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Resistance against infectious pancreatic necrosis exhibits significant genetic variation and is not genetically correlated with harvest weight in rainbow trout (*Oncorhynchus mykiss*)

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ABSTRACT

Infectious pancreatic necrosis (IPN) is one of the most prevalent and economically important diseases in rainbow trout aquaculture (*Oncorhynchus mykiss*). Vaccines as a conventional control measure have shown variable results under production conditions. Genetic improvement for resistance to IPN represents an alternative for the prevention of disease outbreaks. The objective of the present work was to estimate the heritability and genetic correlation for IPN resistance and harvest weight (HW) in rainbow trout. To determine the genetic resistance to the IPN virus, a total of 2278 fingerlings from 58 full-sib families were used, which were challenged with IPN virus to induce the disease. Resistance refers to survival to the disease and was defined as the day of death of each fish. HW was also recorded in 13,241 genetically related individuals from the same population. For the genetic analysis we fitted a bivariate mixed linear model including HW and resistance to IPN as dependent variables; tank:year:sex as a fixed contemporary group and age at harvest as a covariate for HW; and final weight as a covariate for resistance to IPN. The animal effect was included as a random effect for both traits. A random effect associated with common environment was also included for HW. The estimated heritability for IPN resistance was 0.39 ± 0.08 and 0.35 ± 0.06 for HW. Genetic correlation between IPN and HW resistance was not significant (0.05 ± 0.25). The results indicate that the heritability for both traits is moderately high in this population, and that there is no significant genetic correlation between them. The presence of significant genetic variation for both IPN and HW resistance and the absence of genetic correlation between both traits indicate the feasibility of improving them simultaneously by means of artificial selection.

1. Introduction

Rainbow trout is one of the most important species among cultured salmonids, with an estimated world production of 813 thousand tons in 2014, and an estimated value of US$ 3933 million (FAO, 2016). Chile makes an important contribution to the worldwide production of rainbow trout, reaching 152 thousand tons in 2015 (SERNAPESCA, 2016). One of the key factors affecting the profitability and sustainability of this industry is health status, which depends mainly on the control of infectious diseases affecting fish under culture (Yáñez and Martínez, 2010).

Infectious pancreatic necrosis (IPN) virus (*Aquabirnavirus* genus; *Birnaviridae* family) causes an acute viral disease, which generates high mortality in first-feeding juveniles (Roberts and Pearson, 2005) and smolts in the first six months after transfer to seawater (Wolf et al., 1960). Fish surviving the infection can become healthy carriers, which can infect susceptible animals (Wolf et al., 1960; Wolf, 1988), either by vertical and/or horizontal transmission. Vaccination may provide some protection against disease in juvenile fish (Ramstad and Midtlyng, 2008), but control in freshwater environments generally depends on the biosecurity measures and innate resistance of juvenile fish.

From a sustainability and animal welfare perspective and to reduce the negative effects of this disease, the aim should be to increase resistance to IPN through genetic improvement. This strategy is a viable

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alternative for the control of infectious and parasitic diseases in domestic animals (Stear et al., 2001; Yáñez and Martínez, 2010; Yáñez et al., 2014a). Genetic improvement increases the intrinsic potential of animals to resist infection and is a complementary tool in the control of infectious and parasitic diseases. However, in order to succeed in the genetic improvement for resistance to IPN, there must be additive genetic variation of this trait in rainbow trout.

Several studies indicate the presence of significant genetic variation for resistance to pathogens within rainbow trout populations (Rye et al., 1990; Dorson et al., 1995; Slierendrecht et al., 2001; Perry et al., 2004; Henryon et al., 2002, 2005; Silverstein et al., 2009). These results are encouraging, since they allow genetic improvement for resistance to infectious diseases to be feasible. It has also been observed that there is a genetic component associated with IPN resistance in Atlantic salmon with heritabilities ranging from 0.31 ± 0.06 to 0.45 ± 0.07 (Guy et al., 2006, 2009; Wetten et al., 2007; Storset et al., 2007; Kjøglum et al., 2008; Gheyas et al., 2010a, 2010b). For example, a heritability between 0.38 and 0.43 in the marine phase has been reported for IPN resistance (Houston et al., 2008), while in the freshwater phase the value is 0.31 (Houston et al., 2010). In addition, it has been shown that genetic variation for IPN in Atlantic salmon is controlled by a locus of major effect (Houston et al., 2008, 2010; Moen et al., 2009). However, to date there is scarce evidence of genetic variation for resistance to IPN in rainbow trout. Okamoto et al. (1993) demonstrated that the progeny of a rainbow trout strain showed genetic resistance to IPN after a spontaneous outbreak. In addition, the use of low-resolution molecular markers has shown the presence of genomic regions associated with resistance and susceptibility to IPN in rainbow trout (Ozaki et al., 2001). There are no available reports of heritability for IPN resistance in rainbow trout in the scientific literature. The possibilities to improve resistance to IPN depend on the genetic correlations between resistance and other traits of economic interest, such as growth rate. The more favorable the genetic correlations, the easier it is to improve traits simultaneously.

The objective of the present study is to estimate the level of genetic variation for resistance against IPN virus in rainbow trout from survival data obtained from experimental challenges. In addition, we also estimated the genetic correlation between resistance to IPN and body weight at harvest. This information will be useful for planning of disease control strategies through genetic improvement for IPN resistance in rainbow trout.

2. Material and methods

2.1. Experimental population

The fish belong to the genetic nucleus of Aguas Claras SA, and were challenged at the Aquainnovo Center for Research and Aquaculture Transfer (Puerto Montt, Chile). IPN resistance data were obtained from a sib-testing scheme, in which 58 families of full siblings of rainbow trout, with a representation of 17 to 50 fish per family from the 2014 year-class were experimentally challenged against IPN virus. Growth was measured on relatives from year-classes 2008 and 2011, tied through pedigree connections to the challenged fish. There were 2278 and 13,241 fish with records for resistance to IPN and body weight at harvest, respectively. A 3-generation pedigree was used (2008, 2011 and 2014) with a total of 20,529 recorded fish (Table 1). Fertilization of families took between one to five weeks depending on the spawning year. Fertilized ova from each family were incubated separately until hatching. The fish were then marked at about 2 to 7 g with PIT-tags (Passive Integrated Transponders), which were inserted in the abdominal cavity to preserve genealogical information during the challenge test and the grow-out period, for the fish measured for IPN resistance and body weight at harvest, respectively. Fish recorded for growth were then transferred to fresh water sites until smoltification. An average number of 20 to 83 individuals from each family were randomly divided into two fresh water tanks for year-classes 2008 and 2011. After smoltification, fish were transported to sea cages, maintaining the tank distribution established during fresh water rearing. Fish were then reared until market size at approximately 3 kg (on average 25 months post-spawning), in which harvest weight (HW) was recorded for all fish. The variables recorded for the IPN resistance trait were day of death after inoculation and weight at end of challenge. The variables recorded for the growth trait were body weight at harvest, age at harvest, sea cage, year and sex (determined by ultrasound imaging).

Table 1
Summary information of the pedigree from the rainbow trout (*Oncorhynchus mykiss*) breeding population used in the present study by year.

			Number of offspring	
Year	Number of sires	Number of dams	Total number	Mean per full-sib family
2008	18	48	4946	103
2011	48	106	13,305	126
2014	30	58	2278	39
Total	96	212	20,529	97

2.2. Experimental challenge test

Prior to challenge, the sanitary condition of 30 fries from the full-sib families to be experimentally challenged was evaluated randomly. Quantitative real-time PCR was used to detect *Flavobacterium psychrophilum* following the highly sensitive protocol described by Strepparava et al. (2014), which has shown a detection limit of 20 gene copies. Quantitative real-time reverse transcriptase PCR was used to determine the presence of IPN virus following the highly sensitive protocol described by Bowers et al. (2008), which has shown a detection limit of 10 RNA copies. Negative results were obtained for both pathogens. In addition, the latter method was used to confirm the cause of death generated post challenge. All these diagnostic analyses were carried out in the ALAB SA. (Puerto Montt, Chile). At the time of inoculation with IPN, the fish had an average weight of 2.24 (0.71) gr and 154 (15) days of age. The challenge was performed with an IPN virus isolate (virulent Sp serotype) in a 0.25 m^3 tank with fresh water in a recirculation system at an average temperature of 11 °C, oxygen saturation of 95.74% and salinity of 3.46 ppt. The IPN virus isolate (CD-AQ03) was purchased from Centrovet Ltda. (Puerto Montt, Chile). The virus was isolated from affected Atlantic salmon kidney from a Chilean farm (X^{th} Region) in November 2014 using CHSE-214 cell line and then cryopreserved until the preparation of inoculum in RTG-2 cell line.

The challenge was carried out in two stages; i) intraperitoneal inoculation at a rate of 0.05 mL/inoculum fish, at a concentration of $10^{7.82}$ TCID50/mL, determined by the Kärber-Spearman method (Hamilton et al., 1977); and ii) the immersion was then carried out, with 1.1 L of the inoculum diluted in 5 L of water and then poured into the tank which contained 130 L of water and kept at retained flow for 4 h and 17 °C. At the end of the immersion, fresh water was incorporated at 10 °C, causing a thermal shock. Mortality was withdrawn from the tank on a daily basis and each time it occurred, in order to record the day of death for each fish. At the end of the evaluation period (day 63), all surviving fish were euthanized. All the experimental challenge procedures were approved by The Comité Institucional de Cuidado y Uso de Animales (CICUA) from the University of Chile (Certificate N° 17,019–VET-UCH).

2.3. Estimation of co-variance components

To estimate the components of variance and covariance for HW (y_1) and resistance to IPN (y_2), the following bivariate mixed linear model was used: