



## Genetic Parameters for Growth-Related Traits and Survival in Pacific White Shrimp, *Litopenaeus vannamei* under Conditions of High Ammonia-N Concentrations

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### Abstract

Based on restricted maximum likelihood (REML) method under animal model, the genetic parameters in *Litopenaeus vannamei* for growth-related traits on body weight (BW, g), body length (BL, cm), abdominal segment length (ASL, cm), carapace length (CL, cm), and survival (SU) under conditions of 96 hours of ammonia exposure at 14 and 21 weeks of age were estimated, respectively. The heritability estimates for growth-related traits at different growth stages were moderate, ranging from  $0.24 \pm 0.09$  to  $0.30 \pm 0.06$  for 14-week-old shrimp and  $0.26 \pm 0.07$  to  $0.31 \pm 0.06$  for 21-week-old shrimp, respectively. The heritability estimates for survival under conditions of high Ammonia-N concentrations were  $0.13 \pm 0.11$  for 14-week-old shrimp and  $0.17 \pm 0.08$  for 21-week-old shrimp, respectively. Genetic correlations between growth-related traits within age cohorts were generally high (ranging from  $0.74 \pm 0.06$  to  $0.89 \pm 0.16$ ), but for growth-related traits between ages were low (ranging from  $0.14 \pm 0.05$  to  $0.46 \pm 0.09$ ); genetic correlations between growth-related traits and survival under conditions of high Ammonia-N concentrations were all positive (ranging from  $0.25 \pm 0.03$  to  $0.32 \pm 0.05$ ). Our results suggest that selection to improve any single body trait would likely produce correlated responses in the other traits examined and that selecting for growth will cause a positive correlated response in terms of Ammonia-N tolerance; estimates of genetic parameters for Ammonia-N tolerance should be calculated at a later age.

**Keywords:** *Litopenaeus vannamei*, growth-related traits, Ammonia-N tolerance, heritability, genetic correlation.

### Introduction

The white shrimp *Litopenaeus (Penaeus) vannamei* are distributed throughout the Pacific coast from the Gulf of California to northern Peru. It is the major species of penaeid shrimp in the eastern hemisphere and accounts for 30% of the global farmed production of penaeid shrimp (Perez Farfante & Kensley 1997). The intensive culture of penaeid shrimp has been continuously increasing in recent years due to limitations in ponds. In an intensive culture system, ammonia is the most common toxic substance resulting from the excretion of cultured animals and the mineralization of organic detritus such as unconsumed feed and feces at culture late stage. Chen *et al.* (1988) has been reported that the concentration of Ammonia-N (unionized plus ionized ammonia as nitrogen) increases directly with the culture process and can reach levels as high as 46 mg/L in intensive grout-out ponds. The accumulation of ammonia in the shrimp cultured pond water may damage water quality, increase oxygen consumption and Ammonia-N excretion, alter concentrations of

hemolymph protein and free amino acid levels, reduce growth, and induce outbreaks of disease (Wickins 1976; Chen & Lin 1992; Chen *et al.* 1994). Ammonia tolerance is an important resistance trait for shrimp, and genetic improvement is an effective approach to improving the ammonia tolerance of *P. vannamei* in the service of their adaptation to the intensive culture system.

At this time, there are few reports on improving penaeid ammonia resistance through selective breeding, but studies on other resistance traits in aquatic animals remain a hot research field. Li *et al.* (2015) found that the heritability estimates for the cold tolerance of *P. vannamei* were low ( $0.0258 \pm 0.0205$  and  $0.0211 \pm 0.0196$ , respectively based on the cooling degree-hours for each individual and the survival rate of each family at half-lethal time), but they were not significantly different from zero. Thus, further studies on the heritability of cold-tolerance traits are needed. However, significant genetic effects on cold tolerance have been observed in other aquatic animals. Wohlfarth *et al.* (1983) and Cnaani *et al.* (2000) examined tilapia species and their

hybrids and found that a large component of the trait's variance was a result of dominance effects. In Nile tilapia, *Oreochromis niloticus*, Tave *et al.* (1989) and Behrends *et al.* (1990) suggested that cold resistance is controlled by additive genes. In terms of other resistance traits, Cadieu *et al.* (1995) observed high heritability for tolerance to lethal levels of dissolved oxygen ( $=0.56$  when estimated from the dam component and  $0.75$  when estimated from the sire component) in 1- to 2-month-old fingerlings of channel catfish. In post-larvae and juveniles of the Pacific white shrimp *P. vannamei*, the heritability for resistance to hypoxia was high (Ibarra *et al.* 2007). Perry *et al.* (2005) and Zhang *et al.* (2014) observed significant potential for improvement in the upper thermal tolerance of cultured species of turbot. These findings highlight the potential for genetic improvement of resistance traits in cultured aquatic animals. The high-ammonia resistance is an important resistance trait for *P. vannamei* and has not been examined based on assessments of genetic parameters; thus, we examined the genetic parameters for this trait.

Growth-related traits are important targets for selective breeding programs, especially body weight. Body weight is strongly correlated with economic return, is inexpensive and easy to measure, and commonly has a medium level of heritability. Several recent studies have estimated the heritability and genetic correlation for body weight and other growth-related traits in prawns. *P. vannamei* (Pérez-Rostro & Ibarra 2003a, b), and giant freshwater prawns *Macrobrachium rosenbergii* (Kitcharoen *et al.* 2011; Hung *et al.* 2013) showed very high genetic correlations among growth-related traits. The genetic correlation between growth-related traits and resistance traits is an important parameter for designing selective breeding programs and has been studied in many aquatic animals. Li *et al.* (2015) reported negative genetic correlations between these cold-tolerance traits and body weight ( $-0.7702 \pm 0.4583$  and  $-0.8252 \pm 0.4553$ , respectively based on the cooling degree-hours for each individual and the survival rate of each family at half-lethal time) in *P. vannamei*.

This report presents results from the selective breeding program for *P. vannamei* in Guangdong Zhanjiang in terms of genetic variation in harvest weight and resistance to ammonia during two growth stages and also examines their genetic correlations. Increasing the ammonia tolerance in *P. vannamei* could improve the survival rate and reduce economic losses in intensive culture systems.

## Materials and Methods

### Genetic Material and Production of Families

The selective breeding program for *P. vannamei* was conducted at Zhanjiang Haiwei Aquaculture Co.,

Ltd, located in Zhanjiang city, Guangdong province, China. The base population (G<sub>0</sub>) was derived from eight different strains, including three imported populations and five farmed populations in China. After collection, they were subjected to one generation of mass selection for high body weight, with a selection intensity of 1.955, in 2013 before they were used as parents of the base populations. The three imported populations were introduced from Shrimp Improvement Systems (SIS), Kona Bay Marine Resources, and Charoen Pokphand Group, respectively, and the five farmed populations were collected from Kehai NO.1, Zhongxing NO.1, Zhongke NO.1, the Yuehai population, and the Wushi population, respectively. Kehai NO.1, Zhongxing NO.1, Zhongke NO.1, and the Yuehai populations were the cultured populations, which were consecutively selected in China for more than five generations. The Wushi population was from the Zhanjiang cultured population, which was successively cultured in Zhanjiang for more than 10 generations. In 2014, an extra increase in four farmed strains, which had been consecutively selected for more than five generations by Guangdong Haida Group, was introduced to broaden the genetic variation of breeding populations. Finally, 12 different strains were reared in concrete tanks until sexual maturation, when the heaviest males and females were mass selected and used to produce families.

All candidates were tagged with a colored, number-coded ring placed in one eyestalk, after which males were reared in a cement tank and females were reared in another cement tank (after an acclimation period), during which time the vast majority of shrimp molted. The other eyestalk of females was unilaterally ablated using heated wire snips to prompt ovulation. From 70% to 40% of water was exchanged daily, temperature was maintained at 28°C, and salinity ranged from 28 ppt to 32 ppt. The shrimp were fed four times per day with a combination of fresh food, including squid, Nereis, red worms, and oysters. The female maturation stages were monitored daily. Mature females with orange-colored ovaries that occupied a large area of the cephalothorax were preferred. Male ejaculation was immediately analyzed by monitoring spermathecae. Fertilized females were transferred to 250-L individual spawning tanks filled with filtered and disinfected sea water (salinity 30 ppt). Temperature was maintained at between 28°C and 32°C.

### Larvae Culture

A total of 40 full-sib and 20 half-sib families were obtained within 20 days. Spawning typically occurred at night, and newly hatched nauplii were obtained by the next afternoon. All families were hatched individually. Hatched naupii were transferred into separate 250-L larval rearing tanks (LRT) at a

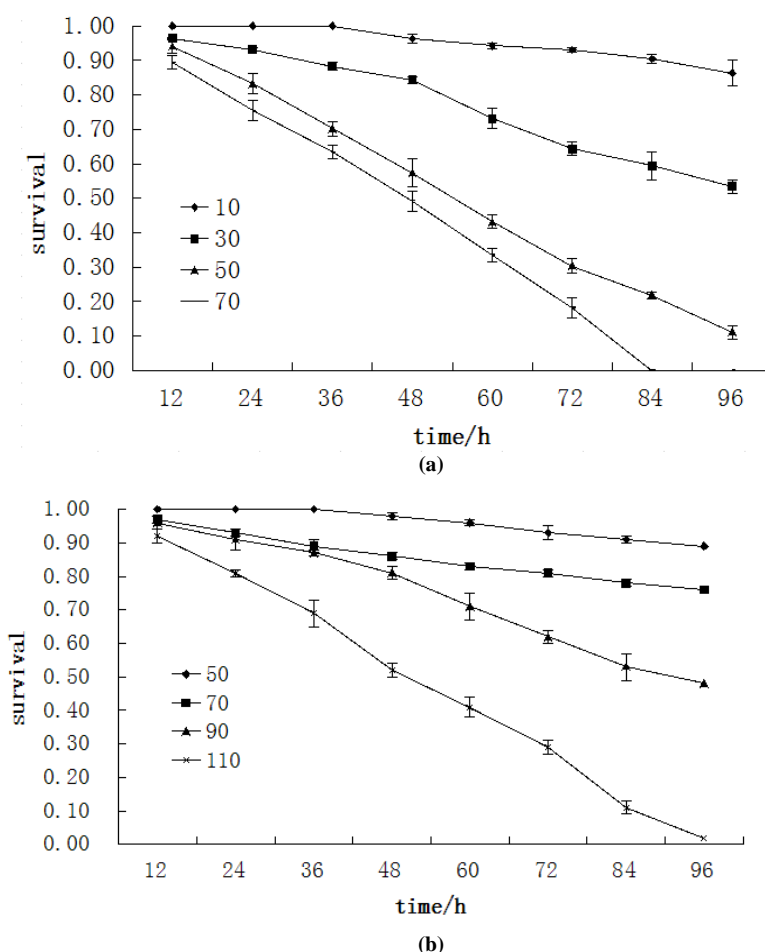
density of 20,000 nauplii per tank. Two replicate LRTs per family were operated using the regular procedures from the hatchery, including a mixed diet of *Chaetoceros* sp., *Artemia* sp., and commercial larval diets. At post-larvae PL-15 (25 days post-spawning), random samples from each family (800 post-larvae per family) were transferred to separate net cages (2.5 m<sup>3</sup>, 1000 µm mesh size) in an outdoor concrete tank for growth.

### Experimental Design

After 3–4 weeks (7 weeks post-hatching) of growth in the outdoor concrete tank, when the total weight per animal was 1 to 2 g, 200 juveniles from each of the 40 families (due to experimental limitations) were individually tagged with a VIE tag, which was injected into the last segment of the animals in each full-sib family. All families could be distinguished and identified based on different VIE color combinations. All tagged shrimp were transferred to an outdoor concrete tank for effect common environment cultivation. After shrimp were cultured for 14 and 21 weeks, a sample of 35 shrimp from each period was randomly selected from every

family for weighing and measuring; data were collected on body weight (BW, g), body length (BL, cm), abdominal segment length (ASL, cm), carapace length (CL, cm), and survival (SU) under conditions of 96 hours of ammonia exposure.

We conducted acute ambient Ammonia-N toxicity pretests before the ammonia tolerance tests. The Ammonia-N solution prepared with ammonium chloride was diluted to different concentrations with three replicates to determine the ammonia concentration in a formal test for the two growth stages. The results showed that the suitable concentration of Ammonia-N in a formal test were 30 mg/L for 14-week-old shrimp and 90 mg/L for 21-week-old shrimp (Figure 1A and Figure 1B). Seawater (33‰) was pumped from the Zhanjiang coast and municipal water was dechlorinated and filtered through a gravel and sand bed by airlifting and aerating for 2 days before use. Trials were conducted in 800L cylindrical polyethylene tanks with median lethal concentrations of Ammonia-N, after which the sample shrimp with eyestalk collar tags were relocated to tank water at 28±0.5°C and pH 8.0±0.2. The testing medium was replaced every 24 h. The experiment lasted for 96 h, and no feed was



**Figure 1.** Line chart of (a) and (b) showing the effect of different concentrations of ammonia-N on the two growth stages of *P. vannamei*. *P. vannamei* reared for 14-week-old in the common environment (a). *P. vannamei* reared for 21-week-old in the common environment (b).

supplied to the animals. The shrimp mortality rate was recorded three times per day, at 00, 08, and 16 h.

### Statistical Analyses

Statistical analyses were performed on a dataset consisting of 2800 pedigree records. All traits were evaluated for normality before further analyses, and raw data were transformed where appropriate. Exploratory analyses using a general linear model (GLM in SAS 9.1) were performed. Body traits approximated normal distributions, and data did not require transformation. A summary of all fixed effects and covariates is provided in Equation 1. The statistical significance of the effects was assessed based on Wald tests using a mixed model in ASReml (Gilmour et al. 2009).

The variance and covariance components for additive genetics, common full-sibs, and residual effects for body and carcass traits were estimated using a restricted maximum likelihood (REML) method. The animal model was written in matrix notation as:

$$y_{imn} = \mu + Age_{imn} + a_m + c_n + e_{imn} \quad (\text{Model 1})$$

where  $y_{imn}$  is a vector obtained as a square root of observed body traits at each growth stage,  $\mu$  is the overall mean,  $Age_{imn}$  is the age at each growth stage as covariate,  $a_m$  is the additive genetic effect,  $c_n$  is the effect common to full-sibs other than additive genetics, and  $e_{imn}$  is the random residual error.

Heritability figures were estimated based on a single-trait model. Phenotypic and genetic correlations were obtained from a series of bi- and trivariate analyses involving all body traits (recorded in all animals) to avoid selection bias (Kennedy 1990). The pedigree included all animals and was traced back to the base population to minimize bias when estimating genetic parameters. The heritability estimate was calculated as  $h^2 = \sigma_a^2 / (\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$ , and the effect common to full-sibs, excluding additive genetics was calculated as  $c^2 = \sigma_c^2 / (\sigma_a^2 + \sigma_c^2 + \sigma_e^2)$ , where  $\sigma_a^2$  is the additive genetic variance,  $\sigma_c^2$  is effect common to full-sibs other than additive genetics variance, and  $\sigma_e^2$  is the residual variance.

A standard threshold (probit) and sire–dam model was used to estimate heritability for survival under conditions of high concentrations of Ammonia-N. The model was written as in ASReml (Gilmour et al. 2009):

$$\lambda_{ijk} = \mu + Sire_i + Dam_j + Age_{ijh} + Common_{ijh} + e_{ijk} \quad (\text{Model 2})$$

$$y_{ijk} = \begin{cases} 0 & \text{if } \lambda_{ijk} \leq 0 \\ 1 & \text{if } \lambda_{ijk} > 0 \end{cases},$$

where  $y_{ijk}$  is the survival status (1=alive, 0=dead) of the  $k$ th shrimp;  $\lambda_{ijk}$  is the underlying liability of  $y_{ijk}$ , which is assumed to be a cumulative standard normal distribution;  $Age_{ijh}$  is the age at each growth stage as covariate;  $\mu$  is the overall mean;  $Sire_i$  and  $Dam_j$  are the additive genetic effects of the  $i$ th sire and the  $j$ th dam, respectively,  $Sire$  or  $Dam \sim (0, A\sigma_{sd}^2)$  ( $\sigma_{sd}^2 = \sigma_s^2 = \sigma_d^2$ ), where  $A$  is the additive genetic relationship matrix among all shrimp; and  $e_{ijk}$  is the random residual error of the  $k$ th individual, with  $e \sim (0, I\sigma_e^2)$ .  $Common_{ijh}$  is the effect common to full-sibs other than additive genetics of  $h$ th family,  $Sire$  or  $Dam \sim (0, A\sigma_c^2)$ . The phenotypic variance was the sum of  $2\sigma_{sd}^2$  and  $\sigma_e^2$  ( $\sigma_p^2 = 2\sigma_{sd}^2 + \sigma_c^2 + \sigma_e^2$ ). Heritability ( $h^2$ ) was computed as the ratio between  $4\sigma_{sd}^2$  and  $\sigma_p^2$  ( $h^2 = 4\sigma_{sd}^2 / \sigma_p^2$ ). The effect common to full-sibs other than additive genetics was computed as the ratio between  $\sigma_c^2$  and  $\sigma_p^2$  ( $c^2 = \sigma_c^2 / \sigma_p^2$ ). The estimates were then adjusted according to Robertson and Lerner (1949), as they were overestimated in this model.

The phenotypic and genetic correlations between body weight and survival rate in Ammonia-N were calculated using bivariate analysis in the ASReml package (Gilmour et al. 2009). The survival variable was set as the first variable using the same model as univariate Model 2. Body weight was set as the second variable, which was similar to the fixed effects and covariate in Model 1. The description for each term in the models was the same as for Model 2 and Model 1.

Z-scores were used to determine whether the heritability estimates were significantly different from one or zero (Nguyen et al. 2007) using the following formula:

$$Z = \frac{x_i - x_j}{\sqrt{(\sigma_i^2 + \sigma_j^2)}}$$

where  $x_i$  and  $x_j$  are the estimates of heritability or correlations and  $\sigma_i$  and  $\sigma_j$  are their respective standard errors. Both  $x_i$  and  $\sigma_i$  were set to zero when testing whether a heritability or correlation estimate was significantly different from zero, whereas  $x_j$  and  $\sigma_j$  were set to one and zero, respectively,

when testing whether a correlation differed significantly from unity. The resulting Z-score was then tested against a large-sample normal distribution.

## Results

Data regarding the survival rates and ambient Ammonia-N tolerance during the two growth stages of *P. vannamei* are shown in Figure 1A and Figure 1B. The white shrimp reared in the common environment for 14-week-old were exposed to four Ammonia-N concentrations (10, 30, 50, and 70 mg/L). The survival rate of shrimp after 96 hours of exposure to 10 mg/L of Ammonia-N concentration was highest, and the survival rates at 50 and 70 mg/L of Ammonia-N were 0 and 0.11, respectively. When the Ammonia-N concentration was 30 mg/L, the survival rate of shrimp was at a middle level, 0.53, where the Ammonia-N concentration was optimal for assessing heritability traits during the first growth stage. The white shrimp reared in the common environment for 21-week-old were exposed to four Ammonia-N concentrations (50, 70, 90, and 110 mg/L). The survival rates of shrimp after 96 hours of exposure at 50 and 70 mg/L of Ammonia-N were high, at 0.81 and 0.96, respectively. However, the survival rate was 0.02 at 110 mg/L Ammonia-N. When the Ammonia-N concentration was 90 mg/L, the survival rate of shrimp was at a middle level, 0.48, which was the optimal concentration for assessing heritability traits for the second growth stage.

The detailed information about growth-related traits from the 40 families is presented in Table 1. The mean body weight (BW) for 14-week-old shrimp and 21-week-old shrimp were 3.87g and 7.93g, respectively; the body length (BL) for 14-week-old shrimp and 21-week-old shrimp were 6.85cm and 8.73cm, respectively. Although each family was established within 20 days and were cultured in a common environment for a long time, a major difference was observed among the growth traits of the 40 families at each time point. For the 21-week-old shrimp, the minimum body weight and body length were 2.63g and 4.14cm, respectively, whereas

their maximum body weight and body length were 13.18g and 10.36cm, respectively. The same situation was found at two growth stages. The coefficients of variation for body weight, which were 59.69% and 60.28%, respectively, was obviously high in both growth stages. The high Ammonia-N concentrations challenge tests were conducted for 96 h, and the mean survival rates for 14-old-shrimp and 21-old-shrimp were 60.14% and 51.09%, respectively. A major difference was observed in the survival rates of the 40 families during 96 h of high Ammonia-N intimidation. The minimum survival rates among the 40 families were 23.45% and 21.47% at the end of the test, whereas the maximum survival rates were 87.43% and 93.62%.

All heritability and the effect common to full-sibs other than additive genetics (C2) estimates for the four growth-related traits and the survival rates under conditions of high Ammonia-N concentrations are presented in Table 2. Moderate levels of heritability were observed for four growth-related traits (0.24±0.09 to 0.30±0.06 for 14-week-old shrimp and 0.26±0.07 to 0.31±0.06 for 21-week-old shrimp). The heritability of the survival rates of *P. vannamei* exposed to experimentally high Ammonia-N concentrations during the two growth stages of shrimp were low (0.13±0.11 for 14-week-old shrimp and 0.17±0.08 for 21-week-old shrimp). Apart from the heritability levels for the trait of SU in 14-week-old shrimp, the other heritability rates at the two age stages differed significantly ( $P < 0.05$ ). The effect common to full-sibs other than additive genetics for growth traits in 14-week-old shrimp varied from 0.06±0.03 to 0.11±0.06. The effect common to full-sibs for growth traits in 21-week-old shrimp varied from 0.05±0.03 to 0.09±0.03. The effect common to full-sibs other than of survival for Ammonia-N tolerance were 0.05±0.02 at 14 weeks old and 0.04±0.02 at 21 weeks old. The effect common to full-sibs other than additive genetics in 14-week-old shrimp for all traits were stronger than those in 21-week-old shrimp.

Genetic and phenotypic correlations between all traits in 14-week-old and 21-week-old shrimp are

**Table 1.** Mean and other descriptive statistics of the growth of the 40 families at different growth age high ammonia-N concentrations tolerance test

Age	Variable	Mean	Minimum	Maximum	Standard deviation	Coefficient of variation(%)
14-week-old	BW (g)	3.87	1.49	7.68	2.31	59.69
	BL (cm)	6.85	5.06	8.51	1.38	20.15
	ASL (cm)	4.79	3.54	6.23	0.96	20.08
	CL (cm)	2.81	2.21	3.44	0.53	8.86
	SU(%)	60.14	23.45	87.43	20.41	33.94
21-week-old	BW (g)	7.93	2.63	13.18	4.78	60.28
	BL (cm)	8.73	6.02	10.36	1.76	20.16
	ASL (cm)	7.95	4.14	7.38	1.34	16.86
	CL (cm)	3.51	2.71	4.21	0.67	19.09
	SU(%)	51.09	21.47	93.62	20.76	40.69

**Table 2.** Heritability estimates ( $h^2$ ) and standard errors (SE) of growth-related traits and survival under conditions of high ammonia-N concentrations at two growth stages.  $C^2$  are common full-sib effects other than additive genetic effects

Age	Traits	$h^2 \pm SE$	$C^2 \pm SE$
14-week-shrimp	BW (g)	0.29±0.07	0.09±0.03
	BL (cm)	0.30±0.06	0.11±0.06
	ASL (cm)	0.28±0.08	0.06±0.03
	CL (cm)	0.24±0.09	0.07±0.04
	SU	0.13±0.11 <sup>NS</sup>	0.05±0.02
21-week-shrimp	BW (g)	0.28±0.09	0.09±0.03
	BL (cm)	0.31±0.06	0.05±0.03
	ASL (cm)	0.28±0.11	0.06±0.02
	CL (cm)	0.26±0.07	0.07±0.04
	SU	0.17±0.08	0.04±0.02

BW, body weight; BL, body length, ASL, abdominal segment length; CL, carapace length; SU, the survival rate of *P. vannamei* exposed to experimentally high ammonia-N concentrations. <sup>NS</sup> not significant; no superscript denotes significance ( $P < 0.05$ ).

shown in Table 3. With the exception of a moderate genetic correlation ( $0.27 \pm 0.04$  to  $0.34 \pm 0.07$ ) between growth-related traits and survival under conditions of high Ammonia-N concentrations, strong and significant genetic ( $0.74 \pm 0.09$  to  $0.89 \pm 0.16$ ) and phenotypic ( $0.55 \pm 0.04$  to  $0.77 \pm 0.13$ ) correlations between growth-related traits were observed in 14-week-old shrimp. With the exception of a moderate genetic correlation ( $0.25 \pm 0.03$  to  $0.31 \pm 0.11$ ) between growth-related traits and survival under conditions of high Ammonia-N concentrations, highly significant genetic ( $0.68 \pm 0.05$  to  $0.85 \pm 0.10$ ) and phenotypic ( $0.53 \pm 0.05$  to  $0.74 \pm 0.08$ ) correlations between growth-related traits were observed in 21-week-old shrimp under conditions of high ammonia-N concentrations. The results for the two growth stages were similar. Genetic and phenotypic correlations of traits in 14-week-old shrimp and 21-week-old shrimp are also shown in Table 3. Strong and significant genetic correlations ( $0.46 \pm 0.09$ ) were observed for body weight during the two growth stages. Other traits showed weaker genetic correlations, ranging from ( $0.14 \pm 0.05$ ) to ( $0.24 \pm 0.08$ ). Phenotypic correlations between two growth stages showed little difference; that for body weight was strongest ( $0.24 \pm 0.08$ ), whereas that for survival was weak ( $0.19 \pm 0.06$ ).

## Discussions

### Heritability of Growth-Related Traits

We found that the estimated heritability values for growth-related traits in *P. vannamei* were moderate, ranging from  $0.24 \pm 0.09$  to  $0.30 \pm 0.06$  in 14-week-old shrimp and from  $0.26 \pm 0.07$  to  $0.31 \pm 0.06$  in 21-week-old shrimp, which was similar to the results reported by Pérez-Rostro *et al.* (2003a) in the same species ( $0.15 \pm 0.16$  to  $0.22 \pm 0.17$  in 17-week-old shrimp and  $0.24 \pm 0.17$  to  $0.32 \pm 0.18$  in 23-week-old shrimp). Weight is an important trait that is strongly correlated with economic returns. The estimates of the heritability of weight at two growth stages were medium and similar ( $0.29$  in 14-week-old shrimp and

$0.28$  in 21-week-old shrimp) in this report, which was lower than that of full-sib heritability ( $0.70 \pm 0.15$ ) for weight in shrimp tagged for 21 weeks, as reported by Argue *et al.* (2002) for *P. vannamei*. Carr *et al.* (1997) reported heritability estimates for the same trait as  $0.42$ , and Arcos *et al.* (2004) reported a heritability estimate of  $0.47$  for weight at 50 g. The differences between those estimates with the present study for the body weight could be from multiple causes of genetic or environmental origin as different populations and growing conditions are involved. Regardless of the differences, it appears that heritability of body weight in *P. vannamei* is moderate or high, indicates a potentially significant response to selection. Our results was also higher than those reported previously:  $0.24 \pm 0.05$  and  $0.17 \pm 0.04$  for harvest weight in ponds and tanks, respectively, at 23 weeks (Gitterle *et al.* 2005);  $0.17 \pm 0.06$  for weight through a restricted-maximum likelihood method after at 29 weeks (Pérez-Rostro & Ibarra 2003b); from  $0.09$  to  $0.11$  for body weight in the presence of WSSV (White spot syndrome virus) at 19 weeks (Caballero-Zamora *et al.* 2014), the probable reason were other research based on f introducing sex as fixed effects, the more acute heritability had produced by eliminating more fixed effects. So we will identification sex at suitable time in next research works.

For other growth-related traits, our heritability estimates for BL, ASL and CL ( $0.30 \pm 0.06$ ,  $0.28 \pm 0.08$  and  $0.24 \pm 0.09$  in 14-week-old shrimp and  $0.31 \pm 0.06$ ,  $0.28 \pm 0.11$  and  $0.26 \pm 0.07$  in 21-week-old shrimp). Pérez-Rostro and Ibarra. (2003a) estimate the heritability of BL and ASL were  $0.15 \pm 0.16$  and  $0.20 \pm 0.17$  at 17-week-old shrimps and  $0.27 \pm 0.16$  and  $0.32 \pm 0.18$  at 32-week-old shrimps. Hung *et al.* (2013) estimated the heritability for CL and AL to be  $0.06 \pm 0.01$  and  $0.07 \pm 0.04$  at 10 weeks of age and  $0.20 \pm 0.08$  and  $0.11 \pm 0.07$  at 18 weeks of age, respectively. There are both different plots and similar plots for the heritability of this traits in present study. The differences could be from different populations, growing conditions, analytical method. But the similar plots showed that considerable additive genetic variance for those growth traits can be used to

**Table 3.** Genetic (left diagonal) and phenotypic correlations between four growth-related traits and survival under conditions of high ammonia-N concentrations in 14-week-old and 21-week-old shrimp

		14-week-old					21-week-old				
		BW	BL	ASL	CL	SU	BW	BL	ASL	CL	SU
14-week-old	BW		0.74±0.07	0.63±0.07	0.55±0.04	0.31±0.06	0.24±0.08				
	BL	0.89±0.16		0.77±0.13	0.65±0.07	0.28±0.04		0.23±0.03			
	AM	0.77±0.04	0.74±0.06		0.74±0.09	0.27±0.03			0.21±0.04		
	ASL	0.74±0.09	0.82±0.07	0.83±0.09		0.34±0.07				0.20±0.05	
	SU	0.31±0.08	0.28±0.03	0.27±0.04	0.32±0.05						0.19±0.06
	BW	0.46±0.09						0.67±0.06	0.59±0.06	0.53±0.05	0.32±0.07
21-week-old	BL		0.24±0.08				0.76±0.05	0.62±0.06	0.62±0.09	0.27±0.04	
	AM			0.14±0.05			0.77±0.11	0.72±0.04	0.74±0.08	0.28±0.06	
	ASL				0.16±0.06		0.68±0.05	0.84±0.08	0.85±0.10	0.33±0.05	
	SU					0.19±0.05	0.31±0.11	0.25±0.03	0.28±0.07	0.29±0.04	
	SU										

BW, body weight; BL, body length, ASL, abdominal segment length; CL, carapace length; SU, survival under high concentration ammonia-N in *P. vannamei*.

improve their growth performance in the future.

### Heritability of Survival under Conditions of High Ammonia-N Concentrations

Few studies have explored the genetic parameters for under survival rates conditions of high Ammonia-N concentrations in *P. vannamei*. Estimate of the heritability of survival under conditions of high Ammonia-N concentrations at two growth stages were both low ( $0.13 \pm 0.11$  in 14-week-old shrimp and  $0.17 \pm 0.08$  in 21-week-old shrimp). Li *et al.* (2015) reported the cold-tolerance heritability of *P. vannamei* to be low ( $0.0258 \pm 0.0205$  and  $0.0211 \pm 0.0196$  using the cooling degree-hours for each individual and the survival rate of each family at half-lethal time, respectively). Baer and Travis (2000) estimated the heritability of thermal tolerance in *H. formosa* as less than 0.15, and Charo-Karisa *et al.* (2005) determined that the heritability of cold tolerance in Nile tilapia was low, 0.09, for temperature-at-death (TAD). An even lower heritability estimate ( $-0.125 \pm 0.095$ ) was reported by Elderkin *et al.* (2004) for the heat tolerance of *Zebira Mussle Veligers*. The low heritability for survival of environment factor tolerance indicated the selection for tolerance traits need more generations and make more effective selectively proposal. The standard error of survival heritability was large at 14-week-old shrimp, which caused that the heritability showed no statistically significant difference from zero, but the heritability at 21-week-old shrimp showed statistically significant difference from zero. Thus, estimating genetic parameter for resistance Ammonia-N in *P. vannamei* at early period is improper.

### The Effect Common to Full-Sibs Other than Additive Genetics

The effect common to full-sibs are caused by possible maternal and non-additive genetic effects, which were confounded in the present study. This effect ranged from  $0.06 \pm 0.03$  to  $0.11 \pm 0.06$  for the body traits of 14-week-old shrimp and from  $0.05 \pm 0.03$  to  $0.07 \pm 0.04$  for that of 21-week-old shrimp. The results show that the effect common to full-sibs other than additive genetics was larger at an early growth age than at an older age, possibly because growth-related additive genes are fully expressed with the extension of growth time. Gitterle *et al.* (2005) reported that the effect common to full-sibs for body weight ranged from  $0.00 \pm 0.05$  to  $0.17 \pm 0.08$  for different batches; if the effect common to full-sibs was large, the heritability was typically small in *P. vannamei* under standard commercial conditions. For other aquatic animals, Hung *et al.* (2013) reported that the effect common to full-sibs other than additive genetics for the same with body traits examined in our study ranged from 0.04 to 0.06 based on a four-analysis model of giant freshwater

prawns (GFPs, *Macrobrachium rosenbergii*); based on the body weight and body length of rainbow trout and Atlantic salmon, the reported effect common to full-sibs other than additive genetics accounted for approximately 5% of the total variation in the rearing period until tagging (Fjalested *et al.* 1996). To improve the accuracy with which genetic parameters are assessed, it is important to design experiments to reduce the effect common to full-sibs other than additive genetics. Possible strategies for doing so in breeding programs include shortening the spawning and nursing periods for full-sib families by upgrading hatchery capacity and improving nursing techniques, tagging the animals at an earlier age, or using molecular techniques for the posterior assignment of parents, which enables common family rearing at a very early stage. This will minimize differences in the length of nursing and grow-out time for full-sib families.

### Phenotypic and Genetic Correlations

Phenotypic and genetic correlations between growth-related traits at two growth periods were both high, with genetic and phenotypic correlations between growth-related traits for 14-week-old shrimp at ( $0.74 \pm 0.06$  to  $0.89 \pm 0.16$ ) and ( $0.55 \pm 0.04$  to  $0.77 \pm 0.13$ ), respectively, and genetic and phenotypic correlations between growth-related traits for 21-week-old shrimp at ( $0.68 \pm 0.05$  to  $0.85 \pm 0.10$ ) and ( $0.53 \pm 0.05$  to  $0.74 \pm 0.08$ ), respectively. The results show that genetic correlations were higher than phenotypic correlations and that the two growth periods were similar. Similarly, Pérez-Rostro *et al.* (2003b) reported high genetic correlations between growth-related traits (ranging from 0.44 to 0.98) compared with those reported previously for *P. vannamei*, but the phenotypic correlation was higher than the genetic correlation, which differs from our findings, the proper reason was environment effects covered up genetic correlation in our study. A high positive phenotypic and genetic correlation for growth-related traits has been observed for other shrimp species, such as *M. rosenbergii* (Hung *et al.* 2014; Kitcharoen *et al.* 2012; Hung *et al.* 2013), as well as different fish species (McKay *et al.* 1986; Myers *et al.* 2001; Silverstein & Hershberger 1994). These outcomes indicate a high genetic correlation of growth-related traits of *P. vannamei*, which is likely controlled by the most same set of genes. Therefore, selection to improve any single growth-related trait would likely produce correlated responses in the other traits examined.

To improve the growth and environment factors tolerance of aquatic animals, many scholars studied the genetic correlation between growth and environment factors tolerance (Moss *et al.* 2011; Moss *et al.* 2013; Argue *et al.* 2002). Charo-Karisa *et al.* (2005) estimated that the genetic correlation between cold tolerance and body weight was stronger



( $0.72 \pm 0.81$  with cooling degree hours) in *Oreochromis niloticus*, but these genetic correlations could not be estimated accurately, as is reflected by the high standard errors and the weak phenotypic correlation ( $0.33 \pm 0.17$  with cooling degree hours). Ma et al. (2007) reported phenotypic correlations between cold tolerance and body weight or length that were positive but weak (0.18 and 0.23, respectively) in red drum *Sciaenops ocellatus*, which suggests that the genetic correlation also be low. Li et al. (2015) reported negative genetic correlations  $-0.7702 \pm 0.4583$  and  $-0.8253 \pm 0.4553$  using the cooling degree-hours for each individual and the survival rate of each family at half-lethal time, respectively), between cold tolerance traits and body weight in *P. vannamei*. In this study, the genetic correlation between survival under high conditions of Ammonia-N concentrations and growth-related traits were  $0.27 \pm 0.04$  to  $0.32 \pm 0.05$  in 14-week-old shrimp and  $0.25 \pm 0.03$  to  $0.31 \pm 0.11$  in 21-week-old shrimp. The correlations between body related traits and survival under conditions of high Ammonia-N concentrations were positive. The sign and magnitude of the genetic correlation between body-related-traits and survival under conditions of high Ammonia-N concentrations found in this study indicate that selecting for growth will cause a positive correlated response in Ammonia-N tolerance.

Estimate the genetic correlations between different growth stages can be dependencies for selection early for a breeding nucleus. In this study, excluding that for body weight ( $0.46 \pm 0.09$ ), the genetic correlations between the same growth trait at 14 and 21 weeks were low (ranging from  $0.14 \pm 0.05$  to  $0.24 \pm 0.08$ ), and the phenotypic correlations were also low (ranging from  $0.19 \pm 0.06$  to  $0.24 \pm 0.08$ ). The correlations reported for body weight in the present study are consistent with those reported by an earlier study evaluating the size and growth traits of *P. vannamei* reared in indoor systems (Pérez-Rostro & Ibarra 2003a); that study estimated genetic correlations to be 0.52 between weeks 17 and 23 and 0.30 between weeks 17 and 29. However, Coman et al. (2010) reported a strong genetic correlation (0.63) for body weight in *Penaeus monodon* between weeks 16 and 24, but the correlation was low (0.15) between weeks 16 and 32. Pérez-Rostro & Ibarra (2003a) also observed a strong genetic correlation between weeks 23 and 29 for body weight (0.77), but the correlation was low (0.30) between weeks 17 and 29 in *P. vannamei*. In fish species, Su et al. 2002 observed a strong genetic correlation between days 280 and 336 for body weight (0.821), but the correlation was low (0.392) between weeks 168 and 224 in *Oncorhynchus mykiss*. These studies show that the genetic correlation between different growth periods for same growth-related traits reflect large differences, indicating that the same trait is expressed by different genes during different growth periods. In our study, the genetic correlations for growth-related-traits between 14 weeks and 21 weeks were weak (although

they were positive), indicating that selection at an early age would not necessarily result in a large correlated response at a later age.

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