

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

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12

13 **Abstract**

14 The Pacific oyster, *Crassostrea gigas*, is one of the most important global aquaculture species
15 due to its potential high growth rates and tolerance of a wide range of environmental conditions;
16 however, most farmers use seed from wild, non-domesticated stocks for production. In this study
17 we estimated genetic parameters for performance traits of oysters from the Molluscan
18 Broodstock Program (MBP), a family-based selective breeding program designed to improve
19 yields of Pacific oysters on the West Coast, USA. Covariance components were obtained using
20 AIREMLF90, a multiple-trait animal model. A total of 15,236 records were analyzed from five
21 generations of selectively bred oysters. Heritability estimates for field traits at harvest were all
22 positive over the five analyzed generations, ranging from 0.58 ± 0.03 (3rd generation) to 0.30 ± 0.04
23 (5th generation) for yield, from 0.55 ± 0.03 (1st generation) to 0.12 ± 0.02 (4th generation) for
24 survival, and from 0.51 ± 0.03 (2nd generation) to 0.40 ± 0.03 (4th generation) for mean individual
25 weight at harvest (growth). Declines in heritabilities for survival and yield in the 4th and 5th
26 generations were perhaps a result of changing ocean conditions due to increased upwelled
27 hypoxic and acidified seawater occurring on the West Coast, USA, that affected the quality of
28 seed from the MBP hatchery/nursery. Realized heritabilities were all positive and medium-to-
29 high across generations, ranging to 0.11 ± 0.42 (for survival in the 3rd generation) to 1.20 ± 0.35
30 (for individual weight in the 5th generation); however, standard errors for realized heritabilities
31 were high (ranging 0.15 to 1.47), especially in the 5th generation. There was a gradual

32 improvement in genetic gains for survival and yield over the five generations of selection,
33 resulting in accumulated gains of +15.7% and +19%, respectively, in the 5th generation;
34 however, little improvement was achieved after the 2nd generation in genetic gain for individual
35 weight, with an accumulated gain in the 5th generation of +11.3%. Realized gains for
36 performance traits were less than predicted by genetic gains. Realized gain in survival
37 consistently improved over the selection period, resulting in an accumulated gain of 11.7% in the
38 5th generation compared to that of wild, non-selected controls; however, gains in individual
39 weight and yield at harvest were not consistent across generations and gains in the 5th generation
40 were -9.8% and 0%, respectively, compared with those of controls, perhaps due to inconsistent
41 genetic quality of control broodstock sampled from wild populations. Across generations, there
42 were positive genetic correlations between yield and both survival (0.38 ± 0.04) and individual
43 weight (0.90 ± 0.01) as well as between survival and individual weight (0.25 ± 0.04). Positive
44 medium-to-high genetic correlations among harvest traits suggest that indirect gains in yield can
45 be achieved by selection for either higher growth or survival.

46

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

48

49 **1. Introduction**

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

56 Shortly after development of hatchery techniques for oyster production in the 1970s,
57 Lannan (1972) reported a positive heritability of 0.37 for growth in adult *C. gigas*. This estimate
58 is similar to that reported in the USA for body weight at harvest (0.31 ± 0.08 ; Evans and Langdon,
59 2006a) and for various growth parameters of *C. gigas* in different parts of Asia: China
60 (0.33 ± 0.03), Japan (0.40 ± 0.02) and South Korea (0.1 ± 0.03) (Li et al., 2011). Estimated
61 heritabilities for growth are strongly affected by rearing conditions and source populations for
62 broodstock, as demonstrated by Langdon et al. (2003) who observed heritability values for yield

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

65 Family-based selection programs for improving desirable traits of the Pacific oyster have
66 been initiated in the USA (Evans and Langdon, 2006b; Langdon et al., 2003), France
67 (Dégremont et al., 2010, 2007), China (Li et al., 2011; Wang et al., 2012) and Australia (Kube et
68 al., 2011), obtaining genetic gains per generation between 7.2% for growth (Li et al., 2011) to
69 25.5% for yield (Langdon et al., 2003). In Australia, Nell et al. (1999, 1996) obtained genetic
70 gains of 4% and 8% for individual weight in the first and second generation of mass selection,
71 respectively, for the rock oyster *Saccostrea commercialis*.

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

78

79 **2. Material and methods**

80 *2.1. Husbandry of MBP families and control groups*

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

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

95 Cohorts were produced by rearing separate families from larval to early juvenile (spat)
96 stages at Oregon State University's facilities at the Hatfield Marine Science Center (HMSC) in
97 Newport, Oregon, USA. Larval and spat cultures for each family were not replicated due to
98 limited space and labor in the hatchery/nursery as well as to meet the goal of maximizing the
99 number of families included in the breeding program. In support of this approach, Evans and
100 Langdon (2006a) reported that variation among replicates during this early phase of family
101 rearing contributed only 1.5%, 0.3% and 5.2% to the total phenotypic variance (10%, 6% and
102 14% expressed in terms of family variance) in yield, average individual weight and survival at
103 harvest, respectively.

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

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

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

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

126

127 2.2. Statistical models

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

132

133 Fig. 1

134

135 Fig. 2

136

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

$$142 \quad y_{ijklmnop} = S_i + TE_j + G_k + Year_l + C_m + (S \times C \times TE)_n + b_1 \gamma_{ijklmnop} + b_2 \gamma_{ijklmnop} + block_o + f_p + e_{ijklmnop} \dots\dots\dots [Eq. 1]$$

143

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

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

157 The model above can be written in matrix form as:

$$158 \quad \mathbf{Y} = \mathbf{X}\boldsymbol{\beta} + \mathbf{Z}\mathbf{a} + \mathbf{e} \quad [\text{Eq. 2}]$$

159 where:

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

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

163 $\boldsymbol{\beta}$ is the vector of fixed effects;

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

$$165 \quad \text{Assume } \begin{bmatrix} \mathbf{Y} \\ \mathbf{e} \end{bmatrix} \sim \mathbf{N}(\mathbf{0}, \mathbf{V}), \quad \mathbf{V} = \begin{bmatrix} \mathbf{G} \otimes \mathbf{A} & \mathbf{0} \\ \mathbf{0} & \mathbf{R} \end{bmatrix} \quad [\text{Eq. 3}]$$

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

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

171

172 Table 1

173

174 2.3. Heritabilities

175 The additive ($\hat{\sigma}_a^2$) and residual ($\hat{\sigma}_e^2$) variances were estimated within and across
 176 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
 177 calculated as $\hat{h}^2 = \hat{\sigma}_a^2 / \hat{\sigma}_p^2$ [Eq. 5].

178 Realized heritability (\hat{h}_r^2) estimates for performance traits were calculated for each cohort
 179 as: $\hat{h}_r^2 = SR/SSD$ [Eq. 6] where \hat{h}_r^2 is the realized heritability (Falconer and Mackay, 1996,

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

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

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

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

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

192

193 2.4. Genetic and realized gains

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

199 calculated as the ratio (percentage) of the regression coefficients (\hat{b}) of the mean of breeding
200 values of MBP families in each generation to least square means (Lsmean) of each trait,
201 according to Maluwa and Gjerde (2007). Therefore, the accumulated genetic gains, expressed as
202 percentage values, in the 5th were calculated as: $\Delta g = \left(\frac{\hat{b} \times 5}{\text{Lsmean in 1}^{st} \text{ generation}} \right) \times 100 [Eq. 9].$

203 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
 204 least square means for performance traits of MPB families and control groups, respectively. Only
 205 cohorts with both MBP families and control groups were used to calculate realized gains.

206

207 **3. Results**

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

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

219

220 Table 2

221

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

228

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

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

236

237 Fig. 3

238

239 Intensities of selection (SSD) used to calculate realized heritabilities were 0.81, 1.24,
 240 1.51 and 0.46, respectively, for the 2nd, 3rd, 4th and 5th generations (Table 2). Realized heritability
 241 values (Fig. 4) for yield, individual weight and survival were medium-to-highly positive and
 242 consistent across generations, except for an apparent increase in the 5th generation, although the
 243 SE values were high for these values. The overall realized heritabilities across generations were
 244 0.55 ± 0.24 , 0.40 ± 0.25 and 0.68 ± 0.22 for the yield, survival and individual weight at harvest,
 245 respectively.

246

247 Fig. 4

248

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

256

257 Table 3

258

259 The predicted accumulated genetic gains for performance traits obtained after five
 260 generations of selection are shown in Fig. 5. The angular coefficients estimated by linear
 261 regression analysis of MBP family breeding values (BLUP) as a function of generation, were
 262 $+0.09$ kg, $+1.89\%$, $+2.27$ g for yield, survival and individual weight at harvest, respectively. The
 263 accumulated genetic gains over these five generations calculated by linear regression, for yield,

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

267

268 Fig. 5

269

270 **4. Discussion**

271 In this study, the number of families was large (1,235 families with 15,236 records; Table
272 1) and this extensive database provided considerable information for estimation of variance
273 components using an animal model. Another important characteristic of this dataset was its
274 spatial and temporal reach. These data covered 17 years of the MBP program (from 1996 to
275 2012) and 11 commercial test sites in Oregon, Washington and California, and Alaska, USA
276 (Fig. 1). This allowed us to obtain accurate estimates of covariance components for performance
277 traits at harvest. The adjusted least square means (Table 2) of performance traits generally
278 increased in MBP families from the 1st to 5th generation, indicating that MBP was successful in
279 improving desirable traits.

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

288

289 *4.1 Heritabilities*

290 Overall heritability estimates for measured field traits (Table 3 and Fig. 3) were high
291 (>0.35), both within and across generations; however, heritabilities for survival for the 4th and 5th
292 generations were lower at 0.12 ± 0.02 and 0.14 ± 0.03 , respectively. These overall consistently
293 positive results indicate that this breeding population of *C. gigas* possessed, and likely still
294 contains, potential for genetic improvement by selection.

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

309

310 Table 4

311

312 Variation in heritabilities for survival were greater than for growth across generations,
313 ranging from 0.12 ± 0.02 to 0.55 ± 0.03 , perhaps indicating that survival was more sensitive to
314 changes in environmental factors across generations than growth. Moderate-to-high realized
315 heritabilities for survival, ranging from 0.34 ± 0.05 from 0.63 ± 0.04 after four generations of mass
316 selection, were reported for *C. gigas* (Dégremont et al., 2015). In another study, narrow-sense
317 heritabilities from 0.47 to 1.08 and realized heritabilities from 0.55 ± 0.18 (3rd generation) to
318 0.98 ± 0.15 (2nd generation) were reported for survival of *C. gigas* by Dégremont et al. (2007,
319 2010). In the USA, realized heritabilities from 0.49 to 0.71 were reported for survival of *C. gigas*
320 by Evans and Langdon (2006a) and in Japan, Usuki (2002) reported narrow-sense heritabilities
321 of 0.77 for this trait.

322 Heritabilities obtained for yield at harvest in this study were high (0.31 ± 0.037 to
323 0.59 ± 0.028). Yield (mean family harvest weight per culture container) compounds both survival
324 and growth into a single metric. It is not a common selected trait in oyster breeding programs;
325 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).

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

362 Lastly, it is possible that the reduction in heritabilities from the 3rd to 4th and 5th
363 generations was due to genetic by environment (GxE) interaction effects in the hatchery and/or at
364 field test sites. Cohorts of the 4th generation were spawned and harvested over a period from
365 2004 to 2009 that corresponded with the onset of intensive hypoxic and acidification events on
366 the West Coast, USA, that started to adversely affected oyster larval production at HMSC in
367 2005 (unpublished observations) and at commercial hatcheries in 2007 (Barton et al., 2012,
368 2015; Fabry et al., 2008; Kurihara, 2008; Lannig et al., 2010). It is possible that these selective
369 pressures resulted in GxE effects on the condition of larvae and seed produced at the MBP
370 hatchery that were carried over into the field. Spat from families that were more sensitive to the
371 stress of upwelled acidified water in the hatchery would be in poorer condition at planting and
372 likely more susceptible to disease and mortality. In addition, conditions in coastal bays, such as
373 Willapa Bay, WA, were also poor for successful collection of natural spat during the period from
374 2006 to 2012 (Barton et al., 2015; Washington Policy Center 2015), perhaps further stressing
375 planted MBP families.

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

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

398

399 4.2. Correlations

400 In general, correlations among performance traits ranged from medium-negative (-
401 0.32 ± 0.10) to high-positive (0.96 ± 0.01 ; Table 3). Yield was positively influenced by survival,
402 with correlation coefficients ranging from 0.21 ± 0.07 to 0.96 ± 0.007 in the 3th and 2nd generations,
403 respectively. A similar wide range of positive correlations were found between yield and
404 individual weights at harvest, ranging from 0.11 ± 0.12 to 0.91 ± 0.01 in the 1st and 4th generations,
405 respectively. The positive relationships between yield and survival found in this study agree with
406 those obtained by Dégremont et al. (2015) in a study on summer mortality of *C. gigas* in France.

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

418

419 4.3. Genetic and realized gains

420 The most important measure in assessing the success of animal breeding programs is
421 genetic gain. Through this measure, one can evaluate the progress and achievements of a
422 breeding program. The positive predicted responses to selection for multiple performance traits
423 estimated in this study indicate that selection by MBP resulted in oysters with improved
424 genotypes for higher field performance. The predicted accumulated (Fig. 5) genetic gains over
425 five generations of selection for harvest traits, calculated by linear regression of the means of
426 breeding values of MBP families as function of generation, were high at +19%, +15.7% and
427 +11.3% for yield, survival and individual weight, respectively. Genetic gains for survival and
428 yield gradually increased over the selection period; however, genetic gain for individual weight
429 at harvest peaked after two generations of selection but did not show further increases in
430 subsequent generations. Unlike genetic improvement programs for many other commercially-
431 farmed animals, food availability for oysters cannot be increased by the farmer in response to
432 higher demands, limiting improvements in growth rates when supplies of suspended natural food
433 particles, such as phytoplankton, are low.

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

443 Overall, realized genetic gains were less than those predicted by genetic gains (Fig. 5).
444 Several factors could be responsible. Environmental conditions at test sites are highly variable,
445 both temporally and spatially; for example, water temperatures ranged from 6.2 to 23.6°C and
446 salinities ranged from 0 to 35.6 psu in the Yaquina Bay over the period from fall 2002 to summer
447 2004 (Evans and Langdon 2006a). Longer-term changes in ocean conditions have also been
448 reported to affect Pacific oysters, for example, meat content of oysters in Willapa Bay, WA,
449 were reported to be influenced by El Niño-southern-oscillation oceanic (ENSO) events

450 (Schoener and Tufts 1987) and, as discussed above, the onset of stronger ocean acidification
451 conditions in 2005-2007 adversely affected larval performance in commercial hatcheries and
452 natural larval settlement in Willapa Bay, WA. Such variability in environmental conditions could
453 result in significant GxE effects on performance traits based on spatial and temporal differences
454 in test site conditions. Evans and Langdon (2006a) reported that GxE effects represented only
455 1.3%, 4.5% and 2.2% of the total phenotypic variance for average weight, survival and yield,
456 respectively, of cohort 13 families planted at four different test sites (Fig. 2). Although this
457 contribution appears to be small compared with reported environmental effects (accounting for
458 90.7%, 22% and 76.2% of total phenotypic variance for growth, survival and yield, respectively),
459 GxE effects of 44.8%, 12.8% and 22.4% for individual weight, survival and yield at harvest, can
460 be estimated when expressed as a proportion of phenotypic variance due to family effect. These
461 latter GxE estimates suggest that individual weight at harvest (growth) is more susceptible to
462 environmental effects than survival, supporting the argument that GxE effects may be
463 responsible for the more limited genetic gain and variable realized response for growth,
464 compared with those obtained for survival over the five generations of MBP selection (Fig. 5).

465 In our study, realized gains for individual weight and yield at harvest for MBP families in
466 the 5th generation declined from values in the 4th generation to -9.8% and 0%, respectively,
467 although realized gain for survival continued an increasing trend to 11.7%, (Fig. 5). These lower
468 realized gains for individual weight and yield in the 5th generation may have been due several
469 factors. Firstly, negative genetic correlations between survival and individual weight in the 1st (-
470 0.32) and 4th (-0.09) generations could have contributed to the negative realized gains for
471 individual weight in the 2nd (-13.4%) and 5th (-9.8%) generations (Table 3) due to reduced
472 selection efficiencies. Selection intensities (SSD) used to calculate realized heritabilities for yield
473 were medium to high (0.81, 1.24, 1.51 and 0.46, respectively, for 2nd, 3rd, 4th and 5th generation,
474 Table 1); however, these estimates cannot explain the negative realized gain in 1st, 2nd and null
475 realized gain in 5th generation (Fig. 5).

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

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

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

494

495 Table 5

496

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

507

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509

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523

524 **References**

- 525 Barton, A., Hales, B., Waldbusser, G.G., Langdon, C.J., Feely, R.A., 2012. The Pacific oyster,
526 *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels:
527 Implications for near-term ocean acidification effects. *Limnol. Oceanogr.* 57, 698–710.
528 doi:10.4319/lo.2012.57.3.0698
- 529 Barton, A., Waldbusser, G.G., Feely, R.A., Weisberg, S.B., Newton, J.A., Hales, B., Cudd, S.,
530 Eudeline, B., Langdon, C.J., Jefferds, I., King, T., Suhrbier, A., McLaughlin, K., 2015.
531 Impacts of coastal acidification on the Pacific Northwest shellfish industry and adaptation
532 strategies implemented in response. *Oceanography* 28, 146–159.
533 doi:10.5670/oceanog.2015.38
- 534 Boudry, P., Collet, B., Cornette, F., Hervouet, V., 2002. High variance in reproductive success of
535 the Pacific oyster (*Crassostrea gigas*, Thunberg) revealed by microsatellite-based parentage
536 analysis of multifactorial crosses. *Aquaculture* 204, 283–296.
- 537 Camara, M.D., 2011. Changes in molecular genetic variation at AFLP loci associated with
538 naturalization and domestication of the Pacific oyster (*Crassostrea gigas*). *Aquat. Living*
539 *Resour.* 43, 35–43.
- 540 Davis, C. V, 2000. Estimation of narrow-sense heritability for larval and juvenile growth traits in
541 selected and unselected sub-lines of eastern oysters, *Crassostrea virginica*. *J. Shellfish*
542 *Res.* 3, 613.
- 543 Dematawewa, C.M., Berger, P.J., 1998. Genetic and phenotypic parameters for 305-day yield,
544 fertility, and survival in Holsteins. *J. Dairy Sci.* 81, 2700–2709. doi:10.3168/jds.S0022-
545 0302(98)75827-8
- 546 Dégremont, L., Bédier, E., Boudry, P., 2010. Summer mortality of hatchery-produced Pacific
547 oyster spat (*Crassostrea gigas*). II. Response to selection for survival and its influence on
548 growth and yield. *Aquaculture* 299, 21–29. doi:10.1016/j.aquaculture.2009.11.017
- 549 Dégremont, L., Ernande, B., Bédier, E., Boudry, P., 2007. Summer mortality of hatchery-
550 produced Pacific oyster spat (*Crassostrea gigas*). I. Estimation of genetic parameters for
551 survival and growth. *Aquaculture* 262, 41–53. doi:10.1016/j.aquaculture.2006.10.025

- 552 Dégremont, L., Nourry, M., Maurouard, E., 2015. Mass selection for survival and resistance to
553 OsHV-1 infection in *Crassostrea gigas* spat in field conditions: response to selection after
554 four generations. *Aquaculture* 446, 111–121. doi:10.1016/j.aquaculture.2015.04.029
- 555 Deng, Y., Fu, S., Du, X., Wang, Q., 2009. Realized Heritability and Genetic Gain Estimates of
556 Larval Shell Length in the Chinese Pearl Oyster *Pinctada martensii* at Three Different
557 Salinities. *N. Am. J. Aquac.* 71, 302–306. doi:10.1577/A08-024.1
- 558 Ernande, B., Clobert, J., McCombie, H., Boudry, P., 2003. Genetic polymorphism and trade-offs
559 in the early life-history strategy of the Pacific oyster, *Crassostrea gigas* (Thunberg, 1795): a
560 quantitative genetic study. *J. Evol. Biol.* 16, 399–414.
- 561 Evans, F., Matson, S., Brake, J., Langdon, C., 2004. The effects of inbreeding on performance
562 traits of adult Pacific oysters (*Crassostrea gigas*). *Aquaculture* 230, 89–98.
563 doi:10.1016/j.aquaculture.2003.09.023
- 564 Evans, S., Langdon, C., 2006a. Effects of genotype×environment interactions on the selection of
565 broadly adapted Pacific oysters (*Crassostrea gigas*). *Aquaculture* 261, 522–534.
566 doi:10.1016/j.aquaculture.2006.07.022
- 567 Evans, S., Langdon, C., 2006b. Direct and indirect responses to selection on individual body
568 weight in the Pacific oyster (*Crassostrea gigas*). *Aquaculture* 261, 546–555.
569 doi:10.1016/j.aquaculture.2006.07.037
- 570 Fabry, V.J., Seibel, B.A., Feely, R.A., Orr, J.C., 2008. Impacts of ocean acidification on marine
571 fauna and ecosystem processes. *ICES J. Mar. Sci.* 65, 414–432.
- 572 Falconer, D.S., Mackay, T.F.C., 1996. Introduction to quantitative genetics. Fourth edition.
573 Addison Wesley Longman, Essex, U.K. 464 pp.
- 574 FAO, 2014. The State of World Fisheries and Aquaculture. Rome. 223 pp.
575 <http://www.fao.org/3/a-i3720e.pdf>. Accessed April. 1st 2016.
- 576 Gjedrem, T., Robinson, N., Rye, M., 2012. The importance of selective breeding in aquaculture
577 to meet future demands for animal protein: A review. *Aquaculture* 350-353, 117–129.
578 doi:10.1016/j.aquaculture.2012.04.008
- 579 He, M., Guan, Y., Yuan, T., Zhang, H., 2008. Realized heritability and response to selection for
580 shell height in the pearl oyster *Pinctada fucata* (Gould). *Aquac. Res.* 39, 801–805.
581 doi:10.1111/j.1365-2109.2008.01889.x
- 582 Henryon, M., Jokumsen, A., Berg, P., Lund, I., Pedersen, P.B., Olesen, N.J., Slierendrecht, W.J.,
583 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance
584 exists within a farmed population of rainbow trout. *Aquaculture* 209, 59–76.
585 doi:10.1016/S0044-8486(01)00729-3
- 586 Hui, W., Jin, L.I.U., Yanhong, L.I., Xiaowen, Z.H.U., Zhigang, L.I.U., 2014. Responses to two-
587 way selection on growth in mass-spawned F₁ progeny of *Argopecten irradians concentricus*
588 (Say). *Chinese J. Oceanol. Limnol.* 32, 349–357. doi:dx.doi.org/10.1007/s00343-014-3153-
589 z
- 590 Ibarra, A.M., Ramirez, J.L., Ruiz, C.A., Cruz, P., Avila, S., 1999. Realized heritabilities and
591 genetic correlation after dual selection for total weight and shell width in catarina scallop

- 592 (*Argopecten ventricosus*). *Aquaculture* 175, 227–241.
- 593 Kong, N., Li, Q., Yu, H., Kong, L.F., 2015. Heritability estimates for growth-related traits in the
594 Pacific oyster (*Crassostrea gigas*) using a molecular pedigree. *Aquac. Res.* 46, 499–508.
595 doi:10.1111/are.12205
- 596 Kube, P., Cunningham, M., Dominik, S., Parkinson, S., Henshall, J., Finn, B., Henshall, J.,
597 Bennett, R., Hamilton, M., 2011. Enhancement of the Pacific oyster selective breeding
598 program. CSIRO Marine and Atmospheric Research, Australia. 177pp.
- 599 Kurihara, H., 2008. Effects of CO₂-driven ocean acidification on the early developmental stages
600 of invertebrates. *Mar. Ecol. Prog. Ser.* 373, 275–284. doi:10.3354/meps07802
- 601 Kvingedal, R., Evans, B.S., Lind, C.E., Taylor, J.J.U., Dupont-Nivet, M., Jerry, D.R., 2010.
602 Population and family growth response to different rearing location, heritability estimates
603 and genotype×environment interaction in the silver-lip pearl oyster (*Pinctada maxima*).
604 *Aquaculture* 304, 1–6. doi:10.1016/j.aquaculture.2010.02.035
- 605 Lallias, D., Taris, N., Boudry, P., Bonhomme, F., Lapègue, S., 2010. Variance in the
606 reproductive success of flat oyster *Ostrea edulis* L. assessed by parentage analyses in
607 natural and experimental conditions. *Genet. Res. (Camb.)* 92, 175–87.
608 doi:10.1017/S0016672310000248
- 609 Langdon, C., Evans, F., Jacobson, D., Blouin, M., 2003. Yields of cultured Pacific oysters
610 *Crassostrea gigas* Thunberg improved after one generation of selection. *Aquaculture* 220,
611 227–244. doi:10.1016/S0044-8486(02)00621-X
- 612 Lannan, J.E., 1972. Estimating heritability for predicting response to selection for the Pacific
613 oyster, *Crassostrea gigas*. *Proc. Natl. Shellfish. Assoc.*, 62, 62-66.
- 614 Lannig, G., Eilers, S., Pörtner, H.O., Sokolova, I.M., Bock, C., 2010. Impact of Ocean
615 Acidification on Energy Metabolism of Oyster, *Crassostrea gigas*—Changes in Metabolic
616 Pathways and Thermal Response. *Mar. Drugs* 8, 2318–2339. doi:10.3390/md8082318
- 617 Li, Q., Wang, Q., Liu, S., Kong, L., 2011. Selection response and realized heritability for growth
618 in three stocks of the Pacific oyster *Crassostrea gigas*. *Fish. Sci.* 77, 643–648.
619 doi:10.1007/s12562-011-0369-0
- 620 Liang, J., Zhang, G., Zheng, H., 2010. Divergent selection and realized heritability for growth in
621 the Japanese scallop, *Patinopecten yessoensis* Jay. *Aquac. Res.* 41, 1315–1321.
622 doi:10.1111/j.1365-2109.2009.02419.x
- 623 Maluwa, A.O., Gjerde, B., 2007. Response to selection for harvest body weight of *Oreochromis*
624 *shiranus*. *Aquaculture* 273, 33–41. doi:10.1016/j.aquaculture.2007.06.014
- 625 Misztal, I., 1997. Estimation of Variance Components with Large-Scale Dominance Models. *J.*
626 *Dairy Sci.* 80, 965–974. doi:10.3168/jds.S0022-0302(97)76021-1
- 627 Misztal, I., 2008. Reliable computing in estimation of variance components. *J. Anim. Breed.*
628 *Genet.* 125, 363–370. doi:10.1111/j.1439-0388.2008.00774.x
- 629 Misztal, I., Varona, L., Culbertson, M., Bertrand, J.K., Mabry, J., Lawlor, T.J., Van Tassel, C.P.,
630 Gengler, N., 1998. Studies on the value of incorporating the effect of dominance in genetic

- 631 evaluations of dairy cattle, beef cattle and swine. *Biotechnol. Agron. Soc. Environ.* 4, 227–
632 233.
- 633 Misztal, I., Tsuruta, S., Strabel, T., Auvray, B., Druet, T., Lee, D.H., 2002. BLUPF90 and related
634 programs (BGF90). *Proc. 7th World Congr. Genet. Appl. Livest. Prod.* 1–2.
- 635 Nell, J.A., Sheridan, A.K., Smith, I.R., 1996. Progress in a Sydney rock oyster, *Saccostrea*
636 *commercialis* (Iredale and Roughley), breeding program. *Aquaculture* 144, 295–302.
- 637 Nell, J.A., Smith, I.R., Sheridan, A.K., 1999. Third generation evaluation of Sydney rock oyster
638 *Saccostrea commercialis* (Iredale and Roughley) breeding lines. *Aquaculture* 170, 195–203.
- 639 Nguyen, T.T.T., Hayes, B.J., Ingram, B. a., 2014. Genetic parameters and response to selection
640 in blue mussel (*Mytilus galloprovincialis*) using a SNP-based pedigree. *Aquaculture* 420–
641 421, 295–301. doi:10.1016/j.aquaculture.2013.11.021
- 642 Norris, A., Foyle, L., Ratcliff, J., 2008. Heritability of mortality in response to a natural pancreas
643 disease (SPDV) challenge in Atlantic salmon, *Salmo salar* L., post-smolts on a West of
644 Ireland sea site. *J. Fish Dis.* 31, 913–920. doi:10.1111/j.1365-2761.2008.00982.x
- 645 Pante, M.J.R., Gjerde, B., McMillan, I., Misztal, I., 2002. Estimation of additive and dominance
646 genetic variances for body weight at harvest in rainbow trout, *Oncorhynchus mykiss*.
647 *Aquaculture* 204, 383–392. doi:10.1016/S0044-8486(01)00825-0
- 648 Schoener, A. and Tufts, D.F., 1987. Changes in oyster condition index with El Niño-Southern
649 Oscillation events at 46 N in an eastern Pacific bay. *Journal of Geophysical Research* 92:
650 doi:10.1029/JC080i013p14429.
- 651 Taris, N., Ernande, B., McCombie, H., Boudry, P., 2006. Phenotypic and genetic consequences of
652 size selection at the larval stage in the Pacific oyster (*Crassostrea gigas*). *J. Exp. Mar. Bio.*
653 *Ecol.* 333, 147–158.
- 654 Toro, J.E., Sanhueza, M.A., Winter, J.E., Aguila, P., Vergara, A.M., 1995. Selection response
655 and heritability estimates for growth in Chilean oyster *Ostrea chilensis* (Philippi, 1845). *J.*
656 *Shellfish Res.* 14, 87–92.
- 657 Usuki, H., 2002. Evaluation of characteristics and preservation of Pacific oyster, *Crassostrea*
658 *gigas*, in view of genetic resources. *Bull. Fish. Res. Agency* 4, 40–104 (in Japanese with
659 English Abstract and tables) <http://www.fra.affrc.go.jp/bulletin/bull/bull04/usuki.pdf>.
- 660 Wada, K.T., 1986. Genetic Selection for Shell Traits in the Japanese Pearl Oyster, *Pinctada*
661 *fucata martensii*. *Aquaculture* 57, 171–176.
- 662 Wang, H., Du, X., Lü, W., Liu, Z., 2010. Estimating the heritability for growth-related traits in
663 the pearl oyster, *Pinctada fucata martensii* (Dunker). *Aquac. Res.* 42, 57–64.
664 doi:10.1111/j.1365-2109.2010.02552.x
- 665 Wang, Q., Li, Q., Kong, L., Yu, R., 2012. Response to selection for fast growth in the second
666 generation of Pacific oyster (*Crassostrea gigas*). *J. Ocean Univ. China* 11, 413–418.
667 doi:10.1007/s11802-012-1909-7
- 668 Washington Policy Center 2015. Ocean acidification update from a shellfish grower on the front
669 line. <https://www.washingtonpolicy.org/blog>; Todd Myers June 19th 2015. Accessed Nov.
670 22nd 2015.

671 Zheng, H., Zhang, G., Liu, X., Zhang, F., Guo, X., 2004. Different responses to selection in two
672 stocks of the bay scallop, *Argopecten irradians irradians* Lamarck (1819). J. Exp. Mar. Bio.
673 Ecol. 313, 213–223. doi:10.1016/j.jembe.2004.04.015

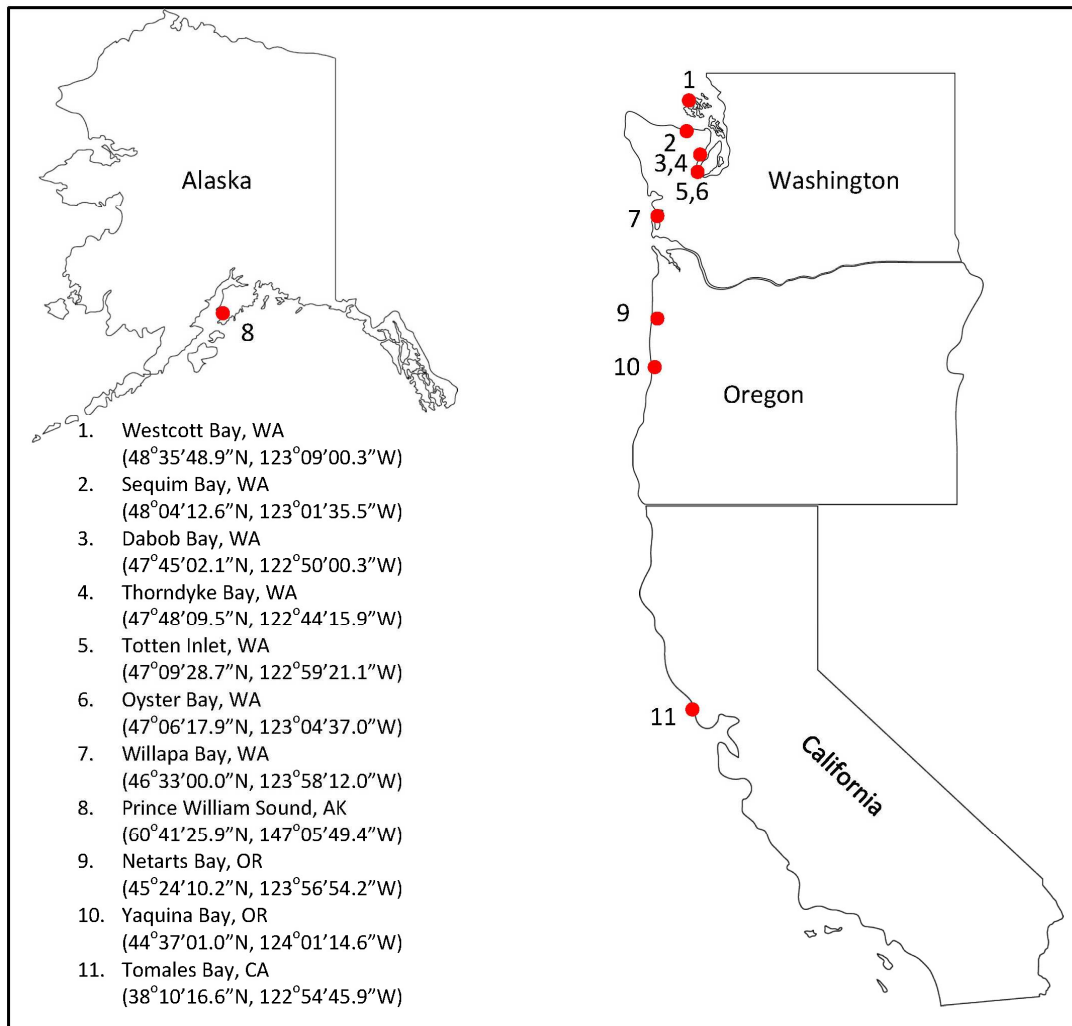


Fig. 1 – Location of test sites for the Molluscan Broodstock Program on commercial oyster farms.

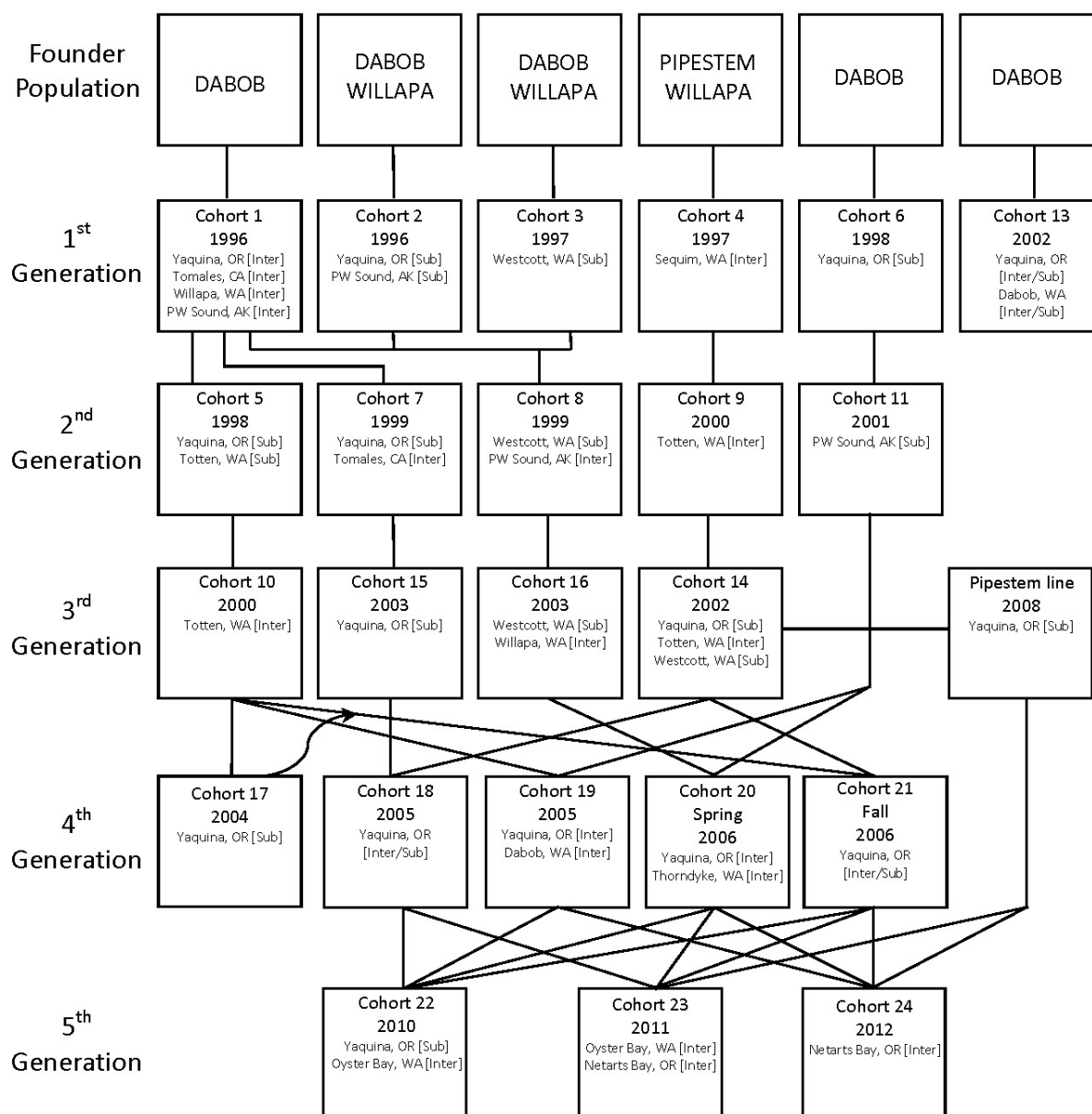


Fig. 2 – Breeding scheme for five generations of cohorts produced by the Molluscan Broodstock Program. Sub: sub-tidal plantings; Inter: intertidal plantings.

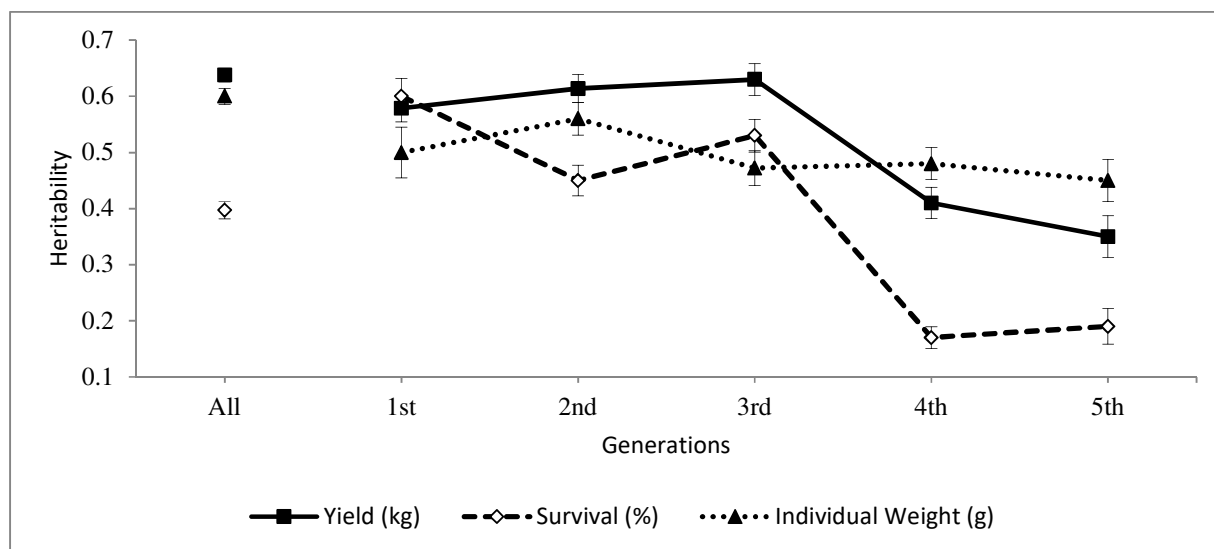


Fig. 3 – Estimates of heritabilities (\pm SE) of performance traits for five generations of the Molluscan Broodstock Program.

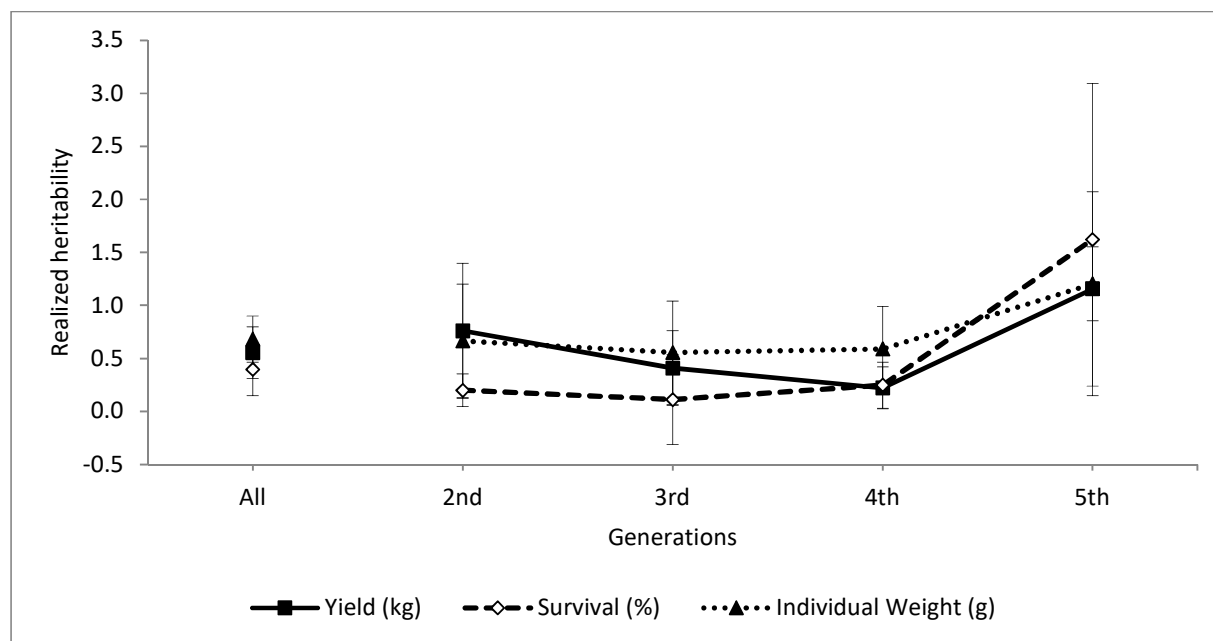


Fig. 4 – Estimates of realized heritabilities (\pm SE) of performance traits for five generations of the Molluscan Broodstock Program.

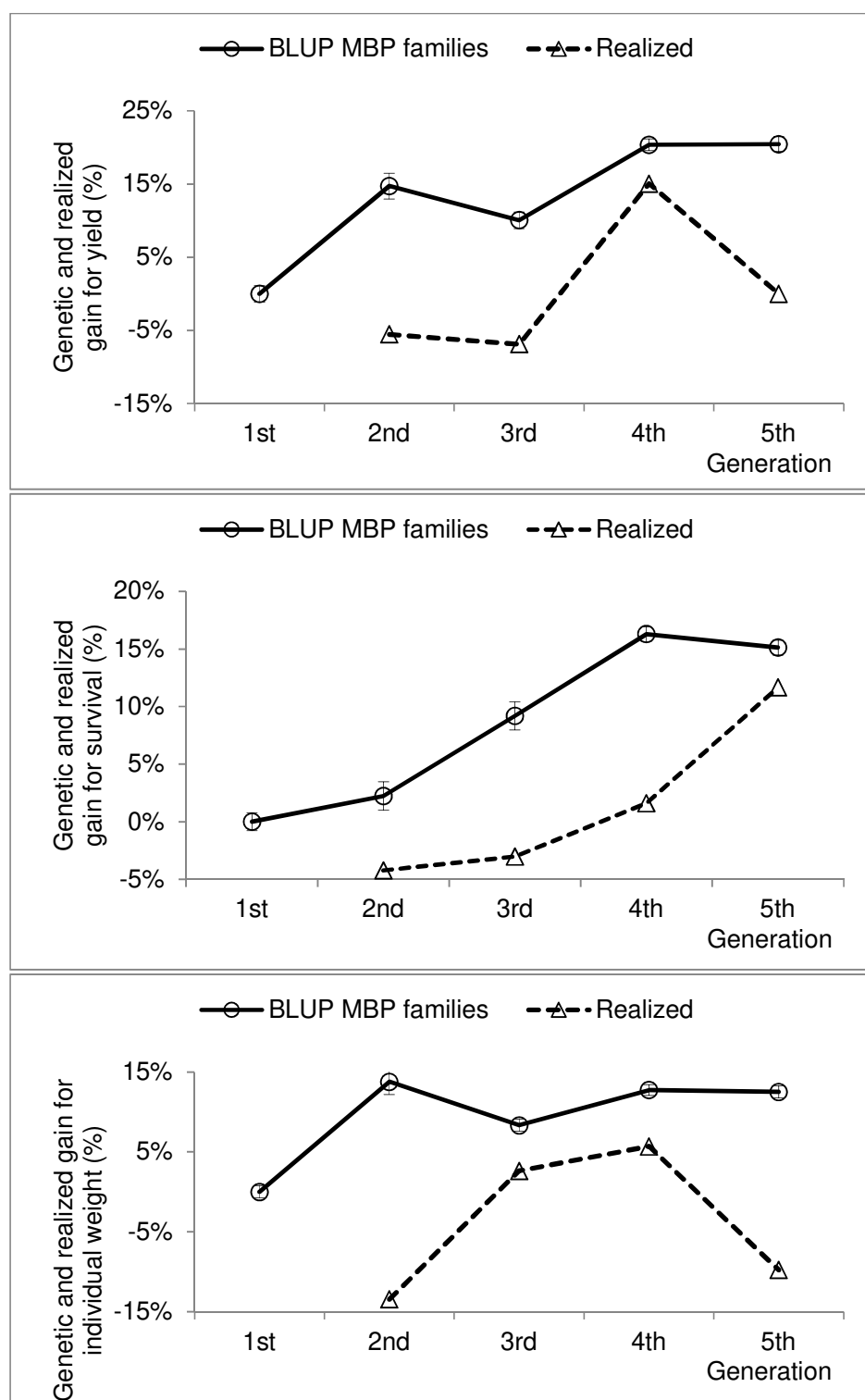


Figure 5 – Genetic gain (\pm SE) of performance traits for families for five generations of the Molluscan Broodstock Program.

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 sites	11	No. of dams [†]	277
No. of tidal exposures	2	No. of years	14
No. of generations	5	No. of cohorts	23
		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\pm1.12	1.81\pm1.42	62.36\pm17.90	58.31\pm22.28	87.99\pm29.52	71.67\pm30.97	36.02\pm51.70	14.26\pm22.62
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\pm0.01	0.38 \pm 0.04	0.90 \pm 0.01	0.53\pm0.02	0.90 \pm 0.02	0.11 \pm 0.12
Survival	0.09 \pm 0.01	0.35\pm0.02	0.25 \pm 0.04	0.83 \pm 0.01	0.55\pm0.03	-0.32 \pm 0.10
Ind. Weight	0.64 \pm 0.01	-0.06 \pm 0.01	0.55\pm0.01	0.26 \pm 0.03	-0.24 \pm 0.03	0.45\pm0.04
	2 nd Generation			3 rd Generation		
Yield	0.56\pm0.02	0.96 \pm 0.01	0.84 \pm 0.03	0.58\pm0.03	0.21 \pm 0.07	0.83 \pm 0.03
Survival	0.89 \pm 0.00	0.40\pm0.03	0.68 \pm 0.05	0.12 \pm 0.02	0.48\pm0.03	0.14 \pm 0.08
Ind. Weight	0.25 \pm 0.02	-0.05 \pm 0.02	0.51\pm0.03	0.62 \pm 0.01	-0.03 \pm 0.02	0.42\pm0.03
	4 th Generation			5 th Generation		
Yield	0.36\pm0.03	0.27 \pm 0.10	0.91 \pm 0.01	0.30\pm0.04	0.70 \pm 0.07	0.90 \pm 0.03
Survival	0.35 \pm 0.02	0.12\pm0.02	-0.09 \pm 0.10	0.76 \pm 0.02	0.14\pm0.03	0.34 \pm 0.12
Ind. Weight	0.64 \pm 0.01	-0.16 \pm 0.02	0.43\pm0.03	0.62 \pm 0.02	0.05 \pm 0.04	0.40\pm0.04

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.