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Changes of heritability and genetic correlations in production traits over time in red abalone (*Haliotis rufescens*) under culture

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Abstract

Red abalone *Haliotis rufescens* is one of the most valuable mollusks in the international market, but it has a low growth rate. A breeding program is being developed to increase its growth rate in Chile. We estimated the changes in direct heritability (h^2), maternal/common environments heritability (m^2) and genetic correlations (r_G) of growth traits (shell length and width, total mass, flesh mass and foot protein as an indicator of meat quality) measured during 2 years (every 4 months) from the juvenile stage (27 months) to the adult harvesting age (51 months), in 60 full-sib red abalone families. Heritabilities for growth traits measured in juveniles and young adults (27–35 months of age), were low (0.07–0.17) and not significant. Initial low h^2 were associated with significant amounts of maternal/common environmental effects ($m^2 = 0.4$). In young adults and abalone near the harvest age (39–51 months of age) h^2 were much higher (0.32–0.75). These results emphasize the importance of multiple estimations of h^2 over time. Among meat quality traits, only the h^2 for the flesh mass for adults at harvesting age was significant (0.15). We observed strong positive r_G (>0.9) between shell sizes (easy to measure) and total and flesh masses (trait more related to market value than shell sizes but harder to measure) for adults at harvesting age. Thus, if the 5% largest 51 month old abalone were selected from the population as broodstock we expect a positively correlated response on flesh mass of 23.4%.

Keywords: Abalone aquaculture, heritability, maternal effects, growth, genetic improvement, *Haliotis rufescens*

Introduction

The application of genetic improvement programs to animal breeding and agriculture is one of the bases of the current high production performance of this industry, which has become highly technical. By contrast, the genetic improvement applied to aquatic animals compared with terrestrial animals is rather limited (Gjedrem 2005). However, the world leading aquaculture industries of salmon, trout, sea bass, tilapia and oyster have developed genetic improvement programs (Robinson & Li 2008). Among the genetic-based improvement approaches applied to fish and invertebrate aquaculture, the most common have been polyploidization, hybridization and artificial selection (Boudry, Barré & Gérard 1997; Elliot 2000). There are increasing efforts to introduce the use of DNA based technologies, as genetic molecular markers to assist selection or hybridization programs (Lucas, Macbeth, Dignan, Knibb & Degnan 2006; Visscher, Hill & Wray 2008; Navarro, Zamorano, Hildebrandt, Ginés, Aguilera & Alfonso 2009). However, these methodologies usually must be integrated within an established breeding program (Lucas *et al.* 2006; Navarro *et al.* 2009) and previous information on the genetic architecture affecting the traits of interest are essential to implement them (Yáñez & Martínez 2010).

Abalone are slow-growing mollusks, with several species that attain an adequate size for commercial exploitation (Hahn 1989). Aquaculture of abalone has generated much interest throughout the world due to their high commercial value and over-exploitation of most wild stocks (Gordon & Cook 2004). In Chile, abalone are not indigenous species, and two species, the red abalone *Haliotis rufescens* and the Pacific abalone *Haliotis discus hannai* were introduced for culture purposes ~20 years ago from California (USA) and Baja California (Mexico), and from Japan, China and Korea respectively (Flores-Aguilar, Gutiérrez, Ellwanger & Searcy-Bernal 2007). In Chile, significant quantities of farmed abalone are produced, with the red abalone being the predominant species (96% of the production; Flores-Aguilar *et al.* 2007). However, red abalone, as other abalone species, only reaches its commercial size in ~4 years, and their slow growth is a main concern for abalone growers. Thus, increasing abalone growth rates through selective breeding is imperative for the industry competitiveness worldwide (Viana 2002).

The success of a selective breeding program depends on the existence of additive genetic variability for the target traits in the population (Falconer & Mackay 1996; Roff 1997). The proportion of total phenotypic variance in a population that is attributable to the additive effect of the genes is the heritability (h^2; Visscher *et al.* 2008). Additionally, response to selection or genetic gain depends on heritability (Falconer & Mackay 1996; Lynch & Walsh 1998). Selection rarely operates upon only one trait at a time, thus it is important to consider undesirable or desirable correlated responses in other traits (Lande & Arnold 1983). Two traits are genetically correlated when variation in one or more genes affects both traits (Krebs, Feder & Lee 1998). When there is a genetic correlation between traits, selection on one trait will indirectly select traits with which it is correlated (Lande & Arnold 1983; Rønning, Jensen, Moe & Bech 2007). Therefore, in a selection program it is essential to identify the size and sign of genetic correlations (r_G) between traits (Rønning *et al.* 2007).

Good results with genetic improvement through selective breeding programs have been obtained for aquatic species (Ponzoni, Hamzah, Tan & Kamaruzzaman 2005), thus we anticipate positive economic outcomes for the abalone industry from such programs. In abalone, selection experiments in the Pacific abalone (*Haliotis discus hannai*) have resulted in 7–21% improvement in growth per generation, which varied according to the age at which selection was applied (Hara & Kikuchi 1989). In the small abalone, *H. diversicolor*, You, Ke, Luo and Wang (2010a) reported that progeny of the 10% largest breeders of two strains grew 4.6–12.8% faster than their respective controls.

Several measurements of the heritability for growth traits have being done in abalone species (Jónasson, Stefansson, Gudnason & Steinarsson 1999; Mgaya 2000; Lucas *et al.* 2006; Kube, Appleyard & Elliot 2007; Li *et al.* 2005; You, Ke, Luo & Wang 2010b). In general, heritability values for shell length have oscillated between low (0.04) to mid-high values (0.56). However, most of these estimates are based on few measures taken over a year, and mostly cover the juvenile to young-adult stages.

A different set of genes and environmental factors can affect the variation of one trait during ontogeny, affecting the predictability of the future performance of individuals based on present data (Visscher *et al.* 2008). Therefore, the availability of genetic parameters such as heritability and genetic correlations during the organism's lifespan is essential for making decisions concerning the design and implementation of selective breeding programs (Mgaya 2000).

Heritability is not always constant for a population (Visscher *et al.* 2008), as a consequence of changes in the magnitude of the genetic variance due, for example, to modifications in allele frequencies (e.g. selective mortalities) or appearance of new variants in the population (e.g. introgression). On the other hand, environmental variance may also change through an animal's lifetime or when the organisms move to a different environment. Environmental and ontogenetic changes along the life span of the individuals can also affect the estimation of heritability, and this aspect can play a central role in the planning of a breeding program. The definition of the age when selection is applied can have impacts on the economic costs of the program, on the genetic progress over time and on the final profit. Therefore, estimating the variance components and h^2 for growth traits throughout the animal's productive cycle will be necessarily to increase the reliability of the estimates and to determine when the selection process must be done to obtain the best selection response.

In this study, we estimated the changes in heritability (h^2), maternal/common environments effects (m^2) and genetic correlations of growth traits, measured during 2 years from the juvenile stage (27 months) to the harvesting adult stage (51 months). Growth traits were measured every 4 months, in 60 full-sib *H. rufescens* abalone families. We further estimated the heritability of the flesh mass and foot protein as a potential indicator of meat quality (Pospiech, Grześ, Mikołajczak, Iwańska & Łyczyński 2007); and genetic correlations among these production traits (growth traits and flesh mass and quality) at two different ages (31 and 51 months), young-adults and harvesting ages respectively.

Materials and methods

Breeding design and animal rearing conditions

Sixty full-sib families were produced using *Haliotis rufescens* abalone broodstock randomly obtained from a base population of 600 adults, which were provided by three different abalone breeding companies (200 abalone per company). The broodstock were conditioned during 4 months in 2000-L tanks with micro-filtered seawater at a temperature between 18 and 19°C and permanent feeding with macroalgae. The mature broodstock were induced to spawn separately using the hydrogen peroxide method (Morse, Duncan, Hooker & Morse 1977), and crossing was conducted following a paternal half-sib nested design. Seawater containing gametes of one male was used to fertilize oocytes from three females randomly chosen from the base population, for a total of 20 males and 60 females. After fertilization, zygotes were transposed in 20 L polycarbonate containers with filtered and UV treated sea water, and washed several times with filtered sea water to eliminate excess of spermatozoa, retaining zygotes on a 60 μm mesh net. After hatching, larvae from each full-sib family were allowed to grow for 5–6 days, with gentle aeration and daily change of water. After that, competent larvae were transferred to 200-L tanks provided with corrugated polycarbonate plates inoculated with wild benthic microalgae for settling.

The entire process of production of families took ~3 months, with three spawning events per month (spaced by 5–10 days between each event). After settling, each full-sib family was cultured separately in 200-L tanks with continuous water flow and constant aeration for the first 14 months, and initially fed with wild benthic microalgae inoculated on corrugated polycarbonate plates. From the seventh month onward, abalone were fed with fresh kelp (*Macrocystis pyrifera*). Upon reaching a size of ≥20 mm shell length (~14 months), the abalone were marked individually with labels attached to the shell with epoxy resin. Subsequently, individuals from different families were mixed and transferred to baskets placed in a 10 000-L raceway-type tank. In the raceway, abalone were maintained during 3 year with continuous water flow, at ambient temperature that varied between ~13 and 20°C during the year, with constant aeration, and were fed twice a week with *M. pyrifera*.

Measures of production traits

Production traits measured were the variation in growth associated measures, i.e., the shell length, width and the total (shell plus soft tissues) and flesh (only soft tissues) masses, and the quality of the foot, measured as its protein content.

From 27 to 51 months of age, each growth trait was measured every 4 months in 15–30 individuals per full-sib family for the 60 abalone families. Flesh mass and foot protein were measured only twice (at 31 and 51 months of age), because for this measure it was necessary to sacrifice the animals. Therefore, the study began with 1400 abalone, and after the second and last measurement periods, 500 individuals were sacrificed for measurement of the flesh meat and protein content of the foot muscle.

Shell sizes were measured using a digital calliper (±0.01 mm). Total and flesh masses were measured using an electronic balance (±0.001 g) after animals had been dried with paper-towel to eliminate water excess. Total protein content was measured in the foot muscle of each abalone in a microplate spectrophotometer EPOCH (BioTek, Winooski, VT, USA) using a Micro-BCA kit and albumin as standard.

Estimation of variance components and heritabilities

All traits were evaluated for normality before analyses were conducted (Kolmogorov-Smirnov test). Mild deviations from normality were detected

in most cases. As a result, analyses were performed both on the raw untransformed data and on the log transformed data for quantitative genetic parameter estimations. Variance components for each production trait were estimated using the animal model with a restricted maximum likelihood approach (Johnson & Thompson 1995), as implemented in ASReml v.3.0 (Gilmour, Gogel, Cullisand & Thompson 2009). We fitted the animal model with random and fixed effects, and covariates that were significant using the following linear model (in matrix notation):

$$y = Xb + Z_a a + Z_m f + e$$

where y is a vector of the observations on 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 e represents the residual effects. X, Z_a and Z_m are the corresponding incidence matrices. The significance of the fixed effects and covariates was estimated using the Wald F statistic. The statistical significance of the maternal/environmental/non-additive random effects and the additive random effects (hence the heritability significance) was estimated by the log-likelihood ratio test (log-LR test). The variables that were evaluated as fixed effects were: (i) the location of the tank in which each full-sib family was held for the first 14 months of life, and (ii) the densities in which the families were held during this period. We also evaluated the effects of the exact age at the time of the measurements as a covariate. As a random factor in the model, we evaluated the direct additive genetic effect as well as the maternal/common environmental effects, because full siblings shared a tank during 14 months and therefore the early common environmental effect was completely confounded with maternal effect. In addition, potential non-additive genetics effects are also confounded with these last effects and cannot be teased out with the experimental design followed here. The direct heritability (h^2) of each trait was calculated as the ratio of the additive genetic variance to the total phenotypic variance (Falconer & Mackay 1996; Kruuk 2004). 'Maternal heritability' m^2 was calculated as the ratio of the maternal/common environment variance to the total phenotypic variance (implicitly including non-additive variance as well).

The potential responses to the selection of each productive trait, for each age, were estimated as $G = i \times \sigma_P \times h^2$, where i is selection intensity (Falconer & Mackay 1996). We used a selection threshold of 5% (i.e. selecting the best 5% of the population as broodstock), which implies a selection intensity (i) of 2.06. This was done on the raw, untransformed data, as it is more easily interpretable than genetic gains on the log transformed scale.

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 production traits for abalone measured at 31 and 51 months of age (i.e., young-adults and adults at harvesting age respectively). 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 two traits to vary and the likelihood of the model with the genetic co-variance fixed to zero (Lynch & Walsh 1998; Wilson, Réale, Clements, Morrissey, Postma, Walling, Kruuk & Nussey 2009). Phenotypic correlations among physiological traits were estimated by Pearson correlation.

Animal research

This study was carried out in strict accordance with the recommendations made by the Canadian Council on Animal Care. The protocol was approved by the Committee of Bio-ethics of the Chilean National Council for Science and Technology.

Results

Growth

At the beginning of the measurements, the abalone population of 27 months of age had an average shell length and total mass of 43.6 (±7.6) mm and 12.2 (±6.6) g respectively. At the end of the experimental period (i.e., after two additional years), the mean length and mass were 68.2 (±10.9) mm and 60.2 (±28.2) g respectively (Fig. 1). The growth rate was consistent along the

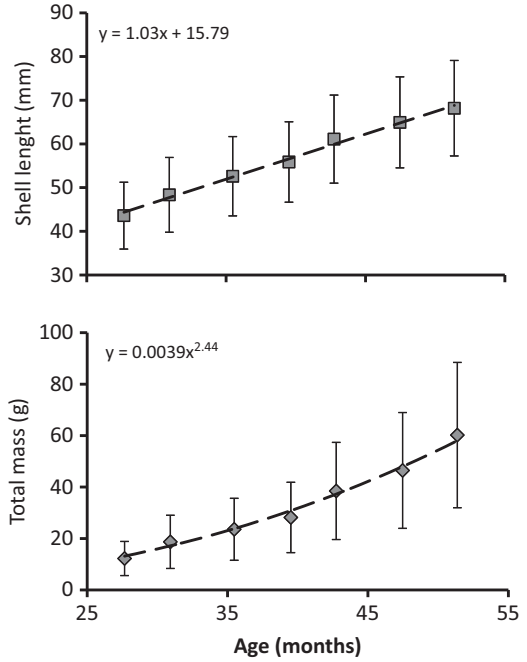

Figure 1 Growth in shell length (mm) and total mass (g) of red abalone *Haliotis rufescens* maintained under culture conditions during 2 years, from 27 to 51 months of age (n = 1400–700).

studied period of 24 months. Length increased linearly in time with a mean increase in 1.04 mm in shell length per month while mass seemed to follow an exponential model.

Changes of direct and maternal heritabilities in growth traits over time

For all the traits at all time points, no fixed effects (i.e., the full-sib tank locations, the family densities and the exact age at the time of the measurements) were significant in the linear mixed model (results not shown). These fixed effects were then dropped and the tested models had just direct additive genetic and maternal/common environmental effects as random effects.

Direct heritabilities (h^2) for growth traits (shell length and width, and total mass) were low and non-significant (h^2 ~0.1) during the three-first measures (i.e., in abalone from 27 to 35 months; Table 1, Fig. 2). These low h^2 coincide with high (~0.4) and significant common environmental/maternal effects (m^2) until animals attained 35 months. Afterwards the maternal heritability m^2 decreased and h^2 increased, stabilizing their levels from 39 until 51 months (i.e., near the harvesting age), at around 0.2 for the h^2 and 0.1 for the m^2, for the length and width. For the total mass, h^2 stabilized around 0.15, but m^2 continued decreasing to near 0.07 (Fig. 2). From 39 to 51 months, maternal heritabilities (m^2) were not significant for all measured traits while direct heritabilities (h^2) estimated without common environmental/maternal effects, became significant (Table 1).

Since m^2 were not significant from 39 months onwards, h^2 were also estimated with a simple or reduced model with just the additive genetic (i.e., animal) effects from this age until 51 months (Table 1). This model estimated higher h^2 for the three growth traits, which ranged (for untransformed data) between 0.50–0.74 for the length, 0.36–0.68 for the width, and 0.34–0.68 for the total mass, with the lowest estimates seen for the oldest abalone (Table 1).

Direct heritabilities for both the raw data and the log transformed data were quite close over all trait-time combinations (Table 1) indicating that the slight deviation from normality that was observed did not result in appreciable estimation bias when using the untransformed data.

Based on h^2 estimates for untransformed data (Table 1), estimates of genetic gains per generation (when selecting the 5% best individuals) varied between ~17% and 26% for the shell length, between ~13% and 24% for the shell width, and between ~16% and 52% for total mass, for abalone between 39 and 51 months of age respectively (Table 1).

Flesh mass and foot protein

Flesh mass increased by 25.5 g in 20 months (Table 2). On the other hand, the proportion of protein in the foot muscle decreased from 184.9 to 122.1 μg mg wet per mass. Heritability of flesh mass at 31 months of age was in the mid-range (0.21) but with a large standard error, and it was not significantly different of zero (log-LR test, $P > 0.05$). In turn, at 51 month h^2 value was lower (0.15), but with lower SE, and it was significantly different to zero (log-LR test, $P < 0.05$; Table 2). Based on h^2 estimates for untransformed data (Table 2), genetic gains per generation for the flesh mass varied between ~13% and 18% (for i = 2.06). Heritabilities of foot protein for both ages were low, with high standard errors and not significantly different from zero (log-LR test, $P > 0.05$). The non-significant heritabilities for

Table 1 Estimates of additive genetic variance (V_A); residual variance (V_R); direct heritability (h^2); expected response to selection (G) (with a selection intensity of 2.06); and percentage of G per generation (G%) of growth traits [i.e., shell length (mm) and width (mm), and total mass (g)] in *Haliotis rufescens* (n = 1400–700), using two models (CE-m and S-m)

Trait	Phenotypic mean (SD)	V_A	V_R	h^2 (SE; Log-transformed data)	h^2 (SE; untransformed data)	G	G%
Length-27 (CE-m)	43.6 (7.6)	3.314	34.70	0.07 (0.24)	0.05 (0.24)	0.87	2.00
Length-31 (CE-m)	48.3 (8.6)	11.43	42.53	0.17 (0.23)	0.15 (0.23)	2.71	5.60
Length-35 (CE-m)	52.6 (9.1)	7.250	51.91	0.11 (0.23)	0.09 (0.22)	1.63	3.10
Length-39 (S-m)	55.9 (9.2)	68.03	24.35	0.75 (0.11)*	0.74 (0.11)*	14.6	26.1
Length-43 (S-m)	61.1 (10.1)	65.77	41.84	0.62 (0.10)*	0.61 (0.10)*	13.1	21.4
Length-47 (S-m)	64.9 (10.4)	66.51	47.98	0.59 (0.10)*	0.58 (0.10)*	12.8	19.7
Length-51 (S-m)	68.2 (10.9)	62.99	61.95	0.49 (0.10)*	0.50 (0.10)*	11.6	17.0
Width-27 (CE-m)	29.7 (5.2)	0.197	17.11	0.03 (0.24)	0.01 (0.23)	0.08	0.25
Width-31 (CE-m)	33.1 (6.0)	3.658	23.23	0.13 (0.21)	0.09 (0.21)	1.21	3.67
Width-35 (CE-m)	36.2 (6.4)	2.641	27.41	0.09 (0.21)	0.06 (0.21)	0.84	2.33
Width-39 (S-m)	39.0 (6.5)	31.39	15.10	0.68 (0.11)*	0.68 (0.11)*	9.48	24.3
Width-43 (S-m)	42.7 (7.1)	28.25	25.25	0.53 (0.10)*	0.53 (0.10)*	7.96	18.6
Width-47 (S-m)	45.5 (7.5)	28.41	30.19	0.48 (0.10)*	0.48 (0.10)*	7.65	16.8
Width-51 (S-m)	49.2 (8.4)	26.17	46.13	0.35 (0.09)*	0.36 (0.09)*	6.34	12.9
Mass-27 (CE-m)	12.2 (6.6)	0.000	28.19	0.00 (0.00)	0.00 (0.00)	0.00	0.00
Mass-31 (CE-m)	18.7 (10.3)	4.217	72.36	0.12 (0.22)	0.04 (0.19)	0.84	4.47
Mass-35 (CE-m)	23.6 (12.0)	5.037	98.09	0.07 (0.23)	0.03 (0.20)	0.86	3.65
Mass-39 (S-m)	28.2 (13.7)	136.7	65.87	0.75 (0.11)*	0.68 (0.11)*	4.42	15.7
Mass-43 (S-m)	38.5 (18.9)	188.2	185.1	0.57 (0.10)*	0.50 (0.10)*	20.1	52.2
Mass-47 (S-m)	46.4 (22.5)	231.4	293.9	0.48 (0.10)*	0.44 (0.09)*	20.8	44.8
Mass-51 (S-m)	60.2 (28.2)	273.8	539.7	0.32 (0.08)*	0.34 (0.09)*	19.8	32.9

*Significant h^2 after log-LR test $P < 0.05$.

CE-m: model that considers as additional random effects the maternal/common environment effects; and S-m: simple model that just include the additive genetic effect as random effect. Models changed depending on the factors that were significant at a specific age. Variances and genetic gains were estimated for untransformed data.

flesh mass and foot protein are probably a function of the small sample size (n = 500 in each age).

Correlations among production traits

Genetic and phenotypic correlations between body traits at 31 and 51 months of age, were all high, significant (>0.9; $P < 0.05$, t-test and log-LR test, respectively) and positive, and with low standard errors for r_G (Table 3). However, genetic correlations between body traits and foot protein contents could not be estimated at 31 months of age due to singularity problems. At 51 months of age, these genetic correlations were highly negative, but had

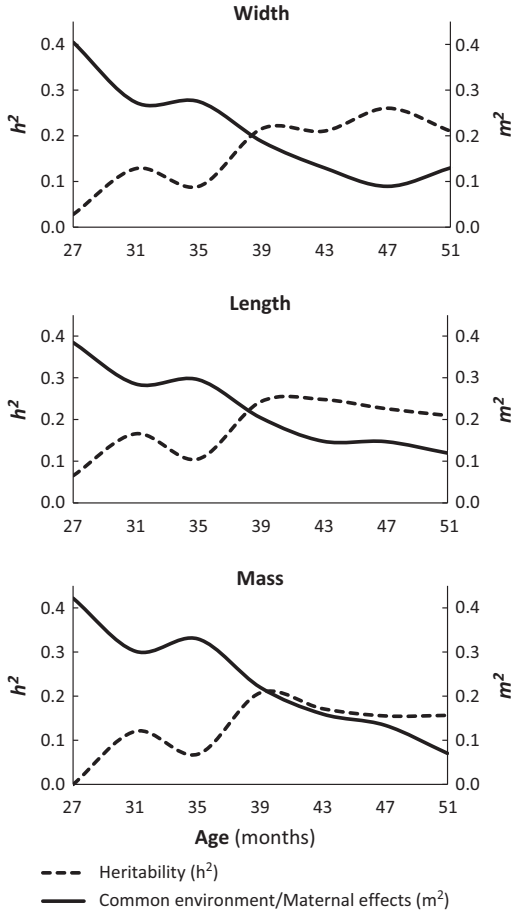

Figure 2 Changes of direct (h^2) and common environment/maternal (m^2) heritabilities in growth traits (Log-transformed) estimated in 60 full-sib families of red abalone *Haliotis rufescens* over 2 years, from 27 to 51 months of age (n = 1400–700).

very high standard errors and were not significant (log-LR test, $P > 0.05$). Phenotypic correlations between body traits and foot protein contents, at both 31 and 51 months, were negative but very low and not significant (Table 3).

Discussion

Our quantitative genetic analyses of 60 full-sib families (20 paternal half-sib families) at different ages in a cultured population of *H. rufescens*, showed that narrow sense heritability (h^2) for production traits changed over their lifespan. Heritabilities for body traits (shell length and width, and total mass) measured in juveniles and young adults (i.e., at 27, 31 and 35 months of age), were low (0.07–0.17) and not significantly different from zero. However, in young adults and abalone near the harvest age (i.e., at 39, 43, 47 and 51 months of age) h^2 of body traits were much higher (0.32–0.75), showing their maximum level at 39 months of age. Increased heritability with age has being reported in shellfishes (Jónasson *et al.* 1999; Coman, Arnold, Wood & Kube 2010; Sun, Huang, Jiang, Yang, Zhou, Zhu, Yang & Su 2015), and has being specifically reported for red abalone, but in animals measured only during juvenile stages (18–24 months old; Jónasson *et al.* 1999). We found that initial low h^2 were associated with significant amounts of maternal/common environmental/non-additive effects translating into a significant maternal heritability m^2 of about 0.4. This is likely a consequence of the culture period from larvae stage to 14 month juvenile as families were maintained in separated tanks

Table 2 Estimates of additive genetic variance (V_A); residual variance (V_R); direct heritability (h^2); expected response to selection (G) (with a selection intensity of 2.06); and percentage of G per generation (G%) of flesh mass (g) and foot protein (μg mg wet per mass) in *Haliotis rufescens*, measured at 31 and 51 months of age (i.e., young-adults and harvesting age, respectively; n = 500 each)

Trait	Phenotypic mean (SD)	V_A	V_R	h^2 (SE; Log-transformed data)	h^2 (SE; untransformed data)	G	G%
Flesh-31 (CE-m)	6.93 (3.72)	2.23	7.89	0.21 (0.26)	0.16 (0.25)	1.27	17.71
Flesh-51 (S-m)	32.4 (13.2)	27.5	148	0.15 (0.07)*	0.16 (0.07)*	4.27	13.18
Foot proteins 31 (S-m)	185 (121)	0.01	14 564	NE	NE	NE	NE
Foot proteins 51 (S-m)	122 (54.4)	43.7	2918	0.02 (0.04)	0.01 (0.04)	0.081	0.75

*Significant h^2 after log-LR test $P < 0.05$.

CE-m: model that considers as additional random effects the maternal/common environment effects; and S-m: simple model that just include the additive genetic effect as random effect. Models changed depending on the factors that were significant at a specific age. Variances and genetic gains were estimated for untransformed data.

Table 3 Genetic (above the diagonal) and phenotypic (below the diagonal) correlations between production traits in the red abalone *Haliotis rufescens* at two ages (31 and 51 months)

	Length	Width	Total mass	Flesh	Foot proteins
Abalone at 31 months					
Length	.....	0.907 (0.146)*	0.994 (0.049)*	0.962 (0.042)*	NE
Width	0.951*	.....	1.036 (0.400)*	0.964 (0.043)*	NE
Total mass	0.946*	0.927*	.....	0.988 (0.019)*	NE
Flesh	0.922*	0.911*	0.962*	.....	NE
Foot proteins	−0.086	−0.071	−0.083	−0.099	.....
Abalone at 51 months					
Length	.....	0.981 (0.008)*	0.986 (0.009)*	0.983 (0.015)*	−0.734 (1.12)
Width	0.962*	.....	0.993 (0.006)*	0.975 (0.017)*	−0.972 (1.38)
Total mass	0.934*	0.946*	.....	0.994 (0.006)*	−0.760 (1.06)
Flesh	0.875*	0.895*	0.944*	.....	−0.192 (0.84)
Foot proteins	−0.011	−0.014	−0.009	0.030	.....

*Significant correlation after log-LR test (r_G) or *t*-test (r_P), $P < 0.05$.

during this period, before tagging and transfer of all individuals to a common tank. These effects might have been truly maternal in origin (e.g., egg size, egg quality, egg provisioning) or may have been related with the type and quality of the food sources growing naturally on the polycarbonate plates that had been inoculated with wild benthic microalgae, or both. The two possible sources as well as non-additive (dominance and epistatis) effects are confounded in the present design. From the 39th month onward, maternal/common environmental effects (m^2) became much smaller and non-significantly different from zero. When fitting the simpler model with just additive genetic effects as random factors from age 39 month onward, the direct heritability was higher at this age and became progressively lower in the next measures (Table 1). This is probably in part an estimation artifact due to the decrease of maternal and common environment effects with time. As clearly seen on Fig. 2, these effects are strong initially but decrease in time. At 39 months of age, the component of variance associated with these maternal/common environment effects was no longer significant, despite being of the same magnitude as the additive genetic effects. When ignoring this component in the simpler model with just the random additive genetic effects, then some of the variance previously attributed to maternal/common environment was attributed to pure additive genetic effect resulting in an apparent inflation of the direct heritability. A similar example of upward bias in h^2 estimates due to unmodelled maternal/common environment effects is provided in Wilson *et al.* (2009) (and supplementary files). This upward bias is likely also present in subsequent measures but as the maternal/common environment effect is decreasing, the potential upward bias is decreasing as well. This indicates that from the age of 39 months onward, the direct heritability estimates provided in Table 1 are probably somewhat upwardly biased, but similarly, the estimates shown on Fig. 2 are probably downwardly biased because the model still includes small non-significant maternal/common environment component of variance. The true heritability probably lies between the two estimates.

The present estimated heritabilities for growth traits in *H. rufescens* are difficult to compare with those obtained in other studies or species, because the reported estimations have been made using very different number of families (thus with contrasting levels of estimation precision), traits used have been measured at different times and at different ages, and for species with different generational times (for examples see Table 4). If we consider those studies that used a suitable number of families (i.e., >30 full-sib families; i.e., Table 4: Jónasson *et al.* 1999; Lucas *et al.* 2006; Deng, Liu, Zhang & Zhao 2007; You *et al.* 2010b), we find that our results (Table 1) for shell length and width, and total mass are higher than most of the previous estimations for comparable ages (i.e., considering generational times). *H. asinina* and *H. diversicolor* have a short grow-out period, attaining market size at ~12 months of age (Lucas *et al.* 2006; You *et al.* 2010b), therefore considering near-harvest ages our estimations are comparable to those obtained for *H. asinina* (Lucas *et al.* 2006), and higher than those of *H. diversicolor* (You *et al.* 2010b; Table 4). Our somewhat higher

Table 4 Estimates of narrow-sense heritabilities (h^2) for growth traits in several abalone species (*Haliotis* spp)

Species	No. of full-sib families analysed	Ages analysed (months)	Heritabilities (h^2)			Authors
			Shell length	Shell width	Total mass	
H. rufescens	100	8, 10, 18, 24	0.08, 0.06, 0.27, 0.34	–	–	Jónasson *et al.* 1999;
H. rubra	14	24, 48	0.07, 0.02	–	0.09, 0.01	Li *et al.* 2005
H. asinina	84	12	0.48 (± 0.15)	0.38 (± 0.13)	0.36 (± 0.13)	Lucas *et al.* 2006;
H. discus hannai	36	0.3, 0.6, 1	0.26- 0.36	0.21- 0.32		Deng *et al.* 2007;
H. laevigata	21	10, 21, 27, 38	0.00, 0.00, 0.00, 0.04 (± 0.10)	–	0.00, 0.00, 0.01 (± 0.05), 0.1 (±0.10)	Kube *et al.* 2007;
H. diversicolor	36–28	0.3, 1.2, 4, 7, 11, 14	0.37, 0.28, 0.15, 0.19, 0.23, 0.21	0.42, 0.31, 0.18, 0.19, 0.27, 0.23	0.34 (11mo), 0.27 (14 mo)	You *et al.* 2010b;
H. tuberculata	7	36	0.37	0.29	0.4	Roussel *et al.* 2013
H. rufescens	60	27, 31, 35, 39, 43, 47, 51	0.07, 0.17, 0.11, 0.75, 0.62, 0.59, 0.49	0.03, 0.13, 0.09, 0.68, 0.53, 0.48, 0.35	0.00, 0.12, 0.07, 0.75, 0.57, 0.48, 0.32	Present study

estimates might be due to the upward bias discussed above. However, it is probable that many of these studies had similar estimation problems in separating maternal/common environment effects from true additive genetic effects due to abalone early rearing challenges. In a previous study in *H. rufescens*, the h^2 for shell length was estimated only for juvenile stages, with the maximal h^2 level (0.34) seen at 24 month (Jónasson *et al.* 1999). At a similar age (27 months old) we observed the lowest level of h^2 for shell length, probably as a consequence of different rearing methodologies that introduce large amounts of common environmental effects in our results. You *et al.* (2010a) reported an average realized heritability for shell length of 0.44 ± 0.06 and 0.11 ± 0.01 in two stocks of *H. diversicolor*, the first product of the cross between wild and cultured populations, and the second a strain maintained for 10 years in aquaculture in Taiwan, emphasizing the negative effects of long time of culture on genetic variability of the populations.

Among previous studies, only You *et al.* (2010b) evaluated the changes in h^2 for growth traits through the abalone life-cycle, from juveniles to near-harvest ages (Table 4). Contrary to our study, their h^2 estimations did not change markedly during this period (~0.2–0.4). However, due to their rearing method (individuals from the same full-sib family were reared together and separate from other full sibs) they were not able to incorporate into their model maternal/common environmental effects, which in our case strongly reduced the h^2 estimations for the early stages.

Higher heritabilities for body traits after the age of 39 months indicate that potential for improving growth rates through selective breeding is good. Our estimations suggest genetic gains per generation from 13% to 26% for the shell length and width, and from 16% to 52% for the total mass can be expected by selecting 5% of the largest or heaviest animals as breeders, at different moments after the 39 months of age. As discussed above, h^2 is probably overestimated somewhat and therefore true genetic gains would probably be slightly lower. There are few selection experiences reported in abalone. In *H. diversicolor*, improvement of growth rate in shell length varied between 5% and 13% selecting the 10% faster growers in two different stocks (You *et al.* 2010a). In our study, the highest gains would be for total mass (~4–21 g per generation), a trait with greater importance for abalone aquaculture. Highest genetic gain for this body trait would be obtained with selection at 43 months of age, and the lowest at 39 months of age, when abalone has attained the market age. However, a complementary bio-economic evaluation would be needed to determine the best age for the selection process.

The growth rate observed in the present study is close to that estimated for wild *H. rufescens* in northern California (Rogers-Bennett, Rogers & Schultz 2007). Different environmental factors have been shown to influence growth rate in abalone, including water temperature (Kelly & Owen 2002; Searle, Roberts & Lokman 2006), amount and quality of food (Nidoo, Manveldt, Ruck & Bolton 2006; Cho & Kim 2012), stocking

density (Capinpin, Toledo, Encena & Doi 1999; Wu, Liu, Zhang & Wang 2009), oxygen concentration (Harris, Maguire, Edwards & Johns 1999), dissolved ammonia (Harris, Maguire, Edwards & Hindrum 1998), stress conditions (Hooper, Day, Slocombe, Handlinger & Benkendorff 2007), and combinations of some of those factors (Tung & Alfaro 2011). In red abalone, growth rate has been related with water temperature, food availability and diseases, like withering syndrome (González, Brokordt & Lohrmann 2012), and the interaction among these factors (Braid, Moore, Robbins, Hedrick, Tjeerdema & Friedman 2005). Jónasson *et al.* (1999) reported monthly gain in shell length of 2.3–2.4 mm per month for juvenile stages between 8 and 24 months of life at temperatures between 16.0 and 17.3°C. These growth rates in shell length are twice those observed in the present study. Optimal temperature range for this species growth has being estimated to be between 17.8°C (Steinarsson & Imsland 2003) and 18.4°C (Díaz, del Río-Portilla, Sierra, Aguilar & Re-Araujo 2000), but it depends upon the size or age of the abalone (Steinarsson & Imsland 2003). In our study, abalone were maintained under variable temperatures (13.3 and 20.2°C), and most of the time at low temperatures (14–16°C). Additionally we worked with ages (27–51 months) where growth rates may be lower. On the other hand, red abalone strains cultured at present in Chile are descendent of individuals introduced 25 years ago from California and Mexico (Flores-Aguilar *et al.* 2007) and have not been the subject of a breeding program. Reduction in maximal shell size and changes in growth rate have been reported for small abalone (*H. diversicolor*) after one decade in a closed culture system (Huang & Hseu 2010), and this kind of effect cannot be excluded in the Chilean population of red abalone.

In the present study, we further estimated the h^2 of traits associated with the abalone meat yield and quality, such as flesh mass and protein content in the foot muscle, which is the commercialized part of the abalone. These estimations were done twice, in the young-adults (31 months old) and adults at harvesting age (51 months old). Foot protein content had a very low and non-significant h^2, for both ages. Heritability for flesh mass for young-adults was 0.21, but with high SE (0.26), and it was not significantly different from zero. Both protein and flesh mass h^2 estimations may have been affected by the small sample size used for these measures. We observed that only the h^2 for the flesh mass for adults at harvesting age was significant ($h^2 = 0.15$), and we estimated an average improvement 4.27 g in flesh mass per individual after one generation of direct selection for this trait. This is important because flesh mass influences market value more than shell sizes (shell length and width). Direct measures of flesh mass are more difficult and need sacrificing the individuals. However, strong positive genetic correlations (>0.9) between shell sizes, total and flesh mass were observed for both young adults and adults at harvest. Genetic correlations among traits arise as a consequence of the pleiotropic effects of genes controlling both traits or due to gametic phase disequilibrium between loci controlling each trait (Falconer & Mackay 1996). When two traits are genetically correlated selection on one of them results in a correlated response of the other. The magnitude of the correlated response to selection (CG_Y) of a trait (Y) as a result of the selection of the other trait (X) is: $CG_Y = i \times h_X \times h_Y \times r_G \times \sigma_{PY}$; where i is the selection intensity, h_X and h_Y are the square root of the heritability of traits X and Y, respectively, r_G is the genetic correlation between the selected traits, and σ_{PY} is the phenotypic standard deviation of trait Y (Falconer & Mackay 1996). Thus, if the 5% heaviest or largest 51 months old abalone are selected from the population as broodstock ($i = 2.063$) we would expect a positively correlated response on flesh mass between 19.3% and 23.4% respectively.

In summary, in this study we found that h^2 for growth traits, strongly changed during the grow-out of *H. rufescens* from juvenile to adult stages, with h^2 being highest for adult stages because of strong maternal/common environment effects in early rearing. These results emphasize the importance of multiple estimations of h^2 over time. Selective breeding programs for this species could be established and should yield commercially important gains. Due to the low additive genetic variation for growth traits in juveniles, early selection would not be recommended. On the other hand, due to their strong genetic correlations, high levels of genetic gains can be attained for total and flesh masses through selection for shell length in near-harvest adults *H. rufescens*.

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