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Evaluation of Growth Performance in Cyclically Mated Populations of Malaysian Giant Prawn, *Macrobrachium rosenbergii*, in Malaysia

(Penilaian Prestasi Tumbesaran secara Klinikal Populasi Ternakan Udang Galah Malaysia, *Macrobrachium rosenbergii*, di Malaysia)

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ABSTRACT

Malaysian giant prawn, *Macrobrachium rosenbergii* (de Man, 1879), is an important crustacean aquaculture candidate globally cultured chiefly in hot climates and seasonally in temperate zones. Although the first hatchery for artificial production of the species was established in Malaysia in 1984, however, its genetic improvement program has just started recently. This study was executed as a part of this improvement project to evaluate morphometric growth related traits of four populations collected from the Western Coast of Peninsular Malaysia and their progenies. The results of the study disclosed significant differences among these populations for all traits that were considered; with Kedah population showing significant ($p < .05$) superiority over the rest. Within the eight successfully cohort progeny families produced, Kedah progeny group was the heaviest at the same level of significance as that of parental groups. Fixed effects of family, sex and their interactions showed high significance ($p < .0001$). Estimated heritability values for the traits mentioned above were moderate to high; i.e. 0.5788 ± 0.2989 , 0.5156 ± 0.2753 , 0.4277 ± 0.2407 and 0.5120 ± 0.2739 for weight, total length, tail length and carapace length, respectively. All mentioned results encourage us to un-hesitantly recommend selection for these traits in future genetic improvement of this species.

Keywords: Cyclical mating; heritability; *Macrobrachium rosenbergii*; Malaysian giant prawn; quantitative traits

ABSTRAK

Udang galah Malaysia, *Macrobrachium rosenbergii* (de Man, 1879), adalah spesies krustasea yang utama dalam akuakultur di peringkat global yang diternak terutamanya di kawasan iklim panas dan musim zon sederhana. Pusat penetasan pertama untuk spesies ini telah ditubuhkan di Malaysia pada tahun 1984, walau bagaimanapun, program penambahbaikan genetiknya baru sahaja dimulakan pada tahun 2009. Kajian yang dilakukan adalah sebahagian daripada projek penambahbaikan untuk menilai perkembangan berkaitan dengan sifat-sifat morfometri daripada empat populasi yang dikumpul dari pesisir pantai Barat Semenanjung Malaysia serta baka mereka. Hasil kajian menunjukkan terdapat perbezaan yang signifikan dalam kalangan populasi bagi semua sifat-sifat yang telah dibincangkan; dengan populasi dari Kedah menunjukkan signifikan ($p < .0001$) kelebihan berbanding yang lain. Antara lapan kelompok keturunan keluarga yang berjaya dihasilkan, sekumpulan progeni dari kedah adalah yang paling berat dengan tahap yang sama signifikan dengan induk. Anggaran nilai perwarisan untuk sifat-sifat yang disebutkan adalah dari sederhana ke tinggi; iaitu 0.5788 ± 0.2989 , 0.5156 ± 0.2753 , 0.4277 ± 0.2407 dan 0.5120 ± 0.2739 masing-masing untuk berat badan, jumlah panjang, panjang ekor dan panjang karapas. Perbezaan sifat yang signifikan yang dianggarkan dalam kalangan populasi induk dan kesan yang tetap antara sumber keluarga yang signifikannya tinggi serta kewarisan yang dianggarkan dari sederhana ke tinggi dalam penyelidikan tentang sifat, mendorong kita untuk tidak ragu-ragu mencadangkan pilihan mereka untuk penambahbaikan genetik spesies ini.

Kata kunci: Keterwarisan; kitaran pengawanan; *Macrobrachium rosenbergii*; sifat kuantitatif; udang galah Malaysia

INTRODUCTION

Malaysian Giant Prawn, *Macrobrachium rosenbergii* (de Man, 1879), which is also known as giant freshwater prawn and locally in Malaysia as udang galah, is an important aquaculture candidate farmed universally in the tropics and subtropics. Its natural distribution includes Western Asia including east coast of India, Bay of Bengal, Gulf of Thailand, Malaysia and the northern Indonesian islands of Sumatra, Java and Kalimantan (Arthur et al. 2004). Genetic

improvement of farmed fish by selection has a paramount advantage over other types of genetic manipulations such as polyploidy and sex reversal by being permanent, i.e. it is fixed in the population or strain once it is achieved (Gjedrem 2005). In general, the response to selection for growth rate in aquatic species is exceptionally high compared to that in the case of terrestrial farm animals. Specifically, genetic response for growth rate in these species with begun selective breeding programs ranged

between 10 and 20% per generation (Eknath et al. 2007; Mahapatra et al. 2006; Ponzoni et al. 2005; Quinton et al. 2005). In addition, these species possess high fecundity that allows application of narrower selection differentials in improvement programs, thus reducing time required to achieve a certain level of improvement. Moreover, economically important quantitative traits for *M. rosenbergii* were reported to be significantly different among populations or strains (Pillai et al. 2011; Thanh et al. 2010, 2009), with heritability estimates ranging from moderate to high (Dinh & Nguyen 2014; Dinh et al. 2013; Kitcharoen et al. 2012; Nugroho et al. 2005). Without such variation breeders and geneticists would not be able to make any progress in genetic improvement.

The culturing of *M. rosenbergii* as an important species under aquaculture in Malaysia has recently experienced fluctuations in production (Annual Fisheries Statistics 2013). This decline may have been caused by high postlarvae (PL) mortalities as a result of high inbreeding levels attributed to sourcing broodstock directly from grow-out ponds based on their readiness to spawn that includes early maturing small females rather than their breeding values (Bhassu 2009; Mather & De Bruyn 2003; Thanh et al. 2010). This latter practice may cause an indirect negative response on weight at harvest. These reasons lead the authors of this paper to study the variation of fresh body weight (WT), total body length (TL), carapace length (CL) and tail length (TA) in four populations of this prawn originally collected from different geographical locations in peninsular Malaysia and produce cohort progenies in order to estimate heritability values of the traits in question.

MATERIALS AND METHODS

COLLECTION AND CONDITIONING OF BROODSTOCK POPULATIONS

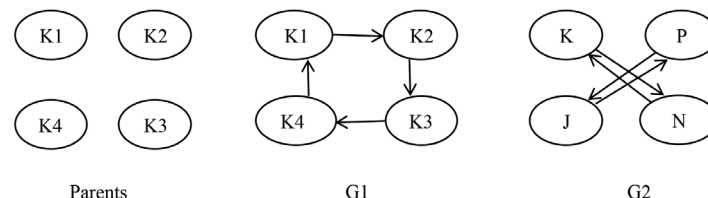
Reasonable numbers of sexually mature *M. rosenbergii* individuals were collected from four different stations of the Marine Aquaculture Institute of the Department of Fisheries (DOF), Malaysia. The stocks were designated

by their states, namely Perak (P), Kedah (K), Negeri Sembilan (N) and Johor (J). Their actual sources were Tapah (4.18° N and 101.27° E), Kota Kuala Muda (5.5833° N, 100.3833° E), Timon (2.44° N, 102.064° E) and Muar (2.58° N, 102.57° E) rivers, respectively. All stocks were transported to a hatchery belonging to the previously mentioned Institute at the National Prawn Fry Production and Research Centre (NAPFRE) located at Pulau Sayak village, (5.62° N and 100.38° E) in June, 2012. They were conditioned in 5000 L cement tanks, supplied with oxygen through a central aeration system and fed mainly on meat of blood cockle (*Anadara granosa*) or squid (*Loligo Spp*) depending upon availability in the market. Water change of 5% was practiced daily in mornings as well as 50% weekly. Economically important morphometric measurements were explicitly taken. WT was measured to the nearest gram by a digital balance (Mettler Toledo, model: B21002-S with two decimal places), while all length measurements were measured in centimeters using a 30 cm ruler.

MATING DESIGN AND REARING OF JUVENILES

Forty selected sires (fathers) and twenty dams (mothers) were chosen from each population to create four different families for each parental group (e.g. J1, J2, J3 and J4 for Johor population) in separate mating tanks according to source in order to guard against failure of PL production. The number of males was meant to be so high in order to make sure that every gravid female would be fertilized, as well as guarding against some females' preference to certain males (New et al. 2009) or many females becoming sexually attractive at the same time. The mating design applied in this study was cyclical. The principle of this design is that male progeny are mated with females of a different group from the one in which they were born. The transfer follows the pattern indicated in the diagram shown in Figure 1. Males are transferred in the directions indicated by the arrows, whereas females stay in the group where they were born. The pattern of transfer varies with the rank of the generation. This design can result in considerably less inbreeding in future generations than random mating (Ponzoni et al. 2012).

In this study we used the progeny of the first generation



* Arrows indicate males' transfer direction.

* Cyclical mating was done for each population *inter se* for the first generation by dividing it into 4 sub-groups (e.g. K1, K2, K3 and K4 for Kedah population).

* For the second generation the mating was done among the sub-groups of the populations Perak, Negeri Sembilan, Kedah and Johor (e.g. P1×J1).

* Only examples of the crosses appear in this schematic diagram.

FIGURE 1. Schematic diagram for the Cyclical mating as designed for four populations of *M. rosenbergii*

to estimate the heritability as it is pure and will not be complicated by combing abilities as the case when crossing among populations are performed (Falconer et al. 1996). Produced larvae were reared in a clear water hatchery system, fed live brine shrimp three times a day plus egg custard as a supplement from the tenth day of larval stocking onwards. This supplement composed of 9 g baby milk powder, 6 pieces of chicken eggs, 2g of a thickening agent and 300 mL of freshwater. Water quality parameters such as dissolved oxygen, water temperature and pH were monitored daily and were assured to be within the normal ranges. These ranges were 28 - 31°C, 5-7 mg/L and 7.0 - 8.5 for water temperature, DO and pH, respectively. Water change was practiced as 10% daily plus 50% weekly once. Immersed heaters of 300Watt were used to keep water temperature in the range mentioned above. Produced PL were raised up in a nursery to early juvenile stage (eight weeks) in separate 5000 L capacity tanks. During this period, feeding rate was 5% of body weight of nursed juveniles and feeding was twice a day at 0800 and 1600, respectively. Dissolved oxygen, water temperature and pH ranges were 5-7 mg/L, 28 - 31°C and 7.0 - 8.5 for DO, water temperature and pH, respectively. This was followed by stocking about 6000 to 9000 fries of 1-2 g body weight of each family cohort, that was further divided into three replicates, in grow-out ponds (1500×30×1.5 m) at Tapah village (4.183° N and 101. 267° E) and were reared separately at 15 individuals per m² and fed formulated feed of 35 percent crude protein.

PROGENY DATA COLLECTION

After 150 days in grow out, samples of 50 individuals of each of three replicates of each of the eight successful cohort families were collected. Then measurements of the same morphometric traits were taken in a similar manner as those of the parental groups described previously.

STATISTICAL ANALYSIS

The general linear model (GLM) of SAS version 9.3 (SAS Institute Inc. 2012) was used to analyze the data. Precisely, multivariate analysis of variance (MANOVA) and mixed model procedures were performed for this purpose. MANOVA procedure was applied to test for the existence of systematic effects of the populations considered that causes mean differences in the traits examined. Then Duncan's multiple- range test was applied to identify which population (s) is/are significantly different for each measured trait. The mixed model procedure was implemented to detect fixed effects of family, sex, replicate and their interactions in the eight progeny families according to the following equation:

$$Y_{ijk} = \mu + C_i + S_j + V_k + (C*S)_{ij} + (C*V)_{ik} + (S*V)_{jk} + e_{ijk},$$

where Y_{ijk} is the measurement of a morphometric body trait, μ is a constant, C_i is the fixed effect of family ($i = 1,2,3 \dots \dots 8$), S_j is the sex ($j = 1,2$), V_k is the replicate effect

($k=1,2,3$), $(C*S)_{ij}$ is the family by sex interaction, $(C*V)_{ik}$ is the family by replicate interaction, $(S*V)_{jk}$ is sex by replicate interaction and e_{ijk} is a random error which is distributed normally.

Furthermore, Duncan's multiple- range test was utilized to test the significance of differences among the groups of progeny families separately.

To estimate heritability of traits in consideration; parents were identified as a group (cohort) in the mating design. Data of the progenies were then used to evaluate their parental groups. Similar to penaeid prawns, heritability through sire side was not different from zero (Benzie et al.1997; Kitcharoen et al. 2012). This may be attributed to the absence of an agreed on correction factor for sex in the considered species as the case in cattle for instance (Chenette & Frahm 1981). Therefore, in this study, it is only estimated through maternal lineage according to Pirchner (1983) equation substituting sire by dam as shown:

$$h^2 = 4 \sigma^2D / \sigma^2D + \sigma^2w,$$

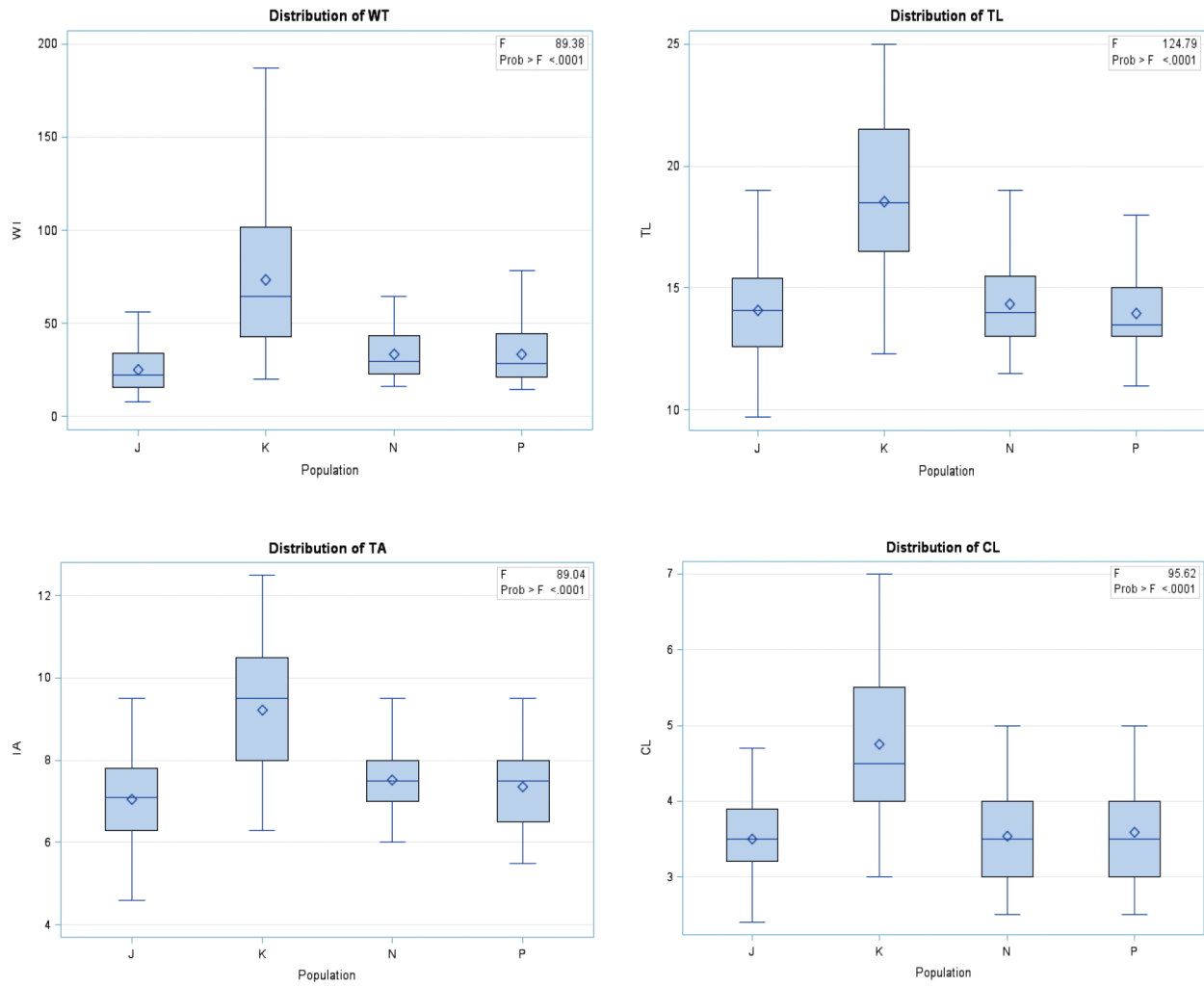
where h^2 is the heritability; σ^2D is the variance due to dams; and σ^2w is the variance within progenies produce by the same dam.

RESULTS AND DISCUSSION

QUANTITATIVE TRAITS OF PARENTAL POPULATIONS

The growth of *M. rosenbergii* is highly skewed with multimodal curves in males and normal distribution in females (Kuris et al. 1987; Nguyen et al. 2007). Males have three major distinct morphotypes: Blue claw, orange claw and small males (Ra'anan & Sagi 1985; Sagi et al. 1988). Blue claw males are socially dominant over the other two morphotypes (Barki et al. 1992) and they are mostly preferred by females for mating (New et al. 2009). This encourage the authors to use only blue claw males, because the breeding period applied was very short.

Figure (2) shows the boxplots for the studied traits in the four parental populations considered. MANOVA showed significant ($p < .0001$) differences among studied populations for all studied traits. The level of significance is always reported without zero before the decimal point according to Field (2009), for p (because it is constrained between 0 and 1). The results of Duncan's multiple range - test are displayed for combined sexed group (as an example) for the populations considered with basic descriptive statistical data on these four parental populations, viz. least square means (LSM), standard errors for males, females. As shown in Table 1, means of morphometric measurements, TL, CL, TA and WT of *M. rosenbergii* were found to differ significantly ($p < .05$) according to population source. Consistently, similar results were reported by Kitcharoen et al. (2010), Munasinghe and Thushari (2010), Pillai et al. (2011) and Thanh et al. (2009).



*Left upper= WT, right upper= TL, left lower= TA, and right lower= CL

FIGURE 2. Boxplot of WT (g), TL, TA and CL of *Macrobrachium rosenbergii* populations of Johor (J), Kedah (K), Negeri Sembilan (N) and Perak (P)

TABLE 1. LSM, standard error and Duncan’s grouping (mixed sexes) of body weight (WT), total length (TL), tail length (TA) and carapace length (CL) for female, male and mixed sexes of Kedah (K), Perak (P), Negeri Sembilan (N) and Johor (J) populations of *Macrobrachium rosenbergii*

Population	No.	LSM	WT (g)	TL (cm)	TA (cm)	CL (cm)
K	100	Female	60.762±3.951	17.996±0.275	9.070±0.138	4.404±0.082
	100	Male	106.810±4.161	19.964±0.289	9.709±0.146	5.402±0.086
	200	Mixed	82.592±3.139a	18.929±0.213a	9.373±0.105a	4.877±0.069a
P	100	Female	25.740±4.489	13.327±0.312	7.009±0.157	3.318±0.093
	100	Male	50.946±5.473	15.541±0.380	8.189±0.192	4.176±0.114
	200	Mixed	35.877±3.803b	14.217±0.258b	7.484±0.127b	3.663±0.084b
N	100	Female	30.292±3.051	14.043±0.211	7.427±0.107	3.377±0.063
	100	Male	53.036±4.805	15.917±0.334	8.121±0.168	4.292±0.100
	200	Mixed	36.829±2.823b	14.578±0.191b	7.625±0.094b	3.638±0.062b
J	100	Female	25.308±6.529	14.250±0.399	7.326±0.200	3.538±0.119
	100	Male	25.874±5.709	13.823±0.454	6.846±0.229	3.408±0.136
	200	Mixed	25.549±4.749c	14.076±0.322b	7.134±0.159c	3.498±0.105b

*a, b, and c refer to Duncan’s grouping among the four populations for traits shown at $p < .05$, *similar letters indicate insignificant difference

QUANTITATIVE TRAITS OF PROGENY GROUPS

Four growth-related quantitative traits were assessed for progenies raised in grow-out ponds. MANOVA for the traits showed significant ($p < .0001$) differences among the progeny groups studied. Table 2 displays LSM and standard errors and Duncan's grouping of these traits in the eight progeny families produced in this study, while Figure 3 represents a histogram illustrating sexual dimorphism in WT means in the above progeny groups as an example for the traits studied, as all of them follow the same pattern.

Kedah progeny group recorded the highest results for all the traits that were investigated. However, one group of Johor progenies was statistically undistinguishable ($p > .05$) from that of Kedah progeny group for both WT and CL.

This finding is similar to those obtained by Thanh et al. (2010) for WT and Kitcharoen et al. (2010) for CL. Moreover, fixed effects of family, sex and their interactions were found to be significant ($p < .001$) for all the traits, while replicate was significant ($p < .05$) only for WT. However, its interactions with family and sex were significant ($p < .001$) except for WT (Table 3), this significance could be as a result of being combined with sex or family that have very high effects. These results concur with the above comparisons among parental groups, thus illustrating genetic differences among studied populations as well

as sexual dimorphism in *M. rosenbergii*, for these traits. Similar results were reported by Pillai et al. (2011), Sethi et al. (2013) and Thanh et al. (2009).

HERITABILITY ESTIMATION

Heritability (h^2) of a trait is precisely defined as the amount of the superiority of the parents above their contemporaries which on average is passed on to the offspring (Dalton 1980). It describes the inheritance strength of the trait in question. However, it is a specific characteristic that is true for a defined group of organisms raised in a specific environment including feeding and shelter. Practically, the most important aspect behind estimating heritability for any trait is its predictive value, (Pirchner 1983) as after the occurrence of fertilization; it would be too late to change the genetic composition of the coming individual, therefore breeders can only select parents of tomorrow from the present generation. For *M. rosenbergii* families of this experiment, Table (4) shows the analysis of variance (ANOVA) of the quantitative traits of the successfully produced family groups (progenies) and their estimated heritability values.

Each estimated value for heritability for each trait as seen in Table 4, falls in the range of moderate to high. These are in line with results obtained by Dinh

TABLE 2. Least square means, standard errors and Duncan's grouping of body weight (WT), total length (TL), tail length (TA) and carapace length (CL) for the eight *Macrobrachium rosenbergii* progeny families

Family	No.	WT(g)	TL(cm)	TA(cm)	CL(cm)
P2P2×P1P1	150	14.267±0.642c	11.059±0.121c	5.435±0.061e	2.831±0.040c
J1J1×J4J4	150	19.186±0.642ab	11.754±0.121b	5.888±0.060c	3.139±0.039ab
J2J2×J1J1	150	13.056±0.609c	10.590±0.115d	5.450±0.057e	2.623±0.038d
J4J4×J3J3	150	13.165±0.636c	10.854±0.120cd	5.926±0.059bc	2.664±0.039d
P4P4×P3P3	150	13.527±0.648c	11.089±0.123c	5.693±0.061d	2.826±0.040c
K4K4×K3K3	150	20.884±0.648a	12.500±0.123a	6.427±0.061a	3.240±0.040a
N1N1×N4N4	150	18.407±0.642b	11.962±0.121b	5.969±0.060bc	2.880±0.039c
N4N4×N3N3	150	20.749±0.604a	12.074±0.114b	6.083±0.056b	3.084±0.037b

*P= Perak, J=Johor, K=Kedah and N=Negeri Sembilan. * a, b, c, d, and e refer to Duncan's grouping at $p < .05$. *similar letters indicate insignificant difference

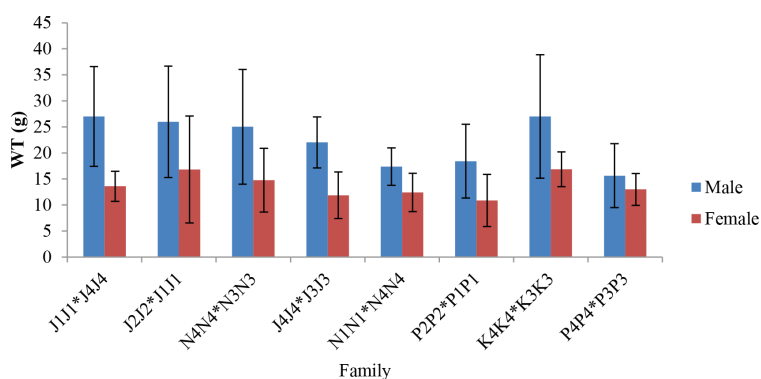


FIGURE 3. A histogram showing weight (WT, g) means of the males (M) and females (F) of the eight progeny families of *M. rosenbergii*

TABLE 3. Analysis of variance (ANOVA) using mixed procedure showing the effect of family, sex, replicate and their interactions on body weight (WT), total length (TL), tail length (TA) and carapace length (CL) of *Macrobrachium rosenbergii*

Trait	Effect	df	F value	p
WT	Family	7	51.63	<.0001
	Replicate	2	3.04	0.0483
	Sex	1	1227.82	<.0001
	Family*sex	7	23.64	<.0001
	Replicate*sex	2	86.24	<.0001
	Family*Replicate	14	1.22	0.2525
TL	Family	7	46.14	<.0001
	Replicate	2	0.38	0.6835
	Sex	1	1152.03	<.0001
	Family*sex	7	13.18	<.0001
	Replicate*sex	2	116.86	<.0001
	Family*Replicate	14	2.20	0.0066
TA	Family	7	40.75	<.0001
	Replicate	2	0.70	0.4968
	Sex	1	1023.91	<.0001
	Family*sex	7	9.09	<.0001
	Replicate*sex	2	134.36	<.0001
	Family*Replicate	14	2.66	0.0008
CL	Family	7	53.80	<.0001
	Replicate	2	0.81	0.4451
	Sex	1	1247.53	<.0001
	Family*sex	7	15.33	<.0001
	Replicate*sex	2	115.55	<.0001
	Family*Replicate	14	2.71	0.0006

*df = degree of freedom, F = F-statistics, P = probability

TABLE 4. Analysis of variance for estimating heritability values of body weight (WT), total length (TL), tail length (TA) and carapace length (CL) of *Macrobrachium rosenbergii* in Malaysia

Trait	Source	DF	Mean square	Heritability estimates
WT	Between families	7	643.54067	0.5788±0.2989
	Within families	392	68.02839	
	Corrected total	399		
TL	Between families	7	20.374425	0.5156±0.2753
	Within families	392	2.425844	
	Corrected total	399		
TA	Between families	7	3.9087286	0.4277±0.2407
	Within families	392	0.5595153	
	Corrected total	399		
CL	Between families	7	2.1350250	0.5120±0.2739
	Within families	392	0.2560015	
	Corrected total	399		

and Nguyen (2014), Dinh et al. (2013), Kitcharoen et al. (2012) and Thanh et al. (2010) for the same species (Dinh & Nguyen 2014). However, higher results were evidenced by Nugroho et al. (2005) showing h^2 estimates as high as 0.84 for body weight and lower estimates as compared to this study were also reported by Luan et al. (2012) and Sun et al. (2013).

CONCLUSION

The results in this study showed significant differences among parental populations for the four traits investigated. Fixed effects of both family and sex and their interactions were found to be significant for all the traits investigated. In addition heritability estimates of the same traits fall in the range of moderate to high. These results encourage

us to un-hesitantly recommend the selection for these quantitative traits for genetic improvement of this species.

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