Version of Record: https://www.sciencedirect.com/science/article/pii/S0044848621002404
Manuscript_0055d37a36dcb9a70bb8751de35f8aad

1 **Title**: Genetic parameters for *Crassostrea virginica* and their application to family-based breeding in the
2 mid-Atlantic, USA

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13 **Abstract**:
14 A family-based breeding program was established at the Aquaculture Genetics and Breeding
15 Technology Center (ABC) at the Virginia Institute of Marine Science beginning in 2004. Over the course
16 of developing the program, data were acquired to determine the optimal way to structure the breeding
17 population for the Eastern oyster, *Crassostrea virginica*, in the Chesapeake Bay estuary, and how best to
18 implement our now-operational 'industrial breeding' program. Traits studied were survival, total weight,
19 meat yield, and shell shape and decisions were based on estimates of heritability, genetic correlations, and
20 genotype by environment interaction for these traits. Post-hoc, we examined the effect of genetic groups
21 and genetic trends. Genetic variation was abundant and additive, with no evidence of large non-additive
22 effects. Age: age correlations were high, allowing abbreviated field deployment to assess market qualities
23 as early as possible. Survival at high and low salinity were poorly correlated, meaning the gene processes
24 that control survival at each salinity zone are different, and therefore survival at high and low salinities
25 were treated as separate traits. Total weight (indicator of growth rate) was moderately correlated between
26 salinity zones and, while there was some commonality in genetic control across salinities (with 70% of

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27 genes in common), total weight was also treated as a separate trait at high and low salinities. Meat yield
28 and shell shape were highly correlated among sites and can therefore be managed as a single trait across
29 all site types. The mass selected lines from our previous work were used as a source of founders, which
30 allowed genetic gains from those lines to be captured in the family program, thus making family-based
31 selection a continuation of previous efforts rather than a restart. The ABC lines have found wide utility
32 not only in the Chesapeake but also in the mid-Atlantic, defined as Rhode Island to South Carolina, USA.
33 Family-based breeding, using the principles encapsulated in this strategy, would be appropriate as a
34 common approach for contiguous breeding efforts for *C. virginica* along the east coast of the US from the
35 Northeast to the Gulf coasts.
36
37 **Keywords**: eastern oyster, heritability, genetic correlation, shellfish, oyster, aquaculture
38
39 **1. Introduction**

40 Breeding birthed aquaculture for the eastern oyster (*Crassostrea virginica*) in the Chesapeake
41 Bay. Long suffering from overfishing and disease mortality (Burreson and Andrews, 1988; Schulte,
42 2017), the fishery of the native *C. virginica* in the Chesapeake was at a nadir in the late twentieth century.
43 There were few efforts to farm oysters commercially because of the presence of oyster diseases,
44 principally MSX-disease caused by *Haplosporidium nelsoni*. Even with the advent of several disease
45 resistant lines, industry was slow to gain momentum. In an ironic twist, it was the failed introduction of
46 the non-native Suminoe oyster, *Crassostrea ariakensis*, that impelled the growth of an industry for the
47 native *C. virginia* in Chesapeake Bay (Allen, 2005).
48 Since the late 1960s, MSX-disease resistant lines have been available through a long-standing
49 "experiment" on heritability of MSX-resistance at the Haskin Shellfish Research Laboratory (HSRL) at
50 Rutgers University, the oldest shellfish breeding program in the world (Haskin and Ford, 1979). In the
51 1990s, disease resistant lines for MSX and Dermo-disease, caused by *Perkinsus marinus*, were also

developed at the Virginia Institute of Marine Science (VIMS) (Ragone Calvo et al., 2003; Degremont et al., 2015a). Despite the development of these lines, the impetus to begin commercial oyster culture was absent, due to a lack of demonstrated profitability and hatchery capacity.

In 1997, the Aquaculture Genetics and Breeding Technology Center (ABC) at VIMS was started to provide a more organized effort to initiate oyster culture, principally through selective breeding. The initial breeding goals were simple: develop oyster lines that were resistant to the two major diseases limiting the success of oyster husbandry. Initially, ABC obtained starting genetic material predominantly from two lines, DEBY and XB. DEBY is a line developed at VIMS from founders sourced from Delaware Bay in New Jersey, USA (Burreson, 1991; Ragone-Calvo et al., 2003). The XB line was developed by S. Allen at the HSRL through the Cooperative Regional Oyster Selective Breeding program, or CROSBreed (hence the abbreviated name: XB) (DeBrosse and Allen, 1996). For both lines, mass selection was applied by breeding survivors of field grow-out in the York River (for DEBY) and Delaware Bay (for XB) where there was consistent MSX-disease exposure. In 2004, despite all this genetic material, and their availability through ABC as brood stock, there was still virtually no oyster aquaculture in the Virginia portion of the Chesapeake Bay.

In 2004, the State of Maryland and the Commonwealth of Virginia proposed the introduction of *C. ariakensis* in an attempt to revitalize the industry in the Chesapeake Bay, which initiated a period of intense research on this subject. In order to accomplish field trials with the non-native, tetraploid *C. ariakensis* were required to make sterile triploid oysters to prevent accidental introduction (Allen, 2005; NRC, 2004). As a control, tetraploid and triploid *C. virginica* were also produced. By the end of the period of non-native research, commercial companies (largely seafood buyers and packers) that had been running the test sites for triploid *C. ariakensis* and *C. virginica* had experienced the crop potential of both species. *C. ariakensis* outgrew *C. virginica* in all salinities, but permission to continue the introduction of *C. ariakensis*, even as a triploid, was denied by regulators. However, the experience (and practice) of growing disease resistant, triploid *C. virginica* convinced many to begin oyster culture. Arguably, the

77 acceptance of *C. virginica* had as much to do with triploidy as disease resistance, but triploid *C. virginica*
78 had to be disease resistant too, or they would not thrive.

79 *C. virginica* aquaculture grew rapidly in the Chesapeake Bay, from less than 1M market oysters
80 sold in 2005 to about 40M in 2014 (Hudson, 2019). With the emergence of the oyster culture industry,
81 ABC focused on building an industrial breeding program for shellfish. By "industrial breeding program,"
82 we mean breeding for the principle purpose of genetic improvement of brood stock to be supplied to the
83 industry. A few other oyster breeding programs of this nature exist. The HSRL program at Rutgers
84 provides brood stock to the industry largely in the Northeast US (Guo et al., 2008) based on the
85 production of both diploid and tetraploid lines known as NEH (Northeast High survival). The HSRL
86 program is based on individual selection and the propagation of derivative lines. The Molluscan Breeding
87 Program (MBP) began in 1996 to provide genetic improvement of *Crassostrea gigas* and a source of
88 brood stock to the oyster industry in the Pacific Northwest US (Langdon et al., 2003). MBP utilizes a
89 modified family breeding program and is based on yield, a product of survival and weight. Modest gains
90 through the MBP were realized early on, but De Melo et al. (2016) reported zero gain in generation 5 and
91 strong genotype by environment (GxE) effects were later noted (De Melo et al., 2018). Reasons for the
92 lack of progress in MBP have recently been posited by Hedgecock and Pan (2021). Still, MBP provides a
93 significant service to the industry in the Pacific northwest of the US by providing brood stock through
94 multiplier populations to commercial hatcheries. In Australia, an industry focused breeding program for
95 Sydney rock oysters, *Saccostrea glomerata*, is operated by New South Wales Department of Primary
96 Industry (Dove et al., 2013) and has evolved from mass selection to a family-based breeding strategy. In
97 New Zealand, the Cawthron Institute began a family breeding program for *C. gigas* in 1999 with a focus
98 on aesthetic traits, although the industry maintained a high reliance on wild caught spat. That changed in
99 2013 with the onset of acute mortalities from the oyster herpes virus, OsHV-1μvar (Paul-Pont et al. 2014)
100 and a shift in the breeding program to address those mortalities (Camara and Symonds, 2014).

101 ABC stylized its family-based breeding approach, beginning in 2004, after the Australian Seafood
102 Industry P/L (ASI) program (Kube et al., 2011), a grower owned company that operates selective

103 breeding for *C. gigas*. Like ASI, ABC's initial goals included establishing economic weights for
104 commercial traits. The adoption of family selection by ABC was partly based on initial estimates of
105 heritability, which showed high heritabilities for most traits but relatively low heritability for general
106 survival, which is a condition suited for family selection (Fjalestad, 2005). Family selection was also
107 deemed appropriate because of the apparent GxE interaction of *C. virginica* throughout an estuarine
108 environment like the Chesapeake Bay (Frank-Lawale et al., 2014). Family breeding is especially powerful
109 with highly fecund animals like oysters, due to the ability to produce large sib-tests, and is considered the
110 state of the art for aquaculture species (Houston, 2017).

111 In this paper, we report the results obtained during the establishment of our family-based
112 approach beginning in 2004 year class from which we obtained our first estimates of heritability, through
113 the creation of founder populations from various sources and the ongoing estimates of heritabilities,
114 genetic correlations, and genetic group effects in ABC's breeding population, up to 2018.

115 **2. Materials and Methods**

116 **2.1 Founder populations and genetic material**

117 Lines DEBY and XB formed the core of initial breeding work at ABC. For both lines, survivors
118 to disease exposure at multiple sites were propagated over several generations of mass selection. Other
119 germplasm was obtained from Louisiana sources (LA – Figure 1) because of their fast growth and
120 inherent resistance to Dermo-disease (Bushek and Allen, 1996; Ragone Calvo et al., 2003; Encomio et al.,
121 2005). Wild oysters from Grand Terre, Camanada Bay, and a selected line from Louisiana State
122 University called OBOY (abbreviated from Oyster Bayou; Leonhardt, 2010) were also incorporated into
123 the VIMS program. By 2004, ABC had founded eight hatchery-reared lines (Frank-Lawale et al., 2014)
124 and by 2006, there were 15. Collectively, these are referred to as "Original HS lines" for the purpose of
125 determining genetic group effects (Figure 1, Table 1). In 2004, ABC started exploring heritability for
126 disease resistance and other traits by producing families from wild Virginia populations; progeny from
127 those genetic groups are designated "Wild Virginia" (Figure 1, Table 1) for genetic group analysis.
128 Starting in 2008, the Original HS lines were consolidated into three major derivative lines, called Super

129 Lines (SL): SL DEBY, SL XB and SL LA. Each of the three Super Lines was selected in both high and
130 low salinity environments (Figure 1). Super Lines selected in high salinity were designated "ABC HS
131 lines" as a genetic group and for low salinity – "ABC LS lines" (Table 1).
132 For the purpose of this paper, low salinity is defined as ~6-15ppt and high salinity – ~15-23ppt.
133 *C. virginica* thrive in higher salinity (Shumway, 1996), up to 40ppt in some places, but 5-25 represents
134 the vast majority of commercial culture in Chesapeake Bay.

135 **2.2 Hatchery and nursery**

136 Between 2004 and 2010, all brood stock oysters for family spawns were allowed to ripen
137 naturally in the York River prior to spawning. From 2013 to 2016, the majority of brood stock was
138 conditioned at the Horn Point Laboratory, University of Maryland Center for Environmental Science,
139 using a flow-through conditioning system. At spawning, conditioned broodstock were brought into the
140 hatchery and maintained within family groups. Individual oysters were shucked and sex determined by
141 gonad biopsy and males and females were separated. Ova were excised by gently scraping the gonad with
142 a clean scalpel blade and rinsing gametes into a beaker using filtered seawater. Eggs were rinsed over a
143 65µm and 20µm sieve and the screened eggs were then counted and assessed for quality. Sperm was
144 excised in a similar fashion and examined under the microscope for density and motility. The mating
145 design was generally 2 males crossed with 2 females (2 x 2), although different designs were used in
146 earlier year classes (Table 1). Eggs for each female were divided equally prior to fertilization by sperm
147 from different males. Sperm dosage was assessed by observing sperm:egg association (e.g., ~10 observed
148 sperm/egg) and fertilization success was assessed by examining the development of zygotes under a
149 microscope.

150 Fertilized eggs were reared in 60L, aerated, larval culture tanks filled with filtered sea water at
151 ~26°C, with each family being reared separately in a single tank. Water was changed every second day
152 and larvae were retained on mesh screens for counting and health assessment. Larvae were fed a daily
153 ration of microalgae in accordance with their stage of development using *Pavlova* sp., *Chaetocerous*
154 *neogracile*, and *Tetraselmis* sp. Larvae were removed from the culture tanks when they reached the

155 pediveliger stage and were deemed competent to set. Competent larvae were harvested over a 4 day
156 period (three harvests: days 0, 2, 4) by holding early setters at 4°C and combining all larvae on day 4 in
157 order to minimize selection pressure within each larval culture (family). Each family was then transferred
158 to individual downwelling systems containing 400µm ground oyster shell. When oysters were large
159 enough to be retained on a 500µm screen (roughly 10 days), families were transferred to a land-based
160 upwelling system, fed with raw water, where each family was allocated to a single upwelling silo. At
161 10mm, families were transferred to culture bags and raised through the fall and winter in the Chesapeake
162 Bay. Families were kept separate at all times.

163 **2.3 Sib Testing in the Field**

164 For field tests, oyster seed was deployed in grow-out units (either bags for rack and bag or
165 baskets for Australian longline culture), with a single family contained in each unit. Families were
166 deployed in replicate units, generally with three replicates per family per site and 100 – 200 oysters per
167 replicate, depending on gear type. Grow-out units were haphazardly placed on a site, meaning there was
168 no blocking structure to the field tests. Tests were deployed during the early spring (March-April)
169 following the year of spawn, and oysters were generally 15-30mm in length. Husbandry at each field site
170 included regular maintenance to remove fouling, eliminate predators, and re-distribute oysters to promote
171 uniform growth within each test unit. Measurements of survival and growth were recorded at ages 1.5 and
172 2.5 years for year classes 2004 – 2010 and at 1.5 years for year classes 2013 – 2018.

173 Field tests were deployed at sites along a salinity gradient in the Chesapeake Bay (Figure 2, Table
174 2). Culture method was rack and bag at all sites until 2014, when Australian long-line systems were
175 established at Horn Point and York River. The Kinsale site was replaced by the Lewisetta site in 2013 but
176 they are adjacent and considered the same environment for analysis.

177 **2.4 Field measurements**

178 Each family grow-out unit was assessed for survival at 1.5 or 2.5 years of age. All oysters were
179 removed from the unit and dead and moribund oysters were discarded. Live oysters remaining were
180 counted and the ratio of remaining oysters to initial count was used to calculate cumulative survival. After

181 survival assessments were complete, a subset of oysters from each family replicate was randomly chosen
182 for assessment of individual weight, shell shape, and meat weight. Each oyster was thoroughly cleaned
183 and fouling organisms removed prior to measurement. Shell length (hinge to bill), height (depth), and
184 width (greatest distance perpendicular to length) were recorded to the closest millimeter using calipers.
185 Width index was calculated as the ratio of width to length. Height index was calculated as the ratio of
186 height to length. Whole weight was recorded to the nearest tenth of a gram. Once external measurements
187 were completed, oysters were opened and all tissue removed. Individual meats were allowed to drain of
188 liquor briefly on a screen prior to recording meat weight. Meat yield was calculated as the ratio of wet
189 meat weight to total weight.

190 **2.6 Genetic groups**

191 Genetic groups were used to account for the fact that founders descend from different gene pools,
192 not a single randomly mated population. Genetic group effects measure the differences between those
193 gene pools as expressed in the ABC family-based program. Defining genetic groups was a two-stage
194 process. First, a model (see section 2.7) was fitted that included 23 genetic groups, representing a small-
195 scale grouping, and that included six wild populations, eight 'original' mass selection lines, and nine mass
196 selection 'Super Lines,' aka ABC HS lines and ABC LS lines (Figure 1). Second, the estimated genetic
197 group effects were then evaluated and condensed to four genetic groups for a final genetic group
198 definition. This re-grouping was done using the predicted values for each trait in each group together,
199 with knowledge of the history of the groups. Groups that had no large differences and which had a similar
200 history were pooled. For example, differences between various wild populations were small and therefore
201 all were combined as a single group that was referred to as 'wild Virginia'. The only results reported here
202 are for the four group structure.

203 **2.7 Genetic analyses**

204 Table 3 summarizes the data used for the analyses. The unit survival counts were converted to
205 binary survival data (dead=0, alive=1) for individual animals and all data were then merged into a single
206 file consisting of individual animal records. Measurements were taken at two ages for five of the nine

207 year classes. Individual measurements comprising weight, meat yield, and shell shape were taken from
208 different animals at each time point due to lethal sampling required for meat weight. Survival data were
209 repeated measurements taken on the same animal since all animals in each unit were counted at each
210 measurement. In total, there were 877,589 individual animal phenotype records in the data file. This
211 comprised of 64,081 animals with weight, meat yield, and shell shape at age 1.5 years; 20,602 animals
212 with those same measurements at age 2.5 years; 813,454 animals with survival records at age 1.5 years;
213 and 318,694 animals with survival records at both 1.5 and 2.5 years.

214 Initial analyses were done for each individual sib test (results not shown) as a data check. These
215 analyses indicated that the phenotypic variances for sib tests were variable, particularly for total weight
216 and meat yield. Consequently, all individual data were standardized by dividing by the phenotypic
217 standard deviation appropriate for each sib test and trait, meaning the analyzed data were in units of
218 phenotypic standard deviations.

219 Data were analyzed using ASReml (Gilmour et al. 2015) to fit multi-variate linear mixed animal
220 models. The terms in the model were:

221 $$Y = \mu + Trial + Unit + Family + Animal + \varepsilon \qquad \text{model 1}$$

222 where Y is a vector of the measured values, as described above, μ is the mean for each trait, *Trial* is the
223 fixed effect of the sib test, *Unit* is the random effect of the grow-out unit within a sib test, *Family* is the
224 random effect of the full-sib family, *Animal* is the random additive genetic effect, and ε is the residual
225 variation. Models specified a separate pedigree file containing the full pedigree structure back to
226 founders. The genetic groups option in ASReml was used, which linked all founders to a genetic group
227 allowing the genetic group effects to be estimated. This pedigree structure was linked to the *Animal* term
228 in the above model. Additive genetic (*Animal*) and residual (ε) terms included inter-trait variance and
229 covariance structures but the inter-trait co-variances were fixed to zero for *Family* and *Unit*. Genetic
230 correlations and their standard errors were estimated from these inter-trait variance and covariance
231 structures.

232 Analyses were done in three stages, all using model 1 but each with different data. First, genetic
233 parameters for each site were estimated using data for each site separately (5 runs) and with site data
234 combined as high and low salinity zones (2 runs). A five trait multivariate model was fitted (total weight,
235 meat yield, width index, height index, survival) and only the age 1.5 year data were used. These analyses
236 used all year classes as a combined population, which was possible due to the genetic links between all
237 year classes. Second, correlations between sites were estimated using a series of bivariate analyses. These
238 analyses were done for all five traits and, for each trait, five pairwise sites comparisons were made (25
239 runs) as well as a combined grouping of low and high salinity sites (5 runs) (see Table 6 for site
240 combinations tested). Correlations could not be estimated for all site combinations because there were
241 insufficient families in common across all sites. As for the first set of analyses, these included all year
242 classes as a single population. Third, correlations between age 1.5 and 2.5 year data were estimated, also
243 using a series of bivariate analyses. Data were available from two sites (Kinsale and York River) and all
244 five traits were analyzed for each site (10 runs). These analyses also combined all year classes as a single
245 population, however, the age 2.5 year data was only available for year classes 2004 to 2010.
246 Heritabilities for all analyses were estimated as:

247 $$h^2 = \sigma^2_a / (\sigma^2_u + \sigma^2_f + \sigma^2_a + \sigma^2_\varepsilon) \qquad \text{model 2}$$

248 where σ^2_u, σ^2_f, σ^2_a, and σ^2_ε are, respectively, the variance components for unit, family, additive genetic,
249 and residual terms in model 1. Standard errors of heritabilities were estimated using the variance
250 component function of ASReml. Genetic correlations and their standard errors were estimated from the
251 inter-trait variance and covariance structures for the Additive genetic (*Animal*) term in model 1. For
252 survival data, the heritability estimates on the observed scale are likely to be underestimates due to the
253 binomial data. Estimates on the underlying scale are a better expression of the true nature of genetic
254 variation and, therefore, values were adjusted using the expression of Dempster and Lerner (1950):

255 $$h^2_u = h^2_o\, (p\,(1-p)) / z^2 \qquad \text{model 3}$$

256 where h^2_u is heritability on the underlying liability scale, h^2_o is heritability on the observed scale (as
257 calculated in model 2), p is the proportion affected (survival), and *z* is the height of the standard normal
258 curve at the threshold point. The values for p were the phenotypic survival averaged across all year
259 classes for the data set being analyzed. Standard errors for heritabilities on the underlying scale were
260 calculated by rescaling estimates on the observed scale by the proportional change in heritability
261 estimates.

262 **2.8 Genetic trends**

263 Genetic trends were calculated to assess the rate of genetic change for each year class and for
264 each trait. This was done by averaging estimated breeding values (EBV, calculated using the analyses
265 described above) of families from each year class. The genetic group effect for wild Virginia was used as
266 the baseline (zero value) and EBV were expressed as deviations from the wild. The operational breeding
267 strategy for ABC has been structured to include two sub-populations (explained in the Discussion
268 section), each with its own breeding objective and with families being selected independently in each.
269 Genetic trends were calculated separately for each sub-population. Total weight, meat yield, width index,
270 and height index were expressed as a percentage change from the baseline using the following
271 relationship:

272 $$EBV_\% = [EBV / (\sqrt{V_a})] . \sqrt{(h^2)} \cdot CV$$

273 where EBV_% is the EBV for each trait expressed in units of percentage gain, EBV is the EBV as output
274 from model 1, V_a is the additive genetic variance, h^2 is our best estimate of the heritability for a particular
275 trait within a sub-population (values in Table 9 were used), and CV is the phenotypic co-efficient of
276 variation for the trait (values in Table 3 were used). Survival was expressed as the expected survival (i.e.
277 not as a percent gain) and was calculated as:

278 $$EBV_Sur = [EBV / (\sqrt{V_a})] . \sqrt{(h^2_{und})} \cdot SD + a$$

279 where EBV_Sur is the EBV for survival expressed as the expected field survival, EBV and V_a are as
280 defined above, h^2_{und} is the underlying heritability for either low salinity or high salinity survival, SD is
281 the phenotypic standard deviation for survival, and *a* is the expected survival assumed for the baseline

282 (wild Virginia) stock. For a binomial, the standard deviation is a function of the incidence and can be
283 calculated as SD = $\sqrt{[i.(1-i)]}$ where i is the survival in these field tests averaged across all year classes.
284 The value for *a* was set at 50%, the grand average for survival of wild Virginia families in 2005 and 2006
285 at both high and low salinities.

286 **3. Results**

287 **3.1 Summary statistics**

288 Mean values for total weight, width index, and height index had very low variation among sites
289 at 18 months, while variation in meat yield tended to be higher at Horn Point (Table 3). Survival had the
290 largest variation among sites and, at 18 months, was lower at York River (mean=69%) and Lynnhaven
291 (72%). Survival was higher at the low salinity sites, Horn Point and Kinsale/Lewisetta, where mean
292 values were 86% and 81%, respectively. At 30 months, mean survival in the York was 24%, while mean
293 survival at the Kinsale/ Lewisetta sites was 49%. Survival and total weight were higher in Lynnhaven.

294 **3.2 Genetic groups**

295 A total of 253 wild Virginia parents contributed to the breeding population, and these
296 contributions occurred over five year-classes from 2004 to 2010 (Table 1). Wild parents were sourced
297 from seven different locations within the Chesapeake Bay. In preliminary analyses (not shown), each
298 geographic location where wild oysters were collected was designated as a separate genetic group,
299 however, there were no large differences in genetic group effect among them for any traits. Therefore, we
300 concluded that the Chesapeake Bay wild populations were undifferentiated with respect to the traits in
301 question and the wild parents were treated as a single genetic group in the final analyses.

302 There was a clear impact of genetic group on performance for total weight and survival, but
303 minimal effects for shell shape traits and meat yield (Table 4). For total weight, large effects of genetic
304 group (29-50%) were seen from the ABC High and Low Salinity lines, which since 2008 had been the
305 subject of mass selection for size (Frank-Lawale et al., 2014). For survival, all the selected lines
306 underwent continuous mass selection for survival (disease resistance) and this exerted large and positive

307 genetic group effects (9-26%). The Original High Salinity lines represent the status of mass selection in
308 the early breeding program in 2004 (9% for survival), while the ABC Low and High Salinity lines (so-
309 called Super Lines) reflect the additional 5 (or so) generations of selection done on the ABC lines (8-26%
310 for survival).

311 **3.3 Genetic variation**

312 **3.3.1 Heritability**

313 Additive genetic variation was present for all traits at all sites (Table 5). The magnitude of
314 heritabilities for traits was generally comparable across sites except for Lynnhaven, where heritabilities
315 were lower for all traits except height index, suggesting this site was subject to factors that increased
316 noise and reduced the clarity of genetic differences. For total weight, heritabilities were moderately high
317 (near 0.3) and ranged between 0.23 – 0.36. For shell shape traits, height index (or cup depth) tended to
318 have stronger genetic expression than width index, although values for both were high to moderately
319 high; width index, 0.29 – 0.38 and height index, 0.19 – 0.31. Heritabilities for meat yield were moderate
320 (0.12 – 0.20) and lower than for other traits.

321 Heritabilities for survival are reported for both the observed and underlying scales, with the
322 assumption that values for the underlying scale are the relevant values for applied breeding. On this scale,
323 values were moderately high (0.23 – 0.35), with the exception of Lynnhaven where there was no
324 significant genetic variation. Heritability estimates for survival were generally consistent within a site and
325 across all year classes, including at the Lynnhaven site. Values for all sites and year classes are not shown
326 but, as an example of the typical pattern, underlying heritabilities at York River from the 2004 to 2018
327 year class were, respectively, 0.19, 0.32, 0.23, 0.12, 0.19, 0.08, 0.15, 0.22, 0.41, 0.23, and 0.20.

328 The Horn Point and York River sites used two different gear types during this study: rack and bag
329 for early year classes and long line for later (see Table 2). The gear types produced distinctively different
330 looking oysters, with shell shape most obviously affected. However, there was no discernible pattern of
331 genetic expression (i.e., heritability or family variance) with different gear types and no apparent pattern
332 in correlations among the sites with different gear. Generally, using a long line grow-out system in the

333 York River gave higher heritabilities for total weight, meat yield, and shell shape and lower heritabilities
334 for survival; however, the long line grow-out at Horn Point produced exactly the opposite pattern.
335 Heritabilities were estimated at both 1.5 and 2.5 years (Table 6). For total weight, the heritability
336 was higher at 2.5 years in both low and high salinity zones. However, this result may be influenced by the
337 1x1 crossing design that predominated in these early year classes, a design that may inflate estimates of
338 additive variance. For survival, heritabilities were lower at 2.5 years, a result that may reflect in
339 increasing causes of mortality over time.

340 **3.3.2 Family variance**

341 Estimates of family variances for each trait and site are reported in Table 5. In this study, family
342 variances represent the combined effects of rearing families separately plus non-additive genetic effects,
343 with those effects being inseparable given there was no replication prior to field deployment. Family
344 effects were mostly statistically significant across all trials and traits but always low. They were highest
345 for total weight and meat yield, explaining between 5% and 8% of total variation, a result that may be due
346 to rearing families in unreplicated units during from the hatchery, nursery, and over-winter stages. Family
347 effects were lower for width and height indices, where they contributed between 0% and 5% of total
348 variation, and also low for survival accounting for 4% of total variation on low salinity sites and no more
349 than 2% of total variation on high salinity sites.

350 **3.3.3 Genetic correlations**

351 Genetic correlations between sites were calculated for four pair-wise comparisons (Table 7).
352 Robust estimates for other site combinations were not possible due to variable and incomplete family
353 deployments across sites. Survival expressed very differently in high and low salinity zones. Within
354 salinity zones, survival was repeatable, with genetic correlations near 1.00. However, genetic correlations
355 between salinity zones were low in the two pair-wise comparisons ($r_g = 0.19$ and 0.57) and in a combined
356 analysis of all sites (0.23). Therefore, survival was deemed to be two traits: low salinity survival and high
357 salinity survival. Genetic correlations for total weight within salinity zone were also high (r_g=0.93 – 1),
358 but only moderate between salinities (r_g=0.70 in a combined analysis), indicating a lack of uniformity in

359 genetic expression across sites. Other traits appear to have consistent expression across salinity zones
360 with genetic correlations being mostly at or near unity (Table 7).
361 Genetic correlations between assessments at age 1.5 and 2.5 years (age from spawning) were
362 estimated for all traits, although only for year classes 2004 to 2010 (Table 6). Values were very high and
363 near unity for total weight, width index, and height index ($r_g \geq 0.96$) and high for meat yield ($r_g \geq 0.80$).
364 For survival, age: age genetic correlations were lower ($r_g = 0.72$ and 0.83 on low and high salinity sites
365 respectively), possibly due to reasons discussed in section 4.3.
366 Genetic correlations among traits were estimated within salinity zones (Table 8), recognizing the
367 important differences between salinity zones, as explained above. There were significant correlations
368 among traits, both favorable and adverse, and trends were similar at low and high salinity. The important
369 correlations were 1) favorable correlations between total weight and survival ($r_g = 0.49$ and 0.59,
370 respectively, on low and high salinity sites); 2) favorable correlations between meat yield and survival
371 ($r_g = 0.40$ and 0.21); 3) high and favorable correlations between width index and height index ($r_g = 0.75$
372 and 0.73); and adverse correlations between total weight and shell shape (ranging between -0.38 for
373 height and -0.50 for width).

374 **3.3.4 Combined site analysis of heritability**

375 The final part of the genetic analysis was to estimate heritabilities in a combined site analysis,
376 grouping low salinity sites (Horn Point and Kinsale/Lewisetta) and high salinity sites (Lynnhaven and
377 York River) (Table 9). This was the logical grouping of the sites based on the genetic parameters that now
378 comprise the underlying breeding strategy of our program (see section 4.3 in Discussion). These
379 parameters are based on the large numbers of parents, families, and progeny, that are spread over multiple
380 year classes and sites and, therefore, are likely to be sound estimates. Overall, significant additive genetic
381 variation was present in all traits. Meat yield had a lower heritability relative to other traits (0.16 – 018)
382 but for all other traits, heritabilities are moderately high (0.24 – 0.37). Furthermore, family variances were
383 consistently low and, while our estimates of family effects conflated non-additive genetic variation with
384 common environment effects from early life stage, genetic variation is predominantly additive.

385 **3.3.5 Genetic trends**

386 A genetic trend measures the progress made in the breeding population for each trait in the
387 breeding objective. These trends roughly correspond to three phases of our program: 1) R&D phase with
388 first family deployments (2004-2010), 2) the founder phase (2013-2014), and the 3) operational phase
389 (2015-2018, and continuing). Genetic trends for high- and low-salinity sub-populations, each with its own
390 breeding objective, are shown separately in Figure 3.

391 In the R&D phase, selections were not targeted to a particular breeding objective and the genetic
392 trends were flat, except for survival, for which genetic trends were upward. The founder phase was
393 characterized by the introgression of various selected lines into the families (see Table 1) in the 2013 and
394 2014 year classes and resulted in a clear rise in the genetic trend for total weight. Operational breeding
395 has been ongoing since 2015, consisting of targeted selection using a selection index based on economic
396 weights. In this period, gains were evident, particularly for low salinity survival. Gains for total weight
397 were more variable in both sub-populations and genetic trends for meat yield and shell shape traits were
398 flat.

399 **4. Discussion**
400
401 **4.1 Building a program**
402
403 In this paper, we reported the results obtained during the establishment of our pedigree-based family
404 breeding program, which began in 2004. Subsequently, we produced families from founder populations
405 from various sources and obtained estimates of heritabilities, genetic correlations, and genetic group
406 effects in ABC's current material, up to 2018. All data analyses were targeted to address two objectives:
407 determine the optimal way to structure the ABC breeding population for *C. virginica* in the Chesapeake
408 Bay estuary, and how best to implement a breeding strategy. The analyses provided an understanding of
409 the genetic architecture of traits important to the oyster culture industry. The major findings that shaped
410 our current operations are the following:

411 • Genetic variation is abundant and additive, with no evidence of large non-additive effects.
412 • Age: age correlations are high, allowing abbreviated field deployments to assess market qualities.

- Survival at high and low salinity are poorly correlated, meaning the genes that control survival at each salinity zone are different, and they are therefore treated as separate traits.
- Total weight (indicative of growth rate) is moderately correlated between salinity zones and, while there is some commonality in genetic control across salinities (~70% of genes appear common), high and low salinity growth are also best treated as separate traits.
- Meat yield and shell shape are highly correlated between sites and can be managed as a single trait across all salinities.
- The use of founders from mass selected lines from our previous work has contributed to genetic gains in the family breeding program, and represents a continuation of previous efforts rather than a restart.

The following discussion steps through the process we followed to build the breeding strategy. That process was an evolution over 15 years and was driven by the use of data to understand the genetic architecture and inform decisions. It involved balancing the seasonal cycles of the animal and logistical planning to ensure the best application of limited resources, while seeking to turn over generations as quickly as possible to maximize gains.

4.2 Genetic groups

The genetic groups analysis allowed us to measure the genetic merit of founder sources and provided empirical evidence of the value of founder sources. The use of genetic groups is common in plant and animal breeding where it is used to account for breed effects or geographic differences among founder sources (Isik et al., 2017). In aquaculture, the use of genetic groups appears less common and our application in *C. virginica* breeding demonstrates the potential and value in this sector. In addition to providing estimates of the genetic merit of a founder and enabling unbiased estimates of genetic parameters (discussed below), it has provided objective measures of the gains made in the mass selection programs that preceded family breeding.

According to this analysis, mass selection on the ABC lines increased high and low salinity survival by 15% and 16%, respectively, and increased total weight by 29% and 50% on high and low

439 salinity sites, respectively. Disease resistance was targeted initially in the mass selection programs and
440 intentional selection on size was instituted later, from 2010-2014. The contribution of germplasm from
441 these mass selected lines demonstrates the value of using them as a source of founders for the family
442 breeding program. The magnitude of the genetic group effects also demonstrates the importance of
443 including these values in models for estimation of genetic parameters. If omitted, genetic group effects
444 would be interpreted as additive genetic effects, upwardly biasing estimates of additive genetic variance.
445 The ABC lines have a long and complicated history, which is not quantitatively documented, and
446 therefore it is not possible to use these data to estimate a rate of gain due to mass selection. Nonetheless,
447 these gains are the result of up to 10 generations of selection.

448 Mass selection on the ABC lines caused a small adverse change for shell shape traits. Shell shape
449 traits were not intentionally targeted in the mass selection program and the observed negative effects are
450 likely due to the negative genetic correlations between the targeted traits (survival and size) and shell
451 shape traits. That is, positive gains for targeted traits also yielded negative gains for shape. Given the
452 magnitude of the adverse correlations between total weight and shell shape, stronger changes in shell
453 shape would have been expected. The fact that the change has been small suggests some selection
454 pressure on shell shape was exerted during mass selection.

455 **4.3 Survival**

456 For shellfish breeding, survival is arguably the most important trait, especially where it can be the
457 difference between success and failure of an industry (Kube et al., 2018). The lack of specificity for the
458 causes of mortality is a confounding problem for oyster breeding and probably any aquaculture species
459 where the environment cannot be controlled. Survival can be categorized as resistance to disease on the
460 one hand or general survival (i.e., robustness) on the other, and clearly could be a combination of both.
461 Few estimates of heritability for resistance to specific oyster diseases are available (Degremont et al.,
462 2015a), but the few that exist are high (Degremont et al., 2015b, $h^2 = 0.49$-0.60; Kube et al., 2018, $h^2 =$
463 0.39-0.49) or judged as high by the realized gain (Degremont et al., 2015c; Dove et al., 2013). ABC has

464 observed but not formally measured, the realized gain to MSX resistance through mass selection of naïve
465 material introduced to the Chesapeake Bay, i.e. Louisiana populations. In these instances, mortality was
466 initially very high (e.g., >90%) but succeeding generations improved dramatically, which is what has
467 been observed for MSX-disease since the onset of such research (Haskin and Ford, 1979). However, we
468 believe that resistance to MSX-disease contributed only a small portion to the heritability for high salinity
469 survival, for two reasons. First, founders were largely from material already strongly selected for
470 resistance to MSX-disease. The genetic groups analysis attests to this, showing significant effects from
471 the addition of ABC lines. Second, there is evidence to suggest that MSX is less prevalent now (Carnegie
472 and Burreson, 2011) owing to resistance developing in natural populations and, therefore, exposure of the
473 families to MSX-disease in the field would be low or intermittent. Because of its low virulence at low
474 salinity (Burreson and Andrews, 1988), no significant exposure to MSX-disease would have occurred at
475 low salinity sites.

476 For the other major disease, Dermo, virulence is also limited to high salinity, and exposure to *P.*
477 *marinus* has been moderately high during our sib tests in the York River (Burreson et al., 1996, Carnegie,
478 2020). However, the etiology of Dermo transmission is different to MSX, in particular because infection
479 is progressive with age (Burreson and Andrews, 1988, Ford and Tripp, 1996). Therefore, while our sib
480 tests have been exposed to the pathogen, mortality is unlikely to have been expressed strongly in 18-
481 month-old animals. Longer sib tests (increased time in the field) would have seriously affected our
482 infrastructure, but more problematic is the difficulty of obtaining a response specifically to *P. marinus*
483 infection. See Proestou et al., 2019 for discussion of challenges to obtaining Dermo-disease resistance.
484 The fact that Dermo-disease worsens with age has afforded us the opportunity to select against disease
485 indirectly, by selecting for faster growth. That is, if commercial animals are harvested before the disease
486 reaches fatal virulence or impairs the animal's condition, then disease resistance is irrelevant. This has
487 been an effective strategy for the culture of oysters for the half shell trade: the vast majority of oysters are
488 larger than market size at 18 months, and in some areas, market size is reached in 12 months. For the
489 spat-on-shell sector of the *C. virginica* industry, this same strategy may be inadequate because growth is

490 slower for on-bottom culture (Congrove et al., 2009). Thus, there is still merit in pursuing Dermo-disease
491 resistance *per se.* While progress in developing Dermo-resistant lines has been reported (Ragone-Calvo et
492 al., 2003), there is still uncertainty about whether so-called resistance to *P. marinus* infection is specific to
493 *P. marinus*, or whether co-resistance to MSX-disease confers greater survival where the diseases co-
494 occur, which is almost everywhere but the Gulf coast of the US. Instead of field tests, we are focusing on
495 lab challenges to sharpen the phenotype and estimate genetic parameters of Dermo resistance (Ben-Horin
496 et al., 2018; Proestou et al., 2019; Proestou and Sullivan, 2020).

497 Mortality, as measured in our ABC trials, seems to be a manifestation of *general* survival, for the
498 reasons above. General survival often has low heritability (Gjedrem 2015), likely because there are
499 manifold reasons affecting it. For the work reported here, observed heritability for survival was low (0.03-
500 0.16), although when corrected as underlying heritability it was mostly moderate, ranging from 0.05-0.35.
501 Our family-based breeding program has shown progress for general survival at both high and low salinity,
502 as evidenced by genetic trends. Since the progress is consistent over time, it suggests the general
503 mechanism for survival in each salinity zone remains similar from year to year.

504 The very low genetic correlation between survival at low and high salinity sites indicated that survival
505 in each salinity zone, as measured in our trials, did not share genetic causation and that survival in each
506 zone needed to be treated as a different trait. In fact, we do not know whether the genes for survival in
507 each salinity zone are different, regulated differently, or partly shared to some degree. The fact is that the
508 high GxE effects on survival posed a fundamental question about breeding in the Chesapeake Bay
509 estuary. Should we breed an "all purpose" oyster with good survival in both salinities, or breed oysters
510 specific to each region using different indices for low and high salinity. We chose the latter even though
511 other major traits were either moderately or highly correlated, and we did so to maximize genetic gains.
512 The alternative was to treat survival as the same trait across all sites and, while survival is heritable when
513 treated as such, the heritability is only 60% of that for each region (h^2_{obs} = 0.09±0.01 compared to
514 h^2_{obs} = 0.15±0.01, Table 9). That is, gains would have been 60% less if treated as a single trait.

515 **4.4 Genetic trends**

516 Genetic trends for the ABC breeding population were estimated from the onset of family breeding
517 in 2004 through to the present day and this period can be characterized by three phases: 1) R&D phase
518 with first family deployments (2004-2010), 2) the founding phase (2013-2014), and 3) the operational
519 phase (2015-2018, and continuing).

520 For the R&D phase, the genetic trends of all traits except survival were flat. For survival, there
521 was a decrease in the genetic trend that corresponded to two year-classes of the introduction of wild
522 Virginia germplasm. It is quite impossible *not* to exert selective pressure for disease resistance or
523 robustness in field deployments, so it is likely that passive selection for survival in the closed population
524 accounted for the observed genetic gain during the early phase of the program.

525 Super Lines (see Introduction, Fig. 1) were then introduced as we transitioned from mass
526 selection for family-based breeding. Super Lines had been selected for survival and growth, primarily
527 using length. (Length and total weight are highly correlated, data not shown). Genetic gains are evident
528 during this period. Operational breeding began in 2015 in which a selection index was chosen and
529 selection proceeded in a targeted and consistent way. Gains are evident, particularly for low salinity
530 survival, which is a primary trait that received a high weighting. Total weight is also a primary trait, but
531 the trend for this is more variable in both sub-populations, possibly due to differences in the application
532 of within-family selection. Choosing individuals from families with high EBV can often be based on the
533 quality of gametes rather than the merit of a trait, for example, size. This makes within family selection
534 haphazard. Genetic trends for meat yield and shell shape traits are flat. Meat yield, which showed some
535 progress, would be expected to be less responsive due to the relatively low heritability and low coefficient
536 of variation. For shell shape, less weight is given these traits; the emphasis they do receive is principally
537 applied to avoid adverse change due to unfavorable correlations with total weight and survival (Table 8).

538 **4.5 Implementing operational breeding**

539 The breeding goal was determined using the desired gains approach. The primary goals were to
540 improve survival and growth rate in equal proportions, and to do so across the entire Chesapeake Bay
541 region as efficiently as possible. Because of the adverse correlations between primary traits and both shell

542 shape and meat yield, secondary goals were to avoid adverse changes. Meat yield is an important trait for
543 the industry, but emphasis on meat yield would have caused significant trade-offs in survival and growth.
544 Instead, meat yield can be improved significantly through triploidy (Degremont et al., 2012, Callam et al.,
545 2016), which is encompassed in our program through commercialization of tetraploid brood stock to
546 hatcheries.

547 Selection traits were chosen using knowledge of genetic parameters and accounting for the
548 genotype by environment effects for survival (section 4.3) and growth rate. In practice, this resulted in
549 survival and growth rate being treated as different traits in each salinity zone, which gave 7 selection traits
550 in all: 1) survival in high salinity, 2) survival in low salinity, 3) total weight in high salinity, 4) total
551 weight in low salinity, 5) meat yield in all salinities, 6) width index in all salinities, and 7) height index in
552 all salinities. Selection was done using two separate selection indices; one for high salinity sites (traits 1,
553 3, 5, 6, 7) and another for low salinity sites (traits 2, 4, 5, 6, 7), and economic weights were assigned to
554 each index in order to meet breeding goals. The breeding population was therefore managed as two sub-
555 populations. Approximately 100 families were produced for each sub-population per year and parents
556 were selected independently for each. However, candidate families were selected on merit, regardless of
557 their sub-population, and the same family could be selected for both sub-populations. An important aspect
558 of that design was to deploy families in each salinity zone, which allowed families to be assessed for each
559 of the 7 selection traits. Thus, there was considerable gene flow between sub-populations. This strategy
560 characterizes our ongoing operations.

561 The breeding strategy now operates as a regular annual cycle. Every year, there are standard
562 operations involving family production, field deployment, measurement, data analysis to calculate EBV,
563 and selection based on the indices described above. Typically, the generation interval for *C. virginica* in
564 our region is two years and in order to produce a single genetic population and, to avoid a disconnection
565 between successive year classes, we use genetic links in every year class, where the genetic links are
566 siblings of previously used brood stock. The EBV models we run are the multi-variate models developed
567 during this study and are not changed. We run two different models for ease of analysis: one for survival,

568 which is a bivariate model with high and low salinity survival, and the other for all other traits, which is a
569 multi-variate model with 5 traits. All data are used for each annual EBV run, which includes all data
570 summarized in Table 3 plus new data generated each year.

571 **4.6 Sustainability of oyster breeding**

572 A review of the literature on breeding programs for shellfish, and oysters in particular, is likely to
573 be a poor indicator of the actual number of extant efforts underway. For example, in a review of
574 aquaculture breeding, Gjedrem et al. (2012) reported three oyster breeding programs worldwide to
575 'service'1.54M tonnes of product. In fact, in 2012, there were four institutional programs in the US alone,
576 and at least three others globally. Private breeding efforts were also underway on at least three continents,
577 with unknown numbers in China. Today, there are likely as many as 17 oyster breeding programs
578 servicing regional industries. 'Industrial breeding programs' are underrepresented in the literature, most
579 likely because they are focused on interactions with industry and not academia.

580 One of the disadvantages of sustaining an industrial breeding program for oysters is the limited
581 nature of the market they serve. On a global scale, aquaculture of *Crassostrea* spp., writ large, accounts
582 for the largest tonnage of product from marine culture (FAO 2014), perhaps aside from seaweed. This
583 vast tonnage is overwhelmingly obtained from wild seed where genetics has no role. The proportion of
584 hatchery production as a function of total oyster production is relatively tiny, and it is this tiny fragment
585 that must sustain breeding efforts.

586 In addition to the limited size of the oyster culture industry that is based around hatchery
587 production, there are biosecurity concerns that limit the transfer of germplasm from one region of the
588 world to another, or even between two regions in the same country (*cf.* Hedgecock, 2012, Carnegie et al.,
589 2016). This has the effect of narrowing the size of the constituency that can support a breeding program.
590 Economic sustainability is the key reason that ABC has been streamlining our breeding strategy: breeding
591 programs are hostage to the resources needed to sustain them.

592 Although the ABC program is based in the Chesapeake Bay, practically it services the mid-
593 Atlantic region of the east coast of the US, essentially extending from Rhode Island through South

Carolina, based on the range of sales from commercial hatcheries to these regions. Despite intentions to widen this region, biosecurity concerns restrict us to this area. Income for the program consists of funding from the Commonwealth of Virginia (fixed at about 60% of the operating budget per year) and revenue derived from the licensing of brood stock, which is based on production of seed and eyed larvae from selected brood stock. Revenues from production are variable and must reach a level each year to fully fund operations at ABC. It often does, and sometimes – like 2020 – does not. The challenge for any oyster breeding program is to optimize the breeding strategy for the available resources, or at least determine the optimum strategy so as to be able to price it for outside or entrepreneurial funding.

In some ways, our quest for an optimal breeding strategy in the Chesapeake Bay is a paradigm for *C. virginica* oyster breeding in general and it is reasonable to think that family-based breeding could serve the entire range of *C. virginica* (Allen et al., 2020). Unlike *C. gigas*, which is globally distributed, *C. virginica* aquaculture shares just one extended range from Canada to Mexico along the east coast of North America. Although there is apparently a need to develop region-specific lines for the industry (Proestou et al., 2016), regional hubs could share specialist staff, data management facilities, together with testing sites to allow gene flow and the flow of information along the entire range of this species.

5.0 Conclusion

Breeding has been an essential component of aquaculture for the Eastern oyster in the Chesapeake Bay and breeding methods have progressively developed since commencement of the ABC program. This paper has documented the progression from mass selection to family-based breeding. Importantly, that progression has been built upon previous work and has captured all prior improvements. The progressive improvement of oyster breeding in the Chesapeake Bay will continue, enabled in part by new genetic technology encompassing disease challenges, polyploid breeding, and genomic selection to provide ongoing development to this breeding program.

6. Acknowledgements

Dr. Mark Camara helped initiate family breeding at ABC with funding the NOAA Oyster Disease Research Program funding. Subsequently, Dr. Lionel Dégremont was responsible for initial ground work

620 for the development of our family breeding infrastructure with funding from NOAA ODRP Grant
621 #NA03OAR4170084 in his role as postdoc and then Breeding Research Manager from 2004-2007. His
622 initiative and leadership with hatchery and field operations were crucial to early steps. Dr. Anu Frank-
623 Lawale continued much of this ground work from 2007-2014 and was responsible, ultimately, for the
624 partnership with CSIRO through funding from NOAA Virginia Sea Grant in 2012. The entire
625 Aquaculture Genetics and Breeding Technology Center team, past and present, have carried the burden of
626 work for the hatchery spawns, field deployments, and evaluations. Scott Cooper and Nigel Valentine at
627 CSIRO built the data system used to manage all data for the ABC family-based breeding program. The
628 functionality and efficiency of this system enabled this work. Special thanks to Joey Matt for providing
629 the figures and Joey and Dr. Chris Hollenbeck for early review of the manuscript. We are also grateful to
630 our industry partners for use of their leased grounds: Rappahannock River Oyster Company, Bevans
631 Oyster Company, and Cliff Love. Work was funded by VIMS and revenues from our licensing program
632 administered through by Jason McDevitt of the William & Mary IP Foundation. Invaluable support from
633 anonymous donors through Fidelity Charitable Trust provided critical assistance during some hard times.
634 This paper is Contribution No. 3994 of the Virginia Institute of Marine Science, William & Mary.

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797 ## Tables

798 Table 1. Summary of the genetic material and population structure for each year class. See Materials and
799 Methods for explanation of Source of Parents categories. The table is subdivided into three phases of the
800 development of the ABC breeding program: R&D phase (2004-2010), founder phase (2013-2014), and
801 operational phase (2015-2018, and still continuing).

Year class	Number families	Number parents	Mating design (♂ x ♀)	Number sites	Source of parents				
					Wild Virginia	Original HS lines	ABC LS lines	ABC HS lines	Families
2004	49	98	1 x 1	2	9%	57%	0%	34%	0%
2005	54	108	1 x 1	2	100%	0%	0%	0%	0%
2006	59	118	1 x 1	2	100%	0%	0%	0%	0%
2007	63	57	4 x 2 [a]	3	7%	0%	0%	0%	93%
2010	128	181	2 x 2 [b]	2	6%	0%	0%	0%	94%
2013	131	170	2 x 2	4	0%	0%	36%	37%	28%
2014	133	163	2 x 2	4	0%	0%	36%	40%	24%
2015	107	142	2 x 2	4	0%	0%	0%	0%	100%
2016	172	194	2 x 2	4	0%	0%	0%	0%	100%
2017	129	166	2 x 2	3	0%	0%	0%	0%	100%
2018	120	146	2 x 2	3	0%	0%	0%	0%	100%

802 [a] For 2007 year class, 20% of parents were crossed as either a 2x2 or 1x1 mating design (♂ x ♀).
803 [b] For 2010 year class, the mating design (♂ x ♀) was a mix of 2x2, 2x1, 1x1, 3x1, and 3x2.
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806 Table 2. Description of the progeny test sites and the grow-out gear used at each site. Also see Figure 2
807 for location abbreviations in parentheses.

Site	Salinity zone	Grow-out gear	Year classes	Salinity (ppt)	Longitude	Latitude
Horn Point (5)	Low	rack & bag long line	2013-2015 2016-2018	6 - 13	38.593	-76.129
Kinsale (4) [a]	Low	rack & bag	2004-2010	9 - 15	38.050	-76.557
Lewisetta (3) [a]	Low	rack & bag	2013-2018	8 - 15	37.989	-76.476
York River (2)	High	rack & bag long line	2004-2014 2015-2018	18 - 23	37.248	-76.501
Lynnhaven River (1)	High	rack & bag	2007-2016	15 - 23	36.894	-76.020

808 [a] Kinsale and Lewisetta are in close proximity and were assumed to be the same site in the analyses.
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810 Table 3. Summary statistics for data at each site. N = number of individual animal measurements,
811 $\overline{x}$ = mean for site across all year classes, CV = coefficient of variation.

Trait	Age (yr)	Lynnhaven			York River			Kinsale/Lewisetta			Horn Point		
		N	$\overline{x}$	CV	N	$\overline{x}$	CV	N	$\overline{x}$	CV	N	$\overline{x}$	CV
Total weight (g)	1.5	7,804	41.2	0.32	27,496	39.2	0.30	19,969	37.6	0.33	8,812	38.7	0.33
Meat yield (ratio)	1.5	5,640	0.15	0.19	21,555	0.17	0.20	14,433	0.17	0.18	8,771	0.22	0.15
Width index (ratio)	1.5	7,789	0.68	0.13	27,479	0.72	0.12	19,938	0.71	0.13	8,812	0.70	0.12
Height index (ratio)	1.5	7,794	0.30	0.16	27,481	0.33	0.14	19,933	0.30	0.16	8,814	0.30	0.14
Survival (proportion)	1.5	93,050	0.72	0.21	309,482	0.69	0.26	311,536	0.81	0.18	99,386	0.86	0.13
Total weight (g)	2.5	1,855	90.5	0.36	9,445	84.1	0.29	9,302	71.6	0.31	-	-	-
Meat yield (ratio)	2.5	1,854	0.12	0.23	4,390	0.09	0.24	6,318	0.12	0.23	-	-	-
Width index (ratio)	2.5	1,854	0.64	0.12	9,440	0.72	0.12	9,292	0.68	0.13	-	-	-
Height index (ratio)	2.5	1,854	0.31	0.17	9,442	0.37	0.14	9,296	0.31	0.16	-	-	-
Survival (proportion)	2.5	4,800	0.56	-	159,940	0.24	-	153,954	0.49	-	-	-	-

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816 Table 4. Genetic group effects for each trait as expressed at low and high salinity sites, with standard
817 errors in parentheses. The percentage values indicate the change in each trait relative to the Wild Virginia,
818 which was used as the population benchmark.

Trait	Genetic Group	Low salinity sites		High salinity sites	
Total weight (g)	Wild Virginia	29.3 (2.0)	0%	37.4 (1.9)	0%
	Original High Salinity lines	28.9 (1.4)	-1%	38.8 (1.4)	4%
	ABC Low Salinity lines	43.9 (1.9)	50%	49.4 (2.4)	32%
	ABC High Salinity lines	41.8 (1.6)	42%	48.1 (1.4)	29%
Meat yield (ratio)	Wild Virginia	0.171 (0.006)	0%	0.135 (0.005)	0%
	Original High Salinity lines	0.182 (0.005)	7%	0.142 (0.004)	5%
	ABC Low Salinity lines	0.179 (0.005)	5%	0.137 (0.005)	1%
	ABC High Salinity lines	0.180 (0.005)	5%	0.139 (0.004)	3%
Width index (ratio)	Wild Virginia	0.747 (0.013)	0%	0.730 (0.013)	0%
	Original High Salinity lines	0.716 (0.009)	-4%	0.691 (0.009)	-5%
	ABC Low Salinity lines	0.691 (0.012)	-7%	0.700 (0.016)	-4%
	ABC High Salinity lines	0.685 (0.010)	-8%	0.710 (0.010)	-3%
Height index (ratio)	Wild Virginia	0.306 (0.007)	0%	0.334 (0.008)	0%
	Original High Salinity lines	0.301 (0.005)	-2%	0.324 (0.006)	-3%
	ABC Low Salinity lines	0.288 (0.007)	-6%	0.327 (0.010)	-2%
	ABC High Salinity lines	0.299 (0.006)	-2%	0.323 (0.006)	-3%
Survival (proportion)	Wild Virginia	0.68 (0.04)	0%	0.59 (0.04)	0%
	Original High Salinity lines	0.74 (0.03)	9%	0.68 (0.03)	15%
	ABC Low Salinity lines	0.86 (0.04)	26%	0.64 (0.06)	8%
	ABC High Salinity lines	0.84 (0.03)	24%	0.74 (0.03)	26%

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821 Table 5. Heritabilities (h^2) and family variances (σ^2_{fam}) for each progeny test site using data pooled across
822 year classes. Family variance is expressed as a proportion of total variance and standard errors are shown
823 in parentheses. h^2_{ob} = heritability on the observed scale, h^2_{und} = heritability on the underlying scale.

		Low salinity sites		High salinity sites	
		Horn Point	Kinsale/ Lewisetta	Lynnhaven	York River
Total weight	h^2	0.36 (0.06)	0.33 (0.04)	0.23 (0.06)	0.29 (0.04)
	σ^2_{fam}	0.08 (0.02)	0.05 (0.01)	0.05 (0.02)	0.07 (0.01)
Meat yield	h^2	0.19 (0.05)	0.20 (0.04)	0.12 (0.05)	0.19 (0.04)
	σ^2_{fam}	0.07 (0.02)	0.04 (0.02)	0.04 (0.02)	0.06 (0.01)
Width index	h^2	0.31 (0.05)	0.23 (0.03)	0.19 (0.04)	0.31 (0.04)
	σ^2_{fam}	0.05 (0.02)	0.03 (0.01)	0.02 (0.01)	0.07 (0.01)
Height index	h^2	0.31 (0.05)	0.29 (0.03)	0.35 (0.05)	0.38 (0.04)
	σ^2_{fam}	0.05 (0.02)	0.03 (0.01)	0.01 (0.01)	0.06 (0.01)
Survival	h^2_{ob}	0.10 (0.02)	0.14 (0.02)	0.03 (0.02)	0.16 (0.02)
	σ^2_{fam}	0.04 (0.01)	0.03 (0.01)	0.01 (0.01)	0.02 (0.01)
	h^2_{und}	0.23 (0.05)	0.35 (0.05)	0.05 (0.03)	0.28 (0.03)

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826 Table 6. Heritabilities (h^2) at ages 1.5 and 2.5 years and genetic correlations (r_g) between those measures,
827 with standard errors in parentheses. Age 2.5 year data was collected on the 2004 to 2010 year classes
828 only.

Trait	Kinsale/Lewisetta (Low salinity)			York River (High salinity)		
	h^2 (1.5 y)	h^2 (2.5 y)	r_g	h^2 (1.5 y)	h^2 (2.5 y)	r_g
Total weight	0.27 (0.06)	0.43 (0.07)	0.98 (0.05)	0.16 (0.05)	0.27 (0.07)	0.96 (0.07)
Meat yield	0.14 (0.06)	0.19 (0.06)	0.87 (0.17)	0.18 (0.05)	0.24 (0.06)	0.80 (0.15)
Width index	0.12 (0.03)	0.13 (0.04)	0.98 (0.1)	0.25 (0.04)	0.27 (0.04)	1.00 (0.02)
Height index	0.14 (0.04)	0.16 (0.05)	0.98 (0.1)	0.29 (0.04)	0.27 (0.04)	1.00 (0.01)
Survival	0.25 (0.03)	0.16 (0.03)	0.72 (0.06)	0.19 (0.02)	0.12 (0.02)	0.83 (0.04)

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831 Table 7. Genetic correlations (r_g) between sites, with standard errors in parentheses, and for all sites
832 pooled by salinity status. Values are shown for pairs of sites where high numbers of common families
833 occurred across sites. No. fam. = count of common families between sites, L=low salinity site, and
834 H=high salinity site.

Site 1	Site 2	No. Fam.	Total weight	Meat yield	Width index	Height index	Survival
York River (H)	Kinsale/Lew (L)	434	0.70 (0.09)	0.89 (0.10)	0.70 (0.09)	1.06 (0.03)	0.19 (0.09)
York River (H)	Lynnhaven (H)	250	0.93 (0.10)	1.05 (0.11)	0.98 (0.08)	0.89 (0.07)	1.04 (0.12)
York River (H)	Horn Point (L)	144	0.84 (0.15)	1.10 (0.09)	1.10 (0.05)	1.06 (0.05)	0.57 (0.14)
Horn Point (L)	Kinsale/Lew (L)	319	1.09 (0.02)	0.93 (0.06)	1.00 (0.06)	1.06 (0.04)	1.01 (0.03)
All high salinity	All low salinity	447	0.70 (0.08)	0.97 (0.08)	1.05 (0.03)	1.06 (0.02)	0.23 (0.08)

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842 Table 8. Genetic correlations between traits (r_g) with standard errors in parentheses. Progeny tests were
843 grouped by salinity of test site and data were pooled across all year classes.

Trait 1	Trait 2	Low salinity sites	High salinity sites
Total weight	Survival	0.49 (0.06)	0.59 (0.06)
Total weight	Meat yield	0.06 (0.10)	-0.06 (0.10)
Total weight	Width index	-0.50 (0.06)	-0.45 (0.06)
Total weight	Height index	-0.38 (0.07)	-0.49 (0.06)
Meat yield	Width index	-0.10 (0.10)	0.01 (0.10)
Meat yield	Height index	0.03 (0.10)	0.10 (0.09)
Meat yield	Survival	0.40 (0.10)	0.21 (0.10)
Width index	Height index	0.75 (0.05)	0.73 (0.05)
Width index	Survival	-0.28 (0.07)	-0.21 (0.07)
Height index	Survival	-0.20 (0.07)	-0.30 (0.06)

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846 Table 9. Heritabilities (h^2) and family variances (σ^2_{fam}) for progeny tests grouped by salinity of test site
847 and with data pooled across all year classes. Family variance is expressed as a proportion of total variance
848 and standard errors are shown in parentheses. h^2_{ob} = heritability on the observed scale, h^2_{und} = heritability
849 on the underlying scale.

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Trait		Low salinity sites	High salinity sites
Total weight	h^2	0.34 (0.04)	0.30 (0.03)
	σ^2_{fam}	0.03 (0.01)	0.04 (0.01)
Meat yield	h^2	0.18 (0.03)	0.16 (0.03)
	σ^2_{fam}	0.05 (0.01)	0.05 (0.01)
Width index	h^2	0.24 (0.03)	0.27 (0.03)
	σ^2_{fam}	0.03 (0.01)	0.05 (0.01)
Height index	h^2	0.27 (0.03)	0.37 (0.04)
	σ^2_{fam}	0.03 (0.01)	0.04 (0.01)
Survival	h^2_{obs}	0.15 (0.02)	0.15 (0.02)
	σ^2_{fam}	0.03 (0.01)	0.02 (0.01)
	h^2_{und}	0.34 (0.04)	0.26 (0.03)

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852 Figures
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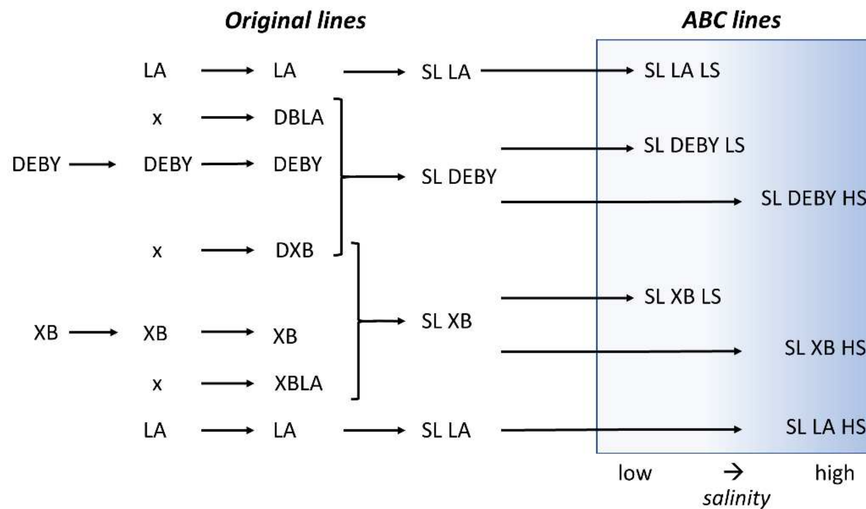

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856 Figure 1: Origin of genetic groups for founder populations. DEBY and XB were closed populations
857 selected for disease resistance. Populations from Louisiana (LA) were introgressed with DEBY and XB
858 starting in early 2000s, producing up to 15 original lines, which became unwieldy. Super Lines
859 (designated SL-) were produced in 2008 and 2009 by crossbreeding original lines. Super Lines were
860 tested in either high or low salinity sites, yielding founder groups ABC high salinity (HS) lines or ABC
861 low salinity (LS) lines.

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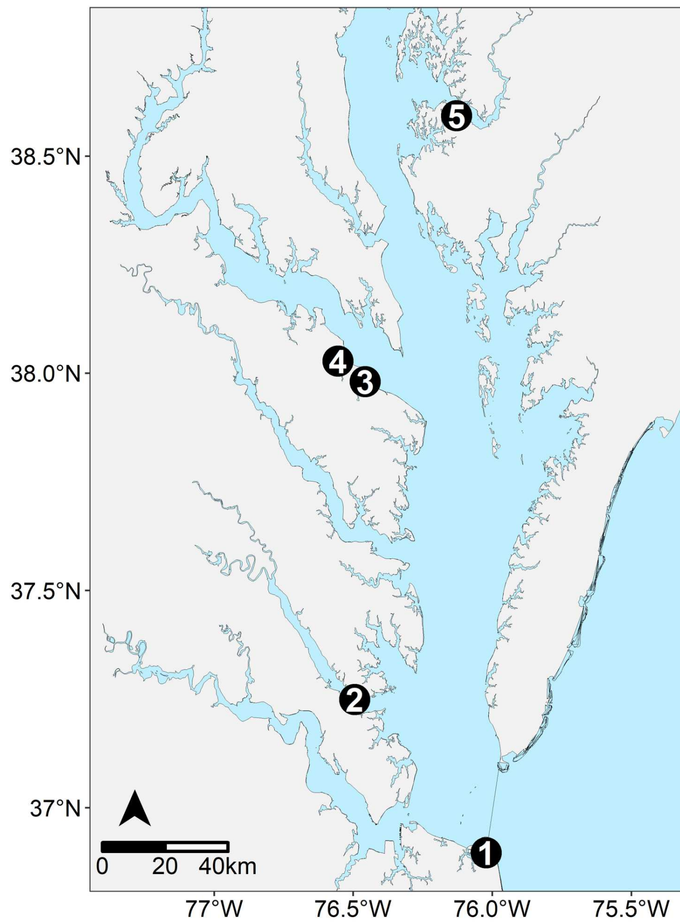

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868 Figure 2: Five field sites spanned a range of salinities in Chesapeake Bay: high salinity sites in the
869 Lynnhaven River (1) and York River (2) and low salinity sites at Lewisetta (3), Kinsale (4), and Horn
870 Point (5). Sites 3 and 4 are considered the same site and so data from both were consolidated for analysis.

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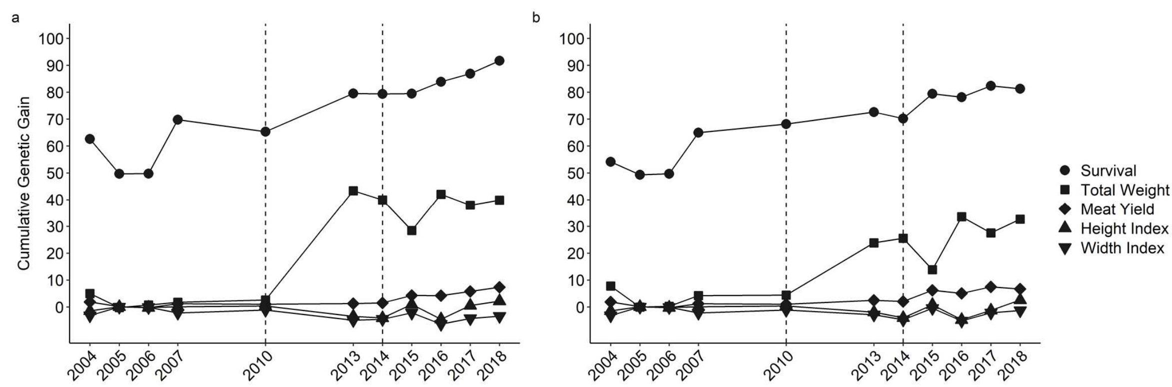

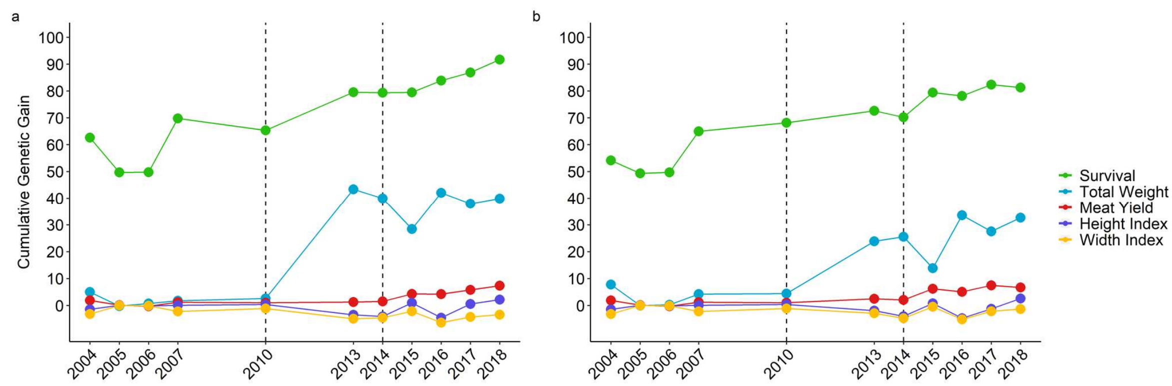

Figure 3: Genetic trends for low- and high- salinity sub-populations (families) during the course of the family breeding program. Dashed lines denote the three phases of the development of the ABC breeding program: R&D phase (2004-2010), founder phase (2013-2014), and operational phase (2015-2018, and still continuing). a – low salinity; b – high salinity.

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