

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/297918185>

Negative genetic correlation between resistance against *Piscirickettsia salmonis* and harvest weight in coho salmon (*Oncorhynchus kisutch*)

Article in *Aquaculture* · March 2016

DOI: 10.1016/j.aquaculture.2016.03.020

READS

114

6 authors, including:



José Manuel Yáñez

University of Chile

28 PUBLICATIONS 96 CITATIONS

SEE PROFILE



Rama Banger

Aquainnovo

15 PUBLICATIONS 46 CITATIONS

SEE PROFILE



Roberto Neira

University of Chile

48 PUBLICATIONS 513 CITATIONS

SEE PROFILE



Negative genetic correlation between resistance against *Piscirickettsia salmonis* and harvest weight in coho salmon (*Oncorhynchus kisutch*)



José M. Yáñez^{a,b}, Rama Bangera^b, Jean P. Lhorente^b, Agustín Barría^a, Marcela Oyarzún^b, Roberto Neira^{b,c}, Scott Newman^d

^a Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santa Rosa 11735, La Pintana, Santiago, Chile

^b Aquainnovo S.A., Talca 60, Puerto Montt, Chile

^c Facultad de Ciencias Agronómicas, Universidad de Chile, Santa Rosa 11315, La Pintana, Santiago, Chile

^d Genus plc, Hendersonville, TN 37075, USA

ARTICLE INFO

Article history:

Received 8 August 2015

Received in revised form 9 March 2016

Accepted 10 March 2016

Available online 12 March 2016

Keywords:

Oncorhynchus kisutch

Body weight

Disease resistance

Heritability

Genetic correlation

ABSTRACT

One of the major infectious diseases affecting coho salmon (*Oncorhynchus kisutch*) aquaculture is the Salmon Rickettsial Syndrome caused by *Piscirickettsia salmonis*. Conventional control measures such as antibiotics and vaccines have shown inconsistent results in production conditions. Thus, genetic improvement for *P. salmonis* resistance represents an alternative for the prevention of outbreaks. In the present study we aimed to determine both the levels of genetic variation for *P. salmonis* resistance (PSR) and genetic co-variation between PSR and harvest weight (HW) in coho salmon. A total of 2606 siblings from 108 maternal full-sib families (60 paternal half-sib families) were challenged against *P. salmonis*. The cumulative mortality rates among families ranged from 5% to 82%, with an average of 39%, indicating considerable phenotypic variation. For the genetic analyses PSR was defined as the day of death for each fish. We also recorded HW in 41,597 genetically related individuals of the challenged fish from the same breeding population. A linear bivariate animal model was used to estimate (co)-variance components and to calculate genetic parameters. Estimated heritabilities for PSR and HW were 0.16 ± 0.04 and 0.41 ± 0.03 , respectively. The genetic correlation between PSR and HW was -0.50 ± 0.13 . The levels of genetic variation detected in the present study indicate that selective breeding for these traits is feasible. However, the magnitude and direction of the genetic correlation between PSR and HW must be taken into account when selecting both traits simultaneously.

Statement of relevance: Negative relationship between resistance and growth in coho.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Chilean coho salmon (*Oncorhynchus kisutch*) aquaculture yielded around 160,000 tons in 2012, representing about 90% of world production for this species (FAO, 2014). The success and sustainability of this industry are highly dependent on the control of infectious diseases. *P. salmonis* is an intracellular bacterium initially isolated from a coho salmon population farmed in Chile (Cvitanich et al., 1991). The infection caused by this pathogen, also called Salmon Rickettsial Syndrome, is currently one of the main issues in Chilean salmon farming (SERNAPECSA, 2015). This is mainly due to the fact that conventional control measures (i.e. vaccines and antibiotics) have not shown to be consistently effective in field conditions (Rozas and Enríquez, 2014).

In addition to antimicrobial drugs and vaccines, selective breeding for resistance against infectious diseases represents a realistic and more sustainable strategy to control disease outbreaks in different livestock and aquaculture species (Bishop and Woolliams, 2014).

For economic and sustainability purposes, resistance to particular pathogens such as *P. salmonis* together with growth-related traits (e.g., harvest weight) must be included in the breeding objective of salmonids (Yáñez and Martínez, 2010; Ødegård et al., 2011; Gjedrem, 2012; Yáñez et al., 2014b). However, in order to include *P. salmonis* resistance into the breeding goal of coho salmon, prior knowledge of the genetic co-variation of *P. salmonis* with other traits of economic importance is required. Recent evidence demonstrates the presence of significant genetic variation for resistance against *P. salmonis* in Atlantic salmon (*Salmo salar*) (Yáñez et al., 2013, 2014c). Another study has evaluated the influence of *P. salmonis* infection over the expression of immune related genes in triploid coho salmon (Correa et al., 2015a). However, levels of genetic variation for *P. salmonis* resistance and the correlation between this trait and harvest weight have not been evaluated up to now in this species. The objective of this work was to assess both the presence and magnitude of genetic variation for *P. salmonis* resistance and the genetic correlation between this trait and harvest weight in coho salmon using a bivariate animal model. The outcome from this work will be helpful in incorporating *P. salmonis* resistance into genetic improvement programs in commercial coho salmon

E-mail address: jmayanez@uchile.cl (J.M. Yáñez).

populations, aimed at providing an alternative for controlling disease outbreaks based on genetically improved eggs.

2. Materials and methods

2.1. Population

This work was carried out in a coho salmon (*Oncorhynchus kisutch*) breeding population from a genetic improvement program run by Pesquera Antares (Puerto Montt, Chile). The breeding nucleus was established in 1997 and consists of two populations depending on the spawning year. Population reproductive management, mating design, fish tagging, rearing conditions and phenotyping strategy for harvest weight (HW) are described in detail by Yáñez et al. (2014a). Briefly, both populations have been selected for harvest weight (HW) over eight generations using *best linear unbiased prediction* (BLUP). Table 1 summarizes pedigree information for the population spawning even years from the breeding nucleus used in the present study. Table 2 shows summary statistics for age at tagging (TA), weight at tagging (TW), age at harvest (HA) and HW records in the population spawning even years, partitioned by year. Overall mean TA, TW, HA and HW across generations were 188 (SD = 24) days, 5.7 (SD = 1.4) g, 604 (SD = 24) days and 3.97 (SD = 1.32) kg, respectively.

2.2. Experimental challenge test

For the experimental challenge against *P. salmonis* we used 2606 fish belonging to 108 maternal full-sib families (60 paternal half-sib families) from the 2012 spawning year from the breeding nucleus described above. To maintain pedigree traceability fish were individually tagged by using Passive Integrated Transponder tags inserted into the abdomen, at an average age of 218 (SD = 3) days and mean weight of 5.5 g (SD = 1.02 g). After tagging the challenge group was communally reared in a single tank for about five months before being transferred to Aquainnovo's Research Station (Lenca River, Xth Region, Chile). The fish were subjected to 117 days of acclimation at the research station in salt water (32 ppt) at an average temperature of 13 °C. After this period, an average of eight individuals (ranging between 1 and 18) from each of the 108 families were distributed into each of the three replicate tanks. Table 3 shows summary statistics of the challenged fish distributed by tank. A sample of 30 fish were previously tested and confirmed to be negative for the presence of Infectious Pancreatic Necrosis virus and Infectious Salmon Anemia virus by RT-PCR, *Flavobacterium psychrophilum* by culture and PCR, and *Renibacterium salmoninarum* by immunofluorescence antibody test (IFAT). All of these diagnostic analyses were carried out at Alab laboratory (Puerto Montt, Chile).

The experimental challenge test against *P. salmonis* was performed following a similar approach to the one described by Yáñez et al. (2013). The test was performed using a pathogenic strain of *P. salmonis* isolated in November 2012 and purchased from ADL

Table 1

Summary information of the pedigree of the population spawning even years from the coho salmon (*Oncorhynchus kisutch*) breeding nucleus used in the present study by year.

Year	Number of Sires	Number of dams	Number of offspring	
			Total number	Mean per family
1998	42	80	8589	109
2000	36	73	6557	90
2002	59	114	9120	80
2004	49	137	10749	78
2006	37	102	10522	103
2008	34	98	8821	90
2010	45	110	8798	80
2012	61	112	15944	142
Total	363	826	79100	96

Table 2

Summary statistics for age at tagging (TA) measured in days (d), weight at tagging (TW) measured in g, age at harvest (HA) measured in days (d) and harvest weight (HW) measured in kg in the population spawning even years partitioned by year.

Variable	Year	n ^a	Mean	SD ^b	CV ^c (%)	Min	Max	
TA (d)	1998	6138	200	3	1.35	196	207	
	2000	6557	177	13	7.06	156	201	
	2002	9120	153	2	1.25	150	158	
	2004	10066	163	2	1.27	159	171	
	2006	9079	185	2	1.30	182	190	
	2008	8821	171	1	0.71	170	174	
	2010	8798	215	2	1.10	211	219	
	2012	15944	218	3	1.44	212	224	
	TW (g)	1998	6138	5.26	1.14	21.65	1.4	12.0
		2000	6557	5.02	1.21	24.06	2.0	12.2
		2002	9120	5.53	1.26	22.77	1.4	11.3
		2004	10065	6.05	1.29	21.40	2.6	12.2
2006		9079	7.40	1.87	25.24	3.1	16.1	
2008		8821	5.90	1.10	18.66	2.7	10.5	
2010		8798	5.42	1.12	20.71	2.2	9.90	
2012		15943	5.32	1.01	19.02	2.6	10.4	
HA (d)		1998	3214	648	6	0.93	630	658
		2000	1561	626	15	2.37	597	656
		2002	4436	627	4	0.65	621	638
		2004	4391	615	8	1.24	600	639
	2006	6387	621	15	2.46	605	655	
	2008	7602	614	2	0.39	609	618	
	2010	6106	582	3	0.5	577	587	
	2012	7900	571	2	0.34	567	574	
	HW (kg)	1998	3214	3.63	0.75	20.62	1.39	5.78
		2000	1561	4.21	1.62	38.41	0.24	7.20
		2002	4436	3.72	1.74	46.92	0.10	7.18
		2004	4391	4.23	1.16	27.37	0.10	7.08
2006		6387	4.90	0.99	20.31	1.11	7.50	
2008		7602	2.47	0.90	36.45	0.05	4.60	
2010		6106	4.39	0.63	14.42	2.49	6.20	
2012		7900	4.44	0.84	18.84	0.20	6.90	

^a Number of fish included in the analysis after removing outliers by interquartile range rule and discarding missing values.

^b Standard deviation.

^c Coefficient of variation.

Diagnostic Chile Ltda, (Puerto Montt, Chile). The *P. salmonis* bacteria were isolated from a kidney sample from rainbow trout and it was identified as a LF-89 strain. Additionally, the inoculum was produced from a

Table 3

Summary statistics for age at tagging (TA) measured in days, weight at tagging (TW), day of death (DD), weight at the beginning of the test (IW) measured in kg, incidence of mortality (IM) as a binary record (0 if the fish survived; 1 if the fish died) and the number of fish per family (FPF) in the group of fish challenged against *P. salmonis* partitioned by tank.

Variable	Tank	n ^a	Mean	SD ^b	CV ^c (%)	Min	Max
TA (d)	1	864	218	3	1.41	212	224
	2	881	218	3	1.40	212	224
	3	861	218	3	1.42	212	224
TW (g)	1	864	5.56	1.06	19.12	3.00	10.0
	2	881	5.55	0.99	17.82	2.90	9.50
	3	861	5.55	1.00	18.06	3.10	0.01
DD (d)	1	864	41	11	26.77	10	50
	2	881	41	8	20.71	11	50
	3	861	43	10	24.16	11	50
IW (kg)	1	864	0.28	0.14	49.37	0.09	0.61
	2	881	0.28	0.14	49.67	0.09	0.62
	3	861	0.28	0.14	49.53	0.09	0.65
IM (binary)	1	864	0.42	0.49	116.44	0	1
	2	881	0.35	0.48	136.13	0	1
	3	861	0.38	0.49	127.55	0	1
FPF (fish)	1	864	8	2.87	35.85	1	15
	2	881	8.23	2.86	34.7	3	18
	3	861	8.05	2.76	34.24	2	14

^a Number of fish included in the analysis after removing outliers by interquartile range rule and discarding missing values.

^b Standard deviation.

^c Coefficient of variation.

solid culture. The TCID₅₀/ml of the concentrated inoculum was 10^{8.5}. This quantity was calculated through the Kärber–Spearman method (Hamilton et al., 1977). We used a preliminary challenge to determine the LD₅₀ dose, testing four different dilutions (1:10, 1:100, 1:1000, 1:10000) from the original inoculum. The LD₅₀ assessment was performed in a random sub-sample of 80 individuals from the same group of fish previously described (20 animals per dilution). A dose of 0.2 ml was inoculated in each fish by means of intraperitoneal (IP) injection. This first test spanned 26 days and a dilution of 1:680 was calculated as the LD₅₀ based on mortality at the end of the testing period, which corresponded to 9.4⁴ infectious particles per fish.

Subsequently, we used 0.2 ml of an inoculum at the LD₅₀ concentration to induce infection by means of IP inoculation on each fish in the definitive challenge test performed. Fish were anesthetized with 30 ppm of benzocaine before IP injection. After infection induction, the animals were randomly divided and maintained in three different communal tanks with salt water (32 ppt) during the test period.

2.3. Records and trait definitions

The challenge test spanned 50 days and mortalities were recorded daily. Initial body weight (IW) was measured at the beginning of the experiment (i.e. at the moment of IP injection) for all fish (Table 3), permitting analysis of the association between IW and PSR. The relationship was studied by fitting a simple linear regression of PSR on IW. There was a significant ($P < 0.05$) and positive effect of IW on PSR ($\beta = 17.89 \pm 1.37$). This result illustrates the actual effect of body weight at the challenge and justifies the relevance of using IW as covariate in the analysis. At day 47, mortalities reached a plateau which was maintained for three days. To confirm *P. salmonis* as the cause of death and discard other pathogens (mentioned in Section 2.2) we carried out necropsy inspection on each dead animal and molecular diagnostics in a sample of fish.

P. salmonis resistance (PSR) was assessed as the challenge–test survival, defined as the day of death (DD), ranging from 1 to 50 depending on the time of the event. Thus fish which showed higher survival time, were the more resistant animals. The value attributed to survivors was 50. In addition, HW was recorded in 41,597 fish connected through pedigree relationships to the challenged full-siblings. Tagged fish were tested for growth in commercial open-sea cages for 19 to 22 months, fed ad libitum by appetite-regulated automatic feeders with commercial feed.

2.4. Statistical analysis

A bivariate linear animal model was used to estimate the variance and co-variance components for PSR (\mathbf{y}_1) and HW (\mathbf{y}_2). The bivariate model was defined as follows:

$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{W}_2 \end{bmatrix} \begin{bmatrix} \mathbf{0} \\ \mathbf{c}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix}$$

where, \mathbf{y}_1 and \mathbf{y}_2 are vectors of phenotypic records for the animals measured for PSR and HW, respectively; \mathbf{b}_1 is the vector of fixed effects for PSR, including tank replicate as a factor and weight at the beginning of the experiment (IW) as a covariate; \mathbf{b}_2 is the vector of fixed effects for HW, including contemporary group of sex:cage:year as a factor and HA as a covariate; \mathbf{u}_1 and \mathbf{e}_1 are vectors of random animal genetic and residual effects, respectively, for PSR and HW; \mathbf{c}_2 is the vector of random common environment effect associated to full-sib families for HW; and \mathbf{X}_1 and \mathbf{Z}_1 are the design matrices for PSR and HW, and \mathbf{W}_2 is the design matrix for HW.

For both traits animal and residual effects, and for HW the common environment effect, were assumed to be random:

$$\begin{aligned} \mathbf{u} &= \begin{bmatrix} \mathbf{u}'_1 & \mathbf{u}'_2 \end{bmatrix}' \sim N(0, \mathbf{G}_0 \otimes \mathbf{A}), \\ \mathbf{c} &= \begin{bmatrix} \mathbf{c}'_2 \end{bmatrix}' \sim N(0, \mathbf{C}_0 \otimes \mathbf{I}_C), \\ \mathbf{e} &= \begin{bmatrix} \mathbf{e}'_1 & \mathbf{e}'_2 \end{bmatrix}' \sim N(0, \mathbf{R}_0 \otimes \mathbf{I}_N), \end{aligned}$$

where \mathbf{A} is the additive genetic relationship matrix among all fish included in the pedigree, \mathbf{I}_C and \mathbf{I}_N are identity matrices of dimension \mathbf{C} and \mathbf{N} , and \otimes indicates the direct product operator. \mathbf{G}_0 and \mathbf{R}_0 denote the 2×2 co-variance matrices of animal additive genetic effect and residual effect, respectively. Given that PSR and HW were recorded on different individuals connected through pedigree relationships, environmental covariance in the \mathbf{R}_0 matrix was set to zero. A random common environment effect associated with full-sib families was assessed in preliminary analyses using a single-trait likelihood ratio test (Lynch and Walsh, 1998). This effect was only significant ($P < 0.05$) for HW. Thus, we only included this effect for HW in the final bivariate model. Therefore, \mathbf{C}_0 represents a 1×1 scalar of common environment effects for HW. The ASREML package (Gilmour et al., 2009) was used to both fit all the models and to estimate variance components.

Prior to fitting the model described above, the interquartile range rule was applied to detect outliers for the dependent variables as well as for the covariates. Outliers were only detected and subsequently discarded for HW ($n = 1024$) and IW ($n = 3$). The total number of records included in the analysis after outlier filtering is shown in Tables 2 and 3.

2.5. Heritabilities and genetic correlations

For both traits, the heritabilities were computed as follows:

$$h_i^2 = \frac{\sigma_{G_i}^2}{\sigma_{G_i}^2 + \sigma_{C_i}^2 + \sigma_{E_i}^2}$$

where, i is PSR or HW, $\sigma_{G_i}^2$ are the additive genetic variance from \mathbf{G}_0 matrix, $\sigma_{C_i}^2$ is the variance explained by the common environment effect associated with full-sib families (only for HW) from \mathbf{C}_0 and $\sigma_{E_i}^2$ are the residual variances from \mathbf{R}_0 matrix. The genetic correlation (r_{xy}) between the two traits, PSR and HW was calculated as follows (Falconer and Mackay, 1996):

$$r_{PSR,HW} = \frac{\sigma_{aPSR,aHW}}{\sqrt{\sigma_{aPSR}^2 \sigma_{aHW}^2}}$$

where, $\sigma_{aPSR,aHW}$ corresponds to the additive genetic covariance between PSR and HW, σ_{aPSR}^2 corresponds to the additive genetic variance of PSR, and σ_{aHW}^2 corresponds to the additive genetic variance of HW.

3. Results

3.1. Experimental challenge test

Fish experimentally infected with *P. salmonis* presented clinical symptoms and lesions typical of the Salmon Rickettsial Syndrome. These symptoms included inappetence and lethargy as well as, skin lesions, pale gills and abdominal swelling. Internally, challenged individuals presented swollen kidneys, splenomegaly, petechiae in visceral organs, and livers with a yellowish tone. Mortalities started to increase rapidly around day 11, reaching an overall total cumulative mortality of 39% at the end of the experiment.

3.2. Phenotypic variation for HW and PSR

Phenotypic variation was demonstrated for HW with an overall mean, standard deviation and coefficient of variation of 3.97, 0.92 kg and 33.2%, respectively, across 41,597 records (Table 2). Overall, minimum and maximum values for HW ranged from 0.05 kg to 7.5 kg. On the other hand, Table 3 shows descriptive statistics for TA, TW, DD, IW, incidence of mortality (IM), and the number of fish per family (FPF) in the group of animals challenged against *P. salmonis* by tank. Overall, DD, IW, IM and FPF across tanks for the challenged fish were 42 (SD = 10) days, 0.28 (SD = 0.14) kg, 38.3 (SD = 48.7) % and 8 (SD = 3) fish per family, respectively.

Kaplan–Meier survival curves (Kaplan and Meier, 1958) were plotted for the three different tanks throughout the test period (Fig. 1). In addition, survival curves were plotted for the best and worst family, as well as the average across families (Fig. 2). Our results demonstrated significant ($P < 0.05$) phenotypic variation for PSR based on Kaplan–Meier survival analysis. Cumulative survival rates ranged from 18% to 95% between families.

3.3. Heritabilities and genetic correlations

Table 4 shows the estimated variance components, heritabilities and genetic correlations between HW and PSR. We identified significant additive genetic variation for both traits. Moderate values of heritability were estimated for HW (0.41 ± 0.03) and PSR (0.16 ± 0.04). The common environment effect associated to full-sib families accounted for only 1.6% of phenotypic variance for HW. In addition, a significant negative genetic correlation between HW and PSR of intermediate value (-0.50 ± 0.13) was detected. Phenotypic correlation was non-estimable due to the fact that PSR and HW were measured on different individuals.

4. Discussion

Previous studies have demonstrated the presence of significant genetic variation for body weight in coho salmon. For example, low to moderate heritability values have been estimated for body weight at harvest ranging between $h^2 = 0.13 \pm 0.04$ and 0.40 ± 0.04 (Neira et al., 2004, 2006). In addition, Gallardo et al. (2010) demonstrated significance of common environment effect on HW in two coho salmon populations from the same breeding nucleus studied by Neira et al. (2006). In this previous study, heritabilities decreased from 0.45 to 0.21 and 0.50 to 0.38 for the even and odd populations respectively, when a common environmental effect was included in the model

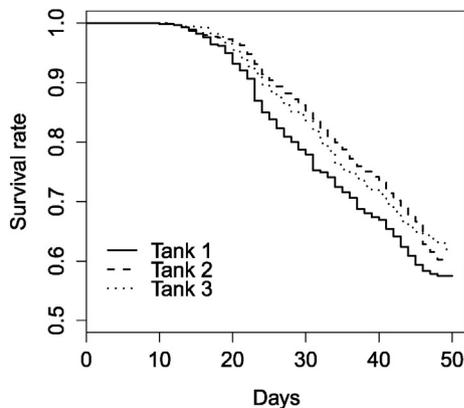


Fig. 1. Kaplan–Meier survival curves of the three tanks (replicates) of an experimental challenge with *Piscirickettsia salmonis* in coho salmon spanning 50 days.

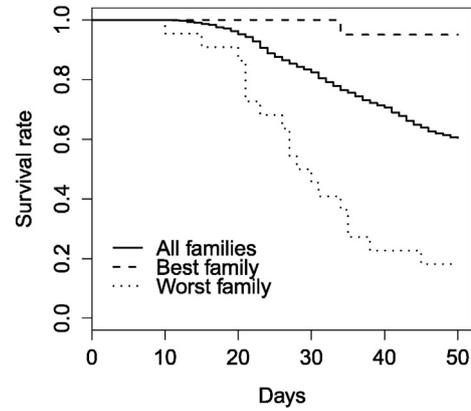


Fig. 2. Kaplan–Meier survival curves of an average of the 108 full-sib families, the best and the worst family after an experimental challenge with *Piscirickettsia salmonis* in coho salmon spanning 50 days.

(Gallardo et al., 2010). In the present study, we also detected a significant common environmental effect for HW associated with common rearing of full siblings before tagging. The magnitude of the effect of common environment expressed as a ratio of the phenotypic variance is slightly smaller ($c^2 = 0.016$) than those reported by Gallardo et al. (2010) ($c^2 = 0.02–0.06$). Heritability for HW presented here ($h^2 = 0.41 \pm 0.03$) is somewhat higher to those values reported by Gallardo et al. (2010) ($h^2 = 0.21$ to 0.38). These results can be explained based on the relative importance of variance components estimated in both studies, indicating both lower residual variation and higher genetic variation for HW in the present study. For instance, the additive genetic variance represented 20.8% and 38%, and the residual variance represented 73.6% and 59.7%, of the total phenotypic variance in the two populations studied by Gallardo et al. (2010). On the other hand, the additive genetic variance represented 42.2% and the residual variance represented 56.3% of the total phenotypic variance in the population included in the present study. The magnitude of additive genetic and residual components explaining total phenotypic variation for HW in a particular year can vary with environmental conditions and heritability may change accordingly (Visscher et al., 2008).

To our knowledge, this is the first work presenting evidence of significant genetic variation for resistance against *P. salmonis* in a coho salmon population. Recent studies have demonstrated significant heritability for resistance against *P. salmonis* in Atlantic salmon (*Salmo salar*) (Yáñez et al., 2013, 2014c). When comparing the magnitude of the heritability values reported in the studies carried out in Atlantic salmon ($h^2 = 0.18 \pm 0.03$), using the same trait definition (day of death) and analyzed using linear models, they correspond well to the value estimated in the present work ($h^2 = 0.16 \pm 0.04$). These results suggest similar levels of both genetic and residual variation for resistance to *P. salmonis* in the analyzed populations of these two related species. Thus, we demonstrate that improving *P. salmonis* resistance in

Table 4

Phenotypic, additive, common environment and residual variance components (σ_p^2 , σ_a^2 , σ_c^2 and σ_e^2 , respectively), heritabilities (h^2) and genetic correlation (r_g) for harvest weight (HW) and *Piscirickettsia salmonis* resistance (PSR) in a coho salmon breeding population (\pm standard errors).

	HW	PSR
σ_p^2	0.64 ± 0.01	91.9 ± 2.97
σ_a^2	0.27 ± 0.02	14.9 ± 3.70
σ_c^2	0.01 ± 0.003	–
σ_e^2	0.36 ± 0.01	77.0 ± 3.05
h^2	0.41 ± 0.03	0.16 ± 0.04
r_g HW	–	–0.50 ± 0.13

coho salmon through selective breeding is achievable as well as in Atlantic salmon (Yáñez et al., 2013, 2014c).

Different strains of *P. salmonis* have been reported (Rozas and Enríquez, 2014). An early phylogenetic analysis based on three rRNA regions and including five isolates (LF-89, ATL-4-91, NOR-92, SLGO-94 and EM-90) demonstrated a slight genetic divergence between them (Mauel et al., 1999). However, EM-90 has been shown to have a slightly higher level of differentiation, when compared to the other isolates, including the reference strain LF-89 (Mauel et al., 1999; Casanova et al., 2003). Recent studies have found three Type IV secretion systems (T4SSs), which are well characterized virulence-associated multiprotein complexes (Waksman and Orlova, 2014), in the genome of a LF-89 strain isolated from coho salmon (Bohle et al., 2014; Pulgar et al., 2015b), while only two were present in a EM-90 strain isolated from Atlantic salmon (Bohle et al., 2014). Other differences at a genomic level were also reported, including indels in *tra* genes and polymorphisms on lipopolysaccharide (LPS) genes (Bohle et al., 2014). These differences are suggested to be related to the variation in virulence of different strains, host range and phenotypic characteristics of the colonies (Bohle et al., 2014). The present study was limited to only one strain of *P. salmonis* (LF-89), which has been well characterized in an early study in coho salmon (Fryer et al., 1992) and shown to be the most virulent, when compared to other two strains (ATL-4-91 and NOR-92) after an experimental challenge in this species (House et al., 1999). Although LF-89 is the most representative and virulent strain affecting coho salmon so far, further studies are required to evaluate if genetic variation detected in this study is present for resistance against other *P. salmonis* strains in this species.

Different molecular mechanisms underlie host resistance to specific pathogens (Glass, 2012). If there is a bacterial change, we can assume that the selected resistance can be either lost or maintained depending on the mechanisms involved in the trait. For instance, if the mechanisms that generate resistance are general and non-strain specific, the resistance can be maintained. However, if the resistance mechanisms are strain specific, we could assume that resistance can be lost. Recent studies have aimed at determining the molecular basis of resistance against *P. salmonis* in Atlantic salmon using genomic techniques. For instance, a study using a transcriptomic approach has found differential expression of genes associated to cellular iron depletion, coupled to low iron levels and bacterial load in the head kidney of resistant fish, which suggest that iron-deprivation is an innate immunity defense mechanism against *P. salmonis* (Pulgar et al., 2015a). Moreover, by means of a genome-wide association analysis, loci associated with resistance against *P. salmonis* have been found in two chromosomes in Atlantic salmon (Correa et al., 2015b). These genomic regions harbor candidate genes associated with different immunological processes. However, the total variance explained by these loci is still low, suggesting that there is a polygenic effect controlling this trait (Correa et al., 2015b). These results suggest that resistance to *P. salmonis* is a complex trait with multiple genetic factors governing the differential response against this pathogen. Further studies involving functional experiments in coho salmon and other salmonid species are needed to better unravel the resistance mechanisms. This information will be helpful to better understand the host-pathogen dynamics and foresee if resistance can be maintained or lost after both exposure and emergence of new pathogenic strains. Hence, more studies are needed to clarify both the epidemiological and ecological effects of improving resistance against *P. salmonis* and other specific pathogens in the long term under farming conditions. With the advent of novel genomic technologies, such as next-generation sequencing and high throughput genome-wide SNP genotyping methods (Houston et al., 2014; Yáñez et al., 2014d, 2016), a better understanding of the genomic basis of disease resistance traits is expected in the near future for aquaculture species (Yáñez et al., 2015).

Results from previous studies have demonstrated positive genetic correlations of low to moderate magnitude between growth-related traits and resistance to the bacterial diseases caused by *Aeromonas*

salmonicida in Atlantic salmon and brook charr (*Salvelinus fontinalis*) (Gjedrem et al., 1991; Perry et al., 2004). In rainbow trout (*Oncorhynchus mykiss*), a genetic correlation not different from zero has also been found between growth and resistance to *F. psychrophilum*, the causative agent of bacterial cold-water disease (Silverstein et al., 2009). In contrast, low and slightly negative genetic correlations ($r_g = -0.15 \pm 0.08$ to -0.19 ± 0.24) between resistance to two bacterial diseases (columnaris diseases and bacterial cold-water disease) and 9 and 12 months body weight have been found (Evenhuis et al., 2015). Similar results were found when assessing the genetic correlations between resistance against viral hemorrhagic septicemia (VHS) and growth-related traits in rainbow trout, with reported values of low to moderately negative magnitude ($r_g = -0.01$ to -0.33), depending on the growth trait analyzed (body weight, body length or feed conversion efficiency) and the age of fish (Henryon et al., 2002). In the same species a positive phenotypic relationship has been found between body weight and resistance to the diseases caused by *F. psychrophilum* and *Yersinia ruckeri*, respectively, however, the relationship between VHS and body weight remained negative for one population and positive for another (Henryon et al., 2005). Bangera et al. (2011) also reported a weak but slightly negative genetic correlation ($r_g = -0.25 \pm 0.16$) between body weight and resistance to vibriosis in Atlantic cod (*Gadus morhua* L.). Furthermore, a recent study has reported a slightly negative genetic correlation ($r_g = -0.19 \pm 0.12$) between resistance to *P. salmonis* and body weight and in Atlantic salmon (Yáñez et al., 2014c). Similarly, we determined a significant negative genetic correlation between PSR and HW of moderately high magnitude. Although there is not a general relationship among growth traits and resistance to specific pathogens in genetic terms, it seems to be a similar direction of the relationship between resistance against *P. salmonis* in coho and Atlantic salmon. These results may imply that selective breeding for faster growth in terms of HW will have a negative effect on *P. salmonis* resistance in these species.

One limitation of the present study is that neither non-Gaussian distributions nor data censoring were considered in the statistical model to analyze DD. This was mainly due to the difficulty of running more sophisticated and appropriate models (i.e. proportional hazard frailty models) in a multi-trait manner, which is needed to estimate genetic correlations between PSR and HW. Nevertheless, a previous study has shown that proportional hazard frailty models (assuming Weibull and Cox distributions) provided only a marginal increase in the predictive ability for *P. salmonis* resistance in Atlantic salmon when defined as DD and the correlation between estimated breeding values (EBVs) from these models and a linear model were greater than 0.98 (Yáñez et al., 2013). Thus, relaxing distributional assumptions and presence of data censoring may have a lower impact on the results presented here. However, these aspects should be further studied.

Genetic correlations among disease resistance traits have been reported in salmonids (For a review see Yáñez et al., 2014b). In general terms, these studies suggest that there is not a clear relationship among genetic resistance to different pathogens. These relationships must therefore be evaluated case by case. Further studies are needed to determine if mechanisms involved in resistance to *P. salmonis* may lead to decreased resistance against other important pathogens affecting coho salmon farming.

From a practical perspective, combined selection for these negatively correlated traits can be improved by the use of molecular information to assist genetic evaluations. The incorporation of molecular information can be particularly advantageous for increasing selection response for economically important traits for which responses are low, due to reduced accuracy of EBVs or unfavorable correlations with other characters with higher heritability (Dekkers, 2007). Another option to efficiently select for PSR and HW simultaneously is to improve for two specific lines in a single-trait manner (i.e. one line selected for PSR and another one for HW). These two lines can be crossed by means of frozen sperm to produce terminal crosses which can generate high genetic

merit animals for production. However, an evaluation of this selection approach should be carefully performed taking technical and economic aspects into account.

5. Conclusion

Our results demonstrate the presence of additive-genetic variation for both harvest weight and resistance to *P. salmonis* in coho salmon, indicating the viability of improving these traits by means of artificial selection. However, we identified a negative genetic correlation of intermediate magnitude between these two commercially important traits. This unfavorable genetic relationship must be accounted for when including them simultaneously into the breeding objective of coho salmon genetic improvement programs.

Acknowledgments

We would like to acknowledge Pesquera Antares S.A. which provided funding for the challenge test and the fish used in this study. This work has been partially funded by grants from CORFO (11IEI-12843), FONDEF – Newton–Picarte (IT1410100) a project funded by CONICYT (Government of Chile) and The British Council (Government of The United Kingdom), and U-inicia Grant, from VID, Universidad de Chile. This research was carried out in conjunction with EPIC4 (Enhanced Production in Coho: Culture, Community, Catch), a project supported by the government of Canada through Genome Canada, Genome British Columbia, and Genome Québec.

References

- Bangera, R., Ødegård, J., Præbel, A.K., Mortensen, A., Nielsen, H.M., 2011. Genetic correlations between growth rate and resistance to vibriosis and viral nervous necrosis in Atlantic cod (*Gadus morhua* L.). *Aquaculture* 317, 67–73.
- Bishop, S.C., Woolliams, J.A., 2014. Genomics and disease resistance studies in livestock. *Livest. Sci.* 166, 190–198.
- Bohle, H., Henríquez, P., Grothusen, H., Navas, E., Sandoval, A., Bustamante, F., Bustos, P., Mancilla, M., 2014. Comparative genome analysis of two isolates of the fish pathogen *Piscirickettsia salmonis* from different hosts reveals major differences in virulence-associated secretion systems. *Genome Announc.* 2, e01219-14.
- Casanova, A., Obreque, J., Gaggero, A., Landskron, E., Sandino, A., Jashes, M., 2003. Electrophoretic analysis of ITS from *Piscirickettsia salmonis* Chilean isolates. *FEMS Microbiol. Lett.* 225, 173–176.
- Correa, K., Filp, M., Cisterna, D., Cabrejos, M.E., Gallardo-Escarate, C., Yáñez, J.M., 2015a. Effect of triploidy in the expression of immune-related genes in coho salmon *Oncorhynchus kisutch* (Walbaum) infected with *Piscirickettsia salmonis*. *Aquac. Res.* 46, 59–63.
- Correa, K., Lhorente, J.P., López, M.E., Bassini, L., Naswa, S., Deeb, N., Di Genova, A., Maass, A., Davidson, W.S., Yáñez, J.M., 2015b. Genome-wide association analysis reveals loci associated with resistance against *Piscirickettsia salmonis* in two Atlantic salmon (*Salmo salar* L.) chromosomes. *BMC Genomics* 16, 854.
- Cvitanič, J.O., Garate, O., Smith, C.E., 1991. The isolation of a rickettsia-like organism causing disease and mortality in Chilean salmonid and its confirmation by Koch's postulate. *J. Fish Dis.* 14, 121–145.
- Dekkers, J.C.M., 2007. Prediction of response to marker-assisted and genomic selection using selection index theory. *J. Anim. Breed. Genet.* 124, 331–341.
- Evenhuis, J.P., Leeds, T.D., Marancik, D.P., LaPatra, S.E., Wiens, G.D., 2015. Rainbow trout (*Oncorhynchus mykiss*) resistance to columnaris disease is heritable and favorably correlated with bacterial cold water disease resistance. *J. Anim. Sci.* 93, 1546–1554.
- Falconer, D.S., Mackay, T.F.C., 1996. *Introduction to Quantitative Genetics*, fourth ed. Longman Group Limited, Harlow, Essex, U.K.
- FAO, 2014. Fisheries and Aquaculture Department [Online]. (Rome. Updated 31 January 2014. <http://www.fao.org/fishery/statistics/global-aquacultureproduction/en>).
- Fryer, J.L., Lannan, C., Giovannoni, S.J., Wood, N.D., 1992. *Piscirickettsia salmonis* gen. nov.: sp. nov. the causative agent of an epizootic disease in salmonid fishes. *Int. J. Syst. Bacteriol.* 42, 120–126.
- Gallardo, J.A., Lhorente, J.P., Neira, R., 2010. The consequences of including non-additive effects on the genetic evaluation of harvest body weight in Coho salmon (*Oncorhynchus kisutch*). *Genet. Sel. Evol.* 42, 19.
- Gilmour, A., Gogel, B., Cullis, B., Thompson, R., Butler, D., Cherry, M., Collins, D., Dutkowsk, G., Harding, S., Haskard, K., 2009. ASReml User Guide Release 3.0. VSN International Ltd., UK, p. 275 (<http://www.vsn.co.uk>).
- Gjedrem, T., 2012. Genetic improvement for the development of efficient global aquaculture: a personal opinion review. *Aquaculture* 344, 12–22.
- Gjedrem, T., Salte, R., Gjoen, H.M., 1991. Genetic variation in susceptibility of Atlantic salmon to furunculosis. *Aquaculture* 97, 1–6.
- Glass, E.J., 2012. The molecular pathways underlying host resistance and tolerance to pathogens. *Front. Genet.* 3, 263.
- Hamilton, M.A., Russo, R.C., Thurston, R.V., 1977. Trimmed Spearman–Kärber method for estimating median lethal concentrations in toxicity bioassays. *Environ. Sci. Technol.* 11, 714–719.
- Henryon, M., Berg, P., Olesen, N.J., Kjær, T.E., Slierendrecht, W.J., Jokumsen, A., Lund, I., 2005. Selective breeding provides an approach to increase resistance of rainbow trout (*Oncorhynchus mykiss*) to the diseases, enteric redmouth disease, rainbow trout fry syndrome, and viral haemorrhagic septicaemia. *Aquaculture* 250, 621–636.
- Henryon, M., Jokumsen, A., Berg, P., Lund, I., Pedersen, P.B., Olesen, N.J., Slierendrecht, W.J., 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. *Aquaculture* 209, 59–76.
- House, M.L., Bartholomew, J.L., Winton, J.R., Fryer, J.L., 1999. Relative virulence of three isolates of *Piscirickettsia salmonis* for coho salmon *Oncorhynchus kisutch*. *Dis. Aquat. Org.* 35, 107–113.
- Houston, R., Taggart, J., Cézard, T., Bekaert, M., Lowe, N., Downing, A., Talbot, R., Bishop, S., Archibald, A., Bron, J., Peman, D., Davassi, A., Brew, F., Tinch, A., Gharbi, K., 2014. Development and validation of a high density SNP genotyping array for Atlantic salmon (*Salmo salar*). *BMC Genomics* 15, 9.
- Kaplan, E.L., Meier, P., 1958. Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* 53, 457–481.
- Lynch, M., Walsh, B., 1998. *Genetics and Analysis of Quantitative Traits*. Sinauer Associates, Sunderland, MA.
- Mauel, M.J., Giovannoni, S.J., Fryer, J.L., 1999. Phylogenetic analysis of *Piscirickettsia salmonis* by 16S, internal transcribed spacer (ITS) and 23S ribosomal DNA sequencing. *Dis. Aquat. Org.* 35, 115–123.
- Neira, R., Díaz, N.F., Gall, G.A.E., Gallardo, J.A., Lhorente, J.P., Manterola, R., 2006. Genetic improvement in coho salmon (*Oncorhynchus kisutch*): I: selection response and inbreeding depression on harvest weight. *Aquaculture* 257, 9–17.
- Neira, R., Lhorente, J.P., Araneda, C., Díaz, N., Bustos, E., Alert, A., 2004. Studies on carcass quality traits in two populations of Coho salmon (*Oncorhynchus kisutch*): phenotypic and genetic parameters. *Aquaculture* 241, 117–131.
- Ødegård, J., Baranski, M., Gjerde, B., Gjedrem, T., 2011. Methodology for genetic evaluation of disease resistance in aquaculture species: challenges and future prospects. *Aquac. Res.* 42, 103–114.
- Perry, G.M., Tarte, P., Croisetiere, S., Belhumeur, P., Bernatchez, L., 2004. Genetic variance and covariance for 0+ brook charr (*Salvelinus fontinalis*) weight and survival time of furunculosis (*Aeromonas salmonicida*) exposure. *Aquaculture* 235, 263–271.
- Pulgar, R., Hödar, C., Travisany, D., Zuñiga, A., Domínguez, C., Maass, A., González, M., Cambiazo, V., 2015a. Transcriptional response of Atlantic salmon families to *Piscirickettsia salmonis* infection highlights the relevance of the iron-deprivation defence system. *BMC Genomics* 16, 495.
- Pulgar, R., Travisany, D., Zuñiga, A., Maass, A., Cambiazo, V., 2015b. Complete genome sequence of *Piscirickettsia salmonis* LF-89 (ATCC VR-1361) a major pathogen of farmed salmonid fish. *J. Biotechnol.* 212, 30–31.
- Rozas, M., Enríquez, R., 2014. *Piscirickettsiosis* and *Piscirickettsia salmonis* in fish: a review. *J. Fish Dis.* 37, 163–188.
- SERNAPESCA, 2015. Situación Sanitaria Salmonicultura Centros Marinos – Año 2014. [online]. Valparaíso, Chile. Updated 27 May 2015. http://www.sernapesca.cl/index.php?option=com_remository&Itemid=246&func=fileinfo&id=11083).
- Silverstein, J.T., Vallejo, R.L., Palti, Y., Leeds, T.D., Rexroad, C.E., Welch, T.J., Wiens, G.D., Ducrocq, V., 2009. Rainbow trout resistance to bacterial cold-water disease is moderately heritable and is not adversely correlated with growth. *J. Anim. Sci.* 87, 860–867.
- Visscher, P.M., Hill, W.G., Wray, N.R., 2008. Heritability in the genomics era—concepts and misconceptions. *Nat. Rev. Genet.* 9, 255–266.
- Waksman, G., Orlova, E.V., 2014. Structural organisation of the type IV secretion systems. *Curr. Opin. Microbiol.* 17, 24–31.
- Yáñez, J.M., Martínez, V., 2010. Genetic factors involved in resistance to infectious diseases in salmonids and their application in breeding programmes. *Arch. Med. Vet.* 42, 1–13.
- Yáñez, J.M., Bassini, L.N., Filp, M., Lhorente, J.P., Ponzoni, R.W., Neira, R., 2014a. Inbreeding and effective population size in a coho salmon (*Oncorhynchus kisutch*) breeding nucleus in Chile. *Aquaculture* 420, S15–S19.
- Yáñez, J.M., Houston, R.D., Newman, S., 2014b. Genetics and genomics of disease resistance in salmonid species. *Front. Genet.* 5, 415.
- Yáñez, J.M., Lhorente, J.P., Bassini, L.N., Oyarzún, M., Neira, R., Newman, S., 2014c. Genetic co-variation between resistance against both *Caligus rogercresseyi* and *Piscirickettsia salmonis*, and body weight in Atlantic salmon (*Salmo salar*). *Aquaculture* 433, 295–298.
- Yáñez, J.M., Bangera, R., Lhorente, J.P., Oyarzún, M., Neira, R., 2013. Quantitative genetic variation of resistance against *Piscirickettsia salmonis* in Atlantic salmon (*Salmo salar*). *Aquaculture* 414, 155–159.
- Yáñez, J.M., Naswa, S., López, M.E., Bassini, L., Cabrejos, M.E., Gilbey, J., Bernatchez, L., Norris, A., Soto, C., Eisenhart, J., Simpson, B., Neira, R., Lhorente, J.P., Schnable, P., Newman, S., Mileham, A., Deeb, N., 2014d. Development of a 200 K SNP array for Atlantic Salmon: exploiting across continents genetic variation. Proceedings of the 10th World Congress on Genetics Applied to Livestock Production, Vancouver, Canada.
- Yáñez, J.M., Naswa, S., López, M.E., Bassini, L., Correa, K., Gilbey, J., Bernatchez, L., Norris, A., Neira, R., Lhorente, J.P., Schnable, P.S., Newman, N., Mileham, A., Deeb, N., Di Genova, A., Maass, A., 2016. Genome wide single nucleotide polymorphism (SNP) discovery in Atlantic salmon (*Salmo salar*): validation in wild and farmed American and European populations. *Mol. Ecol. Resour.* <http://dx.doi.org/10.1111/1755-0998.12503> (Early View).
- Yáñez, J.M., Newman, S., Houston, R.D., 2015. Genomics in aquaculture to better understand species biology and accelerate genetic progress. *Front. Genet.* 6, 128.