



Heritability of energy intake and allocation and their correlated responses to selection on growth traits in cultured juvenile and adult red abalone *Haliotis rufescens*

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ABSTRACT

Increasing abalone growth rates and their physiological efficiency in the use of energy through selective breeding could improve abalone production. High potential responses to selection for growth traits have been estimated in several abalone species; however, the genetic bases underlying individual differences in physiological performance are unknown. The aims of this study were to estimate the heritable variation of physiological traits associated with energy intake and allocation for the red abalone *Haliotis rufescens*; and the potential correlated responses to selection between growth and physiological traits at three developmental stages. Growth traits (shell length and width, and total mass) and physiological traits [energy intake, standard metabolic rate (SMR), and energy losses by ammonia excretion and feces] were measured in individuals belonging to 60 full-sib families in 2-years old juveniles, 3-y old young adults, and 4-y old near-harvest adult ($n = 500\text{--}380$ individuals/age; total $n = 1300$). Food intake showed significant h^2 in juveniles and young adults (0.37 and 0.14, respectively); but near zero h^2 in near-harvest adults. Variation in energy required for SMR showed low but significant h^2 (0.13) in young and near-harvest adults. Variation in energy lost through ammonia excretion showed significant h^2 in juveniles (0.33) and in near-harvest adults (0.14). Heritability for variation in the energy losses through feces was not significant. Heritabilities for growth traits were all moderate to high and significant for young and pre-harvest adults, but were not significant for juveniles. At the young adult stage, food intake was negatively and highly genetically correlated with each of the growth traits (-0.70 to -0.85); and at the near-harvest adult stage both SMR and ammonia excretion showed high-negative genetic correlations with each growth trait (-0.88 to -1.00). Thus, estimated correlated response to selection indicated that if selection was exerted for higher growth (choosing 5% best individuals), we would be indirectly selecting for abalones with lower food intake (correlated responses of -11 to -15%); or lower metabolic demands of (-14 to -24%), if the selection was applied to young or near-harvest adults, respectively. In turn, shell length could be enhanced up to 18% per generation. These results suggest that higher growth in red abalone is genetically associated with a more efficient use of food and a lower metabolic demand. The possibility to reduce food intake or metabolic demands by selecting for faster growing abalones would be a very positive development for the sustainability of this culture.

1. Introduction

Because abalones grow slowly, increasing growth rates by selective

breeding could enhance industry competitiveness worldwide. Measurements of the heritability for production traits (e.g., growth rate, flesh mass) in several abalone species predict responses to selection

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ranging from ~5–10% per generation (Brokordt et al. 2015; Fariás et al. 2017), which augurs well for selective breeding programs for these molluscs. Although these results are quite promising for abalone aquaculture, selective breeding generally focusses on economically important traits such as growth, and less attention has been paid to traits that are actually determining the observed production rates. Growth capacity (both somatic and reproductive) is a function of animal physiological performance, in terms of their efficiency in the use of available energy. Moreover, food expenditure is a critical component for farming profitability and improvement in the animal's efficiency of food use would be of great importance.

The energy balance integrates the basic physiological processes of energy acquisition and allocation. The energy budget, which is an index of the energy available for growth (scope for growth) and reproduction, i.e., individual production, is derived from the energetic components of energy balance. Thus, growth differences of animals that share the same environment, e.g., in culture systems, could reflect in part inter-individual differences in: energy acquisition (i.e., food ingestion, absorption and assimilation); allocation of energy for maintenance (i.e., standard metabolism); losses through nitrogenous waste excretion (e.g., ammonia excretion mainly from protein metabolism) and feces production (i.e., unabsorbed food). Although there is much information on marine mollusc's energetic parameters and its application for ecological and aquaculture studies (e.g., MacDonald and Thompson 1986; Bayne, 1999; Navarro et al. 2000; Zhuang 2005), only some studies have characterized the genetic bases underlying individual differences in physiological performance. These mainly compared selected and not-selected families for faster growth for energy acquisition and metabolic rates (Bayne et al. 1999; Pernet et al. 2008; Tamayo et al. 2014, 2015; Ibarrola et al. 2017).

Among the components of energy allocation, maintenance metabolic rate has been the most studied in animals (Artacho and Nespolo 2009). Maintenance metabolism represents the minimum energy requirements to be alive and is a significant proportion of the overall energy budget (up to 50% in some ectotherms) (Artacho and Nespolo 2009); it is thus considered to be one of the most relevant physiological traits. Heritability of metabolic rates (basal, standard, resting and maximal) has been estimated mainly for vertebrates (Konarzewski et al. 2005; Sadowska et al. 2005; Rønning et al. 2007; Nilsson et al. 2009), and to our knowledge, no information is available for marine molluscs. However, studies done in a terrestrial snail (Czarnecki et al. 2008) and oysters (Bayne 2000, 2004; Pernet et al. 2008) suggest that variation in the standard metabolic rate is partly under genetic control since these studies show a correlated response to selection for fast growth. Thus, maintenance metabolism could be the target of selection, optimizing the energy budget in animals (Alexander 1999; Artacho and Nespolo 2009).

In addition to metabolic rate, physiological processes that deal with energy acquisition such as ingestion rate are of great importance. An individual's capacity or efficiency for food ingestion, absorption and assimilation will determine a significant proportion of the energy available for maintenance, growth and reproduction. The genetic basis underlying the variability of these energy budget components has not been estimated in gastropod molluscs. However, indirect evidence of genetic control upon these traits has been reported for some bivalves, where individuals selected for fast growth showed increased feeding rates or efficiency compared with their unselected counterparts (Bayne 2000, 2004; Tamayo et al. 2014, 2015; Ibarrola et al. 2017).

In spite of the fact that physiological processes associated with energy losses through excretion and feces production are important components of the energy balance of marine molluscs, to our knowledge, the genetic control upon these traits has not been reported. However, indirect estimations of genetic control were assessed through the repeatability levels of these physiological traits in the Pacific abalone *H. discus hannai*; showing significant repeatability values for ammonia excretion (González et al. 2010).

A fundamental principle in evolutionary physiology is the energetic dimension of fitness, which predicts that selection should increase the residual energy available for growth and reproduction (Brown et al. 1993; Alexander 1999). However, to respond to selection, between-individuals variation in energy metabolism components needs to be partly under genetic control. The potential of a trait to respond to selection depends on its additive genetic variation, and narrow-sense heritability (h^2) is the proportion of within-population phenotypic variance that is due to additive genetic variation (Nespolo et al. 2003). Therefore, how much heritable variation a trait possesses will set the magnitude of its intergenerational response to directional selection (Falconer and MacKay 1996). Additionally, when estimating the potential response to selection of the traits of interest, sometimes correlated responses may arise (Lande and Arnold 1983; Rønning et al. 2007). Two traits are genetically correlated when the variation in one or more genes affects both traits (Krebs et al. 1998). Therefore, it is important to estimate the magnitude and direction of the genetic correlations between the traits of interest.

The aim of this study was to estimate the heritable variation of physiological traits associated with energy intake and allocation for the red abalone *Haliotis rufescens*, in order to assess their potential response to selection. We further estimated genetic correlation of these components of the energy budget with growth traits, in order to estimate possible coupled responses to selection. To increase the reliability of the estimates and to determine the optimum time at which the selection process must be done, heritabilities and genetic correlations were estimated at different developmental stages across *H. rufescens* culture life span, such as in juvenile, young adults, and pre-harvest adult.

Red abalone is the most important cultured abalone species in Chile (98% of the production); and this country is the third biggest abalone producer in the world (FAO, 2016). In addition to slow growth, cost and limited availability of natural food are of main concern for abalone growers (O'Mahoney et al. 2014; Zuniga-Jara and Marín-Riffo, 2014). Hence, there is a need for optimizing the use of this resource. Increasing abalone growth rates and their physiological efficiency in the use of energy through selective breeding should improve abalone production.

2. Materials and methods

2.1. Breeding design and animal rearing conditions

Breeding design and animal rearing conditions followed Brokordt et al. (2015). Sixty full-sib families were produced using *Haliotis rufescens* abalone broodstock randomly obtained from a base population of 600 adults provided by 3 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 °C and 19 °C and under permanent feeding with macroalgae. Mature abalones were induced to spawn separately using the hydrogen peroxide method (Morse, 1977). Crossings were conducted following a paternal half-sib nested design where 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 hatching, larvae from each full-sib family were allowed to grow for 5–6 days, and 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 3 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 was initially fed with wild benthic microalgae. From the seventh month onward, abalones 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 their shells with epoxy resin. Then, individuals from different 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. In the raceway, abalones were maintained during 3 years with continuous water flow, constant aeration, at ambient temperature that varied between $\sim 13\text{--}20^\circ\text{C}$ during the year.

2.2. Physiological measurements

In order to assess changes in the heritabilities (h^2) and genetic correlations (r_G) across the abalone culture process, physiological and growth traits (i.e., total mass, shell length and width) were measured at three life stages: juvenile (2 y-old, ~ 40 mm), young adults (3 y-old, ~ 60 mm), and adults at market size (4 y-old, ~ 70 mm). Physiological traits were measured in 5–10 individuals per full-sib family (60 families) in each life stage ($n = 380\text{--}500$ individuals per life stage). Thus a total of 1300 individuals were measured in this study.

Due to the huge time consuming nature of the physiological measurements, components of the energy budget were measured over two days, in groups of ~ 40 abalones, during a period of 4 weeks for each life stage. This factor (date of measurement) was included as a fixed effect in the model for estimating heritabilities (see below for details). Before each measurement period, abalones were acclimated to individual cages and to a near constant temperature of $16 \pm 0.5^\circ\text{C}$ during one week.

Physiological measurement methods followed Gonzalez et al. (2010). For the estimation of food consumption rates, abalones were maintained in individual cages with the same amount of *M. pyrifera*. The consumed food was estimated as the difference between the initial algae mass and the remainder 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.

In order to determine fecal production, 24 h after the feeding measures, abalone were transferred to an open and aerated container containing 0.6 L (0.9 L when animals were 4y-old) of micro filtered ($1\ \mu\text{m}$) sea water and maintained at a near constant temperature ($16 \pm 0.5^\circ\text{C}$) for 12 h. Following this period, the water in the container was filtered and washed with 3% ammonium formate solution to dissolve salts. The feces were then oven dried at 60°C until a constant mass was achieved.

To estimate oxygen consumption (standard metabolic rate, SMR) and ammonia excretion, abalones were placed individually in sealed 0.6 L chambers (0.9 L when animals were 4y-old) filled with micro filtered sea water. Individuals were maintained in these chambers for 1 h at a near constant temperature ($16 \pm 0.5^\circ\text{C}$). The dissolved oxygen and ammonia concentrations were measured using the Winkler (modified by Carpenter 1965) and Solorzano (1969) phenol hypochlorite methods, respectively. The oxygen consumption and the ammonia excretion were estimated by the difference between the initial and final (i.e. after 1 h) concentration of these compounds. We corrected consumption and excretion rates using a control chamber exposed to the same conditions, but without abalone.

Physiological rates were expressed in Joules $\text{g}^{-1}\text{h}^{-1}$ using the following energy conversion values: 1 mL $\text{O}_2 = 4.75$ cal (Thompson and Bayne 1974), 1 mg $\text{NH}_4\text{-N} = 5.94$ cal (Elliot and Davidson, 1975) and 1 g of feces = 2817 cal (Peck et al. 1987). The algae energy value was determined by a Parr isothermal oxygen bomb calorimeter, estimating that 1 g of algae was equivalent to 549 cal. Calories were converted into Joule units (1 cal = 4.184 J).

Following completion of physiological measurements, the total mass of each individual was measured using an electronic balance (± 0.001 g) after animals had been dried with paper-towel to eliminate water excess; and shell length and width were determined using a digital calliper (± 0.01 mm).

2.3. Estimation of phenotypic variance components and genetic estimates

The heritability of each energy budget component were estimated using the full-sib/half-sib design with the restricted estimated maximum-likelihood (REML) procedure (Johnson and Thompson 1995) as implemented in ASReML v.3.0 (Gilmore 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 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 e represents the residual effects. X , Z_a and Z_m are the corresponding incidence matrices. Maternal effects are the non-genetic effects passed by mothers to all their offspring, which could affect the trait(s) of interest. For abalone which do not feed their young or provide other parental care, maternal effects are driven by varying egg quality, in particular variation in energy, vitamins, essential fatty acids and essential amino acid provisioning to the eggs, which in turn affects early survival and growth of the progeny. In most cases, the variables were reasonably well distributed, but in three cases (SMR in juveniles, Total Mass and Shell Width in pre-harvest adults), log transformation was used to normalize the data distribution. In these three cases, the presented results are for the transformed trait.

Using this general model, we fitted a model for each developmental stage after estimating the significance of fixed and random effects. We estimated the significance of fixed effects and covariates using the Wald F statistic as implemented in ASReML 3.0. Statistical significance of the maternal/environmental/non-additive random effects and of the additive random effects (h^2 significance) was estimated by the log-likelihood ratio test (log-LR test). The variables that were evaluated as fixed effects were: 1) location of the tank in which each full-sib family was held for the first 14 months of life, 2) densities in which the families were held during this period, and 3) order in which individuals physiological traits were measured (nested within the year of measure). We also evaluated the effects of the exact age at the time of the measurements. 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, thus we fitted them as common random effects. In addition, potential non-additive genetic effects are also confounded with common environmental/maternal effects and cannot be teased out with the experimental design followed here. The direct h^2 of each trait was calculated as the ratio of the additive genetic variance to the total phenotypic variance V_p , with V_p estimated as the sum of the additive genetic variance and the residual variance (Falconer and Mackay 1996).

From the phenotypic variance components, the coefficients of phenotypic variation ($CV_p = 100\sqrt{V_p}/X$), additive genetic variation ($CV_A = 100\sqrt{V_A}/X$), and residual variation ($CV_R = 100\sqrt{V_p - V_A}/X$) were estimated (Houle 1992). This calculation includes trait means (X), which are considered to be more appropriate for standardising the variances and inferring the evolvability of quantitative traits (Houle 1992). The potential response to the selection for each physiological trait was estimated as $G = i * \sigma_p * h^2$, where i is selection intensity (Falconer and Mackay 1996). This calculation was done in the context of selecting the 5% best individuals as broodstock, with $i = 2.06$. The expected gain response per generation was estimated as $G (\%) = (G/X) * 100$. This calculation was only done for traits with a significant heritability.

A bivariate animal model was used with ASReml version 3.0 (Gilmore et al. 2009) to estimate genetic correlations between physiological traits and growth traits, for each developmental stage. This was only done for traits with significant heritability in the univariate analyses above. 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.

The correlated response of physiological trait y to selection on growth trait x was estimated as $CRy = i \cdot h_x \cdot h_y \cdot r_G \cdot \sigma_{Py}$; where h_x and h_y are the square roots of the heritabilities for the selected trait (x) and correlated traits (y), respectively; r_G is the additive genetic correlation between x and y traits, and σ_{Py} is the standard deviation of the y trait (Falconer and Mackay 1996). The relative correlated response to selection was estimated as $CRy (\%) = (CRy/Xy) \cdot 100$; where Xy is the mean of the correlated physiological trait y .

2.4. Ethics statement

The abalones used in this study were provided by three private abalone breeding companies (Live Seafood Chile S.A., Abalones Chile S.A. and Cultivos Marinos San Cristobal S.A.). Abalone transport from each private company to the Universidad Católica del Norte Aquaculture Center, was permitted and supervised by the National Service of Fisheries of Chile. Ethical approval was not required for this study because no endangered animals were involved.

Animal maintenance and experimental manipulations in this study were carried out in strict accordance with the recommendations in the CCAC (Canadian Council on Animal Care) guidelines on: choosing an appropriate endpoint in experiments using animals for research, teaching and testing. All efforts were made to minimize suffering during animal manipulations and surgery. The protocol for sampling procedures and experimental manipulations were reviewed and approved by the Bioethics Committee of the Centro de Estudios Avanzados en Zonas Aridas (Permit Number: 005–13) and the National Council of Science and Technology of Chile.

3. Results

3.1. Phenotypic variation of physiological and growth traits

Energy intake ($J g^{-1} h^{-1}$) through algae consumption was similar between 2 y-old juvenile and 3 y-old young adults; and lower in 4 y-old pre-harvest adults (ANOVA, $P < 0.01$; Table 1). From this consumed energy, juveniles used more energy for standard metabolism and lost more energy through feces than young and pre-harvest adult abalones; while the latter lost more in ammonia excretion (ANOVA, $P < 0.01$; Table 1). During the 3 year period where physiological parameters were assessed, total mass increased ~4 times and shell length and width increased ~1.5 times (Table 1).

3.2. Genetic variations and heritabilities of physiological and growth traits

The order in which individual's physiological traits were measured (nested within the year of measure) was the only fixed factor that affected significantly the variation of energy intake, and most energy allocation components (Wald F , $P < 0.001$) at the different life stages evaluated. No significant maternal/common environment effects on the variation of these physiological components were detected for either life stage (log-LR test, $P > 0.05$). Therefore, for the estimation of the additive genetic and residual variances and h^2 for the physiological traits at the different life stages, we used a simple linear mixed model with only additive genetic effects fitted as random factor and order of measure fitted as fixed effect. The same simple model was used for the

growth trait mass, length and width at the young adult and pre-harvest adult stage. However, at the juvenile stage, significant maternal/common environment effects were detected and the models included these as random factors in addition to additive genetic effects.

With the exception of food intake and ammonia excretion in juvenile abalones, the coefficients of additive genetic variations (CV_A) for the evaluated physiological traits were much lower than the coefficients of residual variations (CV_R ; Table 1). Most direct heritabilities (h^2) for energy intake and allocation traits varied with abalone developmental stages. The models did not converge reliably in the case of standard metabolic rate in juveniles and feces production in juveniles and young adults, and no estimates of additive genetic variation or heritability could be obtained in these cases (Table 1). Energy incorporated through food consumption showed moderate significant h^2 for juveniles, lower but still significant h^2 for young adults, but fell to near zero values in near-harvest adults. Variation in energy required for standard metabolism showed relatively low but significant h^2 in young and near-harvest adults. Variation in energy lost through ammonia excretion showed moderate and significant h^2 for juveniles, lower but significant h^2 for near-harvest adults, but near zero h^2 for young adults. Heritability for variation in the energy losses through feces could only be estimated for near-harvest adults but was not significant.

On the other hand, production traits showed similar levels of genetic additive and residual variations. Heritabilities for these traits were all moderate to high and significant for young and pre-harvest adults (Table 1). At the juvenile stage, significant maternal/common environment effects were detected for all three traits (result not shown) and heritability of these traits, though of a moderate magnitude were not significant (Table 1).

Based on h^2 estimates, the expected relative genetic gains per generation (G %, when selecting the 5% best individuals) were estimated (Table 1). For physiological traits, food intake showed important levels of potential genetic gain through selection, which varied between 24% for juveniles and 9% in young adults, respectively. Interestingly, young and pre-harvest adults also showed potential gains per selected generation for the SMR of 20% (on the log transformed scale) and 15%, respectively. Substantial potential gains in ammonia excretion were shown as well, 29% for the juveniles and 18% for the pre-harvest adults. For production traits, young adults showed the highest expected genetic gains, especially for the total mass and shell length, which varied between 14 and 40%. An important potential genetic gain of 13% for shell length was observed as well in pre-harvest adults.

3.3. Genetic correlations and estimated correlated response of physiological traits to selection on growth traits

Genetic correlations (r_G) were estimated for traits that presented significant heritabilities; and relative correlated response to selection were estimated between growth and physiological traits that showed significant r_G between them, therefore only young and pre-harvest adults were considered for these analyses (Table 2). At the young adult stage, food intake was negatively and highly correlated with each growth traits. However, r_G between SMR and food intake, and SMR and growth traits were low and not significant at this abalone stage. By contrast, at the pre-harvest adult stage, both SMR and ammonia excretion, were highly and negatively correlated with each growth traits; and positively correlated between them (Table 2). Consequently, high-negative relative correlated responses of food intake to selection on growth traits were observed in the young adult stage. In the pre-harvest stage, energy allocated to SMR and ammonia excretion showed high and negative relative correlated responses to selection on growth traits (Table 2).

4. Discussion

To our knowledge this is the first study to report the existence of

Table 1

Phenotypic means (raw untransformed data) and estimates of coefficients of additive genetic (CV_A) and residual (CV_R) variances, heritabilities (h^2), and expected gain responses to selection per generation [G (%), with a selection intensity of 2.06] for physiological rates ($Jg^{-1}h^{-1}$) [food intake, standard metabolic rates (SMR), ammonia excretion and feces production] and production [total mass (g) and shell length and width (mm)] traits, in red abalone *Haliotis rufescens* at three life stages ($n = 380$ – 500 per stage).

Life stage	Trait	Phenotypic mean (SD) ¹	CV_A	CV_R	h^2	G (%)
Juveniles	Food intake	91.2 (33.8) ^a	19.42	25.36	0.37 (0.11)**	24.33
	SMR	23.0 (16.0) ^a	NE ²	NE	NE	NE
	Ammonia excretion	0.30 (0.16) ^b	26.79	43.9	0.27 (0.10)**	28.74
	Feces production	32.2 (32.4) ^a	NE	NE	NE	NE
	Total mass ³	15.8 (7.9) ^c	22.07	36.76	0.19 (0.27)	ND ⁴
	Shell length ³	45.9 (7.5) ^c	9.15	10.93	0.30 (0.29)	ND
	Shell width ³	31.3 (5.2) ^c	8.06	11.84	0.22 (0.28)	ND
Young adults	Food intake	91.8 (37.5) ^a	11.70	28.69	0.14 (0.09)*	9.10
	SMR (log transf.)	11.8 (7.42) ^c	26.67	69.88	0.13 (0.08)*	19.6
	Ammonia excretion	0.30 (0.20) ^b	17.79	65.83	0.07 (0.07)	ND
	Feces production	15.6 (13.4) ^c	NE	NE	NE	NE
	Total mass	33.6 (15.7) ^b	30.01	35.03	0.42 (0.12)**	40.22
	Shell length	58.3 (9.2) ^b	11.66	10.66	0.54 (0.13)**	17.72
	Shell width	41.0 (6.6) ^b	10.22	12.12	0.42 (0.12)**	13.57
Pre-harvest adults	Food intake	86.3 (48.1) ^b	12.26	50.01	0.06 (0.06)	ND
	SMR	15.3 (9.6) ^b	20.01	52.95	0.13 (0.08)*	14.58
	Ammonia excretion	0.82 (0.50) ^a	22.49	55.08	0.14 (0.08)*	17.51
	Feces production	22.1 (17.5) ^b	21.83	74.00	0.08 (0.07)	ND
	Total mass (log transf.)	55.6 (25.9) ^a	5.83	9.83	0.26 (0.10)**	6.11
	Shell length	65.9 (10.3) ^a	10.03	12.00	0.41 (0.12)**	13.26
	Shell width (log transf.)	47.7 (7.8) ^a	2.27	3.59	0.29 (0.10)**	2.51

¹ Phenotypic means for each trait were compared among life stages through ANOVA and Tukey *a posteriori* multiple comparison (different letter indicates significant pairwise comparison at $P < 0.05$ level).

² NE = not-estimable, the model did not reliably estimate the variance components.

³ Models included random dam effects

⁴ ND = Expected gain per generation not calculated because heritability was not significant.

* Statistical significance at $P < 0.05$.

** Statistical significance at $P < 0.01$, using a log-likelihood ratio test.

heritable variation for food intake and physiological traits associated with energy allocation in a marine mollusc. These genetic estimates can be relevant in the context of a selective breeding program for farmed abalones.

The present study revealed that the variation of food intake is heritable in juveniles (2 y-old, ~40 mm) and in young adults (3 y-old, ~60 mm) *Haliotis rufescens* red abalone, and presents an important potential to respond to selection, especially in juveniles. Traits associated with energy allocation, like the standard metabolic rate (SMR) and ammonia excretion, showed moderate but significant heritable variation in at least two of the three life stages evaluated for red abalone. By contrast, heritability for the variation in feces production could

only be estimated for the pre-harvest adults and was not significant. This probably indicates that this measure is quite difficult to perform accurately particularly for small abalones and noise in the data was probably responsible for the absence of significant heritability estimation. As reported earlier for red abalone (Brokordt et al. 2015), in the present study, heritabilities for growth traits (total mass and shell length and width) were not significant at the juvenile stage, in part because of significant maternal/common environmental effects; but they increased and became significant in young and pre-harvest (4 y-old, ~70 mm) adults.

Interestingly, the food intake was highly but negatively genetically correlated with each of the assessed growth traits in the young adult

Table 2

Genetic correlations and estimated relative correlated responses ($CR\%$) of physiological traits (energy intake and allocation) to selection on growth traits (total mass, and shell length and width) in the red abalone *Haliotis rufescens* at young and pre-harvest adults life stages.

Young adults	Genetic correlations		Correlated response to selection (%)	
	Food intake	SMR	Food intake	SMR
SMR	-0.091 (0.486)	-	-	ND
Total mass	-0.791 (0.144) ^a	-0.097 (0.345)	-12.2	ND
Shell length	-0.849 (0.120) ^a	-0.258 (0.317)	-14.9	ND
Shell width	-0.696 (0.177) ^a	-0.192 (0.345)	-10.8	ND
Pre-harvest adults	Genetic correlations		Correlated response to selection (%)	
	SMR	Ammonia excretion	SMR	Ammonia excretion
Ammonia excretion	0.976 (0.085) ^a	-	16.2	-
Total mass	-0.946 (0.074) ^a	-1.001 (0.039) ^a	-20.3	-24.1
Shell length	-0.895 (0.081) ^a	-0.939 (0.055) ^a	-24.1	-28.3
Shell width	-0.878 (0.093) ^a	-0.978 (0.042) ^a	-13.8	-24.8

$P < 0.05$; SMR = standard metabolic rate; ND = not done, because the estimated genetic correlation was not significantly different from zero.

^a Significant correlation after log-LR test.

stage. Thus estimated correlated response to selection indicated that if selection was exerted for higher growth, we would be indirectly selecting for abalones with lower food intake. These reductions were estimated to be between -11 and -15% , with the highest correlated reduction in food intake when selecting for increased shell length. In turn shell length could be enhanced up to 18% per generation, if the 5% largest young adults are selected as broodstock. It seems counter-intuitive at first glance that growth rate would be negatively genetically correlated with food intake. This probably indicates that feed intake might not be a very good predictor of assimilation efficiency which is probably a primary driver of growth rate. Ibarrola et al. (2017) recently found that fast-growing families of the *Perna canaliculus* mussel showed a higher food processing capacity in the gut than slower growing families. Lower loss of energy by excreted ammonia is associated with higher assimilation efficiency. This is in good agreement with our observations of strong negative genetic correlations between growth traits and both ammonia excretion and standard metabolic rate in pre-adult abalone. Thus our result could be partially associated to a more efficient food processing or assimilation capacity of faster-growing abalone. The present result is potentially very significant for the profitability and sustainability of abalone farming, because two economically relevant aspects such as time to harvest and expenditure in food could be reduced through a selective program for red abalone. Most abalone farms use fresh seaweed (mainly kelp) as feed, but this resource is becoming quite limited because of overexploitation, and this constitutes a bottleneck for increased abalone production (Troell et al. 2006; Cook 2014; Kemp et al. 2015). Thus, the possibility to reduce food intake by selecting abalones is also positive in terms of the sustainability of this culture.

Abalones at the young and pre-harvest adult stages showed heritable variation for SMR, while juveniles and pre-harvest adults also showed heritable variation for ammonia excretion. These results are coherent with our previous finding for the Pacific abalone *H. discus hannai* in which SMR and the energy lost by ammonia excretion were significantly repeatable (the repeatability being the consistency of the between-individuals variation, Senar 1999) (González et al. 2010). Repeatability (R) includes genetic variance, thus it sets an approximate upper limit for heritability (Falconer and Mackay 1996; Dohm 2002). There are no other studies where direct heritability for SMR or energy lost by ammonia excretion has been estimated in marine molluscs; but for the terrestrial snail *Helix aspersa*, SMR has been found to respond to natural selection promoting a reduction of this trait in this species (Artacho and Nespolo 2009).

In the present study, estimated responses to direct selection upon SMR were moderate in young adults and in pre-harvest adults of *H. rufescens*; and for ammonia excretion it was relatively high in juveniles, and moderate in pre-harvest adults. Low, not-significant genetic correlations between SMR and growth traits were observed in young adults. However, in pre-harvest adults both SMR and ammonia excretion were highly genetically correlated with the three growth traits; and interestingly, these genetic correlations were all negative. Consequently, estimated relative correlated responses of SMR and ammonia excretion to selection on growth traits were high and negative, ranging between -14 and -24% , and -24 and -28% , respectively for SMR and ammonia excretion, with the highest correlated response observed when selecting on shell length as growth trait. It should be noted that many of the reported genetic correlations in the present study are very high and are probably inflated. This is most likely driven by unknown measuring biases, probably associated with the observation of a significant fixed effect of the order of measurement on most physiological measures. Overall, these results indicate that if the largest red abalones were selected from the population as broodstock we expect that their progeny will also lose less energy through maintenance metabolism and metabolic waste. This suggests that at this stage a better growth performance in red abalone would be genetically linked with an increased metabolic efficiency. Similarly, Czarnoleski et al.

(2008) found that the main correlated response to artificial selection for large body size in *H. aspersa* was a reduction in energy metabolism; and in the oysters *Crassostrea gigas* and *C. virginica*, animals selected for fast growth for several generations were found to have lower metabolic rates and higher protein turnover efficiency than their unselected counterpart (Bayne 2000, 2004; Pernet et al. 2008). A fundamental principle in evolutionary physiology is the energetic definition of fitness, which anticipates that selection promotes the residual energy available for growth and reproduction (Brown et al. 1993; Alexander 1999). According to this principle, standard, resting or basal metabolic rates are considered as physiological costs, thus should be inversely correlated with fitness associated traits (Nespolo et al. 2013). Our results for red abalone are consistent with this principle, but we also report here that costs due to ammonia excretion follow the same pattern. This is to our knowledge the first study to estimate how this trait would be genetically associated with growth in molluscs.

The possibility to reduce oxygen consumption and ammonia excretion while selecting abalone for fast-growth can be positive, especially for land based abalone farms. Indeed, significant challenges facing the abalone industry included the cost of energy associated with water and air pumping (Kilian 2016). The reduction in abalone demand for oxygen and a decrease in the production of their metabolic waste could reduce the needs for high rates of water exchange and thus the pumping energy cost.

In conclusion, our results indicate that in a selective breeding program for red abalone in Chile, the size of the animals could be substantially increased through selection of growth traits at young and pre-harvest abalone stages (i.e., at 3 and 4-year-old). Furthermore, lower food consumption and lower metabolic demands associated with the increase in size could be expected, if the selection is applied respectively at the age of 3 or 4 years. Overall, our results suggest that higher growth in red abalone is genetically associated with a more efficient use of energy intake and a lower metabolic demand. The fact that these physiological traits associated with energy intake and allocation could be modified in the next generations through indirect selection on growth makes these improvements a practical and worthwhile process to pursue for this industry.

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