



The effects of inbreeding on performance traits of adult Pacific oysters (*Crassostrea gigas*)

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Abstract

Understanding the effects of inbreeding is critical to the long-term viability of shellfish breeding programs, especially as breeders attempt to develop selected lines in hatcheries with small effective population sizes. Inbreeding depression in shellfish is well documented among the offspring of selfed individuals and full-sib crosses. This study was conducted to determine if crossing more distantly related parents would result in measurable inbreeding depression of performance traits in adult *Crassostrea gigas* raised in a commercial intertidal growing environment. Families were created with expected inbreeding coefficients (F) of 0, 0.0625, and 0.203. Average family yield, individual growth rate, and survival were recorded after the first and second growing seasons. Significant inbreeding depression in yield, individual growth rate and survival was observed after the first growing season in families with $F=0.203$ ($P<0.0001$), but not in families with $F=0.0625$ ($P>0.058$). After two growing seasons, significant inbreeding depression of yield and individual growth rate was observed in families with $F=0.0625$ ($P<0.01$) and $F=0.203$ ($P<0.001$). Significant depression of survival at harvest was observed only in families with $F=0.203$ ($P=0.009$). These results emphasize the importance of maintaining pedigree records in shellfish breeding programs to help avoid the deleterious effects of inbreeding depression, even among crosses of distantly related parents.

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1. Introduction

Inbreeding depression of performance characters is pervasive among naturally out-crossing organisms (Lynch and Walsh, 1998; Falconer and Mackay, 1996), including

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many species of shellfish (Langdon et al., 2003; Bucklin, 2002; Launey and Hedgecock, 2001; Naciri-Graven et al., 2000; Bierne et al., 1998; Sheridan, 1997; Wada and Komaru, 1994; Imai and Sakai, 1961). The effects of inbreeding have become more apparent as shellfish breeders attempt to develop selected lines from hatcheries with small effective population sizes (Saavedra, 1997; Hedgecock and Sly, 1990; Newkirk and Haley, 1983). In the context of a breeding program, a small degree of inbreeding is often unavoidable in order to balance maximum selection intensity with the need to retain an adequately large pool of potential parents (Falconer and Mackay, 1996). If the per generation accumulation of inbreeding is small enough ($<2.0\%$), genetic gains made by selection may not be adversely affected (Frankel and Soulé, 1981). Various breeding schemes have been suggested to minimize the accumulation of inbreeding in small domesticated populations, including maintaining a large, randomly mating population, systematic rotational line crossing, and periodically introducing unrelated parents into the breeding program (Kincaid, 1977). Exact implementation of these breeding schemes would depend on many factors (rearing space, labor, etc.), including the target species' sensitivity to low levels of inbreeding.

Most studies examining the effects of inbreeding among oysters have evaluated progeny of one or more generations of full-sib crosses with expected inbreeding coefficients ($F \geq 0.25$) (Bierne et al., 1998; Beattie et al., 1987; Mallet and Haley, 1983; Lannan, 1980; Longwell and Stiles, 1973; Imai and Sakai, 1961) and/or selfed hermaphrodites with $F=0.5$ (Bucklin, 2002; Launey and Hedgecock, 2001; Hedgecock et al., 1995). These studies reported significant inbreeding depression of growth and/or survival during larval and adult stages (for an exception, see Lannan, 1980). The effects of crossing more distantly related parents (such as first cousins) on performance traits in adult *Crassostrea gigas* remain uncertain. Experimental results from other naturally outcrossing species have demonstrated an inverse linear relationship between phenotypic expression and F (Lynch and Walsh, 1998; Falconer and Mackay, 1996). Organisms displaying this linear response to inbreeding range from maize (e.g. Hallauer and Sears, 1973) to mammals (e.g. Templeton and Read, 1983), including humans (Barrai and Cavalli-Sforza, 1964). These findings suggest that any degree of inbreeding ($F > 0$) in oysters could result in depression of performance characters. The resources dedicated to minimizing the accumulation of inbreeding will be dictated, in part, by the sensitivity of the target species to inbreeding. The present study was designed to determine the effects of low levels of inbreeding ($F=0.0625$ and $F=0.203$) on individual growth rate, survival, and yield in adult *C. gigas* grown under commercial conditions.

2. Methods

Adult Pacific oysters (*C. gigas*) from nine pedigreed families were crossed in a 9×9 half-factorial matrix at the Hatfield Marine Science Center (HMSC), Newport, OR, as per Langdon et al. (2003) to produce a total of 41 families. Fig. 1 illustrates the pedigrees of the families used. All families evaluated in this study originated from a naturalized population located in Dabob Bay, WA, USA. These wild individuals, referred to as G_0

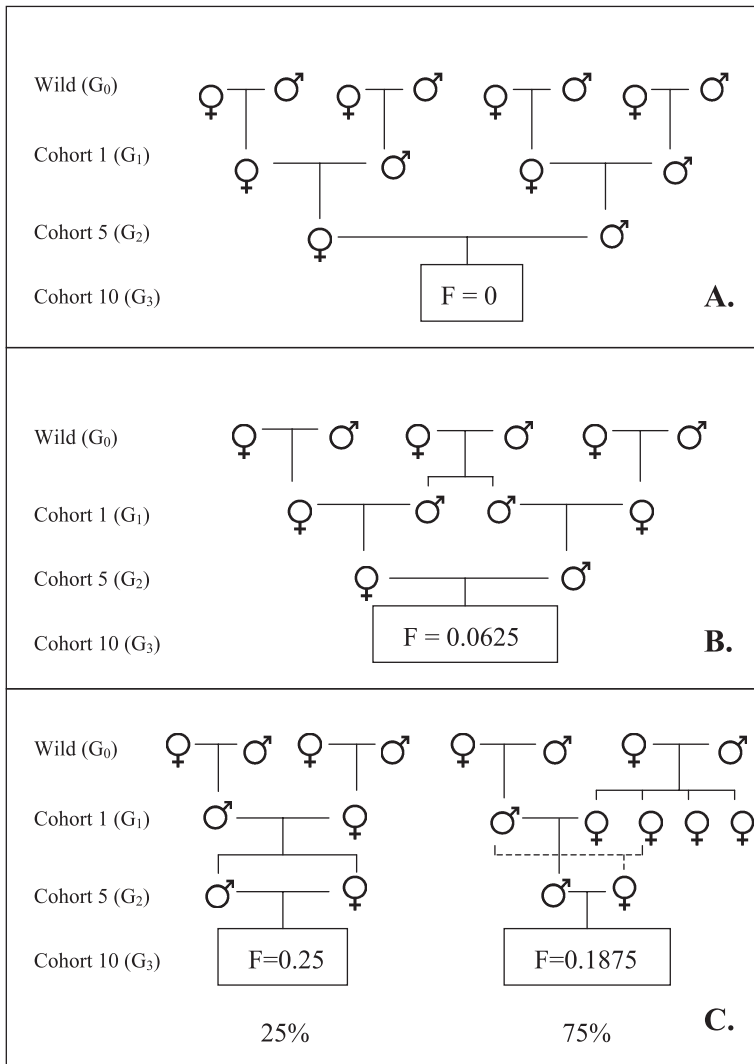


Fig. 1. Pedigrees of families used to generate three levels of inbreeding: (A) $F = 0$, (B) $F = 0.0625$, (C) $F = 0.203$. All families were derived from a naturalized population in Dabob Bay, WA, USA. Wild animals (G₀) were crossed in 1996, G₁ individuals crossed in 1998, and G₂ individuals crossed in 2001.

animals, were pair-spawned to create the G₁ families. Subsequent crosses used to produce the G₂ and G₃ families were conducted by mating one male of a particular family with four females of another family. As indicated in Fig. 1, no inbreeding, relative to the G₀ base population occurred until the G₃ population, which consisted of 19 crosses of unrelated parents ($F = 0$; Fig. 1A), 13 crosses of first-cousins ($F = 0.0625$; Fig. 1B) and 9 crosses of siblings ($F = 0.203$; Fig. 1C). F was estimated based on methods described in Hartl and Clark (1997) and can be interpreted in two ways: (1) as the probability that a specific locus

is homozygous and the alleles are identical by descent (IBD); or (2) as the proportion of all loci in an individual that are IBD. Families developed for this experiment, therefore, had an expected 0%, 6.25% and 20.3% of their total genome IBD, for outbred crosses, first-cousin crosses and siblings crosses, respectively. The estimate of F for crosses among siblings was complicated by the fact that four females were crossed with a single male to produce the G_3 families. As a result, two levels of inbreeding were possible (Fig. 1C): (1) both parents were truly full-sibs, sharing a common mother and father (resulting in progeny with $F=0.25$); or (2) the parents shared a common father (known) and common maternal grandparents (resulting in progeny with $F=0.1875$). For the purpose of this study, it was assumed on average 25% of the offspring had $F=0.25$ and 75% of the offspring had $F=0.1875$, therefore the weighted average F for all sibling crosses was expected to be 0.203. It should be noted, however, that actual levels of homozygosity may differ from expected for several reasons (Vrijenhoek et al., 1990), including unequal contribution from female parents (Gaffney et al., 1992), selection against offspring homozygous for lethal recessives (e.g. Bucklin, 2002; Launey and Hedgecock, 2001; Biene et al., 1998), and the effect of strong inbreeding (e.g. full-sib crosses) on the homozygosity of large chromosomal fragments (Strauss, 1986). Unfortunately, tissue samples were not taken from offspring so actual parental contribution could not be assessed, nor could estimates of homozygosity be made using molecular techniques (McGoldrick and Hedgecock, 1997).

Larval and nursery culture methods followed those outlined by Langdon et al. (2003). One hundred oysters (6.3 mm minimum shell length) were randomly collected from each family, weighed and stocked into each of 10 replicate spat bags (2 mm mesh) in the Spring of 2001. These spat bags were then inserted into larger 6.3 mm mesh grow-out bags. The oysters were placed at an intertidal site (0 m mean low water) in Totten Inlet, Puget Sound, WA. Each family had two replicate oyster bags within each of five blocks, arranged to account for variation in growing environment due to tidal height. Due to low numbers of spat exiting the nursery system, some families had fewer than 10 replicates bags planted in the field.

Data were collected on all families at an interim point in Fall 2001 (157 days after planting) and at the final harvest in Fall 2002 (570 days after planting). Oysters were removed from the 2-mm mesh spat bags at day 157 and stocked directly into the 6.3-mm mesh grow-out bags. At both time periods, oysters were cleaned of fouling organisms and mud, counted, and total oyster bag weight recorded to the nearest gram. These measurements allowed replicated estimates of average individual body weight (g live wt), survival (%) and yield (kg live oyster wt bag⁻¹) to be determined for each family.

Prior to data analysis, average individual body weight, survival and yield were adjusted to account for variation in initial planting weight (Langdon et al., 2003). General linear model (GLM) analysis and linear contrasts (SAS Institute, 2000, Cary, NC, USA) were used to determine if families with a different F differed in phenotypic expression after accounting for variation due to tidal block. Regression analysis was used to determine if the rate of phenotypic depression per unit increase in F was linear or quadratic. Broad-sense genetic correlations between individual body weight and survival at harvest were determined based on the correlations of average family scores for each trait (Lynch and

Walsh, 1998). Normality was assessed for all performance traits using the Kolmogorov–Smirnov test (SPSS, 2000, Chicago, IL, USA).

3. Results

All interim (day 157) performance characters were positively correlated with initial planting weight ($n=404$, $P<0.018$, $r^2=0.027$ to 0.055) and consequently adjusted to a common plant-out weight (Langdon et al., 2003). At harvest, only average family survival ($n=402$, $P<0.001$, $r^2=0.045$) and yield ($n=402$, $P<0.001$, $r^2=0.033$) required adjustment due to variation in initial planting weight. Yield was normally distributed at the interim weighing and at harvest ($P=0.200$). Individual body weight was normally distributed at the interim weighing ($P>0.200$) and normally distributed at the harvest weighing after the removal of a single outlying case ($P>0.090$). Survival was normally distributed at both time periods after a square-root arcsine transformation (Sokal and Rohlf, 1995; $P>0.090$).

Interim average individual body weight, survival and yield are summarized in Table 1. Highly significant block and family effects were detected for all characters (GLM, $P<0.0001$). Blocks higher in the intertidal zone generally exhibited lower survival, lower average body weight and lower yield, than blocks lower in the intertidal zone. Linear contrasts showed that families with $F=0.0625$ did not significantly differ from families $F=0$ for yield ($P=0.062$), individual body weight ($P=0.058$) and survival ($P=0.254$). Families with $F=0.203$, however, performed significantly poorer than families with F of either 0 or 0.0625 for all characters ($P<0.0001$).

Individual body weight, survival and yield at harvest are also summarized in Table 1. Again, highly significant block and family effects were detected for all characters (GLM, $P<0.0001$). Similar to interim measurements, block effects appeared to be a function of tidal height. Linear contrasts showed all three groups of families differed significantly for yield ($P<0.01$) and individual body weight ($P<0.001$). No significant difference between

Table 1
Average yield, individual body weight and survival for families with different estimated inbreeding coefficients (F)

Day	F	Number of families	Yield, kg/bag (S.D.)	Average individual body weight grams (S.D.)	Survival, % (S.D.)
157	0.0	19	0.367 (0.130) ^a	5.571 (1.203) ^a	0.65 (0.171) ^a
157	0.0625	13	0.390 (0.122) ^a	5.771 (1.152) ^a	0.673 (0.159) ^a
157	0.203	9	0.312 (0.121) ^b	5.251 (0.972) ^b	0.581 (0.169) ^b
570	0.0	19	4.507 (1.438) ^a	93.816 (13.707) ^a	0.484 (0.143) ^a
570	0.0625	13	4.128 (1.206) ^b	85.94 (12.297) ^b	0.483 (0.136) ^a
570	0.203	9	3.398 (1.251) ^c	77.478 (13.721) ^c	0.442 (0.144) ^b

Measurements were taken 157 (interim) and 570 (harvest) days after planting on an intertidal commercial grow-out site. Standard deviations (S.D.) are in parentheses. Means with common superscripts do not differ based on linear contrasts (general linear model, SAS; $P=0.05$ level of significance), within each phenotype and time period.

Table 2

Summary table of regression model parameters describing the expression of body weight, survival and yield at harvest as a function of inbreeding coefficient ($n=402$)

Character	Intercept	Slope	r^2	P (slope>0)
Body weight (g)	92.95	- 82.15	0.192	<0.0001
Survival (%)	44.48	- 12.61	0.014	0.020
Yield (kg bag ⁻¹)	4.53	- 5.58	0.101	<0.0001

families with $F=0$ and families with $F=0.0625$ was detected for survival ($P=0.909$). A significant difference in survival existed between families with $F=0.203$ and families with either $F=0$ ($P=0.009$) or $F=0.0625$ ($P=0.019$).

The three levels of expected inbreeding created in this study allowed regression analysis to statistically test whether the measured decrease in phenotype with increased F followed a linear or a quadratic trend. In all cases, phenotype decreased linearly with F ($P<0.024$), while the quadratic term was never significant ($P>0.05$). Simple linear regression model parameters for body weight, survival, and yield are summarized in Table 2. The degree of phenotypic depression with increased F varied among traits. The slopes presented in Table 2 predict a 10% increase in F will result in an 8.80% decrease in individual body weight, a 4.26% decrease in survival and a 12.23% decrease in yield. Fig. 2 illustrates the percent change in phenotypic expression, relative to families with $F=0$, for all traits due to different degrees of inbreeding. Statistically insignificant broad-sense genetic correlations (r_G ; Lynch and Walsh, 1998) at harvest between average family individual body weight and average family survival were observed among families with $F=0$ ($r_G=0.01$, $P=0.968$, $n=19$), $F=0.0625$ ($r_G=-0.262$, $P=0.396$, $n=13$), and $F=0.203$ ($r_G=0.228$, $P=0.569$, $n=9$).

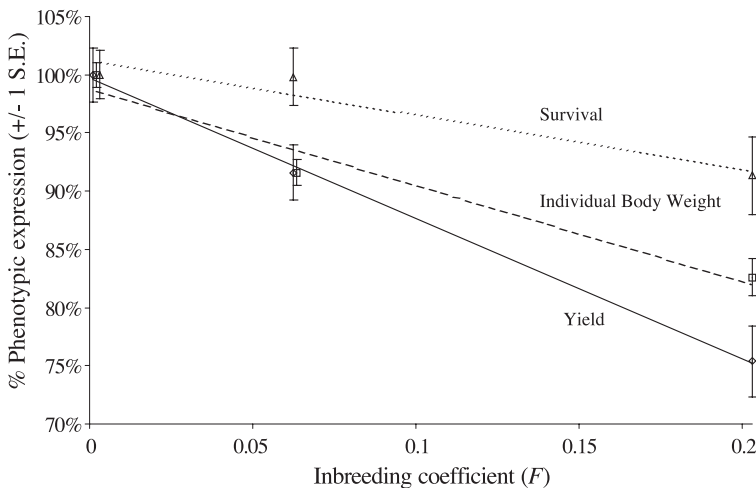


Fig. 2. The effect of inbreeding coefficient (F) on phenotypic expression for body weight, survival and yield relative to families with $F=0$ (phenotypic expression=100%). Regressions lines generated from $n=402$ (see Table 2 for model parameters). Bars represent 1 standard error above and below treatment means.

4. Discussion

These results support previous findings that, like many other naturally outcrossing species, performance traits of Pacific oysters are significantly affected by relatively low levels of inbreeding. Inbreeding depression of all traits at the interim measurement (157 days in the field) was only detected in families with $F=0.203$ (Table 1). At harvest, inbreeding depression was also detected in yield and individual body weight in families with $F=0.0625$. A similar effect of increasing depression of body weight with age in several species of fish was noted by Kincaid (1983) and attributed to the cubic nature of the growth curve magnifying differences in growth rate over time. Additionally, measurement error likely had much more impact when recording weights of small oysters (5–6 g individual body weight) versus larger oysters (75–100 g individual body weight). As a result, this discussion will focus on the effects of inbreeding on phenotypic depression as observed in adult oysters at harvest (570 days in the field).

Traits closely associated with fitness typically display significant depression in response to inbreeding (Lynch and Walsh, 1998; Falconer and Mackay, 1996). Body size, while considered a morphometric trait in endotherms, is more closely associated with fitness in ectotherms, including shellfish, due to the strong relationship between body size and fecundity (Rawson and Hilbish, 1990; Mousseau and Roff, 1987). The average percent reduction of body weight at harvest in families with $F=0.203$ was approximately 17%, consistent with the results of Beattie et al. (1987) who found, on average, a 21% depression in wet body weight in 2-year-old *C. gigas* families with $F=0.25$. The sensitivity of individual body weight in Pacific oysters to inbreeding in this study (8.8% phenotypic reduction per 10% increase in F) tended to be higher than terrestrial species (0.6% to 4.3%; Falconer and Mackay, 1996), but similar to European oysters (10.7%; Naciri-Graven et al., 2000), carp (6%; Moav and Wohlfarth, 1963), rainbow trout (5.12% to 9.3%; Gjerde et al., 1983; Kincaid, 1977; Bridges, 1973), and brook trout (13.8%; Cooper, 1961). The linear relationship between reduction in body weight and estimated F observed in this study is common among naturally outcrossing organisms (Lynch and Walsh, 1998; Falconer and Mackay, 1996) and is consistent with both dominant and over-dominant theories explaining inbreeding depression. Nonlinearity is expected to occur only when epistatic gene action affects inbreeding depression, including the effect of inbreeding on maternal effects (Kincaid, 1983). The linear relationship between inbreeding depression and F suggests that any amount of inbreeding could result in phenotypic depression. However, at low levels of F , depression may not be measurable or, if measurable, may be insignificant when compared to gains made by selection.

Survival is a trait closely associated with fitness (Falconer and Mackay, 1996), and the apparent lack of inbreeding depression among families with $F=0.0625$ and the relatively small amount of depression among families with $F=0.203$, is at first counter-intuitive. The data are consistent, however, with recent findings that oysters carry a very high genetic load (at least 12–14 lethal genes per individual), and that mortality due to IBD lethal recessive genes occurs primarily between larval settlement and 3 months of age (Bucklin, 2002; Launey and Hedgecock, 2001; Bierne et al., 1998). It is possible that by the time oysters from inbred families in this study were planted out in the field, individuals with IBD lethal recessive alleles may have already been purged from the population. If this were the case,

inbred families evaluated in the field would have actual average levels of F (at loci affecting survival) lower than expected had there been no selection against individuals carrying the early-acting lethal IBD alleles. This is consistent with the results observed in this study, where inbred families showed lower than expected depression of survival as adults. The fact that the effect of F on survival was significant (Table 2) suggests either the purging of individuals carrying IBD lethal alleles was incomplete in the nursery (allowing these individual to die in the field) or the genetic correlation between survival in the nursery and survival in the field is < 1 , and late-acting IBD lethal alleles, specifically targeting adult survival, were expressed in the field.

Purging IBD lethal alleles in the nursery is not expected to affect inbreeding depression of body weight in the field, as observed here, if the genes controlling survival segregate independently from genes controlling body weight, and if little or no selection for body weight occurred in the nursery. Under these conditions, deleterious alleles affecting growth rate would remain in the population and be expressed in the field. Independent expression of these two traits in the field is supported by the statistically insignificant genetic correlations (r_G) at harvest between average family individual body weight and average family survival for all levels of inbreeding.

Yield is a composite trait made up of two primary causal components: individual body weight and survival. The effect of inbreeding on yield is therefore a product of the effects of inbreeding on both individual body weight and survival. As expected, the effects of inbreeding depression of individual body weight and survival had a cumulative effect on depression of yield (Fig. 2). For example, based on slopes generated through regression analysis (Table 2), a 10% increase in F , will result in an 8.80% decrease in average body weight and a 4.26% decrease in survival. If the depression of body weight and survival act independently to depress yield, then we would expect a 12.7% depression in yield. This compares favorably with the observed reduction in yield of 12.2% for a 10% increase in F . Any increase or decrease in the sensitivity of the causal components to inbreeding would directly affect the composite character (i.e. yield). The genetic complexity of composite traits such as yield is widely recognized (Ceccarelli et al., 1991; Baker, 1987; Blum, 1985). It is also recognized that as the number of non-interacting causal components of a composite character increase, so does the likelihood of experiencing genotype \times environment interactions (Baker, 1987). Many traits also show increased phenotypic depression for a given level of inbreeding in more stressful environments (Lynch and Walsh, 1998). As a result, it is likely that the effect of inbreeding on composite characters such as yield will be strongly affected by environmental variation, either spatially or temporally.

Understanding the importance of inbreeding in shellfish is essential to the design of efficient long-term breeding strategies. The amount of inbreeding that can be tolerated within a breeding program depends on the species and the character under consideration. Kincaid (1977) reported inbreeding in trout did not become problematic until $F=0.18$, and offered a rotational line crossing design to avoid mating full-sibs but allow the crossing of first-cousins. Frankel and Soulé (1981) suggested a more conservative rate of inbreeding accumulation of 1% per generation, precluding the crossing of double first-cousins (resulting in offspring with $F=0.125$) and first-cousins (resulting in progeny with $F=0.0625$). Data presented here indicate significant inbreeding depression in *C. gigas*

can occur when parents share a common pair of grandparents. A more acceptable rate of inbreeding accumulation would result from crossing individuals that share a common pair of great-grandparents, resulting in progeny with $F=0.0156$. A 7.9% reduction in yield resulting from first-cousin matings ($F=0.0625$) could greatly decrease the expected response to family-based selection in oysters (e.g. 9.5% increase in yield per generation, Langdon et al., 2003). These results suggest that maintaining pedigree records to avoid crossing even distantly related individuals is critical to the long-term sustainability of shellfish breeding programs.

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