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First insight into the heritable variation of the resistance to infection with the bacteria causing the withering syndrome disease in *Haliotis rufescens* abalone

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ABSTRACT

Withering syndrome disease has experienced worldwide spread in the last decade. This fatal disease for abalone is produced by a *rickettsia*-like organism (WS-RLO), the bacterium "*Candidatus* Xenohaliotis californiensis". To evaluate the potential of the red abalone (*Haliotis rufescens*) to improve its resistance to infection by WS-RLO, the additive genetic component in the variation of this trait was estimated. For this, the variation in infection intensity with WS-RLOs and WS-RLOv (phage-infected RLOs) was analyzed in 56 families of full-sibs maintained for three years in a host-parasite cohabitation aquaculture system. A WS-RLO prevalence of 65% was observed in the analysed population; and from the total WS-RLO inclusions 60% were hyperparasited with the phage (WS-RLOv). The decrease in the food ingestion rate was the sole negative effect associated with increasing WS-RLO intensity of infection, suggesting that the high level of WS-RLOv load may have diminished the symptoms of WS disease in the analyzed abalones. The estimated heritabilities were moderate to mid, but significant, varying from 0.21 to 0.23 and 0.36 for WS-RLO and WS-RLOv infections, respectively. This suggests that variation in resistance to infection with WS-RLO may respond to selection in the evaluated red abalone population. Estimated response to selection (G) for the level of infection by WS-RLO indicated that if the 10% of red abalone with the lowest infection level is selected as broodstock, a 90% reduction in the intensity of infection in the progeny can be expected, even with the lowest estimation of heritability (h^2 = 0.21). This strong response would be also due to the large phenotypic variance of this trait. Strong positive correlations, both phenotypic and genotypic, were observed between infection intensities with WS-RLO and WS-RLOv, indicating that selection to increase resistance to one of the types of RLOs will affect resistance in the other in the same direction. This is the first study that demonstrates the existence of additive genetic variation for resistance to WS-RLO in abalone. Consequently, it is possible to increase the resistance to WS-RLO in *H. rufescens* by selective breeding, which can be an economically attractive and environmentally friendly manner to reduce mortalities and growth effects caused by WS in abalone farms.

1. Introduction

Withering syndrome (WS) is a devastating infectious disease that originally affected several abalone species from California (USA) and Baja California (Mexico) coasts. However, in the past decades WS has been spread into several countries by transport of infected abalones to culture centers (Crosson et al., 2014). This is the case for Chile, where WS disease was introduced to culture centers by imported red abalone, *Haliotis rufescens*.

WS is caused by an intracytoplasmic rickettsia-like organism (WS-RLO), "*Candidatus* Xenohaliotis californiensis" (Friedman et al., 2000; Crosson et al., 2014). This bacterium first infects the posterior esophagus of abalone and then induces metaplasia in the digestive gland, transforming the terminal secretor/absorptive acini, which perform the intracellular digestion into transport ducts (Friedman et al., 2014a). This metaplasia generates more tissue for WS-RLO colonization and

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multiplication. The transport ducts cannot perform intracellular digestion, which leads to catabolism of the foot muscle for energy procurement and finally death (Friedman et al., 2000; Braid et al., 2005). Interestingly, in recent years a bacteriophage infecting WS-RLOs has been described for red and black abalones from California (Friedman and Crosson, 2012). WS-RLO inclusions with morphology consistent with phage infection have been also observed in red abalone from Chile since 2004 (Lohrmann, 2010). The presence of phage-infected WS-RLOs (WS-RLOv) was associated with a reduction in pathogenic WS-RLO loads and response to infection and mortality in black abalone *H. cracherodii* (Friedman et al., 2014a).

The host response to infection in natural or farm animal populations is multifactorial and involves the complex interaction between two genomes (the host and the pathogen) and the environment (Caron et al., 2013). It had been long observed that diseases rarely occur in all members of animal populations exposed to pathogens and several studies, mainly in vertebrates, support a key role of the host genetic background in the expression of the disease (Caron et al., 2013). Some abalone species have experienced extended massive mortalities due to WS-RLO infections; however, some populations of this species were less affected by this disease, suggesting genetic variation for susceptibility or resistance to WS (Crosson et al., 2014). Additionally, a species-specific resistance or non-susceptibility to WS-RLO has been described in some abalone species; where *H. discus hannai* seems to be non-susceptible (Gonzalez et al., 2012; González et al., 2014), and *H. corrugata*, *H. fulgens* and *H. diversicolor supertexta* are highly resistant to the infection (Álvarez-Tinajero et al., 2002; Moore et al., 2009; Wetchateng et al., 2010).

Beyond the destruction of wild populations, WS has also had a major impact on aquaculture operations, mainly for the most intensively cultured species, *H. rufescens*, with a long-term decline attributable to disease burden (Moore et al., 2001). Aquaculture production systems favour the transmission and spread of disease because of high animal densities and stressful changing environments (Lafferty et al., 2015; Beardmore and McConnell, 1998). Although WS is treatable with antibiotics such as oxytetracycline (Friedman et al., 2007), this approach is generally only practical for treating small numbers of animals and has putative negative effects for public health (Romero et al., 2012).

While some of the observed variation in natural resistance to infection is related to environmental factors, a significant component of variation appears to be heritable. The approach to improve the animal resistance using selective breeding programs has the advantage that breeding is directional, low cost, easy to implement, and offers ethical advantages (Van Muiswinkel et al., 1999). This strategy has been widely used for fish farming (Leeds et al., 2010; Silverstein et al., 2009) and, among mollusks, for oysters (Simonian et al., 2009; Lynch et al., 2014; Ford and Bushek, 2012; Powell et al., 2011; reviewed by Dégremont et al., 2015a). Including resistance to WS into the breeding objective would require an understanding of the level of additive genetic variation (i.e., heritable variation) for this trait. To the best of our knowledge, no studies aiming to determine additive genetic variation for WS resistance in abalone have been published. In order to obtain the first insight into the heritable variation of the resistance to infection by WS-RLO in red abalone, we have analyzed the associated genetic variation parameters in 56 full-sibs maintained during 3 years under a sympatric host-parasite aquaculture system.

2. Material and methods

2.1. Breeding design and animal rearing conditions

Sixty full-sib families were produced at the Center for Abalone Production of the Universidad Católica del Norte (UCN), Coquimbo, Chile. For this, a non-infected broodstock of 600 *Haliotis rufescens* randomly obtained from three abalone breeding companies (200 abalones per company) was used. Crossing was conducted following a paternal half-sib nested design; where each male (n = 20) was crossed with three females (n = 60) to produce 20 half-sib and 60 full-sib families. The production of the families occurred over ~ 3 months, with three spawning events per month (5–10 days between each event). After settling, each full-sib family was cultured separately in 200-L tanks for the first 14 months. Then 15 abalones per full-sib family were individually marked with labels attached to the shell with epoxy resin; and families were mixed and randomly distributed, at equal densities, in 4 baskets placed in a 10,000-L raceway-type tank. To avoid common environment effects, abalones were remixed randomly among baskets each month. Abalone families were maintained for three years in the raceway (i.e., until they attained the market size of ~ 60 mm shell length) with continuous water flow and constant aeration at room temperature that varied between ~ 13 and 20 °C, and fed with the brown macroalga *Macrocystis pyrifera*.

2.2. Measurements of feeding and body traits

During the 3 years growing period several animals per family were lost due to escapes and label detachments; and a few due to mortalities. Thus we were able to evaluate 3–14 abalone per family from 56 of the initial 60 full-sib created (total n = 473 abalones).

The day before infection analysis, food consumption was measured in each individual from the 56 families (n = 473). For this, abalones were maintained in individual cages with the same amount of food (*M. pyrifera*). The consumed food was estimated as the difference between the initial algal mass and the remnant after 24 h. This estimate was corrected by the algal mass loss in a control cage containing the same amount of algae for the same experimental period but without abalone. Then abalones were sacrificed and soft tissues were dried prior to being weighed using an electronic balance (± 0.001 g). Abalone shell length and width were determined using a digital calliper (± 0.01 mm). Measurements of feeding and body traits lasted 6 days; during which 80 abalones were measured and tissues processed for histological analyses, per day.

2.3. Histological analysis of WS-RLO and WS-RLOv inclusions

WS-RLO and WS-RLOv infection was analyzed in the same adult abalone for which food consumption rate was assessed (n = 473, from 56 full-sib families). Previous analyses indicated the presence of WS-RLO at the UCN Center for Abalones Production. Thus, instead of a controlled challenge assay a natural infection by pathogen-host cohabitation was assessed in this study.

To evaluate the level of infection per abalone from each family, histological analyses were performed. For this, posterior esophagus and digestive gland were placed in tissue embedding cassettes and fixed in Davidson's fluid for 24 h (Shaw and Battle, 1957); then transferred to 70% ethanol and further processed for routine histology. Five μm thick sections were cut, then 200 μm were discarded, and a second section was obtained, which was stained with hematoxylin-eosin only if the first section proved negative for WS inclusions. The slides were analyzed with a Zeiss Axiostar plus microscope, and photographs were taken with a Canon Powershot A 620 camera. Infection intensity was determined counting the total number of bacterial inclusions (WS-RLO and WS-RLOv) per histological slide.

Selected wax blocks were cut at 5 μm, mounted on coverslips, and prepared for scanning electron microscopy (SEM). They were dewaxed in three changes of xylene, passed through three changes of 100% ethanol, critical point dried using CO_2, and ion sputtered with gold (Lohrmann et al., 2002). They were viewed in a JEOL S300 microscope, and photographs were taken.

2.4. Estimation of the components of phenotypic variance and heritability

The heritability and variance components for the WS-RLO and WS-RLOv infection levels were estimated with a restricted, estimated maximum-likelihood (REML) procedure (Johnson and Thompson, 1995) as implemented in ASReml v.3.0 (Gilmour et al., 2009). This procedure involved fitting an individual animal model, i.e., a mixed linear model where the phenotypic response of each individual is separated into an additive genetic component plus other random and fixed effects, as follows (in matrix notation): $y = Xb + Z_a a + Z_m f + e$; where $\boldsymbol{y}$ is a vector of the observations of all individuals; **b** is the vector of fixed effects; **a** is the vector of additive genetic effects (random animal effects or breeding values); **f** is the vector of random effects other than additive genetics (i.e., confounded maternal effects, common environmental effects as well as non-additive genetic effects); and $\boldsymbol{e}$ represents the residual effects. $\boldsymbol{X}$, $\boldsymbol{Z_a}$ and $\boldsymbol{Z_m}$ are the corresponding incidence matrices.

The significance of the fixed effects and covariates were estimated using the Wald F statistic. Statistical significance of maternal/common environment effects and additive random effects (h^2 significance) were estimated by the log-likelihood ratio test (log-LR test). The exact age at the time of the measurement was evaluated as a covariate; and as a fixed effect the date of birth. As random factors in the model, we evaluated the direct additive genetic effects, as well as the maternal/common environmental effects. Because full siblings shared a tank for 14 months, the early common environmental effects were completely confounded with the maternal effects. The h^2 of each trait was calculated as the ratio of the additive genetic variance to the total phenotypic variance (Falconer and Mackay, 1996; Lynch and Walsh, 1998; Kruuk, 2004).

2.5. Estimation of phenotypic and genetic correlations

A bivariate animal model was used with ASReml version 3.0 (Gilmour et al., 2009) to estimate genetic correlations among traits for abalone. The significance of the genetic correlations was estimated using the log-LR test by comparing the likelihood of the model allowing genetic co-variance between the compared traits to vary and the likelihood of the model with the genetic co-variance fixed to zero (Lynch and Walsh, 1998; Wilson et al., 2009). Phenotypic correlations among traits were estimated by Pearson correlation.

3. Results

3.1. Infection intensity

Infection intensity was determined on full-sib families of natural infected abalones by histological analysis. Inclusions with morphology consistent with phage infected RLO (WS-RLOv) were clearly distinguishable from non-infected WS-RLO by its characteristic navy blue color and granular appearance (Fig. 1A and B). Fig. 1C shows two inclusions viewed with scanning electron microscopy (SEM), WS-RLO exhibit a small and regular size; while WS-RLOv are hypertrophied, and highly pleomorphic; being this the first study to illustrate RLO and RLOv morphology using SEM Analyses showed that 65% of the analyzed population was positive to WS-RLO/RLOv and parasite load varied greatly from 1 to 710 inclusions per histological slide (Fig. 2). Although 58% of infected abalones displayed between 1 and 30 bacterial inclusions, a considerable number (15%) of infected abalones had > 120 inclusions. The remaining 27% displayed between 31 and 120 inclusions. Interestingly, of the total bacterial inclusions observed, 60% were infected with the phage hyperparasite.

When data were analyzed by full-sibs, the mean level of infection varied markedly among full and half-sib families; most families contained animals with some degree of WS-RLO/RLOv infections (Fig. 3A). Notably, most animals in some families lacked infection with either

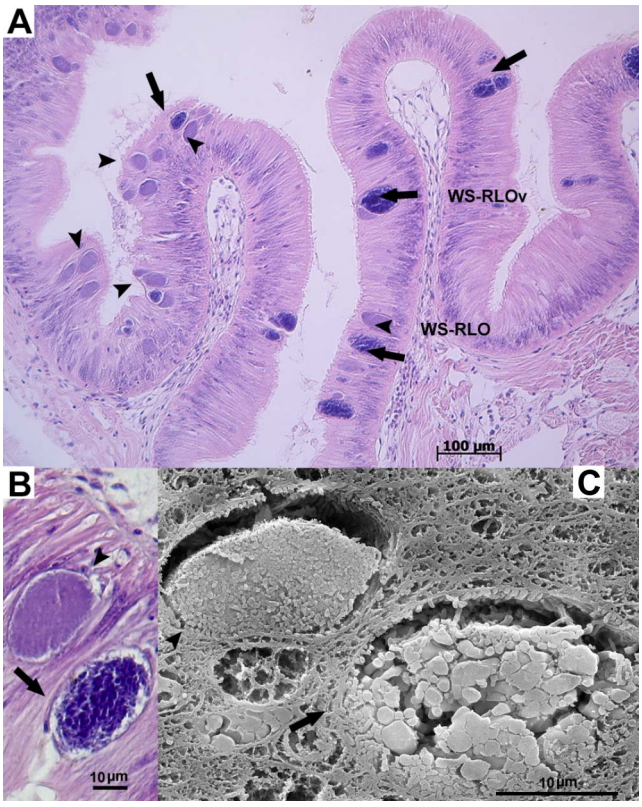

Fig. 1. *Candidatus* Xenohaliotis californiensis (WS-RLO) infecting the posterior esophagus epithelium of the red abalone, *Haliotis rufescens*. (A) Post-esophagus epithelium with several cytoplasmic inclusions (bacterial colonies) of WS-RLO (arrowheads) and inclusions with morphology consistent with phage infection or WS-RLOv (arrows). (B) Detailed micrograph of both inclusions showing differential H & E staining properties of RLOs. Homogenous inclusions correspond to WS-RLO; and coarse inclusions, correspond to inclusions with morphology consistent with phage –infected bacteria (WS-RLOv). (C) Scanning electron microscopy (SEM) of both inclusions (WS-RLO/RLOv).

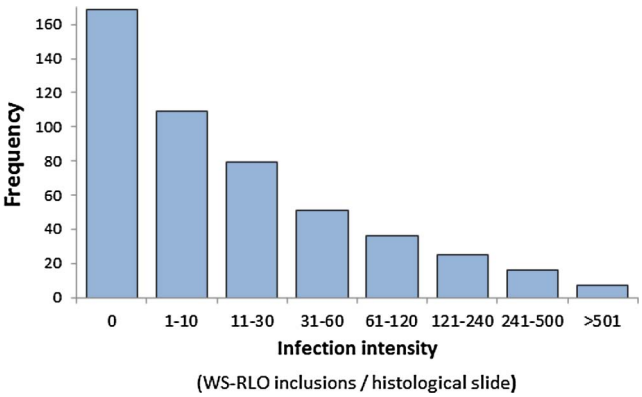

Fig. 2. Frequency of infection intensity with WS-RLO (including RLOv) in *Haliotis rufescens* abalone. Abalones (n = 473) where maintained for 3 years in host-parasite cohabitation culture system.

RLO, suggesting that they were less or non-susceptible to infection (Fig. 3B). In contrast, some other full-sib families where highly susceptible, as indicated by a high degree of infection in most or all animals.

3.2. Genetic analysis

Among the factors evaluated to define the linear mixed model for the analysis of the infection traits (WS-RLO, WS-RLOv and total inclusions), only the age as a covariate had a significant effect, but only for

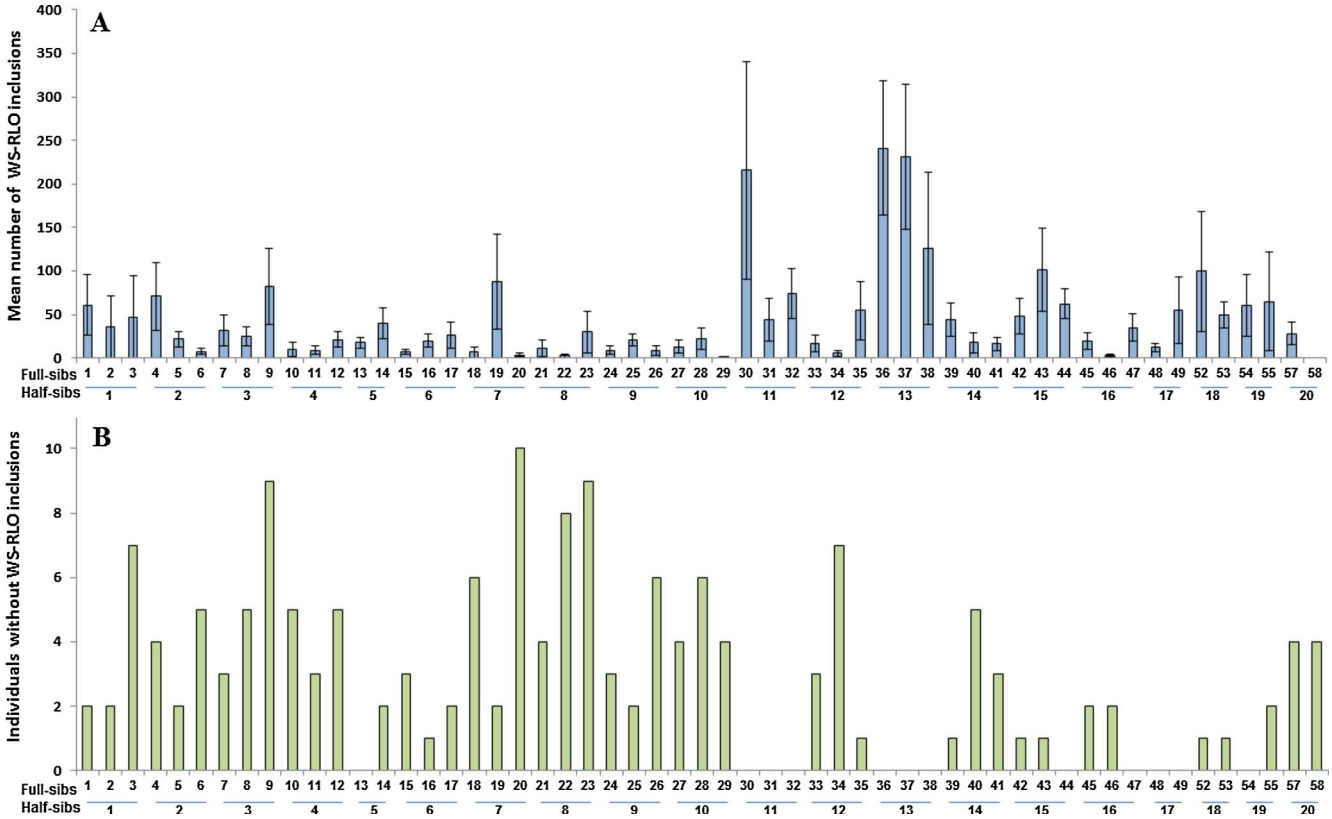

Fig. 3. (A) Variation in the mean number of WS-RLO inclusions per half and full sib family of *Haliotis rufescens* (20 half-sibs and 56 full-sibs; n = 473). (B) Number of abalone without WS-RLO inclusions per half and full sib family. Abalones where maintained for 3 years in host-parasite cohabitation culture system.

Table 1
Phenotypic means and quantitative genetic estimates for the infection level (number of inclusions) with rickettsia-like organisms that produce the Withering Syndrome disease (WS-RLO) in *Haliotis rufescens* abalone. Phenotypic and genetic estimates were also assessed for WS-RLOv (phage-infected WS-RLO) and total number of inclusions.

Trait	Phenotypic Mean (SD)	V_A (SE)	V_R (SE)	V_P (SE)	h^2 (SE)
Simple model					
WS-RLO	16.1 (39.7)	362 (139)	1207 (126)	1570 (109)	0.23 (0.08)*
WS-RLOv	25.2 (60.8)	1314 (405)	2327 (303)	3642 (272)	0.36 (0.10)*
Total inclusions	41.3 (94.8)	3005 (955)	5874 (734)	8880 (655)	0.34 (0.09)*
Age-covariate model					
WS-RLO	16.1 (39.7)	325 (129)	1198 (121)	1524 (105)	0.21 (0.08)*
WS-RLOv	25.2 (60.8)	1290 (400)	2343 (3 0 7)	3633 (271)	0.36 (0.09)*
Total inclusions	41.3 (94.8)	3018 (961)	5624 (724)	8642 (647)	0.35 (0.10)*

Genetic estimates of the additive genetic variance (V_A); residual variance (V_R); phenotypic variance (V_P); and heritability (h^2). WS-RLO and WS-RLOv prevalence was measured in abalone belonging to 56 full-sib families (n = 473 adult abalone). Genetic parameters were estimated using two models: a simple model that only included the additive genetic effect as a random effect; and a model that also included the age as a covariate effect.

the level of WS-RLO load (Wald F, $P < 0.001$). No significant maternal/common environment effects were detected for either infection trait (log-LR test, $P > 0.05$). Therefore, estimates of the variances and h^2 were made through a reduced or simple model that only included the additive genetic effect as a random effect; and through a model that also included the age as a covariate effect (Table 1).

Residual variances (V_R) for infection level by WS-RLO, WS-RLOv and both types of bacterial inclusions, were 2–3 times higher than additive genetic variance for these traits (Table 1). Accordingly, heritabilities (h^2) estimated with both models were of intermediate level, ranging from 0.21 to 0.36, and significant for the three infection traits (log-LR test, P < 0.001).

3.3. Correlations among traits

Phenotypic (r_P) and genetic (r_G) correlations between infection level traits were all high, positive and significant ($P < 0.05$, *t*-test and log-LR test, respectively) (Table 2). Interestingly, strong correlations (both r_P and r_G) were observed between the number of total inclusions and WS-RLOv inclusions (Table 2).

Positive phenotypic correlations between body traits and the three infection level traits were observed ($P < 0.05$). These correlations were low, with the exception of the mass of soft tissues and the WS-RLO level ($P > 0.05$) (Table 2). As expected, negative r_P between food consumption rate and each of the infection levels were observed. However, no other genetic correlations among body or food consumption traits and infection level traits were observed (log-LR test, $P > 0.05$).

4. Discussion

Over the last years, a genetic control on the resistance to "*Candidatus* Xenohaliotis californiensis", the agent causing withering syndrome (WS-RLO) in abalone, has been suggested by differential mortalities or tolerance observed among haliotid species or populations of the same species, after natural or experimental exposition to this bacteria (Crosson et al., 2014; Friedman et al., 2014a). The present study, for the first time, estimated the heritable variation in the infection intensity with WS-RLO. For this, we estimated the heritability (h^2) for the level of infection by WS-RLO or inclusions with morphology

Table 2
Phenotypic (r_P) and genetic (r_G) correlations between infection level with WS-RLOs (number of inclusions of WS-RLO, WS-RLOv, a variant of RLO infected with a phage, and total inclusions), and body and food consumption traits in the red abalone *Haliotis rufescens*.

Traits	Phenotypic correlations			Genetic correlations		
	WS-RLO	WS-RLOv	Total inclusions	WS-RLO	WS-RLOv	Total inclusions
WS-RLOv	0.770 (0.029)*	–	–	0.997 (0.040)*	–	–
Total Inclusions	0.913 (0.019)*	0.964 (0.012)*	–	0.999 (0.018)*	1.00 (0.004)*	–
Mass of soft tissues	0.085 (0.046)	0.091 (0.046)*	0.094 (0.046)*	0.055 (0.303)	0.099 (0.266)	0.085 (0.271)
Total Mass	0.132 (0.046)*	0.171 (0.045)*	0.166 (0.045)*	0.124 (0.277)	0.172 (0.240)	0.160 (0.245)
Shell length	0.135 (0.046)*	0.192 (0.045)*	0.180 (0.045)*	0.224 (0.249)	0.211 (0.217)	0.215 (0.221)
Shell Width	0.137 (0.046)*	0.184 (0.045)*	0.176 (0.045)*	0.247 (0.262)	0.232 (0.229)	0.239 (0.233)
Food consumption	−0.104 (0.046)*	−0.107 (0.046)*	−0.113 (0.046)*	0.078 (0.416)	−0.100 (0.365)	−0.049 (0.370)

* $P < 0.05$

consistent with phage infection (WS-RLOv) in full-sib families of adult red abalone *Haliotis rufescens*. Heritabilities estimated were all moderate to mid and significant; ranging from 0.21 to 0.23 and 0.36 for WS-RLO and WS-RLOv infections, respectively. These results suggest that the variation in the infection levels with WS-RLO is to some extent under genetic control in the red abalone population assessed and thus, this trait may respond to selection. The response to selection (G) for the level of infection by WS-RLO can be estimated as $G = -ih^2\sigma_P$, where i is the selection intensity and σ_P the standard deviation of the number of WS-RLO inclusions (Falconer and Mackay, 1996). If direct selection for lower infection level is exerted at a selection intensity of 1.76 (i.e., selecting the 10% of red abalone with the lowest infection level as broodstock), a 90% reduction in the intensity of infection in the next generation can be expected, even with the lowest estimation of heritability ($h^2 = 0.21$). This strong response would be also due to the large phenotypic variance of this trait. On the other hand, in several families of exposed red abalone, all or most of their full-sibs were negative for WS-RLO; and in several others, most of their full-sibs showed a high number of these bacterial inclusions. In addition to support the genetic basis of resistance to WS-RLO infection, these results suggest that a family based selection for this trait would also be suitable as an alternative to individual selection.

The observed heritable variation and estimated response to selection for the resistance to WS-RLO infection in *H. rufescens* are consistent with observations made on the black abalone *H. cracherodii*, for which it was possible to study a resistant population that remained after a 95–98% mass mortality of this species on San Nicolas Islands, California (USA) (Friedman et al., 2014a). Progeny of surviving abalone from the WS-RLO resistant population showed significantly lower WS-RLO infection intensity in the digestive gland compared to a naïve black abalone population (Friedman et al., 2014a). Crosson et al. (2014) suggested that naturally selected black abalones for WS resistance have some mechanism of genetic origin that decreases the ability of WS-RLOs to proliferate in their digestive gland.

Estimation of genetic variation for resistance to pathogen infection intensity has not been directly estimated for other mollusks. However, heritabilities ranging from 0.49 to 0.60 for mortality caused by the ostreid herpesvirus (OsHV-1), i.e., the disease resistance, were estimated in the oyster *Crassotrea gigas* (Dégremont et al., 2015b). Additionally, a significant response to selection to improve disease resistance was observed in several oyster species, suggesting that resistance to the corresponding disease agents was heritable (reviewed by Dégremont et al., 2015a).

In the present study, results from phenotypic analyses surprisingly showed that body mass and shell size did not decrease with increasing WS-RLO infection level. Conversely, a low but significant positive correlation was observed among body mass and size with infection level. However, a negative effect of the level of infection on the rate of food ingestion was observed. In a previous study, we showed that highly infected red abalone compensated low food ingestion with a higher absorption an assimilation rates (Gonzalez et al., 2012); which may partially explain the absence of a negative effect on body mass. Low temperatures may also explain the absence of disease expression, because most of the time the sea temperature in Coquimbo is below that in which WS is expressed in red abalone (Braid et al. 2005). Alternatively, highly infected abalone may have not showed marked disease symptoms because of a higher prevalence of the inclusions with morphology consistent with phage infection (WS-RLOv) with the increase of WS-RLO intensity. Indeed, we observed a highly positive phenotypic correlation between total WS-RLO inclusions and the WS-RLOv; and 60% of the WS-RLO inclusions presented this RLO variant in the evaluated abalone population. Recently, it was proposed that this bacteriophage may control the pathogenicity of the WS-RLO in the black abalone (Friedman et al., 2014a). According to these authors, WS-RLO pathogenicity may be reduced by the phage by eliminating normal function of its bacterial host, reducing metaplasia, and thus decreasing transport tissue to be colonized by WS-RLO. Friedman et al. (2014a) observed for black abalone that half of the WS-RLO inclusions were phage infected, thus effectively reducing their pathogenic infection load by half. These results are consistent with the observed reduction in the losses due to WS in California abalone farms since the appearance of this bacteriophage (Friedman et al., 2014a). Therefore, the high prevalence of WS-RLOv in the red abalone population of the present study may be another possible cause for the observed tolerance despite the detected high levels of infection.

In addition to a high phenotypic correlation, a very high and positive genetic correlation between WS-RLO and WS-RLOv loads was observed in the present study. Two traits are genetically correlated when variation in one or more genes affects both traits; consequently, selection on one of them results in a correlated response of the other (Price and Langen, 1992; Krebs et al., 1998; Lynch and Walsh, 1998). Thus, present results indicate that the level of infection by WS-RLO and to its phage infected form should respond in a positively correlated form to selection. The infection mechanism of the hyperparasitic phage on the WS-RLOs is unknown, but due to the high genetic correlation in the abalone resistance to infection by both kinds of WS-RLOs it can be inferred that an improvement in resistance to the infection will reduce the host availability and thus, phage proliferation. Accordingly, the beneficial effects of the phage as a control of WS disease could be reduced. A cost/benefit analysis is necessary to determine if the improvement of resistance of abalone to the WS-RLOs infection by a selective breeding or an improvement of their tolerance by a phage therapy (as proposed by Friedman et al., 2014a) are better strategies to control WS-RLO virulence.

In conclusion, this is the first study demonstrating additive genetic variation for resistance to infection with the agent of the withering syndrome disease, "*Candidatus* Xenohaliotis californiensis" (WS-RLO) in an abalone species. As a consequence, the potential for reducing WS-RLO intensity of infection in *H. rufescens* by selective breeding is good and can be an economically attractive and eco-friendly way of

improving resistance to the WS disease in abalone. The new available non-invasive qPCR techniques for the diagnostic of the level of infection by this RLO (Friedman et al., 2014b) should facilitate selection of resistant or non-susceptible abalones or families in a genetic breeding program for abalones. Increasing resistance to WS-RLO infection would further decrease the economic losses associated with slow growth and high mortalities in the abalone farms, but their benefits must be counterbalanced against the advantages that offer the phage therapy as a potential reducing factor of WS-RLOs virulence.

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