Improvements in desirable traits of the Pacific oyster, *Crassostrea gigas*, as a result of five generations of selection on the West Coast, USA.

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Abstract

The Pacific oyster, *Crassostrea gigas*, is one of the most important global aquaculture species due to its potential high growth rates and tolerance of a wide range of environmental conditions; however, most farmers use seed from wild, non-domesticated stocks for production. In this study we estimated genetic parameters for performance traits of oysters from the Molluscan Broodstock Program (MBP), a family-based selective breeding program designed to improve yields of Pacific oysters on the West Coast, USA. Covariance components were obtained using AIREMLF90, a multiple-trait animal model. A total of 15,236 records were analyzed from five generations of selectively bred oysters. Heritability estimates for field traits at harvest were all positive over the five analyzed generations, ranging from 0.58 ± 0.03 (3rd generation) to 0.30 ± 0.04 (5th generation) for yield, from 0.55 ± 0.03 (1st generation) to 0.12 ± 0.02 (4th generation) for survival, and from 0.51 ± 0.03 (2nd generation) to 0.40 ± 0.03 (4th generation) for mean individual weight at harvest (growth). Declines in heritabilities for survival and yield in the 4th and 5th generations were perhaps a result of changing ocean conditions due to increased upwelled hypoxic and acidified seawater occurring on the West Coast, USA, that affected the quality of seed from the MBP hatchery/nursery. Realized heritabilities were all positive and medium-to-high across generations, ranging to 0.11 ± 0.42 (for survival in the 3rd generation) to 1.20 ± 0.35 (for individual weight in the 5th generation); however, standard errors for realized heritabilities were high (ranging 0.15 to 1.47), especially in the 5th generation. There was a gradual

improvement in genetic gains for survival and yield over the five generations of selection, resulting in accumulated gains of +15.7% and +19%, respectively, in the 5th generation; however, little improvement was achieved after the 2nd generation in genetic gain for individual weight, with an accumulated gain in the 5th generation of +11.3%. Realized gains for performance traits were less than predicted by genetic gains. Realized gain in survival consistently improved over the selection period, resulting in an accumulated gain of 11.7% in the 5th generation compared to that of wild, non-selected controls; however, gains in individual weight and yield at harvest were not consistent across generations and gains in the 5th generation were -9.8% and 0%, respectively, compared with those of controls, perhaps due to inconsistent genetic quality of control broodstock sampled from wild populations. Across generations, there were positive genetic correlations between yield and both survival (0.38 ± 0.04) and individual weight (0.90 ± 0.01) as well as between survival and individual weight (0.25 ± 0.04). Positive medium-to-high genetic correlations among harvest traits suggest that indirect gains in yield can be achieved by selection for either higher growth or survival.

Keywords: oyster, heritability, genetic gain, variance components, breeding program

1. Introduction

The Pacific oyster (*Crassostrea gigas*, Thunberg, 1793) is one of the most widely farmed aquaculture species worldwide (FAO, 2014). This shellfish species is fast growing and displays a wide tolerance to different environmental conditions, making it ideal for farming in many regions of the world (FAO, 2014). However, worldwide production is mainly based on unimproved populations or stocks (Gjedrem et al., 2012), likely limiting profitability and expanded production of this species due to the unrealized potential for genetic improvement.

Shortly after development of hatchery techniques for oyster production in the 1970s, Lannan (1972) reported a positive heritability of 0.37 for growth in adult *C. gigas*. This estimate is similar to that reported in the USA for body weight at harvest (0.31 ± 0.08 ; Evans and Langdon, 2006a) and for various growth parameters of *C. gigas* in different parts of Asia: China (0.33 ± 0.03), Japan (0.40 ± 0.02) and South Korea (0.1 ± 0.03) (Li et al., 2011). Estimated heritabilities for growth are strongly affected by rearing conditions and source populations for broodstock, as demonstrated by Langdon et al. (2003) who observed heritability values for yield

ranging from 0.22 to 0.77 for *C. gigas* cultured in different locations in the Pacific Northwest, USA.

Family-based selection programs for improving desirable traits of the Pacific oyster have been initiated in the USA (Evans and Langdon, 2006b; Langdon et al., 2003), France (Dégremont et al., 2010, 2007), China (Li et al., 2011; Wang et al., 2012) and Australia (Kube et al., 2011), obtaining genetic gains per generation between 7.2% for growth (Li et al., 2011) to 25.5% for yield (Langdon et al., 2003). In Australia, Nell et al. (1999, 1996) obtained genetic gains of 4% and 8% for individual weight in the first and second generation of mass selection, respectively, for the rock oyster *Saccostrea commercialis*.

Positive heritability values and genetic gains reported for oysters and a variety of other bivalve species across the world indicate that selection has the potential to improve desirable traits of Pacific oysters. In this study, we report on heritabilities, genetic correlations as well as genetic and realized gains for yield, survival and individual weight at harvest, obtained after five generations (17 years) of selective breeding with 1,235 families of Pacific oysters planted at 11 different sites on the West Coast, USA (Table 1; Supplementary Table S1).

2. Material and methods

2.1. Husbandry of MBP families and control groups

MBP families were selected and produced according to methods described by Langdon et al. (2003). Briefly, parental families were selected based on their yields at harvest. Pair sire/dam matings were carried out between individuals from unrelated families to maintain a low coefficient of coancestry ($\theta_{ij} < 0.1$). Individual parents used for crosses were selected from within each family based on size and shell shape, and were typically from the top third largest oysters per family. From 50 to 100 families were simultaneously produced and included in each cohort. Beginning in the 2nd generation, groups of control crosses were also incorporated in each cohort by pooling 5 to 10 single pair matings among individuals collected from naturalized “wild” populations in the Pacific Northwest. These naturalized stocks were mainly sourced from Willapa Bay (46°33'00.0"N, 123°58'12.0"W) because populations from this bay were primarily used by commercial hatcheries for their broodstock, but some cohorts included progeny from broodstock collected from Dabob Bay (47°45'02.1"N, 122°50'00.3"W) in Washington State

(WA) as well as from Pipestem Inlet (49°01'36.0"N 125°15'36.3"W) on Vancouver Island, British Columbia, Canada.

Cohorts were produced by rearing separate families from larval to early juvenile (spat) stages at Oregon State University's facilities at the Hatfield Marine Science Center (HMSC) in Newport, Oregon, USA. Larval and spat cultures for each family were not replicated due to limited space and labor in the hatchery/nursery as well as to meet the goal of maximizing the number of families included in the breeding program. In support of this approach, Evans and Langdon (2006a) reported that variation among replicates during this early phase of family rearing contributed only 1.5%, 0.3% and 5.2% to the total phenotypic variance (10%, 6% and 14% expressed in terms of family variance) in yield, average individual weight and survival at harvest, respectively.

Larval and spat culture techniques were based on those described by Langdon et al. (2003). Briefly, larvae were raised for 2-3 weeks on an algal diet of *Isochrysis galbana* (Tahitian strain T-ISO) and *Chaetoceros* sp. Larvae were induced to metamorphose using epinephrine. Single spat were reared in upwellers for 2-3 months until they were 3 to 5 mm in size. At this size, they were either transferred to a nursery in Yaquina Bay to grow to 10-15 mm in size before planting at field test sites in the 1st and 2nd generations or, in subsequent generations, directly planted at field test sites at a small size (3 - 5 mm; see Table 2 for initial weights at planting).

Test sites were located on commercial farms in the western coastal states of Alaska, Washington, Oregon and California, USA (Fig. 1). At each site, 35 to 100 spat were placed in grow-out containers (bags or purses at inter-tidal sites or lantern net compartments at sub-tidal sites; Fig. 2; Supplementary Table S1) at densities that were below typical farm densities of adult oysters at harvest. Oyster densities were not adjusted during the plant-out period. Typically, two replicate grow-out containers were randomly placed within each of four or five blocks that were positioned across different tidal heights at inter-tidal sites or at different depths at sub-tidal sites (Langdon et al. 2003).

Spat were initially planted in small-mesh (2 – 4 mm) bags, but as the spat grew, they were transferred to larger-meshed containers to maximize the exchange of water and supply of suspended food. Containers were cleaned of fouling organisms at each bag change and dead spat removed. After 2 to 4 years, depending on grow-out conditions, oysters were harvested when they reached market size, typically with shell lengths of 5 to 10 cm. At harvest, the total weight

of all living oysters (yield) and the number of live oysters per container (survival) were determined and from these values, average individual weights were estimated per family.

2.2. Statistical models

Performance records for family yield (kg), survival (%) and individual mean weight (g) at harvest, as well as initial weight (g) at planting, were obtained for five MBP generations of selectively bred Pacific oysters and 23 cohorts produced between 1996 and 2012 (Fig. 2). The cohorts were planted at 11 sites in Oregon, Washington, California, and Alaska, USA (Fig. 1).

Fig. 1

Fig. 2

After eliminating a total of 86 outlier records (containers of oysters at harvest), using descriptive and graphical analyses to identify biologically improbable values, 15,236 records of field performance remained for statistical analyses (Table 1). These data were analyzed using a linear multiple-trait animal model. Only significant ($P < 0.05$) fixed effects were included in the model, based on analysis of variance. The model can be represented by:

$$y_{ijklmnop} = S_i + TE_j + G_k + Year_l + C_m + (S \times C \times TE)_n + b_1 \gamma_{ijklmnop} + b_2 \gamma'_{ijklmnop} + block_o + f_p + e_{ijklmnop} \dots \dots \dots [\text{Eq. 1}]$$

Where $y_{ijklmnop}$ is the yield, survival and individual harvest weight of family p , planted at site i , at tidal exposure (sub-tidal or intertidal) j , in generation k , year l , spawned in cohort m , cultivated in $block\ o$. Where, S_i is test site; TE_j is tidal exposure (sub-tidal or intertidal); G_k is generation; $Year_l$ is year of spawn; C_m is cohort; $(S \times C \times TE)_n$ is an interaction effect of site, cohort and tidal exposure (sub-tidal or intertidal) used to adjust for planting time and planting duration for each cohort at each site; $block$ refers to planting position relative to tidal height at inter-tidal sites or depth at sub-tidal sties; b_1 is the coefficient of linear regression for yield, survival and individual harvest weight as a function of the initial weight at planting (co-variable); b_2 is the coefficient of linear regression of yield as a function of the density of oysters per

container at harvest; f_p is the random additive genetic effect of family; $e_{ijklmnop}$ is random error. For intra-generational analyses, the fixed effect for “generation” was removed from the model. For genetic estimates related to survival and individual harvest weight, we removed the co-variable “oyster density at harvest” from the model.

The model above can be written in matrix form as:

$$\mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{a} + \mathbf{e} \quad [\text{Eq. 2}]$$

where:

$\mathbf{Y}$ is the vector of observations (yield, survival and individual harvest weight);

$\mathbf{X}$ and $\mathbf{Z}$ are incidence matrix parameters for fixed effects, and random additive genetic effects, respectively.

$\boldsymbol{\beta}$ is the vector of fixed effects;

$\mathbf{a}$ and $\mathbf{e}$ are the vectors of additive genetic and residual random effects, respectively.

$$\text{Assume } \begin{bmatrix} \mathbf{Y} \\ \mathbf{e} \end{bmatrix} \sim \mathbf{N}(\mathbf{0}, \mathbf{V}), \quad \mathbf{V} = \begin{bmatrix} \mathbf{G} \otimes \mathbf{A} & \mathbf{0} \\ \mathbf{0} & \mathbf{R} \end{bmatrix} \quad [\text{Eq. 3}]$$

in which $\mathbf{G}$ and $\mathbf{R}$ are, respectively, the additive genetic and residual matrix; $\mathbf{A}$ is the relationship matrix among all animals in the pedigree file; $\otimes$ is the Kronecker product between matrices.

The covariance components were obtained with the AIREMLF90 program, which uses second derivative REML with extra heuristics (Miszta et al., 2002, Miszta, 2008).

Table 1

2.3. Heritabilities

The additive ($\hat{\sigma}_a^2$) and residual ($\hat{\sigma}_e^2$) variances were estimated within and across generations. Phenotypic variance was calculated as $\hat{\sigma}_p^2 = \hat{\sigma}_a^2 + \hat{\sigma}_e^2$ [Eq. 4]. Heritability ($\hat{h}^2$) was calculated as $\hat{h}^2 = \hat{\sigma}_a^2 / \hat{\sigma}_p^2$ [Eq. 5].

Realized heritability ($\hat{h}_r^2$) estimates for performance traits were calculated for each cohort as: $\hat{h}_r^2 = SR/SSD$ [Eq. 6] where $\hat{h}_r^2$ is the realized heritability (Falconer and Mackay,

1996, Langdon et al., 2003), SR is the standardized response to selection, and SSD is the standardized selection differential.

The standardized response is the least square means trait value for all MBP families, minus the least square means trait for all (unselected) control groups, divided by the standard

deviation among the (unselected) control groups: $SR = \frac{\bar{X}_{MBP} - \bar{X}_{control}}{\sigma_{P(control)}}$ [Eq. 7].

The standardized selection differential was calculated as: $SSD = \frac{\bar{X}_{selected} - \bar{X}_{population}}{\sigma_{P(population)}}$ [Eq. 8].

Where $\bar{X}_{selected}$ is the trait least square means of families from a cohort that were selected to produce the next generation, $\bar{X}_{population}$ is the least square means trait of all the MBP families in the cohort and any additional families that were selected to serve as parents of the next generation. The $\sigma_{P(population)}$ is the standard deviation among families included in the population group mean. Realized heritability estimates ($\pm$ SE) across and within generations were calculated from average cohort values for each generation.

2.4. Genetic and realized gains

Best Linear Unbiased Predictors (breeding values, BLUP) for performance traits were obtained using estimates of heritabilities within and across generations, using the AIREMLF90 program (Misztal et al., 2002, Misztal, 2008). The predicted responses to selection were estimated by linear regression analysis of MBP family breeding values (BLUP) for performance traits (yield, survival and individual weight) as a function of generation. Genetic gains were

calculated as the ratio (percentage) of the regression coefficients ($\hat{b}$) of the mean of breeding values of MBP families in each generation to least square means (Lsmean) of each trait, according to Maluwa and Gjerde (2007). Therefore, the accumulated genetic gains, expressed as

percentage values, in the 5th were calculated as: $Dg = \left(\frac{\hat{b} \times 5}{\text{Lsmean in 1}^{st} \text{ generation}} \right) \times 100$ [Eq. 9].

Realized gains were calculated as: $\frac{(\bar{X}_{MBP} - \bar{X}_{control})}{\bar{X}_{control}} \times 100$ [Eq. 10], where $\bar{X}_{MBP}$ and $\bar{X}_{control}$ are

least square means for performance traits of MPB families and control groups, respectively. Only cohorts with both MBP families and control groups were used to calculate realized gains.

3. Results

Least square means for yield, survival and individual weight at harvest generally increased from the 1st to 5th generation for both MBP families and control groups (Table 2). The overall least square mean values for yields of MBP families and control groups were 2.31 ± 1.12 and 1.81 ± 1.41 kg per container, respectively; equivalent to a 27.6% improvement in overall yields of MBP families compared with control yields.

Initial weights of spat at planting were greater in the 1st and 2nd generations and smaller in subsequent generations. Initial weight was partly influenced by the rearing and planting schedule of the breeding program. In early cohorts, spat were often over-wintered in the nursery and were, therefore, larger at planting in spring compared to spat of later cohorts that were planted in the summer or fall of the same year that they were spawned. Analysis indicated that initial weight had no significant effect ($P > 0.05$) on estimated gains and heritabilities.

Table 2

Estimates of covariance components for performance traits were generally positive across generations (Supplementary Table S2); however, negative values were found between individual weight at harvest and survival in the 1st (-37.45 ± 14.11) and 4th (-5.98 ± 6.93) generations. Negative values were also observed for residual covariance for individual weight and survival in all generations except in 5th generation, suggesting that undetermined environmental factors had opposite effects on each of these traits.

Heritability values (Fig. 3 and Table 3) for yield and individual harvest weights were consistent from the 1st to 3rd generations but estimates for survival were more variable. Heritabilities for all traits decreased from the 3rd to the 4th and 5th generations (Fig. 3). The overall heritability estimates over five generations were 0.59 ± 0.01 , 0.35 ± 0.02 , 0.55 ± 0.01 for

yield, survival and individual harvest weight, respectively. In our study, the total number of records was large (15,236; Table 1) resulting in low standard errors for heritabilities (smaller than 0.04, Fig. 3 and Table 3).

Fig. 3

Intensities of selection (SSD) used to calculate realized heritabilities were 0.81, 1.24, 1.51 and 0.46, respectively, for the 2nd, 3rd, 4th and 5th generations (Table 2). Realized heritability values (Fig. 4) for yield, individual weight and survival were medium-to-highly positive and consistent across generations, except for an apparent increase in the 5th generation, although the SE values were high for these values. The overall realized heritabilities across generations were 0.55 ± 0.24 , 0.40 ± 0.25 and 0.68 ± 0.22 for the yield, survival and individual weight at harvest, respectively.

Fig. 4

Genetic additive correlations (Table 3) for the 2nd, 3rd and 5th generations were all positive and ranged from medium (0.14 ± 0.08) between survival and individual weight in the 3rd generation to high (0.96 ± 0.01) between survival and yield in the 2nd generation. In the 1st and 4th generations, there were negative correlations of -0.32 ± 0.10 and -0.09 ± 0.10 , respectively, between survival and individual weight at harvest. The average correlation estimates for all data records (five generations) were all positive and ranged from 0.25 ± 0.04 between survival and individual weight to 0.90 ± 0.01 between yield and individual weight (Table 3).

Table 3

The predicted accumulated genetic gains for performance traits obtained after five generations of selection are shown in Fig. 5. The angular coefficients estimated by linear regression analysis of MBP family breeding values (BLUP) as a function of generation, were +0.09 kg, +1.89%, +2.27 g for yield, survival and individual weight at harvest, respectively. The accumulated genetic gains over these five generations calculated by linear regression, for yield,

survival and individual weight at harvest were, respectively, +19%, +15.7% and +11.3%. The realized gains across five generation were lower than estimated genetic gains at +0%, +11.7% and -9.8%, respectively, for yield, survival and individual weight at harvest.

Fig. 5

4. Discussion

In this study, the number of families was large (1,235 families with 15,236 records; Table 1) and this extensive database provided considerable information for estimation of variance components using an animal model. Another important characteristic of this dataset was its spatial and temporal reach. These data covered 17 years of the MBP program (from 1996 to 2012) and 11 commercial test sites in Oregon, Washington and California, and Alaska, USA (Fig. 1). This allowed us to obtain accurate estimates of covariance components for performance traits at harvest. The adjusted least square means (Table 2) of performance traits generally increased in MBP families from the 1st to 5th generation, indicating that MBP was successful in improving desirable traits.

Interestingly, performance traits of control groups also improved over the period of this period of the breeding program, perhaps reflecting development of more favorable culture environments in the MBP hatchery/nursery or in the field or both. An alternative explanation for this improved performance of control groups is that wild broodstock collected from bays with extensive oyster farming (Willapa and Dabob Bays) had received genetic contributions from spawns of MBP-derived commercial stocks or by spatial mixing unattached individuals of these stocks through transport by currents and wave action. Genetic analysis of wild populations will be necessary to evaluate this possibility.

4.1 Heritabilities

Overall heritability estimates for measured field traits (Table 3 and Fig. 3) were high (>0.35), both within and across generations; however, heritabilities for survival for the 4th and 5th generations were lower at 0.12 ± 0.02 and 0.14 ± 0.03 , respectively. These overall consistently positive results indicate that this breeding population of *C. gigas* possessed, and likely still contains, potential for genetic improvement by selection.

Heritability estimates for individual weight at harvest were more constant across generations (ranging from 0.40 ± 0.04 to 0.51 ± 0.03) compared to those for other traits; furthermore, these values were higher than realized heritability values (0.00 ± 0.07 ; 0.313 ± 0.08) reported by Evans and Langdon (2006b). Previous studies have reported positive heritability estimates for spat or juvenile growth for oysters and other bivalve species (Table 4). Dégremont et al. (2007) found low narrow-sense estimates for growth, ranging from 0.07 ± 0.07 to 0.15 ± 0.08 for 6 to 8-month-old Pacific oysters planted at three sites in France. A similar low narrow-sense estimate (0.05 ± 0.18) for growth was reported by Ernande et al. (2003) for six-month old Pacific oysters in intensive nursery conditions. Another study reported narrow-sense heritabilities ranging from 0.10 ± 0.05 to 0.51 ± 0.15 for growth (increase in live weight) for seven-month old eastern oysters *Crassostrea virginica* (Davis, 2000). Toro et al. (1995) reported high estimates of realized heritability for growth (live weight) of juvenile (8-month-old animals) *Ostrea chilensis* ranging from 0.35 ± 0.08 to 0.69 ± 0.11 . The estimates from this dataset are, therefore, on the high side of the range of reported growth heritabilities for other species and rearing systems.

Table 4

Variation in heritabilities for survival were greater than for growth across generations, ranging from 0.12 ± 0.02 to 0.55 ± 0.03 , perhaps indicating that survival was more sensitive to changes in environmental factors across generations than growth. Moderate-to-high realized heritabilities for survival, ranging from 0.34 ± 0.05 from 0.63 ± 0.04 after four generations of mass selection, were reported for *C. gigas* (Dégremont et al., 2015). In another study, narrow-sense heritabilities from 0.47 to 1.08 and realized heritabilities from 0.55 ± 0.18 (3rd generation) to 0.98 ± 0.15 (2nd generation) were reported for survival of *C. gigas* by Dégremont et al. (2007, 2010). In the USA, realized heritabilities from 0.49 to 0.71 were reported for survival of *C. gigas* by Evans and Langdon (2006a) and in Japan, Usuki (2002) reported narrow-sense heritabilities of 0.77 for this trait.

Heritabilities obtained for yield at harvest in this study were high (0.31 ± 0.037 to 0.59 ± 0.028). Yield (mean family harvest weight per culture container) compounds both survival and growth into a single metric. It is not a common selected trait in oyster breeding programs; however, on the West Coast, USA, yield is the most important economic trait (Langdon et al.,

2003) and is the primary selection trait for MBP. In previous reported MBP studies, realized heritability estimates for yield were low-to-medium, ranging from 0.01 ± 0.05 to 0.52 ± 0.16 (Langdon et al., 2003) or not significantly different for zero (Evans and Langdon, 2006a).

In our study, heritabilities for all traits decreased from the 3rd to the 4th and 5th generations, especially for survival that decreased from 0.48 in the 3rd generation to 0.12 in the 4th generation (Fig 3.). This is associated with an increase in residual variance and a decrease in genetic variance in these generations (Supplementary Table S2). Possible explanations for this decline were explored by further analysis of the data. Firstly, inbreeding was eliminated as a possibility because the average coefficient of inbreeding was low across all generations, ranging from 0 in the 1st and 2nd generations to 0.0171 in the 3rd generation. The average coefficient of inbreeding ($\pm$ SD) of all MBP families was only 0.002 ± 0.016 , ranging from 0 (1,587 occurrences) to 0.125 (25 occurrences, all in the 3rd generation). These values are likely too small to cause significant inbreeding depression of survival and other performance traits, based on the results of Evans et al. (2004). These researchers reported that after two growing seasons, significant inbreeding depression in yield, individual growth rate and survival were only observed in MBP families with inbreeding coefficients of 0.063, 0.203, and 0.203, respectively. However, we did not determine if there was a significant decrease in genetic diversity from the 3rd to 4th generation, although this seems unlikely due to low inbreeding coefficients and high number of broodstock oysters and families used to produce cohorts in these generations (Supplementary Table S1). In support of this argument, Camara, (2011) compared the genetic diversity of cohorts from the first four MBP generations with those of wild Japanese and USA West Coast naturalized populations and found similar levels of polymorphism; however, some MBP cohorts had lower numbers of private bands, indicating loss of unique alleles.

Secondly, we examined the effect of a reduction in the number of replicate containers planted at field test sites as a source of increased residual variance and reduced heritability values. The number of replicates was reduced from the 3rd to 4th and 5th generations in order to improve the cost efficiency of the breeding program (Supplementary Table S1). In order to address this question, we randomly removed replicates from all families in all cohorts in order to reduce the total number of replicates to four, and re-calculated heritability values. We found that a reduction in the number of replicas had no significant effect on estimates of variance components or heritabilities (Supplementary Table S3).

Thirdly, there was an increase in the representation of test sites in the Yaquina estuary, Oregon, in the 4th and 5th generations (Fig. 2; Supplementary Table S1) and it is possible that this location increased residual variances. However, heritabilities for cohorts planted at non-Yaquina sites also decreased in 4th and 5th generations indicating that the higher use of Yaquina test sites was not a significant causative factor.

Lastly, it is possible that the reduction in heritabilities from the 3rd to 4th and 5th generations was due to genetic by environment (GxE) interaction effects in the hatchery and/or at field test sites. Cohorts of the 4th generation were spawned and harvested over a period from 2004 to 2009 that corresponded with the onset of intensive hypoxic and acidification events on the West Coast, USA, that started to adversely affected oyster larval production at HMSC in 2005 (unpublished observations) and at commercial hatcheries in 2007 (Barton et al., 2012, 2015; Fabry et al., 2008; Kurihara, 2008; Lannig et al., 2010). It is possible that these selective pressures resulted in GxE effects on the condition of larvae and seed produced at the MBP hatchery that were carried over into the field. Spat from families that were more sensitive to the stress of upwelled acidified water in the hatchery would be in poorer condition at planting and likely more susceptible to disease and mortality. In addition, conditions in coastal bays, such as Willapa Bay, WA, were also poor for successful collection of natural spat during the period from 2006 to 2012 (Barton et al., 2015; Washington Policy Center 2015), perhaps further stressing planted MBP families.

Overall, estimates of heritabilities for performance traits at harvest were positive (Fig. 3 and 4), but the values and standard errors were, in general, higher for realized heritabilities (Fig. 4) than for narrow-sense heritabilities (Fig. 3). The greater standard errors for realized heritability estimates can be attributed to the overall smaller number of control groups (168) compared with MPB (1,067) families. Furthermore, the number of control groups was unbalanced among cohorts, ranging from two in cohort 22 to 26 in cohort 9 due to practical limitations in the number of families or groups that could be tested in one cohort; therefore, realized heritability estimates need to be considered with caution. Larger numbers of control groups would have reduced the variance of control group means and increased the accuracy of realized heritability estimates; however, an increase in the number of control groups would have resulted in fewer MBP families per cohort and a potential reduction in long-term genetic diversity.

It should be also noted that our estimates of additive genetic variance could be inflated by common environment (permanent effect of environment) and dominance effects. In fish, Pante et al (2002) found that estimates of additive genetic variance were inflated by dominance effects or full-sib permanent environment effects (common environmental effects). These researchers found that these effects were confounded; however in studies in dairy cattle, beef cattle and swine (Miształ, 1997; Miształ et al., 1998), estimates of additive variance changed very little whether the dominance effect was taken into account or not in their model. In the present study, common environment effects for full-sibs and dominance effects could be “confounded” with additive genetic variance; therefore, these effects may inflate our estimates of heritability and genetic gain.

4.2. Correlations

In general, correlations among performance traits ranged from medium-negative (-0.32 ± 0.10) to high-positive (0.96 ± 0.01 ; Table 3). Yield was positively influenced by survival, with correlation coefficients ranging from 0.21 ± 0.07 to 0.96 ± 0.007 in the 3th and 2nd generations, respectively. A similar wide range of positive correlations were found between yield and individual weights at harvest, ranging from 0.11 ± 0.12 to 0.91 ± 0.01 in the 1st and 4th generations, respectively. The positive relationships between yield and survival found in this study agree with those obtained by Dégremont et al. (2015) in a study on summer mortality of *C. gigas* in France.

Genetic correlations between survival and individual weight at harvest in our study ranged from -0.32 ± 0.10 to 0.68 ± 0.05 in 1st and 2nd generation, respectively, with an average value of 0.25 ± 0.04 across all generations. Negative correlations between these traits can be explained by either growth-limiting competition for food, resulting from oyster filtration rates exceeding food supplies in culture containers, or greater sensitivity to stress in fast-growing families. Antagonistic correlations between survival and yield were reported for Holstein cattle (Dematawewa and Berger, 1998), between disease resistance and body weight, body length and feed conversion efficiencies for rainbow trout (Henryon et al., 2002) and between disease resistance and weight for Atlantic salmon (Norris et al., 2008). Nonetheless, positive correlations between yield and both growth and survival suggest that these latter traits are affected in the same way by genetic factors that control the overall “robustness” of selected families.

4.3. Genetic and realized gains

The most important measure in assessing the success of animal breeding programs is genetic gain. Through this measure, one can evaluate the progress and achievements of a breeding program. The positive predicted responses to selection for multiple performance traits estimated in this study indicate that selection by MBP resulted in oysters with improved genotypes for higher field performance. The predicted accumulated (Fig. 5) genetic gains over five generations of selection for harvest traits, calculated by linear regression of the means of breeding values of MBP families as function of generation, were high at +19%, +15.7% and +11.3% for yield, survival and individual weight, respectively. Genetic gains for survival and yield gradually increased over the selection period; however, genetic gain for individual weight at harvest peaked after two generations of selection but did not show further increases in subsequent generations. Unlike genetic improvement programs for many other commercially-farmed animals, food availability for oysters cannot be increased by the farmer in response to higher demands, limiting improvements in growth rates when supplies of suspended natural food particles, such as phytoplankton, are low.

A review of the literature on selection in bivalve molluscs indicates that it is possible to achieve positive gains in growth-related traits over one-to-three generations of selection, ranging from 2.9 to 23.6%, depending on the species and age of animal (Table 5). Li et al. (2011) reported realized gains for growth at 12 months in three mass selected *C. gigas* stocks that ranged from 7.9 to 12.2%. Similar gains, ranging from 8.4 to 10.4%, were reported after a second generation of mass selection of these stocks (Wang et al., 2012). Dégremont et al. (2015) reported high gains in survival, ranging from 43.9 to 80.6%, after four generations of mass selection. Reports for responses to selection for yield is rare in the literature; however, Langdon et al. (2003) reported values ranging from 0.4 to 25.5% after two generations of selection.

Overall, realized genetic gains were less than those predicted by genetic gains (Fig. 5). Several factors could be responsible. Environmental conditions at test sites are highly variable, both temporally and spatially; for example, water temperatures ranged from 6.2 to 23.6°C and salinities ranged from 0 to 35.6 psu in the Yaquina Bay over the period from fall 2002 to summer 2004 (Evans and Langdon 2006a). Longer-term changes in ocean conditions have also been reported to affect Pacific oysters, for example, meat content of oysters in Willapa Bay, WA, were reported to be influenced by El Niño-southern-oscillation oceanic (ENSO) events

(Schoener and Tufts 1987) and, as discussed above, the onset of stronger ocean acidification conditions in 2005-2007 adversely affected larval performance in commercial hatcheries and natural larval settlement in Willapa Bay, WA. Such variability in environmental conditions could result in significant GxE effects on performance traits based on spatial and temporal differences in test site conditions. Evans and Langdon (2006a) reported that GxE effects represented only 1.3%, 4.5% and 2.2% of the total phenotypic variance for average weight, survival and yield, respectively, of cohort 13 families planted at four different test sites (Fig. 2). Although this contribution appears to be small compared with reported environmental effects (accounting for 90.7%, 22% and 76.2% of total phenotypic variance for growth, survival and yield, respectively), GxE effects of 44.8%, 12.8% and 22.4% for individual weight, survival and yield at harvest, can be estimated when expressed as a proportion of phenotypic variance due to family effect. These latter GxE estimates suggest that individual weight at harvest (growth) is more susceptible to environmental effects than survival, supporting the argument that GxE effects may be responsible for the more limited genetic gain and variable realized response for growth, compared with those obtained for survival over the five generations of MBP selection (Fig. 5).

In our study, realized gains for individual weight and yield at harvest for MBP families in the 5th generation declined from values in the 4th generation to -9.8% and 0%, respectively, although realized gain for survival continued an increasing trend to 11.7%, (Fig. 5). These lower realized gains for individual weight and yield in the 5th generation may have been due several factors. Firstly, negative genetic correlations between survival and individual weight in the 1st (-0.32) and 4th (-0.09) generations could have contributed to the negative realized gains for individual weight in the 2nd (-13.4%) and 5th (-9.8%) generations (Table 3) due to reduced selection efficiencies. Selection intensities (SSD) used to calculate realized heritabilities for yield were medium to high (0.81, 1.24, 1.51 and 0.46, respectively, for 2nd, 3rd, 4th and 5th generation, Table 1); however, these estimates cannot explain the negative realized gain in 1st, 2nd and null realized gain in 5th generation (Fig. 5).

Secondly, each MBP family in the 5th generation was produced from a single pair mating, whereas several single pair matings were pooled for each family in previous generations (Supplementary Table S1). Similar poolings of multiple pair matings were also used to produce control groups in all five generations. Pooling of matings likely results in both greater genetic diversity within each family or control group and selection among progeny could have resulted

in over-representation of high-performing crosses and an increase in the mean family or group performance (Boudry et al., 2002; Taris et al., 2006; Lallias et al., 2010). In future, it would be preferable to compare the performance of MBP families and controls using similar pair mating designs.

Lastly, there is some doubt about of the genetic composition of wild broodstock populations that were sampled to produce control groups. Realized gains are calculated by comparing the performance of selected families with that of controls (see equation 10 above). Variability in the genetic composition of controls among cohorts will add to the variance of estimated realized gains. Furthermore, possible contamination of wild populations with MBP-derived oysters (see discussion above) may have resulted in reductions in performance differences between MBP families and control groups. Ideally, controls with consistent genetic composition should be employed to estimate future realized gains, such as hybrid crosses between highly inbred lines.

Table 5

In summary, the moderate-to-high heritabilities estimated in our study indicate that performance traits in oysters are under a high degree of genetic control and continued gains should be expected through selective breeding. Positive medium-to-high genetic correlations among harvest traits suggest that indirect gains in yield can be achieved by selection for either higher growth or survival. The positive genetic gains obtained for performance traits at harvest corroborate these results. Realized gains were lower than genetic gains, possibly due to large temporal and spatial variation in culture conditions coupled with significant GxE effects limiting the performance benefits of selection from one generation to the next. Modifications of the breeding program to select families in a limited range of less variable environments might improve the prospects for greater long-term realized gains.

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Table 1 – Structure of the data used in analysis.

No. of records	15,236	No. of families [§]	1,274
No. of families with records	1,235	No. of sires [*]	263
		No. of dams [†]	277
No. of sites	11	No. of years	14
No. of tidal exposures	2	No. of cohorts	23
No. of generations	5	No. of blocks at test sites	159

[§] Including parents without records and dummy identities for unknown dams.

^{*} With progeny in the data.

[†] With progeny in the data, including dummy identities assigned for animals with missing dam identities.

Table 2 – Number of MBP families and non-selected control groups, standardized selection differential (SSD) for yield, as well as least square means $\pm$ SD for field performance traits across five generations.

Generation	No. families/groups		SSD for Yield (kg)	Yield (kg)		Survival (%)		Individual Weight (g)		Initial Weight (g)	
	MBP	Control		MBP	Control	MBP	Control	MBP	Control	MBP	Control
ALL	1,067	168	1.09	2.31$\pm$1.12	1.81$\pm$1.42	62.36$\pm$17.90	58.31$\pm$22.28	87.99$\pm$29.52	71.67$\pm$30.97	36.02$\pm$51.70	14.26$\pm$22.62
1 st	293	-	-	2.41 $\pm$ 1.10	-	60.05 $\pm$ 11.41	-	99.97 $\pm$ 25.01	-	82.56 $\pm$ 61.41	-
2 nd	210	79	0.81	1.12 $\pm$ 0.80	1.02 $\pm$ 0.65	52.41 $\pm$ 17.64	57.40 $\pm$ 22.95	36.72 $\pm$ 14.08	42.21 $\pm$ 12.36	34.10 $\pm$ 28.12	22.61 $\pm$ 29.15
3 rd	166	49	1.24	2.41 $\pm$ 1.27	2.65 $\pm$ 1.98	52.24 $\pm$ 16.97	53.72 $\pm$ 26.21	92.88 $\pm$ 13.51	90.26 $\pm$ 16.51	4.81 $\pm$ 2.17	5.67 $\pm$ 3.52
4 th	217	23	1.51	2.52 $\pm$ 0.99	2.19 $\pm$ 0.82	61.64 $\pm$ 16.26	59.74 $\pm$ 16.75	96.61 $\pm$ 16.59	91.68 $\pm$ 9.96	7.40 $\pm$ 3.16	6.27 $\pm$ 3.17
5 th	181	17	0.46	2.90 $\pm$ 0.47	2.85 $\pm$ 0.53	78.89 $\pm$ 9.59	70.98 $\pm$ 7.30	105.33 $\pm$ 17.77	114.16 $\pm$ 18.74	2.74 $\pm$ 1.79	3.90 $\pm$ 0.55

Table 3 – Estimates $\pm$ SE of genetic (above diagonal) and residual (below diagonal) correlations for performance traits and heritabilities (on diagonal) for different generations.

	ALL			1 st Generation		
Traits	Yield (kg)	Survival (%)	Individual Weight (g)	Yield (kg)	Survival (%)	Individual Weight (g)
Yield	0.59$\pm$0.01	0.38 $\pm$ 0.04	0.90 $\pm$ 0.01	0.53$\pm$0.02	0.90 $\pm$ 0.02	0.11 $\pm$ 0.12
Survival	0.09 $\pm$ 0.01	0.35$\pm$0.02	0.25 $\pm$ 0.04	0.83 $\pm$ 0.01	0.55$\pm$0.03	-0.32 $\pm$ 0.10
Ind. Weight	0.64 $\pm$ 0.01	-0.06 $\pm$ 0.01	0.55$\pm$0.01	0.26 $\pm$ 0.03	-0.24 $\pm$ 0.03	0.45$\pm$0.04
	2 nd Generation			3 rd Generation		
Yield	0.56$\pm$0.02	0.96 $\pm$ 0.01	0.84 $\pm$ 0.03	0.58$\pm$0.03	0.21 $\pm$ 0.07	0.83 $\pm$ 0.03
Survival	0.89 $\pm$ 0.00	0.40$\pm$0.03	0.68 $\pm$ 0.05	0.12 $\pm$ 0.02	0.48$\pm$0.03	0.14 $\pm$ 0.08
Ind. Weight	0.25 $\pm$ 0.02	-0.05 $\pm$ 0.02	0.51$\pm$0.03	0.62 $\pm$ 0.01	-0.03 $\pm$ 0.02	0.42$\pm$0.03
	4 th Generation			5 th Generation		
Yield	0.36$\pm$0.03	0.27 $\pm$ 0.10	0.91 $\pm$ 0.01	0.30$\pm$0.04	0.70 $\pm$ 0.07	0.90 $\pm$ 0.03
Survival	0.35 $\pm$ 0.02	0.12$\pm$0.02	-0.09 $\pm$ 0.10	0.76 $\pm$ 0.02	0.14$\pm$0.03	0.34 $\pm$ 0.12
Ind. Weight	0.64 $\pm$ 0.01	-0.16 $\pm$ 0.02	0.43$\pm$0.03	0.62 $\pm$ 0.02	0.05 $\pm$ 0.04	0.40$\pm$0.04

Table 4 – Reported estimates of heritabilities ($h^2 \pm SE$) for different bivalve species and growth traits.

Species	Traits	h^2	Type	Authors
<i>Crassostrea gigas</i>	Shell height	0.149±0.027 0.402±0.024	Realized	Li et al. (2011) [§]
”	”	0.312±0.071 0.457±0.143	”	Wang et al. (2012) [§]
”	”	0.49±0.25	Narrow-sense	Kong et al. (2015)
”	Shell length	0.36±0.19	”	”
”	Shell width	0.45±0.23	”	”
”	Wet individual weight	0.35±0.17	”	”
<i>Pinctada fucata</i>	Shell height	0.713±0.208	Realized	He et al. (2008)
”	Shell width	0.467	”	Wada (1986)
”	Shell convexity	0.350	”	”
<i>Pinctada maxima</i>	Shell width	0.02±0.000	Narrow-sense	Kvingedal et al. (2010)
”	Shell anterior-posterior	0.23±0.03	”	”
”	Tissue weight	0.35±0.07	”	Wang et al. (2010)
”	Shell length	0.64±0.10	”	”
<i>Mytilus galloprovincialis</i>	Total weight (shell + meat)	0.035±0.09	Narrow-sense	Nguyen et al. (2014)
<i>Argopecten irradians</i>	Shell length	0.38±0.06	”	Hui et al. (2014)
”	Body weight	0.22±0.07	”	”

[§]Using three stocks from China, Japan and Korea.

Table 5 – Reported estimates of gain for different bivalve species and traits (excluding Pacific oyster).

Species	Traits	Gain	Type	No. of generations	Authors
<i>Pinctada fucata</i>	Shell height	9.61-21.15% [‡]	Mass selection	2	He et al. (2008)
<i>Pinctada martensii</i>	Shell length	16.60-22.30% [§]	”	1	Deng et al. (2009)
”	”	23.6% [‡]	”	”	”
”	Shell height	14.2-19.9% [§]	”	”	”
”	”	21.2% [‡]	”	”	”
”	Survival	9.7-18.2% [§]	”	”	”
”	”	16.1% [‡]	”	”	”
<i>Saccostrea commercialis</i>	Individual Weight	14-23% [§]	”	3	Nell et al. (1999)
”	”	2.9-8.5% [§]	”	1	Nell et al. (1996)
<i>Patinopecten yessoensis</i>	Shell height	6.60-7.85% [§]	”	”	Liang et al. (2010)
<i>Mytilus galloprovincialis</i>	Total weight (shell + meat)	10% [§]	Family-based	2	Nguyen et al. (2014)
<i>Argopecten irradians</i>	Shell length	7.16% [§]	Mass selection	1	Hui et al. (2014)
”	Body weight	8.16% [§]	”	”	”
”	Shell length	0.14-0.53 [§] mm	”	1	Zheng et al. (2004)
<i>Argopecten ventricosus</i>	Total weight (shell + meat)	16.3% [§]	”	”	Ibarra et al. (1999)

[§] Adult; [‡] Juvenile; [‡] Different ages.

Fig. 1 – Location of test sites for the Molluscan Broodstock Program on commercial oyster farms.

Fig. 2 – Breeding scheme for five generations of cohorts produced by the Molluscan Broodstock Program. Sub: sub-tidal plantings; Inter: intertidal plantings.

Fig. 3 – Estimates of heritabilities ($\pm$ SE) of performance traits for five generations of the Molluscan Broodstock Program.

Fig. 4 – Estimates of realized heritabilities ($\pm$ SE) of performance traits for five generations of the Molluscan Broodstock Program.

Figure 5 – Genetic gain ($\pm$ SE) of performance traits for families for five generations of the Molluscan Broodstock Program.