Heritability of body weight and resistance to ammonia in the Pacific white shrimp *Litopenaeus vannamei* juveniles*

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Abstract   Ammonia, toxic to aquaculture organisms, represents a potential problem in aquaculture systems, and the situation is exacerbated in closed and intensive shrimp farming operations, expecially for *Litopenaeus vannamei*. Assessing the potential for the genetic improvement of resistance to ammonia in *L. vannamei* requires knowledge of the genetic parameters of this trait. The heritability of resistance to ammonia was estimated using two descriptors in the present study: the survival time (ST) and the survival status at half lethal time (SS_{50}) for each individual under high ammonia challenge. The heritability of ST and SS_{50} were low ($0.154\ 4\pm0.044\ 6$ and $0.147\ 5\pm0.040\ 0$, respectively), but they were both significantly different from zero ($P<0.01$). Moreover, these two estimates were basically the same and showed no significant differences from each other ($P>0.05$), suggesting that ST and SS_{50} could be used as suitable indicators for resistance to ammonia. There were also positive phenotypic and genetic correlation between resistance to ammonia and body weight, which means that resistance to ammonia can be enhanced by the improvement of husbandry practices that increase the body weight. The results from the present study suggest that the selection for higher body weight does not have any negative consequences for resistance to ammonia. In addition to quantitative genetics, tools from molecular genetics can be applied to selective breeding programs to improve the efficiency of selection for traits with low heritability.

Keyword: *Litopenaeus vannamei*; heritability; resistance to ammonia; body weight; genetic correlation

1 INTRODUCTION

The Pacific white shrimp, *Litopenaeus vannamei*, whose natural distribution is along the Pacific coast of the western American continent from Mexico to Peru, has been introduced into the Atlantic coast from USA to Brazil and into different Asian countries (Funge-Smith and Briggs, 2005). It is most widely cultured in many parts of the world, and its rapid growth, good survival in high-density culture and disease tolerance make it a good choice for intensive and/or biosecure closed grow-out strategies (Cuzon et al., 2004). However, these culture practices have resulted in the degradation of the culture water as well as a high frequency of diseases in recent years, which seriously affect output and quality of *L. vannamei* (Armstrong, 1979; Colt and Armstrong, 1981; Heath, 1995; Foss et al., 2003; Cuzon et al., 2004; Alagappan et al., 2010).

One of the limiting factors in culture systems is the toxicity of nitrogen waste products, such as ammonia and nitrites (Losordo, 1991) generated by the excretion and ammonification of unconsumed feed, organic wastes and sediments (Wright 1995; Zhao et al., 1997). The situation is exacerbated in closed and intensive shrimp farming operations (Chen et al., 1986, 1988; Chen and Kou, 1993): ammonia

concentrations in the water increase, ammonia excretion by aquatic organisms diminishes, levels of ammonia in the blood and other tissues increase (Colt and Armstrong, 1981) and the metabolic pattern also changes and further affects growth, central nervous system function, immune response, ionic balance, energy metabolism, molting and survival (Heath, 1995; Wicks et al., 2002; Foss et al., 2003; Junkunlo, et al., 2012). There are many published results concerning the toxic effect of ammonia in fish, mollusks and crustaceans (Johnson, 1985; Lewis and Morris, 1986; Chen et al., 1990a, b; Chen and Lin, 1992a, b; Noor-Hamid et al., 1994), and several studies have been carried out to determine the toxicity levels of ammonia in different life stages of penaeid shrimp, such as *Penaeus monodon*, *Marsupenaeus japonicus*, *Fenneropenaeus chinensis*, *Litopenaeus setiferus* and *L. vannamei* (Chin and Chen, 1987; Chen and Lin, 1991a, b; Lin et al., 1993; Alcaraz et al., 1999; Lin and Chen, 2001; Magallón-Barajas et al., 2006), but so far, not on genetic parameters.

Genetic improvement programs can increase the economic efficiency of farmed shrimp (Gjedrem, 2005; Cock et al., 2009). Several selective breeding programs are being developed in *L. vannamei*, and both ANOVA (analysis of variance) and restricted maximum likelihood (REML) methods have been used to estimate the heritability for growth-related, survival, reproduction, resistance to Taura syndrome virus (TSV), resistance to white spot syndrome virus (WSSV) and resistance to hypoxia traits in them (Goyard et al., 1999; Argue et al., 2002; Pérez-Rostro and Ibarra, 2003a, b; Gitterle et al., 2005a, b; Castillo-Juárez et al., 2007; Ibarra et al., 2007; Ibarra et al., 2009; Luan et al., 2012). Ammonia, toxic to aquaculture organisms, represents a potential problem in aquaculture systems; assessing the potential for the genetic improvement of resistance to ammonia in *L. vannamei* requires knowledge of the genetic parameters of this trait. Thus we present here an analysis of the genetic parameters for resistance to ammonia of *L. vannamei* juveniles. Our goal was to estimate the heritability of resistance to ammonia in the Pacific white shrimp (*L. vannamei*) and to reveal the correlation between resistance to ammonia and body weight in them.

2 MATERIAL AND METHOD

2.1 Historical background and shrimp production

All experimental procedures were conducted at the Mariculture Research Station of Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, located in the suburbs of Qingdao (latitude 36°20′32.22″N, longitude120°39′1.93″E, altitude 3.04 m), Shandong province, China. The base population (G_0) was derived from seven commercial *L. vannamei* strains using an incomplete diallel cross experiment in 2011. The seven strains were separately introduced from Shrimp Improvement System Pte. Ltd (SINGAP), Shrimp Improvement System Hawaii LLC (HAWAII), University of Guam, CNAS (GUAMIS), Kona Bay Marine Resources, Waimea Aquatic Lab (KONABA), The Oceanic Institute (OCENAI), Shrimp Improvement System Florida (SISMAM) and High Health Aquaculture Inc (HIGHHA) in June 2011. Their origins were Singap, Oahu, Guam, Kauai, Oahu, Miami and Hawaii, respectively. The pedigrees of individuals in the seven strains were unknown. During subsequent generations (G_1 to G_2), the selection population was closed, and generations were discrete. G_1 to G_2 generations were produced using a full-sib/half-sib mating design. In 2012, 69 sires and 66 dams from the base population (G_0) were used to produce parental shrimps (G_1), which contained 72 full-sib families. A total of 55 full-sib families (consisted of 28 half-sib families) were produced as experimental shrimp (G_2) from 52 sires and 43 dams of G_1. There was an age difference of 24, 39 and 27 days for G_0, G_1 and G_2 generation respectively, which was caused by the different mating and spawning time. The pedigree of all the individuals was known and was used to construct a relationship matrix. The number of sires, dams, full-sib families, half-sib families and the shrimp production scheme is shown in Fig.1.

Each breeding candidate was reared separately in a small net cage (0.25 m×0.25 m×0.25 m) labeled with a four digit identity code. Breeding candidates could then be individually identified using the cage ID. Female and male parents were carefully chosen to maximize mating success. Females with orange colored ovaries that occupied a large area of the cephalothorax were preferred. Males with a healthy appearance and filled spermatophore were obtained for mating with the sexually receptive females. Each full-sib group of fertilized eggs were hatched in separate tanks. After about one day, random samples of approximately 3 000 nauplii from each full-sib family were stocked in separate larvae-culture tanks. Hatched nauplius larvae passed through six nauplius stages, three zoea stages, three mysis stages and
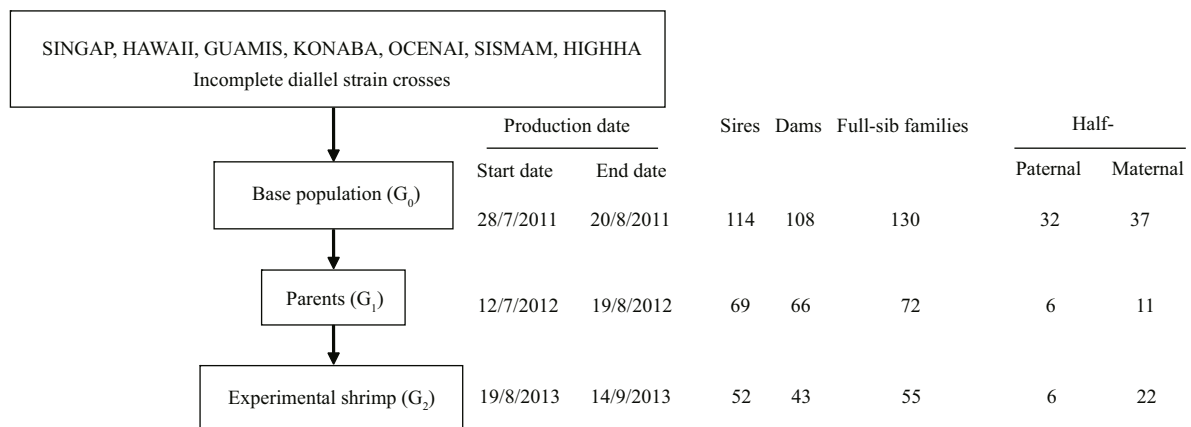
SINGAP, HAWAII, GUAMIS, KONABA, OCENAI, SISMAM, HIGHHA Incomplete diallel strain crosses						Half-	
	Production date		Sires	Dams	Full-sib families	Paternal	Maternal
Base population (G₀)	Start date	End date					
	28/7/2011	20/8/2011	114	108	130	32	37
Parents (G₁)	12/7/2012	19/8/2012	69	66	72	6	11
Experimental shrimp (G₂)	19/8/2013	14/9/2013	52	43	55	6	22

Fig.1 Schematic presentation of production of experimental shrimp from seven commercial *L. vannamei* strains from Singapore and the United States

Abbreviations: SINGAP: strain form Shrimp Improvement System Pte. Ltd; HAWAII: strain form Shrimp Improvement System Hawaii LLC; GUAMIS: strain form University of Guam, CNAS; KONABA: strain form Kona Bay Marine Resources, Waimea Aquatic Lab; OCENAI: strain form OCENAI The Oceanic Institute; SISMAM: strain form Shrimp Improvement System Florida; HIGHHA: strain form High Health Aquaculture Inc.

metamorphosis to post-larva in approximately ten days. Random samples of approximately 200 post-larvae from each family were transferred into larger tanks (3 m³) for separate rearing until the last family reached tagging size (with mean body weight of approximately 3 g, post-larval ages of 73–95 days). Then, the shrimps from each family were tagged using a visible implant elastomer, and each family received a unique tag code.

2.2 Challenge with high ammonia level

After a period of one week of temporary rearing to recover from tagging stress, 36 tagged individuals per family (totally 52 full-sib families) were assigned equally and randomly to four 3 m² concrete tanks. Then, the shrimps were exposed to high ammonia concentration of 62.23 mg/L, that was the half lethal concentration for approximately 120 h according to our simple pre-experiment. An ammonia stock solution was prepared by adding NH_4Cl (Aldrich, Milwaukee, WI, USA). The water temperature was kept at 24±1°C during the experiment, and dissolved oxygen (DO), pH, and total ammonia were measured once a day, using a WTWR multi 340i meter and HACH kits. Dissolved oxygen was maintained no less than 5 mg/L; pH ranged between 7.9–8.3; ammonia ranged between 61.33–63.33 mg/L. Tanks were cleaned daily by suction to remove feces. Water that was removed during cleaning was replaced with clean water with the same concentration of NH_4Cl. Tanks were observed once each hour for any shrimp mortality. Death was defined as the point at which shrimp lost balance, fell on their side, ceased body

movements and lost their response to external stimulation. Throughout the experiment, dead shrimp were removed from the tanks at the beginning of each hour with a scoop net, and their tag, body weight (BW) and death time were recorded, which enabled us to quantify the survival time (ST) and survival status (1=alive,0=dead) at the half lethal time (SS_{50}) for each individual. Shrimp were fed feedstuff (Haid Dachuan #1, Guangdong Haid Group Co., Ltd.) four times a day according to their nutritional needs until the first death occurred.

2.3 Data analysis

An analysis of the descriptive statistics was conducted using the MEANS procedure (SAS Institute Inc., 2005). A complete pedigree in this breeding program from G_0 was included in the below analysis. The common environment effect was omitted in the present analysis because of the short period of communal rearing.

The estimation of heritability for BW was performed using the ASReml package (Gilmour et al., 2009). The individual animal model was used to estimate the genetic parameters for BW of the shrimps challenged with high concentration ammonia. Age at the end of the experiment showed a linear relationship with body weight, and was fitted as a covariate in the model. All the fixed effects and the covariate were statistically significant ($P<0.01$). The fitted model was:

$$Y_{ijk}=\mu+\text{Tank}_i+b\times\text{Age}_j+a_k+e_{ijk}, \qquad \text{(Model 1)}$$

where Y_{ijk} is the observed BW of the a_k individual; μ

is the overall mean; $Tank_i$ is the fixed effect of ith tank; b is the regression coefficient; Age_j is the covariate of the jth individual age when the experiment ended; a_k is the random additive genetic effect of the kth individual and e_{ijk} is the random residual effect associated with observation ijk. The phenotypic variance (σ_p^2) was the sum of all variance components: $\sigma_p^2 = \sigma_a^2 + \sigma_e^2$. The heritability was calculated as the ratio between animal genetic variance and the total phenotypic variance: ($h^2 = \sigma_a^2/\sigma_p^2$).

The resistance to ammonia was expressed as ST and SS_{50} in the present study. The individual animal model was used to estimate genetic parameters for ST of the shrimps challenged with high concentration ammonia. Body weight showed a linear relationship with ST ($P<0.01$), and was fitted as a covariate in the model. The fixed effect of tank was also statistically significant ($P<0.01$). The fitted model was:

$$Y_{ijk} = \mu + Tank_i + b \times BW_j + a_k + e_{ijk}, \qquad \text{(Model 2)}$$

where Y_{ijk} is the observed ST of the kth individual; μ is the overall mean; $Tank_i$ is the fixed effect of ith tank; BW_j is the covariate of the jth body weight; b is the regression coefficient; a_k is the random additive genetic effect of the kth individual and e_{ijk} is the random residual effect associated with observation ijk. The phenotypic variance (σ_p^2) was the sum of all variance components: $\sigma_p^2 = \sigma_a^2 + \sigma_e^2$. The heritability was calculated as the ratio between animal genetic variance and the total phenotypic variance ($h^2 = \sigma_a^2/\sigma_p^2$).

A standard threshold (probit) and a sire-dam model were used in this study to estimate the heritability for SS_{50}. The model was written as in ASReml (Gilmour et al., 2009):

$$\lambda_{ijklm} = \mu + Tank_i + b \times BW_j + Sire_k + Dam_i + e_{ijklm}, \text{ (Model 3)}$$

$$y_{ijklm=} \begin{cases} 0 \text{ if } \lambda_{ijklm} \leq 0 \\ 1 \text{ if } \lambda_{ijklm} < 0 \end{cases},$$

where y_{ijklm} is the survival status (1=alive, 0=dead) of the mth shrimp; λ_{ijklm} is the underlying liability of y_{ijklm}, assumed to be a cumulative standard normal distribution; μ is the overall mean; $Tank_i$ is the fixed effect of the ith test tank; BW_j is the covariate of the jth body weight; b is the regression coefficient; $Sire_k$ and Dam_i are the additive genetic effects of the kth sire and the h^2 dam, 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_{ijklm} is the random residual error of the mth individual, with $e(\hat{0}, I\sigma_e^2$. The residual variance of λ was assumed to be 1. The phenotypic variance was the sum of $2\sigma_{sd}^2$ and σ_e^2: $\sigma_p^2 = \sigma_{sd}^2 + \sigma_e^2$.

Heritability (h^2) was computed as the ratio between $4\sigma_{sd}^2$ and σ_p^2 ($h^2 = 4\sigma_{sd}^2/\sigma_p^2$). The estimates then were adjusted according to Robertson and Lerner (1949), as they were overestimated in this model.

The phenotypic and genetic correlations between BW and ST were estimated using bivariate animal model with ASReml package (Gilmour et al., 2009). The bivariate animal model is the extension of Model 1 and Model 2, and the description for each term in the models was same as that for Model 1 and Model 2. The phenotypic and genetic correlations of BW/ SS_{50} and ST/ SS_{50} were calculated using bivariate analysis of ASReml package (Gilmour et al., 2009). The SS_{50} was set as the first variable, which has the same model as the univariate Model 3. BW and ST were set as the second variable, respectively, with the same fixed effects and covariate of Model 1 and Model 2. The description for each term of the models was same as that for Model 1, Model 2 and Model 3.

The Z-score was used for testing whether the heritability estimates were significantly different from each other and whether the heritability estimates and genetic correlations were significantly different from zero (Nguyen et al., 2007).

$$Z = \frac{x_i + x_j}{\sqrt{\sigma_i^2 + b_j^i}},$$

where x_i and x_j are the heritability estimates from different models or genetic correlations; σ_i^2 and σ_j^2 are their respective standard errors. Both x_j and σ_j were set to be zero when testing whether an estimate was significantly different from zero. The resulting z-score was then tested against a large sample normal distribution.

3 RESULT

3.1 Descriptive statistics

A total of 1 849 individuals' records were obtained and analyzed in the present study, and 23 individuals were lost, which might be the result of cannibalism during the experiment process. The numbers of the samples, simple means, minimum and maximum, standard deviation and coefficient of variation for BW, ST and survival rate for each family at half lethal time (SR_{50}) are given in Table 1. The means of BW, ST and SR_{50} were 3.13 g, 157.66 h and 50.03%, respectively. The SR_{50} of each family at half lethal time had the highest coefficient of variation, followed by the BW and lowest in the ST. During the high

Table 1 Number of samples/families (*n*), mean, minimum, maximum, standard deviation, and coefficient variation for BW, ST and SR$_{50}$

Traits	*n*	Mean	Minimum	Maximum	Standard deviation	Coefficient variation (%)
BW (g)	1 849	3.13	0.98	6.59	0.83	26.52
ST (h)	1 849	157.66	65	218	33.09	20.99
SR$_{50}$ (%)	52	50.03	20.51	87.5	19.38	31.82

Table 2 Variance components, heritability for BW, ST and SS$_{50}$

Traits	Model	Variance components			Heritability
		σ_a^2 (σ_{sd}^2)	σ_e^2	σ_p^2	$h^2 \pm SE$
BW	1	0.194 8	0.489 8	0.684 6	0.284 6±0.067 6*
ST	2	59.611 2	326.365	385.98	0.154 4±0.044 6*
SS$_{50}$	3	0.065 5	1.000 0	1.131 0	0.147 5±0.040 0*

* Estimate is very significantly different from zero (*P*<0.01); variance component terms are as follows: σ_a^2 is the additive genetic variance; σ_{sd}^2 is the mean of sire and dam variance; σ_e^2 is the random residual error variance; σ_p^2 is the phenotypic variance.

ammonia level challenge, mortality started gradually at 65 h, but increased dramatically at approximately 90 h and complete mortality occurred at 218 h (Fig.2).

3.2 Variance components and heritability for BW, ST and SS$_{50}$

Estimates of variance components, heritability for BW, ST and SS$_{50}$ and their standard errors are given in Table 2. The heritabilities of BW, ST and SS$_{50}$ under high ammonia level challenge were 0.284 6±0.067 6, 0.154 4±0.044 6 and 0.147 5±0.040 0, respectively, and they were all significantly different from zero (*P*<0.01). Moreover, the heritabilities of ST and SS$_{50}$, which indicated the resistance to high ammonia, were not significantly different from each other.

3.3 Correlation analysis among BW, ST and SS$_{50}$

The phenotypic correlation and genetic correlation among BW, ST and SS50 are given in Table 3. There were significant positive phenotypic and genetic correlations between ST and SS$_{50}$ (0.784 7±0.176 2 and 0.865 1±0.232 4 respectively, *P*<0.01)). A significant positive phenotypic correlation was also shown between BW and resistance to ammonia (0.427 3±0.133 5 and 0.234 0±0.024 7 for BW/SS$_{50}$ and BW/ST, respectively). The genetic correlations between them were even stronger (0.427 6±0.165 9 for BW/SS$_{50}$ and 0.827 2±0.121 6 for BW/ST). These results indicated moderate to high positive correlations

Table 3 Correlation analysis based on phenotypic value and breeding value among three traits

Traits	SS$_{50}$	ST	BW
SS$_{50}$	1	0.784 7±0.176 2**	0.427 3±0.133 5**
ST	0.865 1±0.232 4**	1	0.234 0±0.024 7**
BW	0.427 6±0.165 9**	0.827 2±0.121 6**	1

** Estimate was very significantly different from zero (*P*<0.01); phenotypic correlations were above the diagonal, genetic correlation were under the diagonal.
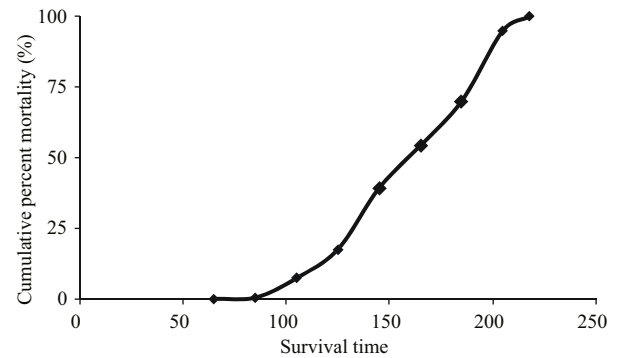

Fig.2 Cumulative mortality of *L. vannamei* at different times during the high ammonia level challenge

between the resistance to ammonia and BW.

4 DISCUSSION

Half lethal condition appeared at approximately 150 h under the ammonia concentrate of 62.23 mg/L in the present study. It showed a fairly higher resistance to ammonia than that of previous studies in *L. vannamei* and *F. chinensis* (Sun et al., 2002; He et al., 2008). This might be a result of the different temperature, salinity and post-larval age used in different experiments. It has been proven in many studies that a decrease in temperature and an increase in age and salinity improved the resistance of penaeids to the toxic effects of ammonia, and the effect also depended on different species (Young-Lai et al., 1991; Lin et al., 1993; Frías-Espericueta et al., 1999; Racotta and Hernández-Herrera, 2000; Kir et al., 2004).

Although the biochemical and metabolic response to ammonia toxicity has been studied for shrimp species, differences between individual and population capabilities to survive after toxic ammonia events have not been studied. Estimates of heritabilities for ammonia tolerance of *L. vannamei* using ST and SS$_{50}$ were 0.154 4±0.044 6 and 0.147 5±0.040 0, respectively. Those are much lower than the values of TSV resistance in this species (0.28±0.14), tolerance to low-oxygen (0.23) and low-pH (0.23) in *Larimichthys crocea* (Argue et al., 2002; Wang et al.,

2009), but is similar to those of cold tolerance (0.08±0.17) in *Oreochromis niloticus*, upper thermal tolerance (0.026±0.034) in *Scophthalmus maximus* and WSSV resistance (0.14±0.12) in *Fenneropenaeus chinensis* (Charo-Karisa et al., 2005; Liu et al., 2011; Kong et al., 2012).The common environment effect was omitted from the generalized linear mixed model in the present study, because the juveniles were with extremely short communal rearing period and lack of gene ties (only 25 half-sib families in 52 full-sib families), which may have contributed to the fact that the common environment variances could not be partitioned effectively (Gitterle et al., 2005a, b; Krishna et al., 2011; Luan et al., 2012). Thus, the heritability estimates might be overestimated with a confounding effect between the additive and non-additive genetic and maternal effects included in the c^2 effect (Pante et al., 2002; Rutten et al., 2005; Castillo-Juárez et al., 2007; Nguyen et al., 2007). Therefore, accurate estimates of heritability for ammonia might be lower than the results obtained in the present study and a more precise estimate should be obtained to enhance the accuracy of breeding.

Resistance to ammonia as a trait was described in two ways in the present study: ST and SS_{50}. The estimates obtained using ST and SS_{50} were basically the same, and the *Z*-test also showed that they were not significantly different from each other. There were also high positive phenotypic and genetic correlations (nearly equal to 1) between them, indicating a strong relationship between the two descriptors of resistance to ammonia. All of these results suggested that both the ST and SS_{50} could be used as suitable descriptors of resistance to ammonia. More research is needed to confirm this conclusion, and once it is confirmed, SS_{50} may have widespread applications in select breeding programs of tolerance or resistance traits, as this would not only save resources and improve work efficiency but also keep the individuals with high tolerance or resistance alive so that they can be used as parent shrimps.

The effect of size on tolerance traits has been reported in this species. It showed a negative correlation between TSV resistance, WSSV resistance and the body weight of *L. vannamei* in early studies (Argue et al., 2002; Gitterle et al., 2005a, b). However, there showed a strong positive correlation between resistance to ammonia and body weight in our research, which is consistent with the result that resistance to ammonia increases with ontogenetic development (Barajas et al., 2006). This means that

resistance to ammonia can be enhanced by the improvement of husbandry practices that increase body weight. The results from the present study suggest that the selection for higher body weight does not have any negative consequences for resistance to ammonia.

In addition to quantitative genetics, tools from molecular genetics, such as gene clone and RNA-sequencing, which can help us to find the genes that play crucial roles in ammonia tolerance, can be applied to selective breeding programs to improve the efficiency of selection for traits with low heritability (Moore et al., 1999). To date, several genes might play crucial roles in stress tolerance, for example, heat shock proteins genes (HSPs) (Parsell and Lindquist, 1993), superoxide dismutase (SOD) (Lynch and Kuramitsu, 2000), metallothionein (MT) (Coyle et al., 2002) and cytochrome P450 (Anzenbacher and Anzenbacherová, 2001) and Aquaporin (AQP) (Peaydee et al., 2014) have been studied and some of them were proved can help shrimps to resist ammonia stress. With the development of RNA-sequencing, more and more ammonia resistant related genes and molecular markers will be identified and applied to shrimps selective breeding programs of improving the resistance to ammonia.

5 CONCLUSION

Overall, the present results indicate that the heritability estimate for ammonia resistance was low in *L. vannamei* juveniles, but it was significantly different from zero. Consequently, genetic gain for ammonia resistance still could be obtained in future by increasing the selection intensity in our selective breeding program. In addition, it has strongly positive correlation between ammonia resistance and body weight, suggesting that ammonia resistance and body weight could be improved simultaneously in our breeding program. Moreover, heritability estimates for ammonia resistance at different growth stages and genetic correlation with other economic traits should be investigated in the future studies.

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