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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44   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 ± 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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72   INTRODUCTION

73   As in any intensive animal production system, infectious diseases are one of the main

74   threats affecting the success and sustainability of aquaculture (Yáñez *et al.* 2014a). In the

75   case of salmonid production, one of the major pathogens affecting productivity is the

76   facultative intracellular bacteria *Pisciricketssia salmonis,* etiological agent of salmonid

77   rickettsial syndrome (SRS). This bacterium was first identified in 1989 in Chile, in a farmed

78   coho salmon (*Oncorhynchus kisutch*) population (Cvitanich *et al.* 1991). Since then,

79   mortalities resulting from SRS have been also identified in Atlantic salmon (*Salmo salar)*

80   and rainbow trout (*Oncorhynchus mykiss*) in several countries, such as Scotland, Ireland,

81   Norway and Chile (Fryer and Hedrick 2003). In Chile, SRS was responsible for 20.7, 67.9

82   and 92.6% of the mortalities associated with infectious diseases in *S. salar*, *O. kisutch* and

83   *O. mykiss*, species respectively (Sernapesca 2018). To date, strategies for *P. salmonis* control

84   and treatment are mainly based on vaccines and antibiotics. The effectiveness of both

85   approaches has not been adequate (Rozas and Enríquez 2014). Therefore, it has been

86   estimated that economic losses due SRS mortalities, reached up to US$450 million in Chile

87   in 2012 (Camussetti *et al.* 2015). However, variables such as laboratory diagnosis screening

88   expenses or loss of quality of the harvested fish and products were not considered, implying

89   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 e*t 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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127   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 ±

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^3

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$_{50}$) 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.

152

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   myDesignTM 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 < $1\text{x}10^{-6}$). 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.

170

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
$$\boldsymbol{H}^{-1}=\boldsymbol{A}^{-1}+\begin{bmatrix} 0 & 0 \\ 0 & \boldsymbol{G}^{-1}\text{-}\boldsymbol{A}_{22}^{-1} \end{bmatrix}$$

179   Where A^{-1} is the inverse of the numerator relationship matrix, considering all the phenotyped

180   animals, A_{22}^{-1} is the inverse of the pedigree-based relationship matrix considering only the

181   genotyped animals, and G^{-1} is the inverse genomic relationship matrix. The following model

182   was used for GWAS analysis:

183
$$y = X\beta + Za + e$$

184 Where y is the vector of phenotypes (for TS and BS), β 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 X and 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 σ_a^2 is the additive genetic variance estimated using the H matrix, and σ_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.

209

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.

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219   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 ± 7.86 days. At the end of the

224   experimental challenge the proportion of non-survivor fish was 0.59 ± 0.49. Cumulative

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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 ± 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 ± 0.04 and 0.34 ± 0.04 were

233   estimated for TD and BS, respectively. Furthermore, a high genetic correlation was found

234   between both traits (-0.96 ± 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 ($\sim 64.68\%$). 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).

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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 (*il11*). 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).

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272   On *Omy29*, *MAPK12* was found; previous studies described that MAPK12 is

273   involved on the signaling pathways responsible for TNF-α 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 *TNFα*, 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; Bangera *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 >= 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

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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

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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).

375

376   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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395   AUTHORS' CONTRBUTIONS

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

Trait	Mean	SD	CV(%)	Min	Max
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

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

Trait	$\sigma_a^{2\,a}$	$\sigma_e^{2\,b}$	$h^2(SD)^c$
TD	25.95	28.92	0.48(0.04)
BS	6.27×10^{-2}	1.21×10^{-1}	0.34(0.04)

[a] Additive genetic variance

[b] Residual variance

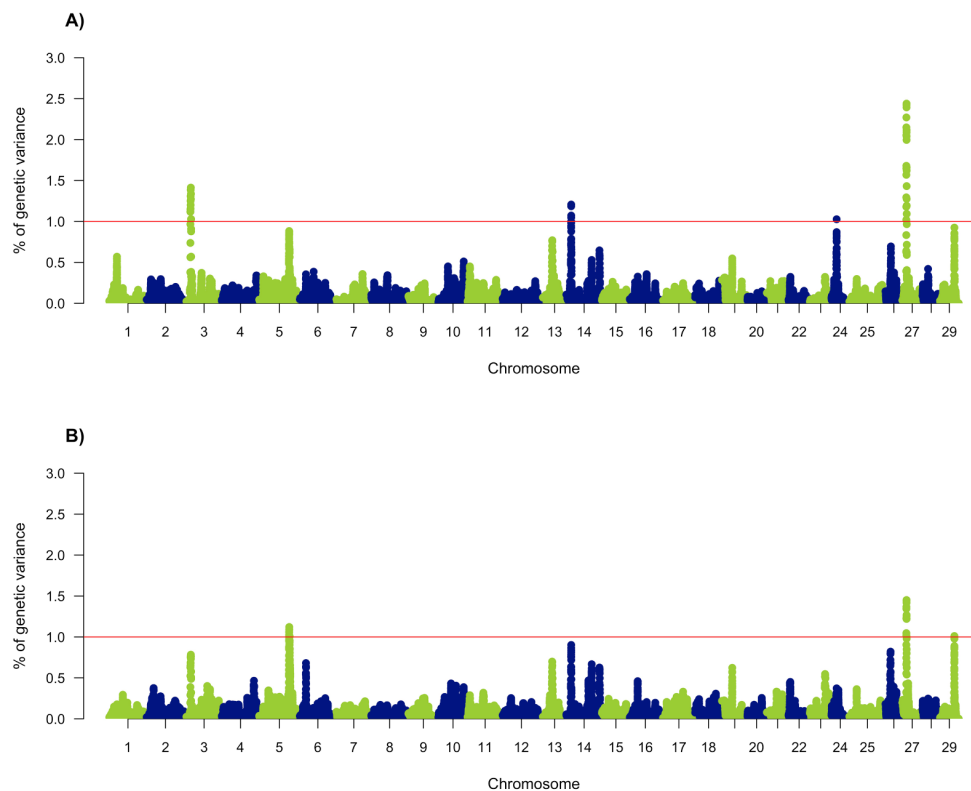[c] Heritability and standard deviation

646 **Table 3.** Top markers associated with *Piscirickettsia salmonis* resistance defined as TD and

647 BS in rainbow trout, using ssGWAS,

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

648 [a] Chromosome.

649 [b] Percentage of genetic variance.

650 [c] Summary of the genes located within 1Mb window.

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