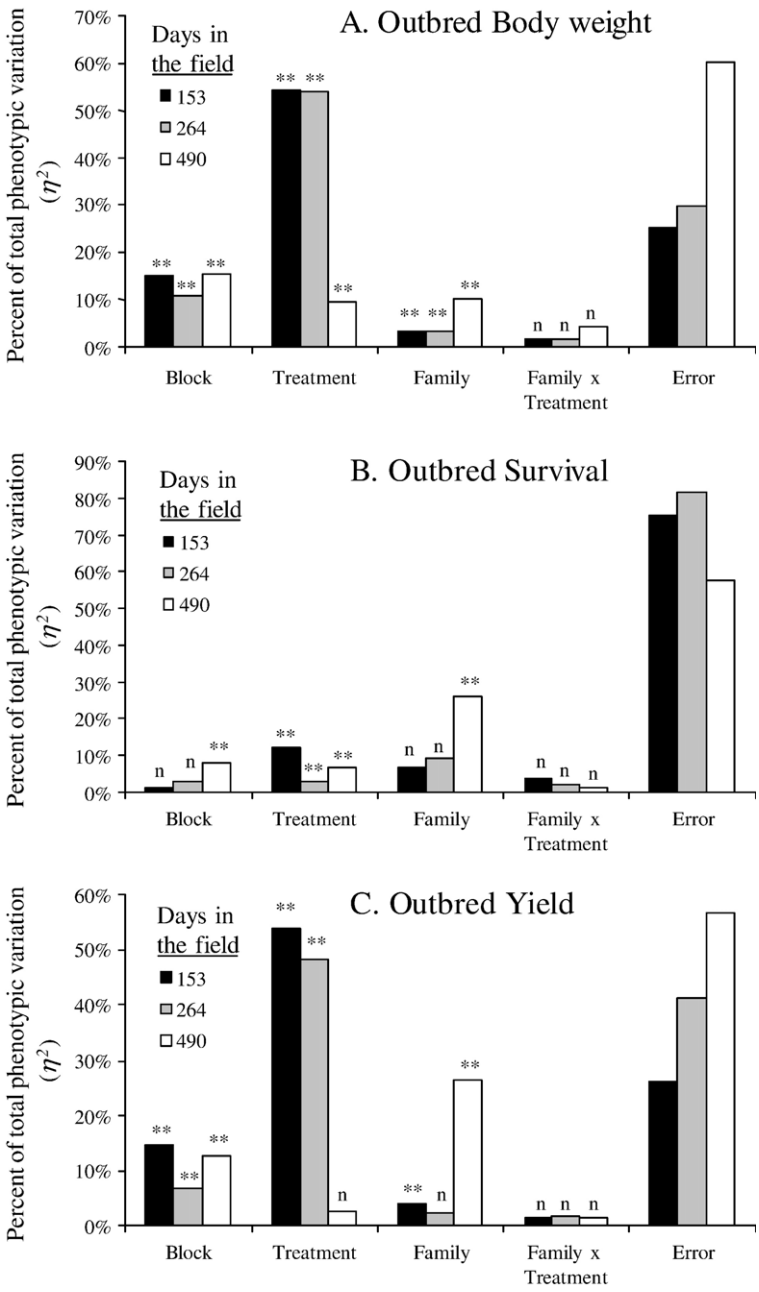


Fig. 4. Percent of total phenotypic variation among outbred families explained by block, family, nursery environment ("Treatment"), family × nursery environment, and experimental error for (A) individual body weight, (B) survival, and (C) yield. Sum of squares were used to estimate $\eta^2$ values for the three measurement periods (153, 264 and 490 days in the field). Significance of treatment effects within each sampling period is indicated by "ns" ($P>0.05$), "*" ($0.05>P>0.01$), and "**" ($P<0.01$) as determined by analysis of variance (see Table 2).

environment interaction present at harvest ($P=0.019$; Table 2). The significance of the block effect was typically due to the increased body weight in the shallowest block for both inbred and outbred families.

Figs. 4A and 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, respectively. 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.

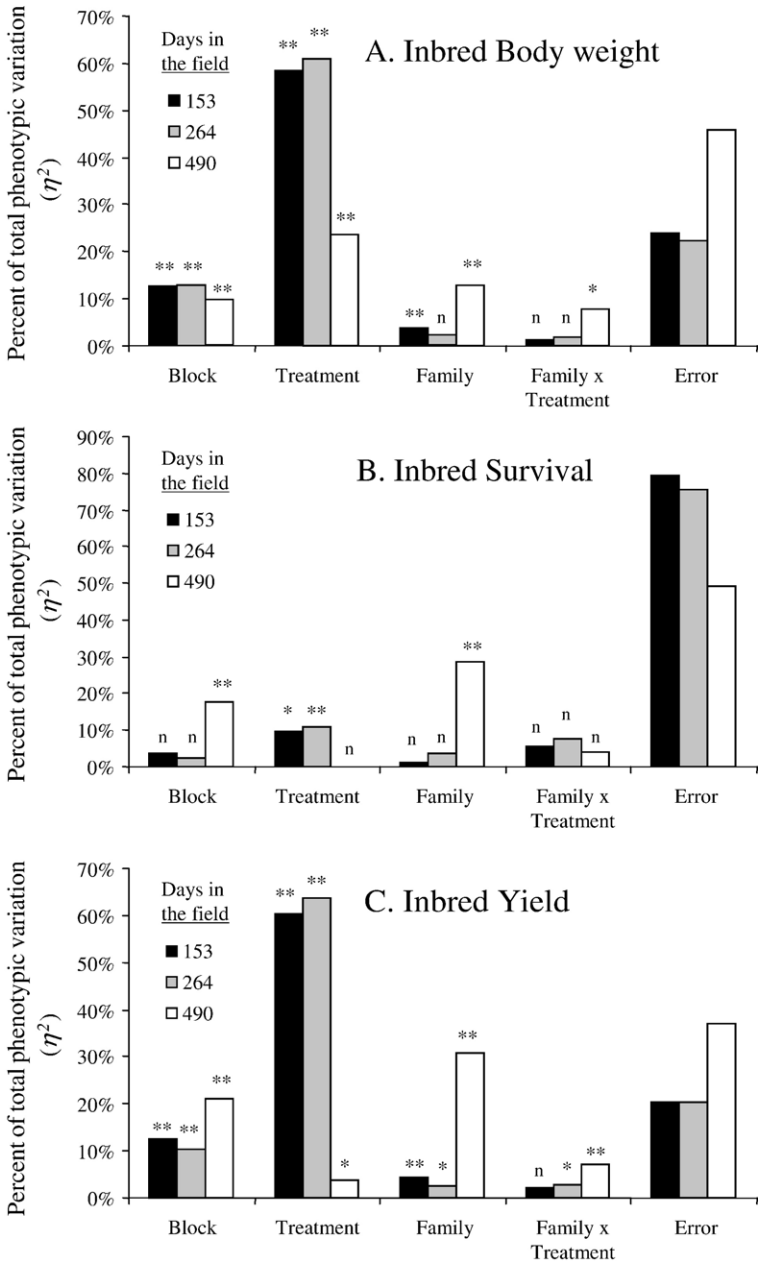


Fig. 5. Percent of total phenotypic variation among inbred families explained by block, family, nursery environment ("Treatment"), family × nursery environment, and experimental error for (A) individual body weight, (B) survival, and (C) yield. Sum of squares were used to estimate $\eta^2$ values for the three measurement periods (153, 264 and 490 days in the field). Significance of treatment effects within each sampling period is indicated by "ns" ($P>0.05$), "*" ($0.05>P>0.01$), and "**" ($P<0.01$) as determined by analysis of variance (see Table 2).

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 (Figs. 2B and 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). At harvest, after the mortality event, all main effects were significant ($P<0.0023$). The family × nursery treatment interaction, however, remained non-significant ($P=0.9464$).

Among inbred families, only the 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 the 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 (Figs. 4B and 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 in Figs. 2C and 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 2). Yield of outbred families at harvest was affected by block ($P<0.001$) and family ($P<0.001$) but not family × 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 × 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, again, 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 (Figs. 4C and 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.

## 4. Discussion

This study demonstrates that 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 the 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.

Parallel growth trajectories among all treatments after day 153, for both inbred and outbred families (Figs. 2A and 3A), and the significant effect of nursery feeding regime on body weight even after 490 days in the field (Table 2), suggest little or no compensatory weight gain in the field 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 siblings 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, dependent upon the age at which food restriction is applied, the duration of food restriction and

the severity of restriction (e.g., Lay et al., 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.

Family significantly affected survival at harvest for both inbred and outbred families (Table 2), suggesting survival may be, in part, under genetic control. These findings are consistent with the published results from the other researchers. 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). It is interesting to note that average treatment survival among animals exposed to the low feeding regime in the nursery was significantly higher than the survival in both the medium and high feeding regimes (Fig. 2). Two possible explanations for this trend are offered below.

First, it is possible that large animals at plant-out remained larger in the field, 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., 2004), some researchers have suggested that 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., 2004) 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 the observations in the present study, where greater mortality at harvest occurred among the larger oysters from the high-ration nursery treatment compared to the smaller oysters from the low-ration nursery treatment. 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 50 g) 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 experienced 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 Hale, 1997; O'Steen, 1998; Lay, 2000; Pahkala et al., 2001; Lee and Petersen, 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). 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."

Yield is a commercially important character in shellfish aquaculture (Hedgecock et al., 1997; Langdon et al., 2003; Pacific Shellfish Institute, 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, was reversed during the second growing season, when survival became the dominant component of yield. As a result, among outbred families, the treatment with the smallest animals but the highest survival (the "low" nursery feeding treatment) became the highest yielding treatment (although it did not differ significantly 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. This tradeoff resulted in no net nursery treatment effect

on harvest yield (Table 2). 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. Similarly, it is unclear how culture method (e.g. subtidal versus intertidal) may effect these results.

Evaluating inbred and outbred families allowed us to consider the effects of overall genomic homozygosity on sensitivity to permanent environmental effects. Inbred families behaved differently from outbred families in several ways. First, individual body weight and yield of inbred families were significantly affected by genotype × nursery environment interactions (Table 2), where rank changes occurred under the most stressful ("low") nursery feeding regime. 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 1). The fact that these correlations occurred only at harvest suggests that inbred and outbred families differed in their response to the mortality event during the second growing season. Lastly, inbreeding depression primarily affected harvest body weight (Outbred = 85 g; Inbred = 55 g), with little effect on harvest survival (outbred = 40%; inbred = 44%). Evans et al. (2004) attributed a similar pattern in a different cohort of *C. gigas* to the purging of individuals homozygous for early acting lethal recessive genes from the population during the larval 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. It is worth emphasizing that these results are based on only three, non-randomly selected, inbred families and conclusion inferred to a large population should be made with caution.

It is important for breeders to consider the relative effects of block, family, nursery environment, and family × 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, 1996). The more traditional approach of using mean squares to estimate variance components is, strictly speaking, only applicable to random effects. Again, 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 (Figs. 4 and 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 effect-size ($\eta_p^2$; $SS_{Family}/[SS_{Family} + SS_{Error}]$) suggests that this is true even as the relative contribution of unexplained error increases over time for body weight and yield. 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).

## 5. Conclusions

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 × 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 (i.e. genotype) 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 size-specific mortality or conditioning to nutrient stress as juveniles.

## Acknowledgements

The authors wish to acknowledge the contributions of Dave Jacobson, Sean Matson, John Brake, Ebru Onal, and Anja Robinson in the hatchery and field. Support for this research was supplied in part from the USDA-CSREES Molluscan Broodstock Program and the 2001 Anja Robinson Shellfish Fellowship. The authors also wish to thank Oregon Oyster Farm for the generous use of their facilities.

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