

1 **Single-step genome-wide association study for resistance to *Piscirickettsia salmonis* in**  
2 **rainbow trout (*Oncorhynchus mykiss*)**

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24 **GWAS for *Piscirickettsia salmonis* resistance in rainbow trout**

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## ABSTRACT

45 One of the main pathogens affecting rainbow trout (*Oncorhynchus mykiss*) farming is the  
46 facultative intracellular bacteria *Piscirickettsia salmonis*. Current treatments, such as  
47 antibiotics and vaccines, have not had the expected effectiveness in field conditions. Genetic  
48 improvement by means of selection for resistance is proposed as a viable alternative for  
49 control. Genomic information can be used to identify the genomic regions associated with  
50 resistance and enhance the genetic evaluation methods to speed up the genetic improvement  
51 for the trait. The objectives of this study were to i) identify the genomic regions associated  
52 with resistance to *P. salmonis*; and ii) identify candidate genes associated with the trait. We  
53 experimentally challenged 2,130 rainbow trout with *P. salmonis* and genotyped them with a  
54 57 K SNP array. Resistance to *P. salmonis* was defined as time to death (TD) and as binary  
55 survival (BS). Significant heritabilities were estimated for TD and BS ( $0.48 \pm 0.04$  and  $0.34$   
56  $\pm 0.04$ , respectively). A total of 2,047 fish and 26,068 SNPs passed quality control for  
57 samples and genotypes. Using a single-step genome wide association analysis (ssGWAS) we  
58 identified four genomic regions explaining over 1% of the genetic variance for TD and three  
59 for BS. Interestingly, the same genomic region located on *Omy27* was found to explain the  
60 highest proportion of genetic variance for both traits (2.4 and 1.5% for TD and BS,  
61 respectively). The identified SNP in this region is located within an exon of a gene related  
62 with actin cytoskeletal organization, a protein exploited by *P. salmonis* during infection.  
63 Other important candidate genes identified are related with innate immune response and  
64 oxidative stress. The moderate heritability values estimated in the present study show it is  
65 possible to improve resistance to *P. salmonis* through artificial selection in the current  
66 rainbow trout population. Furthermore, our results suggest a polygenic genetic architecture

67 and provide novel insights into the candidate genes underpinning resistance to *P. salmonis*  
68 in *O. mykiss*.

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## INTRODUCTION

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As in any intensive animal production system, infectious diseases are one of the main threats affecting the success and sustainability of aquaculture (Yáñez *et al.* 2014a). In the case of salmonid production, one of the major pathogens affecting productivity is the facultative intracellular bacteria *Piscirickettsia salmonis*, etiological agent of salmonid rickettsial syndrome (SRS). This bacterium was first identified in 1989 in Chile, in a farmed coho salmon (*Oncorhynchus kisutch*) population (Cvitanich *et al.* 1991). Since then, mortalities resulting from SRS have been also identified in Atlantic salmon (*Salmo salar*) and rainbow trout (*Oncorhynchus mykiss*) in several countries, such as Scotland, Ireland, Norway and Chile (Fryer and Hedrick 2003). In Chile, SRS was responsible for 20.7, 67.9 and 92.6% of the mortalities associated with infectious diseases in *S. salar*, *O. kisutch* and *O. mykiss*, species respectively (Sernapesca 2018). To date, strategies for *P. salmonis* control and treatment are mainly based on vaccines and antibiotics. The effectiveness of both approaches has not been adequate (Rozas and Enríquez 2014). Therefore, it has been estimated that economic losses due SRS mortalities, reached up to US\$450 million in Chile in 2012 (Camusetti *et al.* 2015). However, variables such as laboratory diagnosis screening expenses or loss of quality of the harvested fish and products were not considered, implying that the economic impact could be even higher.

90           Therefore, selective breeding could be a feasible alternative to enhance disease  
91 resistance; reducing mortality rates from *P. salmonis*, as well as improving animal health and  
92 productivity (Bishop and Woolliams 2014; Yáñez and Martínez 2010). However, the main  
93 requisite to include a trait into a genetic program is the presence of significant additive  
94 genetic variance within the population (Falconer and Mackay 1996). Previous studies  
95 estimated heritability values ranging from 0.11 to 0.41 for *P. salmonis* resistance in Atlantic  
96 salmon and coho salmon (Yáñez *et al.* 2013; Yáñez *et al.* 2016a; Barría *et al.* 2018). In the  
97 case of rainbow trout, Yoshida *et al.* (2018a) estimated heritabilities ranging from 0.39 to  
98 0.57 for resistance to *P. salmonis* using day of death and 0.54 to 0.62 for binary survival as  
99 trait definitions. Altogether, these results demonstrate the possibility of improving this trait  
100 by means of artificial selection in different salmonid species.

101           The development of next generation sequencing technologies has facilitated the  
102 identification of thousands of single nucleotide polymorphisms (SNPs) segregating along the  
103 genome of several animals, including aquaculture species (Yáñez *et al.* 2015). Thus, using a  
104 genotyping by sequencing (GBS) approach in conjunction with genome-wide association  
105 studies, some authors evaluated genomic regions associated with resistance to bacterial  
106 infections in aquaculture species (Liu *et al.* 2015; Palti *et al.* 2015a; Palaiokostas *et al.* 2016;  
107 Barría *et al.* 2018). However, in salmonid species, the use of SNP panels has been the most  
108 used alternative for genotyping a high number of individuals with thousands of genetic  
109 variants simultaneously. This has been made simpler by the development of high density  
110 SNP arrays for Atlantic salmon (Houston *et al.* 2014; Yáñez *et al.* 2016b) and rainbow trout  
111 (Palti *et al.* 2015b). The use of these SNP panels have also allowed the comparison of the  
112 accuracy of estimated breeding values (EBV) using genomic selection to pedigree-based  
113 genetic evaluations for resistance to infectious diseases in Atlantic salmon (Ødegård *et al.*

114 2014; Tsai *et al.* 2016; Bangera *et al.* 2017; Correa *et al.* 2017), coho salmon (Barría *et al.*  
115 2018) and rainbow trout (Vallejo *et al.* 2016; Vallejo *et al.* 2017a; Yoshida *et al.* 2018a;  
116 2018b). SNP arrays have also enabled the dissection of the genetic architecture of resistance  
117 to bacterial diseases in salmonids. For instance, genomic regions and candidate genes  
118 associated with resistance to *P. salmonis* in Atlantic and coho salmon (Correa *et al.* 2015;  
119 Barría *et al.* 2018), and bacterial cold water disease (BCWD) in rainbow trout (Vallejo *et al.*  
120 2017b) have been identified.

121 To date there are no studies aimed at identifying genomic regions or candidate genes  
122 associated with resistance to *P. salmonis* in rainbow trout populations. Therefore, the main  
123 objectives of the current study were to i) identify genomic regions associated with resistance  
124 to *P. salmonis* in a farmed rainbow trout population, and ii) identify candidate genes  
125 associated with the trait.

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## MATERIALS AND METHODS

### 128 **Population and experimental challenge**

129 The population used in this study was rainbow trout (*Oncorhynchus mykiss*) year-  
130 class 2011 bloodstock, owned by Aguas Claras (Puerto Montt, Chile) and belonging to a  
131 genetic improvement program run by Aquainnovo (Puerto Montt, Chile). This population  
132 was artificially selected for growth, appearance-related traits and carcass quality for three  
133 generations. For a detailed description about rearing conditions and population management  
134 please see Flores-Mara *et al.* (2017), Rodríguez *et al.* (2018) and Neto *et al.* (2019)

135 Fish from 105 full-sib families (48 half-sib families) with an average weight of  $7.0 \pm$   
136 1.5 g, were PIT-tagged for individual traceability of families. After tagging, fish were

137 maintained in a single tank until they were transferred to Aquainnovo's Aquaculture  
138 Technology Center Patagonia in August 2012. Fish were acclimated for 20 days in a 15m<sup>3</sup>  
139 tank, prior to experimental challenge. A random sample of fish were selected to evaluate the  
140 sanitary status of the population, i.e. qRT-PCR for Infectious Salmon Anemia virus (ISAV),  
141 Infectious Pancreatic Necrosis virus (IPNV), and *Renibacterium salmoninarum*, and culture  
142 for *Flavobacterium spp.* Later, a total of 2,130 juveniles (with an average of 23 individuals  
143 per family and ranging from 17 to 27 fish per family), were intraperitoneally (IP) injected  
144 with 0.2ml of a lethal dose (LD<sub>50</sub>) of the LF-89 strain of *P. salmonis* inoculum. Post injection,  
145 fish were equally distributed into three different tanks, considering similar family distribution  
146 into each replicate (with 5 to 9 fish per family in each tank). Environmental parameters were  
147 measured throughout the challenge and the experimental challenge continued until the  
148 mortality curve showed a plateau. Daily mortality was recorded, and body weight was  
149 measured for each fish at time of death or at the end of the experiment (FW). Surviving fish  
150 were euthanized and body weight was also recorded. Fin clips from all fish were sampled  
151 and stored in 95% ethanol at -80°C until they were genotyped.

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### 153 **Genotyping**

154 The genomic DNA from the sampled fin clips was extracted using a commercial kit  
155 (DNeasy Blood & Tissue Kit, Qiagen), following the manufacturer's instructions.  
156 Genotyping was performed using a commercial 57K SNP array (Affymetrix® Axiom®  
157 myDesign™ SNP) developed by the National Center for Cool and Cold water Aquaculture  
158 at USDA (Palti *et al.* 2015b).

159 Quality control (QC) was assessed through Affymetrix's Axiom Analysis Software,  
160 using default settings. Then, a second QC using Plink software (Purcell *et al.* 2007), was

161 applied to remove SNPs with a genotype call rate lower than 0.90, minor allele frequency  
162 (MAF) < 0.01 and deviated from Hardy-Weinberg Equilibrium ( $p < 1 \times 10^{-6}$ ). Individuals with  
163 a call rate lower than 0.90 were also removed from further analyses.

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#### 165 **Trait definition**

166 Resistance to *P. salmonis* was defined as time to death (TD), measured in days, with  
167 values ranging from 1 until the end of challenge test. Additionally, resistance to *P. salmonis*  
168 was also defined as binary survival (BS), with a value of 1 or 0 based on if the fish died or  
169 survived until the end of the challenge.

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#### 171 **Genomic-Wide association study**

172 A single-step GWAS (ssGWAS) analysis was performed to identify genomic regions  
173 associated with resistance to *P. salmonis*. This approach considered fish with both  
174 phenotypes and genotypes and also individuals with phenotypes but no genotypes in the  
175 analysis (Wang *et al.* 2012). The pedigree and genotypic data in ssGWAS are connected  
176 through the H matrix. Thus, the H matrix combines both the pedigree and the genomic  
177 relationship matrices (Aguilar *et al.* 2010). Thus, the inverse of the H matrix is:

$$178 \quad \mathbf{H}^{-1} = \mathbf{A}^{-1} + \begin{bmatrix} 0 & 0 \\ 0 & \mathbf{G}^{-1} - \mathbf{A}_{22}^{-1} \end{bmatrix}$$

179 Where  $\mathbf{A}^{-1}$  is the inverse of the numerator relationship matrix, considering all the phenotyped  
180 animals,  $\mathbf{A}_{22}^{-1}$  is the inverse of the pedigree-based relationship matrix considering only the  
181 genotyped animals, and  $\mathbf{G}^{-1}$  is the inverse genomic relationship matrix. The following model  
182 was used for GWAS analysis:



183 
$$y = \mathbf{X}\beta + \mathbf{Z}a + e$$

184 Where  $y$  is the vector of phenotypes (for TS and BS),  $\beta$  is the vector of fixed effects (tank as  
185 factor and final body weight as a covariate),  $a$  is the vector of random effects,  $e$  is the vector  
186 of residuals, and  $\mathbf{X}$  and  $\mathbf{Z}$  are the incidence matrices for fixed and random effects,  
187 respectively. A linear model and a threshold model were used for TD and BS, respectively.  
188 Both trait definitions were fitted using BLUPF90 statistical software (Misztal *et al.* 2016).  
189 Thus, AIREML and THRGIBBS1F90 were used for TD and BS, respectively. For the latter,  
190 a total of 200,000 Markov Chain Monte Carlo (MCMC) iterations were used, the first 20,000  
191 were discarded as burn-in iterations and from the remaining 180,000 samples, we saved one  
192 from every 50. Therefore, the analyses included 3,600 independent samples.

193 For TS and BS, the heritability was estimated as follows:

194 
$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_e^2}$$

195 Where  $\sigma_a^2$  is the additive genetic variance estimated using the H matrix, and  $\sigma_e^2$  is the residual  
196 variance.

197 To identify genomic regions associated with each trait, we estimated the percentage  
198 of the genetic variance (PGV) explained by windows of 20 adjacent SNPs. Then, if a 20 SNP  
199 window explained more than 1% of the PGV, we considered that region as associated with  
200 resistance to *P. salmonis*.

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202 **Candidate genes**

203           The candidate genes were identified by searching 500kb up and downstream from the  
204 SNP explaining the highest proportion of PGV within each associated window. For this  
205 purpose, we used the last version of the *Oncorhynchus mykiss* reference genome  
206 (GCA\_002163495.1). The criteria for selecting candidate genes lies in the function of the  
207 protein that encodes each gene found, mainly related to immune response, DNA repair, stress  
208 response and similar pathways.

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#### 210 **Data availability**

211           The raw genotypes and phenotype data are available from the online repository  
212 figshare (<https://figshare.com/s/221a39319b236d46f9fc>). Table S1 contains all genes located  
213 within 1Mb window surrounding the SNPs explaining the highest proportion of genetic  
214 variance and is available at [10.6084/m9.figshare.7883342](https://doi.org/10.6084/m9.figshare.7883342).

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## RESULTS

#### 220 **Descriptive statistics and heritabilities**

221           Summary statistics for resistance to *P. salmonis* measured as TD and as BS and for  
222 FW are shown in Table 1. The first death was recorded on day 10 post intraperitoneal  
223 injection; the last on day 32. Average TD was  $23.26 \pm 7.86$  days. At the end of the  
224 experimental challenge the proportion of non-survivor fish was  $0.59 \pm 0.49$ . Cumulative

225 mortality among all 105 families ranged from 7.7 to 100%, indicating considerable  
226 phenotypic variation for resistance to *P. salmonis* in the current rainbow trout population.  
227 Cumulative mortality within each replicate tank was 59.4, 65.1 and 64.7%. Mortality peaked  
228 on days 12, 15 and 19 post injection. Average final body weight was  $173.80 \pm 52.27$  g. This  
229 trait ranged considerably among challenged fish, with a minimum of 46.10g and maximum  
230 448g.

231 Variance components for TD and BS are shown in Table 2. Significant heritability  
232 values were estimated for both trait definitions. Thus,  $0.48 \pm 0.04$  and  $0.34 \pm 0.04$  were  
233 estimated for TD and BS, respectively. Furthermore, a high genetic correlation was found  
234 between both traits ( $-0.96 \pm 0.01$ ).

235

### 236 **Genome-wide association study**

237 From all genotyped animals, 2,047 passed quality control (representing 97.10% of the  
238 total). A total of 26,068 SNPs remained in the set for further analyses (~ 64.68%). The Figure  
239 1 shows the Manhattan plot for resistance to *P. salmonis* measured as TD and BS. We  
240 identified four genomic regions associated with resistance as TD. These regions were located  
241 on *Omy3*, *Omy14*, *Omy24* and *Omy27*. For BS, we identified three genomic regions  
242 associated with the trait. These were found on *Omy5*, *Omy27* and *Omy30*. Interestingly, the  
243 genomic region located on *Omy27* was found to be associated with resistance to *P. salmonis*  
244 for both TD and BS. In both cases, this common genomic region explains the highest  
245 proportion of genetic variance for each trait, with 2.4 and 1.5% for TD and BS, respectively.  
246 The SNP explaining the highest proportion of the genetic variance (Affx-88923370) is the  
247 same for both TD and BS (Table 3).

248 Using the *O. mykiss* reference genome (GCA\_002163495) we identified candidate  
249 genes associated with resistance to *P. salmonis*. Table 3 shows a summary of the genes  
250 located proximate to the SNPs explaining the highest proportion of the genetic variance  
251 within each genomic region.

252 Among the candidate genes flanking the most important SNP on *Omy3* for TD, we  
253 found *Gluthatione S-transferease kappa 1 (gstk1)* and Interleukin-11 (*ill1*). These genes are  
254 involved in the response to oxidative stress and the immune response to bacterial infections,  
255 respectively (Oruc *et al.* 2004; Wang *et al.* 2005). On *Omy14*, we found the *Toll-like receptor*  
256 *4 (tlr4)* gene, which has been suggested to act as a bacteria sensor (Palti 2011). On *Omy24*,  
257 we found *alpha-2-macroglobulin-like (a2m)*, which is part of a broad-spectrum protease  
258 inhibitor, and it has been suggested that plays a role in the defense against *Cryptobia*  
259 *salmositica* on rainbow trout (Zuo and Woo 1997).

260 Also, we found *POU class 2 associating factor 1 (pou2af1)*, which has been described  
261 as a coactivator of transcription factors that regulate Ig expression of B cells (Teitell 2003),  
262 and *NF-kappa-B inhibitor zeta-like (nfkbiz)*, a regulator of process like the pathogen  
263 recognition, phagocytosis and production of cytokines by dendritic cells (Rozas-Serri *et al.*  
264 2017).

265 For BS, on *Omy5* we found *fas ligand (faslg)*, whose protein has been suggested as  
266 an important mediator of anti-bacterial innate immune response, by inducing apoptosis of  
267 target cells and recruiting phagocytic cells (Kaur *et al.* 2004). On the same chromosome we  
268 found *Peroxiredoxin-6-like (prdx6)*, one of the six different isoforms that conforms the  
269 peroxiredoxins group, which are antioxidants proteins that protect cells from oxidative  
270 damage and is likely to be involved in protective response against a bacterial infection in  
271 *Scophthalmus maximus* (Zheng *et al.* 2010).

272 On *Omy29*, *MAPK12* was found; previous studies described that *MAPK12* is  
273 involved on the signaling pathways responsible for  $TNF-\alpha$  secretion from rainbow trout  
274 macrophages, there for in innate immunity (Roher *et al.* 2011). *Glutaminase kidney isoform*,  
275 *mitochondrial-like (gls)* was also found on *Omy29*, which family proteins, generally forms a  
276 part of enzymes that plays a role in nucleotide, amino acid and urea biosynthesis (Kumada *et*  
277 *al.* 1993).

278 On *Omy27* we found genes related with innate immune response regulation, *NF-kB*  
279 activation by *TNF $\alpha$* , and some molecules related with metabolic process and apoptosis.  
280 However, the SNP explaining the highest proportion of genetic variance is located within an  
281 exon of the gene *Smoothelin protein 2 (Smtnl2)* which remains poorly characterized both in  
282 humans and fishes, but it is believed that participates in actin cytoskeleton organization.

283 The complete list of genes located within the 1Mb window flanking the SNPs  
284 explaining the highest proportion of genetic variance, within each genomic region associated  
285 with resistance to *P. salmonis*, is shown in Table S1.

286

## 287 DISCUSSION

288 In the current study we show significant genetic variation for resistance to *P. salmonis*  
289 in a farmed rainbow trout population. A moderate to high heritability was estimated for  
290 resistance as TD (0.48) and BS (0.34). These estimates are higher than those reported in  
291 previous studies carried out for resistance to other bacterial diseases in aquaculture species,  
292 with heritabilities ranging from 0.22 to 0.38 (Ødegård *et al.* 2006; Palaiokostas *et al.* 2016;  
293 Vallejo, *et al.* 2017b). In the case of *P. salmonis* resistance, several studies have evaluated  
294 the presence of genetic variation in different salmonid species. Thus, similar estimates have

295 been shown for Atlantic salmon, when using pedigree or genomic data, with values ranging  
296 from 0.19 to 0.39 (Yáñez *et al.* 2013; Yáñez, *et al.* 2014b; Correa *et al.* 2015; Bangerla *et al.*  
297 2017). In the case of coho salmon, heritability estimates range from 0.16 to 0.27 when  
298 resistance is defined as a linear or binary trait (Yáñez, *et al.* 2016a; Barría, *et al.* 2018).

299         Recent studies in rainbow trout, using different pedigree and genome-based genetic  
300 evaluation approaches, estimate heritabilities ranging from 0.39 to 0.57 for TD and from 0.54  
301 to 0.62 for BS (Yoshida, *et al.* 2018a); values which are within the range of our estimations.  
302 Moreover, our results suggest a higher effect of the additive genetic component on the  
303 phenotypic variance for resistance to *P. salmonis* in rainbow trout when compared to *S. salar*  
304 and *O. kisutch*, which would imply potentially faster genetic progress for the improvement  
305 of resistance to *P. salmonis* by means of artificial selection in the rainbow trout population  
306 used in the present study.

307         The effect of the genetic architecture of a trait (among other variables) on the accuracy  
308 of breeding values obtained through genomic selection (GS) is widely known (Daetwyler *et*  
309 *al.* 2008; Goddard 2009). Previous studies in salmonid species (Atlantic salmon and coho  
310 salmon), suggest that resistance to *P. salmonis* is a polygenic trait (Correa *et al.* 2015; Barría,  
311 *et al.* 2018). Based on the 26K SNPs which passed QC, our study similarly suggests a  
312 polygenic nature for resistance to *P. salmonis* resistance in rainbow trout (*i.e.* no QTL  
313 explaining  $\geq 10\%$  of the genetic variance). Thus, it is expected that, when compared with a  
314 pedigree-based Best Linear Unbiased Predictor (BLUP) method, a genomic BLUP approach  
315 for GS would have an increase in accuracy of breeding values over a Bayesian approach  
316 (Habier *et al.* 2007; Hayes *et al.* 2009) for the current rainbow trout population. Nonetheless,  
317 as predicted by Yoshida, *et al.* (2018b) this was true only at low SNP densities (*i.e.* 0.5 to 10  
318 K). When 20K and 27K were used, Bayes C outperformed GBLUP accuracies. The authors

319 suggested that this could be due to an oligogenic architecture of the resistance trait, or that  
320 Bayes C had higher effectiveness in capturing the linkage disequilibrium between the SNPs  
321 and a QTL when more SNPs were used.

322 Resistance to bacterial infections implies a modulation of the host immune response  
323 to inhibit or reduce the replication rate of the pathogen (Doeschl-Wilson and Kyriazakis  
324 2012). The infection process carried out by *P. salmonis* uses clathrin for internalization and  
325 then the actin cytoskeleton for vacuole generation (Ramírez *et al.* 2015). Similar pathways  
326 have been observed in other mammalian intracellular gram-negative bacteria (Manon *et al.*  
327 2012; Valencia-gallardo *et al.* 2015). Within the region associated with TD on *Omy3* we  
328 identified a gene coding for the receptor DC-SIGN related with the immune response and  
329 expressed on macrophage and dendritic-cell surfaces (Ahmed *et al.* 2015). It has been  
330 previously described that *Mycobacterium tuberculosis*, interferes with the Toll-like receptor  
331 signaling by DC-SIGN, inhibiting interleukin-12 production (Gorvel *et al.* 2014), a  
332 proinflammatory cytokine, which plays a key role in the performance of phagocytes in teleost  
333 fish (Alvarez *et al.* 2016).

334 As mentioned before, endocytosis mediated by clathrin is the main pathway used by  
335 *P. salmonis* for cell invasion. Clathrin recruits, among other cell components, AP-2; which  
336 is regulated by NECAP-1 (Ritter *et al.* 2013), a gene flanking the SNP explaining the highest  
337 proportion of genetic variance in *Omy3* for resistance measured as TD. Similarly, on this  
338 chromosome we also found the gene *glutathione S-transferase kappa 1 (gstk1)* (GTS), which  
339 is member of the glutathione S-transferase family (GST), involved in cellular detoxification,  
340 and expressed in cells to reduce oxidative stress-related damage (Morel and Aninat 2011), a  
341 consequence of *P. salmonis* infection (Rozas and Enríquez 2014), and differentially  
342 expressed in Atlantic salmon after *P. salmonis* exposure (Rise *et al.* 2004). A candidate gene

343 related to resistance as measured by BS, was found on *Omy5*, the *fas ligand* gene (*faslg*) is a  
344 member of the TNF superfamily. The Fas/FasL pathway is essential for immune system  
345 regulation, including apoptosis induced by T cell activation and by cytotoxic T lymphocytes  
346 (Siegel *et al.* 2000).

347 For both resistance trait definitions, the same chromosome and identical SNP was  
348 identified as the marker explaining the highest genetic variation for resistance, which makes  
349 this QTL as an interesting region in rainbow trout. Within this region we found the gene  
350 *phosphatidylinositol transfer protein alpha* (*pitpna*), which belongs to the  
351 phosphatidylinositol family (ptdlns) (Piscatelli *et al.* 2016), and is responsible for  
352 phospholipid transfer between cellular membranes (Thornbrough *et al.* 2016), which in turn  
353 are regulators of cell signal transduction, membrane trafficking and cytoskeleton  
354 organization (Hilbi and Haas 2012). The latter process is affected by *P. salmonis* once inside  
355 the macrophages (Ramírez *et al.* 2015). Similar to *P. salmonis*, *Legionella pneumophila* also  
356 replicates inside macrophages, and manipulates the vesicle generation inside the cell by  
357 joining with ptdlns 5 (Hilbi and Haas 2012).

358 Additionally, in this region we found the gene *nlr family card domain containing 3*  
359 (*nlrc3*). Previously, Álvarez *et al.* (2017), described a higher differential expression of *nlrc3*  
360 in rainbow trout in response to bacterial lipopolysaccharides (lps), specifically in the skin,  
361 liver and gills. This pattern has also been observed in Atlantic salmon during an infection  
362 with *P. salmonis* (Tacchi *et al.* 2011), and is therefore a likely mechanism used by this  
363 bacteria to evade the immune response.

364 The gene *tapsain* (*tap*) is also involved in the immune response, transporting cytosolic  
365 peptides generated by the proteasome to load on MHC class I (Procko *et al.* 2005). On



366 *Omy27*, we found a gene that encodes a protein related to tapsain (TAPBPR), which  
367 negatively regulates *tap*; generating a reduction in immune response efficiency (Boyle *et al.*  
368 2013).

369 We expect that in the near future, the identification and validation of causative  
370 mutations affecting some of the candidate genes presented here, by means of functional  
371 studies, will provide a better understanding of resistance against this and other infectious  
372 diseases in rainbow trout and other salmonid species. These studies will be facilitated through  
373 international collaborative initiatives such as the Functional Annotation of All Salmonid  
374 Genomes, FAASG (Macqueen *et al.* 2017).

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## CONCLUSIONS

377 To the best of our knowledge this is the first report identifying candidate genes related  
378 to resistance to *P. salmonis* in a farmed rainbow trout population. Genes likely related with  
379 resistance were identified close to SNPs explaining the highest proportion of genetic  
380 variance. Furthermore, we identified the same genomic region associated with resistance  
381 using both a linear and binary trait. Our results show that this trait is controlled by multiple  
382 genes each with a small effect. Therefore, a genomic selection approach is suggested as the  
383 best method to improve this trait by means of artificial selection.

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394

#### 395 AUTHORS' CONTRIBUTIONS

396 RM-N assessed the GWAS analyses, genes identification and contributed with discussion.  
397 AB wrote the initial version of the manuscript and contributed with discussion. PC  
398 contributed with discussion. MEL contributed with initial analysis. LB performed DNA  
399 extraction. JPL contributed with study design. JMY conceived and designed the study and  
400 supervised the work of RM-N. All authors reviewed and approved the manuscript.

401

402 Animal ethics approval

403 Experimental challenge was approved by the Comité de Bioética Animal from University of  
404 Chile (Certificate Number 17041-VET-UCH)

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#### 406 **References**

407 Aguilar, I., I. Misztal, D. L. Johnson, A. Legarra, S. Tsuruta *et al.*, 2010 Hot topic: A  
408 unified approach to utilize phenotypic, full pedigree, and genomic information for  
409 genetic evaluation of Holstein final score. *J. Dairy Sci.* 93: 743–752.

410 Ahmed, Z., T. Kawamura, S. Shimada, and V. Piguet, 2015 The Role of Human Dendritic  
411 Cells in HIV-1 Infection. *J. Invest. Dermatol.* 135: 1225–1233.

412 Alvarez, C., F. A. Gomez, L. Mercado, R. Ramirez, and H. Marshall, 2016 *Piscirickettsia*

- 413 salmonis Imbalances the Innate Immune Response to Succeed in a Productive  
414 Infection in a Salmonid Cell Line Model. PLoS One 11: 1–14.
- 415 Álvarez, C. A., F. Ramírez-cepeda, P. Santana, E. Torres, J. Cortés *et al.*, 2017 Insights into  
416 the diversity of NOD-like receptors : Identification and expression analysis of NLRC3  
417 , NLRC5 and NLRX1 in rainbow trout. Mol. Immunol. 87: 102–113.
- 418 Banger, R., K. Correa, J. P. Lhorente, R. Figueroa, and J. M. Yáñez, 2017 Genomic  
419 predictions can accelerate selection for resistance against *Piscirickettsia salmonis* in  
420 Atlantic salmon (*Salmo salar*). BMC Genomics 18: 121.
- 421 Barria, A., K. A. Christensen, G. M. Yoshida, K. Correa, A. Jedlicki *et al.*, 2018 Genomic  
422 predictions and genome-wide association study of resistance against *Piscirickettsia*  
423 *salmonis* in coho salmon ( *Oncorhynchus kisutch* ) using ddRAD sequencing. G3  
424 Genes Genomes Genet. 4231: g3.200053.2018.
- 425 Bishop, S. C., and J. A. Woolliams, 2014 Genomics and disease resistance studies in  
426 livestock. Livest. Sci. 166: 190–198.
- 427 Boyle, L. H., C. Hermann, J. M. Boname, K. M. Porter, P. A. Patel *et al.*, 2013 Tapasin-  
428 related protein TAPBPR is an additional component of the MHC class I presentation  
429 pathway. Proc. Natl. Acad. Sci. 110: 3465–3470.
- 430 Camussetti, M., A. Gallardo, D. Aguilar, and J. Larenas, 2015 Análisis de los costos por la  
431 utilización de quimioterápicos y vacunas en la salmonicultura. Salmonexpert 4: 46–49.
- 432 Correa, K., R. Banger, R. Figueroa, J. P. Lhorente, and J. M. Yáñez, 2017 The use of  
433 genomic information increases the accuracy of breeding value predictions for sea  
434 louse (*Caligus rogercresseyi*) resistance in Atlantic salmon (*Salmo salar*). Genet. Sel.  
435 Evol. 49: 15.
- 436 Correa, K., J. Lhorente, M. Lopez, L. Bassini, S. Naswa *et al.*, 2015 Genome-wide

437 association analysis reveals loci associated with resistance against *Piscirickettsia*  
438 *salmonis* in two Atlantic salmon (*Salmo salar* L.) chromosomes. *BMC Genomics* 16:  
439 854.

440 Cvitanich, J., O. Garate, and C. E. Smith, 1991 The isolation of a rickettsia-like organism  
441 causing disease and mortality in Chilean salmonids and its confirmation by Koch's  
442 postulate. *J. Fish Dis.* 14: 121–146.

443 Daetwyler, H. D., B. Villanueva, and J. A. Woolliams, 2008 Accuracy of Predicting the  
444 Genetic Risk of Disease Using a Genome-Wide Approach. *PlosOne* 3:.

445 Doeschl-Wilson, A. B., and I. Kyriazakis, 2012 Should we aim for genetic improvement in  
446 host resistance or tolerance to infectious pathogens? *Front. Genet.* 3: 1–2.

447 Falconer, D. S., and T. F. C. Mackay, 1996 *Quantitative Genetics*.

448 Flores-mara, R., F. H. Rodríguez, R. Bangera, J. P. Lhorente, R. Neira *et al.*, 2017  
449 Resistance against infectious pancreatic necrosis exhibits significant genetic variation  
450 and is not genetically correlated with harvest weight in rainbow trout (*Oncorhynchus*  
451 *mykiss*). *Aquaculture* 479: 155–160.

452 Fryer, J. L., and R. P. Hedrick, 2003 *Piscirickettsia salmonis*: a Gram-negative intracellular  
453 bacterial pathogen of fish. *J. Fish Dis.* 247: 251–262.

454 Goddard, M., 2009 Genomic selection: Prediction of accuracy and maximisation of long  
455 term response. *Genetica* 136: 245–257.

456 Gorvel, L., J. Textoris, R. Banchereau, A. Ben Amara, W. Tantibhedhyangkul *et al.*, 2014  
457 Intracellular Bacteria Interfere with Dendritic Cell Functions: Role of the Type I  
458 Interferon Pathway. *PLoS One* 9: 1–11.

459 Habier, D., R. L. Fernando, and J. C. M. Dekkers, 2007 The impact of genetic relationship  
460 information on genome-assisted breeding values. *Genetics* 177: 2389–2397.

- 461 Hayes, B. J., P. M. Visscher, and M. E. Goddard, 2009 Increased accuracy of artificial  
462 selection by using the realized relationship matrix. *Genet. Res. (Camb)*. 91: 47–60.
- 463 Hilbi, H., and A. Haas, 2012 Secretive Bacterial Pathogens and the Secretary Pathway.  
464 *Traffic* 13: 1187–1197.
- 465 Houston, R. D., J. B. Taggart, T. Cézard, M. Bekaert, N. R. Lowe *et al.*, 2014 Development  
466 and validation of a high density SNP genotyping array for Atlantic salmon (*Salmo*  
467 *salar*). *BMC Genomics* 15: 90.
- 468 Kaur, H., L. Jaso-Friedmann, and D. L. Evans, 2004 Single base oligodeoxyguanosine  
469 upregulates Fas ligand release by nonspecific cytotoxic cells. *Developmental and*  
470 *Comparative Immunology* 28: 571-579.
- 471 Kumada, Y., D. R. Benson, D. Hillemann, T. J. Hosted, D. A. Rochefort and C. J.  
472 Thompson, 1993 Evolution of the glutamine synthetase gene, one of the oldest  
473 existing and functioning genes. *Proc. Natl. Acad. Sci.* 90: 3009-3013.
- 474 Liu, S., R. L. Vallejo, Y. Palti, G. Gao, D. P. Marancik *et al.*, 2015 Identification of single  
475 nucleotide polymorphism markers associated with bacterial cold water disease  
476 resistance and spleen size in rainbow trout. *Front. Genet.* 6: 1–10.
- 477 Macqueen, D. J., C. R. Primmer, R. D. Houston, B. F. Nowak, L. Bernatchez *et al.*, 2017  
478 Functional Annotation of All Salmonid Genomes ( FAASG ): an international  
479 initiative supporting future salmonid research , conservation and aquaculture. *BMC*  
480 *Genomics* 18: 1–9.
- 481 Manon, R., N. Abed, F. Namdari, I. Virlogeux-Payant, P. Velge *et al.*, 2012 The different  
482 strategies used by *Salmonella* to invade host cells, pp. 339–364 in *Salmonella –*  
483 *Distribution, Adaptation, Control Measures and Molecular Technologies.*
- 484 Misztal, I., S. Tsuruta, D. Lourenco, Y. Masuda, I. Aguilar *et al.*, 2016 Manual for

- 485 BLUPF90 family of programs. 125.
- 486 Morel, F., and C. Aninat, 2011 The glutathione transferase kappa family. *Drug Metab. Rev.*  
487 43: 281–291.
- 488 Neto, R., G. M. Yoshida, J. Paul, L. José, and M. Yáñez, 2019 Genome-wide association  
489 analysis for body weight identifies candidate genes related to development and  
490 metabolism in rainbow trout ( *Oncorhynchus mykiss* ). *Mol. Genet. Genomics*.
- 491 Ødegård, J., T. Moen, N. Santi, S. A. Korsvoll, S. Kjøglum *et al.*, 2014 Genomic prediction  
492 in an admixed population of Atlantic salmon (*Salmo salar*). *Front. Genet.* 5: 1–8.
- 493 Ødegård, J., I. Olesen, B. Gjerde, and G. Klemetsdal, 2006 Evaluation of statistical models  
494 for genetic analysis of challenge test data on furunculosis resistance in Atlantic salmon  
495 (*Salmo salar*): Prediction of field survival. *Aquaculture* 259: 116–123.
- 496 Oruc, E.O., Y. Sevgiler, and N. Uner, 2004 Tissue-specific oxidative stress response in fish  
497 exposed to 2,4-D and azinphosmethyl. *Comparative Biochem. Physiol. Part C*, 137:  
498 43-51.
- 499 Palaiokostas, C., S. Ferrarreso, R. Franch, R. D. Houston, and L. Bargelloni, 2016 Genomic  
500 prediction of resistance to pasteurellosis in gilthead sea bream ( *Sparus aurata* ) using  
501 2b-RAD sequencing. *G3 Genes Genomes Genet.* X: 1–8.
- 502 Palti, Y., 2011 Toll-like receptors in bony fish : From genomics to function. *Dev. Comp.*  
503 *Immunol.* 35: 1263–1272.
- 504 Palti, Y., G. Gao, S. Liu, M. P. Kent, S. Lien *et al.*, 2015b The development and  
505 characterization of a 57K single nucleotide polymorphism array for rainbow trout.  
506 *Mol. Ecol. Resour.* 15: 662–672.
- 507 Palti, Y., R. L. Vallejo, G. Gao, S. Liu, A. G. Hernandez *et al.*, 2015a Detection and  
508 Validation of QTL Affecting Bacterial Cold Water Disease Resistance in Rainbow

- 509 Trout Using Restriction-Site Associated DNA Sequencing. PLoS One 10: e0138435.
- 510 Piscatelli, H. L., M. Li, and D. Zhou, 2016 Dual 4- and 5-phosphatase activities regulate  
511 SopB-dependent phosphoinositide dynamics to promote bacterial entry. Cell.  
512 Microbiol. 18: 705–719.
- 513 Procko, E., G. Raghuraman, D. Wiley, M. Raghavan, and R. Gaudet, 2005 Identification of  
514 domain boundaries within the N-termini of TAP1 and TAP2 and their importance in  
515 tapasin binding and tapasin-mediated increase in peptide loading of MHC class I.  
516 Immunol. Cell Biol. 83: 475–482.
- 517 Purcell, S., B. Neale, K. Todd-brown, L. Thomas, M. A. R. Ferreira *et al.*, 2007 PLINK : A  
518 Tool Set for Whole-Genome Association and Population-Based Linkage Analyses.  
519 Am. J. Hum. Genet. 81: 559–575.
- 520 Ramírez, R., F. A. Gómez, and S. H. Marshall, 2015 The infection process of *Piscirickettsia*  
521 *salmonis* in fish macrophages is dependent upon interaction with host-cell clathrin and  
522 actin. FEMS Microbiol. Lett. 362: 1–8.
- 523 Rise, M. L., S. R. M. Jones, G. D. Brown, K. R. von Schalburg, W. S. Davidson *et al.*, 2004  
524 Microarray analyses identify molecular biomarkers of Atlantic salmon macrophage  
525 and hematopoietic kidney response to *Piscirickettsia salmonis* infection. Physiol.  
526 Genomics 20: 21–35.
- 527 Ritter, B., S. Murphy, H. Dokainish, M. Girard, M. V Gudheti *et al.*, 2013 NECAP 1  
528 Regulates AP-2 Interactions to Control Vesicle Size , Number , and Cargo During  
529 Clathrin-Mediated Endocytosis. PLoS One 11: e1001670.
- 530 Rodríguez, F. H., G. Cáceres, J. P. Lhorente, S. Newman, R. Bangera *et al.*, 2018 Genetic (   
531 co ) variation in skin pigmentation patterns and growth in rainbow trout. Animals 1–8.
- 532 Roher, N., A. Callol, J. V. Planas, F. W. Goetz and S. A. Mackenzie, 2011 Endotoxin

- 533 recognition in fish results in inflammatory cytokine secretion not gene expression.  
534 *Innate Immunity* 17(1): 16-28.
- 535 Rozas, M., and R. Enríquez, 2014 Piscirickettsiosis and *Piscirickettsia salmonis* in fish: a  
536 review. *J. Fish Dis.* 37: 163–188.
- 537 Rozas-Serri M., A. Peña, L. Maldonado, 2017 Transcriptomic profiles of post-smolt  
538 Atlantic salmon challenged with *Piscirickettsia salmonis* reveal a strategy to evade the  
539 adaptive immune response and modify cell-autonomous immunity. *Developmental*  
540 *and Comparative Immunology* 81: 348-362.
- 541 Sernapesca, 2018 Informe Sanitario de Salmonicultura en Centros Marinos.
- 542 Siegel, R. M., F. K. Chan, H. J. Chun, and M. J. Lenardo, 2000 The multifaceted role of  
543 Fas signaling in immune cell homeostasis and autoimmunity. *Nat. Immunol.* 1: 469–  
544 474.
- 545 Tacchi, L., J. E. Bron, J. B. Taggart, C. J. Secombes, R. Bickerdike *et al.*, 2011 Multiple  
546 tissue transcriptomic responses to *Piscirickettsia salmonis* in Atlantic salmon (*Salmo*  
547 *salar*). *Physiol. Genomics* 43: 1241–1254.
- 548 Teitell, M. A., 2003 OCA-B regulation of B-cell development and function. *Trends*  
549 *Immunol.* 24: 546–553.
- 550 Thornbrough, J. M., A. Gopinath, T. Hundley, and M. J. Worley, 2016 Human Genome-  
551 Wide RNAi Screen for Host Factors That Facilitate *Salmonella* Invasion Reveals a  
552 Role for Potassium Secretion in Promoting Internalization. *PlosOne* 11: e0166916.
- 553 Tsai, H.-Y., A. Hamilton, A. E. Tinch, D. R. Guy, J. E. Bron *et al.*, 2016 Genomic  
554 prediction of host resistance to sea lice in farmed Atlantic salmon populations. *Genet.*  
555 *Sel. Evol.* 48: 47.
- 556 Valencia-gallardo, C. M., N. Carayol, and G. T. Van Nhieu, 2015 Microreview



- 557 Cytoskeletal mechanics during *Shigella* invasion and dissemination in epithelial cells.  
558 *Cell. Microbiol.* 17: 174–182.
- 559 Vallejo, R. L., T. D. Leeds, B. O. Fragomeni, G. Gao, A. G. Hernandez *et al.*, 2016  
560 Evaluation of genome-enabled selection for bacterial cold water disease resistance  
561 using progeny performance data in rainbow trout: Insights on genotyping methods and  
562 genomic prediction models. *Front. Genet.* 7: 1–13.
- 563 Vallejo, R. L., T. D. Leeds, G. Gao, J. E. Parsons, K. E. Martin *et al.*, 2017a Genomic  
564 selection models double the accuracy of predicted breeding values for bacterial cold  
565 water disease resistance compared to a traditional pedigree-based model in rainbow  
566 trout aquaculture. *Genet. Sel. Evol.* 49: 17.
- 567 Vallejo, R., S. Liu, G. Gao, B. O. Fragomeni, A. G. Hernandez *et al.*, 2017b Similar genetic  
568 architecture with shared and unique quantitative trait loci for bacterial cold water  
569 disease resistance in two rainbow trout breeding populations. *Front. Genet.* 8: 1–15.
- 570 Wang, T., J. W. Holland, N. Bols, and C. J. Secombes, 2005 Cloning and expression of the  
571 first nonmammalian interleukin-11 gene in rainbow trout *Oncorhynchus mykiss*.  
572 *FEBS J.* 272(5): 1136-47.
- 573 Wang, H., I. Misztal, I. Aguilar, A. Legarra, and W. M. Muir, 2012 Genome-wide  
574 association mapping including phenotypes from relatives without genotypes. *Genet.*  
575 *Res. (Camb).* 94: 73–83.
- 576 Yañez, J. M., R. Bangera, J. P. Lhorente, A. Barria, M. Oyarzun *et al.*, 2016 Negative  
577 genetic correlation between resistance against *Piscirickettsia salmonis* and harvest  
578 weight in coho salmon (*Oncorhynchus kisutch*). *Aquaculture* 459: 8–13.
- 579 Yañez, J. M., R. Bangera, J. P. Lhorente, M. Oyarzún, and R. Neira, 2013 Quantitative  
580 genetic variation of resistance against *Piscirickettsia salmonis* in Atlantic salmon

- 581 (Salmo salar). Aquaculture 414–415: 155–159.
- 582 Yañez, J. M., R. D. Houston, and S. Newman, 2014 Genetics and genomics of disease  
583 resistance in salmonid species. Front. Genet. 5: 1–13.
- 584 Yañez, J. M., J. P. Lhorente, L. N. Bassini, M. Oyarzún, R. Neira *et al.*, 2014 Genetic co-  
585 variation between resistance against both *Caligus rogercresseyi* and *Piscirickettsia*  
586 *salmonis*, and body weight in Atlantic salmon (*Salmo salar*). Aquaculture 433: 295–  
587 298.
- 588 Yañez, J. M., and V. Martinez, 2010 Genetic factors involved in resistance to infectious  
589 diseases in salmonids and their application in breeding programmes. Arch. Med. Vet.  
590 42: 1–13.
- 591 Yañez, J. M., S. Naswa, M. E. Lopez, L. Bassini, K. Correa *et al.*, 2016 Genomewide  
592 single nucleotide polymorphism discovery in Atlantic salmon (*Salmo salar*): validation  
593 in wild and farmed American and European populations. Mol. Ecol. Resour. 16:  
594 1002–1011.
- 595 Yañez, J., S. Newman, and R. D. Houston, 2015 Genomics in aquaculture to better  
596 understand species biology and accelerate genetic progress. Front. Genet. 6: 1–3.
- 597 Yoshida, G. M., R. Bangera, R. Carvalheiro, K. Correa, R. Figueroa *et al.*, 2018a Genomic  
598 prediction accuracy for resistance against *Piscirickettsia salmonis* in farmed rainbow  
599 trout. G3 Genes Genomes Genet. 8: 719–726.
- 600 Yoshida, G. M., R. Carvalheiro, F. H. Rodríguez, and J. P. Lhorente, 2018b Genomics  
601 Single-step genomic evaluation improves accuracy of breeding value predictions for  
602 resistance to infectious pancreatic necrosis virus in rainbow trout. Genomics 1–6.
- 603 Zheng, W., Y. Hu, M. Zhang, L. Sun, 2010 Analysis of the expression and antioxidative  
604 property of a peroxiredoxin 6 from *Scophthalmus maximus*. Fish & Shellfish

605 Immunology 29: 305-311.

606 Zuo, W., and P. T. K. Woo, 1997 Natural anti-proteases in rainbow trout, *Oncorhynchus*  
607 mykiss and brook charr, *Salvelinus fontinalis* and the in vitro neutralization of fish  
608 alpha 2-macroglobulin by the metalloprotease from the pathogenic haemoflagellate,  
609 *Cryptobia salmositica*. *Parasitology* 114: 375–81.

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630 **Table 1.** Summary statistics for time to death (TD), binary survival (BS) and final weight  
 631 (FW) measured in 2,130 rainbow trout individuals.

<b>Trait</b>	<b>Mean</b>	<b>SD</b>	<b>CV(%)</b>	<b>Min</b>	<b>Max</b>
TD	23.26	7.86	33.27	10	32
BS	0.59	0.49	0.83	0	1
FW	173.80	52.27	30.07	46.10	448

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634 **Table 2.** Genetic parameters and heritabilities for resistance to *Piscirickettsia salmonis* as  
 635 time to death (TD) and binary survival (BS).

<b>Trait</b>	$\sigma_a^2$ <sup>a</sup>	$\sigma_e^2$ <sup>b</sup>	$h^2$ (SD) <sup>c</sup>
<b>TD</b>	25.95	28.92	0.48(0.04)
<b>BS</b>	6.27x10 <sup>-2</sup>	1.21x10 <sup>-1</sup>	0.34(0.04)

636 <sup>a</sup> Additive genetic variance

637 <sup>b</sup> Residual variance

638 <sup>c</sup> Heritability and standard deviation

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646 **Table 3.** Top markers associated with *Piscirickettsia salmonis* resistance defined as TD and  
 647 BS in rainbow trout, using ssGWAS,

<b>Ranking</b>	<b>Name</b>	<b>Chr<sup>a</sup></b>	<b>Pos (Bp)</b>	<b>PGV<sup>b</sup></b>	<b>Genes<sup>c</sup></b>
<b>Time to death</b>					
1	Affx-88923370	27	9998276	2.43	<i>usp2, nlrc3, tap, pitpna</i>
2	Affx-88916453	3	14818380	1.41	<i>stl2, aicda, il11, gstk1</i>
3	Affx-88922612	14	10975036	1.21	<i>tlr4, tax1bp1, satb1</i>
4	Affx-88927397	24	11828385	1.02	<i>a2m, pou2af1, nfkbiz</i>
<b>Binary survival</b>					
1	Affx-88923370	27	9998276	1.50	<i>usp2, nlrc3, tap, pitpna</i>
2	Affx-88951679	5	68055053	1.12	<i>faslg, prdx6, plpp6</i>
3	Affx-88908715	29	32519588	1.01	<i>mapk12, gls</i>

648 <sup>a</sup> Chromosome.

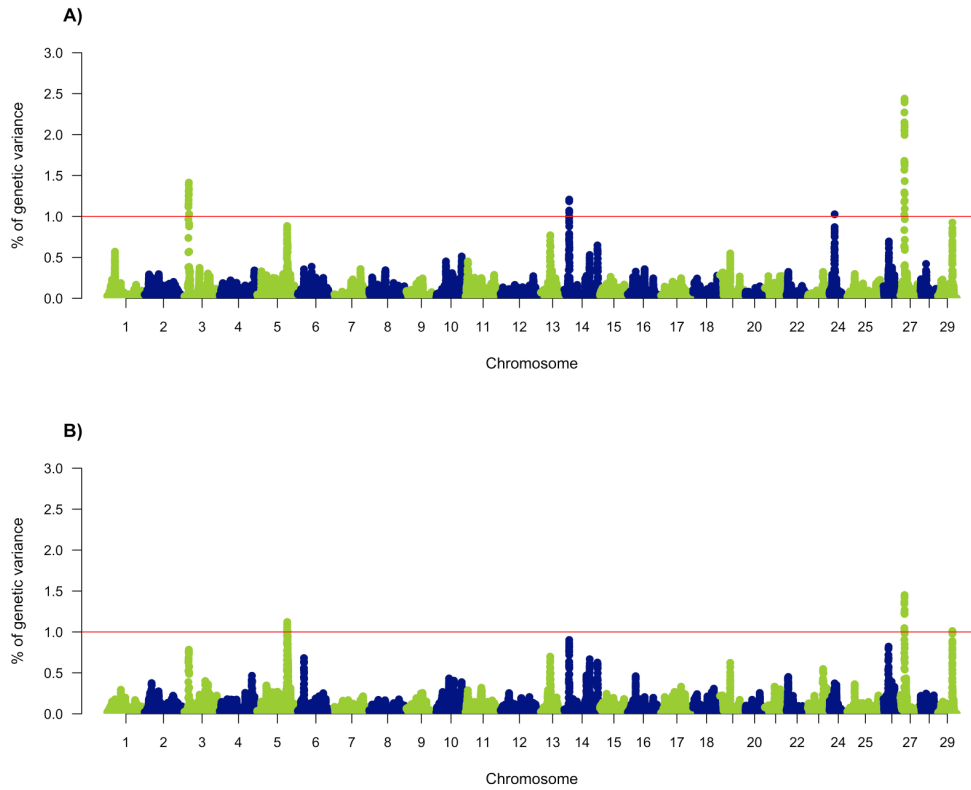
649 <sup>b</sup> Percentage of genetic variance.

650 <sup>c</sup> Summary of the genes located within 1Mb window.

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655 **Figure 1.** Genomic association analysis for resistance to *Piscirickettsia salmonis* in  
656 rainbow trout (*Oncorhynchus mykiss*). Resistance was defined as time to death (A) and as  
657 binary survival (B).