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Growth performance and white spot syndrome virus resistance in families of kuruma shrimp (*Marsupenaeus japonicus)*

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Present study reveals the variation in wet weight at harvest (4 months in pond) and resistance to white spot syndrome virus (WSSV) in the Kuruma shrimp *Marsupenaeus japonicus*. Eight families obtained from gravid female shrimp from wild and two inbred families were used. Representatives from each family were challenged with White Spot Syndrome virus. Survival curves in a challenge experiment revealed no difference between wild and inbred families. However, one family each from wild and inbred had higher median survival compared to the rest. Analysis of the wet weight at harvest revealed sexual dimorphism for growth with females weighing heavier than their male counterparts. Heritability was estimated sex-wise for harvest weight using full-sib correlation method. The estimates indicate that harvest weight would respond readily to selection.

[**Keywords:** Kuruma shrimp, heritability, White Spot Syndrome virus]

Introduction

The Kuruma shrimp-*Marsupenaeus japonicus* is fast gaining importance as a species that holds a lot of potential in shrimp production. The shrimp are exported live and a transit time of upto 36 hours has been reported[1]. Kuruma shrimp command prices of $ 180 a kg (live) in Japan where they are considered the Champagne of Prawns[2]. A selection programme in this species was started in late nineties in Australia[3]. This programme is now at generation 10 and is executed by the private shrimp industry[4]. There is a report indicating that resistance to White Spot Syndrome virus in Kuruma shrimp developed 3 or 4 weeks after exposure to the virus and it persisted for another month at a temperature of 24°C[5].

In India, there is very little information regarding the performance of Kuruma shrimp especially the culture aspects. In the CIBA hatchery, during 2003, both the species viz. tiger shrimp and inbred kuruma shrimp were being maintained for the usual experiments. However, a severe infection of WSSV resulted in the mortality of all the tiger shrimp. Surprisingly, it was observed that the kuruma shrimp maintained in the same room were not affected by this disease and no mortality was reported. We wanted to ascertain whether this was due to the kuruma shrimp resisting the infection. To test this hypothesis, an experiment was planned to study the resistance of inbred stock and wild kuruma shrimps to White Spot Syndrome virus (WSSV) as also their growth performance in pond conditions.

Materials and Methods

Collection of wild gravid adult females

Eight gravid female Kuruma shrimp were caught off the Chennai coast in November/December 2006 and brought to the Institute hatchery at Muttukadu, situated 30 kms away from Chennai. The shrimp were conditioned in the hatchery and maintained in aerated sea water having a salinity of more than 30 ppt. Daily in the morning, water exchange was carried out to the extent of 80% and live polychaete worms provided. Later during the day, squid meat and in the night, fresh clam meat was provided. All the eight females spawned and the spawns were collected and kept family-wise in 200 l FRP tanks. At the post larval stage, samples from each family were collected and checked for WSSV through PCR. All samples tested negative. The progeny from these 8 dam families were utilized for the experiment.

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At CIBA, an inbred stock was being maintained by breeding full-sibs from a single family for 7 generations. In each generation, the matings were performed between the full-sibs. Two families from two matings of the inbred stock were chosen for performance comparison. These two inbred families were also kept for larviculture and subsequently used for the challenge test experiment as also the culture trial.

In the second fortnight of January 2007, approximately one thousand post larvae per family were randomly selected and distributed into two 500 l FRP tanks. Each tank therefore contained 500 post larvae. The age of the post larvae at stocking ranged from PL 4 to PL 28. A layer of clean sand was provided at the bottom of the tank. The larviculture entailed daily exchange of sea water @ 20%. The sea water was initially passed through a 5 µ 20 inches candle filter and thereafter sterilized through a UV unit before being poured into the tanks. Continuous aeration was provided. Formalin treatment was given once a fortnight @ 50 ppm for 30 minutes. Once a week, the tank was cleaned thoroughly after removing the water and sand completely. A commercial feed for post larvae was provided 6 times daily at an interval of 4 hours. Water quality parameters like salinity, pH and temperature were monitored and recorded regularly.

The juveniles were sampled in February and May 2007. In the month of August, the juveniles were collected and tagged with visible implant elastomer tags. Thus, the post larvae had spent approximately about 7 months in FRP tanks. Before tagging, each shrimp was weighed in an electric balance and wet weight recorded. Four colours viz. red, green blue and orange were used in different combinations. The dorsal, ventral left and ventral right portions of the sixth abdominal segment were used for injecting the dye using a tagging equipment[6]. About 12 tagged shrimps from each family were randomly selected and transported to the wet laboratory of CIBA, Santhome for carrying out the challenge test experiment. The rest of the tagged shrimp (408 nos) were stocked into a pond for monitoring growth.

The challenge test was initiated by estimating the LD_{50} dose of the White Spot Syndrome Virus using the method of Reed and Muench[7]. The WSSV virus stock for the challenge experiment was prepared from the tissues of WSSV infected *Penaeus monodon* stored in -20°C. The infected tissue was minced and centrifuged. The supernatant was collected, filtered and confirmed for the presence of virus by PCR which resulted in an amplified product of 640 bp. This virus stock was subjected to 10 fold dilutions. An amount of 0.1 mL of the virus dilutions in the range of 10^{-1} to 10^{-4} was inoculated by intramuscular route into *Penaeus monodon* of 2-3 g size. From this experiment, a dilution of $10^{-3.41}$ was obtained as LD_{50} dose. This virus dilution was used for the subsequent challenge experiment carried out in 8 wild and 2 inbred families of Kuruma Shrimp. A tagged family from wild served as a negative control. The challenge test was carried out in the wet laboratory on 12 shrimp per family distributed into two replicates of 6 each. The virus was injected intramuscularly using a sterile disposable syringe. The challenged shrimp were housed in 6 lt plastic containers supplied with pressure sand filtered sea water and continuous aeration. The experiment was terminated when no continuous mortality was observed for 5 days.

In the first fortnight of August 2007, the tagged shrimp of all the ten families were stocked in a pond of size 600 m^2. The growth in pond was monitored from DOC 40 when sampling was carried out for the first time. Thereafter, sampling was carried out regularly once a fortnight. A commercial feed was provided to the shrimp four times in a day. The water quality parameters were also recorded.

In January 2008, the entire tagged shrimp in the pond were harvested and wet weight of each shrimp recorded (Table 1 & 2). It was possible to obtain individuals from all the 8 wild and 2 inbred families. The number of adults retrieved ranged from 6 to 33 in the eight wild families, and 5 & 7 in the two inbred families respectively (Table 3).

Statistical Analyses

The means and standard errors were computed using standard statistical procedures[8].

Table 1—Average wet weight at tagging in different families (200 DOC)

Family	Mean ± SE (g)	N	CV (%)
1	5.43 ± 0.29	25	27.07
2	4.24 ± 0.25	41	37.48
3	4.94 ± 0.28	30	31.58
4	4.97 ± 0.28	31	30.84
5	6.42 ± 0.33	34	29.72
6	5.46 ± 0.26	37	28.49
7	4.63 ± 0.13	97	27.41
8	3.23 ± 0.14	57	32.54
9*	2.54 ± 0.28	62	54.72
10*	2.00 ± 0.21	102	43.91

*inbred stocks

Table 2—Descriptive statistics of harvest data

Number of observations	115
Number of families	9
Average family size	13
Body Weight	
Mean (g)	25.93
Standard Deviation	5.13
Standard error	0.48
Coefficient of Variation (%)	19.80

Table 3—Least-squares means of body weight at harvest

Effect	Body weight ± SE (n)
Sex (P<0.001)	
Male	21.79 ± 0.46 (53)
Female	29.47 ± 0.48 (62)
Family	
F1	25.65 ± 1.10 (6)
F2	25.64 ± 1.00 (6)
F3	24.66 ± 0.92 (6)
F4	23.84 ± 1.44 (3)
F5	25.45 ± 0.79 (11)
F6	27.41 ± 0.78 (12)
F7	24.94 ± 0.48 (33)
F8	25.30 ± 0.52 (29)
F9	27.07 ± 0.97 (7)
F10	26.32 ± 0.96 (5)

Kaplan-Meier survival curves for different families were compared using Mantel-Cox test in *GraphPad Prism 5*[9]. The wet weight at harvest was analysed using a general linear model in ASReml[10] using the syntax given in appendix.

The linear model equation in matrix form is:

$$y = Xb + Zu + e$$

where y is a 115 × 1 observation vector

b is a 2 × 1 vector of 2 levels of fixed effects male and female

u is a 10 × 1 vector of 10 levels of random effects (families)

e is a 115 × 1 vector of random residual terms

X is a known design matrix of order 115 × 2 which relates to the records in y to the fixed effects of each sex

Z is a known design matrix of order 115 × 10 which relates to the records in y to the random effects in families

Results

Stocking weight

The weight at tagging ranged from 3.23 to 6.42g in the families from wild whereas the inbred stocks weighed 2.00 and 2.54 g. The coefficient of variation ranged from 27.07 to 54.72%. During larviculture in FRP tanks, the water temperature ranged from 28.3° to 31.1°C; the pH ranged from 7.54 to 8.14 and the salinity ranged from 18 to 31.45‰.

Challenge test studies

It was observed that 100% mortality in the WSSV inoculated families occurred between 71-159 hrs post inoculation. No significant difference in the mortality rate was observed between the wild and inbred stocks. In the inbred families, 100% mortality was observed within 135 hrs post inoculation. No mortality was observed in the control. Screening of WSSV challenged shrimp showed 36% samples to be Ist step PCR positive. An interesting observation was that one wild family (F 6) and one inbred family (F 10) had one and two survivors respectively at the termination of the experiment. When a PCR was carried out for checking WSSV in these three shrimp, they tested negative.

For survival analysis, the mortality data was recorded as 0 (live) and 1 (dead). The survival of families from wild and inbred was compared and it was observed that there was no significant difference between the wild and inbred stock as far as the resistance to WSSV was concerned. The Kaplan-Meier survival curves are depicted in Figure 1.

The median survival was 61.5 hours in most of the wild and one inbred family. One family (F 6) exhibited a median survival of 63 whereas one wild family (F 10) exhibited a median survival of 85.5. These two families had a higher survival time compared to the rest of the families.

Harvest after 153 DOC

The stocking density in the pond was 0.68 m^2 and the survival from stocking to harvest was about 34% which is quite low. A total of 118 tagged shrimp from

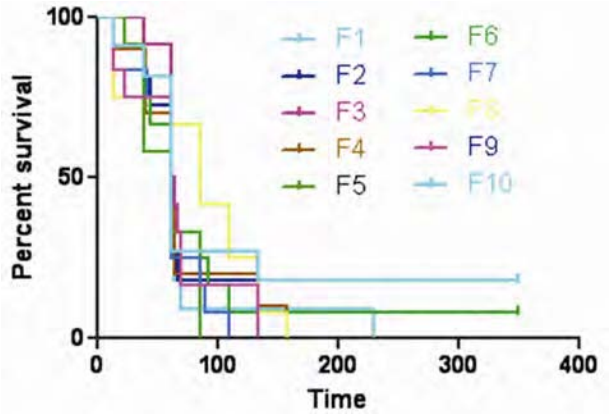

Figure 1—Kaplan-Meier Survival Curve comparing wild families and inbred families

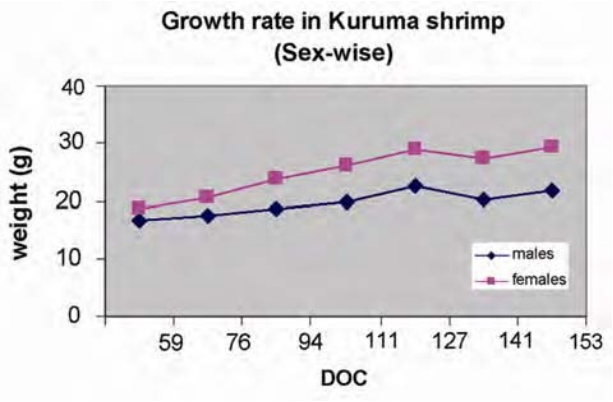

Figure 2—Growth of kuruma shrimp (sex-wise)

all the 10 families and 21 tagged shrimp (from a family which was used as a control for the challenge-test) were retrieved from the pond during harvest. The average wet weight in the two sexes over the period of sampling has been depicted graphically in Figure 2. During the culture period in pond, the water temperature ranged from 27.8° to 31.7°C; the pH ranged from 7.44 to 8.12 and the salinity ranged from 19.3 to 28.4‰. Whenever there was a drop in pH, lime was added to enhance the pH.

Discussion

A perusal of weight at tagging of the juveniles reveal that the inbred stock weighed less (2.00 and 2.54 g) compared to the individuals in other families (3.23 to 6.42 g). This could probably be due to the effect of inbreeding. The post larvae had been maintained in 500 l FRP tanks for about 7 months. At tagging, the weights ranged from 2.0 g (inbred) to 6.42 g in the juveniles (from wild families) and the coefficient of variation for wet weight ranged from 27.07 to 54.72% which clearly indicated that this trait is amenable to selection.

Regarding the resistance of the juveniles to WSSV, there appeared to be no difference between the inbred and wild families. However, two wild families exhibited longer survival values (63.0 and 85.5 hrs). Also, one shrimp from an inbred family and two from another wild family survived the challenge test after the experiment was terminated, and they were checked for the presence of WSSV by PCR. The results were negative for the presence of WSSV from these two shrimp. This could possibly be due to the shrimp developing resistance to the virus after an initial exposure. Wu *et al.*[5] (2002), challenged Kuruma shrimp with WSSV in an effort to study the onset and duration of resistance in experimental survivors. The shrimp were again re-challenged at 1-4 weeks and 1-3 months post initial exposure (PIE). Among the survivors, 10% of them sampled on the third day and after one month PIE exhibited presence of virus, but all survivors sampled after second and third month PIE tested negative through nested-PCR. Flegel and Pasharawipas[11] (1998) hypothesized that tiger shrimp which survive an initial infection to WSSV were found to have 'tolerance' to the virus, exhibited high virus titres, and the authors termed this phenomenon as 'viral accommodation'. However, Wu *et al.*[5] (2002) opined that the resistance observed in their experiment could not be attributed to this phenomenon as the survivors from a re-challenge conducted at 30 days PIE did not exhibit high virus titres. The authors further observed that resistance to WSSV developed 3 or 4 weeks after exposure and this persisted for another month at 24°C.

The mean wet weight at harvest was 25.93 g with a coefficient of variation of 19.80%. The wet weight is comparable to that reported by Preston *et al.*[3] (1999), who reported an average wet weight of 25 g at harvest. The value of coefficient of variation is comparable to that reported by Gjedrem[12] (1997), who reported values ranging from 20 to 35% in fish and shellfish. This value of coefficient of variation for growth rate indicates that this trait is amenable to selection and if selected for, would respond readily. The average harvest weight in inbred families was 27.68g compared to 25.73 g in wild families. However, it is difficult to draw any conclusion from this due to low number of observations in the inbred families. In general, the female shrimp were heavier than their male counterparts indicating sexual dimorphism in growth. (Figure 2). Such a dimorphism has been reported in penaeid shrimp[13-17]. Gopal *et al.*[15] (2010), in their study observed that the weight of tiger shrimp at the onset of sexual dimorphism was 15-20 g in pond culture. In the present study too, a similar pattern was observed.

The estimate of heritability for harvest weight in males was 0.80 ± 0.36 whereas in females it was 0.32 ± 0.23. The standard error of the estimate for females is on the higher side indicating that this estimate has to be used with caution. However, the estimate from males was high indicating that wet weight would readily respond to selection. Hetzel *et al.*[18] (2000) estimated the heritability by regressing the offspring mean on mid-parent mean of 6 month body weight in Kuruma shrimp (0.27 ± 0.08). The

realised heritability in their study ranged from 0.16 to 0.31 and averaged 0.26. In the present study, our estimates are towards the higher side due to the fact that the full-sib estimates may be biased due to maternal and dominance effects.

In the present study, an effort was made to ascertain the possibility of using Kuruma shrimp for a selection programme targeting growth and resistance to WSSV. The heritability of harvest weight indicates that growth could be a trait which is amenable to selection. There appears to be no difference between the wild and inbred stock for resistance to WSSV. However the longer survival times after WSSV challenge in two families and the absence of WSSV in two survivors, indicate that *M. japonicus* has some innate capacity to survive WSSV. However, further studies are warranted to find out whether resistance to WSSV is under genetic control amenable for family based selection.

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