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THESIS

A STUDY ON HERITABILITY AND GENETIC IMPROVEMENT OF GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii* de Man, 1879)

NISSARA KITCHAROEN

A Thesis Submitted in Partial Fulfillment of
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Nissara Kitcharoen 2011: A Study on Heritability and Genetic Improvement of Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879). Doctor of Philosophy (Aquaculture), Major Field: Aquaculture, Department of Aquaculture. Thesis Advisor: Professor Uthairat Na-Nakorn, Ph.D. 79 pages.

In this study heritability was estimated for growth related traits of Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879) before and after morphological sexual differentiation. Estimation was made on data from 16 full-sib and 8 half-sib families. The variance estimation was performed using a univariate mixed linear animal model. Variance components were analyzed following an animal model using a Restricted Maximum Likelihood procedure (REML) employing average information (AI) algorithm. Heritability estimates (h^2) varied considerably with ages. Based on mixed sex data, h^2 for carapace length (CL; 0.35 ± 0.15) and body weight (BW; 0.26 ± 0.13) at 2 months old were higher than those estimated at 5 months old. However, when data were sorted by sex, h^2 estimated from data of females were higher than those of males for CL (0.26 ± 0.16 *vs.* 0.10 ± 0.06), BW (0.28 ± 0.17 *vs.* 0.12 ± 0.08), body length (BL; 0.40 ± 0.17 *vs.* 0.11 ± 0.07), total length (TL; 0.47 ± 0.18 *vs.* 0.11 ± 0.07), and claw length (ClL; 0.29 ± 0.16 *vs.* 0.03 ± 0.04). The same trend was observed for traits at 6 months old in both bulk and individual rearing. In the second experiment, an empirical evidence was provided that selection made from early maturing female Giant Freshwater Prawns yielded offspring that grew faster than those of females selected from the later maturing batches. Sixteen full sibling families were produced and separately reared. When they reached maturation, gravid females were removed and separately divided into four batches according to time to maturity. The within family selection, with a 10% selection proportion on body length, was then performed within each batch. The females were simultaneously mated with males from different families within a batch. Growth comparison of offspring between batches showed that the offspring of the females selected from the first batch were larger in carapace length (CL) and body weight (BW) than those from later batches. In the third experiment, a genetic trend analysis was performed on the data from a total of 2,236 GFP in 3 generations of which the within family selection was performed targeting female body length at 7 months of age. Mean breeding values of body length increased 0.37 in females but was not changed for males. These results imply that of, selection to improve growth traits performed on female Giant Freshwater Prawn will result in positive genetic response in females.

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Nissara Kitcharoen

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LIST OF ABBREVIATIONS

ANOVA	=	Analysis of variance
BLUP	=	Best Linear Biased Prediction
BC male	=	Blue claw male
BL	=	Body length
BW	=	Body weight
CL	=	Carapace length
ClL	=	Claw length
C.V.	=	Coefficient of variation
°C	=	Degree Celsius
F	=	Inbreeding coefficient
FS	=	Full-sib
GFP	=	Giant Freshwater Prawn
g	=	Gram
h^2	=	Heritability
HS	=	Half-sib
mm.	=	Millimeter
MME	=	Mixed Model Equation
ppt	=	Part per thousand
%	=	Percentage
PL	=	Post larva
TL	=	Total length
S.D.	=	Standard deviation
S.E.	=	Standard error

A STUDY ON HERITABILITY AND GENETIC IMPROVEMENT OF GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii* de Man, 1879)

INTRODUCTION

Aquaculture of Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879) is of economically importance, e.g., average annual production of more than 200,000 tonnes as of 2008 (Food and Agriculture Organization [FAO], 2010) with three major contributors, PR China, Thailand and India (New, 2005). Despite a long culture history, only a little studies had been advocated to broodstock management and genetic improvement of Giant Freshwater Prawn (e.g., Uraiwan *et al*., 2002; Thanh *et al*., 2009) and only a few genetically improved strains have been established [e.g., Charoenpokphand strain, Anonymous (2001)].

There are a number of limiting factors preventing expansion of Giant Freshwater Prawn culture. A differential growth rate between male and female necessitates partial harvesting and eventually expanding a culture period to 8 to 12 months (Limsuwan and Jantharattakul, 2004). Hierarchical size differences among male morphotypes accompanying with aggressive behavior of dominated males remarkably reduces stocking density of this species (35 prawns/square meter) comparing to those of marine shrimp (approximately 50 shrimps/square meter) (Limsuwan and Jantharattakul, 2004). Moreover, territorial behavior of dominated males coupled with differential sizes of male morphotypes has led to skewed distribution of body sizes towards smaller size (comprising of 50% small and 10% large prawns; Malecha, 1983; Ranjeet and Kurup, 2002).

Efforts have been made to improve culture technologies, namely, feed, culture systems and farm management but only a little had been advocated to broodstock management and genetic improvement of Giant Freshwater Prawn (e.g., Uraiwan *et al*., 2002; Charoentawee *et al*., 2007; Karaket *et al.*, 2011). On the contrary, some

practices could have had adverse impacts on the stocks. For examples, Doyle *et al*. (1983) reported un-intentional negative selection for growth with selection differential estimated as 2.6 g/generation whereby, farmers (in Thailand) selected gravid females from the late maturing prawn instead of the early matured which may grow faster. As yet, there has been no empirical data to support or oppose to this report while the farmers continue the old practices until now (FAO, 2002; Limsuwan and Jantharattakul, 2004). Therefore, it is urgent to find out supporting information for broodstock management and selective breeding to improve production related traits of this species.

Selective breeding programs require basic information to enhance efficiency of the program, especially in a long term. Heritability refers to a proportion of additive genetic variance to a total variance (Falconer and Mackay, 1996) and it is essential for planning efficient selection programs. Heritability (h^2) has been estimated for growth of juvenile Giant Freshwater Prawn wherein differences between sex was demonstrated, e.g., heritability of 11 months old prawn was higher for females (0.35 ± 0.15) than for males of which h^2 was not different from zero ($P > 0.05$; Malecha *et al*., 1984). The h^2_{S+D} estimated in 5 months old prawn were -0.018 ± 0.014 and 0.122 ± 0.074 respective to length and weight of males while, it was 0.060 ± 0.054 and 0.030 ± 0.041 respective to length and weight of females (Uraiwan *et al*., 2002)]. As such, efficiency of selection performed after sex differentiation may be compromised due to the confounding of male morphotypes. Therefore, h^2 before sex differentiation is of interest because it may provide a hint to conduct efficient selection without confounding from male morphotypes, providing that the h^2 is remarkable. Moreover, the estimation of h^2 previously mentioned, employed statistical models disregarding systematic errors, e.g., effects of age, sex, pond-cage, farm, feed, etc. (Gjedrem and Olesen, 2005), which could not be avoid.

At present Mixed Model Equation, MME (Henderson, 1975) has been widely used for the estimation of h^2 (Mrode, 2005). The MME model, considered as BLUP (Best Linear Unbiased Prediction; Henderson, 1975), is capable of separating random and systematic errors and hence is expected to give precise estimation of h^2.

Therefore, in the present study MME was used to estimate h^2 of growth related traits in Giant Freshwater Prawn. Besides, a study was also conducted to provide the empirical data on the results of selecting females from the early maturing *versus* the late maturing females. The results are useful for improvement of broodstock management practices of Giant Freshwater Prawn Thai farmers to change their practice of Giant Freshwater Prawn.

OBJECTIVES

1. To estimate heritability (h^2) and correlations of growth traits of Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879) before sex differentiation and morphotype differentiation, in 2 different rearing conditions.

2. To compare response of Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879) to selection for growth performed on different maturing batches of females.

3. To estimate the selection response on growth of Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879) after two generations of selection by genetic trend analysis.

LITERATURE REVIEW

1. Growth pattern and morphology of Giant Freshwater Prawn

The growth pattern of Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879), according to New and Singholka (1985), is shown in the Figure 1. There are four stages in a life cycle of Giant Freshwater Prawn, egg (1), larva (2), post-larva (3) and adult (4). The larva requires brackish water for survival until reaching the post-larval stage which was through development of 11 stages of metamorphosis by molting (FAO, 2002). The post-larva which already resembles adult morphology will then be transferred to freshwater.

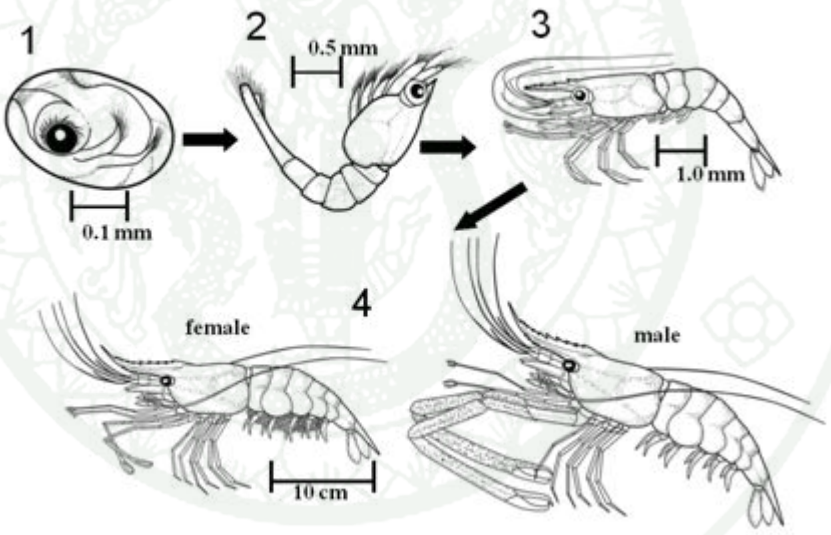

Figure 1 The growth pattern of Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879)

Morphological differentiation between sexes in Giant Freshwater Prawn occurs as early as two months after reaching a post-larval stage when some male prawns develop a genital pore at the base of the 5th periopod (walking leg). A testis has not been developed until about 67 days after reaching a post-larval stage when an appendix masculina is observed at the inner rim of the 2nd pleopod (swimming leg) of male prawns (Rungsin *et al*., 2006). In general, a majority of the prawns shows sexual

differentiation at 67 days after reaching a post-larval stage. At this stage male and female prawns are recognized by a position of a genital pore, at the base of 3rd and 5th periopod in female and male, respectively.

Adult male prawns can be divided into three distinctive morphological types, blue claw-, orange claw- and small-male, bases on claw size and claw color. Among which the blue claw males show strong territorial behavior and thus dominate in feeding and reproduction. These have led to skewed distribution of body sizes towards smaller size (comprising of 50% small-male, 40% orange claw-male and 10% blue claw-male) (Malecha, 1983; Ranjeet and Kurup, 2002).

2. Heritability and estimation of heritability

2.1 Heritability

Heritability (h^2) is a proportion of additive genetic variance to a total variance (Falconer and Mackay, 1996) and was precisely called “narrow sense heritability”. In other words, this value shows the proportion of phenotypic variance that can be transmitted from parents to offspring in predictable and reliable manner (Tave, 1986).

Heritability is one of the most useful parameters for selective breeding programs. The heritability varies between 0 and 1. In aquatic organisms, the heritability values of 0 to 0.15 are considered as low, 0.15 to 0.3 are moderate and the values higher than 0.3 are considered as high heritability (Tave, 1986). It is important to know the value of the heritability when planning a breeding program as well as when predicting the response to selection. Furthermore, heritability is useful for developing a selection index which enables an efficient selection for multiple traits. For example, 25% response for growth rate and 18.4, 3.6% response for resistance to Taura Syndrome Virus were achieved from a selective breeding program for Pacific white shrimp, *Litopenaeus vannamei,* based on an index weighted equally for both traits (Argue *et al*., 2002).

It is of concern that heritability values varies among species, populations and rearing environments ,e.g., age, pond types, feed, management, etc. (Falconer and Mackay, 1996).

2.2 Estimation of heritability

Among a few methods for estimating heritability, namely, sib analysis, regression of offspring on parents, etc. (Falconer and Mackay, 1996), sib analysis yields the most precise estimation. It estimates heritability based on variance components obtained from analysis of variance among sibs. The reliability of heritability obtained from this method is justified by the standard error (S.E.) of the estimation. The number of sire and dam is an important factor of the reliability of heritability and could be calculated as $2 / h^2$ (Falconer and Mackay, 1996). In general, the number of mating pairs should be 20 to 30 (Robertson, 1959).

The estimations of h^2 of important traits of Giant Freshwater Prawn are shown in Table 1. The heritability of growth pattern variation in juvenile Giant Freshwater Prawn ware estimated by Malecha *et al*. (1984) using full and half-sib families in an unbalanced nested design, comprising 16 sires mated with 5, 4, 3 and 2 dams per sire in 3, 3, 3 and 7 replicates, respectively. The narrow sense heritabilities for juvenile were 0.35±0.05 for females and not statistically different from zero for males. This study indicated that selection for growth improvement in Giant Freshwater Prawn should be performed on female prawns.

Moreover, Meewan (1993) estimated the heritabilities of growth in relation to morphotypic transformation of Giant Freshwater Prawn based on 16 full-sib and 8 half-sib families. The heritability of carapace length at 23 weeks was 0.4±0.2 based on paternal, 0.13±0.07 base on maternal, and 0.26 ±0.11 based on full-sib analysis. The heritability of morphotype transformation at 31 weeks from orange claw males to blue claw males were 0±0.04, 0.73±0.08, 0.37±0.02; from small males to orange claw after removing bulls (large blue claw males) were 0.21±0.06, 0.56±0.05, 0.39±0.03 for paternal, maternal and full-sib analysis, respectively. This

study shows that the genetic improvement of freshwater prawn should be performed by selection methods.

Uraiwan *et al*. (2002) estimated heritability on growth rate of Giant Freshwater Prawn by sib analysis. The experiment was carried out in cages with mixed sexes rearing during 1991 to 1992 and in concrete pond with separated sex rearing during1996 to 1997. Under the cage condition, heritability of length and weight at five months of age estimated by nested analysis of variance using 16 full-sib and 8 half-sib families were –0.018±0.014 and 0.122±0.074 for male prawn and 0.060±0.054 and 0.030±0.041 for female prawn, respectively. Under pond condition, heritabilities estimated at six months of age, based on 17 full-sib families were 0.156±0.077 for length and 0.142±0.096 for weight of male prawn and 0.254±0.080 for length and 0.272±0.210 for weight of female prawn.

Heritability estimation by sib analysis employs statistical models disregarding systematic errors (e.g., effects of age, sex, pond-cage, farm, feed, etc. (Gjedrem and Olesen, 2005) which are difficult to avoid. The Mixed Model Equation: MME (Henderson, 1975) approach, which is considered as BLUP (Best Linear Unbiased Prediction) is capable of separating random and systematic errors and hence is expected to give precise estimation of heritability. Variance components are analyzed using Restricted Maximum Likelihood procedure (REML; Patterson and Thompson, 1971). Then heritability is calculated following a formula $h^2 = \sigma^2_a/\sigma^2_p$ where σ^2_a = additive genetic variance and σ^2_p = phenotypic variance. The calculation is facilitated by computer packages such as ASREML (Gilmour *et al*., 2002), VCE (Kovac and Groeneveld, 2003), PEST (Groeneveld, 1990), etc.

Despite the well documented advantage of BLUP over the ANOVA based analysis (Gjedrem and Olesen, 2005), the application of BLUP in Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879) has never been reported before.

In addition, heritability estimates for growth related traits have been reported in some of Penaeid species (Table 2) including *Penaeus vannamei* (Carr *et*

al., 1997; Argue *et al*., 2002; Perez-Rostro and Ibara, 2003; Gitterle *et al*., 2005; Castillo-Juárez *et al*., 2007), *P. monodon* (Benzie *et al*., 1997; Jarayabhand *et al*., 1998; Kenway *et al*., 2006) and *P. japonicus* (Hetzel *et al*., 2000).

Table 1 Heritability (h^2 ± S.E.) for growth related traits in the Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879)

Traits	Paternal	Maternal	Full-sib analyses	References
Female weight at 5 months old	0.34±0.24	0.22±0.23	0.28±0.13	Malecha *et al.* (1984)
Male weight at 5 months old	0.20±0.29	-0.236±0.11	-0.18±0.10	Malecha *et al.* (1984)
Female weight at 11 months old	0.35±0.29	0.35±0.28	0.35±0.15	Malecha *et al.* (1984)
Male weight at 11 months old	-0.14±0.25	0.19±0.19	0.02±0.13	Malecha *et al.* (1984)
Carapace length	0.40 ±0.2	0.13 ±0.07	0.26 ±0.11	Meewan (1993)
Morphotypes transformation (from orange to blue claw at 31 weeks old)	0 ±0.04	0.73 ±0.08	0.37 ±0.02	Meewan (1993)
Morphotypes transformation (from small male to orange claw and blue claw)	0.21±0.06	0.56±0.05	0.39±0.03	Meewan (1993)
Male body length at 5 months old			0.16±0.08	Uraiwan *et al.* (2002)
Male body weight at 5 months old			0.14±0.10	Uraiwan *et al.* (2002)
Female body length at 5 months old			0.25±0.08	Uraiwan *et al.* (2002)
Female body weight at 5 months old			0.27±0.21	Uraiwan *et al.* (2002)

Table 2 Heritability (h^2 ± S.E.) for growth related traits in commercially important Penaeid shrimps

Species/traits	Heritability	References
P. vanamei;		
Weight at about 11 g.	0.42±0.15	Carr *et al*. (1997)
Weight at about 23 g.	0.84±0.43 (raceway), 1.19±0.59 (pond)	Argue *et al*. (2002)
Total length at harvest size	0.227±0.07	Perez-Rostro and Ibarra (2003)
Abdominal length at harvest size	0.237±0.07	Perez-Rostro and Ibarra (2003)
Cephalothorax length at harvest size	0.177±0.06	Perez-Rostro and Ibarra (2003)
Total weight at harvest size	0.177±0.06	Perez-Rostro and Ibarra (2003)
Abdominal weight at harvest size	0.187±0.06	Perez-Rostro and Ibarra (2003)
Cephalothorax weight at harvest size	0.157±0.06	Perez-Rostro and Ibarra (2003)
Width of the first abdominal segment at harvest size	0.147±0.05	Perez-Rostro and Ibarra (2003)
Harvest weight	0.24±0.05 for line 1 and 0.04±0.02 for line 2	Gitterle *et al*. (2005)
Body weight at harvest size	0.24±0.14 to 0.35±0.18 for univariate models	Castillo-Juárez *et al*. (2007)
Body weight at harvest size	0.37±0.06 to 0.45±0.09 for multivariate models	Castillo-Juárez *et al*. (2007)

Table 2 (Continued)

Species/traits	Heritability	References
P. monodon;		
Total length at 6 weeks	0.08±0.10 (Sire Component) 0.59 ±0.30 (Dam Component)	Benzie *et al.* (1997)
Total length at 10 weeks	0.12±0.02 (Sire Component) 0.56±0.03 (Dam Component)	Benzie *et al.* (1997)
Wet weight at 6 weeks	0.12±0.07 (Sire Component) 0.30±0.11 (Dam Component)	Benzie *et al.* (1997)
Wet weight at10 weeks	0.10±0.002 (Sire Component) 0.39±0.004 (Dam Component)	Benzie *et al.* (1997)
Total length at 25 days	0.153±0.06	Jarayabhand *et al.* (1998)
Total length at 65 days	0.266±0.037	Jarayabhand *et al.* (1998)
Wet weight at 65 days	0.053±0.029	Jarayabhand *et al.* (1998)
Growth rate at 16 weeks	0.56±0.04	Kenway *et al.* (2006)
Growth rate at 30 weeks	0.55±0.07	Kenway *et al.* (2006)
Growth rate at 40 weeks	0.45±0.11	Kenway *et al.* (2006)
Growth rate at 54 weeks	0.53±0.14	Kenway *et al.* (2006)
P. japonicus;		
Weight at 6 months	0.234 (realized heritability)	Hetzel *et al.* (2000)

3. Selection and selection methods

3.1 Selection

A selection program aims at altering mean of a targeted trait(s) in offspring by selecting desired parents. The difference of phenotypic mean between the offspring of the selected parents and the population prior to selection represents response to selection (R). The selection response depends on heritability and a selection differential (S) which denotes the difference between phenotypic mean of selected parents and the population mean (Falconer and Mackay, 1996).

3.2 Selection methods

Among simple selection methods, namely individual selection or mass selection, family selection, within family selection, combined selection, etc., efficiency of family selection is well recognized [e.g., Atlantic salmon, *Salmo salar*, for growth rate, age at sexual maturity, improved resistance to diseases and a number of traits related to product quality (Gjedrem, 2000; Gjerde, 1986; Gjerde and Korsvoll, 1999; Flynn *et al*., 1999), Pacific White Shrimp, *P. vanamei* for resistance to TSV and WSSV (Argue *et al*., 2002; Gitterle *et al.* 2006)]. A family selection refers to a selection method in which family groups are ranked according to the mean performance of each family and the whole family is saved or discarded (Lush, 1947). A family selection efficiently improves traits even with low heritability. However, the disadvantage of this method is requirement of large facilities and other resources.

A within family selection aims at selecting individuals with the best performance from all families. The benefit of this method over a family selection is reducing number of ponds and labors required. Besides, a within family selection is especially advantageous when there is a large component of environmental variance common to members of the same family (Uraiwan and Dolye, 1986). However, a within family selection has low efficiency compared to the most other selection methods (Gall and Huang, 1988 a, b). After one generation of a within family

selection in Giant Freshwater Prawn, female prawn of selected line at six months of age were significantly ($P < 0.01$) larger by length and weight than those of the control line and those of the parental lines wherein selection responses were 6 and 12 %, and 5 and 16 % for length and weight, respectively (Uraiwan *et al.*, 2002).

The selection methods mentioned above are performed based on a phenotypic mean, thus the selection efficiency may be compromised due to confounding of environmental effects on parental performances. The BLUP (Best Linear Unbiased Prediction) selection is one of the efficient methods to select by ranking of Expected Breeding Value (EBV) which is estimated base on individual phenotypic records, of all animals in a population. EBV reflects the additive genetic variance of a trait and hence can be transmitted from parents to offsprings. The selection based on EBV ranking, therefore, efficiently enhances selection response.

Application of the BLUP selection in aquatic animals was first started in Nile tilapia whereby the Mixed Model Equation (MME) was employed for estimation of EBV of body weight at 98 days (Gall and Bakar, 2002). A 40% selection response was reported within 3 generations by the mass selection based on EBV. The within family selection base on EBV in Nile tilapia resulted in 2.2 g weight gain per generation while inbreeding was efficiently minimized to 0.525% per generation with only 19 full-sib families (Bolivar and Newkirk, 2002). In Coho salmon (*Oncorhynchus kisutch*) Neira *et al.* (2006) reported a selection response on weight at harvesting was 13.9% per generation comparing to the controlled population. However, after 4 generations of selection, inbreeding coefficient increased to 9.5%.

4. Estimation of response to selection

The selection response can be estimated using simple methods such as a comparison between mean of the offspring of the selected parents with that of the unselected control population. However the precision of this method is hampered by the fact that inbreeding in the controlled population may inflate selection response while genetic drift and un-intentional selection of the control population may either

inflate or under estimate of selection response (Falconer and Mackay, 1996). The bidirectional selection is designed assuming equal response between positively and negatively selected lines. As such, the selection response equals the difference between means of the positively and negatively selected line divided by 2. However, it is always true that the positive and negative response are unequal. Wherein the selection response of the low line is always higher than that of the high line, for examples, the response to one generation of selection for growth in Kuruma prawn, *Penaeus japonicus* was 8.3% for high line and 13.1% for low line (Hetzel *et al*., 2000).

Fortunately, the genetic trend analysis of EBV in each generation was proposed as an alternative way to estimate selection response (Sorensen and Kennedy, 1984, 1986). From a ranking of individual EBV in a population, the EBV larger than a mean of the population was designated with a positive sign and *vice versa*. The mean of a population equals zero or close to zero. Then a within generation mean EBV of a population will be subjected to the genetic trend analysis. As a result, the trend will reflect progress of a selection (Bourdon, 2000). For example, in Nile tilapia (Bolivar and Newkirk, 2002), there was no control lines in the selection program therefore, the genetic trend analysis for estimation of selection response was used. Progress in a rainbow trout selection program was also assessed by estimating genetic trend in growth, maturing age and skeletal deformation (Kause *et al.*, 2005). Rezk *et al*. (2009) selected Nile tilapia to improve harvest weight and compared two ways to estimate selection response. They reported a superiority of the genetic trend analysis of EBV over the traditional estimation of selection response (the difference between the selected and control lines).

MATERIALS AND METHODS

1. Experiment 1: Estimation of heritability and the correlation among growth traits of Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879) before sex and morphotype differentiation in two rearing conditions

1.1 Preparation of brooders and mating

The 200 Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man, 1879) brooders were collected from Pasaak Chonlasidth Dam, Lop Buri province. They were delivered to Fish Genetics Laboratory on 26th April 2006 and were individually reared in 75-l plastic buckets until they were proven to be free of virus [MrNV (*M. rosenbergii* nodavirus) and XSV (extra small virus)] which cause the white tail disease (Bonami *et al*., 2005). Only the virus free broodstocks were transferred to aquaria (65 × 60 × 45 cm3) where they were individually reared. The ovarian development was daily observed and the mating was initiated when a female with fully developed ovaries (a reproductively competent female) molted. A blue claw male was randomly taken and placed in the aquarium stocked with a newly molted reproductively competent female. After mating the male was removed and it would be used for mating with the second female. The mating had been performed until 23rd July, 2007 when a total of 9 males was successfully mated to 15 females at a ratio of one male to one or two females following a single pair mating scheme in which one female was used twice. As such, 7 paternal and 1 maternal half-sib families, and 16 full-sib families were produced (the detail of the mating plan was shown in Table 3)

Within 24 hours after mating the mated female released eggs which subsequently attached to its pleopods. Hatching was commenced within 23 days.

Table 3 Identification number of males (sire) and female (dam) Giant Freshwater Prawn used for the hierrachical mating, length of incubation, hatching date, date reaching a post larval stage, and length of larval development of the offspring in each family

Sire	Dam	Length of incubation(days)	Hatching date	Date reaching a post larval stage	Length of larval development (days)
(1) LM13	(1) LF55	22	6-Feb-07	23-Feb-07	18
	(2) LF56	23	10-Feb-07	26-Feb-07	17
(2) LM21	(3) LF68	21	26-Feb-07	15-Mar-07	18
	(4) LF37	20	4-Mar-07	18-Mar-07	17
(3) LM09	(5) LF13	19	5-Mar-07	23-Mar-07	19
	(6) LF63	20	5-Mar-07	23-Mar-07	19
(4) LM23	(7) LF10	19	14-Mar-07	4-Apr-07	22
	(8) LF43	20	15-Mar-07	4-Apr-07	21
(5) LM01	(9) LF58	19	6-May-07	23-May-07	18
	(10) LF33	19	7-May-07	24-May-07	18
(6) LM02	(11) LF56	19	9-May-07	26-May-07	18
	(12) LF34	19	20-May-07	-	-
(7) LM03	(13) LF59	19	12-May-07	1-Jun-07	21
	(14) LF06	20	13-May-07	1-Jun-07	20
(8) LM04	(15) LF11	19	19-May-07	6-Jun-07	19
	(16) LF39	19	21-May-07	-	-
(9) LM09	(17) LF61	20	29-Jul-07	17-Aug-07	20
	(18) LF58	20	13-Aug-07	31-Aug-07	19
Mean	-	**19.83**	-	-	**19**

1.2 Larval rearing

Each family of the larvae was separately reared in 250 l tank at about 80,000 larvae/tank. They were subsequently fed with brine shrimp (*Artemia* spp.) 3

times daily during day 2-7; *Artemia* plus steamed egg during day 7-14; steamed egg and pelleted feed from day 14 until reaching the post-larval stage (which took 17-22 days at water temperature of 31°C), and pelleted feed from PL-7 (7 days after reaching the PL stage) onward. The post-hatched larvae were first reared at the salinity of 13-15 ppt. Then the salinity was gradually reduced to 0 ppt at 1 week after reaching the PL stage. Water was 30 to 50% replaced every day.

At 2 weeks after reaching the PL stage, the post larvae were transferred to 2 types of rearing (communal-within each family, and individual rearing). The pooled full-sib family rearing was performed in concrete tanks ($2 \times 1 \times 0.5$ m^3) wherein each pond was stocked with 200 prawns /family with two replicates.

Individual rearing was performed at 5 months old when 20 individuals (10 males and 10 females) were solitarily stocked in a round plastic box (20 cm diameter) floating in a $2 \times 1 \times 0.5$ m^3 concrete tank. The individual rearing was continued until morphotypes were distinguishable (6 to 7 months old).

The PL were fed with a commercially available pelleted feed (Charoenpokphand), 40% crude protein for one to two months old PL; 38% crude protein for the PL older than two months. Water was 30 to 50% replaced every other day during the first two months of rearing, and once a week in the later rearing period until the end of the experiment.

1.3 Measurements

The measurements were done for the pooled full-sib rearing at 2, 5 and 6 months of age while the individual rearing prawns were measured at 6 months old. Whereby, 40 prawns were sampled from each replicate and measured for carapace length (CL: from an ocular lobe to a carapace groove), body length (BL: from a carapace groove to a telson), claw length (ClL: length of the propodus part of the biggest claw), total length (TL: from end of a rostrum to a telson), and body weight (BW). The measurement features of Giant Freshwater Prawn are shown in Figure 2.

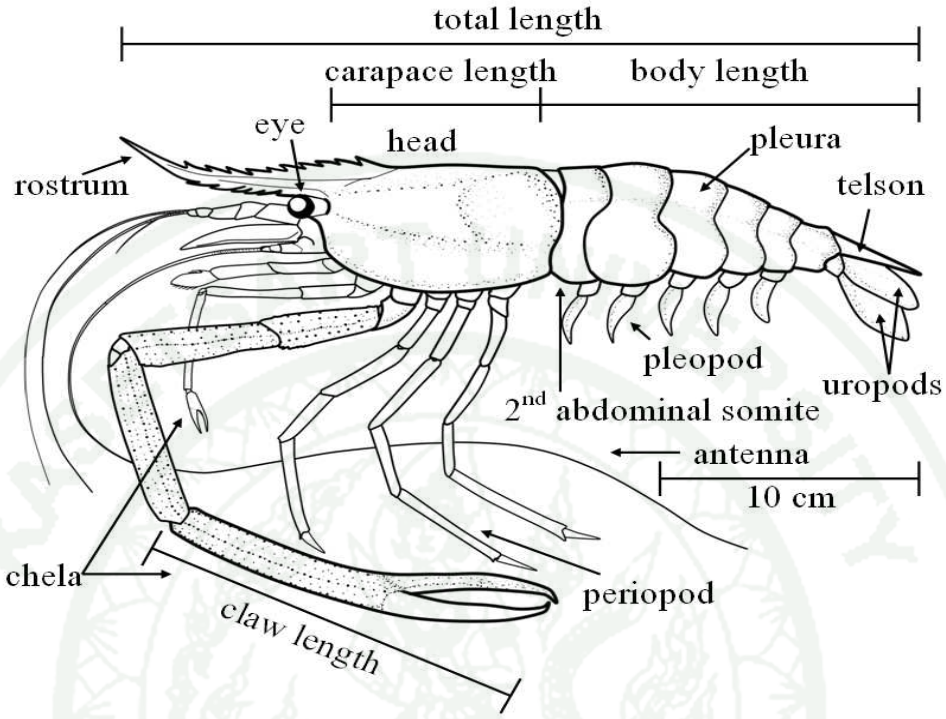

Figure 2 The measurement features of Giant Freshwater Prawn

1.4 Data analyses

The data were analyzed for descriptive statistics using the program SAS (SAS, 2003). Then variance component estimation was performed using a univariate mixed linear animal model. The model is written in matrix notation as follows.

$$\boldsymbol{y} = \boldsymbol{Xb} + \boldsymbol{Za} + \boldsymbol{Wc} + \boldsymbol{e} \quad (1)$$

where;

y is a vector of observations (at 2 months old: carapace length-CL and body weight-BW; at 5 and 6 months old: CL, BW, body length-BL, claw length-ClL and total length-TL**,** ***b*** is a vector of fixed effects (at 2 months old: hatching month-HM; at 5 months old: HM and sex; at 6 months old: HM, sex and rearing conditions-

RC), $\boldsymbol{a}$ is a vector of random effect of animal additive genetic effects, $\boldsymbol{c}$ is a vector of random effect of common environment effect including pond environments for each full-sib family, $\boldsymbol{e}$ is a vector of random effect of residual effects, $\boldsymbol{X}$, $\boldsymbol{Z}$ and $\boldsymbol{W}$ are incident matrices assigning the observations to levels of $\boldsymbol{b}$, $\boldsymbol{a}$ and $\boldsymbol{c}$, respectively.

The assumption was as follow:

$$\begin{bmatrix} \boldsymbol{y} \\ \boldsymbol{a} \\ \boldsymbol{c} \\ \boldsymbol{e} \end{bmatrix} \sim \boldsymbol{NID}, \left(\begin{bmatrix} \boldsymbol{X\beta} \\ \boldsymbol{0} \\ \boldsymbol{0} \\ \boldsymbol{0} \end{bmatrix}, \begin{bmatrix} \boldsymbol{ZGZ'+WCW'+R} & \boldsymbol{ZG'} & \boldsymbol{WC'} & \boldsymbol{R} \\ \boldsymbol{GZ'} & \boldsymbol{G} & \boldsymbol{0} & \boldsymbol{0} \\ \boldsymbol{CW'} & \boldsymbol{0} & \boldsymbol{C} & \boldsymbol{0} \\ \boldsymbol{R} & \boldsymbol{0} & \boldsymbol{0} & \boldsymbol{R} \end{bmatrix} \right) \quad (2)$$

when

$\boldsymbol{G} = \boldsymbol{A}\boldsymbol{\sigma}_{\boldsymbol{a}}^{\boldsymbol{2}}$; $\boldsymbol{A}$ is a numerator relationship matrix (Henderson, 1975), $\boldsymbol{C}$ ($\boldsymbol{I}\boldsymbol{\sigma}_{\boldsymbol{c}}^{\boldsymbol{2}}$) is a common environmental matrix and $\boldsymbol{R}$ ($\boldsymbol{I}\boldsymbol{\sigma}_{\boldsymbol{e}}^{\boldsymbol{2}}$) is a residual variance matrix.

Variance components were analyzed following the animal model using Restricted Maximum Likelihood procedure (REML) employing average information (AI) algorithm. Then the variance components were used for calculations of heritability following a formula $h^2 = \sigma_a^2/\sigma_p^2$. The calculation was performed using the computer package ASREML (Gilmour *et al*., 2002).

Unfortunately, the available dataset did not allow for multivariate analyses, therefore the genetic correlations between traits were calculated based on the correlation between individuals' breeding values of particular traits using Proc CORR in SAS software (SAS, 2003).

2. Experiment 2: Comparison of growth between offspring of Giant Freshwater Prawn selected from different maturing batches

2.1 Preparation of brooders and establishing the parental generation

This experiment was conducted during 25th September 2007 to 30th September 2008. The 16 full-sib families described in 1.1 were reared until maturation which occurred at 7 months of rearing.

2.2 Separation of gravid females into four batches and selection

Due to the fact that once reaching maturation, a female will develop eggs and released them to the pleopods where the eggs are attached, regardless of mating. The presence of eggs at the female's pleopods (the female was called "gravid female") was considered as a sign of maturation. When the first gravid female was observed in each family, it was removed from the tank and separately kept in an aquarium (25 cm × 50 cm × 25 cm water depth). Likewise, the next gravid females of the same family were continuously collected. Due to a small number of the gravid females appeared in some families, the collection time was expanded to two months in order to have a sufficient number of females for the selection (at least 20 females/family). Prior to selection, the immature and gravid females were individually measured for CL, BL, TL and ClL to the nearest mm. Then each female batch was subjected to a within family selection (proportion of selection = 10% on body length). Then the subsequent harvests and selection were performed during 8th, 9th and 10th to 11th month. This resulted in four batches of selected females.

2.3 Comparison of offspring of the four batches of females

2.3.1 Mating

After the selected gravid females discarded the eggs, they were reared until they developed eggs and became reproductively competent females again.

Then each of them was mated with a blue-claw male taken at random from a different family following the rotational crossing scheme (Kincaid, 1977). It should be noted that due to very low heritability of growth of the male Giant Freshwater Prawn (Malecha, 1983; Malecha *et al*., 1984; Kitcharoen *et al*., 2011), using a random male would not affect the result of the selection.

Due to necessity to synchronize the mating between the four female batches, only the gravid females available at that time were used. This resulted in obtaining 17, 18, 10 and 1 full-sib offspring for batch 1- 4, respectively.

2.3.2 Experimental design and measurements

To compare growth performance between offspring of the four female batches, each family of offspring was separately reared in concrete tanks and each tank represented a replication. The nursing and rearing procedures were the same as previously described. Measurement was done at 2 months after reaching the PL stage whereby, 40 prawns/tank were sampled and measured for carapace length and body weight.

2.3.3 Data analyses

The Monte-Carlo randomisation test (500,000 randomisations at 99% confidence limit) was performed to compare CL, BL, and TL of the immature and mature females from each maturation batch. The same test was employed to test the difference in CL and BW between the two-month-old offspring of different spawning batches. The analyses were facilitated by the PopTools™ program (Hood, 2002).

In order to test a hypothesis of whether farmers who grow the offspring from the selected brooders of the first maturing batch will get higher average growth rates than (1) if they took PLs at random from all batches, or (2) if they took PLs from the last batch, which represents the perceived farmer behavior,

the PopTools was used to generate 50,000 random sets of data. The *P*-value of the first hypothesis is the fraction of the 50,000 random samples in which the mean of the first 680 (representing batch 1 offspring) exceeds the overall mean by the amount actually observed (observed difference between batch 1 and the overall mean). Likewise, the *P*-value of the hypothesis 2 is the fraction of the 50,000 random samples in which the mean of the first 680 individuals exceed the mean of the last batch.

3. Experiment 3: Estimation of a genetic trend of Giant Freshwater Prawn selected for growth for two generations

3.1 The selection experiment

The selection experiment was a continuation of the experiment 2 whereby the first generation of selection was conducted by selecting large females from the first maturing batch (17 selected females out of 47 females matured in the first batch). Subsequently, each female was mated with a randomly taken male from different family. The offsprings of each family were separately reared in concrete tanks (2 × 1 × 0.5 m^3). Feeding and water management were as described in 1.2.

At 7 months of age the within family selection on body length was applied to the 17 full-sib families. According to the previous results, selection should be performed on early matured females, therefore approximately 10% of the longest females in each family were selected resulting in 2 to 3 selected females/family. Then each selected female was mated with a randomly taken male from different family following the rotational crossing scheme. However, due to highly fluctuated water temperature between days and nights during April to June, 2009 that caused mass mortality in a majority of the full-sib families, only offspring of 8 full-sib families survived. The offsprings were reared at a stocking density of 200 prawns/tank (2 × 1 × 0.5 m^3) with the same feeding and water management regimes as described in 1.2. At 7 months of age, body length of all offsprings was measured to the nearest millimeters and the 10 % within family selection on body length of females was

performed. Then the selected females were individually mated with randomly taken males following rotational crossing (Kincaid, 1977). The larvae from each family were separately reared and a within family selection was applied as in generation 1. The 10 % biggest females and random males from each family were mated following a single pair mating scheme resulting in 8 full-sib families from 6 males and 6 females (generation 3). The larvae from each family were separately reared as described for the previous generations.

3.2 Data recording and Measurements

In each generation the individual pedigree, maturation status and growth traits were recorded at 7 months of age on all mature females and forty prawns (20 males and 20 immature females) that were randomly sampled from each family. The growth traits were CL, BL, ClL, TL, and BW.

3.3 Data analyses

The dataset composed of individual pedigree and growth traits measured from 2,236 Giant Freshwater Prawn (941, 963 and 333 Giant Freshwater Prawn from generations 1, 2 and 3, respectively). The descriptive statistics of the dataset were calculated using the procedure MEANS in the statistical software SAS (SAS, 2003). Variance component estimation was performed using a univariate mixed linear animal model as detailed in 1.4 with slight modification (the fixed effects (b) included sex and maturation status).

Selection differentials (S) were computed within families, as the average difference in BL between the selected prawn and its respective family mean. The standardized selection differential (actual intensity of selection, *i*) was computed by dividing the selection differential by the phenotypic standard deviation of body length of the seven months old offspring.

There was no unselected control in this experiment. The selection response on growth of Giant Freshwater Prawn after two generations of selection was estimated by a genetic trend analysis (Sorensen and Kennedy, 1984, 1986) in three generations of mean breeding value.

The estimation of variance components for a breeding value (EBV) estimation was carried out using Restricted Maximum Likelihood (REML) fitting of an animal model. The calculation was facilitated by a computer package ASREML (Gilmour *et al*., 2002).

Unfortunately, the available dataset did not allow for multivariate analyses. Therefore, the genetic correlations between traits were calculated based on the correlation between individuals' breeding values of particular traits using Proc CORR in SAS software (SAS, 2003).

The inbreeding coefficient for an individual was computed from the pedigree data using the algorithm by Meuwisen and Luo (1992). The coefficient of inbreeding for a specific generation was computed as the mean of the inbreeding coefficients of all individuals. The rate of inbreeding in a generation was computed according to the formula by Falconer and Mackay (1996):

$$(F_t - F_{t-1}) / (1 - F_{t-1}) \quad (3)$$

where,

F_t is the inbreeding coefficient in generation t and F_{t-1} is the inbreeding coefficient in generation $t-1$.

The calculations were implemented using the Pedigree viewer software (Kinghorn, 2010).

RESULTS AND DISCUSSION

Results

1. Experiment 1: Estimation of heritability and correlations of growth traits of Giant Freshwater Prawn before sex differentiation and morphotype differentiation in two rearing conditions.

Overall, the length of incubation time and larval development were very consistent among families (Table 3). Descriptive statistics for carapace length, body length, total length, claw length and body weight at different stages are shown in Table 4. It was obvious that variance on growth traits increased with ages. Male prawns grew faster than female prawns and the variance on growth traits was larger. In general, the pooled full-sibs prawn which was directly stocked in tanks grew faster than individually reared prawns. It was noteworthy that the latter group showed larger variation than the communally reared prawns except for body weight.

1.1 Heritability

Heritability of growth related traits varied considerably with ages. At two months after reaching PL stage, $h^2$of carapace length (CL, 0.35 ± 0.15) and body weight (BW, 0.26 ± 0.13) was high. At five months, h^2 of CL and BW based on mixed sex data was lower than those estimated at two months old (Table 5).

However, when data were sorted by sex, h^2 calculated from females data was as high as the estimation at two months [h^2 was 0.26 ± 0.16 for CL, 0.28 ± 0.17 for BW, 0.40 ± 0.17 for body length (BL), 0.47 ± 0.18 for total length (TL), and 0.29 ± 0.16 for claw length (ClL)] and was especially higher than those of males (h^2 was for 0.10 ± 0.06 for CL, 0.12 ± 0.08 for BW, 0.11 ± 0.07 for BL, 0.11 ± 0.07 for TL, and 0.03± 0.04 for ClL). The same trend was observed at six months for both rearing methods (bulk and individual rearing) whereby h^2 estimated from female data was

higher than those of males. However, the estimation made from individual rearing showed high and unacceptable standard errors. Notably, the proportion of c^2 to total variance for all estimation was small (ranged 0 to 0.08).

1.2 Correlation

Most of the phenotypic and genotypic correlations between growth related traits at 2, 5 and 6 months were high and significantly higher than 0, excepted for the correlations between ClL and other traits at five months calculated from male (0.33 to 0.52; Table 6).

Table 4 Mean, standard deviation, coefficient of variation (C.V.) for carapace length, body length, total length, claw length and body weight of Giant Freshwater Prawn at 2, 5 and 6 month old by sex and rearing condition; 1= individual rearing, 2= bulk rearing

			Carapace length				Body length			Total length			Claw length			Body weight		
Age	Sex	Rearing condition	No. of records	Mean (g)	S.D.	C.V. (%)	Mean (mm)	S.D.	C.V. (%)	Mean (mm)	S.D.	C.V. (%)	Mean (mm)	S.D.	C.V. (%)	Mean (mm)	S.D.	C.V. (%)
2 months	unsex	-	1,280	7.47	1.64	21.98	-	-	-	-	-	-	-	-	-	0.36	0.21	58.45
5 months	unsex		1,280	18.68	5.43	29.10	39.15	8.60	21.97	79.08	17.83	22.55	16.70	8.31	49.79	5.71	4.45	77.94
	male	-	640	19.10	5.71	29.89	39.67	8.92	22.48	80.45	18.35	22.81	17.72	9.88	55.79	6.32	5.20	82.30
	female	-	640	18.25	5.11	28.02	38.62	8.25	21.36	77.71	17.20	22.14	15.63	6.10	39.02	5.11	3.45	67.53
6 months	over all	-	1,824	20.63	5.69	27.60	42.24	9.04	21.40	84.65	19.46	22.99	19.23	10.47	54.43	7.85	8.52	108.56
	unsex	1	556	20.44	5.15	25.19	42.05	8.16	19.40	83.95	17.27	20.57	20.36	9.27	45.51	8.80	12.08	137.22
	unsex	2	1,268	20.71	5.92	28.56	42.32	9.40	22.21	84.96	20.34	23.95	18.65	10.99	58.94	7.44	6.33	85.13
	male	1	263	21.17	5.18	24.47	42.78	7.61	17.79	85.60	16.40	19.15	22.72	10.34	45.51	9.19	11.86	129.12
	female	1	293	19.78	5.04	25.46	41.40	8.58	20.73	82.47	17.92	21.73	18.16	7.51	41.36	8.46	12.28	145.20
	male	2	632	21.42	6.47	30.22	42.87	9.85	22.98	86.44	21.33	24.67	21.35	13.05	61.12	8.27	7.68	92.88
	female	2	636	20.00	5.21	26.06	41.78	8.90	21.31	83.48	19.22	23.02	15.93	7.51	47.12	6.60	4.46	67.53

Table 5 Heritability (h^2 ± S.E.) and ratio of common environmental variance/total variance (c ratio ± S.E.; in parentheses) for carapace length, body length, total length, claw length and body weight of Giant Freshwater Prawn at 2, 5 and 6 months after reaching the PL stage, by sex and rearing condition; 1= individual rearing, 2 = bulk rearing

Age	Sex	Rearing condition	Carapace length	Body length	Total length	Claw length	Body weight
2 months	unsex	-	0.35±0.15 (0.033±0.019)	-	-	-	0.26±0.13 (0.028±0.018)
5 months	unsex		0.12±0.75 (0.022±0.017)	0.18±0.12 (0.018±0.016)	0.16±0.08 (0.007±0.012)	0.02±0.05 (0.033±0.022)	0.11±0.08 (0.043±0.024)
	male	-	0.10±0.06 (0.000±0.000)	0.11±0.07 (0.003±0.017)	0.11±0.07 (0.000±0.000)	0.03±0.04 (0.000±0.000)	0.12±0.08 (0.011±0.022)
	female	-	0.26±0.16 (0.080±0.050)	0.40±0.17 (0.029±0.035)	0.47±0.18 (0.007±0.029)	0.29±0.16 (0.053±0.041)	0.28±0.17 (0.104±0.058)

Table 5 (Continued)

Age	Sex	Rearing condition	Carapace length	Body length	Total length	Claw length	Body weight
6 months	unsex	1	0.24±0.13	0.20±0.11	0.18±0.10	0.15±0.10	0.20±0.71
			(0.000±0.000)	(0.000±0.000)	(0.000±0.000)	(0.000±0.000)	(0.000±0.000)
		2	0.15±0.08	0.16±0.08	0.15±0.08	0.01±0.02	0.07±0.04
			(0.006±0.012)	(0.001±0.010)	(0.003±0.011)	(0.015±0.023)	(0.003±0.010)
	male	1	0.10±0.10	0.03±0.06	0.01±0.05	0.05±0.09	0.20±0.49
			(0.000±0.000)	(0.000±0.000)	(0.000±0.000)	(0.000±0.000)	(0.000±0.000)
	female	1	0.42±0.19	0.46±0.20	0.47±0.20	0.27±0.15	0.26±3.09
			(0.000±0.000)	(0.000±0.000)	(0.000±0.000)	(0.000±0.000)	(0.000±0.000)
	male	2	0.05±0.05	0.05±0.05	0.06±0.06	0.00±0.00	0.03±0.04
			(0.008±0.021)	(0.014±0.022)	(0.015±0.023)	0.011±0.017)	(0.002±0.018)
	female	2	0.54±0.19	0.52±0.19	0.51±0.19	0.10±0.07	0.33±0.14
			(0.000±0.000)	(0.000±0.000)	(0.000±0.000)	(0.010±0.071)	(0.000±0.000)

Table 6 Phenotypic correlation(r_P; below diagonal) and genotypic correlation (r_G above diagonal) for carapace length (CL), body length (BL), total length (TL), claw length (ClL) and body weight (BW) of Giant Freshwater Prawn at 2, 5 and 6 months old after reaching the PL stage by sex

Age	Sex group	Traits					
2 months	unsex		CL	BL	TL	ClL	BW
		CL	-	-	-	-	0.93
		BL	-	-	-	-	-
		TL	-	-	-	-	-
		ClL	-	-	-	-	-
		BW	0.91	-	-	-	-
5 months	male		CL	BL	TL	ClL	BW
		CL	-	0.98	0.89	0.33	0.92
		BL	0.96	-	0.96	0.44	0.96
		TL	0.92	0.95	-	0.52	0.95
		ClL	0.83	0.84	0.84	-	0.42
		BW	0.87	0.88	0.87	0.81	-
5 months	female		CL	BL	TL	ClL	BW
		CL	-	0.97	0.95	0.77	0.90
		BL	0.95	-	0.98	0.80	0.92
		TL	0.91	0.97	-	0.80	0.91
		ClL	0.86	0.86	0.83	-	0.71
		BW	0.83	0.89	0.90	0.78	-
6 months	male		CL	BL	TL	ClL	BW
		CL	-	0.99	0.96	0.82	0.96
		BL	0.97	-	0.97	0.79	0.97
		TL	0.96	0.96	-	0.74	0.99
		ClL	0.86	0.84	0.83	-	0.74
		BW	0.73	0.72	0.71	0.67	-
6 months	female		CL	BL	TL	ClL	BW
		CL	-	0.98	0.97	0.79	0.58
		BL	0.97	-	0.98	0.79	0.56
		TL	0.95	0.97	-	0.78	0.53
		ClL	0.83	0.82	0.81	-	0.52
		BW	0.58	0.57	0.57	0.54	-

2. Experiment 2: Comparison of growth between offspring of Giant Freshwater Prawn selected from different maturing batches

The first maturation occurred at 6-7 months after reaching the PL stage and involved 18.18 to 21.43% of the females in each family (mean = 19.46 ± 1.81). The second maturing batch, which was observed in month 8, included 31.49 ± 1.14% of all females (range 29.79 to 33.68%), whereas 14.11 ± 0.91% matured in month 9 (range 12.63 to 16.00%) and 17.66 ± 2.31% (range 14.74 to 22.67%) matured during months 10 to 11. However, during months 10 to 11, some females had not reached maturation. The proportion (%) of maturing female Giant Freshwater Prawn in each family is shown in Appendix Table A1.

The matured females were larger than the immature individuals of the same sex, as shown by the significantly higher ($P < 0.001$; Table 7) CL, BL, and TL for every maturing batch. It is likely that there was a threshold size for maturation when the females reached maturation at CL (26.22 to 26.65 mm), BL (53.18 to 53.96 mm), and TL (103.88 to 108.30 mm; Table 7).

The Monte-Carlo randomisation test revealed significant differences in the mean CL and BW among the offsprings of the selected females from different batches (Table 8). Whereas the offspring of the first batch were the heaviest in BW, their CL was longer than those of the second and fourth batches but was not significantly different from that of the third batch.

The offspring of the first-maturing batch grew at least 6.00% (CL) and 14.94% (BW) faster than the offspring of random females ($P = 0.000$) and at least 53.28% (CL) and 127.27% (BW) faster than those of late-maturing females ($P = 0.000$).

Table 7 Mean carapace length (CL), body length (BL) and total length (TL) of the mature and immature female Giant Freshwater Prawns in each maturing batch. Means (column for the same maturing batch superscripted with different letters) are significantly different ($P < 0.001$; n = sample size)

Maturing Batch	Maturation	n	CL	BL	TL
1	Mature	312	26.22^{a}	53.56^{a}	105.46^{a}
	Immature	309	21.36^{b}	44.49^{b}	87.55^{b}
2	Mature	277	26.20^{a}	53.96^{a}	108.30^{a}
	Immature	197	21.98^{b}	44.49^{b}	87.89^{b}
3	Mature	142	26.31^{a}	53.52^{a}	103.88^{a}
	Immature	104	24.18^{b}	49.40^{b}	96.57^{b}
4	Mature	20	26.65^{a}	53.18^{a}	105.59^{a}
	Immature	98	23.27^{b}	47.06^{b}	91.77^{b}

Table 8 Mean carapace length (CL) and body weight (BW) of Giant Freshwater Prawn offspring of the selected females from four different maturing batches. Means in a column superscripted with different letters are significantly different ($P < 0.05$). The upper and lower values provided by the Monte-Carlo randomisation test are offset in parentheses.

Maturing Batch	Number of full-sib	CL (mm)	BW (gm)
1	17	10.76 ± 0.10^{a}	1.00 ± 0.02^{a}
		(10.49 – 11.03)	(0.94 – 1.06)
2	18	9.66 ± 0.10^{b}	0.79 ± 0.02^{b}
		(9.39 – 9.93)	(0.74 – 0.84)
3	10	10.39 ± 0.13^{a}	0.86 ± 0.03^{b}
		(10.08 – 10.70)	(0.79 – 0.92)
4	1	7.02 ± 0.43^{d}	0.44 ± 0.08^{c}
		(6.39 – 7.02)	(0.33 – 0.44)

3. Experiment 3: Estimation of a genetic trend of Giant Freshwater Prawn selected for growth for two generations

3.1 Descriptive statistics, heritability and correlation

Descriptive statistics for carapace length, body length, total length, claw length and body weight at 7 months of age are shown in Table 9. It was obvious that male prawns had higher BW than females while means of CL and BL were similar but the variance was always larger for males in all traits. ClL of male was significantly higher than that of females (P<0.01). Most of the phenotypic correlations of all growth related traits at 7 months were statistically significant and tremendously varied (Table 10) both in females (mean 0.62 ± 0.11; ranged 0.46-0.85) and males (mean0.69 ± 0.12; ranged 0.40-0.85).

Heritability for growth trait estimated from the individual data across three generations is shown in Table 10. The heritability for CL and BL at 7 months old of male prawns were nil (0.00 ± 0.00 and 0.00 ± 0.00, respectively) while those of the females (0.03 ± 0.15 and 0.09 ± 0.22 respective to CL and BL) were statistically significant (P<0.01). The genotypic correlations of all growth related traits at 7 months varied between 0.43-0.93 in females and 0.34-0.69 in males (Table 10).

Table 9 Mean, standard deviation (S.D.), coefficient of variation (C.V.) for carapace length (CL), body length (BL), claw length (ClL), total length (TL) and body weight (BW) of Giant Freshwater Prawn at 7 months of age by sex and generation, N = sample size

Gen1	Females				Males			
	N	Mean	S.D.	C.V.	N	Mean	S.D.	C.V.
CL	621	23.68	5.37	22.66	320	23.97	7.30	30.47
BL	621	46.08	9.11	18.57	320	46.85	11.78	25.15
ClL	352	21.22	8.17	38.52	269	31.85	24.78	77.82
TL	621	96.14	19.16	19.93	319	90.31	27.45	30.39
BW	621	11.12	6.12	55.01	320	12.56	12.41	98.82
Gen2	Females				Males			
	N	Mean	S.D.	C.V.	N	Mean	S.D.	C.V.
CL	606	24.56	5.27	21.45	357	25.71	6.85	26.62
BL	606	46.83	7.92	16.91	357	49.10	15.60	31.77
ClL	518	23.82	7.85	32.97	318	32.92	15.91	48.32
TL	543	91.00	15.91	17.48	357	92.96	22.58	24.29
BW	606	11.02	4.26	38.66	357	13.91	9.36	67.27
Gen3	Females				Males			
	N	Mean	S.D.	C.V.	N	Mean	S.D.	C.V.
CL	155	24.57	3.40	13.84	177	25.75	7.35	28.53
BL	155	48.72	6.26	12.85	177	48.90	11.02	22.53
ClL	145	24.42	5.39	22.05	148	31.67	17.83	56.30
TL	155	98.07	14.20	14.48	177	99.66	22.85	22.93
BW	155	9.54	3.54	37.13	177	11.96	9.72	81.27
Overall	Females				Males			
	N	Mean	S.D.	C.V.	N	Mean	S.D.	C.V.
CL	1382	24.17	5.15	21.33	854	25.07	7.17	28.59
BL	1382	48.05	8.38	17.45	854	48.22	13.40	27.80
ClL	1195	22.73	7.86	34.58	735	32.28	19.94	61.78
TL	1319	94.25	17.55	18.62	853	93.36	24.77	26.54
BW	1382	10.90	5.14	47.13	854	13.00	10.69	82.23

Table 10 The heritability ± S.E. of the growth traits (in the diagonal) phenotypic (below the diagonal) and genetic (above the diagonal) correlations of carapace length (CL), body length (BL), claw length (ClL), total length (TL) and body weight (BW) of Giant Freshwater Prawn at 7 months of age by sex

Females	CL	BL	ClL	TL	BW
CL	0.03±0.15	0.57	0.43	0.69	0.83
BL	0.60	0.09±0.22	0.93	0.68	0.75
ClL	0.51	0.46	0.08±0.01	0.44	0.67
TL	0.58	0.85	0.63	0.13±0.23	0.78
TW	0.66	0.69	0.67	0.51	0.00±0.00
Males	CL	BL	ClL	TL	BW
CL	0.00±0.00	0.75	0.31	0.37	0.69
BL	0.72	0.00±0.00	0.36	0.42	0.57
ClL	0.71	0.60	1.13±0.95	0.64	0.46
TL	0.77	0.70	0.40	0.51±0.37	0.34
TW	0.85	0.70	0.72	0.71	0.00±0.00

Table 11 Mean selection differentials (*S*) and selection intensities (*i*) in each generation

Generation	Mean BL Female ±S.D. (mm)	Mean BL Selected Female ±S.D. (mm)	*S* (mm)	*i*
Gen1	46.08±9.11	60.43±6.57	14.35	1.58
Gen2	46.83±7.92	60.36±3.47	13.53	1.71
Gen3	48.72±6.26	-	-	-

3.2 Selection differentials and selection intensities

Mean selection differentials (*S*) and selection intensities (*i*) in each generation are presented in Table 11. The selection intensities were relatively high, corresponding to about 2% of the population being selected.

3.3 Predicted genetic response

Average estimated breeding values per generation are presented in Table 12. A positive genetic response for body length was observed in only females (on average, 0.37 mm. per generation) while it was not significant different from zero in males. The same trend was observed for other growth traits except for total length wherein male also showed a positive response.

3.4 Inbreeding

The parents used to form the base population were assumed to be un-related, thus the average inbreeding coefficient for the first generation was zero. After 2 generations of selection, the inbreeding coefficient was 1.03 % and the average rate of inbreeding was 0.91 % per generation (Table 13).

Table 12 Estimated mean breeding values (EBV; standard deviation in parentheses) for carapace length (CL), body length (BL), total length (TL), claw length (ClL) and body weight (BW) in each generation of Giant freshwater Prawn at 7 months of age by sex

Females	CL	BL	TL	ClL	BW
Gen1	0.015	0.037	0.148	0.107	1.44×10^{-7}
	(0.112)	(0.451)	(0.728)	(1.444)	(9.45×10^{-7})
Gen2	0.058	0.065	0.724	0.184	4.71×10^{-7}
	(0.134)	(0.473)	(0.703)	(3.587)	(9.82×10^{-7})
Gen3	0.145	0.781	1.401	2.568	9.22×10^{-7}
	(0.078)	(0.459)	(0.720)	(1.522)	(8.49×10^{-7})
Males	CL	BL	TL	ClL	BW
Gen1	0.000	0.000	-0.433	0.693	1.03×10^{-8}
	(0.000)	(0.000)	(10.542)	(10.542)	(9.82×10^{-7})
Gen2	0.000	0.000	0.055	2.982	3.90×10^{-7}
	(0.000)	(0.000)	(5.022)	(5.022)	(5.40×10^{-7})
Gen3	0.000	0.000	-0.508	7.062	9.75×10^{-8}
	(0.000)	(0.000)	(6.005)	(7.070)	(6.44×10^{-7})

Table 13 The inbreeding coefficient and rate of inbreeding in each generation of selected lines of Giant Freshwater Prawn

Generation	Inbreeding coefficient	Rate of inbreeding
Gen0	0.000	0.000
Gen1	0.000	0.000
Gen2	0.015	0.015
Gen3	0.037	0.022
Average	0.013	0.009

Discussion

1. Estimation of heritability, h^2 and correlations of growth traits of Giant Freshwater Prawn before sex differentiation and morphotype differentiation at two different rearing conditions

In this study, the effects of embryonic development period (time during egg incubation at female bellies) and larval development periods (time between hatching to post-larva) were not included in the model for h^2 estimation because of the indifference of those traits between families. Our observation was supported by Malecha *et al*. (1984), Meewan (1993) and Uraiwan *et al*. (2002).

Although the h^2 of growth related traits of crustacean tremendously varied, but the majority indicated intermediate heritabilities in the range of 0.20 to 0.84 [e.g., in *Penaeus vannamei* (Carr *et al*., 1997; Argue *et al*., 2002; Perez-Rostro and Ibarra, 2003; Gitterle *et al*., 2005; Castillo-Juárez *et al*., 2007), *P. japonicus* (Hetzel *et al*., 2000), *P monodon* (Benzie *et al*., 1997; Jarayabhand *et al*., 1998; Kenway *et al*., 2006)]. This was true for the estimations made at two months (mixed sex) and five and six months estimated from females Giant Freshwater Prawn in the present study, despite of low h^2 estimates in males. The h^2 estimates of growth related traits of Giant Freshwater Prawn reported here together with those reported by Malecha *et al*. (1984; $h^2 = 0.35 \pm 0.15$), Uraiwan *et al*. (2002; $h^2 = 0.254 \pm 0.080$ and 0.272 ± 0.210) and a report on the high additive genetic variance of Giant Freshwater Prawn based on a diallel crossing (Thanh *et al*., 2010) may imply that the h^2 for growth of Giant Freshwater Prawn is moderate to high in general.

Heritability of growth related traits of mixed sex Giant Freshwater Prawn declined with age (e.g., h^2_{F+M} for carapace length at two and five months of PL stage were 0.35 ± 0.15 and 0.12 ± 0.75, respectively) which was in accordance with those reported by Malecha *et al* (1984); Meewan (1993) and Uraiwan *et al*. (2002). This may be partly explained by an aggressive behavior of particularly male prawn which differentiates into three morphotypes (Meewan 1993; Ranjeet and Kurup 2002;

Karplus and Hulata 2005; Thanh *et al.*, 2009) at about five months after reaching the PL stage. The differentiation of morphotypes enhanced variation in growth capacity especially of males.

Heritability of growth related traits in Giant Freshwater Prawn was sexually dimorphic, high in females and low in males (e.g., $h^2$of carapace length at five months after PL stage was 0.10 ± 0.06 for male and 0.26 ± 0.16 for female). A similar finding was also reported by Malecha *et al*. (1984). Likewise, Thanh *et al*. (2009) reported that growth variation, and relative frequencies among male morphotypes of Giant Freshwater Prawn masked the among strains growth difference for male while it was pronounced in females. Despite the limited numbers of studies, sexual dimorphic heritabilities were also reported for other crustacean, [for example, h^2 for growth related traits was higher for the immature male than female in Red Swamp Crawfish, *Procambarus clarkii* (Girard), Lutz and Woltress, 1989], and fishes (El-Ibiary and Joyce, 1978; Crandell and Gall, 1993).

Despite the well documented advantage of BLUP over the ANOVA based analysis (Gjedrem and Olesen, 2005), the application of BLUP to aquatic animals for only beeb well documented during the last decade [e.g., Chinook salmon, *Oncorhynchus tshawytscha* (Winkelman and Peterson, 1994a,b) Nile tilapia, *Oreochromis niloticus* (Gall and Bakar, 2002; Charo-Karisa *et al*., 2006), Pacific white shrimp, *Litopenaeus vannamei* (Gitterle *et al*., 2005; Castillo-Juárez *et al*., 2007), Coho salmon, *Oncorhynchus kisutch* (Neira *et al*., 2006), common carp, *Cyprinus carpio* L. (Wang and Li, 2007), Atlantic salmon, *Salmo salar* (Powell *et al*., 2008)]. The present study was the first attempt to employ BLUP for estimating of h^2 in the Giant Freshwater Prawn. According to the prior information obtained from ANOVA (data not shown), major factors affecting the present estimation of h^2 were hatching months, tanks, effect of sex at five and six months, and rearing conditions (bulk and individual rearing). Therefore, these factors were incorporated into the model in order to separate the effects of these factors from genetic effect (Gjerdrem and Olesen, 2005) and thus enhanced precision of the estimation. As a consequence, the standard errors (S.E.) of the current estimation was smaller than S.E. of the

ANOVA based estimation of the same trait [e.g., $h^2$of female body length at five months without incorporating sex as a fixed effects was 0.06 ± 0.05 (Uraiwan *et al*., 2002) comparing to h^2 of 0.40 ± 0.17 in this study]. It is noteworthy that, in our study, the variation of hatching time (months) was large (covering a period of 5 months), but with the BLUP approach, precision of the study was improved over the studies with less variation of spawning time [e.g., within a week (Malecha *et al*., 1984; Meewan, 1993)].

It was our concern that the number of families (16 full-sib families) used in the present study was smaller than those employed in a majority of the studies [e.g., 50 full and half-sib families nested with 16 sires (Malecha *et al*., 1984); 430 full-sib family in Pacific white shrimp, *Litopenaeus vannamei* (Gitterle *et al*,. 2005)]. However, it meets the range of the recommended numbers [e.g., 20 to 30 families (Robertson 1959); n = $2/h^2$ for full-sib families and n = $4/h^2$ for half-sib families (Falconer and Mackay 1996)]. The weakness dues to a consequence of small number of families could be partly compensated by relatively large family size (80 prawns/family for the pooled reared Giant Freshwater Prawn and 40 prawns/family for the individually reared Giant Freshwater Prawn). As a result of this study, regarding standard error (S.E.), obtained better precision of h^2 of female and male body weight at five months (h^2 = 0.28 ± 0.17 and 0.12 ± 0.08 respectively) than Malecha *et al.* (1984) based on 50 full-sib families (h_S^2= 0.34 ± 0.24 and – 0.24 ± 0.11 for female and male respectively).

Based on our experimental design that each family was reared in duplicate, our animal model considered the random common environmental effect in addition to the random additive genetic effect and they were assumed to have no covariation. As such, the effect of common environment was theoretically removed and hence resulted in a more congruent estimation (Winkelman and Peterson, 1994a). Thus, h^2 in this present study can be considered without any confounding effect of common environmental effect (c^2). However, it is of concern that the limited population size and the small genetic relationship available for the analyses may cause high SEs of the h^2 estimates in the present study.

2. Growth comparison of Giant Freshwater Prawn offspring selected from different maturing batches of females

This study provided empirical data supporting the estimation by Doyle *et al*. (1983) that the selection of gravid females from the first maturing batch results in fast growing offspring relative to those obtained from selection on the late maturing females. Hence, the result demonstrated that this method of broodstock recruitment in Thailand was incorrect. The farmers preferred to select brooders from late maturing females because they were relatively large. However, the present study showed that the late maturing females grew slower than early maturing females and produced slower growing offspring. Therefore, the broodstock practise resulted in a negative selection for growth. Our result is supported by the study reported by Ra'anan *et al*. (1991), who showed that female Giant Freshwater Prawn matured when they reached a threshold size range. Such a threshold was clearly shown in the present study, regardless of age, where first maturing females had carapace lengths of 26.22 to 26.65 mm, body lengths of 53.18 to 53.96 mm, and total lengths of 103.88 to 108.30 mm. The relationship of maturing time and size was also reported in other crustaceans, for example, the fiddler crab *Uca cumulanta* (Pralon and Negreiros-Fransozo, 2008), and implied that fast growing individuals matured earlier. In addition, the correlation between growth rate and maturing time has been well documented in fishes, for example, Atlantic cod, *Gadus morhua L*. (Holdwal and Beamish, 1985); cod, *Hippoglossus hippoglossus* (Godo and Hang, 1999); and Atlantic salmon, *Salmo salar* (Baum *et al*., 2004; Guerrero-Tortolero and Bromage, 2008).

Although growth of the offspring declined consistent with the first timing batch of maturing females, the offspring from the third batch of matured females exhibited better growth than those from the second and fourth batches. This may have been due to the presence of early, first maturing females missed during initial size selection and passed to a portion of the third female batch. After their eggs are discarded, females spawned again after two molting cycles, a period of four weeks (Okumura and Aida, 2001; FAO, 2002).

Although the maturation of Giant Freshwater Prawn females was relatively size specific (Ra'anan *et al.,* 1991), the frequency of the size distribution was normal, allowing for selection to be performed. A concern, however, was that using early maturing prawns as brooders might result in the selection of prawns that stop growing at an early age. Ra'anan *et al*. (1991) showed that even though the growth rates of females declined and did not cease after maturation, the faster growing females retained their growth potential as compared to the slow growing females.

3. Estimation of response to selection on growth of Giant Freshwater Prawn after two generations of selection

In general, male prawns grew faster than females and the variance of growth traits was larger because of hierarchical size differences among male morphotypes accompanied with aggressive behavior of dominated males. Furthermore, territorial behavior of dominated males coupled with variable sizes of male morphotypes has caused skewed distribution of body sizes towards smaller size (Malecha, 1983; Ranjeet and Kurup, 2002; Karplus and Hulata, 2005).

The h^2 estimated at 7 months old showed the same trend of sex differential characteristics as was observed in the previous estimation in base population of Giant Freshwater Prawn at five and six months of age (Kitcharoen *et al*., 2011), despite of a high standard error of the h^2 at 7 months old. The high standard error may be a result of confounding effects of body size differences between male morphotypes and different maturation status in females. Heritability was zero in body weight of female prawn which was probably affected by the excessive weight of eggs presented in some (gravid) mature females and not in the others. However, according to Kitcharoen *et al*. (2010) we should select the female at this time because the early maturing females grow faster than the late maturing. It is recommended that selection for growth should be performed on body length rather than on weight.

Phenotypic and genotypic correlation between BL and other traits were relatively high. It indicated a possibility that a selection for increasing body length in

females may result in increasing other growth related traits. The empirical data obtained in this study supported this statement wherein selection aiming at increasing body length in female also have a positive response in other traits, e.g., TL, CL and BW.

It is of concern that the correlation of carapace length and body length was high. Therefore, a selection for increasing body length or total length may increase carapace length which is undesirable.

Actually, the rotational mating scheme was effective in minimizing inbreeding. This mating scheme also used to be manageable even with limited facilities and it integrated well with the within-family selection method where a complete pedigree was maintained. However with the initial 16 families used in the rotational mating, inbreeding was expected to occur only after five generations but due to losses of some families, especially in the last generation, the inbreeding rate tremendously increased. Inbreeding was first detected in generation two in this study (F = 0.01 in generation 2 and 0.04 in generation 3). A similar result on inbreeding was reported in a selection line of Nile tilapia that started with 19 families where inbreeding was first observed in generation four (Bolivar and Newkirk, 2002). Overall, the increase in inbreeding coefficient in this study is acceptable regarding to a recommendations made by Tave (1999) (e.g., 5 to 10% /generation).

In fact, there are limitations in applying a within-family selection method, namely, it cannot be applied for traits with fatal measurement and it is inefficient for traits with low heritability (Bolivar *et al*., 1994; Bolivar and Newkirk, 2002). For this study, the within family selection was used to eliminate a large component of environmental variance common to member of a family resulted from variation in management and other environment. The success of managing the within-family selection in this study has confirmed that this selection method could still be useful in a breeding program with limited facilities as in the case of Uraiwan *et al*. (2002).

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The conclusions are drawn as follow:

1. The heritability estimates on growth traits of Giant Freshwater Prawn at 2 months of age (before sex differentiation) is high while the estimates at 5 and 6 months of age (before morphotypes differentiation) in two rearing conditions is high only in female prawns. The results illustrated that genetic improvement to increase growth rate of Giant Freshwater Prawn may be achieved through selection method, especially selection on female prawn.

2. A growth comparison of offspring between batches showed that the offspring of the females selected from the first batch were larger in carapace length (CL) and body weight (BW) than those from later batches.

3. The heritability estimates of growth traits of Giant Freshwater Prawn at 7 months of age were relatively high only in female prawns. To improve growth traits of Giant Freshwater Prawn, a selection performed in female prawns will result in positive genetic response in females.

Recommendations

1. High heritability of the growth related traits suggested that a simple mass selection, selection is up on individual's performance, may efficiently improve the growth related traits of the Giant Freshwater Prawn with a concern on high probability of inbreeding accumulation (Falconer and Mackay, 1996). Nonetheless, this method has been efficiently used in marine shrimp (e.g., 17% and 14% increase respective to survival rate and weekly weight gain, FCR reduced by 19%, over 11 generations of the Pacific white shrimp, *Litopenaeus vannamei*, De Donato *et al*.,

2005) and other aquatic organisms (e.g., 29% and 21% increase body weight in three generation of Kansas and Marion strains of channel catfish, *Ictalurus punctatus*, Rezk *et al*., 2003) with minimal adverse impacts from inbreeding.

2. A selection may be performed on mixed sexes prawns at two months after reaching a PL stage because heritability is relatively high. However, it is of concern that the growth profile of the selected individuals may change as they grow up. According to our unpublished data, high correlation between growth related traits at two months and seven months after reaching the PL stage (r = 0.70 for CL, $P < 0.001$; r = 0.60 for BW, $P < 0.001$) of the 135 tagged individually reared prawns were detected. Therefore, it is also recommended that a selection to improve growth of Giant Freshwater Prawn may be performed at two months after reaching the PL stage.

3. A selection performed after sex differentiation should be exerted on females, by selecting on either carapace length, body length or total length. According to the present study, body weight is not a good target for a selection due to relatively low h^2. It is of concern that the correlation of carapace length and other traits was high. Therefore, a selection for increasing body length or total length may increase carapace length which is undesirable.

4. High h^2 estimate for carapace length indicated a possibility of selection for smaller head. However, other growth related traits may also decline as suggested by high positive correlation between these traits and carapace length.

5. The offspring of the females selected from the first batch were larger in carapace length (CL) and body weight (BW) than those from the later batches. Therefore, selection on maturing female should be performed on the first batch of maturation.

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APPENDICES

Appendix A

Maturation of giant freshwater prawn female results

Appendix Table A1 The proportion (%) of maturing female Giant Freshwater Prawn in each family of 2nd experiment.

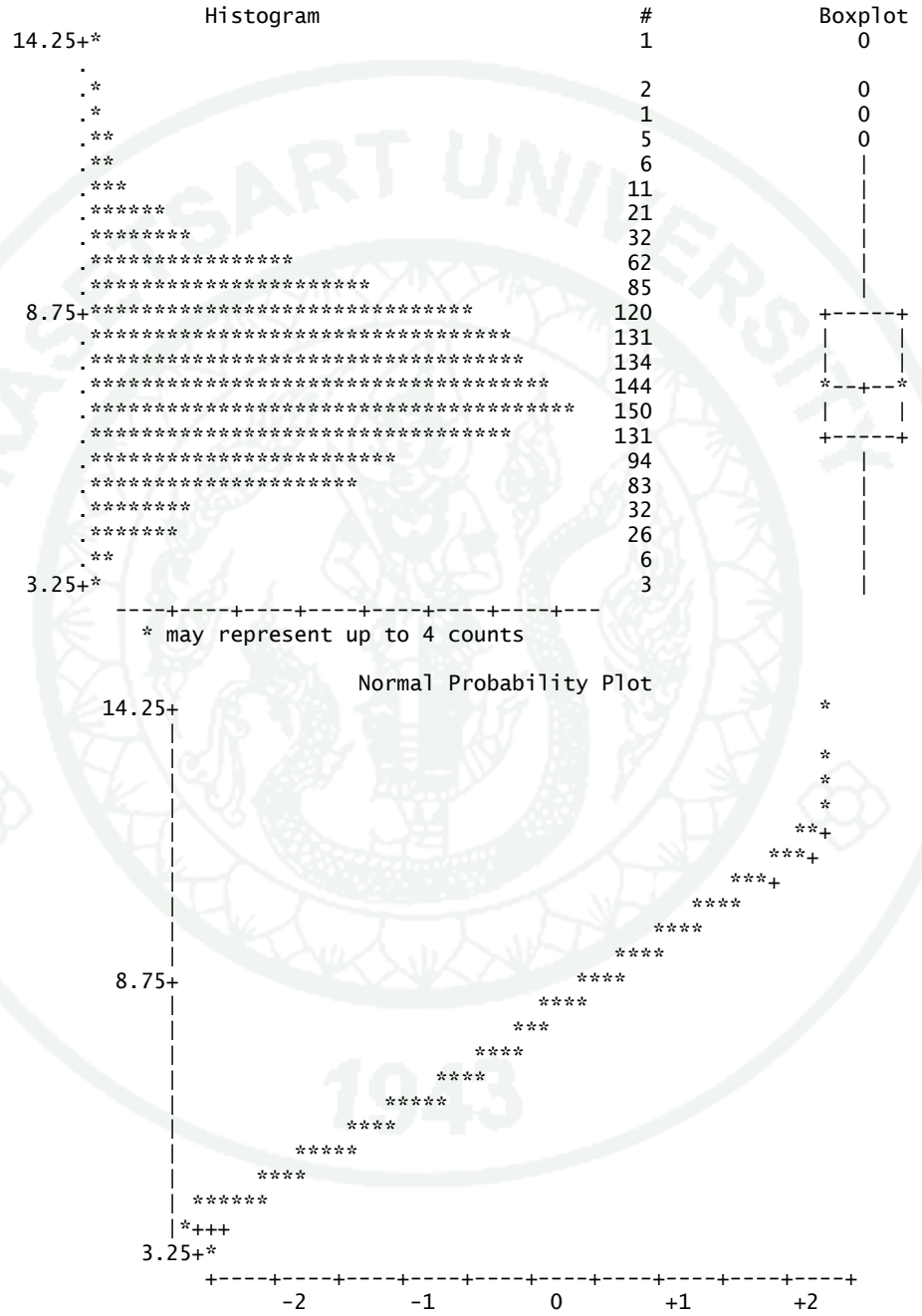
Family	1st batch	2nd batch	3rd batch	4th batch
1	20.35	33.33	16.00	22.67
2	20.65	32.94	15.29	21.18
3	20.69	30.53	12.63	16.84
4	18.60	30.00	14.44	21.11
5	20.78	33.68	12.63	16.84
6	18.48	32.26	13.98	15.05
7	17.10	30.53	13.68	15.79
8	21.43	31.87	14.29	19.78
9	20.52	30.34	14.61	16.85
10	20.43	30.43	13.59	16.30
11	20.21	31.58	13.68	16.84
13	20.25	31.58	14.74	14.74
14	14.29	31.37	12.75	15.69
15	18.18	31.82	14.20	19.32
17	19.15	29.79	14.36	17.02
18	20.31	31.87	14.84	16.48
Mean	19.46	31.49	14.11	17.66
S.D.	1.81	1.14	0.91	2.31

Appendix B

Statistical methods for genetic evaluation

The UNIVARIATE Procedure for Genetic Analysis

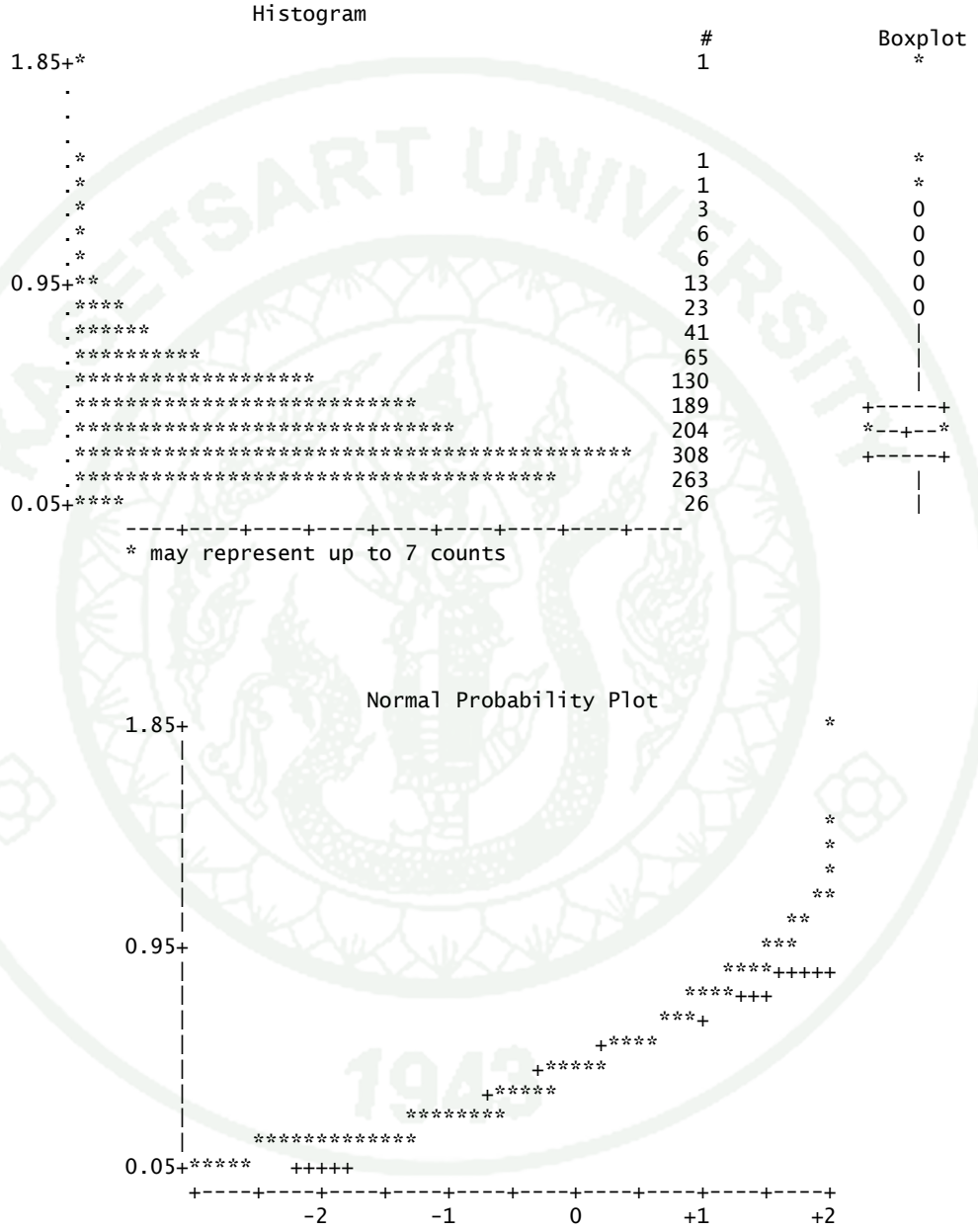
Variable: CL2M



Appendix Figure B1 Histogram, box plot and normal probability plot for CL at 2 months old.

The UNIVARIATE Procedure for Genetic Analysis

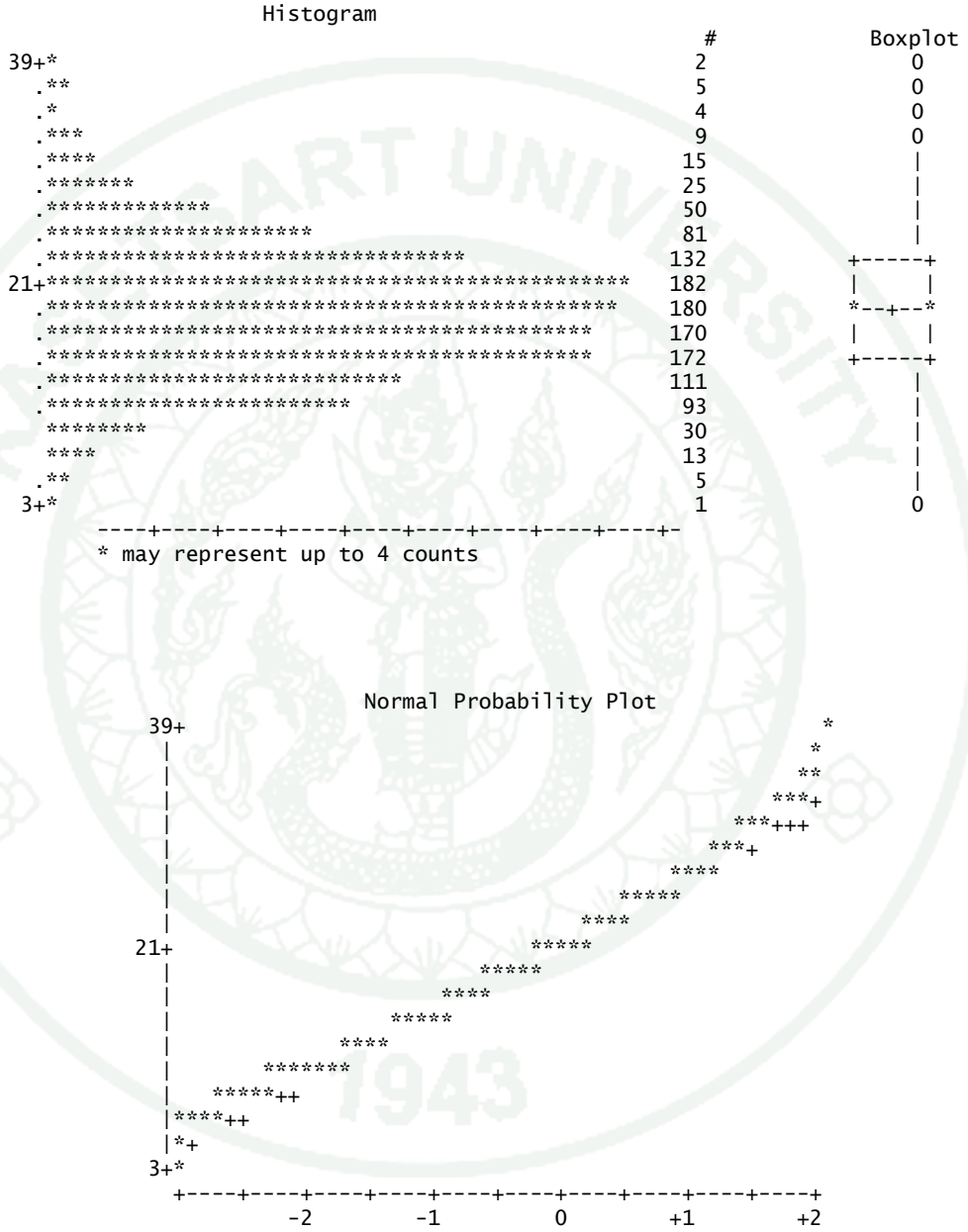
Variable: BW2M



Appendix Figure B2 Histogram, box plot and normal probability plot for BW at 2 months old.

The UNIVARIATE Procedure for Genetic Analysis

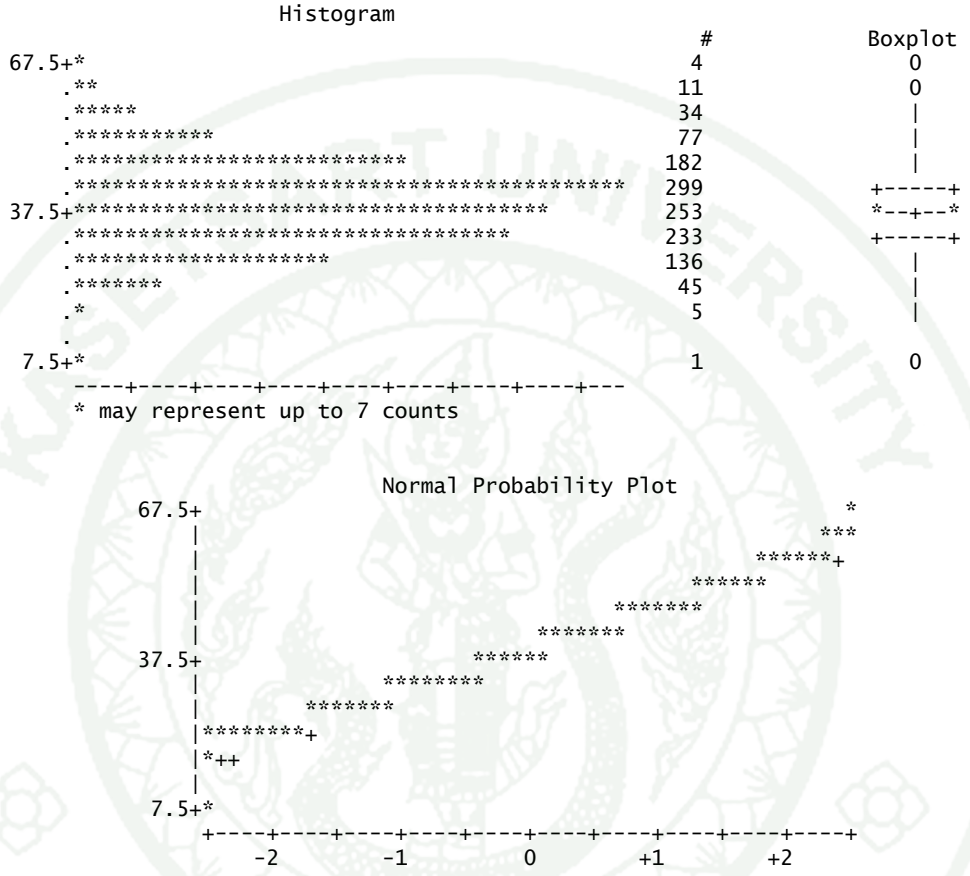
Variable: CL5M



Appendix Figure B3 Histogram, box plot and normal probability plot for CL at 5 months old.

The UNIVARIATE Procedure for Genetic Analysis

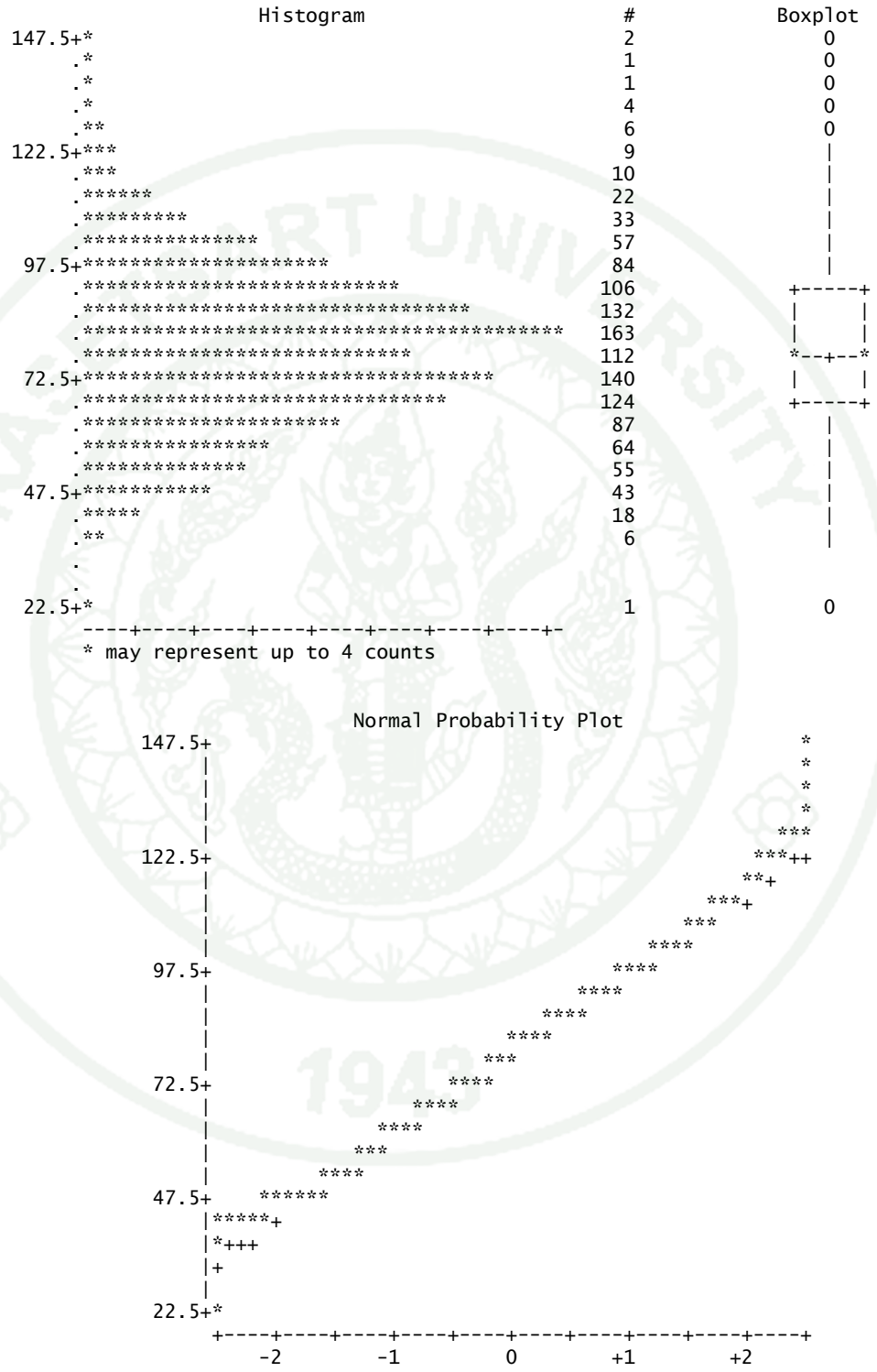
Variable: BL5M



Appendix Figure B4 Histogram, box plot and normal probability plot for BL at 5 months old.

The UNIVARIATE Procedure for Genetic Analysis

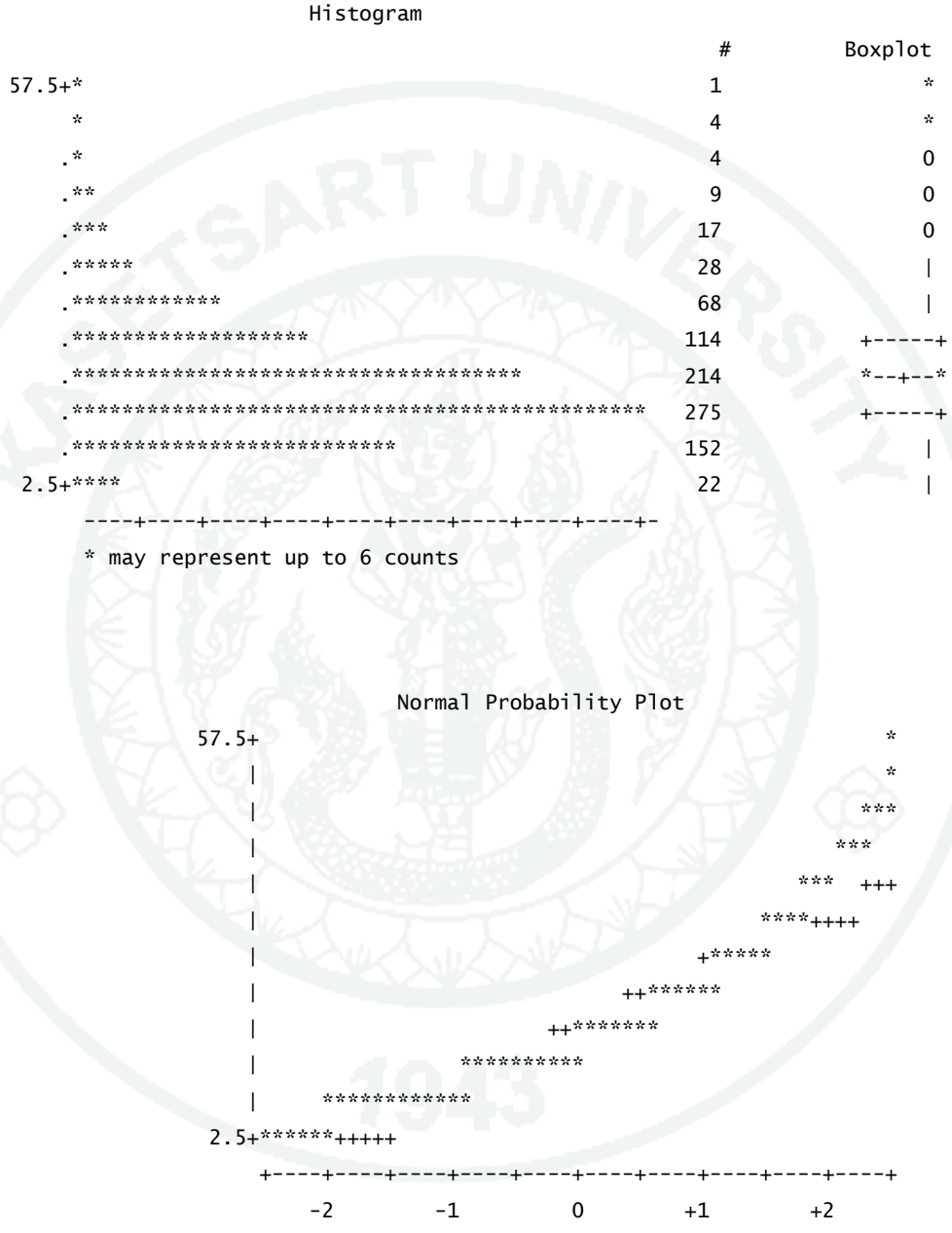
Variable: TL5M



Appendix Figure B5 Histogram, box plot and normal probability plot for TL at 5 months old.

The UNIVARIATE Procedure for Genetic Analysis

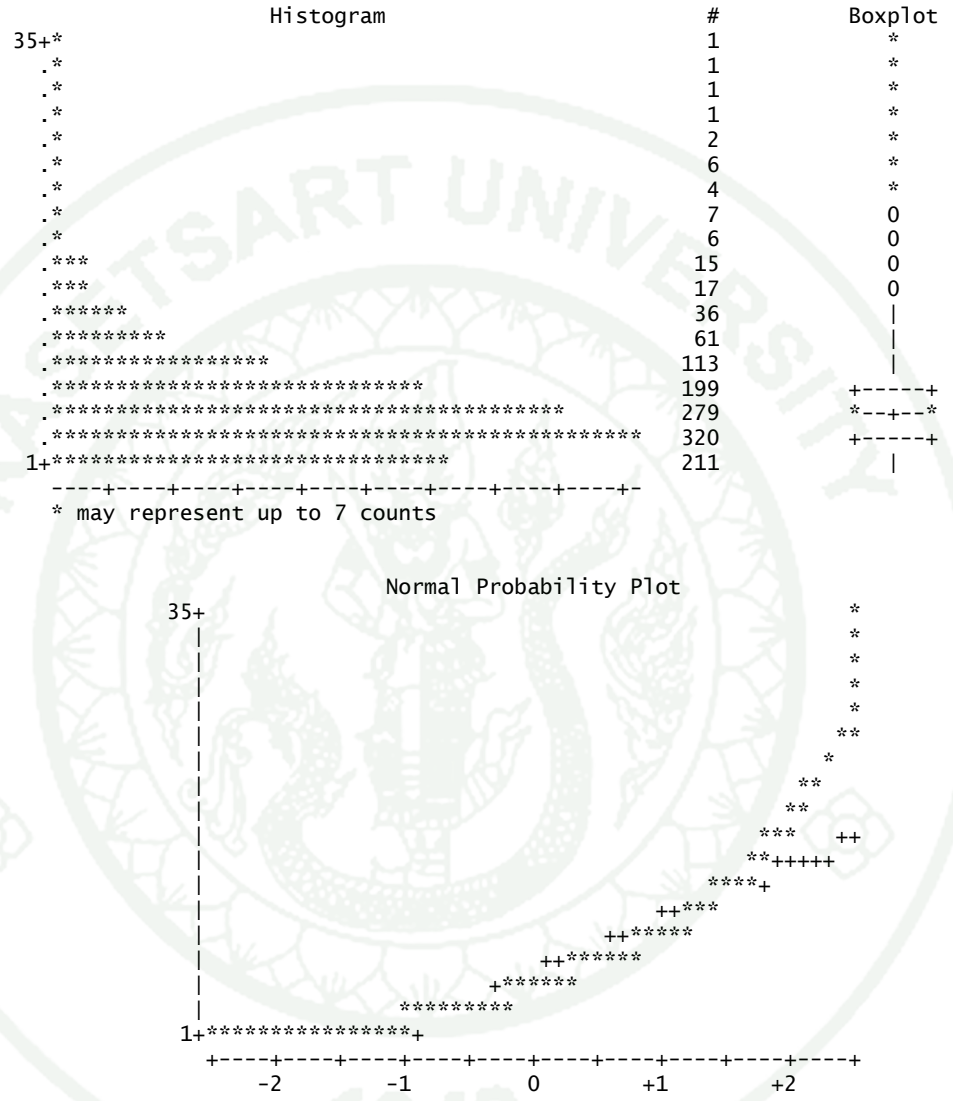
Variable: ClL5M



Appendix Figure B6 Histogram, box plot and normal probability plot for ClL at 5 months old.

The UNIVARIATE Procedure for Genetic Analysis

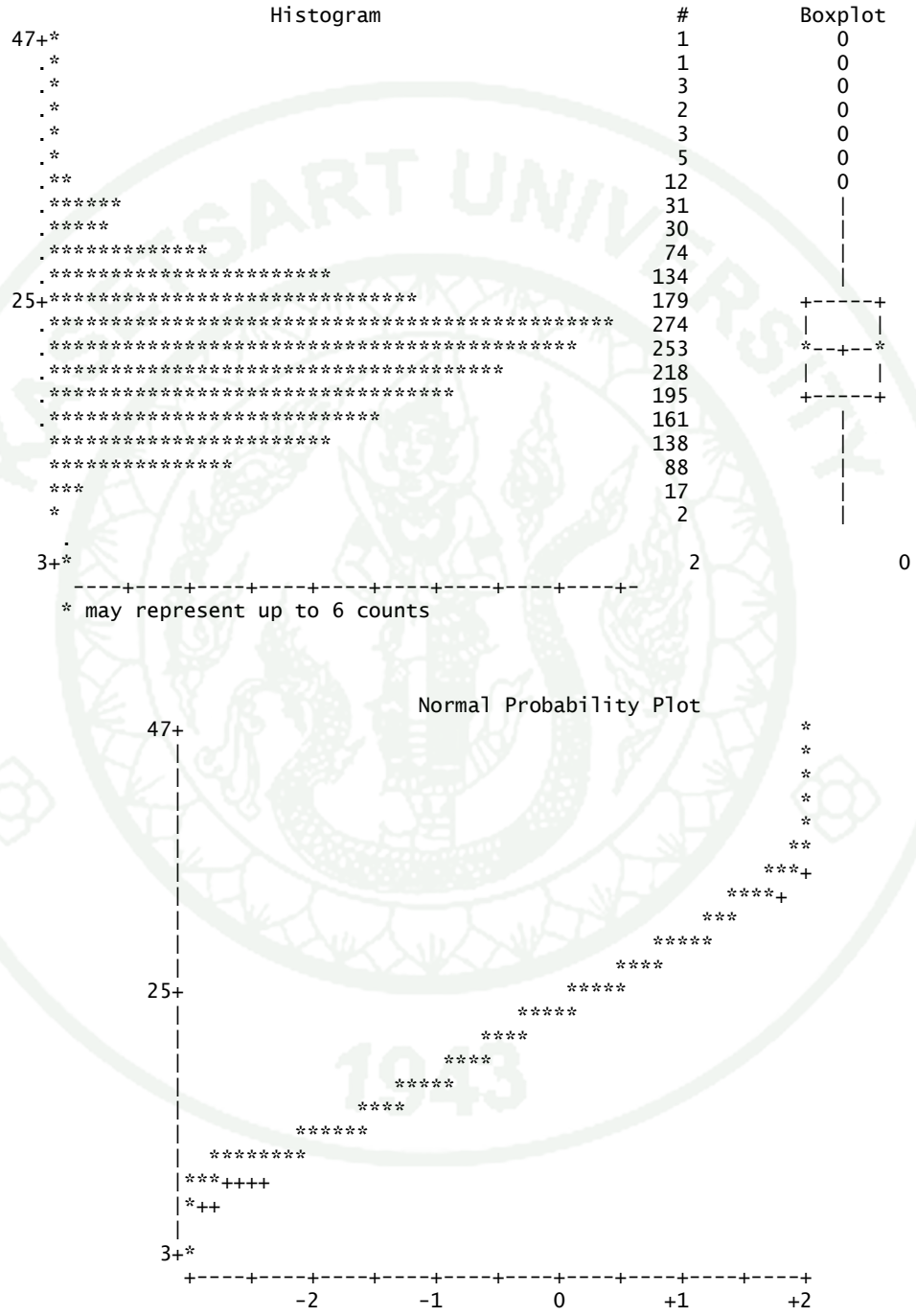
Variable: BW5M



Appendix Figure B7 Histogram, box plot and normal probability plot for BL at 5 months old.

The UNIVARIATE Procedure for Genetic Analysis

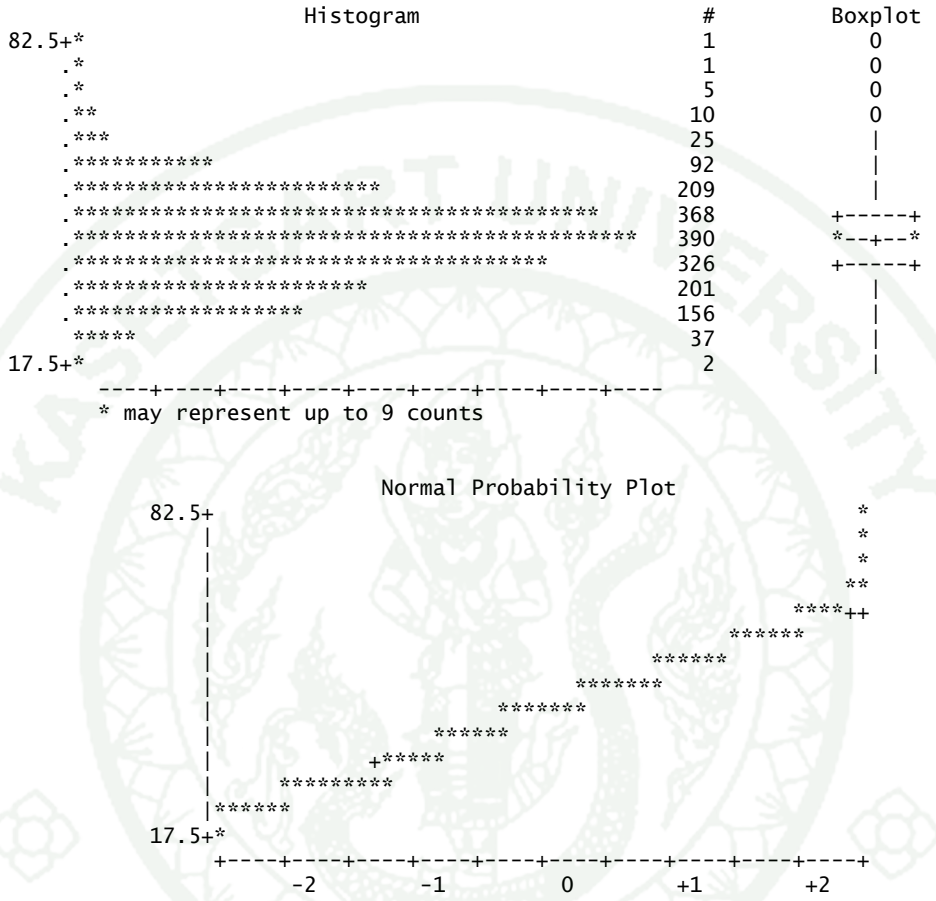
Variable: CL6M



Appendix Figure B8 Histogram, box plot and normal probability plot for CL at 6 months old.

The UNIVARIATE Procedure for Genetic Analysis

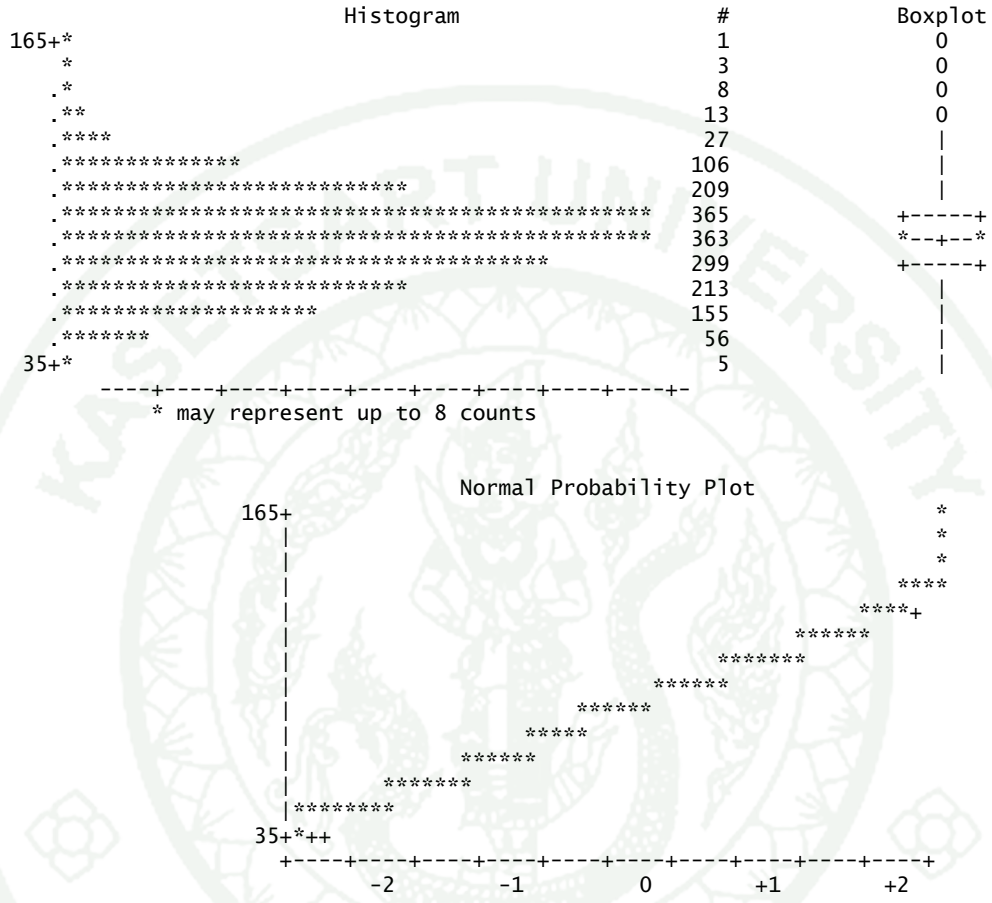
Variable: BL6M



Appendix Figure B9 Histogram, box plot and normal probability plot for BL at 6 months old.

The UNIVARIATE Procedure for Genetic Analysis

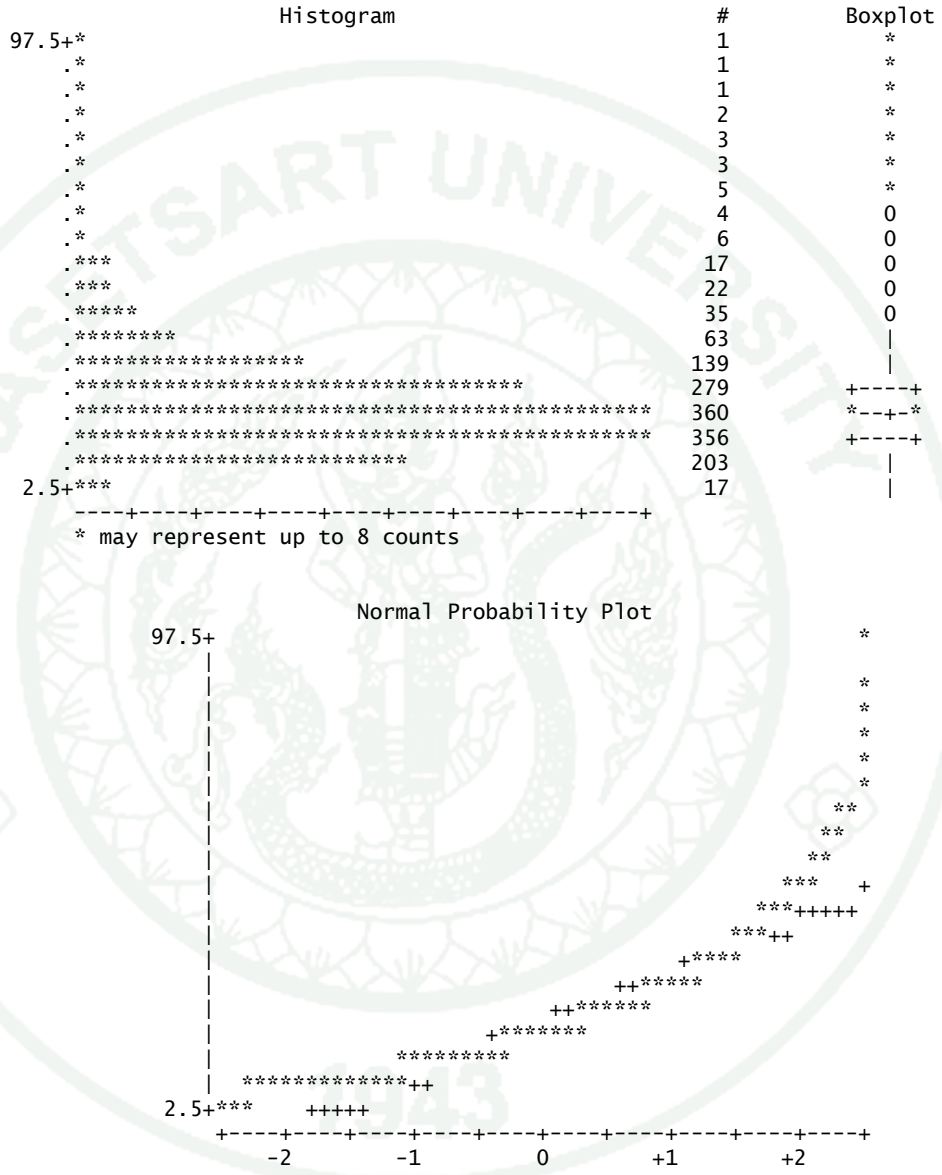
Variable: TL6M



Appendix Figure B10 Histogram, box plot and normal probability plot for TL at 6 months old.

The UNIVARIATE Procedure for Genetic Analysis

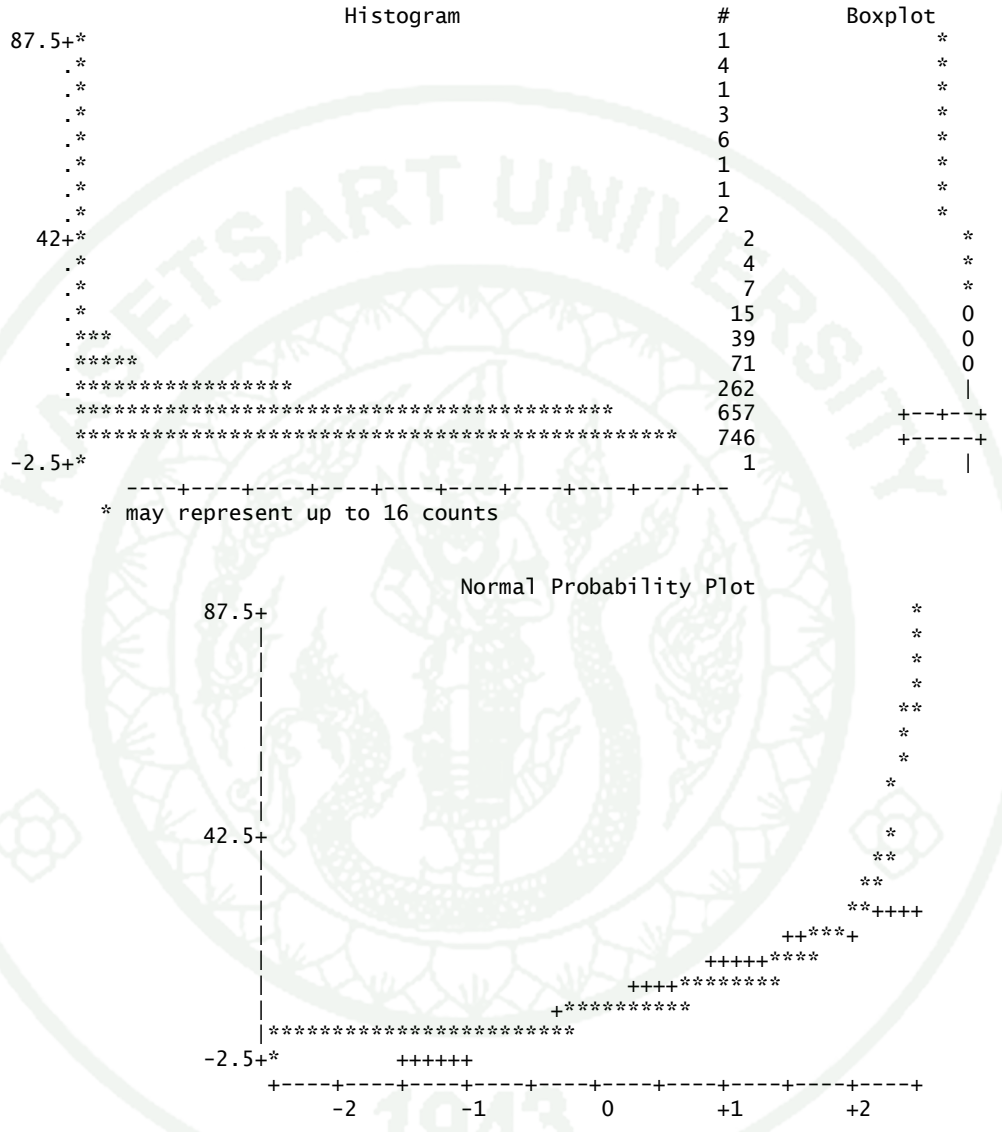
Variable: ClL6M



Appendix Figure B11 Histogram, box plot and normal probability plot for ClL at 6 months old.

The UNIVARIATE Procedure for Genetic Analysis

Variable: BW6M



Appendix Figure B12 Histogram, box plot and normal probability plot for BW at 6 months old.

The SAS program for descriptive statistic

```
Proc univariate plots data=CL2M;
var CL TW;
RUN;
proc mixed data=CL2M;
 class HM Tank Sire Dam Animal;
 model CL = HM ;
 random Animal Tank;
 lsmeans HM ;
 RUN;
 QUIT;

proc mixed data=BW2M;
 class HM Tank Sire Dam Animal;
 model BW = HM ;
 random Animal Tank;
 lsmeans HM ;
 RUN;
 QUIT;

proc univariate;
Var CL BL TL ClL TW;
RUN;

proc mixed data=CL5M;
 class HM Sex Tank Sire Dam Animal;
 model CL = HM Sex ;
 random Animal Tank;
 lsmeans HM Sex ;
 RUN;
 QUIT;

proc univariate;
Var CL BL TL ClL TW;
RUN;

proc mixed data=CL6M;
 class HM Sex Con Tank Sire Dam Animal;
 model CL = HM Sex Con;
 random Animal Tank;
 lsmeans HM Sex Con;
 RUN;
 QUIT;
```

The ASReml programs for genetic evaluation

The .as file for CL at 2 months old:

Prawn - Research Fishery 200801

```
Anim  !P          # Animals with records
Sire  !P          # Sire of the animal
Dam   !P          # Dam of the animal
Tank  !I
HM    !I
CL
TW

C:\ASREML\nissara-CL2M20080619.ped !ALPHA !MAKE !SKIP 1 !REPEAT
C:\ASREML\nissara-CL2M20080619.dat !SKIP 1 !MAXIT 50 !ASUV

 CL ~ HM !r Anim Tank
```

The .pin file for CL at 2 months old:

```
###############################
# Single Trait : Direct only #
###############################
P Total 1 + 2+ 3
H Heritability 1 4
```

The .as file for CL at 5 months old:

Prawn - Research Fishery 200806

```
 Anim  !P              # Animals with records
 Sire    !P            # Sire of the animal
 Dam   !P              # Dam of the animal
 Tank   32
HM     8
Sex    2
 CL
 BL
 TL
 ClL
 TW
C:\ASREML\nissara-CL5MM20080619.ped !ALPHA !MAKE !SKIP 1 !REPEAT
C:\ASREML\nissara-CL5MM20080619.dat !SKIP 1 !MAXIT 50 !ASUV

 CL   ~ HM  Sex  !r Anim Tank
```

The .pin file for CL at 2 months old:

```
##############################
# Single Trait : Direct only #
##############################
P Total 1 + 2+ 3
H Heritability 1 4
```

The .as file for CL at 6 months old:

Prawn - Research Fishery 200806

```
Anim  !P          # Animals with records
Sire  !P          # Sire of the animal
Dam   !P          # Dam of the animal
Tank  32

Sex   2
Con   2
CL
BL
TL
ClL
TW
C:\ASREML\nissara-CL6M20080620.ped !ALPHA !MAKE !SKIP 1 !REPEAT
C:\ASREML\nissara-CL6M20080620.dat !SKIP 1 !MAXIT 50 !ASUV

CL  ~ HM Sex Con !r Anim Tank
```

The .pin file for CL at 2 months old:

```
################################
# Single Trait : Direct only #
################################
P Total 1 + 2 + 3
H Heritability 1 4
```

The .as file for BL at 7 months old:

Prawn - Research Fishery 2010

```
 Anim   !P       # Animals with records
 Sire     !A       # Sire of the animal
 Dam    !A       # Dam of the animal
 YHM   !I  13
 YHM-Gr !I  13
 Tank    !I 41
 Sex     !I 2
 CL
 BL
 TL
 ClL
 TW
 Mature  !I 2
 Sex-Mat !I 3
C:\ASREML\nissara-BL7MGen0-2F-20101125.dat !ALPHA !MAKE !SKIP 1
!REPEAT
C:\ASREML\nissara-BL7MGen0-2F-20101125.dat !SKIP 1 !MAXIT 50 !EXTRA 5
!ASUV
 BL  ~ mu Sex-Mat !r Anim Tank
```

The .pin file for CL at 7 months old:

```
################################
# Single Trait : Direct only #
################################
P Total 1 + 2 + 3
H Heritability 1 4
```

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Kitcharoen, N., W. Rungsin, S. Koonawootrittiron and U. Na-Nakorn. 2011. Heritability for growth traits in Giant freshwater prawn, *Macrobrachium rosenbergii* (de Mann 1879). **Aquacult. Res.** In press.