

1 **Genome-wide association and genomic prediction of growth traits in the European flat**
2 **oyster (*Ostrea edulis*)**

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36 ABSTRACT

37 The European flat oyster (*Ostrea edulis*) is a bivalve mollusc that was once widely distributed
38 in Europe and represented an important food resource for humans for centuries. Populations
39 of *O. edulis* experienced a severe decline across their biogeographic range mainly due to
40 anthropogenic activities and disease outbreaks. To restore the economic and ecological
41 benefits of European flat oyster populations, extensive protection and restoration efforts are
42 in place within Europe. In line with the increasing interest in supporting restoration and oyster
43 farming through the breeding of stocks with enhanced performance, the present study aimed
44 to evaluate the potential of genomic selection for improving growth traits in a European flat
45 oyster population obtained from successive mass-spawning events. Four growth-related
46 traits were evaluated: total weight (TW), shell height (SH), shell width (SW) and shell length
47 (SL). The heritability of the growth traits was moderate-low, with estimates of 0.45, 0.37, 0.22,
48 and 0.32 for TW, SH, SW and SL, respectively. A genome-wide association analysis revealed a
49 largely polygenic genetic architecture for the four growth traits, with two distinct QTLs
50 detected on chromosome 4. To investigate whether genomic selection can be implemented
51 in flat oyster breeding at a reduced cost, the utility of low-density SNP panels (down to 100
52 SNPs) was assessed. Genomic prediction accuracies using the full density panel were high
53 (>0.83 for all traits). The evaluation of the effect of reducing the number of markers used to
54 predict genomic breeding values revealed that similar selection accuracies could be achieved
55 for all traits with 2K SNPs as for a full panel containing 4,577 SNPs. Only slight reductions in
56 accuracies were observed at the lowest SNP density tested (i.e. 100 SNPs), likely due to a high
57 relatedness between individuals being included in the training and validation sets during
58 cross-validation. Overall, our results suggest that the genetic improvement of growth traits in
59 oysters is feasible. Nevertheless, and although low-density SNP panels appear as a promising
60 strategy for applying GS at a reduced cost, additional populations with different degrees of
61 genetic relationship should be assessed to derive estimates of prediction accuracies to be
62 expected in practical breeding programmes.

63

64 **Keywords:** *Ostrea edulis*, oyster, GWAS, Genomic selection, growth, Aquaculture

65

66 1. INTRODUCTION

67 The European flat oyster (*Ostrea edulis*) was an abundant native bivalve species and an
68 important fishery resource in much of Europe up to the 19th century (Pogoda 2019). However,
69 populations of *O. edulis* experienced a severe decline across their biogeographic range due to
70 a range of detrimental factors including overfishing and habitat degradation (Thurstan et al.
71 2013), the subsequent invasion of non-native species (e.g. slipper limpet, *Crepidula fornicata*)
72 (Preston et al. 2020; Helmer et al. 2019) and pathogenic diseases (Robert et al. 1991; Sas et
73 al. 2020). The continuous decimation of native populations in the Atlantic and Mediterranean
74 seas led to significant changes in oyster production, which progressively shifted towards
75 farming (Korringa 1976), and eventually to the cultivation of different species including
76 *Crassostrea angulata* (Oelig and Uf 2000) and the non-indigenous Pacific oyster (*Crassostrea*
77 *gigas*) (Grizel and Héral 1991; Walne and Helm 1979). The Pacific oyster was introduced into
78 Europe for aquaculture purposes owing to its favourable production traits, such as a faster
79 growth rate and higher resistance to the main diseases affecting *C. angulata* and *O. edulis*

80 (Renault et al. 1995; Grizel and Héral 1991). Worldwide oyster production is now dominated
81 by the Pacific oyster (97.7%), while the production of the European flat oyster remains stably
82 low, constituting just ~0.2% of global production (FAO 2019). Despite the demand for shellfish
83 continues to increase (Botta et al. 2020), the level of *O. edulis* production is stagnant. One of
84 the main factors that hinders the growth of the industry is the lack of a substantial and steady
85 supply of oyster seed (i.e. juveniles) (see Colsoul et al. (2021) for a review). Hence, the
86 optimization of oyster larval production in hatcheries and spatting ponds is key for future
87 European flat oyster aquaculture, as well as for restoration projects, which are also expected
88 to rely on sustainable sources of juveniles for restocking (Pogoda et al. 2020). Importantly,
89 the artificial propagation of flat oyster seed will facilitate the application of selective breeding
90 programmes. Although selective breeding programmes are typically used to improve
91 aquaculture production, they could also benefit the ecological restoration of *O. edulis*. If
92 desirable traits such as disease resistance are found to have a strong genetic component, then
93 increased resistance to life-limiting diseases – such as bonamiosis (Culloty et al. 2004; Naciri-
94 Graven et al. 1998) – could potentially be achieved while maintaining the adaptive potential
95 (i.e. genetic diversity) of restored populations.

96 Selective breeding in oysters has mainly focused on improving meat yield and quality, disease
97 resistance, survival and growth (Toro and Newkirk, 1990; Allen et al., 1993; Ragone Calvo et
98 al., 2003; Ward et al., 2005; Dégremont et al., 2015; De Melo et al., 2016; Proestou et al.,
99 2016; Camara et al., 2017; Zhang et al., 2019), with a recent interest in nutritional content
100 and shell shape (Grizzle et al., 2017; Liu et al., 2019; Meng et al., 2019; Wan et al., 2020; He
101 et al., 2022). Among these traits, growth is comparatively simple to assess and consequently
102 select for using phenotypic information. Although the direct comparison of heritability
103 estimates from different studies is difficult (e.g. due to intrinsic differences between
104 populations), estimates for growth rate in oysters tend to be moderate (e.g. 0.26 and 0.31 –
105 De Melo et al. (2016) and Evans and Langdon (2006), respectively). As a result, fast-growing
106 lines of oysters have been developed for some of the main commercial species, such as the
107 Pacific (*C. gigas*) (Zhang et al. 2019), Portuguese (*C. angulata*) (Vu et al. 2020), American (*C.*
108 *virginica*) (Varney and Wilbur 2020) and Sydney rock (*Saccostrea glomerata*) (Fitzer et al.
109 2019) oyster. Initial attempts to genetically improve the European flat oyster *O. edulis*
110 resulted in a 23% increase in growth rate compared to an unselected (control) line (Newkirk
111 and Haley 1982). This striking genetic response was not replicated in a second generation of
112 selection, possibly due to unintentional inbreeding (Newkirk and Haley 1983). Indeed, even
113 relatively modest levels of inbreeding have been shown to significantly affect performance
114 traits in oysters (Evans et al. 2004), highlighting the importance of an adequate management
115 of genetic diversity in hatchery-derived stocks. Moreover, oysters and bivalves in general,
116 appear to have a high genetic load (see for a review Plough (2016)) and, therefore, may be
117 particularly susceptible to inbreeding depression. Hence, the incorporation of genomic tools
118 into shellfish breeding schemes will be key for balancing genetic gain with population diversity
119 in order to sustain the long-term progress for traits under selection.

120 A vast array of genomic tools and resources have become available for genetic research and
121 breeding applications in oysters. For example, for economically relevant species,
122 chromosome-level genome assemblies (Peñaloza et al. 2021; Qi et al. 2021; Modak et al.
123 2021; Li et al. 2021), SNP arrays (Gutierrez et al. 2017; Qi et al. 2017; Lapegue et al. 2014) and
124 medium-density linkage maps (Gutierrez et al. 2018; Li et al. 2018; Jones et al. 2013; Wang et
125 al. 2016; Yin et al. 2020) have been produced. These resources have been applied to examine

126 the genetic basis of growth (Gutierrez et al. 2018; Jones et al. 2014; He et al. 2021), low salinity
127 tolerance (McCarty et al. 2021), disease resistance (Gutierrez et al. 2018; Yang et al. 2022)
128 and nutritional content (Meng et al. 2019). For the European flat oyster, high-quality genomes
129 have recently been released (Boutet et al. 2022; Gundappa et al. 2022), which along with
130 available high-throughput genotyping techniques (e.g. SNP arrays and genotype-by-
131 sequencing approaches), provide the opportunity for gaining insight into the genomic
132 architecture of relevant production traits. Most of the traits of economic importance in
133 aquaculture species have a polygenic architecture (Zenger et al. 2019). For polygenic traits
134 (i.e. those controlled by many loci), the application of predictive techniques such as genomic
135 selection (GS) may enable a faster genetic gain than conventional pedigree-based selection.
136 GS is a method based on genome-wide markers in which the effect of all loci are
137 simultaneously used for predicting the estimated breeding values (EBV) of selection
138 candidates (Meuwissen et al. 2001), and has shown major potential in aquaculture species,
139 where it can be used to characterise variation within and between large families of potential
140 breeders. However, commercial application to aquaculture production is largely limited to
141 the major finfish and crustacean species (e.g. salmonids, Nile tilapia, tropical shrimp)
142 (Lillehammer et al. 2020; Boudry et al. 2021; Zenger et al. 2019). Studies into the feasibility of
143 applying genomic selection schemes in oyster breeding programmes have shown that for
144 growth (Vu et al. 2021b; Gutierrez et al. 2018), edibility (Vu et al. 2021b), low salinity tolerance
145 (McCarty et al. 2021), and disease resistance traits (Vu et al. 2021b; Gutierrez et al. 2020),
146 greater genetic gains could be achieved through GS compared to traditional breeding.
147 Nevertheless, the practical application of GS as a selection strategy will likely depend on how
148 cost-effective it is compared to pedigree-based methods. The development of feasible
149 alternatives for reducing genotyping costs, such as using affordable low-density genotyping
150 tools that yield similar accuracies than higher-density panels, will be critical for the potential
151 of GS to be realized by oyster breeding programmes.

152 In line with the increasing interest in supporting oyster culture and restoration through the
153 breeding of stocks with enhanced performance, the overall aim of this study was to evaluate
154 the potential of GS for the genetic improvement of growth and growth-related
155 (morphometric) traits in the European flat oyster. First, the heritability of total weight, shell
156 length, shell width and shell height was estimated for a hatchery-derived population
157 genotyped using a ~15K SNP array. Second, a genome-wide association (GWAS) analysis was
158 conducted to dissect the genetic architecture of the measured traits. Last, to evaluate
159 whether GS may be an effective and cost-effective strategy for improving traits associated
160 with oyster growth, the accuracy of genomic predictions using reduced density SNP marker
161 panels was assessed.

162

163 **2. MATERIALS AND METHODS**

164 **2.1 Field experiment**

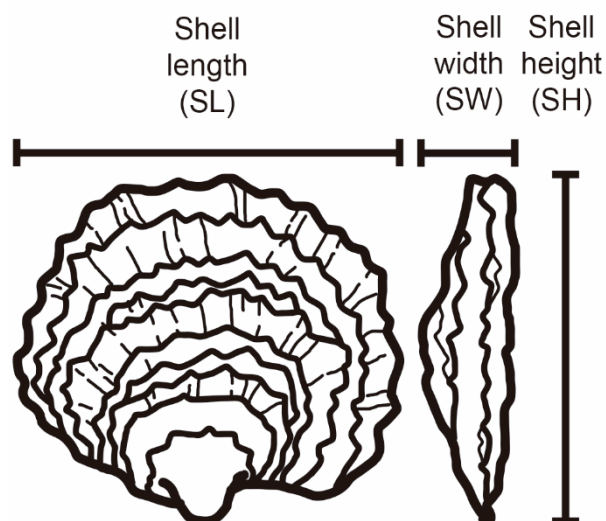
165 The European flat oyster population used in this study was generated in a UK-based hatchery
166 (Sea Salter Morecombe hatchery) by mass spawning of approximately 40 broodstock parents
167 over several spawning events. The resulting F1 generation was then deployed to Lochnell
168 oysters (56.494° N, 5.459° W) and grown for six months in ortac grow-out cages. Animals were
169 then transferred to the Institute of Marine Sciences at the University of Portsmouth (UK), and
170 maintained in a flow-through system until deployment. During this holding period, ~1,000

171 randomly selected oysters were individually tagged and their first phenotype measurements
172 recorded (see 'Phenotypes' section below). Prior to deployment, animals were cleaned of
173 fouling, washed in fresh water and dried. Embossed plastic tags with unique identifier codes
174 were attached to their shell with epoxy resin glue. Animals were returned to aquaria within
175 the hour. Oysters were placed in Aquamesh® cages (L 0.55 m x W 0.55 m x D 0.4 m) (GT
176 Products Europe Ltd) at a density of 200 oysters per cage, and deployed 1 metre below
177 floating pontoons at Port Hamble Marina (MDL) in the River Hamble (50.861° N, 1.312° W) in
178 January 2019. Mortalities were documented monthly and dead oysters – i.e. those with
179 empty or gaping shells – were removed from the experiment. General disease status was
180 assessed on subsets of oysters throughout the experiment by histology and *in situ*
181 hybridisation using an adaptation of available methods (Fabioux et al. 2004; Montagnani et
182 al. 2001). In addition, the presence of *Bonamia ostreae*, a protozoan parasite that causes a
183 lethal infection of flat oyster haemocytes (Pichot et al. 1979), was assessed by qPCR following
184 Robert et al. (2009). The prevalence of *B. ostreae* infections was negligible; hence disease
185 status had a minor influence on the assessment of growth traits in the experimental
186 population. After 10 months of growth under field conditions, gill tissue was dissected from
187 individuals alive at the end of the study and preserved in molecular grade absolute ethanol
188 (Fisher Scientific) for genetic analysis.

189

190 2.2 Phenotypes

191 Four growth-associated traits were measured at three time points over the course of 10
192 months: total weight (TW, the weight of an individual oyster including the shell), shell length
193 (SL, the maximum distance between the anterior and posterior margins), shell height (SH, the
194 maximum distance between the hinge to the furthest edge), and shell width (SW, the
195 maximum distance at the thickest part of the two shell valves) (Figure 1). Weight was
196 recorded in grams up to one decimal place. Shell measurements were taken with traceable
197 digital callipers (Fisher Scientific) with 0.02 mm precision. Oysters were cleaned and defouled
198 before measurements were taken.



199

200

201 **Figure 1.** Nomenclature of the growth-related morphometric traits measured in this study.

202

203 **2.3 DNA extraction**

204 Total DNA was isolated from gill tissue following a CTAB (cetyltrimethylammonium bromide)-
205 based extraction protocol (details in Gutierrez et al. (2017)). The integrity of the extracted
206 DNA was assessed by agarose gel electrophoresis, while DNA quality was verified on a
207 Nanodrop ND-1000 (Thermo Fisher Scientific) spectrophotometer by checking the 260/280
208 and 260/230 ratios. All samples had 260/280 and 260/230 values ≥ 1.85 and ≥ 1.96 ,
209 respectively.

210

211 **2.4 SNP genotyping and Quality Control**

212 Whole-genome genotyping of $\sim 15\text{K}$ SNPs was carried out by IdentiGEN (Dublin, Ireland) using
213 the combined-species Affymetrix Axiom oyster SNP-array (Gutierrez et al. 2017). Signal
214 intensity files were imported to the Axiom analysis Suite v4.0.3.3 software for quality control
215 (QC) assessment and genotype calling. Genotypes were generated using the default
216 parameter settings for diploid species, resulting in 11,808 SNPs typed for 870 individuals. To
217 assess the reproducibility of genotype calls, five DNA samples from the same individual were
218 genotyped independently on three different arrays, and their genotype concordance
219 evaluated through an identity-by-state (IBS) analysis. The genotype concordance rate among
220 replicates was 99.7%, demonstrating a high reproducibility of the genotyping assays. The
221 flanking region of these markers were mapped to the *O. edulis* chromosome-level genome
222 assembly (Gundappa et al. 2022). Of the 11,808 SNPs, 10,025 had uniquely mapping probes
223 and were retained for downstream analysis. A total of 1,539 markers (15.4%) were
224 monomorphic in the population under study. QC was conducted using Plink v2.0 (Chang et al.
225 2015). SNP variants were retained for further analysis if they had a call rate $>95\%$ and a minor
226 allele frequency (MAF) >0.05 . These filters removed 4,391 SNPs (leaving a total of 5,634 SNPs),
227 of which the majority were filtered out based on the MAF threshold (i.e. were monomorphic
228 or near-monomorphic in this population). Given that significant sub-clustering was detected
229 in the data (Figure S2), possibly due to a high variance in the reproductive success of
230 broodstock parents and/or temporal variation in spawning, a k-means clustering method was
231 used to assign individuals into groups. Deviations from Hardy-Weinberg Equilibrium (HWE)
232 were tested separately in each of the three genetic clusters identified by the analysis. SNP
233 markers showing significant deviations (HWE p-value $< 1e-10$) in two of the three clusters
234 were excluded from the analysis. Sample QC included removing individual oysters with a
235 missingness above 5% and high heterozygosity (i.e. more than three median absolute
236 deviations from median). Finally, a principal component analysis (PCA) was performed using
237 a set of $\sim 3.5\text{K}$ SNPs for which no pair of markers within a window of 200 kb had a $r^2 > 0.5$. The
238 top five PCs, which explain 47% of the variance (considering 20 PCs), were fitted in the model
239 to account for the effect of population structure. The final dataset comprised 840 samples
240 genotyped at 4,577 genome-wide SNPs.

241

242 **2.5 Genetic parameter estimation**

243 Genetic parameters for growth-related traits were estimated by fitting the following
244 univariate linear mixed model in GEMMA v0.95alpha (Zhou and Stephens 2012):

$$245 \quad (1) \quad y = \mu + Xb + Zu + e$$

246 Where \mathbf{y} is the vector of observed phenotypes; $\boldsymbol{\mu}$ is the overall mean of the phenotype in the
247 population; \mathbf{b} is the vector of fixed effects to be fitted (the first five principal components
248 were included as covariates); \mathbf{u} is the vector of the additive genetic effects; \mathbf{X} and \mathbf{Z} are the
249 corresponding incidence matrices for fixed and additive effects, respectively; and \mathbf{e} is a vector
250 of residuals. The following distributions were assumed: $\mathbf{u} \sim \mathbf{N}(\mathbf{0}, \mathbf{G}\sigma_u^2)$ and $\mathbf{e} \sim \mathbf{N}(\mathbf{0}, \mathbf{I}\sigma_e^2)$.
251 Where σ_u^2 and σ_e^2 are the additive genetic and residual variance, respectively, \mathbf{G} is the
252 genomic relationship matrix and \mathbf{I} is the identity matrix. The heritability of growth-related
253 traits was estimated as the ratio of the additive genetic variance to the total phenotypic
254 variance.

255 Bivariate animal linear models were implemented to estimate the genetic (co)variance
256 between TW, SL, SH and SW. Each bivariate analysis was fitted with the same top 5 PCs
257 mentioned above. Subsequently, genetic correlations among traits were measured as the
258 ratio of the covariance of two traits to the square root of the product of the variance for each
259 trait. Phenotypic correlations between traits were calculated using the Pearson correlation
260 coefficient.

261

262 **2.6 Genome-wide association study (GWAS)**

263 To identify SNPs in the flat oyster genome correlated with variation in growth-related traits,
264 a GWAS was performed by implementing the same model described previously in the GEMMA
265 software. SNPs were considered significant at the genome-wide level if their likelihood ratio
266 test P-values surpassed a conservative Bonferroni-corrected significance threshold ($\alpha/4,577$
267 = $1.09\text{e-}5$). To derive a threshold for chromosome-wide (suggestive) significance, α was
268 divided by the average number of SNPs per chromosome ($\alpha/457 = 1.09\text{e-}4$). The single-marker
269 P-values obtained from GEMMA were plotted against their chromosome location using the R
270 package qqman v 0.1.4 (Turner 2017). To assess the inflation of the association statistics, the
271 genomic control coefficient lambda λ_{GC} was calculated following (Devlin and Roeder 1999).
272 Candidate genes were searched within 100 kb of the most significant SNP loci using BEDOPS
273 v2.4.26 (Neph et al. 2012).

274

275 **2.7 Genomic Prediction**

276 To evaluate the accuracy of genomic selection, a 5-fold cross validation approach - animals
277 split into training (80%) and validation (20%) sets - was used on a population of 840 oysters
278 genotyped for 4,577 informative SNP markers. To reduce stochastic effects arising from
279 individual sampling, each analysis was repeated 10 times. For each replicate, animals were
280 randomly partitioned into five subsets (each subset contained 168 individuals). TW, SL, SH
281 and SW phenotypes recorded in individuals allocated to one of the subsets (validation set)
282 were masked. The breeding values of the validation set were then predicted based on the
283 information from the remaining four subsets (training sets) using model (1). The model was
284 fitted using the AIREMLF90 module from BLUPF90. The accuracy of genomic predictions was
285 calculated as follows:

$$286 \quad Accuracy = \frac{r_{gEBV,y}}{h}$$

287

288 where $r_{gEBV,y}$ is the correlation between the predicted and the actual phenotypes of the
289 validation set, while h is the square root of the heritability of the trait estimated as described
290 above.

291

292 **2.8 Evaluation of the effect of SNP density on genomic predictions**

293 To assess the effect of SNP density on the accuracy of genomic predictions of growth-related
294 traits, SNP panels of varying sizes were randomly sampled from the final pool of QC-filtered
295 array markers ($n = 4,577$ SNPs). Panels of the following densities were evaluated: 4K, 3K, 2K,
296 1K, 500, 400, 300, 200 and 100 SNPs. To build the lower-density panels, markers were
297 randomly sampled from the full QC-filtered SNP dataset in proportion to chromosome lengths
298 using the R package CVrepGPAcalc v1.0 (Tsairidou 2019; Tsairidou et al. 2020). To account for
299 sampling bias, 10 SNP panels were generated for each of the SNP densities. The average
300 genomic prediction accuracies of the different low-density panels were compared against the
301 equivalent accuracy values obtained with the full panel.

302

303 **2.9 Data Availability**

304 The phenotype data used in the current study can be found in Mendeley Data,
305 <https://doi.org/10.17632/sdtjyys7gr.1>.

306

307 **3. Results and discussion**

308 **3.1 Growth traits and heritability**

309 Improvement of growth rate is typically one of the first traits to be included as a selection
310 target in breeding programmes across many farmed species. In this study, oyster growth rate
311 was assessed in a hatchery-derived oyster population that was translocated to a growing site
312 and monitored for 10 months. The experimental population had a lower genome-wide
313 heterozygosity ($H_o=0.27$; $H_e=0.22$) compared to the values reported by (Vera et al., 2019)
314 ($H_o>0.31$) for a diverse set of flat oyster populations genotyped with the same array. An
315 overall mortality of 14% was observed during the field trial, among which the majority (36%)
316 occurred during a summer month (July). At the end of the experimental period, the *O. edulis*
317 population had the following growth means and standard deviations: +15.7 g (SD = 5.8), 50.8
318 mm (SD = 7.3), 12.9 mm (SD = 2.3) and 45.8 mm (SD = 8.9), for TW, SH, SW, SL, respectively
319 (Table 1). The phenotypic correlation was found to be the highest ($r > 0.8$) between two pairs
320 of traits: (i) TW and SH, and (ii) TW and SL (Figure 2).

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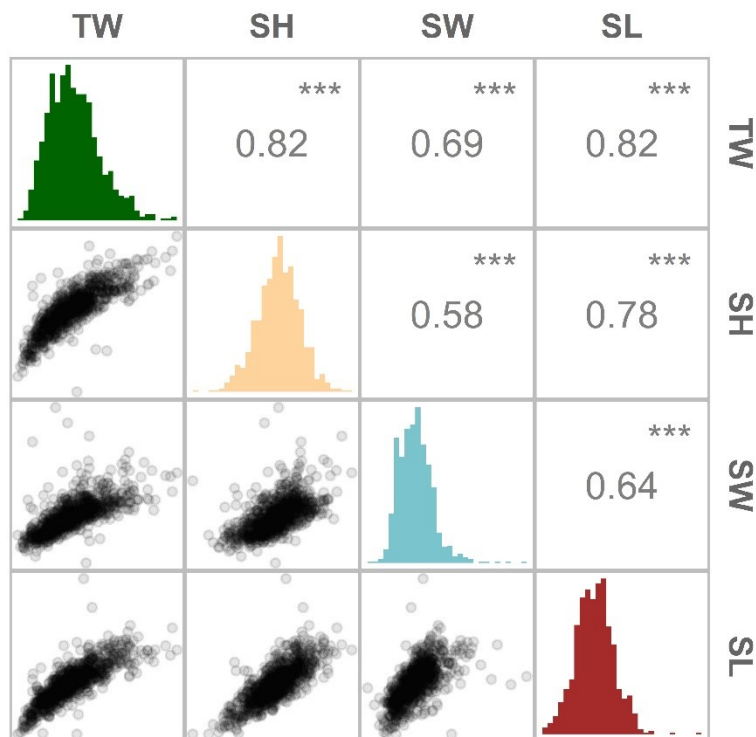
327 **Table 1.** Summary statistics of the phenotypic data (SD: Standard deviation; CV: coefficient of
 328 variation).

Trait	Unit	Mean	Min	Max	SD	CV (%)
Total weight	g	15.7	4.0	38.5	5.8	36.8
Shell height	mm	50.8	22.9	76.1	7.3	14.4
Shell width	mm	12.9	6.5	27.7	2.3	18.2
Shell length	mm	45.8	22.2	94.4	8.9	19.5

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332

333 **Figure 2.** Distribution and magnitude of the phenotypic correlations between growth-related
 334 traits in *Ostrea edulis*. Pearson's correlation between traits (above the diagonal), histogram
 335 of trait distribution (diagonal) and scatterplots comparing two traits (below the diagonal). TW
 336 (total weight), SH (shell height), SW (shell width) and SL (shell length). *** indicates p -values
 337 < 0.001 .

338

339

340

341 For the European oyster population under study, the heritability estimates of these growth-
342 related traits were in the moderate range of 0.22 (for SW) to 0.45 (for TW) (Table 2).
343 Consistent with similar studies carried out in related oyster species (Xu et al. 2017; Vu et al.
344 2020), heritability estimates based on SNP markers were higher for total weight than for
345 growth-related morphometric traits (i.e. shell height, shell width and shell length). The
346 estimation of heritability for total weight (herein referred to as TW) was similar to those
347 reported for nine- month-old Portuguese oysters ($h^2 = 0.45$) and a two-year old Pacific oyster
348 strain ($h^2 = 0.42$) (Vu et al. 2021b; Xu et al. 2017). Total weight, as measured in this study, is
349 a composite phenotype made up of the animal's shell and soft tissue weights, in addition to
350 the weight of any pallial fluid - thus is not a direct reflection of meat yield. Nevertheless, in *C.*
351 *angulata*, a positive genetic correlation (0.63) has been found between TW and soft tissue
352 weight (Vu et al. 2021b), suggesting that selecting for TW - a trait easier to measure - could
353 lead to improvements in meat yields. Such indirect improvements of correlated traits have
354 been reported in a Portuguese oyster line selected only for harvest weight. While the
355 achieved average selection response for total weight at harvest was 5.8% per generation,
356 genetic gains were also observed for soft tissue weight, with indirect gains reaching a 1.2%
357 increase per generation (Vu et al. 2020). For the shell-related traits examined in this study
358 (SH, SW and SL), heritability estimates were in line with previous studies (Gutierrez et al. 2018;
359 Yuehuan et al. 2017), and ranged from 0.22 to 0.37. Traditionally, the focus on shell
360 morphometric traits was to improve oyster growth. Nevertheless, in recent years, oyster shell
361 shape is increasingly being viewed as an attractive goal for selective breeding due to its
362 growing importance for consumers (Mizuta and Wikfors 2019). The perceived attractiveness
363 of an oyster shell can be represented as a secondary trait derived from a ratio between
364 primary (shell dimension) traits, such as the shell width index (Kube et al. 2011). Given that
365 significant heritable variation was observed for the three examined morphometric traits,
366 strategies for homogenizing particular shell shapes may be feasible in *O. edulis*.

367

368

369 **Table 2.** Estimates of heritability (h^2) and standard error on the diagonal and pairwise genetic
370 correlations (below the diagonal) for growth-related traits in a European flat oyster
371 population.

Trait	Total weight	Shell height	Shell width	Shell length
Total weight	0.45 (0.06)			
Shell height	0.99	0.37 (0.06)		
Shell width	0.96	0.90	0.22 (0.05)	
Shell length	0.95	0.93	0.88	0.32 (0.06)

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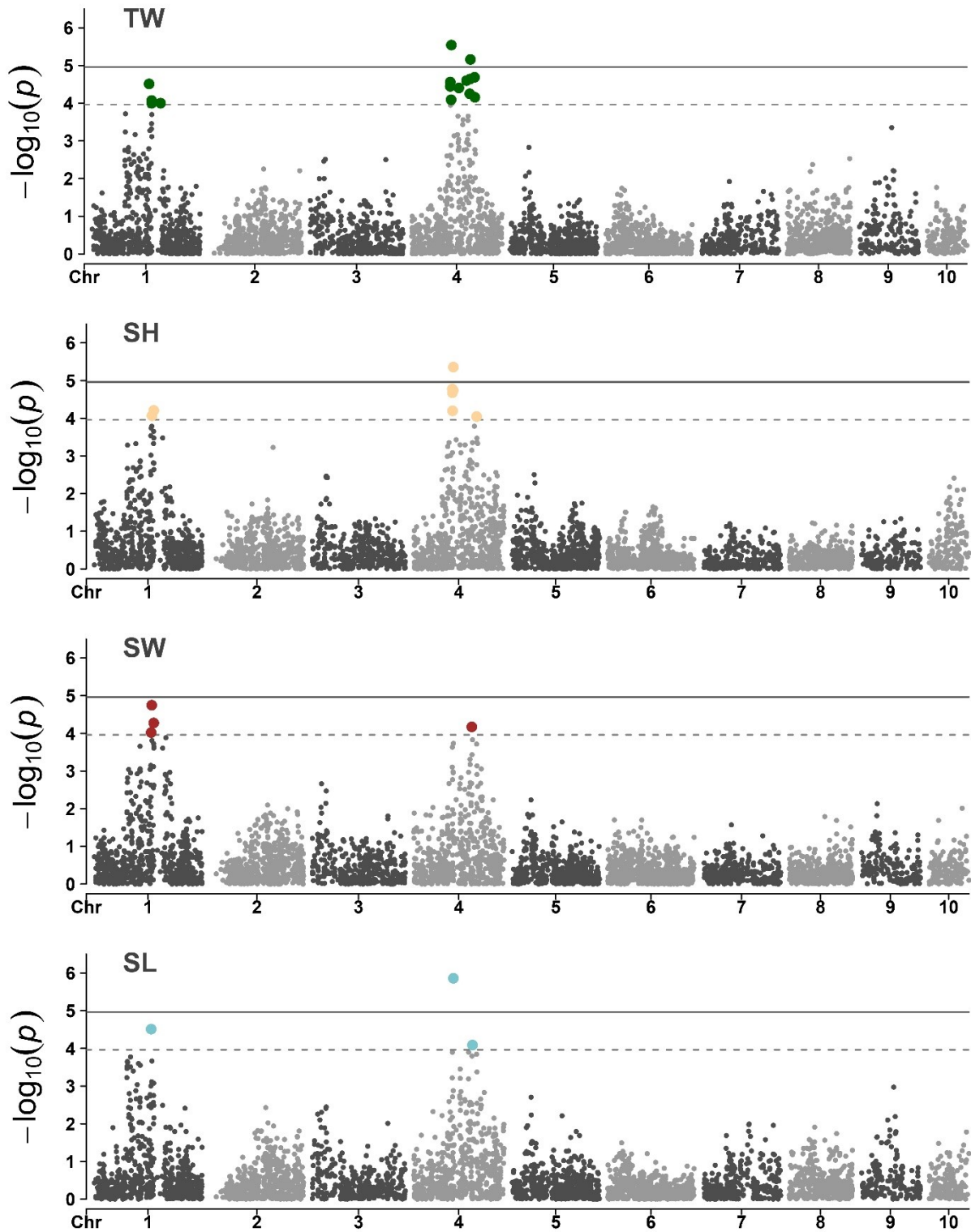
375

376 3.2 Genome-wide association analysis for growth-related traits

377 A GWAS of ~4.5K SNPs passing the filtering criteria were genotyped on 840 oysters with
378 phenotypic records to gain insight into the genetic basis of growth rate variation in *O. edulis*.
379 Three of the four examined traits showed association signals surpassing the genome-wide
380 level of significance (Figure 3). The genomic inflation factor lambda of the GWAS analysis were
381 close to the desired value ($\lambda=1$) (see Figure S1), indicating that population structure was
382 adequately accounted for by the model. For TW, the GWAS identified two putative
383 quantitative trait loci (QTLs) on chromosome 4 associated with the trait. The presence of two
384 separate QTLs is supported by the low linkage disequilibrium observed between the most
385 significant SNPs at each locus (pairwise $r^2 < 0.1$). An additional 13 suggestive loci were also
386 identified, of which nine were located in the vicinity of the two abovementioned genome-
387 wide hits and four were found on chromosome 1 (Table S1). The SNP showing the strongest
388 association with TW (AX-169174635) explained 3% of the phenotypic variance. This lead SNP
389 was also found to be significantly associated with SH and SW. For SL, no SNP reached a
390 genome-wide significance level, although a few of the same markers showing associations
391 with TW, SH and SW surpassed the threshold for suggestive significance. The complete
392 overlap of GWAS hits across the different traits suggests a high degree of shared genetic
393 control among them, consistent with the high positive genetic correlations observed (Table
394 2). Overall, the GWAS results indicate that growth-related traits in *O. edulis* are influenced by
395 many small-effect loci, exhibiting a polygenic architecture, but that two regions on
396 chromosome 4 may have a moderate effect on these traits.

397 The marker showing the most significant association with TW, SH and SW is located in the
398 exon of a gene annotated as a *N4BP2* (NEDD4 Binding Protein 2)-like protein (Gene ID:
399 *FUN_017843*; Gundappa et al. 2022). The predicted protein product of this gene contains an
400 AAA domain, hence can bind and hydrolyse ATP (Lupas and Martin 2002). Proteins with these
401 domains have been shown to be involved in several mechanical cell processes, including
402 protein folding. Further characterization of this *N4BP2*-like protein would help better
403 understand the genetic component of growth variation in oysters. Nevertheless, considering
404 that the candidate allele on *N4BP2* explained a small percentage of the phenotypic variance,
405 independent oyster populations should first be evaluated to confirm the validity of the
406 association signal. A second genome-wide significant association – detected only in the TW
407 GWAS – was located in the exon of an uncharacterized gene (*FUN_018833*) whose product
408 shares a high sequence identity (>90%) with similarly uncharacterized proteins in *C. gigas* and
409 *C. virginica* (NCBI accession numbers XP_011433755 and XP_022325737, respectively).
410 Additional genes within the two genomic regions (+/- 100 kb) showing significant associations
411 with flat oyster growth traits are shown in Table S2. Given that the SNPs identified in this
412 study had a small effect on the traits in question, GS would be an effective approach for
413 increasing genetic gains from selection.

414



415

416 **Figure 3.** Manhattan plots of the GWAS for growth-related traits in a European flat oyster
417 population. Solid lines indicates the threshold value for genome-wide significance. Dashed
418 lines indicate the threshold for a suggestive (chromosome-level) significance.

419

420

421 3.3 Genomic selection

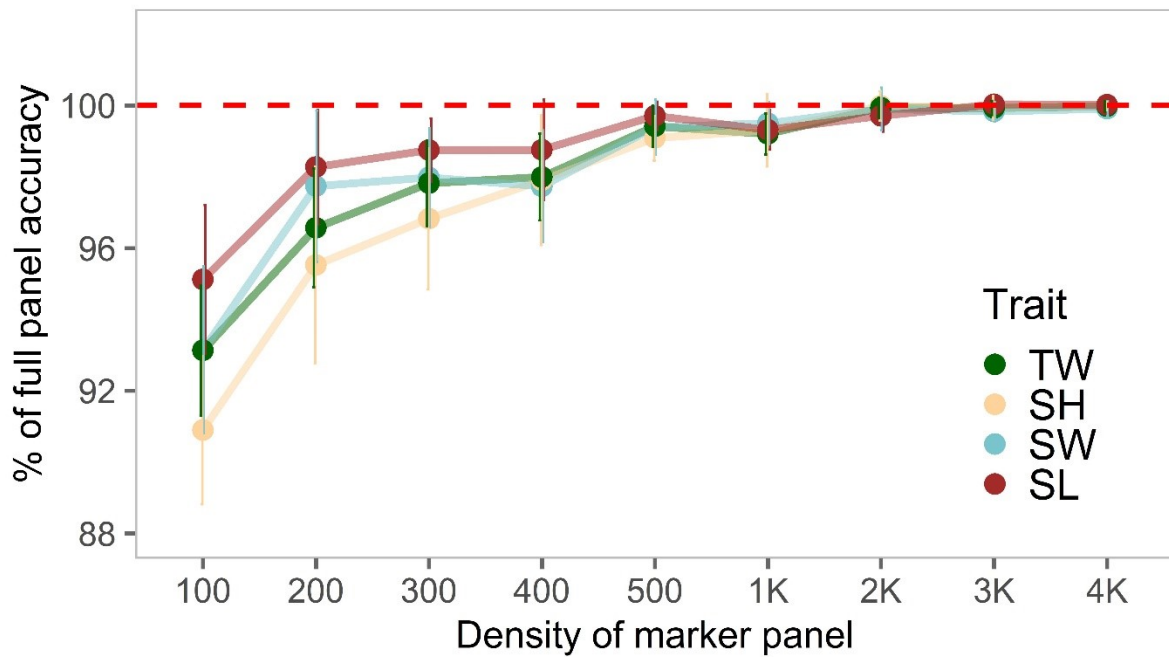
422 The incorporation of genetic markers into breeding programmes requires a previous
423 understanding of the genetic architecture of the targeted trait(s). In the *O. edulis* population
424 under study, the genetic contribution to the observed variation in growth-related traits was
425 largely polygenic in nature. For the improvement of polygenic traits, genomic selection has
426 been shown to be superior to alternative marker-aided selection due to genome-wide
427 markers capturing a higher proportion of the genetic variation in a trait compared to
428 individual QTL-targeted markers. Consequently, by means of applying GS, higher predictions
429 have been achieved for several production traits in a wide range of commercially important
430 aquaculture species (reviewed in Houston et al. (2020)). Despite GS not yet being widely
431 operational in oyster breeding programmes (Boudry et al. 2021), studies have demonstrated
432 the potential of incorporating genome-wide information into selection schemes in these taxa.
433 In the Pacific oyster, Gutierrez et al. (2018) showed that prediction accuracies for growth-
434 traits increased 25-30% when the genetic merit of individuals was estimated from SNP
435 markers using the Genomic Best Linear Unbiased Prediction (GBLUP) model (VanRaden 2008)
436 compared to a classical pedigree-based approach (PBLUP). Similar results were reported in
437 the Portuguese oyster, as prediction accuracies increased 7-42% for growth-related traits
438 when EBVs obtained by GBLUP were compared to those obtained by PBLUP (Vu et al. 2021a).
439 Since the flat oyster population under study derived from a mass-spawning event, the
440 pedigree structure was unknown. Therefore, comparisons between pedigree and genome
441 based methods for estimating breeding values (e.g. GBLUP and Bayesian approaches) could
442 not be performed.

443 One of the major barriers of implementing GS is the high number of markers required to
444 accurately predict EBVs and the cost of genotyping these markers (Goddard and Hayes 2007).
445 Therefore, the design of a strategy to reduce the cost of genotyping is critical for the extensive
446 adoption of genomic prediction approaches in aquaculture breeding programmes. One such
447 strategy involves genotyping the minimum number of markers required to achieve maximal
448 accuracy, which by definition is equal to that obtained with a full panel of markers. As shown
449 by Kriaridou et al. (2020) for different aquaculture species, the use of low-density SNP panels
450 has the potential to achieve similar EBV accuracies as when using medium density genotype
451 datasets of around 7-14K SNPs. The authors estimated that only 1,000 to 2,000 SNPs are
452 required to achieve maximal accuracy. These results were shown to be consistent across a
453 range of traits (e.g. disease resistance, growth) and species (e.g. Pacific oyster, Atlantic
454 salmon) showing robustness to differences in family structure, genotyping approach, trait
455 heritability and the underlying genetic architecture. In agreement with these findings,
456 maximal accuracy was attained herein for all the assessed growth-related traits at a minimum
457 density of 2K SNPs, with only a slight decline in accuracy observed at the lower densities
458 evaluated (Figure 4A). Consequently, a reduction in the costs of applying GS for improving
459 growth traits in *O. edulis* can be achieved by means of exploiting low-density SNP panels.
460 Although low-density panels might not accurately capture the genetic resemblance among
461 individuals within a population, and therefore show reduced genetic variance estimations and
462 EBV predictions when compared with high density panels, their use has been widely
463 evaluated and suggested for different aquaculture species and traits. Furthermore, studies
464 have shown that low-density panels can achieve higher accuracies than the classical pedigree-
465 based approach, being a feasible alternative to identify candidates with the highest genetic
466 merit. For example, in rainbow trout (*Oncorhynchus mykiss*) Vallejo et al. (2018) showed that

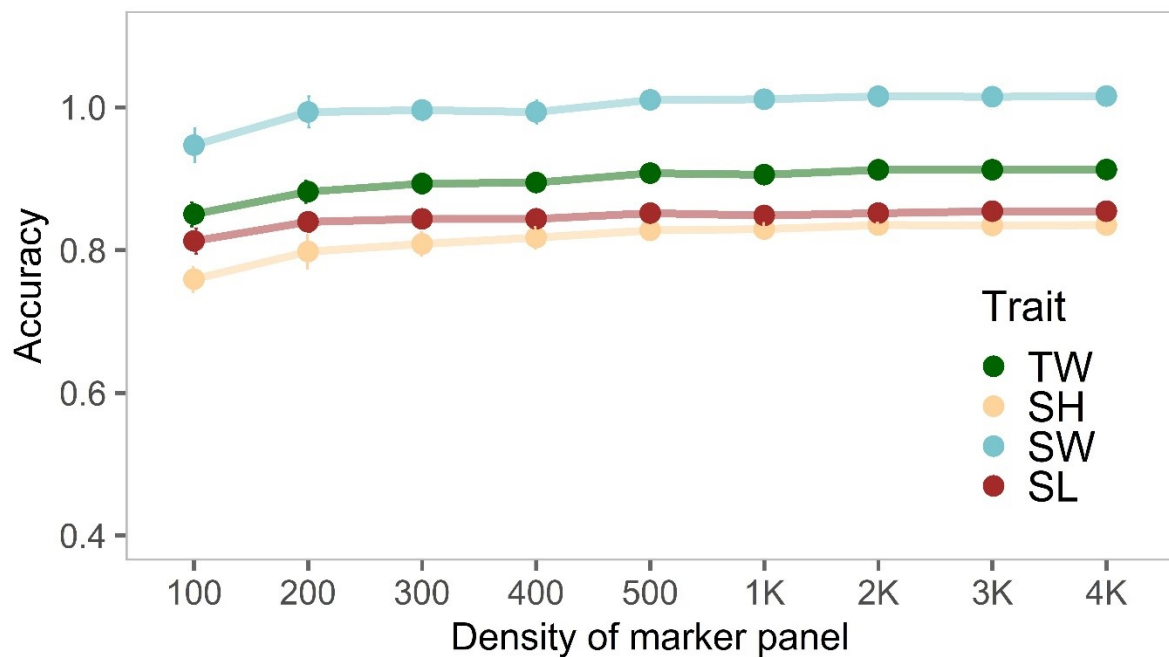
467 at least 200 SNPs could exceed PBLUP accuracies for bacterial cold water disease resistance.
468 Whilst Al-Tobasei et al. (2021) found a similar trend when using between 500-1000 SNPs for
469 fillet yield traits. To date, the utilization of low-density panels to decrease the cost of genomic
470 evaluations has also been tested in several aquaculture species, including Atlantic salmon
471 (*Salmo salar*) (Correa et al. 2017; Tsai et al. 2016), rainbow trout (Yoshida et al. 2018; Al-
472 Tobasei et al. 2021), and Nile tilapia (*Oreochromis niloticus*) (Barría et al. 2021; Yoshida et al.
473 2019), suggesting that the development of cost-effective strategies for applying GS will be key
474 for shaping modern aquaculture breeding programs.

475

A



B



476

477 **Figure 4.** Evaluation of the effect of SNP density on genomic predictions of growth related
478 traits in a European flat oyster population. (A) Percentage of the maximum genomic
479 prediction accuracy achieved using different lower density SNP panels. Values were
480 calculated by dividing the mean accuracy (averaged over ten replicates) estimated at each
481 nominal SNP density by the accuracy obtained using the full SNP dataset. (B) Average genomic
482 prediction accuracy values obtained for oyster growth traits at different panel densities.

483

484 Although our results highlight the possibility of reducing the genotyping costs associated with
485 genomic prediction approaches, caution should be taken as even for the smallest marker
486 density (i.e. 100 SNPs), prediction accuracies (averaged over 10 replicates) were high and
487 close to the value obtained with the full marker panel. By using only 100 SNPs the estimated
488 decrease in the accuracy of genomic breeding values (GEBVs) was of 5% for SL, 7% for TW and
489 SW, and 10% for SH (Figure 4A). These values highly exceed those reported in the literature
490 for aquaculture species, where reductions >20% were estimated for panels with 100 SNPs
491 compared to a complete dataset (Kriaridou et al. 2020). The relative stability of GEBVs
492 observed across different marker densities (Figure 4B) is likely explained by the underlying
493 genetic structure of the dataset. For this study, 40 potential parents were placed in the same
494 tank and spawned during successive events. The genetic analysis of the progeny revealed that
495 the population was dominated (70% of the sample size; n=589) by a group of highly related
496 individuals (Figures S2-S3), suggesting there was a large variance in reproductive success
497 among breeders, as previously reported in mass spawning of oysters (Lallias et al. 2010). In
498 the context of GS, the inclusion of highly related animals in the training and validation sets
499 results in only a small number of markers being required to capture the haplotype effects, as
500 related animals share longer haplotypes (Hickey et al. 2014). The fact that in the current study
501 animals grouped in the reference and validation data sets were highly related would have
502 likely increased the accuracy of predicted gEBV, even when animals are genotyped at low
503 density, as also shown by Fraslin et al. (2022) in Atlantic salmon. Moreover, since the high
504 accuracies predicted in the flat oyster population could have also been affected by factors
505 such as a low effective population size (Lee et al., 2017) and the observed structure (Werner
506 et al. 2020), additional populations with different genetic background should be assessed to
507 obtain estimates to be expected in practical breeding programs. Future work focused on
508 evaluating the extent to which low-density panels and alternative strategies (e.g. genotype
509 imputation) can be used to reduce genotyping costs will be key for the cost-effective
510 exploitation of GS by oyster breeding programmes.

511

512 **4. Conclusion**

513 Growth-related traits in *O. edulis* had moderate-low heritability estimates, ranging from 0.22
514 (for SW) to 0.45 (for TW). High genetic correlations were identified between all traits (>0.9);
515 hence, TW - a trait easier to measure - can potentially be used as a proxy phenotype for
516 improving the three examined morphometric traits (SH, SW and SL). The GWAS results
517 revealed that growth traits were largely polygenic, but with two distinct QTLs on chromosome
518 4 reaching genome-wide significance. Prediction accuracies were high for all traits (>0.83),
519 with minimal differences observed when comparing estimates obtained using different
520 marker densities. Altogether, these results suggest that the high prediction accuracies found
521 in this study could have been influenced by the uneven family structure of the experimental
522 population. Although low-density SNP panels appear as a promising cost-effective GS
523 strategy, additional populations with different degrees of genetic relationship should be
524 assessed to derive estimates of prediction accuracies to be expected in practical breeding
525 programmes in oysters.

526

527

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537

538 6. References

- 539 Al-Tobasei, R., Ali, A., Garcia, A.L.S., Lourenco, D., Leeds, T., and Salem, M. (2021). Genomic
540 predictions for fillet yield and firmness in rainbow trout using reduced-density SNP panels.
541 *BMC Genom.* 22:92. doi: 10.1186/s12864-021-07404-9.
- 542 Allen, S.K., Gaffney, P.M., and Ewart, J.W. (1993). Genetic Improvement of the Eastern Oyster for
543 Growth and Disease Resistance in the Northeast. *Northeastern Regional Aquaculture Center.*
544 210
- 545 Barría, A., Benzie, J.A.H., Houston, R.D., De Koning, D.J., and de Verdal, H. (2021). Genomic Selection
546 and Genome-wide Association Study for Feed-Efficiency Traits in a Farmed Nile Tilapia
547 (*Oreochromis niloticus*) Population. *Front. Genet.* 12: 737906. doi:
548 10.3389/fgene.2021.737906.
- 549 Botta, R., Asche, F., Borsum, J.S., and Camp, E.V. (2020). A review of global oyster aquaculture
550 production and consumption. *Mar. Policy* 117:103952. doi: 10.1016/j.marpol.2020.103952.
- 551 Boudry, P., Allal, F., Aslam, M.L., Bargelloni, L., Bean, T.P., Brard- Fudulea, S., et al. (2021). Current
552 status and potential of genomic selection to improve selective breeding in the main
553 aquaculture species of International Council for the Exploration of the Sea (ICES) member
554 countries. *Aquac.* 20:100700. doi: 10.1016/j.aqrep.2021.100700.
- 555 Boutet, I., Alves Monteiro, H.J., Takeuchi T, Bonnivard, E., Farhat, S., Riso, R., Salaun, B., Andersen,
556 A., Toullec, J.-Y., Lallier, F., Flot, J.F., Guiglielmoni, N., Guo, X., Allam, B., Espinoza E.,
557 Marbouty, M., Koszul, R., and Tanguy, A. (2022) Chromosomal assembly of the flat oyster
558 (*Ostrea edulis* L.) genome as a new genetic resource for aquaculture. Evolutionary
559 applications. XX-XX-XX
- 560 Camara, M.D., Yen, S., Kaspar, H.F., Kesarcodi-Watson, A., King, N., Jeffs, A.G., et al. (2017).
561 Assessment of heat shock and laboratory virus challenges to selectively breed for ostreid
562 herpesvirus 1 (OsHV-1) resistance in the Pacific oyster, *Crassostrea gigas*. *Aquaculture* 469,
563 50-58. doi: 10.1016/j.aquaculture.2016.11.031.
- 564 Chang, C.C., Chow, C.C., Tellier, L.C., Vattikuti, S., Purcell, S.M., and Lee, J.J. (2015). Second-
565 generation PLINK: rising to the challenge of larger and richer datasets. *GigaScience* 4:7. doi:
566 10.1186/s13742-015-0047-8.
- 567 Colsoul, B., Boudry, P., Pérez-Parallé, M.L., Bratoš Cetinić, A., Hugh-Jones, T., Arzul, I., et al. (2021).
568 Sustainable large-scale production of European flat oyster (*Ostrea edulis*) seed for ecological
569 restoration and aquaculture: a review. *Rev. Aquac.* 13, 423-1468. doi: 10.1111/raq.12529.
- 570 Correa, K., Bangera, R., Figueroa, R., Lhorente, J.P, and Yáñez, J.M. (2017). The use of genomic
571 information increases the accuracy of breeding value predictions for sea louse (*Caligus*
572 *rogercresseyi*) resistance in Atlantic salmon (*Salmo salar*). *Genet. Sel. Evol.* 49:15. doi:
573 10.1186/s12711-017-0291-8.

- 574 Culloty, S.C., Cronin, M.A., and Mulcahy, M.F. (2004). Potential resistance of a number of
575 populations of the oyster *Ostrea edulis* to the parasite *Bonamia ostreae*. *Aquaculture* 237,
576 41-58. doi: 10.1016/j.aquaculture.2004.04.007.
- 577 De Melo, C.M., Durland, E., and Langdon, C. (2016). Improvements in desirable traits of the Pacific
578 oyster, *Crassostrea gigas*, as a result of five generations of selection on the West Coast, USA.
579 *Aquaculture* 460, 105- 115. doi: 10.1016/j.aquaculture.2016.04.017.
- 580 Dégremont, L., Nourry, M., and Maurouard, E. (2015). Mass selection for survival and resistance to
581 OsHV-1 infection in *Crassostrea gigas* spat in field conditions: response to selection after
582 four generations. *Aquaculture* 446, 111-121. doi: 10.1016/j.aquaculture.2015.04.029.
- 583 Devlin, B., and Roeder, K. (1999). Genomic control for association studies. *Biometrics* 55, 997-1004.
584 doi: 10.1111/j.0006-341x.1999.00997.x.
- 585 Evans, F., Matson, S., Brake, J., and Langdon, C. (2004). The effects of inbreeding on performance
586 traits of adult Pacific oysters (*Crassostrea gigas*). *Aquaculture* 230, 89-98. doi:
587 10.1016/j.aquaculture.2003.09.023.
- 588 Evans, S., and Langdon, C. (2006). Effects of genotype × environment interactions on the selection of
589 broadly adapted Pacific oysters (*Crassostrea gigas*). *Aquaculture* 261, 522-534. doi:
590 10.1016/j.aquaculture.2006.07.022.
- 591 Fabioux, C., Huvet, A., Lelong, C., Robert, R., Pouvreau, S., Daniel, J.Y., et al. (2004). Oyster vasa-like
592 gene as a marker of the germline cell development in *Crassostrea gigas*. *Biochem. Biophys.*
593 *Res. Commun.* 320, 592-598. doi: 10.1016/j.bbrc.2004.06.009.
- 594 FAO (2019). Cultured Aquatic Species Information Programme. *Ostrea edulis*, edited by F.F. Division.
595 Fitzter, S.C., McGill, R.A.R., Torres Gabarda, S., Hughes, B., Dove, M., O'Connor, W., et al. (2019).
596 Selectively bred oysters can alter their biomineralization pathways, promoting resilience to
597 environmental acidification. *Glob. Chang. Biol.* 25, 4105-4115. doi: 10.1111/gcb.14818.
- 598 Fraslin, C., Yáñez, J.M., Robledo, D., and Houston, R.D. (2022). The impact of genetic relationship
599 between training and validation populations on genomic prediction accuracy in Atlantic
600 salmon. *Aquaculture Reports* 23, 101033. doi: doi.org/10.1016/j.aqrep.2022.101033.
- 601 Goddard, M.E., and Hayes, B.J. (2007). Genomic selection. *J. Anim. Breed. Genet.* 124, 323-330. doi:
602 10.1111/j.1439-0388.2007.00702.x.
- 603 Grizel, H., and Héral, M. (1991). Introduction into France of the Japanese oyster (*Crassostrea gigas*).
604 *ICES Mar. Sci. Symp.* 47, 399- 403. doi: 10.1093/icesjms/47.3.399.
- 605 Grizzle, R.E., Ward, K.M., Peter, C.R., Cantwell, M., Katz, D., and Suvillan, J. (2017). Growth,
606 morphometrics, and nutrient content of farmed eastern oysters, *Crassostrea virginica*
607 (Gmelin), in New Hampshire, USA. *Aquac. Res.* 48, 1525-1537. doi: 10.1111/are.12988.
- 608 Gundappa, M. K., Peñaloza, C., Regan, T., Boutet, I., Tanguy, A., Houston, R. D., Bean, T. B., &
609 Macqueen, D. J. (2022). A chromosome level reference genome for European flat oyster
610 (*Ostrea edulis* L.). Evolutionary applications. XX-XX-XX
- 611 Gutierrez, A.P., Matika, O., Bean, T.P., and Houston, R.D. (2018). Genomic Selection for Growth
612 Traits in Pacific Oyster (*Crassostrea gigas*): Potential of Low-Density Marker Panels for
613 Breeding Value Prediction. *Front. Genet.* 9:391. doi: 10.3389/fgene.2018.00391.
- 614 Gutierrez, A.P., Symonds, J., King, N., Steiner, K., Bean, T.P., and Houston, R.D. (2020). Potential of
615 genomic selection for improvement of resistance to ostreid herpesvirus in Pacific oyster
616 (*Crassostrea gigas*). *Anim. Genet.* 51, 249-257. doi: 10.1111./age.12909.
- 617 Gutierrez, A.P., Turner, F., Gharbi, K., Talbot, R., Lowe, N.R., Peñaloza, C., et al. (2017). Development
618 of a Medium Density Combined-Species SNP Array for Pacific and European Oysters
619 (*Crassostrea gigas* and *Ostrea edulis*). *G3(Bethesda)* 7, 2209-2218. doi:
620 10.1534/g3.117.041780.
- 621 He, X., Li, C., Qi, H., Meng, J., Wang, W., Wu, F., et al. (2021). A genome-wide association study to
622 identify the genes associated with shell growth and shape-related traits in *Crassostrea gigas*.
623 *Aquaculture* 543:736926. doi: 10.1016/j.aquaculture.2021.736926.

- 624 He, X., Wu, F., Qi, H., Meng, J., Wang, W., Liu, M., et al. (2022). Whole-genome resequencing reveals
625 the single nucleotide polymorphisms associated with shell shape in *Crassostrea gigas*.
626 *Aquaculture* 547:737502. doi: 10.1016/j.aquaculture.2021.737502.
- 627 Helmer, L., Farrell, P., Hendy, I., Harding, S., Robertson, M., and Preston, J. (2019). Active
628 management is required to turn the tide for depleted *Ostrea edulis* stocks from the effects
629 of overfishing, disease and invasive species. *PeerJ* 7:e6431. doi: 10.7717/peerj.6431.
- 630 Hickey, J.M., Dreisigacker, S., Crossa, J., Hearne, S., Babu, R., Prasanna, B.M., et al. (2014). Evaluation
631 of Genomic Selection Training Population Designs and Genotyping Strategies in Plant
632 Breeding Programs Using Simulation. *Crop Sci.* 54, 1476-1488. doi:
633 10.2135/cropsci2013.03.0195.
- 634 Houston, R.D., Bean, T.P., Macqueen, D.J., Gundappa, M.K., Jin, Y.H., Jenkins, T.L., et al. (2020).
635 Harnessing genomics to fast-track genetic improvement in aquaculture. *Nat. Rev. Genet.* 21:
636 389-409. doi: 10.1038/s41576-020-0227-y.
- 637 Jones, D.B., Jerry, D.R., Khatkar, M.S., Moser, G., Raadsma, H.W., Taylor J.J., et al. (2014).
638 Determining genetic contributions to host oyster shell growth: Quantitative trait loci and
639 genetic association analysis for the silver-lipped pearl oyster, *Pinctada maxima*. *Aquaculture*
640 434, 367-375. doi: 10.1016/j.aquaculture.2014.08.040.
- 641 Jones, D.B., Jerry, D.R., Khatkar, M.S., Raadsma, H.W., and Zenger, K.R. (2013). A high-density SNP
642 genetic linkage map for the silver-lipped pearl oyster, *Pinctada maxima*: a valuable resource
643 for gene localisation and marker-assisted selection. *BMC Genom.* 14:810. doi: 10.1186/1471-
644 2164-14-810.
- 645 Korrynga, P. (1976). Farming the flat oyster of the genus *Ostrea*. Amsterdam: ElsevierScientific.
- 646 Kriaridou, C., Tsairidou, S., Houston, R.D., and Robledo, D. (2020). Genomic Prediction Using Low
647 Density Marker Panels in Aquaculture: Performance Across Species, Traits, and Genotyping
648 Platforms. *Front Genet.* 11:124. doi: 10.3389/fgene.2020.00124.
- 649 Kube, P., Cunningham, M., Dominik, S., Parkinson, S., Finn, B., Henshall, J., et al. (2011).
650 Enhancement of the Pacific Oyster Selective Breeding Program. Australia: FRDC and Seafood
651 CRC.
- 652 Lallias, D., Taris, N., Boudry, P., Bonhomme, F., and Lapègue, S. (2010). Variance in the reproductive
653 success of flat oyster *Ostrea edulis* L. assessed by parentage analyses in natural and
654 experimental conditions. *Genet. Res.* 92, 175-187. doi: 10.1017/S0016672310000248
- 655 Lapegue, S., Harrang, E., Heurtebise, S., Flahauw, E., Donnadiou, C., Gayral, P., et al. (2014).
656 Development of SNP-genotyping arrays in two shellfish species. *Mol. Ecol. Resour.* 14, 820-
657 830. doi: 10.1111/1755-0998.12230.
- 658 Lee, S.H., Clark, S., and van der Werf, J.H.J. (2017). Estimation of genomic prediction accuracy from
659 reference populations with varying degrees of relationship. *PLoS One* 12(12), e0189775. doi:
660 10.1371/journal.pone.0189775.
- 661 Li, A., Dai, H., Guo, X., Zhang, Z., Zhang, K., Wang, C., et al. (2021). Genome of the estuarine oyster
662 provides insights into climate impact and adaptive plasticity. *Commun. Biol.* 4:1287. doi:
663 10.1038/s42003-021-02823-6.
- 664 Li, C., Wang, J., Song, K., Meng, J., Xu, F., Li, L., et al. (2018). Construction of a high-density genetic
665 map and fine QTL mapping for growth and nutritional traits of *Crassostrea gigas*. *BMC*
666 *Genom.* 19:626. doi: 10.1186/s12864-018-4996-z.
- 667 Lillehammer, M., Banger, R., Salazar, M., Vela, S., Erazo, E.C., Suarez, A., et al. (2020). Genomic
668 selection for white spot syndrome virus resistance in whiteleg shrimp boosts survival under
669 an experimental challenge test. *Sci. Rep.* 10:20571. doi: 10.1038/s41598-020-77580-3.
- 670 Liu, S., Li, L., Zhang, S., Wang, W., Yang, J., and Zhang, G. (2019). Heritability estimates for nutritional
671 quality-related traits of the Pacific oyster, *Crassostrea gigas*. *J. World Aquacult. Soc.* 50, 738-
672 748. doi: 10.1111/jwas.12588.
- 673 Lupas, A.N., and Martin, J. (2002). AAA proteins. *Curr. Opin. Struct. Biol.* 12, 746-753. doi:
674 10.1016/s0959-440x(02)00388-3.

- 675 McCarty, A.J., Allen, Jr., S.K., and Plough, L.V. (2022). Genome-wide analysis of acute low salinity
676 tolerance in the eastern oyster *Crassostrea virginica* and potential of genomic selection for
677 trait improvement. *G3 (Bethesda)* 12:jkab368. doi: 10.1093/g3journal/jkab368.
- 678 Meng, J., Song, K, Li, C., Liu, S., Shi, R., Li, B., et al. (2019). Genome-wide association analysis of
679 nutrient traits in the oyster *Crassostrea gigas*: genetic effect and interaction network. *BMC*
680 *Genom.* 20:625. doi: 10.1186/s12864-019-5971-z.
- 681 Meuwissen, T.H., Hayes, B.J., and Goddard, M.E. (2001). Prediction of total genetic value using
682 genome-wide dense marker maps. *Genetics* 157, 1819-1829. doi:
683 10.1093/genetics/157.4.1819.
- 684 Mizuta, D.D., and Wikfors, G.H. (2019). Seeking the perfect oyster shell: a brief review of current
685 knowledge. *Rev. Aquac.* 11, 586-602. doi: 10.1111/raq.12247.
- 686 Modak, T.H., Literman, R., Puritz, J.B., Johnson, K.M., Roberts, E.M., Proestou, D., et al. (2021).
687 Extensive genome-wide duplications in the eastern oyster (*Crassostrea virginica*). *Philos.*
688 *Trans. R. Soc. Lond., B, Biol. Sci.* 376:20200164. doi: 10.1098/rstb.2020.0164.
- 689 Montagnani, C., Le Roux, F., Berthe, F., and Escoubas, J.M. (2001). Cg-TIMP, an inducible tissue
690 inhibitor of metalloproteinase from the Pacific oyster *Crassostrea gigas* with a potential role
691 in wound healing and defense mechanisms(1). *FEBS Lett.* 500, 64-70. doi: 10.1016/s0014-
692 5793(01)02559-5.
- 693 Naciri-Graven, Y., Martin, A.G., Baud, J.P., Renault, T., and Gérard, A. (1998). Selecting the flat oyster
694 *Ostrea edulis* (L.) for survival when infected with the parasite *Bonamia ostreae*. *J. Exp. Mar.*
695 *Biol. Ecol.* 224, 91-107. doi: 10.1016/S0022-0981(97)00171-8.
- 696 Neph, S., Kuehn, M.S., Reynolds, A.P., Haugen, E., Thurman, R.E., Johnson A.K., et al. (2012). BEDOPS:
697 high-performance genomic feature operations. *Bioinformatics* 28, 1919-1920. doi:
698 10.1093/bioinformatics/bts277.
- 699 Newkirk, G.F., and Haley, L.E. (1982). Progress in Selection for Growth Rate in the European Oyster
700 *Ostrea edulis*. *Marine Ecology Progress Series* 10, 3.
- 701 Newkirk, G.F., and Haley, L.E. (1983). Selection for growth rate in the European oyster, *Ostrea edulis*:
702 Response of second generation groups. *Aquaculture* 33, 149-155. doi: 10.1016/0044-
703 8486(83)90396-4.
- 704 Oelig, and G. Uf, (2000). Present Status of the French Aquaculture. *Aquaculture Science* 48 (2):243-
705 248.
- 706 Peñaloza, C., Gutierrez, A.P., Eöry, L., Wang, S., Guo, X., Archibald, A.L., et al. (2021). A chromosome-
707 level genome assembly for the Pacific oyster *Crassostrea gigas*. *GigaScience* 10:giab020. doi:
708 10.1093/gigascience/giab020.
- 709 Pichot, Y., Comps, M., Tige, G., Grizel, H., and Rabouin, M.A. (1979). Recherches sur *Bonamia ostreae*
710 gen. n., sp. n., parasite nouveau de l' huitre plate *Ostrea edulis* L. [France]. *Rev. Trav. Inst.*
711 *Peches Marit.* 43, 131-140.
- 712 Plough, L.V. (2016). Genetic load in marine animals: a review. *Curr. Zool.* 62, 567-579. doi:
713 10.1093/cz/zow096.
- 714 Pogoda, B. (2019). Current Status of European Oyster Decline and Restoration in Germany.
715 *Humanities* 8:9. doi: 10.3390/h8010009.
- 716 Pogoda, B., Boudry, P., Bromley, C., Cameron, T.C., Colsoul, B., Donnan, D.W., et al. (2020). NORA
717 moving forward: Developing an oyster restoration network in Europe to support the Berlin
718 Oyster Recommendation. *Aquat. Conserv.* 30, 2031-2037. doi: 10.1002/aqr.3447.
- 719 Preston, J., Fabra, M., Helmer, L., Johnson, E., Harris-Scott, E., and Wendy, I.W. (2020). Interactions
720 of larval dynamics and substrate preference have ecological significance for benthic
721 biodiversity and *Ostrea edulis* Linnaeus, 1758 in the presence of *Crepidula fornicata*. *Aquat.*
722 *Conserv.: Mar. Freshw. Ecosyst.* 30, 2133-2149. doi: 10.1002/aqc.3446
- 723 Proestou, D.A., Vinyard, B.T., Corbett, R.J., Piesz, J., Allen, S.K., Small J.M., et al. (2016). Performance
724 of selectively-bred lines of eastern oyster, *Crassostrea virginica*, across eastern US estuaries.
725 *Aquaculture* 464:17-27. doi: 10.1016/j.aquaculture.2016.06.012.

- 726 Qi, H., Li, L., and Zhang, G. (2021). Construction of a chromosome-level genome and variation map
727 for the Pacific oyster *Crassostrea gigas*. *Mol. Ecol. Resour.* 21, 1670-1685. doi:
728 10.1111/1755-0998.13368.
- 729 Qi, H., Song, K., Li, C., Wang, W., Li, B., Li, L., et al. (2017). Construction and evaluation of a high-
730 density SNP array for the Pacific oyster (*Crassostrea gigas*). *PLoS One* 12:e0174007. doi:
731 10.1371/journal.pone.0174007.
- 732 Ragone Calvo, L.M., Calvo, G.W., and Burreson, E.M. (2003). Dual disease resistance in a selectively
733 bred eastern oyster, *Crassostrea virginica*, strain tested in Chesapeake Bay. *Aquaculture* 220,
734 69-87. doi: 10.1016/S0044-8486(02)00399-X.
- 735 Renault, T., Cochenec, N., and Grizel, H. (1995). *Bonamia ostreae*, parasite of the European flat
736 oyster, *Ostrea edulis*, does not experimentally infect the Japanese oyster, *Crassostrea gigas*.
737 *Bull. Eur. Ass. Fish Pathol.* 15:78.
- 738 Robert, M., Garcia, C., Chollet, B., Lopez-Flores, I., Ferrand, S., François, C., et al. (2009). Molecular
739 detection and quantification of the protozoan *Bonamia ostreae* in the flat oyster, *Ostrea*
740 *edulis*. *Mol. Cell. Probes* 23, 264-271. doi: 10.1016/j.mcp.2009.06.002.
- 741 Robert, R., Borel, M., Pichot, Y., and Trut, G. (1991). Growth and mortality of the European oyster
742 *Ostrea edulis* in the Bay of Arcachon (France). *Aquat. Living Resour.* 4, 265-274. doi:
743 10.1051/alr:1991028.
- 744 Sas, H., Deden, B., Kamermans, P., zu Ermgassen, P.S.E., Pogoda B., Preston, J., et al. (2020). *Bonamia*
745 infection in native oysters (*Ostrea edulis*) in relation to European restoration projects. *Aquat.*
746 *Conserv.: Mar. Freshw. Ecosyst.* 30, 2150-2162. doi: 10.1002/aqc.3430.
- 747 Thurstan, R.H., Hawkins, J.P., Raby, L., and Roberts, C.M. (2013). Oyster (*Ostrea edulis*) extirpation
748 and ecosystem transformation in the Firth of Forth, Scotland. *J. Nat. Conserv.* 21, 253-261.
749 doi: 10.1016/j.jnc.2013.01.004.
- 750 Toro, J.E., and Newkirk, G.F. (1990). Divergent selection for growth rate in the European oyster
751 *Ostrea edulis*: response to selection and estimation of genetic parameters. *Mar. Ecol. Prog.*
752 *Ser.* 62, 219-227. doi: 10.3354/meps062219.
- 753 Tsai, H.Y., Hamilton, A., Tinch, A.E., Guy, D.R., Bron, J.E., Taggart, J.B., et al. (2016). Genomic
754 prediction of host resistance to sea lice in farmed Atlantic salmon populations. *Genet.* 48:47.
755 doi: 10.1186/s12711-016-0226-9.
- 756 Tsairidou, S., 2019 CVrepGPAcalc. <https://github.com/SmaragdaT/CVrep/tree/master/CVrepGPAcalc>
757 [Accessed March 15, 2020].
- 758 Tsairidou, S., Hamilton, A., Robledo, D., Bron, J.E., and Houston, R.D. (2020). Optimizing Low-Cost
759 Genotyping and Imputation Strategies for Genomic Selection in Atlantic Salmon. *G3*
760 (*Bethesda*) 10, 581-590. doi: 10.1534/g3.119.400800.
- 761 Turner, S. (2018). qqman: an R package for visualizing GWAS results using Q-Q and manhattan plots.
762 *Journal of Open Source Software*, 3(25), 731. <https://doi.org/10.21105/joss.00731>
- 763 Vallejo, R.L., Silva, R.M.O., Evenhuis, J.P., Gao, G., Liu, S., Parsons, J.E., et al. (2018). Accurate
764 genomic predictions for BCWD resistance in rainbow trout are achieved using low-density
765 SNP panels: Evidence that long-range LD is a major contributing factor. *J Anim Breed Genet.*
766 doi: 10.1111/jbg.12335.
- 767 VanRaden, P.M. (2008). Efficient Methods to Compute Genomic Predictions. *J. Dairy Sci.* 91, 4414-
768 4423. doi: 10.3168/jds.2007-0980.
- 769 Varney, R.L., and Wilbur, A.E. (2020). Analysis of genetic variation and inbreeding among three lines
770 of hatchery-reared *Crassostrea virginica* broodstock. *Aquaculture* 527:735452. doi:
771 10.1016/j.aquaculture.2020.735452.
- 772 Vera, M., Pardo, B.G., Cao, A., Vilas, R., Fernández, C., Blanco, A., et al. (2019). Signatures of selection
773 for bonamiosis resistance in European flat oyster (*Ostrea edulis*): New genomic tools for
774 breeding programs and management of natural resources. *Evolutionary applications* 12(9),
775 1781-1796. doi: 10.1111/eva.12832.

- 776 Vu, S.V., Gondro, C., Nguyen, N.T.H., Gilmour, A.R., Tearle, R., Knibb, W., et al. (2021a). Prediction
777 Accuracies of Genomic Selection for Nine Commercially Important Traits in the Portuguese
778 Oyster (*Crassostrea angulata*) Using DArT-Seq Technology. *Genes* 12:210. doi:
779 10.3390/genes12020210.
- 780 Vu, S.V., Knibb, W., Gondro, C., Subramanian, S., Nguyen, N.T.H., Alam, M., et al. (2021b). Genomic
781 Prediction for Whole Weight, Body Shape, Meat Yield, and Color Traits in the Portuguese
782 Oyster *Crassostrea angulata*. *Front. Genet.* 12:661276. doi: 10.3389/fgene.2021.661276.
- 783 Vu, S.V., Knibb, W., Nguyen, N.T.H., Vu, I.V., O'Connor, W., Dove, M., et al. (2020). First breeding
784 program of the Portuguese oyster *Crassostrea angulata* demonstrated significant selection
785 response in traits of economic importance. *Aquaculture* 518:734664. doi:
786 10.1016/j.aquaculture.2019.734664.
- 787 Walne, P.R., and Helm, M.M. (1979). Introduction of *Crassostrea gigas* into the United Kingdom,
788 (Cambridge, MIT Press), 83-105.
- 789 Wan, S., Li, Q., Yu, H., Liu, S., and Kong, L. (2020). Estimating heritability for meat composition traits
790 in the golden shell strain of Pacific oyster (*Crassostrea gigas*). *Aquaculture* 516:734532. doi:
791 10.1016/j.aquaculture.2019.734532.
- 792 Wang, J., Li, L., and Zhang, G. (2016). A High-Density SNP Genetic Linkage Map and QTL Analysis of
793 Growth-Related Traits in a Hybrid Family of Oysters (*Crassostrea gigas* × *Crassostrea*
794 *angulata*) Using Genotyping-by-Sequencing. *G3 (Bethesda)* 6,1417-1426. doi:
795 10.1534/g3.116.026971.
- 796 Ward, R.D., Thompson, P.A., Appleyard, S.A., Swan, A.A., and Kube, P.D. (2005). "Sustainable Genetic
797 Improvement of Pacific Oysters in Tasmania and South Australia", in: *FRDC Project 2000/206*.
798 CSIRO Marine and Atmospheric Research.
- 799 Werner, C.R., Gaynor, R.C., Gorjanc, G., Hickey, J.M., Kox, T., Abbadi, A., et al. (2020). How
800 population structure impacts genomic selection accuracy in cross-validation: implications for
801 practical breeding. *Front. Plant Sci.* 11: 592977. doi: 10.3389/fpls.2020.592977.
- 802 Xu, L., Li, Q., Yu, H., and Kong, L. (2017). Estimates of Heritability for Growth and Shell Color Traits
803 and Their Genetic Correlations in the Black Shell Strain of Pacific Oyster *Crassostrea gigas*.
804 *Mar. Biotechnol. (NY)* 19, 421-429. doi: 10.1007/s10126-017-9772-6.
- 805 Yang, B., Zhai, S., Zhang, F., Wang, H., Ren, L., Li, Y., et al. (2022). Genome-wide association study
806 toward efficient selection breeding of resistance to *Vibrio alginolyticus* in Pacific oyster,
807 *Crassostrea gigas*. *Aquaculture* 548:737592. doi: 10.1016/k.aquaculture.2021.737592.
- 808 Yin, X., Arias-Pérez, A., Kitapci, T.H., and Hedgecock, D. (2020). High-Density Linkage Maps Based on
809 Genotyping-by-Sequencing (GBS) Confirm a Chromosome-Level Genome Assembly and
810 Reveal Variation in Recombination Rate for the Pacific Oyster *Crassostrea gigas*. *G3*
811 *(Bethesda)* 10, 4691-4705. doi: 10.1534/g3.120.401728.
- 812 Yoshida, G.M., Banger, R., Carvalheiro, R., Correa, K., Figueroa, R., Lhorente J.P., et al. (2018).
813 Genomic Prediction Accuracy for Resistance Against *Piscirickettsia salmonis* in Farmed
814 Rainbow Trout. *G3 (Bethesda)* 8, 719-726. doi: 10.1534/g3.117.300499.
- 815 Yoshida, G.M., Lhorente, J.P., Correa, K., Soto, J., Salas, D., and Yañez, J.M. (2019). Genome-Wide
816 Association Study and Cost-Efficient Genomic Predictions for Growth and Fillet Yield in Nile
817 Tilapia (*Oreochromis niloticus*). *G3 (Bethesda)* 9, 2597-2607. doi: 10.1534/g3.119.400116.
- 818 Yuehuan, Z., Wu, X., Qin, Y., Xiao, S., Ma, H., Li, J., et al. (2017). Sustained response to selection of
819 growth traits to the third generation for two strains of Kumamoto oyster *Crassostrea*
820 *sikamea*. *Journal of Fishery Sciences of China* 24, 1161-1167. doi:
821 10.3724/SP.J.1118.2017.16350.
- 822 Zenger, K.R., Khatkar, M.S., Jones, D.B., Khalilisamani, N., Jerry, D.R., and Raadsma, H.W. (2019).
823 Genomic Selection in Aquaculture: Application, Limitations and Opportunities With Special
824 Reference to Marine Shrimp and Pearl Oysters. *Front. Genet.* 9:693. doi:
825 10.3389/fgene.2018.00693.

826 Zhang, J., Li, Q., Xu, C., and Han, Z. (2019). Response to selection for growth in three selected strains
827 of the Pacific oyster *Crassostrea gigas*. *Aquaculture* 503:34-39. doi:
828 10.1016/j.aquaculture.2018.12.076.
829 Zhou, X., and Stephens, M. (2012). Genome-wide efficient mixed-model analysis for association
830 studies. *Nat. Genet.* 44, 821-824. doi: 10.1038/ng.2310.

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