

Article

Prospects for Genetic Improvement in Objective Measurements of Body Colour in Pacific Whiteleg Shrimp (*Litopenaeus vannamei*)

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Abstract: Body colour, together with growth and survival, are traits of commercial importance in Pacific whiteleg shrimp (*Litopenaeus vannamei*). However, heritability estimates for objective measurements of body colour are not available in Whiteleg shrimp species, including *L. vannamei*. Further, the effect of genotype by environment interactions ($G \times E$) on this trait (i.e., the objective measures of body colour) and its genetic associations with growth are not known in this species. The present study presented the first attempt at understanding the genetic architecture of this complex character (body colour) that is of economic significance to the shrimp aquaculture sector world-wide. Specifically, we investigated the quantitative genetic basis of shrimp colour, while using the measurement tool (colorimeter) for a Whiteleg shrimp population reared in two contrasting environments. A total of 5464 shrimp had the objective measurements of body colour (lightness, yellowness, and redness) and growth trait records (weight, length and width). They were the offspring of 204 dams and 197 sires. The restricted maximum likelihood mixed model analysis showed that there were heritable additive genetic components for all of the measurements of shrimp colour, with the heritability (h^2) ranging from 0.11–0.55. The h^2 estimates for redness and yellowness traits differed between the two environments ($h^2 = 0.66$ – 0.82 in Khanhhoa vs. 0.00 – 0.03 in Haiphong). However, the heritability for colour traits was moderate (0.11–0.55) when the two environments were combined. There is existence of (co)-genetic variances between the studied traits. The genetic correlations of body traits with redness or yellowness colour of the shrimp were moderate and positive (a^* : 0.13–0.32 for redness and b^* : 0.19–0.40 for yellowness). The effect of $G \times E$ interactions on shrimp colours could be important, as the genetic correlations for these traits between the two environments were low (-0.41 to 0.16). Our results showed that the genetic improvement for body colour can be achieved through direct selection and the increased redness colour is also expected to have favorable impacts on growth traits. Breeding programs to improve shrimp colour should account for the effects of environmental factors.

Keywords: body colour; colorimeter; heritability; genetic correlation; selection and whiteleg shrimp

1. Introduction

The Pacific whiteleg shrimp (*Litopenaeus vannamei*) is an important crustacean specie that is widely cultured in many countries, especially in Asia and Latin America, where it accounts for about 70% of

total crustacean production. Body colour is a trait of commercial importance in crustacean species [1,2]. Many environmental factors largely influence this trait, including light and water temperature [3]; water quality, e.g., the effect of copper [4]; substrate colour [5]; dietary astaxanthin levels [6]; and, rearing tank colour schemes [7]. Darker colour changes in crustaceans are well documented as a result of changing environments [8]. Although many studies have shown abundant evidence that shrimp colour can be improved through the manipulation of environmental factors, quantitative genetic basis of this trait while specifically considering objective measurements of body colour is limited in shrimp species [3]. To date, only two studies reported heritability for body colour of banana shrimp based on visual assessment of dark or light colour [1] and Pacific blue shrimp while using the $L^*a^*b^*$ system [9]. The results from these studies suggested that either dark colour of raw or red colour of cooked shrimp could positively respond to genetic selection. Improved redness/or darkness of the shrimp will increase the product quality and consumer acceptance to achieve greater economic return for farmers and shrimp producers [7]. For example, one unit improvement of body colour (\$2 increase per unit) can create the value of US\$1.8 million for the national shrimp sector in Australia [1].

We used a colorimeter to make objective measurements of body colour for heritability analysis in efforts to genetically improve this important character in *L. vannamei*. To date, there is no published estimate of heritability for objective measurements of shrimp colour. This is partially due to the unavailability of efficient measurement methods. Besides the subjective measurements of shrimp colour by observation, previous studies in laboratory have used the roche colour card, digital camera [6,7,10], or a combination of both roche colour card and laminated paper for hue measurements [11,12], and/or using computer tomography [13]. Some other methods can measure pigmentation, such as high performance liquid chromatography; thin layer liquid chromatography; spectrophotometry; colorimetry; digital imaging acquisition; and, processing [14]. However, none of the above methods, except FRU colorimeter WR10, are cost-effective in enabling a large-scale routine data recording in commercial breeding programs for Whiteleg shrimp.

In the present study, a colorimeter was used for body colour measurements on individual shrimps that were reared in two contrasting environments to enable the estimation of genotype by environment interaction ($G \times E$) effects. Understanding the magnitude of the $G \times E$ effects not only helps the design, but also the optimization of genetic improvement programs for this species [15]. In the past three decades, a total 127 $G \times E$ studies have been conducted in 38 different aquatic species in different environments [16]. These studies exclusively focused on body and carcass traits that were recorded in selective breeding programs for various aquaculture species, including *L. vannamei* [17–19]; *Penaeus monodon* [20]; Red Tilapia *Oreochromis spp.* [21]; Atlantic salmon (*Salmo salar*) [22]; or, yellowtail kingfish [23]. However, published information regarding genetic correlations for body colour in diverse-cultured environments is not available in any penaeid shrimp species. Furthermore, the genetic relationships of the objective measurements of body colour with morphometric traits have not been estimated in all crustaceans. Therefore, the estimation of genetic parameters for body colour and its association with growth traits is necessary for obtaining fundamental parameters to broaden the breeding objectives for Whiteleg shrimp *L. vannamei*.

Here, the main aim of this study was to understand quantitative genetic basis of the objective measurements of body colour and their genetic associations with morphometric traits in *L. vannamei* shrimp reared under different culture environments. The specific objectives of the present study were (a) to estimate heritability for body colour while using a FRU colorimeter WR10; (b) to examine genetic association between body colour and morphometric traits; and (c) to investigate the genotype by environment interaction for body colour traits.

2. Materials and Methods

2.1. Source of Shrimp and Production of the Families

The shrimp that were used in this study originated from the genetic lines of the on-going breeding program initiated by the Research Institute for Aquaculture No.1 (RIA1), Vietnam [24,25]. In 2014, 88 female and 88 male broodstocks were selected to form the base population (G_0). A total of 1051 shrimp were harvested after 90 days of grow-out in a pond and mature shrimp were selected for the next generation. In 2015, a total of 204 dams and 197 sires were recruited to produce the next generation (G_1); family origin was considered to avoid the mating between sibs. Broodstock parents were kept in black fiberglass tanks ($3\text{ m} \times 2\text{ m} \times 1.7\text{ m}$) for conditioning with a density of nine individuals/ m^2 [26]. The shrimp breeders were fed at the rate of 15–18% of total body mass per day, including about 40% squid, 15% polychaete worms, 40% oysters, and 5% commercial maturation diet [27] during 25 days. Mating one male with one or two females carried out artificial reproduction and they spawned after 1–4 h. The eggs were then collected, washed with iodine, and then placed back in the 180 L containers, where they hatched with 24 h of aeration condition. After 36–40 h, the larvae from each family were separately grown in a 400 L tank while using standard shrimp rearing practices. Stocking density in the first phase of larval rearing was 100 Nauplius/L and postlarvae/L. Postlarvae were fed a combination of 0.4–0.6 g synthetic food (Spirulina, Prippak and Lansy)/ m^3 and 30,000–50,000 cells/mL of the *Chaetoceros* algae. In the latter stage, they were fed an amount of 0.6–1.2 g artificial food (Frippak and Lansy)/ m^3 and then combined with *Artemia* nauplii. At post-larvae 15, each family was transferred and then grown in an elliptical-fiberglass tank of 2.5 m^3 with constant aeration and the density reduced from 600 to 200 juveniles per tank. After 45 days from post-hatching, the shrimp reaching the size of approximately 2.5 g/individual were chosen for the experiments.

2.2. Grow-Out Environments

The experiment was conducted in two different locations (Northern and Southern Vietnam) from March to June 2016 in cement ponds ($20\text{ m} \times 25\text{ m} \times 1.5\text{ m}$). A total of 12,000 shrimp from 112–119 families used in the $G \times E$ experiment were tagged by injecting six different colours of visible implant elastomer (VIE), as described by Hung et al. [28]. The tagged shrimp of each family were split, randomly, into two groups (each group/environment). A total of 6000 tagged shrimp in the first group were stocked at the Northern National Broodstock Center for Mariculture, Research Institute for Aquaculture No.1 (RIA 1) in Haiphong, Vietnam (Latitude: 20.7; longitude: 106.9). Another group was cultured at the National Center for Marine culture, Research Institute for Aquaculture No.3 (RIA 3) in Khanhhoa, Vietnam (Latitude: 12.6; Longitude: 109.2). The weather conditions recorded at two environments were: Water temperature ranging from 24–28 °C for Haiphong and 26–30 °C for Khanhhoa. The main difference between the two culture environments was salinity level that ranged from 25–30‰ in Haiphong and 30–35‰ in Khanhhoa. The average dissolved oxygen was 4–7 mg/L (Haiphong) and 5–7 mg/L (Khanhhoa). Prior to stocking, all of the shrimp were weighed and they were initially stocked at a density of 12 shrimp per m^2 . During the grow-out period, the shrimp at the two environments were fed 5–20% of their body weight. The feeding included a commercial pellet (CP 9003-P and HI-PO 7701) containing 35% crude protein, 8–10 g vitamins, and 4–5 g minerals/kg food; shrimp were fed four times per day (about 6 a.m., 11 a.m., 6 p.m. and 10 p.m.). Water was exchanged at the rate of 85% per month in both environments.

2.3. Measurements

2.3.1. Body Traits Record

After a grow-out period of 90 days (March–June 2016), a total of 5464 animals had phenotypic information recorded (2778 and 2691 shrimps at Haiphong and Khanhhoa, Vietnam, respectively). At harvest, approximately 5% of the marked animals failed to read the VIE tags. The data were

separately collected for each environment and each individual shrimp, including: harvested weight (g, total live body weight at harvest), while using a digital scale; body lengths (mm), using a standard ruler. Figure S1 presents details regarding the measurement of body dimensions (lengths and widths). In addition to body traits, sex, deformity, and maturation traits were also recorded on individual shrimp at harvest. The overall number of observations, raw means, units, standard deviation (SD), and coefficient of variation (CV) for colour traits (as described below), body weight (WT); body length (LG); and, abdomen length (WD) are shown in Table 1.

Table 1. Basic statistics of body colour L* (lightness), a* (redness), and b* (yellowness) values as well as descriptively morphometric traits for samples of *L. vannamei* shrimp grown in both environments.

Traits	Unit	n	Mean	SD	CV (%)
Body Colour					
L*		5460	27.5	8.7	31.9
a*		5451	4.6	3.7	80.3
b*		5455	5.1	4.3	84.6
Morphometric					
WT	g	5464	21.0	5.6	26.5
LG	mm	5464	144.9	12.8	8.9
WD	mm	4429	88.3	7.9	9.0

2.3.2. Colour Trait Analysis

At harvest, the shrimp of each family were separated and stored in a room temperature. Objective measurements of shrimp colour were made using FRU Colorimeter WR10 8 mm (<http://www.colorinstrument.cn/index.php/list/index/id/25.html>). Three colour coordinates characterized the colour points. L* is the lightness coordinate ranging from no reaction for black ($L^* = 0$) to perfect diffuse reaction for white ($L^* = 100$); a* is the redness coordinate ranging from negative values for green to positive values for red colour; b* is the yellowness coordinate ranging from negative values for blue and positive values for yellow ($b^* = 0$, neutral colour).

2.3.3. Maturation

The maturation of each individual was determined by external observations following the recognition of AA Vaca and J Alfaro [29]. The mature status of each shrimp was coded as 1 and immature shrimp was coded as 0.

2.4. Statistical Analysis

A total of 5464 data records collected from the two environments were available for statistical analysis. Preliminary analysis using general linear model (GLM procedure in SAS 9.3 [30] examined systematic fixed effects to include in the final statistical models for each trait. The analysis of co-variance components was performed using ASReml 3 [31]. We conducted two different analyses: (i) the data from the two environments were combined; and, (ii) each environment was separately analysed and a detailed description of the mixed model followed.

2.5. Modelling Genetic Variation when the Two Environments were Combined

A linear mixed model (Equation (1)) was used to estimate the heritability and genetic correlations for all of the traits studied. The model included the fixed effects of sex, testing environment or ambient factors, and harvest age as a linear covariate. The random effects are the additive genetics of individual

shrimp and the common full-sib effects (also called as maternal and common environmental effects). In a matrix notation, the mixed model is written as:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Za} + \mathbf{Wc} + \mathbf{e} \quad (1)$$

where, $\mathbf{y}$ is the vector of observations for phenotypic value of traits studied; $\mathbf{b}$ is vector of fixed effects, namely sex (female or male), culture environment (HP and KH), and age from birth to harvest; $\mathbf{a}$ is vector of random additive genetic effects, $a \sim N(0, \sigma_a^2 \mathbf{A})$ where $\mathbf{A}$ is the numerator relationship matrix calculated from the pedigree; $\mathbf{c}$ is vector of random common family effects, $c \sim N(0, \mathbf{I}\sigma_c^2)$; $\mathbf{e}$ is vector of residual environmental random effects, $e \sim N(0, \mathbf{I}\sigma_e^2)$; and, $\mathbf{X}$, $\mathbf{Z}$, and $\mathbf{W}$ are the corresponding incidence matrices relating observation ($\mathbf{y}$) to the levels of fixed effects ($\mathbf{b}$), additive genetic effects ($\mathbf{a}$), and common full-sib effects ($\mathbf{c}$).

Under the (1), $\text{var}(\mathbf{a}) = \mathbf{G} = \mathbf{A}\sigma_a^2$. The remaining effects are assumed to be distributed as $\text{var}(\mathbf{e}) = \mathbf{R} = \mathbf{I}\sigma_e^2$, $\text{var}(\mathbf{c}) = \mathbf{W} = \mathbf{I}\sigma_c^2$, in which $\mathbf{I}$ is an identity matrix with ones on the diagonal. The expectations of all the random effects are zero, $\text{cov}(\mathbf{a}, \mathbf{e}) = 0$ and $\text{cov}(\mathbf{a}, \mathbf{c}) = 0$. While assuming normality, we have mixing model for the best linear unbiased estimator of estimable functions of $\mathbf{b}$ and the best linear unbiased prediction of $\mathbf{a}$ and $\mathbf{c}$ is:

$$\begin{bmatrix} \hat{b} \\ \hat{a} \\ \hat{c} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{X} & \mathbf{X}'\mathbf{Z} & \mathbf{X}'\mathbf{W} \\ \mathbf{Z}'\mathbf{X} & \mathbf{Z}'\mathbf{Z} + \mathbf{A}^{-1}\alpha_1 & \mathbf{Z}'\mathbf{W} \\ \mathbf{W}'\mathbf{X} & \mathbf{W}'\mathbf{Z} & \mathbf{W}'\mathbf{W} + \mathbf{I}\alpha_2 \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \\ \mathbf{W}'\mathbf{y} \end{bmatrix} \quad (2)$$

with $\text{var}(\mathbf{y}) = \mathbf{ZAZ}'\sigma_a^2 + \mathbf{WI}\sigma_c^2\mathbf{W}' + \mathbf{R}$; where $\alpha_1 = \sigma_e^2/\sigma_a^2$ and $\alpha_2 = \sigma_e^2/\sigma_c^2$.

Heritability (h^2) and common full-sib effect (c^2) were estimated from univariate analysis as:

$$h^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2}; \quad c^2 = \frac{\hat{\sigma}_c^2}{\hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2}$$

where σ_a^2 is the additive genetic variance; σ_c^2 is common full-sib variance; and, σ_e^2 is the residual (error) variance. Genetic and phenotypic correlations were estimated using bivariate models, and they were calculated as:

$$r = \frac{\sigma_{12}}{\sqrt{\sigma_1^2} \sqrt{\sigma_2^2}}$$

where σ_{12} is the estimated additive genetic or phenotypic covariance between the two traits, (σ_1^2) and σ_2^2 are the additive genetic or phenotypic variances of traits 1 and 2, respectively. The multivariate analyses included the final harvest traits to minimize any possible bias in the genetic parameter estimates. The correlations (r) ranged from -1 to $+1$; a value of $+1$ indicates a perfect positive association and a value of -1 indicates perfect negative association.

2.6. Separate Analysis of Each Environment

The data were separately analysed for each environment in addition to the combined analysis of the two environments, as described above. For this analysis, to examine the effect of $G \times E$ effect, the characters measured in each environment were treated as different traits to obtain genetic correlations for the same traits between the testing environments. There was no residual covariance between the traits, as they were measured on animals in different environments. The model is as described above (1), with one exception was that the fixed effect of environment was omitted from the statistical model.

2.7. Threshold Generalized Linear Mixed Model

As maturation of the shrimp was recorded as a binary trait, threshold generalized mixed models (GLMM) that assumed that the data followed a binomial distribution with a probit link function were used [32]. The threshold model that was fitted to analyze these traits was the same as described in Equation (1). In this case, heritability was calculated by using the variance of the probit link function.

$$h^2 = \frac{\sigma_A^2}{\sigma_s^2 + \sigma_c^2 + \sigma_e^2}$$

where σ_A^2 is additive genetic variance, σ_c^2 is common full-sib variance, and $\sigma_e^2 = 1$.

2.8. Significance Test of the Heritability Estimates and Genetic Correlations

The Z-score was used to test whether the heritability and correlations estimates were significantly different between the two environments or whether the estimates were significantly different from zero, while using the following formula:

$$z = \frac{x_i - x_j}{\left(\sigma_i^2 + \sigma_j^2\right)^{0.5}}$$

where x_i and x_j are the estimates of heritability, maternal effects, or genetic correlations for the two traits, and σ_i and σ_j are their respective standard errors. x_i and σ_j are both set to zero or one when testing whether a correlation is significantly different from unity. The resulting z-score was then tested against a large sample normal distribution.

3. Results

3.1. Pedigree Information and Data Characteristics

The data analysed here were collected from 5464 individual shrimps that were offspring of 197 sires and 204 dams (Table 2). Representatives of total 84 families were reared in both environments (Khanhhoa and Haiphong), although the numbers of full-sib and half-sib families were greater at Khanhhoa than at Haiphong. The basic statistics for colour and morphometric traits are shown in Table 1 above. The coefficient of variation for L* (lightness, CV = 31.9%) was smaller than that for redness (CV = 80.3%) and (yellowness, CV = 84.6%). The CV of body colour traits (redness and yellowness) was two-fold greater than that for body weight. In contrast, the CV of body length and abdominal width was relatively low (8.9% and 9.0%, respectively). The average body weight of the shrimp was 21 g at harvest.

Table 2. Pedigree structure in testing environments—Numbers of sires, dams, estimated number of records per full-sib and half-sib family in the complete data set at 135 days post-hatching for *L. vannamei* within both environments.

Pedigree Information	Khanhhoa	Haiphong	Total
Number of sires	113	84	197
Number of dams	119	85	204
Number of half-sib families	65	27	92
Number of full-sib families	194	112	306
Number of common sires in both environments *	84		

* Common sires used to produce families for testing in both environments.

3.2. Environmental Effects on Shrimp Colour

The effects of culture environment on shrimp colour were statistically significant ($p < 0.001$). Shrimp that were cultured in Khanhhoa tended to have lighter colour and yellower hues than those reared in Haiphong. The magnitude of the difference in red colour between the two environments was smaller than that for lightness and yellowness (Figure S2).

3.3. Heritability and Common Full-Sib Effects

Heritability (h^2) and common full-sib effects (c^2) that were estimated for colour traits of commercial interest are presented for each environment and across the two environments in Table 3. The h^2 estimates for the objective measurements of body colour differed between the environments (0.27–0.82 in Khanhhoa vs. 0.00–0.03 in Haiphong). The heritability for colour traits was moderate when the two environments were combined in our analysis (0.11, 0.55, and 0.33 for lightness, redness, and yellowness, respectively). The common full-sib effects were small (0–2% of total phenotypic variance) when the analysis was separately conducted for each environment or when the two environments were analysed together.

Table 3. Heritability (h^2) and common full-sib effects (c^2) within two environments (Haiphong in the North and Khanhhoa, Southern Vietnam) for body colour (lightness, redness and yellowness) and morphometric traits include body weight (WT), body length (LG) and abdomen length (WD), and maturation trait recorded. Standard error (SE) in parentheses.

Location	Khanhhoa		Haiphong		Both Environments	
Traits	h^2	c^2	h^2	c^2	h^2	c^2
Body Colour						
L*	0.27 (0.04)	0.00 (0.00)	0.01 (0.00)	0.00 (0.00)	0.11 (0.04)	0.01 (0.02)
a*	0.66 (0.15)	0.01 (0.01)	0.00 (0.00)	0.01 (0.01)	0.55 (0.06)	0.01 (0.01)
b*	0.82 (0.07)	0.00 (0.00)	0.03 (0.02)	0.00 (0.00)	0.30 (0.04)	0.01 (0.00)
Morphometric						
WT	0.62 (0.13)	0.18 (0.07)	0.17 (0.08)	0.00 (0.00)	0.17 (0.05)	0.06 (0.03)
LG	0.61 (0.13)	0.07 (0.06)	0.11 (0.02)	0.00 (0.00)	0.18 (0.06)	0.01 (0.01)
WD	0.66 (0.14)	0.07 (0.06)	0.19 (0.04)	0.00 (0.00)	0.14 (0.05)	0.06 (0.03)
Maturity						
Maturity ¹	0.11 (0.05)	0.01 (0.02)	0.05 (0.02)	0.00 (0.00)	0.17 (0.03)	0.00 (0.00)
Maturity ²	0.04 (0.09)	0.15 (0.06)	0.08 (0.07)	0.01 (0.04)	0.07 (0.05)	0.04 (0.03)

(¹) = linear mixed model and (²) = probit threshold model.

The heritabilities for body traits (weight, length, and width) were moderate to high and the estimates were greater in Khanhhoa than the Haiphong environment. The common full-sib effects (c^2) accounted for 1 to 18% of total phenotypic variance for body traits. Linear mixed and threshold model analyses both showed that the maturity is lowly heritable ($h^2 = 0.04$ – 0.11). The c^2 effect for sexual maturity ranged from 0 to 15% of the total variance.

3.4. Genetic Correlations among Traits

Table 4 presents the genetic and phenotypic correlations among the traits studied. The genetic correlations among body colour traits (lightness, redness, and yellowness) were high and positive (0.79 to 0.85). Interestingly, L* (lightness) showed negative genetic correlations (−0.14 to −0.28) with body traits (weight, length, and width). However, redness and yellow hues were both genetically correlated positively with weight, length, and width (0.13–0.40). The genetic correlation estimates of body colour traits and maturity were not significant, due to their high standard errors.

As expected, the genetic correlations among the morphometric body traits (weight, length, and width) were highly positive and close to one (0.94 to 0.98). In a similar manner, the phenotypic correlations among these traits were high and positive (0.65 to 0.77). There were also strong genetic associations between the maturity and body traits (weight, length and width, $r_g = 0.72$ to 0.89) (Table 4).

Table 4. Genetic (r_g , below diagonal) and phenotypic (r_p , above diagonal) correlations among the traits (n = 5464). Estimates and standard error (SE).

Trait	WT	LG	WD	L*	a*	b*	Maturity
WT	-	0.68	0.77	-0.07	0.05	0.01	0.29
LG	0.96 (0.01)	-	0.65	0.03	0.06	-0.01	0.26
WD	0.98 (0.01)	0.94 (0.02)	-	-0.07	0.01	-0.04	0.26
L*	-0.25 (0.11)	-0.14 (0.12)	-0.28 (0.11)	-	0.09	0.06	-0.06
a*	0.24 (0.10)	0.32 (0.09)	0.13 (0.10)	0.79 (0.06)	-	0.96 (0.01)	0.01
b*	0.32 (0.10)	0.40 (0.09)	0.19 (0.11)	0.85 (0.05)	0.85 (0.05)	-	-0.01
Maturity	0.89 (0.18)	0.76 (0.20)	0.72 (0.26)	-0.36 (0.25)	0.25 (0.27)	0.24 (0.26)	-

The detailed traits are abbreviated as Tables 2 and 3. Table 4 omits all SE of the phenotypic correlations equal to 0.02.

3.5. Genotype by Environment Interaction

The genetic correlations between colour trait expressions in different environments were negative for lightness ($r_g = -0.41$), but positive for redness and yellowness (0.08 and 0.16) (Table 5). The between-environment genetic correlation estimates significantly differed from one, which suggested that the $G \times E$ effect is potentially important for body colour. However, the standard errors of the estimates were relatively large (s.e. = 0.14 to 0.44), likely due to characteristics of the data measured while using the Colorimeter. A negative correlation indicates that, as the values of one variable increase in one environment, the values of the other variable decrease in another environment.

Table 5. Genetic correlation (r_g) and standard error (SE) for body colour trait of *L. vannamei* shrimp between testing environments.

Trait	Genetic Correlation	S.E
Body colour	-	-
L*	-0.41	0.22
a*	0.08	0.14
b*	0.16	0.44

L* = lightness; a* = redness; b* = yellowness.

4. Discussion

Obtaining accurate genetic parameters (heritability and correlations) for novel traits, namely the objective measurements of body colour in shrimp, is crucial in broadening the breeding objectives for Whiteleg shrimp. This study presented the first attempt at understanding quantitative genetic basis of shrimp colour. We showed that there are prospects for improving shrimp colour by selection. The standard errors of the heritability estimates for colour traits were small; this could be a result of the relatively large sample size included in our multivariate analysis. The population structure with half-sib families (albeit only 27 in Haiphong vs. 65 in Khanhhoa environment) enabled the separation of the additive genetic components from other non-additive effects, such as common full-sib variances. By testing the representatives of shrimp families in different environments, we were also able to estimate the effect of genotype by environment interaction for colour traits. The knowledge that was gained from this study not only helps our understanding of the genetic architecture of body colour under different environments, but it also suggests the possibilities for simultaneous improvement of redness colour and growth traits in selective breeding programs. As follows, we discuss our main

findings with emphasis on the genetic basis of colour traits that are potential phenotypes of commercial values for future genetic improvement programs in *L. vannamei*. A reference to other penaeid shrimp species was also made, due to the economic importance of the shrimp sector worldwide.

4.1. Heritability for Body Colour

The first important finding of this study was that the objective measurements of body colour (lightness, redness, and yellowness) exhibited substantial heritable genetic variation ($h^2 = 0.11$ to 0.55) to allow scope for selection to improve this trait in commercial breeding programs for Whiteleg shrimp. However, the heritability (h^2) estimates for colour traits varied with culture environments. This suggests that the response to selection is environment-dependent (theoretical prediction at 5–11%/generation in Khanhhoa, but low or close to zero in Haiphong). Furthermore, the h^2 that were estimated in both environments of this study were similar to those that were reported for visual dark/light colour observations in banana shrimp (*Fenneropenaeus merguensis*) ($h^2 = 0.03$ – 0.55) [1] and blue shrimp *Litopenaeus stylirostris* (0.41 – 0.59) [9]. The findings in shrimps were generally in line with the heritabilities that were reported for flesh colour score in Atlantic salmon (*Salmo salar*), ranging from 0.14 [22,33] to 0.33 [34] and 0.04 – 0.27 in rainbow trout or coho salmon [35,36]. In our study, the difference in the h^2 estimates for body colour between the two environments is as expected, because environmental factors largely influence this trait. The additive genetic variance or the genetic correlations between environments can change under different rearing conditions [21]. In summary, this study indicated that a large proportion of the genetic variance component contributing to colour traits was heritable and, thus, efforts enhancing genetic improvement for these traits are desired in commercial breeding programs for this species.

4.2. Common Full-Sib Effects

Non-additive genetic effects, such as common full-sib or maternal and environmental effects, can be important for quantitative traits in animals [37]. The common full-sib family effect (c^2) that was estimated for body colour traits in this study was small (1–6%), but it was larger in Khanhhoa than the Haiphong environment. This was likely due to the smaller number of half-sib families in the later than the former environments (27 vs. 65, respectively). By contrast, the c^2 effects ranged from 0 to 18% for body traits. Our result is consistent with the published information in other shrimp populations, such as $c^2 = 0.02$ – 0.03 in the study of Castillo-Juarez et al [18]. The low common full-sib variance estimates in this study can be explained, because carcass and quality traits are less affected by the c^2 than growth-related traits [38]. The inclusion of the c^2 effects in our statistical models avoided an overestimation of the heritability estimated here. In studies where the common full-sib environmental effect was omitted, genetic variance was inflated due to the confounding of non-additive effects. The moderate common full-sib effects were observed for growth traits, likely because the full-sib group sizes in this study are large and the c^2 effects arose from separate rearing of each full-sib family prior to tagging (about 135 days post-hatching).

It is suggested that accounting for common environmental variances is needed for increasing the accuracy of genetic parameter estimates and, thus, selection response.

4.3. Genetic Correlations Between Growth Traits and Colours

Genetic correlations between the body and colour traits are of interest because almost all of the breeding programs in aquaculture species have exclusively focused on improving growth and we do not know if selection for increased growth has positive or negative impact on quality traits, such as shrimp colour.

The estimates of genetic correlations between the body colour and growth traits in our study showed that, on the one hand, selection for reduced light colour may lessen the growth and maturity. On the other hand, both growth and red-yellow colour can be simultaneously improved due to the moderate and positive correlations between the two traits. Similar to our results, the previous report

of Nguyen et al. [1] in banana shrimp (*Fenneropenaeus merguensis*) indicates that genetic correlation between a dark/red colour and body traits were high and positive. In fish, positive genetic correlations between flesh colour and growth traits have been reported for salmonids [39–41] or tilapia [42]. However, in red tilapia (*Oreochromis spp.*), the correlation between body traits and body colour was moderate and negative (−0.47 to −0.25) [43].

Our results showed that, during the grow-out, the fast growth of White leg shrimp was genetically associated with increased redness colour. The strong positive correlations of body colour with the morphometric traits observed here, together with those reported in the literature, suggest that body colour and growth-related traits can both be simultaneously improved in selective breeding programs for whiteleg shrimps [1].

4.4. Genetic Correlations among Measurements of Body Colours

The high and positive genetic correlations among the three measures of body colour indicate that selection for improved redness can result in favourable changes in light colour or yellow hues. Hence, the breeding goal for improved shrimp colour in whiteleg shrimp can use any of the objective measurements that were studied here. An increased a^* value of raw shrimp is expected to improve the red colour of cooked counterparts, as evidenced by the high genetic correlation ($r_g = 0.64$) between the two traits in banana shrimp [1]. However, Enez et al. [9] reported a negative genetic correlation between the blue colour on raw shrimp and red colour of cooked *L. stylirostris*. This suggests that further study is needed to understand the genetic relationships between colour traits on raw and cooked *L. vannamei* before defining the breeding objective for improved colour traits in this species. The breeding goal also depends on market niche or local consumer preference (e.g., light colour according to [44], but a dark color of raw and red colour of cooked shrimp, e.g., in Australia and Asia [7]). Published information regarding correlations among objective measurements of body colour are not available in crustacean species to compare with our study. Studies in rainbow trout (*Salmo gairdneri*) showed that genetic correlations of body traits with meat colour score were low, but positive [35], and redness/yellowness colour displayed a curvilinear relationship with an increasing carotenoid concentration in the flesh, while the lightness value was associated with decreased carotenoid content [45].

4.5. Genotype by Environment Interaction

The impacts of environmental factors on shrimp colour are well documented in the literature [6]. For instance, the first report of M Fingerman and DW Tinkle [46] revealed that the white pigment of the daggerblade grass shrimp (*Palaemonetes pugio*) and the eastern grass shrimp (*Palaemonetes paladosus*) became more concentrated with an increased temperature. A recent study of A Bhandiwad and S Johnsen [47] showed that changes in the salinity and temperature levels significantly affected bright coloured tissue in the grass shrimp (*P. pugio*). At higher salinities (30‰) and temperatures (27 °C), dark colour significantly increased ($p < 0.001$) [47]. Furthermore, a large proportion of shrimps had orange/red colour if temperatures and salinities of water are maintained at about 28 °C and 32‰ in dark culture environments [7].

However, to date, the magnitude of the $G \times E$ interaction effect on colour traits is not known in any crustacean species. In this study, a multi-trait linear mixed model analysis showed that the effect of $G \times E$ interaction could be important, with the between-environment genetic correlation estimates ranging from negative −0.41 for lightness to positive 0.16 for yellowness. The significant $G \times E$ effect on shrimp colour calls for the conduct of separate breeding programs to improve this trait in each production environment [48]. Previous reports in shrimp species only examined the $G \times E$ effects on growth related traits [17,19,21,28,49]. Provided with the strong $G \times E$ effects on colour traits here, selection in multiple environments, such as in both the nucleus and production environments should be conducted to address the re-ranking effect (ranking of animals differently between the environments), because this can help to improve the accuracy and precision in the assessment of both genetic and environmental influences. Alternatively, the selection for improved colour in the nucleus (e.g., in Khanhhoa) can

produce genotypes for commercial production (i.e., Haiphong), although the achieved genetic gain is not fully expressed due to the low between-environment genetic correlations for colour traits.

Our findings also suggest that the effects of $G \times E$ interaction should be accounted for in statistical models to analyse the quantitative colour traits in genetic evaluation programs. This way, the genetic gain that is achieved from the selection program in the nucleus can be captured in production systems [50].

5. Conclusions

The heritability for measurements of body colour while using colorimeter was estimated for the first time in *L. vannamei* shrimp. There was significant difference in the heritability estimates for colour traits between culture environments. The genetic correlation values between body colour (lightness, redness and yellowness) and growth traits (body weights and lengths) predict that selection for increased harvest weight would result in favourable changes in colour of the shrimp, and vice versa (i.e., reducing lightness and increasing redness and yellowness). However, the estimates of genetic correlations between colour traits that were measured in different environments were low, which indicated that the genotype by the environment effect was important for shrimp colour.

In summary, objective measurements of body colour would effectively respond to selection and breeding programs aiming to improve this trait should take the genotype into consideration by the environment interaction effect to maximise the animal productivity in commercial production systems.

Supplementary Materials: The following are available online at <http://www.mdpi.com/2077-1312/7/12/460/s1>, Table S1. Significance of fixed effects (F-statistic value). Figure S1. Calculation of body traits, from left to right: LG is total body length (mm, distance from eye orbit to tip of telson); WD is abdomen length (mm, distance from the hind margin of the carapace to tip of telson). Figure S2. Environmental effects on body colour score of whiteleg shrimp *L. vannamei* (Least square means after corrections for the fixed effects).

Author Contributions: C.T.G. conducted the study, participated in data analysis and prepared the draft manuscript, W.K. read and approved the manuscript, T.T.M. and N.H.N. conducted the study, provided experimental materials and participated in data collection and preparation of the manuscript, and N.H.N. (last author) conceived the study, designed the experiment, analysed the data, and prepared and finalised the manuscript. All the authors approved the manuscript before submission.

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Conflicts of Interest: There are no competing interest.

Abbreviations

L* = Lightness, a* = Redness and b* = Yellowness; WT = body weight, LG = body length; WD = abdomen length.

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