animal

Genetic (co)variation in skin pigmentation patterns and growth in rainbow trout

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From a physiological-behavioral perspective, it has been shown that fish with a higher density of black eumelanin spots are more dominant, less sensitive to stress, have higher feed intake, better feed efficiency and therefore are larger in size. Thus, we hypothesized that genetic (co)variation between skin pigmentation patterns and growth exists and it is advantageous in rainbow trout. The objective of this study was to determine the genetic relationships between skin pigmentation patterns and BW in a breeding population of rainbow trout. We performed a genetic analysis of pigmentation traits including dorsal color (DC), lateral band (LB) intensity, amount of spotting above (SA) and below (SB) the lateral line, and BW at harvest (HW). Variance components were estimated using a multi-trait linear animal model fitted by restricted maximum likelihood. Estimated heritabilities were 0.08 ± 0.02, 0.17 ± 0.03, 0.44 ± 0.04, 0.17 ± 0.04 and 0.23 ± 0.04 for DC, LB, SA, SB and HW, respectively. Genetic correlations between HW and skin color traits were 0.42 ± 0.13, 0.32 ± 0.14 and 0.25 ± 0.11 for LB, SA and SB, respectively. These results indicate positive, but low to moderate genetic relationships between the amount of spotting and BW in rainbow trout. Thus, higher levels of spotting are genetically associated with better growth performance in this population.

Implications

In the present study, we found evidence establishing a positive correlation between harvest weight and skin pigmentation patterns, including melanin-based spottiness and intensity of the lateral band (LB). Therefore, selection on growth will not impact negatively skin patterns or conversely. The positive relationship between skin pigmentation patterns and BW, and their link with different types of physiological-behavioral individual responses (i.e. coping styles) must be further investigated.

Introduction

Aquaculture has an increasingly important role in global protein production for human consumption, reaching 70.5 million tons in 2012 (Food and Agriculture Organization (FAO), 2016), with rainbow trout (*Oncorhynchus mykiss*) being one of the most cultivated species. Rainbow trout was the second largest species of salmonid produced in 2014,

with an estimated world production of 812 939 tons, valued in the US $3631 million (FAO, 2016). Salmonids are particularly known for their wide genetic variation, life history and their adaptability to different places and environments. An example of variation among individuals is the amount of skin spottiness. In vertebrates, darker eumelanic individuals have been reported to be sexually more active, more aggressive, larger in size, less sensitive to stressful factors, and with a better immune function than lighter individuals of the same population (Ducrest *et al.*, 2008). In addition, it appears that melanin-based skin spots in rainbow trout is associated with various behavioral (feeding and activity), morphological and physiological characteristics, such as dominance and food consumption, characteristics that have also been associated to different coping styles: low response (LR) and high response (HR) individuals (Kittilsen *et al.*, 2009a). High feed efficiency and feeding motivation of LR fish might have an impact on economically important traits (e.g. harvest weight (HW)), making this kind of fish more desirable for farming purposes. Measuring levels of cortisol to identify LR fish can be expensive and labor-intensive.

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Pigmentation patterns can be indicative of LR type fish, given the relationship between both the number and size of melanin-based skin spots and the individual's coping style (Kittilsen *et al.*, 2009a). Thus, to identify and select fish that are more suitable for farming conditions in a simpler manner, the relationship between pigmentation patterns and other desirable characteristics can be exploited.

The success of selective breeding is influenced by levels of additive genetic variation present for the trait and by the genetic correlations with other relevant traits included in the breeding goal, which must not be disadvantageous (Falconer and Mackay, 1996). Quantitative genetic studies in several species of farmed fish have analyzed characteristics such as growth, body size, age at maturity and meat quality (Gjedrem, 2012). Furthermore, previous studies have identified the presence of significant genetic variation for skin color and a melanin-based spottiness in rainbow trout, indicating the potential for improving these traits by means of selective breeding (Kause *et al.*, 2003 and 2004). The same previous studies have investigated the relationships of skin color and spottiness with growth and body shape in a Finnish population of rainbow trout (Kause *et al.*, 2003 and 2004) and as the genetics of skin color can be environment- (Kause *et al.*, 2004) and population-dependent, we considered necessary to re-evaluate these links in Chilean farmed rainbow trout. These authors did not find significant correlations between body composition and growth with skin color patterns. They judged skin color in terms of commercial influence, which means that silvery color with few spots is desirable. In the present study, skin color is judged in terms of supposed beneficial effects on growth performance, which means that intense coloration and spottiness are advantageous.

In this study, we hypothesized that genetic (co)variation between skin pigmentation patterns and growth exists and it is positive in rainbow trout. The objective of this study was to determine the genetic relationships between skin pigmentation patterns and BW in a breeding population of rainbow trout, to show that selection on growth will not impact negatively skin patterns or conversely. For this, we assess the levels of quantitative genetic variation for different traits related with skin pigmentation patterns in rainbow trout, including dorsal color (DC), the intensity of LB, melanin-based spottiness above (SA) and below (SB) the LB. The genetic correlation among these traits and HW were also determined

to demonstrate the potential relationship between skin pigmentation patterns and a growth-related trait.

Material and methods

Population and traits

This work was carried out in a rainbow trout (*O. mykiss*) breeding population from a genetic improvement program established in 1998 by Aguas Claras S.A. in Puerto Montt, Chile (Yoshida *et al.*, 2018a and 2018b). A total of four generations representing the years 1998, 2001, 2004 and 2007 were included in the study, reflecting a total of 40 429 pedigree-recorded fish. Each generation with a mean of 90 families were generated according to a nested design in which one male was mated on average to three females. Eggs from each family were incubated independently in one tank per family. Thus, eyed-eggs were transferred to family tanks for hatching. Every year during May fish from each family were passive integrated transponder tagged at about 5 to 7 g to keep pedigree traceability. Table 1 summarizes pedigree structure for the breeding population across years. Fish were then transferred to fresh water facilities to begin smoltification. An average number of 60.5 (SD = 5.7) to 205.2 (SD = 58.1) individuals from each family, depending on generation, were randomly divided into two fresh water tanks. After smoltification, fish were transferred to sea cages, keeping the tank distribution defined during fresh water rearing. The farming process finished at an average of 22 months post-spawning and at ~ 3.7 kg, in which HW and skin color scores were recorded for all fish. Within the same year, fish were harvested within a period of 1 to 2 months. Four skin color pattern scores were generated to visually classify fish into different categories. Dorsal color was divided into three categories according to the color at the back (blue (0), gray (1) or light green to brown (2)), intensity of the LB (none (0), tenuous (1), median (2) or marked (3)) and intensity of SA and SB the LB were divided into four categories according to the number of melanin spots and intensity (none (0), limited (1), median (2) and abundant (3)). The skin color scores were always given by the same person within generations. The descriptions of the different categories for each score are shown in Table 2. The dataset was comprised phenotypes for a total of 20 542, 20 560, 20 541, 20 541 and 19 615 records for DC, LB, SB, SA and HW, respectively.

Table 1 *Pedigree structure of the breeding population of rainbow trout (*Oncorhynchus mykiss*) by year*

| Years | Number of sires | Number of dams | Number of offspring | | | |
			Total number	Mean/family (SD)	Minimum/family	Maximum/family
1998	25	112	6774	60.5 (5.7)	59	120
2001	21	50	10 440	205.2 (58.1)	100	303
2004	32	105	8634	90.3 (1.4)	89	90
2007	43	94	14 581	155.2 (22.2)	151	304
Total	121	361	40 429	113.9 (16.0)	59	304

Table 2 *Appearance scores for each skin color category for dorsal color (DC), intensity of lateral band (LB) and intensity of spots above (SA) and below (SB) lateral band in a rainbow trout (*Oncorhynchus mykiss*) population*

Traits	Appearance scores			
	0	1	2	3
DC	Blue	Gray	Light green or brown	
LB	None	Tenuous	Median	Marked
SB	None	Limited	Median	Abundant
SA	None	Limited	Median	Abundant

Statistical analysis

To estimate the variance and co-variance components for DC (y_1), LB (y_2), SA (y_3), SB (y_4) and HW (y_5), we used the following multi-trait linear animal model:

$$
\begin{bmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \end{bmatrix} = \begin{bmatrix} X_1 & 0 & 0 & 0 & 0 \\ 0 & X_2 & 0 & 0 & 0 \\ 0 & 0 & X_3 & 0 & 0 \\ 0 & 0 & 0 & X_4 & 0 \\ 0 & 0 & 0 & 0 & X_5 \end{bmatrix} \begin{bmatrix} b_1 \\ b_2 \\ b_3 \\ b_4 \\ b_5 \end{bmatrix} + \\
\begin{bmatrix} Z_1 & 0 & 0 & 0 & 0 \\ 0 & Z_2 & 0 & 0 & 0 \\ 0 & 0 & Z_3 & 0 & 0 \\ 0 & 0 & 0 & Z_4 & 0 \\ 0 & 0 & 0 & 0 & Z_5 \end{bmatrix} \begin{bmatrix} u_1 \\ u_2 \\ u_3 \\ u_4 \\ u_5 \end{bmatrix} \\
+ \begin{bmatrix} W_1 & 0 & 0 & 0 & 0 \\ 0 & W_2 & 0 & 0 & 0 \\ 0 & 0 & W_3 & 0 & 0 \\ 0 & 0 & 0 & W_4 & 0 \\ 0 & 0 & 0 & 0 & W_5 \end{bmatrix} \begin{bmatrix} c_1 \\ c_2 \\ c_3 \\ c_4 \\ c_5 \end{bmatrix} + \begin{bmatrix} e_1 \\ e_2 \\ e_3 \\ e_4 \\ e_5 \end{bmatrix}
$$

where y_1, y_2, y_3, y_4 and y_5 are vectors of phenotypic records for DC, LB, SA, SB and HW, respectively; b_i the vectors of fixed effects for trait i ($= 1, 2, 3, 4$ and 5), all including the contemporary group of sea cage:sex:year as factor and harvest age (HA) as covariate; u_i, c_i and e_i the vectors of random animal genetic effects, common environment effects related to full-sib families and residual effects, respectively, for trait i; and X_i, Z_i and W_i the design matrices for trait i.

For all traits, the animal, common environment, and residual effects were assumed random:

$$u = \begin{bmatrix} u_1' & u_2' & u_3' & u_4' & u_5' \end{bmatrix}' \sim N(0, G_0 \otimes A),$$

$$c = \begin{bmatrix} c_1' & c_2' & c_3' & c_4' & c_5' \end{bmatrix}' \sim N(0, C_0 \otimes I_C),$$

$$e = \begin{bmatrix} e_1' & e_2' & e_3' & e_4' & e_5' \end{bmatrix}' \sim N(0, R_0 \otimes I_N)$$

where A is the additive genetic relationship matrix constructed from the pedigree records, I_C and I_N are identity matrices with dimension C and N, respectively, $\otimes$ indicates the Kronecker product. G_0, C_0 and R_0 are 5×5 co-variance matrices of animal additive genetic, common environment and residual effects, respectively. The random common environment

effect related to full-sib families was assessed preliminarily based on a likelihood ratio test carried out by means of fitting single-trait models. This effect was statistically significant ($P < 0.05$) for all traits. The ASREML software (Gilmour *et al.*, 2009) was used to fit the multi-trait model described above to estimate the variance and co-variance components for DC, LB, SA, SB and HW.

Heritability and genetic correlations

For each trait i ($= 1, 2, 3, 4$ and 5) the heritability (h_i^2) and common environment (c_i^2) were calculated as follows:

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

$$c_i^2 = \frac{(\sigma_{C_i}^2)}{(\sigma_{G_i}^2 + \sigma_{C_i}^2 + \sigma_{E_i}^2)}$$

where $\sigma_{G_i}^2$, $\sigma_{C_i}^2$ and $\sigma_{E_i}^2$ are the additive genetic, common environment and residual variances from G_0, C_0, and R_0 matrices, respectively. The phenotypic (r_p) and genetic (r_g) correlations between traits x and y were determined as (Falconer and Mackay, 1996):

$$r_{xy} = \frac{\sigma_{x,y}}{\sqrt{\sigma_x^2 \sigma_y^2}}$$

where $\sigma_{x,y}$ is the additive genetic or phenotypic co-variance between x and y, σ_x^2 the additive genetic or phenotypic variance of x and σ_y^2 is the additive genetic or phenotypic variance of y.

Results

Phenotypic variation

We found a considerable phenotypic variation for skin color scores. The number of records, absolute and relative frequencies of the skin color scores for DC, LB, SA and SB recorded at harvest time are shown in Table 3. For DC the relative frequencies varied between 1.6% to 7.2%, 3.9% to 40% and 52.8% to 94.6% across the 4 years for categories 0, 1 and 2, respectively. Considerable phenotypic variation was also present for HW. Table 4 shows the number of records and descriptive statistics for HW and HA divided by year. Overall, the mean (and standard deviation) for HW and HA were 3.4 ± 1.10 kg and 682.92 ± 73.13 days, respectively, across 19 615 records measured during the 4 years. The minimum and maximum values for HW and HA across the 4 years ranged from 0.3 to 7.2 kg and 564 and 818 days, respectively.

Table 3 *Sample size (n), absolute frequency (AF) and relative frequency (RF) for each skin color category for dorsal color (DC), intensity of lateral band (LB) and intensity of spots below (SB) and above (SA) lateral band in a rainbow trout (*Oncorhynchus mykiss*) population per year*

Appearance scores			0		1		2		3	
Traits	Year	n	AF	RF	AF	RF	AF	RF	AF	RF
DC	2001	2715	174	6.4	998	36.8	1543	56.8	-	-
	2004	3635	58	1.6	140	3.9	3437	94.6	-	-
	2007	5134	226	4.4	1530	29.8	3378	65.8	-	-
	2010	9076	649	7.2	3632	40.0	4795	52.8	-	-
LB	2001	2715	1240	45.7	847	31.2	518	19.1	110	4.1
	2004	3635	1518	41.8	1356	37.3	531	14.6	230	6.3
	2007	5134	814	15.9	2059	40.1	1236	24.1	1025	20.0
	2010	9058	6994	77.2	1903	21.0	76	0.8	85	0.9
SB	2001	2715	419	15.4	1225	45.1	739	27.2	332	12.2
	2004	3633	929	25.6	1968	54.2	638	17.6	98	2.7
	2007	5134	1385	27.0	2348	45.7	991	19.3	410	8.0
	2010	9059	1692	18.7	6731	74.3	412	4.5	224	2.5
SA	2001	2715	27	1.0	214	7.9	584	21.5	1890	69.6
	2004	3635	123	3.4	701	19.3	1306	35.9	1505	41.4
	2007	5133	138	2.7	855	16.7	1220	23.8	2920	56.9
	2010	9058	30	0.3	131	1.4	607	6.7	8290	91.5

Table 4 *Summary statistics for harvest weight (HW) and harvest age (HA) in a rainbow trout (*Oncorhynchus mykiss*) population per year*

Traits	Year	n[a]	Mean	SD	CV	Min	Max
HW (kg)	2001	2942	4.12	1.35	32.77	0.4	7.1
	2004	3577	4.3	1.06	24.75	0.3	7.2
	2007	5117	3.52	0.78	1.54	0.73	5.9
	2010	7979	2.67	0.55	0.79	0.4	4.3
HA (days)	2001	2981	580.97	11.71	2.02	564	610
	2004	3638	660.72	13.13	1.99	632	681
	2007	5134	627.32	5.73	0.91	614	642
	2010	8079	765.87	8.82	1.15	743	818

Min = mínimum; Max = máximum.
[a]Number of fish included in the analysis after removing outliers by interquartile range rule and discarding missing values.

Heritabilities and common environment effect

The estimated variance components and heritabilities for DC, LD, SA, SB and HW are shown in Table 5. We identified significant additive genetic variation for all analyzed traits. Moderate values of heritability were estimated for LB (0.17 ± 0.03), SA (0.17 ± 0.04) and HW (0.24 ± 0.04). A low and a relatively high heritability were found for DC (0.08 ± 0.02) and SB (0.44 ± 0.04), respectively. Regarding skin color traits, the common environment effect ranged from 3.4% to 11.4% of phenotypic variance for DC and SA, respectively. In addition, the common environment effect for HW accounted for 8.7% of the phenotypic variance.

Phenotypic and genetic correlations

The phenotypic and genetic correlations between DC, LD, SA, SB and HW are shown in Table 6. All the genetic correlations were significant and positive, except for the genetic correlations between DC and SA and DC and HW, which were not significantly different from zero. The highest and the lowest significant genetic correlations were found between SB and SA (0.73 ± 0.07) and SB and HW (0.25 ± 0.11), respectively. Thus, the magnitude of all of the significant genetic correlations were from moderate to high values. All the phenotypic correlations were significant and positive, except for the phenotypic correlations between DC and HW, which was significantly different from zero of low magnitude and negative, and SB and HW that was not significantly different from zero.

Discussion

The genetic analysis performed in the present study revealed significant genetic variation for skin color traits. There are few previous studies aimed at determining heritability values for skin color traits in farmed rainbow trout. For instance, low to moderate heritability values for skin color, ranging from 0.13 ± 0.03 to 0.29 ± 0.10, were found when the trait was scored in three different categories: silver shining, dark silver and dark color (Kause *et al.*, 2003 and 2004). In addition, moderate to high heritability for melanin-based skin spottiness, ranging from 0.45 ± 0.12 to 0.6 ± 0.05, were found when the trait was categorized as few/little, moderate and many/large spots (Kause *et al.*, 2003 and 2004). In the present study, we found a low heritability for DC (scored gray, blue or light green to brown), moderate values for intensity of the LB and spottiness above it (SA), and a moderately high heritability for spottiness below the LB (SB) (Table 5). These results confirm the existence of genetic variation for skin color-related appearance traits in farmed populations of rainbow trout using different trait definitions involving different areas of the skin surface. It is important to mention that, there is a high variation between years for DC, with an extreme frequency distribution in 2004, with 94% of fish in the same category. This could be a bias due to the person recording the trait or a high environmental effect on this trait during this particular year and may be explaining the low heritability value for DC. We also found a significant common environment effect for all skin color traits, which can be associated with the separate rearing of full-sib families before tagging, ranging from $c^2 = 0.03$ to 0.11. This common environment effect was also present for skin color traits in previous studies, however, the range of values for this effect was slightly smaller ($c^2 = 0.01$ to 0.04) (Kause *et al.*, 2003 and 2004).

Previous studies have demonstrated the existence of significant genetic variation for BW in rainbow trout. For example, low to moderate heritability values have been estimated for BW ranging from 0.16 ± 0.02 to 0.37 ± 0.04 (Henryon *et al.*, 2002; Pante *et al.*, 2002; Haffray *et al.*, 2012; Janhunen *et al.*, 2012; Sae-Lim *et al.*, 2015; Flores-Mara *et al.*, 2017). Here, we found heritability for BW at harvest of moderate magnitude (0.23 ± 0.04) and within the range previously reported. The presence of a significant common

Table 5 *Estimate variance components and heritabilities for dorsal color (DC), intensity of lateral band (LB) and intensity of spots above (SA) and below (SB) lateral band and weight at harvest (HW) in a rainbow trout (*Oncorhynchus mykiss*) population*

Traits	$h^2 \pm SE$[a]	$c^2 \pm SE$[b]	σ_a^2[c]	$\sigma_c^2 \pm SE$[d]	$\sigma_e^2 \pm SE$[e]	$\sigma_p^2 \pm SE$[f]
DC	0.08 ± 0.02	0.03 ± 0.01	0.03 ± 0.01	0.01 ± 0.01	0.28 ± 0.01	0.31 ± 0.01
LB	0.17 ± 0.03	0.07 ± 0.01	0.10 ± 0.02	0.04 ± 0.01	0.44 ± 0.01	0.57 ± 0.01
SB	0.44 ± 0.04	0.05 ± 0.01	0.25 ± 0.03	0.03 ± 0.01	0.29 ± 0.02	0.57 ± 0.01
SA	0.17 ± 0.04	0.11 ± 0.01	0.08 ± 0.02	0.05 ± 0.01	0.31 ± 0.01	0.44 ± 0.01
HW	0.23 ± 0.04	0.09 ± 0.03	0.11 ± 0.02	0.03 ± 0.01	0.35 ± 0.01	0.49 ± 0.01

[a]Heritability (h^2).
[b]Effect of the common environment (c^2).
[c]Additive variance (σ_a^2).
[d]Common environment variance (σ_c^2).
[e]Residual variance (σ_e^2).
[f]Phenotypic variance (σ_p^2).

Table 6 *Phenotypic correlations (below diagonal) and genetic correlations (above diagonal) for dorsal color (DC), intensity of lateral band (LB) and intensity of spots above (SA) and below (SB) lateral band and weight at harvest (HW) in a rainbow trout (*Oncorhynchus mykiss*) population*

Traits	DC	LB	SB	SA	HW
DC	–	0.62 ± 0.13	0.39 ± 0.11	0.29 ± 0.16	-0.11 ± 0.15
LB	0.13 ± 0.01	–	0.53 ± 0.10	0.57 ± 0.13	0.42 ± 0.13
SB	0.13 ± 0.01	0.28 ± 0.01	–	0.73 ± 0.07	0.25 ± 0.11
SA	0.12 ± 0.01	0.22 ± 0.01	0.40 ± 0.01	–	0.32 ± 0.14
HW	-0.09 ± 0.01	0.13 ± 0.01	0.04 ± 0.02	0.04 ± 0.01	–

environment effect for BW measured at different times in salmonid species, which might lead to an overestimation of the genetic variance for this trait in a particular population if not included in the analysis has also been demonstrated previously. Here, we also found a significant effect associated with the common environment for HW due to the common rearing of full siblings before tagging. The magnitude of the common environment effect for HW expressed as a ratio of the phenotypic variance is slightly higher ($c^2 = 0.09$) than those previously reported for rainbow trout: $c^2 = 0.02$ to 0.08 (Pante *et al.*, 2002; Janhunen *et al.*, 2012; Sae-Lim *et al.*, 2015; Flores-Mara *et al.*, 2017), and coho salmon and other salmonid species from the same genus, $c^2 = 0.02$ to 0.06 (Gallardo *et al.*, 2010; Dufflocq *et al.*, 2017; Yáñez *et al.*, 2016a).

Phenotypic and genetic correlations among skin color traits and spotting were mostly positive and of moderate to high magnitude, except for the correlation between DC and SA which was not significantly different from zero (Table 6). These results indicate positive genetic relationships among different skin color traits. Such correlations suggest either positive pleiotropy among the different skin color traits or linkage disequilibrium between the regions controlling them. The pleiotropic effects of key regulators of melanogenesis have been previously suggested as responsible of a widespread association between melanin-based coloration and other behavioral and phenotypic traits in vertebrates, such as growth (Ducrest *et al.*, 2008). From a genetic improvement perspective, these results also indicate that skin color traits can be simultaneously improved by artificial selection.

In the present study, we used mixed linear models to estimate genetic parameters on the observed scale for color skin patterns traits, which are traits of a categorical nature. This can have implications on the genetic parameters estimation. For instance, Kause *et al.* (2003 and 2004) found that the heritability values of skin color patterns presented in the underlying scale and estimated using a threshold model were always higher than the heritability values presented on the observed scale and estimated using a linear model. Thus, the heritability values for the categorical traits presented here may be underestimated if compared to values obtained using threshold models. We used a linear multi-trait approach to assess the genetic relationships between all the skin color pattern traits and BW. This approach was chosen in order to compare the results against the previous studies by Kause *et al.* (2003 and 2004), in which the authors did not fit multi-trait threshold models because of computing limitations. The genetic correlations presented here are on the observed scale, and they can be directly compared against those obtained by Kause *et al.* (2003 and 2004).

The amount of melanin-based spots and the intensity of the LB were positively correlated with HW. However, the correlation between DC and HW was not significantly different from zero. Kause *et al.* (2003) found that correlations among skin color patterns and conformational body traits, including condition factor, body shape and body mass measured at 2 and 3 years of age were not significantly different from zero. It is important noting that when a fish has few or many spots after smoltification, these will remain almost invariable during growth and under different rearing

environments (fresh water and sea) while the skin color may be more flexible (Kause *et al*., 2004). It has also been reported that rapid growth is correlated with the silvery appearance of salmonid smolts (Rodgers *et al*., 1987). Thus, our results differ from previous studies and indicate that melanin-based spottiness and intensity of LB have a positive genetic relationship with growth in rainbow trout. These differences may be due to the fact that we used a different population of farmed fish, implying a different genetic background and consequently different allele frequencies and linkage disequilibrium between causative variants controlling skin color patterns and growth. It is also important noting that we used a larger dataset compared to previous studies, which can have had an impact on statistical power to detect significant correlations between skin color patterns and BW. Another potential explanation for the differences between the present and previous studies are differences in trait definitions; for instance, Kause *et al*. (2004) used body shape, skin color and skin spots as appearance traits each with three scoring categories, Kause *et al*. (2003) used the same trait definitions plus body mass measured at age of 2 and 3 years and condition factor, while in the present study we used appearance scores with three categories for DC and four categories for intensity of LB and intensity of spots above and below LB and BW measured at harvest. The differing results between this work and previous studies must be considered, and further evidence must be carefully evaluated before making general conclusions on the relationships between skin color patterns and growth in salmonids.

The positive relationship between skin pigmentation patterns and BW, and their link with different types of physiological-behavioral individual responses (i.e. coping styles) must be further investigated (Castanheira *et al*., 2015). A previous study have shown that rainbow trout with more melanic pigmentation have a reduced cortisol response (Kittilsen *et al*., 2009b). Lower cortisol release associated with LR individuals has also been reported to be associated with other beneficial characteristics for aquaculture such as higher feed intake and feed motivation (Øverli *et al*., 2002), higher disease resistance (Kittilsen *et al*., 2012) and ease in routine formation (Ruíz-Gomez *et al*., 2011). On the other hand, HR individuals, that have lighter pigmentation, have expressed higher mortality and lower capacity to cope with multiple stressors (Fevolden *et al*., 2003), such as those present in aquaculture systems. Feed intake differences have also been reported between LR and HR individuals. For example, Kittilsen *et al*. (2009a) reported a generally quicker resumption of normal feeding behavior in spotted (LR) salmon after confronted with a stressor (isolation), and also less time moving during acute stress, decreasing energy loss. Based on the literature, it seems that there is an association between skin and response to farm conditions, which lead us to support the hypotheses that the favorable genetic correlation between skin color patterns and growth might be explained by genetic links between coping styles and both appearance and growth traits. Thus since in our study, there is a moderate correlation between skin color and growth, it is

possible that selection for growth will impact positively or at least not negatively on response to farm conditions. However, this hypothesis should be confirmed by estimating genetic correlations between growth and response to farm conditions. Thus, the genetic relationship between skin color patterns and different coping styles (HR and LR), and its potential impact on growth-related traits in farmed rainbow trout still remains to be proven in further studies.

The commercial implications of skin color and spottiness need to be taken into account; since they are important production traits that affect consumers' acceptance (Colihueque, 2010). Although to our knowledge no studies have addressed Chilean consumers perception of these traits, because one of the main markets is the Japanese one, this external aspect is critical since Japanese consumers demand specimens with few spots and silvery (Taub and Palacios, 2003). Thus, the correlated response of selection for growth towards more spotted fish could have a negative commercial impact for Chilean exports of rainbow trout products to the Japanese market.

In the present study, we found evidence establishing a positive correlation between HW and skin pigmentation patterns, including melanin-based spottiness and intensity of the LB. Melanin has been linked to the physiological stress response and behavior in different vertebrates (Ducrest *et al*., 2008). For example, the melanin-like pigment is positively correlated with dominance in mammals, birds and salmonids (Kittilsen *et al*., 2009b; Backström *et al*., 2015), suggesting that those salmon most densely spotted are more aggressive, and more dominant, so that the feed consumption after the stress is not affected. In addition, it has been reported that those salmon with a large number of skin spots are more resistant to stress and ectoparasites (Kittilsen *et al*., 2009a; Øverli *et al*., 2014). Alternatively, Pérez *et al*. (2012), in an *in vitro* assay with *Cichlasoma dimerus* showed that the concentration of melanin is involved in the regulation of skin color in the fish and is also responsible for regulating the expression and synthesis of growth hormone. Furthermore, melanin can regulate food intake in mammals and fish, acting as an appetite stimulant (Takahashi *et al*., 2004; Yamanome *et al*., 2005). Our results clearly show that higher pigmentation is genetically associated with higher HW in trout. If this increased BW is genetically associated with the effect that melanin triggers at the physiological and cellular level in farmed rainbow trout still remains to be proven.

Further studies aimed at unraveling the molecular mechanisms of the genetic relationships between skin pigmentation patterns and growth found in this study are needed. With the advent of high throughput genome-wide single nucleotide polymorphism genotyping methods, already available for salmonid species (Houston *et al*., 2014; Palti *et al*., 2015; Yáñez *et al*., 2016b), and next-generation sequencing technologies, a better understanding of the molecular basis of complex traits at the genomic level is expected in the near future (Yáñez *et al*., 2015). In fact, genomic technologies have recently allowed the identification of genomic regions involved in growth traits in different

salmonid salmonid species by means of genome-wide association studies (Gutierrez *et al.*, 2015; Tsai *et al.*, 2015; Gonzalez-Pena *et al.*, 2016; Yoshida *et al.*, 2017). The discovery of functional variants for this and other traits could be facilitated by the international initiative on Functional Annotation of All Salmonid Genomes (Macqueen *et al.*, 2017). Further studies are required in order to identify genomic regions associated to skin pigmentation patterns. These studies will be crucial to confirm and provide further insights into the genetic relationship between skin pigmentation patterns and HW. Further research should aim study in detail the association between these traits and the implications they can have for response to stress and fish welfare in aquaculture systems.

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Declaration of interest
The authors declare no conflict of interest.

Ethics statement
The research protocol was approved by bioethics committee of Chile University.

Software and data repository resources
The data sets and programs used in the current study are available from the corresponding author on reasonable request.

References

Backström T, Heynen M, Brännäs E, Nilsson J and Magnhagen C 2015. Dominance and stress signalling of carotenoid pigmentation in Arctic charr (*Salvelinus alpinus*): lateralization effects? Physiology and Behavior 138, 52–57.

Castanheira MF, Conceicao LEC, Millot S, Rey S, Bégout ML, Damsgard B, Kristiansen T, Höglund E, Øverli Ø and Martins CIM 2015. Coping styles in farmed fish: consequences for aquaculture. Reviews in Aquaculture 7, 1–19.

Colihueque N 2010. Genetics of salmonid skin pigmentation: clues and prospects for improving the external appearance of farmed salmonids. Reviews in Fish Biology and Fisheries 20, 71–86.

Ducrest AL, Keller L and Roulin A 2008. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends in Ecology & Evolution 23, 502–510.

Dufflocq P, Lhorente JP, Bangera R, Neira R, Newman S and Yáñez JM 2017. Correlated response of flesh color to selection for harvest weight in coho salmon (*Oncorhynchus kisutch*). Aquaculture 472, 38–43.

Falconer DS and Mackay TFC 1996. Introduction to quantitative genetics, 4th edition. Essex, UK.

Food and Agriculture Organization (FAO) 2016. The state of world fisheries and aquaculture contributing to food security and nutrition for all. FAO, Rome, Italy.

Fevolden SE, Røed K and Fjalestad K 2003. A combined salt and confinement stress enhances mortality in rainbow trout (*Onchorhynchus mykiss*) selected for high stress responsiveness. Aquaculture 216, 67–76.

Flores-Mara R, Rodríguez FH, Bangera R, Lhorente JP, Neira R, Newman S and Yáñez JM 2017. Resistance against infectious pancreatic necrosis exhibits significant genetic variation and is not genetically correlated with harvest weight in rainbow trout (*Oncorhynchus mykiss*). Aquaculture 479, 155–160.

Gallardo JA, Lhorente JP and Neira R 2010. The consequences of including non-additive effects on the genetic evaluation of harvest body weight in Coho salmon (*Oncorhynchus kisutch*). Genetics Selection Evolution 42, 19.

Gilmour AR, Gogel BJ, Cullis BR, Thompson R and Butler D 2009. ASReml user guide release 3.0. VSN International Ltd, Hemel Hempstead, UK.

Gjedrem T 2012. Genetic improvement for the development of efficient global aquaculture: a personal opinion review. Aquaculture 344, 12–22.

Gonzalez-Pena D, Gao G, Baranski M, Moen T, Cleveland BM, Kenney PB, Vallejo RL, Palti Y and Leeds TD 2016. Genome-wide association study for identifying loci that affect fillet yield, carcass, and body weight traits in rainbow trout (*Oncorhynchus mykiss*). Frontiers in Genetics 7, 203.

Gutierrez AP, Yáñez JM, Fukui S, Swift B and Davidson WS 2015. Genome-wide association study (GWAS) for growth rate and age at sexual maturation in Atlantic salmon (*Salmo salar*). PLoS ONE 10, e0119730.

Haffray P, Bugeon J, Pincent C, Chapuis H, Mazeiraud E, Rossignol MN, Chatain B, Vandeputte M and Dupont-Nivet M 2012. Negative genetic correlations between production traits and head or bony tissues in large all-female rainbow trout (*Oncorhynchus mykiss*). Aquaculture 368, 145–152.

Henryon M, Jokumsen A, Berg P, Lund I, Pedersen PB, Olesen NJ and Slierendrecht WJ 2002. Genetic variation for growth rate, feed conversion efficiency, and disease resistance exists within a farmed population of rainbow trout. Aquaculture 209, 59–76.

Houston RD, Taggart JB, Cézard T, Bekaert M, Lowe NR, Downing A, Talbot R, Bishop SC, Archibald AL, Bron JE, Penman DJ, Davassi A, Brew F, Tinch AE, Gharbi K and Hamilton A 2014. Development and validation of a high density SNP genotyping array for Atlantic salmon (*Salmo salar*). BMC Genomics 15, 90.

Janhunen M, Kause A, Vehviläinen H and Järvisalo O 2012. Genetics of micro-environmental sensitivity of body weight in rainbow trout (*Oncorhynchus mykiss*) selected for improved growth. PLoS ONE 7, e38766.

Kause A, Ritola O and Paananen T 2004. Breeding for improved appearance of large rainbow trout in two production environments. Aquaculture Research 35, 924–930.

Kause A, Ritola O, Paananen T, Eskelinen U and Mäntysaari E 2003. Big and beautiful? Quantitative genetic parameters for appearance of large rainbow trout. Journal of Fish Biology 62, 610–622.

Kittilsen S, Ellis T, Schjolden J, Braastad B and Øverli Ø 2009b. Determining stress-responsiveness in family groups of Atlantic salmon (*Salmo salar*) using non-invasive measures. Aquaculture 298, 146–152.

Kittilsen S, Johansen I, Braastad B and Øverli Ø 2012. Pigments, parasites and personalitiy: towards a unifying role for steroid hormones? PLoS ONE 7, e34281.

Kittilsen S, Schjolden J, Beitnes-Johansen I, Shaw J, Pottinger T, Sørensen C, Braastad BO, Bakken M and Øverli Ø 2009a. Melanin-based skin spots reflect stress responsiveness in salmonid fish. Hormones and Behavior 56, 292–298.

Macqueen DJ, Primmer, C, Houston R, Nowak B, Bernatchez L, Bergseth S, Davidson WS, Gallardo-Escárate C, Goldammer T, Guiguen Y, Iturra P, Kijas JW, Koop B, Lien S, Maass A, Martin S, McGinnity P, Montecino M, Naish K, Nichols K, Ólafsson K, Omholt S, Palti Y, Plastow G, Rexroad C 3rd, Rise M, Ritchie R, Sandve SR, Schulte P, Tello A, Vidal R, Vik JO, Wargelius A and Yáñez JM 2017. Functional Annotation of All Salmonid Genomes (FAASG): an international initiative supporting future salmonid research, conservation and aquaculture. BMC Genomics 18, 484.

Øverli Ø, Nordgreen J, Mejdell C, Janczak A, Kittilsen S, Johansen IB and Horsberg TE 2014. Ectoparasitic sea lice (*Lepeophtheirus salmonis*) affect behavior and brain serotonergic activity in Atlantic salmon (*Salmo salar L.*): perspectives on animal welfare. Physiology and Behavior 132, 44–50.

Øverli Ø, Pottinger TG, Carrick TR, Øverli E and Winberg S 2002. Differences in behavior between rainbow trout selected for high- and low-stress responsiveness. Journal of Experimental Biology 205, 391–395.

Palti Y, Gao G, Liu S, Kent MP, Lien S, Miller MR, Rexroad CE 3rd and Moen T 2015. The development and characterization of a 57K single nucleotide polymorphism array for rainbow trout. Molecular Ecology Resources 15, 662–672.

Pante MJR, Gjerde B, McMillan I and Misztal I 2002. Estimation of additive and dominance genetic variances for body weight at harvest in rainbow trout, Oncorhynchus mykiss. Aquaculture 204, 383–392.

Pérez SD, Cánepa M, Fossati M, Fernandino J, Delgadin T, Canosa L, Somoza GM and Vissio PG 2012. Melanin concentrating hormone (MCH) is involved in the regulation of growth hormone in *Cichlasoma dimerus* (Cichlidae Teleostei). General and Comparative Endocrinology 176, 102–111.

Rodgers JD, Ewing RD and Hall JD 1987. Physiological changes during seaward migration of wild juvenile coho salmon (*Oncorhynchus kisutch*). Canadian Journal of Fisheries and Aquatic Science 44, 452–457.

Ruíz-Gomez ML, Huntingford FA, Øverli Ø, Thörnqvist PO and Höglund E 2011. Response to environmental change in rainbow trout selected for divergent stress coping styles. Physiology and Behavior 102, 317–322.

Sae-Lim P, Kause A, Janhunen M, Vehviläinen H, Koskinen H, Gjerde B, Lillehammer M and Mulder HA 2015. Genetic (co)variance of rainbow trout (*Oncorhynchus mykiss*) body weight and its uniformity across production environments. Genetics Selection Evolution 47, 1.

Takahashi A, Tsuchiya K, Yamanome T, Amano M, Yasuda A, Yamamori K and Kawauchi H 2004. Possible involvement of melanin-concentrating hormone in food intake in a teleost fish, barfin flounder. Peptides 25, 1613–1622.

Taub S and Palacios S 2003. La acuicultura en Chile. Techno-Press, Santiago de Chile. 326 pp.

Tsai HY, Hamilton A, Tinch AE, Guy DR, Gharbi K, Stear MJ, Matika O, Bishop SC and Houston RD 2015. Genome wide association and genomic prediction for growth traits in juvenile farmed Atlantic salmon using a high density SNP array. BMC Genomics 16, 969.

Yamanome T, Amano M and Takahashi A 2005. White background reduces the occurrence of staining, activates melanin-concentrating hormone and promotes somatic growth in barfin flounder. Aquaculture 244, 323–329.

Yáñez JM, Bangera R, Lhorente JP, Barría A, Oyarzún M, Neira R and Newman S 2016a. Negative genetic correlation between resistance against *Piscirickettsia salmonis* and harvest weight in coho salmon (*Oncorhynchus kisutch*). Aquaculture 459, 8–13.

Yáñez JM, Naswa S, López ME, Bassini L, Correa K, Gilbey J, Bernatchez L, Norris A, Neira R, Lhorente JP, Schnable PS, Newman S, Mileham A, Deeb N, Di Genova A and Maass A. 2016b. Genomewide single nucleotide polymorphism discovery in Atlantic salmon (*Salmo salar*): validation in wild and farmed American and European populations. Molecular Ecology Resources 16, 1002–1011.

Yáñez JM, Newman S and Houston RD 2015. Genomics in aquaculture to better understand species biology and accelerate genetic progress. Frontiers in Genetics 6, 128.

Yoshida GM, Bangera R, Carvalheiro R, Correa K, Figueroa R, Lhorente JP and Yáñez JM 2018a. Genomic prediction accuracy for resistance against *Piscirickettsia salmonis* in farmed rainbow trout. G3: Genes, Genomes, Genetics 8, 719–726.

Yoshida GM, Carvalheiro R, Rodriguez F, Lhorente JP and Yáñez JM 2018b. Single-step genomic evaluation improves accuracy of breeding value predictions for resistance to infectious pancreatic necrosis virus in rainbow trout. Genomics (In Press). https://doi.org/10.1016/j.ygeno.2018.01.008.

Yoshida GM, Lhorente JP, Carvalheiro R and Yáñez JM 2017. Bayesian genome-wide association analysis for body weight in farmed Atlantic salmon (*Salmo salar* L.). Animal Genetics 48, 698–703.