



Effect of inbreeding on survival, WSSV tolerance and growth at the postlarval stage of experimental full-sibling inbred populations of the Chinese shrimp *Fenneropenaeus chinensis*

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ABSTRACT

Inbreeding effects on growth, survival and anti-WSSV (White Spot Syndrome Virus) traits were studied in experimental full-sibling inbred populations of Chinese shrimp *Fenneropenaeus chinensis* at three levels of inbreeding coefficient ($F = 0.25$, $F = 0.375$, $F = 0.50$) from a Chinese shrimp selection program. The body weight of the control group ($F \approx 0.00$) was significantly different from those of the three levels of inbreeding groups at three postlarval stages (PL₈₀, PL₁₀₀ and PL₁₄₀). At PL₁₄₀, an increasing inbreeding depression of growth was observed with increasing inbreeding coefficient. The inbreeding depression was found to be -10.4% at $F = 0.25$, -16.61% at $F = 0.375$ and -23.68% at $F = 0.50$. There was also a tendency for inbreeding depression for body weight to increase with age. The estimated coefficient of body weight of PL₁₄₀ were -4.16% , -4.43% and -4.74% per 10% increase of inbreeding coefficient of $F = 0.25$, $F = 0.375$, $F = 0.50$, respectively. During growout, inbreeding depression of survival ranged from -0.98% to -12.44% , although the effects were not significant. Over all levels of inbreeding the average inbreeding depression was found to be -5.95% for survival of PL₈₀, -5.51% for PL₁₀₀ and -6.71% for PL₁₄₀. The estimated average inbreeding depression coefficient of survival ranged from -1.27% to -1.77% per 10% increase of inbreeding coefficient of F , lower than the estimate obtained for growth. The effects of inbreeding on survival time after challenge with White Spot Syndrome Virus (WSSV) were not significant, as there was no significant difference in survivorship among the control and the three levels of inbreeding coefficient groups. The inbreeding depression of the three levels ranged from -0.68% to -2.22% , and the estimated inbreeding depression coefficient of survival time ranged from -0.27% to -0.44% per 10% increase of inbreeding coefficient of F . This study demonstrates that inbreeding has a negative effect on the economic traits, especially on growth, thus highlighting the need to maximize the genetic diversity in selective breeding programs.

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1. Introduction

Chinese shrimp, *Fenneropenaeus chinensis*, is mostly distributed in the Yellow Sea, Bohai Sea in China and the west coast of the Korean Peninsula (Liu, 1959). It is an important marine aquaculture species in China (Wang et al., 2006). With the demand for shrimp products growing, there is a need to dramatically increase the supply. Since many natural shrimp stocks are declining, increased production must come from aquaculture. The approach to sustain long-term shrimp industry development is to develop selected breeds and carry out genetic improvement for important traits. Through selection of performance traits such as growth, survival and disease resistance, selective breeding programs can make shrimp much more adaptive to commercial culture conditions and meet the needs of the breeders.

With the development of breeding technologies, numerous selective breeding programs for penaeid shrimp have been initiated over the last decade (Argue et al., 2002; Clifford and Preston, 2006; Clifford et al., 2003; Dixon et al., 2008; Li et al., 2007; Moss et al., 2005; Sellars et al., 2012; Wyban, 2000). However, shrimp farming is still in the early stages of selective breeding compared to terrestrial farming (Thodesen et al., 2005).

Inbreeding is referring to situations in which relatives are mated and the homozygosity of genes in a population is being increased. Inbreeding is unavoidable in captured populations because of their restricted population size, and can lead to decrease in genetic variation of the population and the subsequent depression of performance traits (Bierne et al., 2000; Falconer and Mackay, 1996). The inbreeding coefficient (F) is a measure of inbreeding and can be defined as the probability that two alleles at any given locus carried by an individual are derived from an identical ancestral gene (Falconer and Mackay, 1996). Inbreeding depression is the effect of inbreeding measured as the decline in performance of a quantitative trait (Falconer and Mackay, 1996; Lynch and

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Walsh, 1998; Miller, 1994). Inbreeding will usually reduce the performance of shrimp and other aquaculture species, especially if the accumulation of inbreeding happens too fast (Evans et al., 2004; Pante et al., 2001a; Rye and Mao, 1998). Additionally, the magnitude of inbreeding depression may vary considerably depending on the species and the trait examined (Bentsen and Olesen, 2002; Bondari and Dunham, 1987). Therefore, there is a need to assess the effect of inbreeding on the production traits (Pante et al., 2001b).

F. chinensis have been selectively bred for at least ten generations (Wang et al., 2010). Zhang et al. (2008) demonstrated an improved growth of 13% in response to selection in a concrete pond environment over one generation. Although inbreeding was minimized by ensuring that no closely related animals were allowed to mate with each other, the accumulation of inbreeding was inevitable.

The present study examines the effects of different levels of inbreeding on growth and survival, and the amount of inbreeding depression for the growth, survivorship and disease resistant traits in the Chinese shrimp *F. chinensis* for inclusion in the selective breeding programs of the future.

2. Materials and methods

2.1. Populations under investigation

The Yellow Sea Fisheries Research Institute (YSFRI) has developed a new highly efficient breed of Chinese shrimp *F. chinensis* through several generations of selective breeding that combines favorable traits in growth rate, survivorship and White Spot Syndrome Virus (WSSV) resistance. In 2004, a base population was established using animals from two domesticated breeds developed by YSFRI and animals originated from a wild population in South Korea and three wild populations in China. There are complete pedigree records for the breeding population. In order to restrict the accumulation of inbreeding (goal of <1% increase/generation), no closely related animals are allowed to mate with each other.

2.2. Full-sibling mating and control group

Full-sibling (brother–sister) matings were used to produce inbred groups from families of the breeding population. Each year, full-sibling families of the breeding population were mated to obtain their offspring (F1 generation) by artificial insemination. In the second year, full-sibling families of F1 generation were mated to obtain their offspring (F2 generation). In the third year, F3 generations were obtained. By repeating the process over three successive generations, three groups with different levels of inbreeding coefficient ($F = 0.25$, $F = 0.375$, and $F = 0.50$) were obtained in the third year. Control group was established in the third year by strict mating of males and females sampled from base population of known pedigree, and all had an average inbreeding coefficient (F) of below 1%. In an attempt to eliminate the effects of spawning age and the common environmental effects on trait so as to increase the precision of the estimation, families spawned on the same day were chosen, and the number of families established for each level of inbreeding and control group was 8, 7, 8 and 9, respectively.

A standardized procedure for family production was used in larvae rearing. Each inbred full-sibling and control group of fertilized eggs were hatched in separate tanks. The eggs hatched after about 2 days, and a random sample of about 3000 nauplii from each inbred full-sibling family and control group were stocked in separate larval-culture tanks. Hatched larvae passed through six nauplius stages, three zoea stages, three mysis stages and metamorphosed to postlarvae in 3 weeks. A random sample of about 200 post-larvae from each family was transferred into separate larger tanks (3 m³) for rearing. After 4 weeks, shrimp from each family were tagged using a visible implant

elastomer (VIE). At the termination of each trial, shrimp were identified (by family) from the tag codes, counted, and individually weighed.

2.3. Trait measurement

2.3.1. Growth and survival

As soon as the shrimp had been tagged at the age of 60 days (PL₆₀), a random sample of 50 tagged test shrimp per family was stocked in each of the two 100-m² concrete ponds located at the YSFRI breeding station. We assessed the growth of the animals by recording the body weight of all individuals alive, when the animals had reached post-larval ages of 80 days (PL₈₀), 100 days (PL₁₀₀), and 140 days (PL₁₄₀). Survival rate was also assessed during each of the three grow-out stages by dividing the number of shrimp stocked in each concrete pond at the beginning and the number found alive at the three grow-out stages.

2.3.2. WSSV-resistance

When individuals reached a body size suitable for VIE tagging (average 2.0 g), a total of 1600 animals, including random sample of 50 tagged test shrimp from each inbred family and control family was challenged with White Spot Syndrome Virus (WSSV) in a common biosecure test environment. All juveniles of every family were distributed evenly into two 20 m² tanks after tagging. The water temperature ranged from 27 to 29 °C, and salinity was 29‰. Viral-challenge was measured by survival time of tagged shrimp after being infected with a proportional amount of WSSV relative to their body weight. After starving the shrimp for 24 h, each shrimp was fed with 10⁴ copies of WSSV g⁻¹ body weight, and dead shrimp were recorded at 30-min intervals after the infection. After oral infection, the two tanks were maintained as usual, including exchanging water once a day, feeding shrimp three times a day.

2.4. Analysis

For body weight and survival time challenged with WSSV, the following mixed linear mixed model was fitted to estimate the least square means of the inbreeding and control groups by the MIXED procedure in the SAS software.

$$y_{ijkl} = \mu + \text{Group}_i + \text{Tank}_j + \text{Age}_l + \text{Family}_k(\text{Group}_i) + e_{ijkl}$$

Where y_{ijkl} is the observed value of the l th shrimp of the inbreeding or control groups; μ is the overall mean; Group_i is the fixed effect of the i th group (inbreeding and control groups); Tank_j is the fixed effect of the j th tank (two tanks); Age_l is the covariates of age from the l th shrimp; $\text{Family}_k(\text{Group}_i)$ is the random effect of the k th family nested within the i th group; and e_{ijkl} is the random residual error of the l th individual.

For survival, the following generalized linear mixed model was fitted to estimate the marginal means of the inbreeding and control groups by the GLMM procedure, in the SAS software.

$$\Pr(y_{ijkl} = 1) = \Pr(\lambda_{ijkl} > 0) = \Phi(\mu + \text{Group}_i + \text{Tank}_j + \text{Family}_k(\text{Group}_i))$$

Where y_{ijk} is survival status (1 = alive, 0 = dead) of the i th shrimp; λ_{ijkl} is the underlying liability of y_{ijkl} , assumed to be cumulative standard normal distribution (Φ); μ is the overall mean; Group_i is the fixed effect of the i th group (inbreeding and control populations); Tank_j is the fixed effect of the j th tank (two tanks); $\text{Sire}_k(\text{Group}_i)$ is the random effect of the k th family nested within the i th group.

Statistical comparisons were assessed using the least significant difference (LSD) multiple comparison test.

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