

1 **A genome-wide association study for host resistance to Ostreid**

2 **Herpesvirus in Pacific oysters (*Crassostrea gigas*)**

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22 **Running tittle:** GWAS for OsHV resistance in oyster  
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42 **Abstract**

43 Ostreid herpesvirus (OsHV) can cause mass mortality events in Pacific oyster  
44 aquaculture. While various factors impact on the severity of outbreaks, it is clear that  
45 genetic resistance of the host is an important determinant of mortality levels. This  
46 raises the possibility of selective breeding strategies to improve the genetic  
47 resistance of farmed oyster stocks, thereby contributing to disease control.

48 Traditional selective breeding can be augmented by use of genetic markers, either  
49 via marker-assisted or genomic selection. The aim of the current study was to  
50 investigate the genetic architecture of resistance to OsHV in Pacific oyster, to identify  
51 genomic regions containing putative resistance genes, and to inform the use of  
52 genomics to enhance efforts to breed for resistance. To achieve this, a population of  
53 ~1,000 juvenile oysters were experimentally challenged with a virulent form of OsHV,  
54 with samples taken from mortalities and survivors for genotyping and qPCR  
55 measurement of viral load. The samples were genotyped using a recently-developed  
56 SNP array, and the genotype data were used to reconstruct the pedigree. Using  
57 these pedigree and genotype data, the first high density linkage map was  
58 constructed for Pacific oyster, containing 20,353 SNPs mapped to the ten pairs of  
59 chromosomes. Genetic parameters for resistance to OsHV were estimated,  
60 indicating a significant but low heritability for the binary trait of survival and also for  
61 viral load measures ( $h^2$  0.12 – 0.25). A genome-wide association study highlighted a  
62 region of linkage group 6 containing a significant QTL affecting host resistance.  
63 These results are an important step towards identification of genes underlying  
64 resistance to OsHV in oyster, and a step towards applying genomic data to enhance  
65 selective breeding for disease resistance in oyster aquaculture.

66

67 **Introduction**

68

69 A specific genotype of the ostreid herpesvirus (OsHV-1- $\mu$ var) has been suggested to  
70 be the main cause of periodic mass mortality events in farmed Pacific oysters  
71 (*Crassostrea gigas*) worldwide (Segarra *et al.* 2010), with other contributing factors  
72 potentially including *Vibrio* bacterial infection and elevated temperature (Petton *et al.*  
73 2015; Malham *et al.* 2009). Given that Pacific oysters account for 98% of global  
74 oyster production, which was estimated at ~0.6M tons in 2015, this pathogen is a  
75 significant problem for global aquaculture. Due to the current lack of effective options  
76 to prevent or control disease outbreaks (e.g. no option for vaccination and limited  
77 evidence of effective biosecurity) improving host resistance to OsHV-1 via selective  
78 breeding has become a major target.

79 A significant additive genetic component has been described for survival during  
80 OsHV-1 infection, with estimated heritability values ranging from 0.21 to 0.63  
81 (Azéma *et al.* 2017; Camara *et al.* 2017; Dégremont *et al.* 2015a). Substantial efforts  
82 are being made to establish selective breeding programs for *C. gigas* with OsHV-1  
83 resistance as the primary target trait. An encouraging response to selection for  
84 resistance has been observed in *C. gigas* spat after four generations of mass  
85 selection (Dégremont *et al.* 2015b). Modern selective breeding programs for  
86 aquaculture species can facilitate the simultaneous selection of multiple traits,  
87 including those not possible to measure directly on selection candidates. Genomic  
88 tools can facilitate this process, allowing for increase in selection accuracy and rate  
89 of genetic gain for target traits, with improved control of inbreeding (Houston 2017).  
90 Further, these tools allow investigation of the genetic architecture of key production

91 traits, opening up possibilities for downstream functional studies to discover genes  
92 contributing directly to genetic variation. Putative QTL affecting host resistance to  
93 OsHV-1 have been identified using a linkage mapping approach (Sauvage *et al.*  
94 2010), but genome-wide association approaches have not previously been  
95 performed in oysters and offer a substantially higher marker density and mapping  
96 resolution.

97 SNP arrays are enabling tools for genetic analysis and improvement of  
98 complex traits in farmed animal species. In the past few years, many genomic  
99 resources have been developed for *C. gigas* and include a reference genome  
100 assembly (Zhang *et al.* 2012), and a moderate number of genetic markers, such as  
101 microsatellites (Li *et al.* 2003; Sekino *et al.* 2003; Sauvage *et al.* 2009) and SNPs  
102 (Fleury *et al.* 2009; Sauvage *et al.* 2007; Wang *et al.* 2015). Additionally, low to  
103 medium density linkage maps have been developed, containing both microsatellites  
104 and SNPs (Li and Guo 2004; Sauvage *et al.* 2010; Hedgecock *et al.* 2015; Hubert  
105 and Hedgecock 2004). Importantly, the recent development of medium and high  
106 density SNP arrays for oysters (Gutierrez *et al.* 2017; Qi *et al.* 2017) raises the  
107 possibility of rapidly collecting genotype data for many thousands of SNP markers  
108 dispersed throughout the genome. These tools therefore facilitate development of  
109 high density linkage maps and high resolution genome-wide association studies into  
110 the genetic architecture of traits of economic interest. In addition, such genome-wide  
111 genotyping platforms enable testing of genomic selection approaches which are  
112 increasingly common in aquaculture breeding, with encouraging empirical data  
113 supporting the advantage over pedigree-based approaches (Tsai *et al.* 2015; Tsai *et*  
114 *al.* 2016; Vallejo *et al.* 2017; Dou *et al.* 2016; Correa *et al.* 2017).

115           The aim of this study was to investigate the genetic architecture of resistance  
116    to OsHV-1 infection in *C. gigas* using a large immersion challenge experiment  
117    followed by a GWAS to identify loci associated with the trait, and the relative  
118    contribution of these loci to the heritability of the trait.

119

120   **Methods**

121   *Source of oysters and disease challenge*

122   Oysters used in this study were obtained from multiple crosses of parents provided  
123   by Guernsey Sea Farms (UK) and reared at Cefas facilities. These comprised three  
124   pair crosses that were created at Cefas (from 3 sires and 2 dams) and each reared  
125   separately, while the rest of the crosses (from 14 sires and 14 dams) were obtained  
126   as spat from Guernsey Sea Farms and combined into a mixed culture tank at Cefas.  
127   Oysters were held at 20 +/- 2 °C during post-settlement and fed with a combination of  
128   *Isocrysis*, *Tetraselmis*, *Chaetoceros*, *Pavlova* sp., and 'Shellfish Diet 1800' until they  
129   reached an appropriate size at approximately eight months of age. A subsample of  
130   approximately 1,000 oysters were then transferred to a new tank at 20 +/- 2 °C for  
131   two days for acclimation. An aliquot of the oyster herpes virus OsHV-1 µvar  
132   (amplified by two passages through *C. gigas*, purified by filtration and whole genome  
133   sequenced for confirmation using Illumina MiSeq technology) was then added to the  
134   water tank at an end concentration of 2.49x10<sup>7</sup> copies /ml (empirically assessed by  
135   qPCR) with continuous flow. The challenge lasted for 21 days, by which time  
136   mortality rate had returned to baseline levels, and mortalities and survivors were  
137   snap-frozen and stored for DNA extraction.

138

139 *Phenotypic measurements*

140 Survival was coded as binary trait i.e. 0 (mortality) or 1 (survival). The viral  
141 count of all samples was determined by qPCR according to (Martenot *et al.* 2010),  
142 with the addition of a plasmid based standard curve cloned for absolute  
143 quantification. The estimated copy number was then divided by the weight of the  
144 animal (mg) to obtain a measure of the viral load. Viral load values were then  
145 normalised by transformation to the logarithmic scale for further analyses.

146 *SNP array genotyping*

147 Genomic DNA was extracted from the whole oyster (minus the shell) using the  
148 RealPure genomic DNA extraction kit (Valencia, Spain), quantified on Qubit and the  
149 DNA integrity was checked on a 1% agarose gel. Genotyping was carried out at  
150 Edinburgh Genomics using the recently developed Affymetrix SNP array for oysters  
151 (Gutierrez *et al.* 2017). After considering available DNA quality and quantity, only  
152 897 samples were retained for genotyping (33 parents + 864 challenged offspring).  
153 After quality control (QC) using the Axiom Analysis Suite v2.0.0.35, 854 samples  
154 were retained following the “best practices workflow” with a sample and SNP call  
155 threshold of 90 resulted in 23,388 SNPs classified as good quality  
156 (‘PolyHighResolution’ and ‘NoMinorHom’ categories), from ~40K putative available  
157 for *C.gigas* on the array, and retained for downstream analyses.

158 *Linkage mapping*

159 Linkage maps were constructed using Lep-map 3 (Rastas 2017). Families used for  
160 the generation of this map were assigned using Cervus (Kalinowski *et al.* 2007) as  
161 described by Gutierrez *et al.* (2017), and further confirmed through the IBD module  
162 in Lep-map3. Putative erroneous or missing parental genotypes were re-called using

163 the “ParentCall2” module. Linkage groups were identified using the  
164 “SeparateChromosomes2” module using a LodLimit=60 and distortionLod=1. Data  
165 were then filtered to remove markers from families showing deviations expected  
166 Mendelian segregation ratios (“dataTolerance=0.001”) and used with the  
167 “OrderMarkers2” module to order the markers in the linkage groups. Individuals  
168 showing excessive recombination were also removed from the data as this indicated  
169 a potential problem with genotyping or family assignment for this individual. The  
170 estimated genome coverage of the map was calculated as  $c = 1 - e^{-2dn/L}$ , where d is  
171 the average spacing of markers, n is the number of markers, and L is the length of  
172 the linkage map (Bishop *et al.* 1983). Only full sibling families were used for the  
173 construction of the linkage maps.

174 *Model and heritability estimation*

175 Genetic parameters for the resistance traits were estimated using a linear mixed  
176 model approach fitting animal as a random effect using ASReml 4 (Gilmour *et al.*  
177 2014) with the following model:

178

179 
$$\mathbf{y} = \mathbf{X} + \mathbf{Z}\mathbf{u} + \mathbf{e}$$

180

181 where  $\mathbf{y}$  is the observed trait,  $\mathbf{u}$  is the vector of additive genetic effects,  $\mathbf{e}$  is the  
182 residual error, and  $\mathbf{X}$  and  $\mathbf{Z}$  the corresponding incidence matrices for fixed effects  
183 and additive effects, respectively. The (co)variance structure for the genetic effect  
184 was calculated either using pedigree (**A**) or genomic (**G**) matrices (i.e.  $\mathbf{u} \sim N(0, \mathbf{A}\sigma_a^2)$   
185 or  $N(0, \mathbf{G}\sigma_a^2)$ ), where  $\mathbf{G}$  is the genomic kinship matrix and  $\sigma^2$  is the genetic variance.

186 Hence, the narrow sense heritability was estimated by the additive genetic variance  
187 and total phenotypic variance, as follows:

188

189 
$$h^2 = \sigma^2_a / \sigma^2_p$$

190

191 where  $\sigma^2_a$  is the additive genetic variance and  $\sigma^2_p$  is the total phenotypic variance  
192 which is a sum of  $\sigma^2_a + \sigma^2_e$ . Heritability on the observed binary scale obtained for  
193 survival was converted to the underlying liability scale according to Dempster and  
194 Lerner (1950). The genomic relationship matrix required for the analysis was  
195 obtained according to (VanRaden 2008) using the GenABEL package (Aulchenko *et*  
196 *al.* 2007) and inverted using a standard 'R' function.

197 *Genome-wide association studies*

198 The GWAS was performed using the GenABEL package (Aulchenko *et al.* 2007) in  
199 R. The genotype data were filtered as part of quality control by using the  
200 *check.markers* module to retain SNPs with a MAF > 0.01, call rate >0.90 and allow a  
201 deviation from Hardy-Weinberg Equilibrium  $< 1 \times 10^{-5}$ , leaving 16,223 filtered SNPs  
202 for downstream analyses. Association analyses were run using the family-based  
203 score test for association (FASTA) using the mmscore function (Chen and Abecasis  
204 2007) with the mixed linear model (MLM) approach used to avoid potential false  
205 positive associations derived from population structure. Genotype data were used to  
206 calculate the genomic kinship matrix which was fitted in the model alongside the top  
207 four principal components as covariates to account for population structure.  
208 Additionally, the GWAS was run using the Efficient Mixed-Model Association  
209 eXpedited (EMMAX) software (Kang *et al.* 2010) to perform a form of validation test

210 for SNPs identified as significant in the GenABEL analysis. The genome-wide  
211 significance threshold was set to  $3.08 \times 10^{-6}$  as determined by Bonferroni correction  
212 ( $0.05 / N$ ), where N represents the number of QC-filtered SNPs across the genome,  
213 while the suggestive threshold was set as  $3.08 \times 10^{-5}$  ( $0.5/N$ ), i.e. allowing 0.5 false  
214 positive per genome scan.

215 *Identification of candidate genes*

216 To identify candidate genes potentially underlying the identified QTL for further  
217 study, the location of the most significant SNPs on individual contigs and scaffolds  
218 was recorded on the *C. gigas* genome v9 assembly (GCA\_000297895.1) (Zhang *et*  
219 *al.* 2012). The sequences of these scaffolds / contigs were then aligned (using a  
220 custom-built blastn database) with the *C. gigas* gene annotation database. Contig  
221 and scaffold sequences for significant SNPs were also aligned using blastn and  
222 blastx (using non-redundant protein sequences) from the NCBI database.

223 *Data availability*

224 Linkage map including all mapped markers and their position is given in File S1.  
225 Genotype data corresponding to all informative markers for all the individuals  
226 involved in this study is given in File S5.

227 **Results**

228 *Challenge outcome and trait heritability*

229 At the end of the 21 day disease challenge, 749 oysters had survived while 251 had  
230 died during the experiment. From the latter, 71 oysters had no body tissue at the

231 moment of their removal, leaving 181 mortalities suitable for downstream analyses.

232 Therefore, overall mortality was approximately 25 %, but in the subset of oysters

233 available for genotyping the mortality was ~18%.

234 A total of 23 full sibling families were identified using the family assignment software.

235 The largest comprised 231 individuals, and the smallest only two individuals. The

236 vast majority of offspring were assigned to a unique parent pair, but a total of seven

237 individuals were assigned to only one parent (five only to a dam and two only to a

238 sire). Making use of the pedigree information, the estimated heritability on observed

239 scale was 0.13 (0.06), corresponding to a value of 0.25 on the underlying liability

240 scale (Table 1). These estimates were slightly lower when using the genomic kinship

241 matrix, with  $0.08 \pm 0.03$  and 0.17 for the observed and liability scale respectively. For

242 viral load, heritability based on pedigree was estimated at  $0.19 \pm 0.08$  and  $0.13 \pm$

243 0.05 for genomic matrix. (Table 1).

244 Table 1. Estimated heritabilities for survival and viral load in challenged populations.

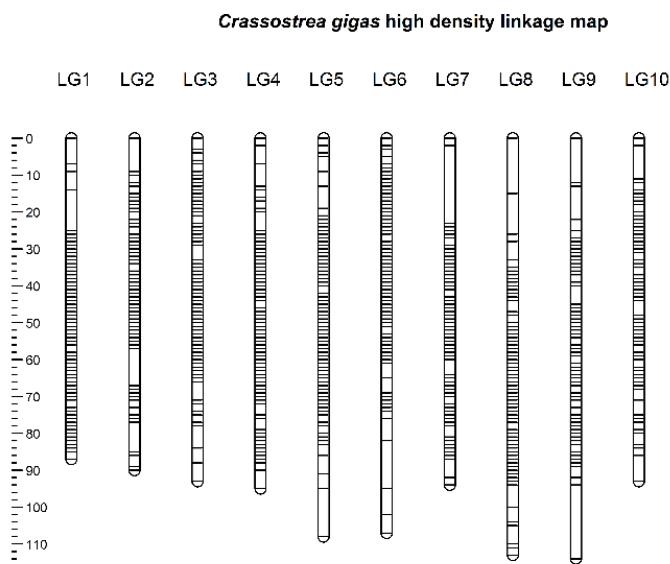
<b>Trait</b>	<b>Method</b>	<b>Heritability (s.e)</b>
Survival	Observed binary scale (G)	0.078 (0.037)
	Underlying liability scale (G)	0.168
	Observed binary scale (P)	0.13 (0.058)
	Underlying liability scale (P)	0.25
Viral load	Log transformed viral load(G)	0.127 (0.05)
	Log transformed viral load (P)	0.19 (0.08)

245

246 *Linkage map*

247 The linkage mapping was performed using the 23 full sibling families comprising 809  
248 progenies and 31 parents. On average 10% of the markers showed evidence of  
249 segregation distortion ( $p < 0.001$ ) in at least one family with at least ten progenies,  
250 leaving 21,087 maternally informative markers and 20,528 paternally informative  
251 markers for map construction (Table S1).

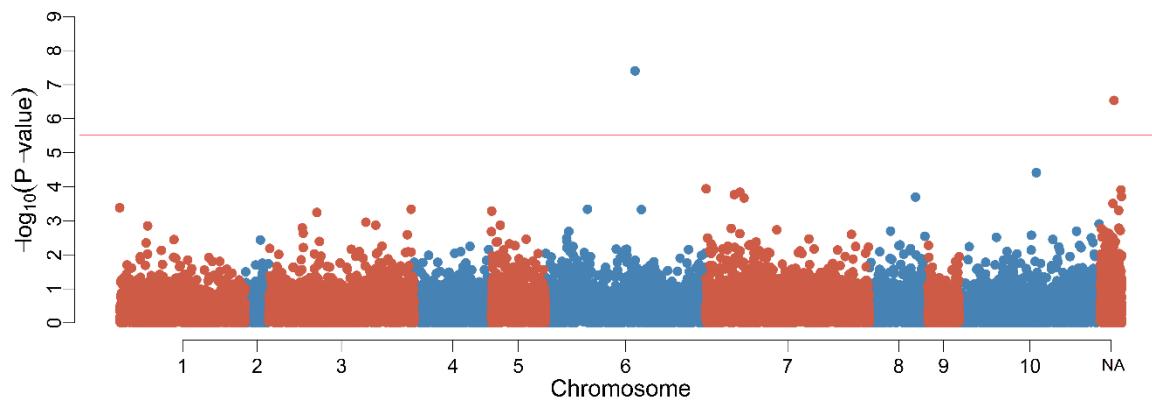
252 The linkage map contains 20,353 SNPs distributed on 10 LGs (in accordance with  
253 the *C. gigas* karyotype) as shown in Figure 1, with a length of 951 cM for the male  
254 map and 994 cM for the female map. The ~20K mapped SNPs correspond to 1,921  
255 scaffolds and 149 contigs, according to the latest oyster genome assembly  
256 (GCA\_000297895.1, Zhang *et al.* 2012, File S1). These scaffolds and contigs  
257 containing mapped SNPs covered approximately 87% of the reference genome  
258 length.



259

260 Figure 1. Distribution of SNP markers on the linkage map.

261 Linkage groups were labelled according to Hedgecock *et al.* (2015) to keep  
262 consistency across *C.gigas* linkage maps. Our medium density oyster array contains  
263 464 of the SNPs mapped by Hedgecock *et al.* (2015). From these, 307 were mapped  
264 in the current study and their new linkage group assignment fully agrees with their  
265 previous assignment (File S2). Likewise, we observed that approximately 38 % (734  
266 out 1,921) of the scaffolds with informative markers show evidence of errors in the  
267 assembly, due to assignment to at least two distinct LGs in our map (File S3). As  
268 expected, the number of LGs associated with scaffolds was positively correlated with  
269 scaffold length (Figure S1).



270

271 Figure 2. Manhattan plot the GWAS for survival. The position of the SNPs on the X  
272 axis is calculated according to the linkage map. "NA" represent a chromosome that  
273 contains markers not assigned to any linkage group. Horizontal red line indicates the  
274 genome-wide significance threshold.

## 275 *Association analyses*

276 The GWAS for the binary survival trait using the FASTA approach identified two  
277 markers showing a genome-wide significant association with the trait (both also

278 significant using EMMAX, with an additional two SNPs significant using EMMAX  
279 only), as shown in Table 2, Figure 2 and File S4. Of the ten markers showing the  
280 most significant association in the two approaches, four markers are linked to LG 6  
281 but they do not map to the same scaffold, nor are they close together on the linkage  
282 map. The proportion of phenotypic variation explained by the top ten markers ranged  
283 between 0.019 and 0.047, which implies a polygenic architecture to host resistance,  
284 albeit the LG 6 QTL potentially explains a large proportion of the genetic variance  
285 given the low heritability estimates.

286 Table 2. The top ten markers associated with survival.

SNP ID	LG (position cM)	LG nearest marker (position cM)	Scaffold (position bp)	A1	A2	GenABEL	EMMAX	PVE	Nearest Gene
AX-169184215	LG 6 (42.46)	-	scaffold241 (824,662)	T	G	3.94E-08*	4.74E-10*	0.0473	CORO1B
AX-169192574	Unassigned	LG 6 (54.61)	scaffold1827 (350,776)	A	G	2.91E-07*	7.79E-08*	0.0411	MYO10
AX-169208860	Unassigned	LG 1 (54.37)	scaffold714 (58,763)	G	A	0.000124	5.72E-07*	0.0224	CYP1A1
AX-169209993	LG 7 (9.48)	-	scaffold1599 (493,016)	T	C	0.000115	1.56E-06*	0.0231	D2R
AX-169207075	LG 5 (47.54)	-	scaffold57 (142,065)	C	T	0.004125	1.09E-05	0.0122	IFT88
AX-169210119	Unassigned	LG 6 (29.41)	scaffold198 (583,825)	T	C	0.000194	2.25E-05	0.0206	RANBPM
AX-165319118	LG 5 (25.77)	-	scaffold43494 (138,038)	G	A	0.000519	4.82E-05	0.019	KPNA1
AX-169158711	LG 6 (42.59)	-	scaffold109 (558,765)	G	A	0.000468	6.48E-05	0.0183	CASP
AX-169199571	LG 10 (42.24)	-	scaffold186 (320,367)	C	T	3.85E-05	7.02E-05	0.0247	AP1AR
AX-169168346	LG 3 (43.83)	-	scaffold1785 (251356)	G	T	0.000568	8.37E-05	0.018	KIF6

287 \* Genome-wide significant ( $p < 0.05$ ) markers. A1 & A2, major and minor allele. PVE, phenotypic  
288 variation explained by the SNP. The physical position of the SNPs on the Scaffolds are given  
289 according to the Pacific oyster reference assembly (Genbank ID GCA\_000297895.1).

290 The GWAS for the trait of viral load detected two markers showing significant  
291 genome-wide association with both FASTA and EMMAX, with an addition eight  
292 SNPs identified as significant using EMMAX only (Table 3, Figure S2 and File S4).  
293 The SNP showing the most significant association is located in LG 8, however, no  
294 other markers are located in the same LG. While most of the markers significantly  
295 associated with the trait were not mapped, the nearest mapped SNPs according to

296 their position on the genome scaffolds suggests that three SNPs are located on LG  
297 6. Therefore, it is plausible that there is at least one QTL on LG 6, and this QTL may  
298 affect both viral load and the binary trait of survival. The proportion of phenotypic  
299 variation in viral load explained by the top ten markers ranged between 0.0209 and  
300 0.037.

301 **Table 3. Top ten markers associated with viral load.**

SNP ID	LG (position cM)	LG nearest marker (position cM)	Scaffold (position bp)	A1	A2	GenABEL	EMMAX	PVE	Gene
AX-169203956	LG 8 (0)	-	scaffold501 (742,989)	C	T	6.54E-07*	3.47E-09*	0.037	FBN2
AX-169210119	Unassigned	LG 6 (29.41)	scaffold198 (583,825)	T	C	9.09E-07*	9.33E-08*	0.0349	RANBPM
AX-169172429	Unassigned	LG 4 (57.01)	scaffold713 (269,794)	T	G	3.72E-06	9.36E-07*	0.0314	B3GALNT2
AX-169192982	Unassigned	LG 6 (43.46)	scaffold1093 (208,087)	C	T	9.61E-06	1.54E-07*	0.0284	SKI
AX-169167580	Unassigned	LG 6 (36.95)	scaffold1763 (82,048)	A	G	1.65E-05	8.91E-07*	0.0277	CLEC16A
AX-169199878	Unassigned	LG 8 (71.91)	scaffold536 (135,453)	T	C	2.11E-05	1.42E-06*	0.0269	TNR
AX-169203386	Unassigned	LG 10 (52.04)	scaffold1301 (188,626)	C	T	2.27E-05	1.13E-06*	0.0266	RAPGEF2
AX-169196070	Unassigned	LG 1 (57.32)	scaffold433 (1,082,890)	G	A	3.62E-05	1.69E-06*	0.0261	CARS
AX-169199276	LG 7 (6.68)	-	scaffold128 (550,765)	G	T	3.70E-05	8.44E-07*	0.0243	SMARCA5
AX-169194053	LG 4 (19.39)	-	scaffold728 (174,857)	G	T	0.000148	2.22E-06*	0.0209	TNIK
AX-169193982	LG 1 (47.02)	-	scaffold41452 (35,018)	A	G	6.79E-05	6.03E-06	0.0243	U/P <sup>a</sup>
AX-169192459	Unassigned	LG 1 (22.66)	scaffold447 (373,487)	G	A	7.28E-05	9.00E-06	0.0232	SCARF2

302 \* Genome-wide significant (p<0.05) markers. A1 & A2, major and minor allele. PVE, phenotypic  
303 variation explained by the SNP. <sup>a</sup> U/P indicates uncharacterized protein. The physical position of the  
304 SNPs on the Scaffolds are given according to the Pacific oyster reference assembly (Genbank ID  
305 GCA\_000297895.1).

## 306 **Discussion**

### 307 **Heritability of OsHV-1 resistance**

308 Estimates of heritability observed for survival to OsHV-1 challenge in the current  
309 study were low to moderate (0.078-0.25) in comparison to other recent studies that  
310 have analysed resistance to OsHV-1, where estimates have ranged from 0.21 to  
311 0.63 (Dégremont *et al.* 2015a; Azéma *et al.* 2017; Camara *et al.* 2017). Mortality  
312 resulting from OsHV-1 exposure in our challenge was relatively low, reaching ~ 25%

313 in the overall challenge. The mortality level in the genotyped samples was lower  
314 (~18%), although it is not clear if the dead oysters found with no tissue were affected  
315 by the virus or were abnormal at the time of the exposure. It is possible that the  
316 population studied may have high level of innate resistance to OsHV-1, considering  
317 the low mortality level in ~8 month old oysters compared to the mortalities typically  
318 observed due to OsHV-1 exposure in spat and juvenile oysters (Azéma *et al.* 2017).  
319 Oysters from these families also showed lower mortality levels compared to other  
320 batches of oyster spat when using a more established single animal bath OsHV-1  
321 challenges (data not shown), which would support the possibility of a relatively  
322 resistant sample of animals.

323 ***Linkage map***

324 The linkage map construction resulted in 10 linkage groups that correspond to the  
325 number of chromosomes of *C. gigas*, successfully mapping ~20K SNPs. The highest  
326 density linkage map for *C. gigas* to date was described by Hedgecock *et al.* (2015)  
327 and contains ~1.1K SNPs and microsatellites. Therefore, the linkage map presented  
328 in the current study is an improvement to existing resources offering an advance for  
329 oyster genomics with potential in assisting future mapping studies, particularly those  
330 using the medium density SNP array.

331 Family assignments were rigorously tested to avoid pedigree errors in the  
332 construction of the linkage maps. Distortions from the expected Mendelian  
333 segregation were found in ~10 % of the SNPs in the larger families ( $p < 0.001$ )  
334 (Table S1). Moderate levels of segregation distortion have been commonly observed  
335 in oysters (Jones *et al.* 2013; Hedgecock *et al.* 2015; Guo *et al.* 2012) and bivalves  
336 in general (Saavedra and Bachère 2006). In the current study, distorted markers

337 were included for the linkage group assignment, but were filtered out for the  
338 determination of the order in the LG. It has been argued that distorted markers can  
339 affect marker ordering, albeit the effect on map construction has been shown to be  
340 minor (Hackett and Broadfoot 2003; Guo *et al.* 2012).

341 A measure of the quality of the linkage map was given by overlap with a previous  
342 linkage map described by Hedgecock *et al.* (2015). Several hundred SNPs were

343 successfully re-mapped to the same LG, indicating correct LG definition.

344 Accordingly, assembly errors observed by Hedgecock *et al.* (2015) were also  
345 observed in our high-density linkage map, where almost ~40% of the mapped  
346 scaffolds were assigned to more than one LG (File S1). This linkage map should be  
347 able to provide a good base for the identification of assembly errors and the potential  
348 re-assembly of the genome, which seems like a requirement to maximise its utility for  
349 future genomics research in this species.

### 350 **GWAS and associated genes**

351 The association analyses for OsHV-1 survival and viral load suggest that both traits  
352 are likely to be impacted by multiple genomic regions, albeit the putative QTL on LG  
353 6 potentially explains a large proportion of the genetic variation. Accordingly, GWAS  
354 for survival found SNPs surpassing the genome-wide threshold on LG 6, and SNPs  
355 surpassing the suggestive threshold on LG 1, LG 5, & LG 7 (Figure 2, Table 2 and  
356 File S4). For the trait of viral load, markers showing a genome-wide significant  
357 association were located in LG 8, LG 6LG 10 & LG 4, and suggestive association  
358 found in LG 1 & LG 7(Table 3 and File S4). The only previously published study  
359 describing genomic regions associated to summer mortality resistance found  
360 significant QTL in LG V, VI, VII & IX (which correspond to LG 6, LG 7, LG 8 & LG 10

361 in our map) in different families (Sauvage *et al.* 2010). It is noteworthy that LG 6  
362 contains genome-wide significant SNPs for both survival and viral load (and was  
363 previously detected by Sauvage *et al.* 2010). In addition, a single SNP (AX-  
364 169210119) reached genome-wide significant level for viral load, and the suggestive  
365 level for survival. While this SNP was not mapped directly, the nearest mapped SNP  
366 was linked to LG 6.

367 Numerous genes were identified from the genomic regions flanking the most  
368 significant SNPs impacting the resistance traits. While the limits defined for  
369 screening flanking regions of significant SNPs were defined practically (i.e. the contig  
370 / scaffold to which the SNP maps), these genes may represent candidates for future  
371 validation, resequencing and functional testing. The SNP showing an association  
372 with both survival and viral load (AX-169210119) was located in the RAN Binding  
373 Protein 9-like gene which has recently been linked to the interferon gamma signalling  
374 pathway (Zhang *et al.* 2017), and also in viral adhesion and its replication in host  
375 cells (Yang *et al.* 2015). Another gene located near a significant SNP (AX-  
376 169184215) is a Coronin gene (CORO1B), from a family of genes that have multi-  
377 faceted roles in immune response (Tokarz-Deptula *et al.* 2017). Finally, the actin  
378 motor protein Myo10 gene is located near AX-169192574, and this gene encodes a  
379 protein which is essential for release of Marburgvirus particles from host cells  
380 (Kolesnikova *et al.* 2007). These and other genes may form the basis for  
381 downstream functional studies to assess their function in host response to virus in  
382 oysters. In addition, validation studies are required in independent populations to  
383 assess the robustness of the observed association between the significant SNPs  
384 and OsHV-1 resistance in oysters. Further, from a practical breeding perspective,

385 these SNPs may have potential for marker-assisted or genomic selection to improve  
386 host resistance in farmed oyster populations.

387 **Conclusion**

388 This study is the first to report GWAS using the a high density SNP panel Pacific  
389 oysters, and was enabled by the recent development of a SNP array (Gutierrez *et al.*  
390 2017). Heritability of resistance to OsHV-1 in oysters was significant, but low to  
391 moderate in magnitude. The fact that this heritability was detected using both the  
392 pedigree and genomic relationship matrix implies that selective breeding and  
393 genomic selection for resistance is likely to be effective. Using the genotype data, a  
394 high-density linkage map was constructed for *C. gigas*, and the GWAS identified  
395 numerous markers showing a genome-wide significant association with the traits.  
396 The most encouraging QTL was located on LG 6, reaching genome-wide  
397 significance for the binary trait of survival, with some evidence of a significant  
398 association with viral load. Future analyses will test candidate genes identified by the  
399 GWAS, verify trait-associated SNPs in independent populations, and test genomic  
400 selection as a tool to enhance host resistance to this problematic pathogen for oyster  
401 aquaculture.

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