



## Short communication

# First insight into the heritable variation and potential response to selection of phototaxis and locomotion behavior associated to the light/dark stimuli in the abalone *Haliotis discus hannai*



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## ABSTRACT

Abalones are especially susceptible to environmental lighting conditions. This factor greatly affects crucial biological process such as feeding rates, energy balance, physiological stress status, and consequently, growth and survival of farmed abalone. Most of these effects have been studied in the economically valuable abalone *Haliotis discus hannai*. The use of specific photoperiods, and/or light qualities and intensities, have been proposed as managing strategies to increase its production; however, for extensive off-shore or in intensive land-based farming systems, lighting conditions are not likely to be easily managed. Despite the great importance of the biological responses to the light/dark stimuli for abalone farming production, to the best of our knowledge the genetic control upon the variation associated behavioral traits have not been studied. Therefore, the aim of this study was to estimate the heritable variation and potential responses to selection for the phototaxis [i.e., displacement towards (positive) or against (negative) the light source] and locomotion behaviors associated to the intensity of the response (i.e., crawling speed and displacement distance) to the light/dark stimuli in juvenile *H. discus hannai*. Genetic and phenotypic correlations between these traits were also estimated. Results showed moderate but significant heritable variations for phototaxis ( $h^2 = 0.15$ ) and locomotion responses ( $h^2 = 0.18–0.37$ ); and significant positive genetic correlations among them. Expected gain responses to selection per generation (with a selection intensity of 2.06, i.e., selecting 5% of the individuals from a population) were moderate for phototaxis variation (16%) and high for locomotion responses variation (33–67 or 36–73%, depending on the model used for the estimations). As a consequence, the potential for reducing (or incrementing, depending on the breeding goal) the reactivity or the sensibility to the light stimulus by selective breeding is good, and can be an attractive way of indirectly improving growth, survival and general welfare of farmed *H. discus hannai*.

## 1. Introduction

Light is an environmental factor that controls directly or indirectly several crucial biological processes of cultured organisms, such as ingestion, growth, development and locomotion behavior (e.g., Boehlert, 1981; Begtashi et al., 2004; Garcia-Esquivel et al., 2007; Gao et al., 2016a; Gao et al., 2018). Abalones are especially susceptible to light,

and in their natural environment they live in boulder crevices, caves or cavities that produce a dark atmosphere (Shepherd, 1973). Under culture environment, a marked phototaxis and locomotion behavior of *Haliotis discus hannai* associated to light quality and dark environments was recently shown, where abalones preferred dark and long wavelengths (i.e., red and orange) to natural or short-lengths lights (Gao et al., 2016b). Interestingly dark acclimated abalones showed similar

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responses to light than light acclimated individuals (Gao et al., 2016b), suggesting a possible genetic control of this behavior in *H. discus hannai*.

Abalone's susceptibility to light strongly impacts their food intake, metabolic efficiency and thus growth rate performance under farming environment (Garcia-Esquivel et al., 2007; Ahmed et al., 2008; Gao et al., 2018). For example, juvenile *H. discus hannai* cultured under 20–24 h of darkness showed the highest food intakes, food conversion efficiency and growth rates; and those cultured under only 8 h of darkness showed the lowest survival rates (Gao et al., 2018). Considering these results, it has been proposed that abalone should be cultured under at least 4:20 h of light-dark (or similar wave-length) photoperiod, as a managing strategy to increase its production (Gao et al., 2016a, 2016b, 2016c; Gao et al., 2018). Nevertheless, for extensive off-shore abalone cultivation or even in intensive land-based farming systems, it is not likely that the photoperiod or light intensity can be managed in such a way.

Despite the great importance of the biological responses to the light/dark stimuli for abalone farming production, to the best of our knowledge, the genetic control upon the variation of phototaxis and related behavioral traits has not been reported. Therefore, the aim of this study was to estimate the heritable variation and potential responses to selection for the phototaxis [i.e., displacement towards (positive) or against (negative) the light source] and locomotion behaviors associated to the intensity of the response (i.e., crawling speed and displacement distance) to the light/dark stimuli in juvenile Pacific abalone *H. discus hannai*. This abalone is cultivated in Chile and exported to Asian countries where is also cultivated and has a tremendous economic importance (Mardones et al., 2013). The existence of additive genetic variability for responses to light/dark would allow selecting less susceptible and/or reactive abalones to this controlling factor, opening an opportunity to manage the associated biological performance in farmed Pacific abalone.

## 2. Material and methods

### 2.1. Breeding design and animal rearing conditions

Breeding design and animal rearing conditions followed Brokordt et al. (2015, 2018). Forty full-sib families were produced using *Haliotis discus hannai* abalone broodstock randomly obtained from the Center for Abalone Production of the Universidad Católica del Norte, Coquimbo, Chile. The broodstock was conditioned 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 following Morse et al. (1977). Crossings were conducted following a paternal half-sib nested design where gametes of one male were used to fertilize oocytes from four females, for a total of 10 males and 40 females. Competent larvae were transferred to 200-L tanks provided with corrugated polycarbonate plates inoculated with wild benthic microalgae for settling. After settling, each full-sib family was cultured separately in 200-L tanks with continuous water flow and constant aeration for the first 7 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  $\geq 15$  mm shell length ( $\sim 8$  months), abalones were marked individually with labels attached to their shells with epoxy resin. Individuals from different families were then mixed and randomly distributed, at equal densities, in baskets placed in a 10,000-L raceway-type tank. To avoid common environment effects, abalones were re-mixed randomly among baskets each month. In the raceway, abalones were maintained with continuous water flow, constant aeration, at ambient temperature that varied between  $\sim 16$ – $20$  °C during 4 month, until reaching  $\sim 20$  mm shell length.

### 2.2. Experiment for phototaxis and locomotion responses to light/dark stimuli

The phototaxis and locomotion responses to the light/dark stimuli were evaluated on 200 juvenile abalones belonging to the 40 full-sib families, with 5 individuals per family. This family size is within the range recommended by Lynch and Walsh (1998) when no information on  $h^2$  for the trait is available prior to the study. Behaviors were evaluated for each individual on an acrylic plate of  $20 \times 20$  cm, 3 mm thick; marked on its back with a line perpendicular to the light source, which divided the plate into two equal parts. In each of the vertices of the plate a magnet was glued on its back face. An aquarium of 20 L capacity ( $40 \times 25 \times 20$  cm, respectively length, width, and high) was used, where four magnets were fixed on the bottom, coinciding with the vertices of the acrylic plate. These allowed fixing the plate in a stable position in the water column. The light source came from an incandescent white light bulb (40 watts) mounted on a lamp specially designed for these purposes. Half of the aquarium was covered with a black box in such a way that the direct incident light reached half of the plate, leaving the other half in darkness.

The experiments were carried out during 9 days in summer season. Each juvenile abalone was randomly taken from the tank containing all the individuals from the different families. Each individual was placed in the center of the plate facing both the lightened and dark halves of the plate (Fig. 1). Preliminary experiments showed that responses to the light/dark stimuli were consistent within each juvenile abalone ( $n = 20$  individual, each tested 3 times). These preliminary experiments also showed that after 4 min faster abalones reached the limit (edges) of the experimental plate. Thus, the behaviors performed by each abalone from the different families were recorded during 4 min. At the conclusion of each individual trial, the plate was removed from the aquarium, the abalone carefully removed with a spatula, and the plate was carefully washed with fresh water to eliminate residues from the previously measured organism that could affect the response of the next

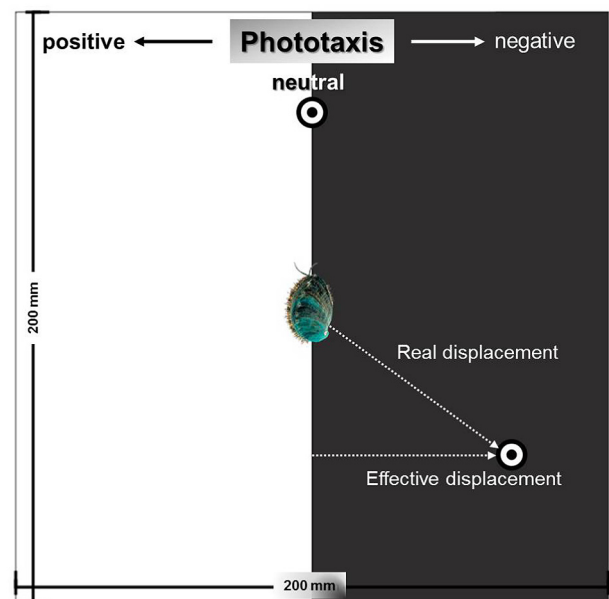


Fig. 1. Behavioral traits recorded in juvenile *Haliotis discus hannai* in response to the light/dark stimuli. Phototaxis: negative (i.e., crawling towards darkness), positive (i.e., crawling towards lightness), or a neutral (i.e., no obvious reaction) response to the light/dark stimuli. Real displacement (mm) and displacement speed ( $\text{mm s}^{-1}$ ), considering the distance traversed by the abalone from the center of the plate until stopping. Effective displacement (mm) and displacement speed ( $\text{mm s}^{-1}$ ), considering the effective distance from the middle of the plate from which the abalone moved away until stopping.

experimental individual.

For each abalone the following traits recorded were (Fig. 1): 1) Phototaxis response, coded as either a negative response (i.e., crawling towards darkness), a positive response (i.e., crawling towards lightness), or a neutral response (i.e., no obvious response, the individual remains in the place in which was placed on the plate). 2) Real displacement (in mm), which was the distance that the abalone traversed after crawling from the center of the plate until stopping. 3) Real displacement speed (in  $\text{mm s}^{-1}$ ), which was the speed for the real displacement. 4) Effective displacement (in mm), which was the effective distance from the middle of the plate from which the abalone moved away until stopping. 5) Effective displacement speed (in  $\text{mm s}^{-1}$ ), which was the speed adjusted for the effective displacement. For each displacement and displacement speed, the direction of the movement (i.e., negative if was towards darkness; positive if was towards lightness) was included as a covariate.

### 2.3. Estimation of phenotypic variance components and genetic estimates

The heritability of each trait was 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 (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  $y$  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. With the exception of the phototaxis response the variables followed a normal distribution. Phototaxis was coded as 1 (negative phototaxis), 2 (neutral phototaxis) and 3 (positive phototaxis), and was analyzed in two different ways. In a first approximation, it was simply analyzed as a normally distributed trait following the same model as the other traits. It was also analyzed with a Generalized Linear Mixed Model (GLMM) with a Logit link, treating phototaxis as an ordered multinomial trait.

Using this general model, we fitted a model for each trait after estimating the significance of fixed and random effects. Significances of fixed and covariate effects were estimated using the Wald F statistic as implemented in ASReml 3.0. Statistical significances of the maternal/environmental/non-additive random effects and of the additive random effects ( $h^2$  significance) were estimated by the log-likelihood ratio test (log-LR test), except for the GLMM model where this cannot be done. The location of the tank in which each full-sib family was held for the first 7 months of life was evaluated as a fixed effect. The date in which individuals traits were measured was evaluated as a covariate; as well as the direction of the movement (negative or positive in relation to the light source) for displacement and displacement speed traits. 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 7 months, the early common environmental effects were completely confounded with the maternal effects, thus we fitted them as common random effects. 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 ( $V_A$ ) variance and the residual variance ( $V_R$ ) (Falconer and Mackay, 1996). In the case of the GLMM analysis of phototaxis as a multinomial trait, it is not possible to truly estimate the residual variance, but the implicit residual variance on the underlying logistic distribution is  $\pi^2/3$  and this was used for heritability estimation (Gilmour et al., 2009). The potential response to the selection for each trait was estimated as  $G = i * \sigma_p * h^2$ ; where  $i$  is the selection intensity

(Falconer and Mackay, 1996). This estimation was done assuming a selection of 5% of individuals as broodstock, i.e., with  $i = 2.06$ . The expected gain response per generation was estimated as  $G (\%) = (G/X) * 100$ . This was not done in the case of phototaxis treated as multinomial trait.

A bivariate animal model was used with ASReml version 3.0 (Gilmour et al., 2009) to estimate genetic correlations ( $r_G$ ) between traits. 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). Genetic correlations involving phototaxis were only done with phototaxis treated as approximately normally distributed. The phenotypic correlations between traits were estimated by Pearson correlation; and their significance estimated by a  $t$ -test (R Core Team, 2016).

### 2.4. Ethics statement

The abalone brooders used in this study were obtained from a population of Pacific abalone imported from Japan by the Universidad Católica del Norte, following the Chilean laws and rules for introduction of alien hydrobiological species, as supervised by the Chilean National Service of Fisheries (SERNAPESCA). 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.

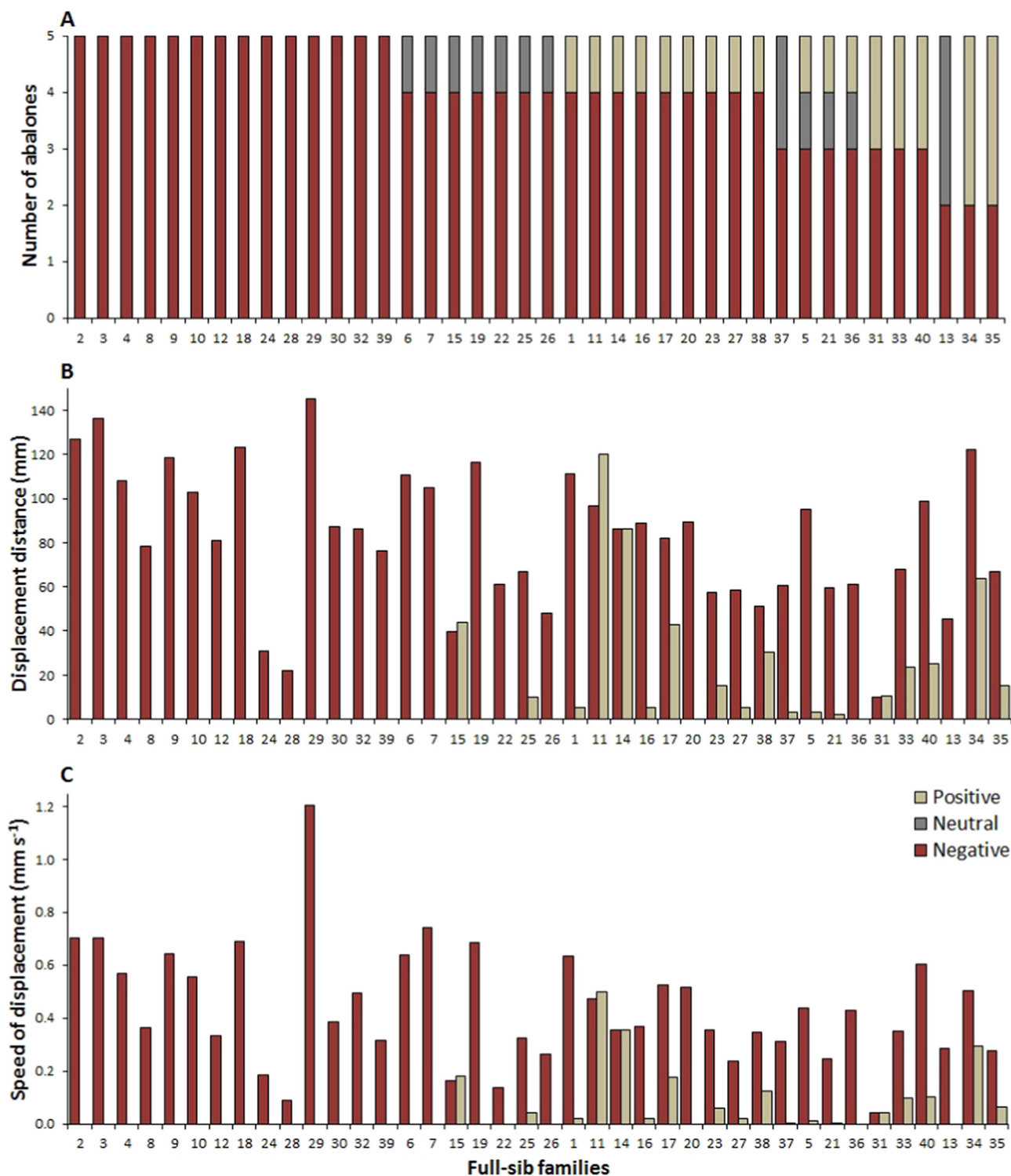
## 3. Results

### 3.1. Phenotypic variation of phototaxis and locomotion responses to light/dark stimuli

Among tested abalones, 79% showed a negative phototaxis, 11% a positive phototaxis, and 10% were neutral to the light/dark stimuli. None of the full-sib families were completed positive or neutral against the light stimulus (Fig. 2A). Real and effective displacements were higher in individuals that showed negative phototaxis (respectively,  $84.4 \pm 4.4$  and  $56.5 \pm 3.3$  mm) compared with those that showed positive phototaxis (respectively,  $30.4 \pm 7.8$  and  $16.5 \pm 4.2$  mm) ( $t$ -test,  $P < 0.0001$ ). Real and effective displacement speeds were also higher in individuals that showed negative phototaxis (respectively,  $0.46 \pm 0.03$  and  $0.30 \pm 0.02 \text{ mm s}^{-1}$ ) than in those that showed positive phototaxis (respectively,  $0.13 \pm 0.03$  and  $0.07 \pm 0.02 \text{ mm s}^{-1}$ ) ( $t$ -test,  $P < 0.0001$ ). These traits associated with the intensity of the response showed marked variations among full-sib families (Fig. 2B, C).

### 3.2. Heritabilities, expected gain responses to selection and genetic correlations of phototaxis and locomotion responses to the light/dark stimuli

The date in which individuals were measured as a covariate affected significantly the variation of phototaxis, and displacement and displacement speed traits (Wald F,  $P < 0.001$ ). These last locomotion traits were also affected by the direction of the movement (negative or positive in relation to the light source) as covariates. No other significant fixed or maternal/common environment effects were detected for any of the traits (respectively, Wald F and log-LR tests,  $P > 0.05$ ). Therefore, for the estimation of the additive genetic and residual variances and  $h^2$ , we used a simple linear mixed model with the additive genetic effects fitted as random factor, and the date of measure fitted as a covariate effect (Model 1). The date of measure was also used as a



**Fig. 2.** Variation in the mean response to the light/dark stimuli in full-sib families (40 full-sibs,  $n = 200$ ) of juvenile *Haliotis discus hannai*. (A) Frequency of phototaxis response per full-sib families. Registered responses were positive (displacement towards the light source); negative (displacement against the light source); or neutral (no obvious response, i.e., the individual remains in the place in which was placed). (B) Mean real displacement distance (mm) per full-sib towards or against the light source. (C) Mean real speed of displacement ( $\text{mm s}^{-1}$ ) per full-sib towards or against the light source.

covariate when analyzing phototaxis as a multinomial trait. An additional model including also the direction of the movement fitted as a covariate effect (Model 2) was used for variances and  $h^2$  estimations for locomotion traits (Table 1).

Additive genetic variations ( $V_A$ ) for the evaluated traits were lower than the residual variations ( $V_R$ ; Table 1). Estimated direct heritabilities ( $h^2$ ) were moderate, but all were significantly different from zero.

Similar heritability estimates were obtained for phototaxis with the normal approximation and with the multinomial model. Expected gain responses to selection per generation ( $G\%$ ; selection intensity of 2.06) were moderate for phototaxis, and high for locomotion responses (Table 1). Similar genetic estimates were observed for the variation of locomotion responses after using model 1 or 2.

Genetic and phenotypic correlations between phototaxis and



**Table 1**

Phenotypic means ( $\pm$  SD), estimates of additive genetic ( $V_A$ ) and residual ( $V_R$ ) variances, heritabilities ( $h^2 \pm$  SE), and expected gain responses to selection per generation [G (%), with a selection intensity of 2.06] for phototaxis and locomotion responses against light/dark stimuli (real and effective displacements and displacement speeds), in juvenile Pacific abalone *Haliotis discus hannai* (40 full-sib families; total  $n = 200$ ).

Trait	Phenotypic mean (SD)	$V_A$	$V_R$	$h^2$ (SE)	G (%)
Model 1					
Phototaxis (normal approximation)	1.32 (0.68)	0.069	0.380	0.15 (0.11)*	16.1
Phototaxis (multinomial model)	1.32 (0.68)	0.593	–	0.15	–
Real displacement (mm)	71.2 (57.9)	953	2257	0.30 (0.14)*	48.7
Effective displacement (mm)	47.0 (42.3)	311	1387	0.18 (0.12)*	33.1
Real displacement speed ( $\text{mm s}^{-1}$ )	0.38 (0.38)	0.047	0.092	0.34 (0.15)*	67.4
Effective displacement speed ( $\text{mm s}^{-1}$ )	0.25 (0.27)	0.021	0.049	0.30 (0.15)*	64.3
Model 2					
Real displacement (mm)	71.2 (57.9)	885	1985	0.31 (0.15)*	47.8
Effective displacement (mm)	47.0 (42.3)	327	1233	0.21 (0.14)*	36.3
Real displacement speed ( $\text{mm s}^{-1}$ )	0.38 (0.38)	0.049	0.085	0.37 (0.16)*	72.0
Effective displacement speed ( $\text{mm s}^{-1}$ )	0.25 (0.27)	0.023	0.045	0.34 (0.17)*	73.3

Model 1 included random dam effect and the date of measure fitted as a covariate effect. Model 2 included random dam effect and the direction of the movement as a covariate effect.

\* Statistical significance at  $P < 0.05$ , using a log-likelihood ratio test.

**Table 2**

Genetic (above the diagonal) and phenotypic (below the diagonal) correlations ( $\pm$  SE) between phototaxis (normal approximation) and locomotion responses to the light/dark stimuli traits in juvenile Pacific abalone *Haliotis discus hannai* (40 full-sib families; total  $n = 200$ ).

	Phototaxis	Real displacement	Effective displacement	Real displacement speed	Effective displacement speed
Phototaxis	–	0.733 (0.246)*	0.714 (0.302)*	0.677 (0.281)*	0.606 (0.326)*
Real displacement	0.611 (0.056)*	–	0.988 (0.082)*	0.990 (0.028)*	0.884 (0.097)*
Effective displacement	0.558 (0.059)*	0.924 (0.027)*	–	1.064 (0.061)*	0.983 (0.041)*
Real displacement speed	0.510 (0.061)*	0.916 (0.029)*	0.846 (0.038)*	–	0.987 (0.024)*
Effective displacement speed	0.471 (0.063)*	0.861 (0.036)*	0.919 (0.028)*	0.939 (0.024)*	–

For the bivariate analyses a model that included random dam effect and the date of measure fitted as a covariate effect was used.

\* Statistical significance at  $P < 0.05$ , using a log-likelihood ratio test for genetic correlation, or  $t$ -test for phenotypic correlations.

locomotion responses to the light/dark stimuli were all positive, high and significant ( $> 0.5$ ;  $P < 0.05$ ,  $t$ -test and log-LR test, respectively) (Table 2). However, these genetic correlations should be considered with caution because treating phototaxis as a normally distributed trait is only a crude approximation and because the number of measured individuals was not very high.

#### 4. Discussion

Phenotypic analysis showed a predominance of a negative phototaxis in juvenile *Haliotis discus hannai*, an observation that coincides with previous studies for this and other abalone species (Garcia-Esquivel et al., 2007; Ahmed et al., 2008; Gao et al., 2016a, 2016b, 2016c). In the natural environment, selection would have favored the development of behaviors of light evasion to avoid or decrease predation risk (Hayashi, 1988), limiting their feeding activity to the hours of darkness. Nevertheless, from the present results, we would like to highlight that 21% of abalones showed either a positive or neutral response to the light source. This non-negligible level of low reactivity to the light could be a result of several generations of culturing this abalone species under non-natural light conditions (Morikawa and Norman, 2003). Because selection operates on the variation of the trait, present results on phototaxis would open the possibility for artificial selection upon this trait. Furthermore, abalones with positive phototaxis were slower and moved a shorter distance than abalone with negative phototaxis, a behavior that suggests a lower reactivity or intensity in the response to the light stimulus. Interestingly, among abalones with negative phototaxis a marked inter-family variation in these traits was observed, with some full-siblings being very reactive and others very unreactive to the light stimulus. The intensity of the response to light could also be an interesting trait to select, since sensitivity to this stimulus could affect feeding rate and other physiological

traits of *H. discus hannai* (Gao et al., 2016a, 2016b, 2016c; Gao et al., 2018). However, for a trait to be selected, it must not only present phenotypic variation, but this variation must be heritable.

In this study, heritabilities, genetic correlations and potential responses to selection of phototaxis and locomotion responses, as measures of reactivity and intensity of the response to the light stimulus, were estimated for the first time for a mollusk. Results showed significant heritable variations for both phototaxis and locomotion responses, i.e., displacements and speeds of displacements (real and effective) in juvenile *H. discus hannai*, and positive genetic correlations among these traits were observed. Expected gain responses to selection per generation were moderate for phototaxis variation (16%) and high for locomotion responses variation (33–67 or 36–73%, depending on the model used). These gains were estimated with a selection intensity of 2.06, which is when selecting 5% of the target individual from the population.

The high potential responses to the selection obtained for the phototaxis and the intensity of the locomotion responses to the light stimulus, offer the possibility of improving these traits by, for example, a selective breeding program for the Pacific abalone. This improvement could be applied to increase the productivity of intensive farming of this species, or for the purpose of restocking natural over-exploited populations with seeds produced in hatcheries. For the improvement of the production of farmed abalones, the objective of the genetic program should be to reduce the light sensitivity of abalones, either through the selection of abalones with positive phototaxis or with a lower intensity of response to light. Because these traits present positive genetic correlations among them, the selection on one of them would generate a correlated response on the other in the same direction (Krebs et al., 1998; Lynch and Walsh, 1998). The decrease in sensitivity to light could imply improvements in the culture productivity by increasing the time for foraging (thus, food intake rates), the physiological efficiency in the

use of the ingested food (i.e., a greater assimilation), a decrease in physiological stress caused by the light; and consequently an increase in growth rates (and, therefore, decrease in production cycle time) and survival. Each of these biological aspects have been shown to be directly or indirectly affected by light in abalones (Gao et al., 2016a, 2016b, 2016c; Gao et al., 2018). However, future studies should look at genetic correlations between phototactic behaviors and growth; which in the present study was not possible because growth traits showed very low non-significant heritabilities.

On the other hand, a decrease in sensitivity to light by abalones produced by several generations under lighting conditions during culture could decrease the abalone's natural adaptation of nocturnal activity, and hiding in crevices during the day to avoid predation (Schiel and Welden, 1987; Hayashi, 1988; Morikawa and Norman, 2003). This can be especially critical for repopulation strategies with seeds produced in hatchery (Morikawa and Norman, 2003). Our results indicate that a genetic improvement program with the aim of increasing the abalone's reactivity to the light would be a potential strategy for the production of seeds for the purpose of repopulating natural stocks.

In conclusion, this is the first study demonstrating heritable variation and potential response to selection for behavioral traits associated with reactivity to the light/dark stimuli in an abalone species, and for mollusks in general. As a consequence, the potential for reducing (or increasing, depending on the breeding goal) this reactivity/sensitivity by selective breeding is good, and this can be an economically attractive way of indirectly improving growth, survival and general welfare of farmed *H. discus hannai*. Future research should explore molecular markers with significant effects on the phenotypic variation of light reactivity and susceptibility, in order to assist and shorten the required time to achieve genetic improvement goals.

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