

# **Genetic improvement of giant freshwater prawn in Vietnam**

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A thesis submitted in fulfillment of requirements for the degree of

**Doctor of Philosophy**

**2013**

## **Keywords**

Giant freshwater prawn, GFP, *Macrobrachium rosenbergii*, heritability, genetic correlations, phenotypic correlations, selection response, body traits, carcass weight traits, combined family selection, sexual dimorphism, social effect, genotype by age interaction.

## **Statement of Original Authorship**

I hereby declare that this thesis has been composed entirely by myself and is a result of my own investigations. It has neither been accepted nor submitted for any other degree except where due reference is made. All sources of information have been duly acknowledged.

QUT Verified  
Signature

Dinh Hung

April 2013

# Works Published or Submitted for Publication by the Author

## Incorporated into the Thesis

### Statement of Contribution to Jointly Authored Works in the Thesis

1. Hung, D., Coman, G., Hurwood, D., and Mather, P. (2012). Experimental assessment of the utility of VIE tags in a stock improvement program for giant freshwater prawn (*Macrobrachium rosenbergii*) in Vietnam. *Aquaculture Research*, 43, 1471–1479

This manuscript is incorporated as Chapter 2 of this thesis. The first author is responsible for the research, analysis and interpretation of data, and written work of this manuscript. The co-authors provided conceptual, logistical and editorial support.

2. Hung, D., Vu, N. T., Nguyen, N. H., Ponzoni, R. W., Hurwood, D., and Mather, P. (2012a). Quantitative genetic parameter estimates for body and carcass weight traits in a culture stock of giant freshwater prawn (*Macrobrachium rosenbergii*) selected for fast growth. *Aquaculture*, in review.

This manuscript is incorporated as Chapter 3 of this thesis. The first author is responsible for the research, analysis and interpretation of data, and written work of this manuscript. The co-authors provided conceptual, logistical and editorial support.

3. Hung, D., Vu, N. T., Nguyen, N. H., Ponzoni, R. W., Hurwood, D., and Mather, P. (2012b). Genetic response to combined family selection for improved mean body weight in giant freshwater prawn (*Macrobrachium rosenbergii*) in Vietnam. *Aquaculture*, in review.

This manuscript is incorporated as Chapter 4 of this thesis. The first author is responsible for the research, analysis and interpretation of data, and written work of this manuscript. The co-authors provided conceptual, logistical and editorial support.

4. Hung, D., Nguyen, N. H., Hurwood, D., and Mather, P. (2012c). Quantitative genetic parameters for body traits at different ages in a cultured stock of giant freshwater prawn (*Macrobrachium rosenbergii*) selected for fast growth. *in prep.*

This manuscript is incorporated as Chapter 5 of this thesis. The first author is responsible for the research, analysis and interpretation of data, and written work of this manuscript. The co-authors provided conceptual, logistical and editorial support.

## Acknowledgements

First and foremost, my sincere thanks go to my supervisors Prof. Peter Mather and Dr. David Hurwood at the Science and Engineering Faculty, Queensland University of Technology (QUT), Australia for their supervision, advice, guidance and suggestions throughout the study program and significant contributions to the planning and writing of the thesis and also their friendly attitude. Without their supervision this thesis would not have been completed.

I would like to express my gratitude to an Australian government based AusAID program for providing a postgraduate scholarship to me to conduct research in Australia. The chance to do a PhD research in Australia has been one of the most valuable opportunities in my career and I therefore feel grateful to AusAID and all the staff in the AusAID Liaison Office at the QUT who have provided me with necessary support for my research and fieldwork, supporting me in my efforts to complete this journey from the beginning till the end.

I am grateful to the National Breeding Center for Southern Freshwater Aquaculture, a part of the Research Institute for Aquaculture N.2 (RIA2) that offered facilities and technical assistance to conduct breeding and grow-out experiments. The research could not have been completed without the support from my colleagues in the Research Institute for Aquaculture No.2, Vietnam. Their extensive assistance has been one of the most important factors in the completion of this thesis. At the risk of offending the rest, I would like to name but a few: Drs. Nguyen Van Hao, Pham Van Khanh, Nguyen Minh Thanh, Nguyen Van Sang, Mr. Nguyen Thanh Vu, Mr. Trinh Quoc Trong, Mr. Nguyen Trung Ky and Mrs. Kieu Thi Thu Nga for their support for various aspects of this study. I would also like to thank Mr. Nguyen Van Ut, who culture giant freshwater prawn at Tam Nong ward, Dong Thap province for supporting the field culture test in his own 13 ha farm.

I would like to acknowledge Drs. Raul Ponzoni, Nguyen Hong Nguyen and Alex Safari (World Fish Center, Penang, Malaysia) for kindly organizing my visit to WorldFish Center for analysis of the quantitative genetic component data. They also assisted in interpretation of data and preparation of manuscripts. Special thanks are given to Dr. Greg Coman (CSIRO Marine Atmospheric Research, Cleveland, Australia) for assistance with interpretation of data in the first paper on tagging technique.

My friends in Brisbane have provided me with all the special fun and joys that friendship can bring to one. Thank you to special Vietnamese families, namely Nguyen-Thu, Khanh-Kien, Nin-Cuong, Huyen-Chien, Huyen-Hau, and Thanh-Thanh. Together they have established a small community in Brisbane that I have really enjoyed being a part of in my life. The weekly dinners they cook for me have provided me with nutrition and energy to fill with my sleepless nights working on the thesis.

I would like to give gratitude to my parents and parents-in-law who always encourage me during a long period of study. On a personal note, I would like to recognize the sacrifices made by my wife, Le Thi Kim Hong, to get me through this journey. Without her unconditional love and patient support during this journey, there would have been no thesis completed. I owe her much. My last words, but most important of all, are for my little son Dinh Nhat Quang, who has brightened every one of my days during the four years in Brisbane.

## Abstract

The giant freshwater prawn (*Macrobrachium rosenbergii*) or GFP is one of the most important freshwater crustacean species in the inland aquaculture sector of many tropical and subtropical countries. Since the 1990's, there has been rapid global expansion of freshwater prawn farming, especially in Asian countries, with an average annual rate of increase of 48% between 1999 and 2001 (New, 2005). In Vietnam, GFP is cultured in a variety of culture systems, typically in integrated or rotational rice-prawn culture (Phuong *et al.*, 2006) and has become one of the most common farmed aquatic species in the country, due to its ability to grow rapidly and to attract high market price and high demand. Despite potential for expanded production, sustainability of freshwater prawn farming in the region is currently threatened by low production efficiency and vulnerability of farmed stocks to disease. Commercial large scale and small scale GFP farms in Vietnam have experienced relatively low stock productivity, large size and weight variation, a low proportion of edible meat (large head to body ratio), scarcity of good quality seed stock. The current situation highlights the need for a systematic stock improvement program for GFP in Vietnam aimed at improving economically important traits in this species.

This study reports on the breeding program for fast growth employing combined (between and within) family selection in giant freshwater prawn in Vietnam. The base population was synthesized using a complete diallel cross including 9 crosses from two local stocks (DN and MK strains) and a third exotic stock (Malaysian strain - MY). In the next three selection generations, matings were conducted between genetically unrelated brood stock to produce full-sib and (paternal) half-sib families. All families were produced and reared separately until juveniles in each family were tagged as a batch using visible implant elastomer (VIE) at a body size of approximately 2 g. After tags were verified, 60 to 120 juveniles chosen randomly from each family were released into two common earthen ponds of 3,500 m<sup>2</sup> pond for a grow-out period of 16 to 18 weeks. Selection applied at harvest on body weight was a combined (between and within) family selection approach. 81, 89, 96 and 114 families were produced for the Selection line in the F0, F1, F2 and F3 generations, respectively. In addition to the Selection line, 17 to 42 families were produced for the Control group in each generation. Results reported here are based on a data set consisting of 18,387 body and 1,730 carcass records, as well as full pedigree

information collected over four generations. Variance and covariance components were estimated by restricted maximum likelihood fitting a multi-trait animal model.

Experiments assessed performance of VIE tags in juvenile GFP of different size classes and individuals tagged with different numbers of tags showed that juvenile GFP at 2 g were of suitable size for VIE tags with no negative effects evident on growth or survival. Tag retention rates were above 97.8% and tag readability rates were 100% with a correct assignment rate of 95% through to mature animal size of up to 170 g. Across generations, estimates of heritability for body traits (body weight, body length, cephalothorax length, abdominal length, cephalothorax width and abdominal width) and carcass weight traits (abdominal weight, skeleton-off weight and telson-off weight) were moderate and ranged from 0.14 to 0.19 and 0.17 to 0.21, respectively. Body trait heritabilities estimated for females were significantly higher than for males whereas carcass weight trait heritabilities estimated for females and males were not significantly different ( $P > 0.05$ ). Maternal and common environmental effects for body traits accounted for 4 to 5% of the total variance and were greater in females (7 to 10%) than in males (4 to 5%). Genetic correlations among body traits were generally high in both sexes. Genetic correlations between body and carcass weight traits were also high in the mixed sexes. Average selection response (% per generation) for body weight (transformed to square root) estimated as the difference between the Selection and the Control group was 7.4% calculated from least squares means (LSMs), 7.0% from estimated breeding values (EBVs) and 4.4% calculated from EBVs between two consecutive generations. Favourable correlated selection responses (estimated from LSMs) were detected for other body traits (12.1%, 14.5%, 10.4%, 15.5% and 13.3% for body length, cephalothorax length, abdominal length, cephalothorax width and abdominal width, respectively) over three selection generations. Data in the second selection generation showed positive correlated responses for carcass weight traits (8.8%, 8.6% and 8.8% for abdominal weight, skeleton-off weight and telson-off weight, respectively). Data in the third selection generation showed that heritability for body traits were moderate and ranged from 0.06 to 0.11 and 0.11 to 0.22 at weeks 10 and 18, respectively. Body trait heritabilities estimated at week 10 were not significantly lower than at week 18. Genetic correlations between body traits within age and genetic correlations for body traits between ages were generally high. Overall our results suggest that growth rate responds well to the application of family selection and carcass weight traits can also be improved in parallel, using this approach. Moreover, selection for high growth rate in



GFP can be undertaken successfully before full market size has been reached. The outcome of this study was production of an improved culture strain of GFP for the Vietnamese culture industry that will be trialed in real farm production environments to confirm the genetic gains identified in the experimental stock improvement program.

## **Note on Thesis Preparation**

Chapter 2 to 5 of this thesis are presented as either published papers or as manuscripts submitted for publication. As such, there is some necessary repetition of information in the General Introduction and General Discussion and Conclusion when compared with the Introductions and Discussions of the data chapters, Chapters 2 to 5. Figures and Tables in each chapter are re-initialized to maintain each chapter as an independent research paper. Data chapters have their individual references lists. The references list at the end of the thesis refers to Chapters 1 and 6.

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# CHAPTER 1: General Introduction

## 1.1. Role of aquaculture in food security worldwide

According to FAO (2010b), the world human population is growing at an average of 1.6% per year and had grown from 4.4 billion in 1980 to 6 billion by 2000 and is now 6.8 billion. It is estimated that by 2015 it will reach more than 7 billion and by 2050 the world population will be over 9 billion. Data shows that most of the growth has been, and will continue to be, in developing nations with populations in sub-Saharan Africa expected to grow the fastest (up 108%, 910 million people), and east and south east Asia's the slowest (up 11%, 228 million). Rapid population growth, combined with rising average incomes and increased urbanization with associated shifts in diet towards more nutritious and higher quality food, is expected to result in almost a doubling of the demand for food. This analysis indicates that the world already faces a serious food crisis, and this is expected to worsen in coming years. More food must be produced otherwise some 370 million people will be hungry in 2050 and this will represent almost 5% of the total human population in developing nations. Estimates also indicate that that no less than 1.02 billion people are currently undernourished (FAO, 2010b) and that they overwhelmingly live in developing nations, largely concentrated in parts of Asia and in Africa south of the Sahara. Around two billion people also suffer from micronutrient deficiencies, primarily vitamin A, iodine and iron (UNSCN, 2004), making these the most common and often under-appreciated nutritional problems facing human populations.

Many human populations, particularly those in developing nations depend on fish as part of their daily diets (FAO, 2010a). For them, fish and fish products represent an affordable source of animal protein that is often cheaper than alternative animal protein sources, but is also preferred and forms an important component of local and traditional staple food cultures. Food fish, whether wild-caught or farmed, play an important role in human nutrition and global food supply, particularly in traditional diets and for food security of the poor. Fish currently represents the major source of animal protein (contributing more than 25% of the total animal protein supply) for approximately 1.25 billion people in 39 nations worldwide, including 19 sub-Saharan countries (FAO, 2009a) and in excess of 50% of the protein intake for 400 million people in the poorest African and South Asian countries. Aquatic species are also important sources of many

nutrients, including high quality protein, vitamin A, vitamin D, vitamin E, iodine and selenium. Evidence is increasing that consumption of fish can enhance brain development and learning in children, protect vision and eye health, and offers protection from cardiovascular disease and some cancers. Fats and fatty acids present in fish, particularly the long chain n-3 fatty acids (n-3 polyunsaturated fatty acids (PUFAs)), are highly beneficial to health and these compounds are difficult to obtain from other food sources. Of particular importance are eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA).

Total fisheries production (capture fisheries and aquaculture) reached approximately 142 million tons in 2008 (FAO, 2009a). Of this, 115 million tons was used as human food, providing an estimated per capita supply of about 17 kg, which is an all-time high with the remainder going to non-food uses (e.g. livestock feed, fishmeal for aquaculture etc.). Aquaculture now accounts for 46% of total food fish supply, representing an increase from 43% in 2006. In 2008, global production of food fish from aquaculture, including finfish, crustaceans, molluscs and other aquatic animals for human consumption, reached 52.5 million tons. The relative contribution of aquaculture compared with total fish production from capture fisheries continues to grow, rising from 34.5% in 2006 to 36.9% in 2008. In the period 1970–2008, production of food fish from aquaculture increased at an average annual rate of 8.3%, while the world population grew at an average of 1.6% per year. For several years wild fish and shellfish capture fisheries have been stable at approximately 90 million tonnes per year, and harvests of seaweeds at approximately one million tonnes (FAO, 2009b). It has been suggested however, that maximum potential fish yield from the world's oceans has probably already been reached. For aquaculture the situation is different. Aquaculture production of fish and shellfish has grown by an average of 7.7% per annum over the last decade (FAO, 2009b) and production from freshwater now contributes 59.9% to world aquaculture production by quantity and 56.0% by value. Marine aquaculture (both in sea and pond culture) accounts for 32.3% of world production by quantity and 31% by value with brackish water production representing only 7.7% of world production in 2008, but 13% of total value. This apparent anomaly reflects the prominence of relatively high-valued crustaceans and finfishes that are cultured in brackish water. While farmed crustaceans only account for less than half of total crustacean global production, culture of penaeid species of prawn in 2008 contributed 73%. Introduction of the exotic white-leg shrimp (*Litopenaeus vannamei*) to Asia for culture has promoted a boom in farming of this species in China, Thailand,

Indonesia and Vietnam over the last decade, resulting in an almost complete shift from farming the indigenous giant tiger prawn (*Penaeus monodon*) to this introduced species in southeast Asia. A ban on introduction and culture of white-leg shrimp was lifted in 2008 in India, and this is likely to also have a major impact on development of shrimp culture here.

## **1.2. Development of improved farm breeds**

The purpose of animal breeding is not to genetically improve individual animals because once an individual is conceived, it is too late to change the genotype of that animal but to improve the culture population and so to improve future generations of individuals. To achieve this, animal breeders apply two basic tools: artificial selection and controlled mating.

The idea behind selection is simply to allow individuals with the best genes to reproduce so that the next generation has, on average, more desirable genes than does the current generation of individuals. Those individuals with the best sets of alleles are considered to have the best breeding values and they are, from a genetic point of view, the individuals with the greatest value as parents. In selection, we try to choose parents with the best breeding values – the individuals that will contribute the best genes to the next generation. The result of a successful selection program is then, to genetically improve future generations of the culture population by increasing over time the proportion of desirable allelic forms of genes in that population.

For a selection program, a group of high performing males and females are identified to be parents for mating, the approach is to match specific males and females from the selected group of animals to produce the next generation. There are many different methods used for mating animals, and each method can be defined by a set of mating rules referred to as the mating system. There are three reasons for using mating systems: (1) to produce offspring with extreme breeding values, (2) or to make use of complementarity, (3) or to obtain hybrid vigour. Extreme phenotypes can be obtained by mating parents with extreme breeding values (high\*high and low\*low). If an animal of intermediate phenotype is desired, mating individuals from opposite phenotypic extremes is one approach that can be used. Parental genotypes may be quite different, and in many cases, neither one is optimal, but the mating is complementary because the offspring provide an intermediate and optimal phenotype.

### **1.3. Strategies for improving the quality of culture lines in aquaculture**

#### ***1.3.1. Crossbreeding***

Crossbreeding is the mating of unrelated individuals, and will tend to increase heterozygosity. By increasing the frequency of heterozygous genotypes, recessive alleles are less likely to be expressed and so the fitness of the population may be increased. Crossbreeding can be used effectively to achieve the following goals:

- To improve the whole production system by crossing complementary breeds.
- To produce animals of intermediate performance from extreme parental phenotypes.
- To upgrade to a different pure breed.
- As a step in creating a new synthetic or composite breed.
- To introduce a single novel gene into an existing breed.
- To take advantage of heterosis.

Heterosis, or “hybrid vigour,” is defined as the superior performance of an offspring over the average of the parental breeds (Dunham, 2007; Hedgecock and Davis, 2007). This can have a marked effect on the profitability of production. Heterosis is often greatest when crossing two parents of totally unrelated ancestry. Hybrid vigour may be exhibited in a variety of traits including; better survival rate, growth rate and/or reproductive rate of crossbred individuals (Hedgecock and Davis, 2007). Heterosis is exploited in some plant and animal breeding systems (Goyard *et al.*, 2008), but has not been widely used in stock improvement for aquaculture species. Two exceptions include Nile tilapia in the Philippines and channel catfish in the USA. In a large scale trial, two different crosses, *O. aureus* x *O. spilurus* and *O. mossambicus* x *O. niloticus* showed 22 and 25% heterosis for body weight, respectively (Tayamen *et al.*, 2002). Hybrid catfish (channel catfish x blue catfish) also showed 30% heterosis (Dunham, 2007). The amount of heterosis that is maintained in a population will depend however, on the type of crossbreeding system the producer employs. In general, crossbreeding is largely a strategy for testing out various genetic combinations and there are no simple rules about which parental combinations are likely to perform best.

### ***1.3.2. Artificial selection***

An individual's total genetic value,  $G$ , is the sum of two different components namely; additive and non-additive genetic values. Additive genetic value is heritable and inherited from parents to offspring while non-additive genetic value is not. The additive genetic value, denoted as  $A$ , represents the sum of additive genetic effects from each locus and is referred to as an individual's value. The non-additive genetic value is comprised of the dominance genetic value,  $D$ , and represents the sum of all genetic effects from each locus that result from interactions within a locus and the epistatic genetic value,  $I$ , represents the sum of all interactions between loci. Total genetic value can, therefore, be written as the sum of additive and non-additive effects:  $G = A + D + I$  (Gjedrem, 2005).

A selection program is carried out to identify and select individuals with better additive genetic merit for traits of interest as parents for the next generation, and to continue this over several generations to improve population performance for these traits (Bourdon, 2000; Falconer and Mackay, 1996). The effect of selection will be to change gene frequencies that is observed as a change in the population phenotypic mean of the traits. It is also, necessary however, to minimise inbreeding in the population during selection. One effect of inbreeding is to increase the frequency of homozygous genotypes that may lead to the expression of recessive alleles. Most deleterious or disease conditions are inherited as a recessive phenotype so inbred populations, therefore, may suffer from a reduction in fitness. Many selection methods have been trialed in fish, and all aim to estimate true additive genetic merit and to apply this. The most common selection methods used in aquatic species include individual selection, family selection and combined selection. These approaches are described in some detail below:

#### ***Individual selection***

Individual selection (commonly referred to as - mass selection) is based only on the phenotype/performance of individuals in the breeding program. This is a very popular method of selection used in terrestrial farm animal breeding as well as in many aquaculture species. Individual selection is a simple method with many advantages for implementation, such as low cost and potentially rapid response, but serious drawbacks may result due to environmental and age differences, and uncontrolled inbreeding. In addition, individual selection can only measure



and select individuals that survive, so it can be problematical for selection of certain types of trait including; meat quality or disease resistance, that often show low heritabilities or for which individuals need to be killed/infected for assessment. Mass selection applied to fish species is more practical for traits of intermediate or high heritability ( $h^2$ ), including growth rate ( $h^2$  values often 0.2 - 0.4). To apply individual selection it is very important to keep all individuals in the same production environment and to consolidate other factors at any stage of the life cycle. It is also very important with this selection method to try to keep population size ( $N_e$ ) as high as possible. Doing this, will reduce errors and delay potential for inbreeding depression and thus potentially increase genetic gains (Gjedrem, 2005).

### ***Family selection***

In family selection, families are maintained separately and individuals within families are tagged as early as possible before they are communally stocked and reared. The main advantage of communal rearing is to eliminate environmental differences between families, enabling breeders to overcome one of the main challenges in family selection. This allows animal breeders to deal better with traits where animals need to be killed for assessment or where  $h^2$  is low including for example; threshold traits, carcass quality traits and disease resistance traits. The relative efficiency of family selection will depend however, on the number of individuals available in each family or family size. The larger the family size, the closer will be the phenotypic and genetic means. The relatively high cost however, of this approach can be a major issue since families are normally reared separately up to tagging.

### ***Between-family selection***

Between-family selection predicts the mean breeding value of each family from its phenotypic mean. Families are treated as homogeneous groups so that each family member has the same estimated breeding value. Families are selected as whole groups and so individuals used as parents are chosen at random from superior families. In general, the rate of response is slow when selecting on family means (Falconer and Mackay, 1996). When heritability and common environmental variation is low however, rates of response can be much higher than under individual selection. This method also allows for selection of traits that may only be measured on slaughtered animals, e.g. flesh colour or fat content. The mean of records collected from

slaughtered animals can be used to estimate breeding values for remaining surviving family members. Similar advantages exist for disease resistance traits.

### ***Within-family selection***

Within-family selection predicts the breeding value of an individual by the deviation of its phenotype from its family mean. Animals that exceed their family mean by a specified amount are selected as parents. This method has the greatest value when environmental effects are common to members of a family while being different among families, e.g. families kept in separate tanks or pens. Without a need to replicate family tanks, this method reduces the size of the facility required to run a breeding program, and with particular mating strategies, can help to reduce accumulated rate of inbreeding.

### ***Combined selection***

If more than one selection method is used in a breeding program, this approach is called combined selection. The aim is to maximize the rate of genetic gain. The advantages of combined selection are to combine and optimize available sources of information that can be useful for breeding value estimation including information on individuals, information about full-sibs and half-sibs, progenies and pedigree information. It is therefore often considered to be the most effective selection method available. Increasing the number of records from different relatives increases the accuracy of prediction above that gained from using other methods. The general method used to predict breeding values from information taken from many different relatives is known as BLUP (Best Linear Unbiased Prediction) (Gjedrem, 2005). In addition because we use information from different relatives, the accuracy of prediction is increased by the capacity of BLUP to correct for environmental effects. The most popular combined selection methods are combined between-family and within-family selection that make use of information on both family deviations and mean phenotypic values of individuals (Bourdon, 2000).

### ***Marker-assisted selection***

The usefulness of molecular information in genetic improvement programs for particular target species will depend on advances made in four main areas of research: molecular genetics (genetic markers and linkage maps), genes and quantitative trait loci (QTL) detection and genetic

evaluation systems, and marker-assisted selection. So far, partial genetic maps have been constructed for only a limited number of aquatic species namely tilapia (Kocher *et al.*, 1998; Lee *et al.*, 2005), common carp (Sun and Liang, 2004), rainbow trout (Young *et al.*, 1998; Sakamoto *et al.*, 1999; Nichols *et al.*, 2003), Atlantic salmon (Moen *et al.*, 2004), kumura prawn (Moore *et al.*, 1999; Liu *et al.*, 2003), black tiger shrimp (Wilson *et al.*, 2002) and channel catfish (Liu *et al.*, 2003). Only few studies however, have examined QTLs affecting cold tolerance (Cnaani *et al.*, 2003) and salinity tolerance in Tilapia (Lee, 2003), cold tolerance in common carp (Sun and Liang, 2004), infectious pancreatic necrosis in rainbow trout (Ozaki *et al.*, 2001), infectious salmon anaemia in Atlantic salmon (Moen *et al.*, 2004), thermal tolerance (Perry *et al.*, 2005), development rate (Sundin *et al.*, 2005) and pyloric caeca number (Zimmerman *et al.*, 2005) in rainbow trout. To date, no causative mutations or candidate genes have been identified that control performance or production traits in any aquatic species. Hence, the potential for direct genotype- assisted selection (GAS) or introgression assisted selection (IAS) can not be realized for aquatic species at this stage, although in theory the IAS method could be carried out if informative markers were available. By contrast, several direct DNA tests have been developed in plants and animals; in both cases, the application has focused on direct genetic markers. Based on linkage markers published for aquaculture species, there are two possible uses for marker-assisted selection (MAS): in cross populations between inbred lines, and within strains (Dekkers, 2004). For each of these approaches, three strategies can be employed, namely: 1) selection on estimated breeding values (EBV) derived from markers alone (MAS), 2) selection on marker-based EBV first and then on polygenic EBV, and 3) index selection that combines both QTL-EBV and polygenic- EBV (COMB).

#### **1.4. Study species - *Macrobrachium rosenbergii***

Giant freshwater prawn (*Macrobrachium rosenbergii*) or GFP is one of approximately 200 species of freshwater prawn in the *G. Macrobrachium* that occur naturally in most tropical and subtropical regions on all continents except for Europe and Antarctica. Previous names for *M. rosenbergii* have included *Palaemon carcinus*, *P. dacqueti* and *P. rosenbergii* and it was not until 1959 that its present scientific name, *Macrobrachium rosenbergii* (De Man, 1879) became universally accepted. Recent molecular and systematic studies have suggested however, that this taxon is polyphyletic and actually consists of two closely related species that have disjunct

natural distributions either side of Huxley's Line in southeast Asia (de Bruyn *et al.*, 2004; Wowor and Ng, 2010) *M. rosenbergii* is indigenous to south and southeast Asia, northern Oceania, and some western Pacific islands. Until 2000, the only farmed *Macrobrachium* species was GFP (*M. rosenbergii*, also known as the Malaysian prawn). More recently however, China has begun farming the oriental river prawn (*M. nipponense*) in large quantities, and India now farms a small quantity of monsoon river prawn (*M. malcolmsonii*). In 2003, these three species accounted for all farmed freshwater prawns, approximately two-thirds *M. rosenbergii* and one-third *M. nipponense*. *M. rosenbergii* is the largest species in the genus where males can reach a body size of 32 cm and the females can grow to 25 cm or greater in length.

#### **1.4.1. GFP biology and lifecycle**

GFP have a hard outer carapace that must be shed regularly in order for individuals to grow. This process is called "molting". Because of a requirement for periodic molts, growth occurs in stepped increments, rather than continuously. There are four distinct stages in the GFP life cycle namely; egg, larva, postlarva (PL) and adult. Mating results in deposition of a gelatinous mass of semen (referred to as a spermatophore) on the underside of the thoracic region of the female's body (placed between the walking legs). Successful mating requires ripe females, that have just completed their pre-mating moult (usually occurs at night) when they are soft-shelled to mate with hard-shelled males. Within a few hours of copulation, eggs are extruded through the gonopores and guided by the ovipositing setae (stiff hairs), that occur at the base of the walking legs that are then moved to the brood chamber. During this process the eggs are fertilized by semen attached to the exterior of the female's body. The eggs are held in the brood chamber (stuck to the ovigerous setae) and kept aerated by vigorous movements of the female parent's swimmerets. The length of time that eggs are carried by female freshwater prawns varies but is not normally longer than three weeks. After mating, females need to move to estuarine areas as their larvae require brackish water for early survival and development (Ismael and New, 2000). As the eggs hatch, a process that is normally completed for the whole brood within one or two nights, the larvae are dispersed by rapid movements of the females's abdominal appendages. GFP larvae are planktonic and swim actively tail first, ventral side uppermost (i.e. upside down) and larvae require brackish water to complete their metamorphosis to the juvenile stage. Larvae that hatch in freshwater will die unless they reach brackish water within a few days. There are a

number of microscopically distinct stages during the larval life of GFP, that last several weeks. GFP larvae in hatcheries have been observed to complete their larval life in as little as 16 days but reaching this stage can take much longer, depending on water temperature and other factors. Larvae eat continuously and, in nature, their diet includes principally zooplankton (mainly minute crustaceans), very small worms, and the larval stages of other aquatic invertebrates. On completion of their larval life, freshwater prawns metamorphose into postlarvae (PL). From this point onwards they resemble miniature adult prawns and move mainly by crawling rather than by free-swimming. When they do swim it is usually in a normal (dorsal side uppermost) way and in a forward direction. Rapid predator avoidance is also achieved by contracting the abdominal muscles combined with rapid movement of the tail fan. Postlarvae are tolerant of a wide range of salinities, which is a general characteristic of most freshwater prawn species. Postlarvae begin to migrate upstream into freshwater conditions within one or two weeks after metamorphosis and are soon able to swim against rapidly flowing currents and to crawl over stones at the shallow edges of rivers and in rapids. They can also climb vertical surfaces and even move across land, provided there is abundant moisture available. In addition to using the foods available to them as larvae, they now utilize larger pieces of organic material, both of animal and vegetable origin. Postlarval freshwater prawns are omnivorous and, as they grow, their natural diet can include aquatic insects and their larvae, algae, nuts, grain, seeds, fruits, small molluscs and crustaceans, fish flesh and the offal of fish and other animals. They can also be cannibalistic. Although freshwater prawns require brackish water in the initial stages, most of their lifecycle is spent in turbid, riverine systems.

#### ***1.4.2. Male social dominance hierarchy in *M. rosenbergii****

Male GFP are classified by their external phenotypic characteristics (morphotype) into three basic morphotypes documented by Kuris *et al.* (1987). They include, blue claw males (BC), orange claw (OC) and small (SM) males. BC males are generally the largest individuals possessing very long chelipeds that are deep blue in colour. BC males are dominant and defend territories but grow relatively slowly. OC males are also relatively large and have long chelipeds (but shorter than BC males) and are usually orange in colour. OC males are not territorial, have poorer mating success and show faster growth rates than BC males. SM males are small and have short claws that are generally not pigmented and are translucent. SM males are subordinate, non-

territorial and only mate with females using an opportunistic mating behaviour. Males progress from SM to OC to BC phenotype and have a strict dominance hierarchy with territorial BC males socially dominant over OC males that in turn, are dominant over SM males. Presence of BC males inhibits growth of SM males and delays metamorphosis of OC males into the BC phenotype. OC males can continue to grow until they are larger in size than the largest BC male in their neighborhood before transforming into the BC form (Karplus, 2005). Suppression of growth via social dominance when coupled with the physical constraint on the number of territories that can “fit” into the bottom area of a pond, limits the total potential prawn biomass that is theoretically possible at the end of a production cycle.

### 1.4.3. Giant freshwater prawn aquaculture

While *M. rosenbergii* is indigenous to south and southeast Asia, parts of Oceania and some Pacific islands, it has also been introduced into many tropical and subtropical regions of the world because it is favoured for aquaculture. Following its introduction to Hawaii from Malaysia in 1965, where the pioneer work of Ling (1969) was translated into a method for mass production of postlarvae (PL) by Fujimura and Okamoto (1972), this species has been introduced subsequently to almost every all continents for aquaculture. Global annual production of freshwater prawns in 2003 was approximately 280,000 tons (New, 2005), of which China produced approximately 180,000 tons, followed by India and Thailand that each produced approximately 35,000 tons. Other major producer countries include; Taiwan, Bangladesh, Brazil, Ecuador and Vietnam.

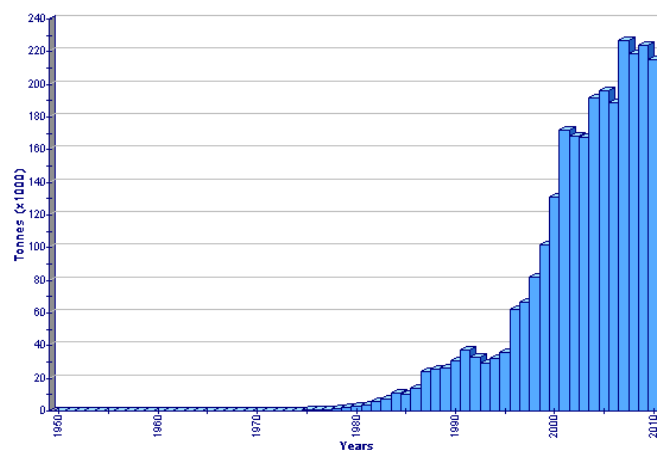


Figure 1: Global GFP aquaculture production for (FAO Fishery Statistic, 2011)

In addition, there are also valuable wild fisheries for *M. rosenbergii*, for example in Bangladesh, India, and several countries in southeast Asia. Global production data for GFP shown in Figure 1 do not include the considerable production from Viet Nam, which is included in the category 'freshwater prawns, shrimps nei'.

### **1.5. GFP aquaculture in Vietnam**

GFP is now one of the most important crustaceans produced in inland aquaculture in Vietnam. Three major production systems are practiced in Vietnam; namely integrated rice-prawn culture, and alternative rice-prawn culture types 1 and 2 (Phuong *et al.*, 2006). Recently, the rice-prawn culture type 1 model has become the most widely practiced system because it shows high productivity and good economic returns. In this model, post-larvae (PL) are usually stocked between April and May at a stocking density of 10 to 12 PLs per m<sup>2</sup> and adults are harvested in December prior to the winter-spring rice crop. This model was developed with the aim to utilize favourable conditions in flooded areas across the Mekong delta in Vietnam. Culture ponds used in this model are rice fields surrounded by a fine mesh fence supported by bamboo poles pitched around field dykes. Ponds are usually large in size and range from 1 to 5 ha in size, most commonly between 3 and 4 ha. Water depth in GFP ponds depends on the flood level so it will vary among years and between months within a year. Generally, water depth ranges from approximately 1.5 m at PL stocking to as much as 7 m at the peak of the flood season and decreases to approximately 2.0 m when adult GFP are ready for harvest. Water depth is usually from 3 to 5 m for almost 4 months and this constitutes the main culture period. In this production model, while natural food plays an important role, supplementary feeding with commercial pellets is also required because pond biomass is large. GFP has become a favoured aquaculture species in Vietnam because growth rate is relatively high and the product is attractive to both domestic and export markets.



Figure 2a: Rice-prawn culture



Figure 2b: Grading for different markets



Figure 2c: Transporting for processing



Figure 2d: Live transportation

In spite of significant potential to expand GFP production in Vietnam, sustainability of freshwater prawn farming in many parts of southeast Asia is currently threatened by low production efficiency because there are no improved culture strains available to the industry and existing farmed stocks are vulnerable to disease (Thanh, 2009). Commercial large scale and small scale GFP farms in Vietnam are experiencing many problems including relatively low productivity, large size and weight variation, a low proportion of edible meat (large head to body ratio), scarcity of good quality seed stock and high cost of seed production. Overall, production yield at the farming level is approximately one half that achieved under research conditions, primarily due to poor survival rates (50 - 60%). This may be explained, in part, by large size variation of juveniles at stocking (Tidwell *et al.*, 2005). The major constraint for farmers in Vietnam currently, is a lack of high quality seed stock. GFP culture stocks used for farming in Vietnam in the past were imported from Hawaii, although they originally were sourced from southeast Asia. After many years of farming unimproved stocks, productivity declines have been



a real concern for the industry. In addition, current breeding practices used in hatcheries are not appropriate for producing high quality seed. Firstly, only a very limited number of breeders are commonly employed in hatcheries. Secondly, long term use of brooders and low replacement rate of brood stock (mainly from on-farm stock) have impacted performance of GFP, as observed in several prawn culture sites around the country, that is likely due to the effects of high rates of inbreeding. New (2005) also commented that routine sourcing of GFP brood stock from grow-out ponds rather than from the wild, in all probability results in high levels of inbreeding accumulating over time and this was believed to be one of the major reasons for growth rate declines in Thailand. The common practice of selecting brood stock based on their readiness to spawn may also lead to a loss of performance as the practice exerts an indirect negative effect on weight at harvest (Mather and de Bruyn, 2003). The same study also hypothesised that while genetic attributes of culture stocks worldwide are currently unknown, many factors are in play that suggests that genetic diversity in cultured GFP stocks may be low and declining.

The current situation in Vietnam and elsewhere in Asia, highlights the need for a systematic stock improvement program for GFP aimed at improving economically important traits in this species. Potential to improve culture stocks however, will in part, depend on the levels of genetic variation available in quantitative traits of interest. For GFP, substantial genetic variation has been reported for growth rate in females but not in males (Malecha *et al.*, 1984; Kitcharoen *et al.*, 2011). If genetic control of growth is sexually dimorphic in GFP, improvement in performance could potentially be achieved by a selection program directed at females. This hypothesis merits further study however, because heritability estimates came from only a small number of families: 50 full- and half-sib families nested within 16 sires (Malecha *et al.*, 1984) or 8 sires and 16 dams (Kitcharoen *et al.*, 2011). In addition, both studies were conducted under laboratory (rather than field conditions) where experimental animals showed only very small weight gains across the experimental period. Hence, heritability estimates were probably biased however, due to maternal genetic and common environmental effects as well as being confounded by non-additive genetic factors. In marine penaeid shrimp species, recent work using residual maximum likelihood methods of analysis have shown that genetic variation for harvest weight or growth-related traits (the main determinant of enterprise efficiency) is considerable, with heritability estimates ranging from 0.10 to 0.60 (Gitterle, Rye *et al.*, 2005). This indicates potential for a high response to selection and is supported by experimental results from a number

of studies where realized direct response to selection for harvest weight or growth-related performance ranged from 10 to 24% per generation in freshwater redclaw crayfish *Cherax quadricarinatus* (McPhee *et al.*, 2004), in yabby *Cherax destructor* (Jerry *et al.*, 2005) and in some shrimp species - *Penaeus japonicus*, *P. stylirostris*, *Litopenaeus vanamei* (Hetzl *et al.*, 2000; Goyard *et al.*, 2002; Argue *et al.*, 2002; De Donato *et al.*, 2005).

Selection for harvest weight or growth-related performance can also result in favourable correlated genetic responses being achieved for other economically important traits (e.g. total length, abdominal width etc). This is consistent with genetic correlations being evident between production traits as reported by Pérez-Rostro and Ibarra (2003b). It is noticeable however, that survival rate of either larvae or postlarvae decreased by 2 to 3% but this was not considered significant (Argue *et al.*, 2002). The authors also found a negative genetic correlation (- 0.46) between harvest weight and resistance to Taura Syndrome Virus. In contrast, several studies have reported positive genetic correlations between growth rate and disease resistance to bacterial or fungal infections in fish (Standal and Gjerde, 1987; Gjedrem *et al.*, 1991; Nilsson, 1992) and in the marine shrimp, *L. vannamei* (Gitterle, Rye *et al.*, 2005). In general, previous studies have reported that estimates of genetic correlations between body weight and survival in aquaculture species are generally positive and moderate in magnitude, indicating that selection for fast growth rate will likely result in a positive correlated response in overall survival rates (Gitterle, Rye *et al.*, 2005; Gjedrem *et al.*, 1991)

### **1.6. Selection program for fast growth rate in GFP in Vietnam**

A selective breeding program for GFP commenced in Vietnam in 2007. Three strains including two native Vietnamese strains (Mekong and Dong Nai) and an exotic strain (Malaysian strain) from Malaysia were sampled and taken to the National Breeding Center for Southern Freshwater Aquaculture (NABRECSOFA), under the Research Institute for Aquaculture No.2 in Vietnam (RIA2). Brood stock for the two native strains were collected from two geographically independent natural drainage basins in Vietnam the Mekong River and the Dong Nai River (Thanh, 2009) as juveniles and sub-adults (5 to 10 g individuals). The Mekong River has a very extensive drainage basin so brood stock were collected from the two largest branches namely the Tien and Hau Rivers. Wild GFP were collected at different localities (upstream, middle and in the lower sections of both rivers) from sites with different ecological conditions (freshwater and

brackish water) in a number of different provinces (An Giang, Dong Thap, Soc Trang, Ben Tre and Tien Giang provinces) and at different times of the year. To maximize diversity, brood stock from the Dong Nai River were collected in the same way as for the Mekong strain. The Malaysian strain (juveniles) was provided by the WorldFish Center in Malaysia.

To generate the F0 generation (year 2008), a complete 3 x 3 diallel cross involving a total of 9 crosses was carried out in order to establish a synthetic base population for selection. In later generations: F1 (year 2009), F2 (year 2010) and F3 (year 2011), matings were made between genetically unrelated brood stock (to minimize inbreeding) to produce full-sib and (paternal) half-sib families. All families were reared separately until tagging size of approximately 2 g. At tagging, all juveniles in each family were tagged as a batch using visible implant elastomer (VIE) tags as described by (Hung *et al.*, 2012). Two tags of 5 to 6 different colours were applied to individual prawns to maintain pedigree records. After tags were verified, 60 to 120 juveniles chosen randomly from each family were released into two common earthen ponds for grow-out over approximately 16 to 18 weeks. At harvest, six body traits were measured on each individual including body weight (BW), body length (BL), cephalothorax length (CL), abdominal length (AL), cephalothorax width (CW) and abdominal width (AW). In addition, male morphotype, female reproductive status, tag code, sex and culture pond were also recorded at harvest. Selection was based on body weight at harvest and a combined-family (between-family and within-family) selection approach was employed. After data analysis, individuals with the highest breeding values were chosen as brood stock (the Selection line) for the next generation. The Control line in each generation consisted of individuals that had a mean (or as close as possible to the mean) EBV in that generation.

### **1.7. Objectives of the current study**

The overall objective of this study was to trial family selection to improve growth rate of a GFP culture strain in Vietnam. The long-term aim was to develop a high yielding GFP strain for the local culture industry in the Mekong Delta, with improved growth rate and better survival that is well adapted to local culture environments.

Specific objectives of the current study included:

- To develop protocols for producing families and tagging of juveniles for use in a family selection program.
- To establish a synthetic base population with high levels of genetic diversity for genetic selection.
- To estimate heritabilities and phenotypic and genotypic correlations for traits of economic importance (in particular, body and carcass weight traits).
- To conduct a combined (between and within) selective breeding program to improve growth rate of the synthetic stock.
- To measure direct and correlated genetic responses in specific traits of economic importance.
- To estimate heritabilities and phenotypic and genotypic correlations for traits of economic importance (in particular, body traits) at different ages.

**CHAPTER 2. Experimental assessment of the utility of VIE tags in a  
stock improvement program for giant freshwater prawn  
(*Macrobrachium rosenbergii*) in Vietnam**

- Presented at The 10<sup>th</sup> International Symposium on Genetic in Aquaculture ‘Role of aquaculture genetics in addressing global food crisis’ 22-26 June 2009, Bangkok, Thailand
- Published in Aquaculture Research (2012). Aquaculture Research (43) pages 1471–1479.

## **Preface to Chapter 2**

The current study was initiated to test the efficiency of using Visible Implant Elastomer (VIE) as an external tagging method for juvenile giant freshwater prawn (GFP, *Macrobrachium rosenbergii*). The first experiment was conducted to assess the application of VIE at different juvenile body size, at different potential tagging places, using different number of tags applied on an individual and different colors. The second and the third experiments were conducted to test the efficiency of the method in a real, large-scale selection program on GFP in Vietnam. Results were very positive and suggested that VIE tags can be successfully applied in GFP juvenile at 2 g with high success in growth, survival, tag retention rates and correct assignment.

**Experimental assessment of the utility of VIE tags in a stock improvement program  
for giant freshwater prawn (*Macrobrachium rosenbergii*) in Vietnam**

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## **Abstract**

An important requirement of many breeding programs for aquaculture species is the ability to identify organisms individually or at least by family. While a variety of external and internal tagging methods have been developed that can provide efficient identifications systems, most have specific drawbacks. The present study assessed the efficiency of an internal tagging method that can be applied to family selection programs in crustacean species. Experiments were conducted to test the efficacy of applying VIE tags to juvenile giant freshwater prawn (GFP). The first experiment assessed performance of VIE tags in juvenile GFP of different size classes and stability of tags placed in different positions in the abdomen with different numbers of tags implanted. The second experiment applied VIE tags in a long term, large scale, field based farming experiment. The third experiment tested the reliability of the system. Results showed that juvenile GFP at 2 g were of suitable size for VIE tags with no negative effects evidence on growth and survival. Tag retention rates were above 97.8% in all experiments and tag readability rates were 100% with a correct assignment rate of 95% through to mature animal size of up to 170 g.

**Keywords:** *Macrobrachium rosenbergii*, VIE tags, family selection, blind test.



## 1. Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*), commonly referred to GFP, is one of the most important freshwater decapod crustacean species in the inland aquaculture sector in many tropical and subtropical countries. Data from 2005 (FAO, 2007) show that GFP comprised 15% of both total production (over 473,000 tons) and total value (over US\$ 1.8 billion) of all farmed shrimp and prawn, globally (New *et al.*, 2008). This is significant, considering that all GFP are produced in Asia, while marine shrimp are cultured worldwide. Sustainability of this industry is currently threatened however, by low production efficiency and vulnerability of farmed stocks to disease (Thanh *et al.*, 2009). Family selection is employed commonly in stock improvement programs as it can reduce potential for developing inbreeding depression and can provide better estimates of genetic parameters than can be achieved with individual selection. A major constraint associated with adopting a family selection approach in some species however, is availability of an appropriate tagging system.

Tagging crustaceans can be a problem because they moult (i.e. discard their exoskeleton) periodically to grow. As a result, moulting can lead to loss of external tags affixed to the exoskeleton. External tagging systems have advantages of being relatively low cost, are easy to apply and do not require sophisticated equipment for identity detection. External tags however, are often relatively large in size and can often affect growth, health and/or survival of tagged organisms (Claverie and Smith, 2007; Soula *et al.*, 2007; Navarro *et al.*, 2006). As a result, many types of external tags applied successfully in fish are inappropriate for crustaceans. Internal tags in contrast, are generally smaller in size, do not interfere with the moulting process, and are not lost during ecdysis. A major disadvantage however, is that external visual detection can be difficult (Isely and Stockett, 2001).

The Visible Implant Elastomer (VIE) tag developed by Northwest Marine Technology (Shaw Island, WA, USA) is a coloured polymer supplied in liquid form that cures to a flexible solid with the addition of a curing agent. This system has been applied successfully for tagging a variety of species including earthworms (Butt and Lowe, 2007; Butt *et al.*, 2009), European eels (Imbert *et al.*, 2007), amphibians (Heemeyer *et al.*, 2007; Jennifer *et al.*, 2007), cephalopods (Zeeh and Wood, 2009), finfish (Doupé *et al.*, 2003; Astorga *et al.*, 2005; Jensen *et al.*, 2008; Woods and Martin-Smith, 2004), some shrimp species (Godin *et al.*, 1996; Pillai *et al.*, 2007;

Fuller et al., 2009; Coman et al., 2010a), crayfish (Mazlum, 2007) and lobster (Woods and James, 2003; Ashley and Jean-Paul, 2006; Uglem *et al.*, 1996). The VIE has also been shown to last for extended periods of time (Butt and Lowe, 2007; Godin *et al.*, 1996) with little or no negative effects on biological functions assessed under both laboratory and field culture conditions (Ashley and Jean-Paul, 2006; Davis *et al.*, 2004; Frisch and Hobbs, 2006). The VIE tags also have advantages of showing high retention rates (Pillai *et al.*, 2007; Uglem *et al.*, 1996), being easy to apply, being cost effective (Fuller *et al.*, 2009), and can be applied even to very small organisms (Jensen *et al.*, 2008). Furthermore, they can be easily identified with the naked eye (although better results can be achieved under UV light) (Uglem *et al.*, 1996) without the need to sacrifice tagged animals. The aim of the current study was to assess the effectiveness of VIE tags for application in a family selection program for GFP.

## **2. Methods**

### **2.1. Tagging protocol**

Visible implant elastomer tags were prepared according to the manufacture's instructions. Tags were implanted anteriorly using a 0.3 cc syringe into the epidermal layer on the lateral side of juvenile GFP. Elastomer was then injected gradually as the needle was withdrawn. Juvenile GFP did not require anaesthesia during the tagging process.

### **2.2. Culture conditions**

Following implantation, tagged animals were either maintained in labelled fibreglass 1200 litre tanks with aeration, and fed with commercial pellets for a week allowing all juvenile GFP to moult at least once (Experiment 1) or released immediately into ponds (Experiment 2). After stocking, tagged animals were reared in hapas (Experiment 1) or ponds (Experiment 2) and were fed with an appropriate sized commercial pelleted feed. Environmental rearing conditions (i.e. DO, pH, temperature and nitrite) were monitored weekly to ensure suitable conditions were met.

### **2.3. Experiment 1**

In experiment 1, two size classes of juvenile GFP were tested. 1,062 juvenile GFP in the *1 gram class (1g)* with a mean body weight of  $1.01 \pm 0.011$  g were used (817 tagged, 245 untagged)

as controls). An additional 1,292 juvenile GFP, weighing  $2.10 \pm 0.017$  g in the *2 gram class (2g)* were also assessed (962 tagged, 330 controls). Juvenile GFP were tagged with a single VIE implant placed on the left-hand side in either the sixth or first segments (coded as group 1 and group 2, respectively) or given tags in both segments (coded as group 3). Surviving animals were checked after 7 days. Survival rates and tag retention rates, defined as survival rates and tag retention rates at tagging, were scored. For each size class, 100 tag-retained juvenile GFP from each of the 3 groups and the control group (400 organisms in total) were collected at random to stock into a 100 m<sup>2</sup> hapa located in an earthen pond for a further 70 day growth period trial. At harvest, all GFP were weighed to the nearest 0.1 g and tags were checked.

## **2.4. Experiment 2**

A total of 6,793 juvenile GFP in the *2g class* weighing  $2.10 \pm 0.018$ g (0.8-4.1 g) were derived from 116 full- and half-sib families and were tagged using the described tagging protocol. Families used in this experiment were generated in a family selection stock improvement program conducted at Research Institute for Aquaculture No.2 (RIA2) in the south of Vietnam which aims to improve the growth rate of GFP. To have sufficient tag codes, two VIE tags (red, orange, blue, yellow or pink) were employed, either on the left- or right-hand side of the first and sixth segments. Tagged animals were then stocked into two ponds of 3,500 m<sup>2</sup> (Pond A) and 800 m<sup>2</sup> (Pond B) at a stocking density of approximately two organisms per square meter. An additional 1,000 untagged juvenile GFP, belonging to the same *2g class*, were stocked in Pond A as a control group. Experimental GFP were harvested after 112 days and 92 days in Ponds A and B, respectively. At harvest, animals were weighed, and then grouped based on their body weights namely; small size (< 20 g, n=1,174), medium size (20 - 40 g, n=1,524) and large size (>40 g, n=1,124) groups for comparison of tag retention rate among groups.

## **2.5. Experiment 3**

Experiment 3 constituted a *blind test* to assess the reliability of the tagging method if tags had been scored by different scorers. 300 adult GFP with a mean body weight of  $38.7 \pm 0.45$  g (12.2 - 89.4 g) each with two tags retained were harvested from Pond A in Experiment 2 were employed. Experimental GFP were grouped based on their body weights namely; small size (< 30 g), medium size (30-50 g) and large size (>50 g) group for testing the effect of body size on

tag scoring. Five observers with different levels of tag scoring experience were asked to score and record the identity of tags independently for each GFP organism. Observers were divided into 3 groups; advanced, moderate and basic. Advanced observers participated in the original tagging process and had extensive experience working with tag scoring. Moderate observers did not participate in tagging but had had one day training in tag scoring before the test. Basic observers received a brief training and practiced tag scoring under supervision of an advanced observer just before the test. Finally, a sixth person retrieved the tags surgically from the GFP to record the 'true' tag code. Consistency of the recorded tag codes from the five observers, as compared to the 'true' tag codes, was used to assess reliability of the VIE tagging method.

## **2.6. Data analysis**

Survival rate was calculated as the percentage of GFP from each group surviving to the point of evaluation compared with the total number of GFP stocked initially. Tag retention rate was calculated as the percentage of surviving GFP with tag(s) compared with total surviving GFP. Readability rate was calculated as the percentage of GFP in which tag(s) were readable over total number of GFP that had retained tag(s). Correct tag scoring rate was calculated as the percentage of tag or unique tag code correctly scored.

Body weights are presented in the current study as means  $\pm$  SE (Standard error). Differences in body weight within size class between treatment groups were analysed using one-way analysis of variance (one-way ANOVA). Differences in survival rates, tag retention rates, tag scoring rates and correct tag scoring rates among treatment groups were tested using a Pearson's  $\chi^2$ -test (2-tailed). The frequency difference between two groups was tested using pairwise comparison of proportions. Significant differences between treatments were determined from the ANOVA F and the Pearson's  $\chi^2$  statistics set at  $\alpha = 0.05$ . Analyses were performed using the computer software package SPSS student version PASW v. 18 (licensed to QUT University by SPSS Australia Pty Ltd, Australia).

## **3. Results**

### **3.1. Experiment 1**

#### ***Survival rate***

At tagging, different tagging groups did not significantly affect survival rates in either the *1g* class ( $\chi^2(3) = 7.8, P = 0.51$ ) or the *2g* class ( $\chi^2(3) = 4.3, P = 0.23$ ) (Table 1). Survival rates of tagged groups were high (90.1 - 98%) and were not different from controls. After the growth period however, there were significant differences between survival rates among the *2g* class treatment groups, but no differences were observed for the *1g* class ( $\chi^2(3) = 10.6, P = 0.01$  and  $\chi^2(3) = 3.9, P = 0.27$  respectively). Survival rates of tagged GFP were generally higher for the *2g* class organisms than for the *1g* class organisms ( $\chi^2(1) = 6.1, P = 0.014$ ) but were not different from controls. Comparisons of survival rates between the two size classes were performed for each group. Higher survival rates were found in *2g* class GFP for group 2 ( $\chi^2(1) = 17.5, P < 0.0001$ ). No significant differences were observed however, in the other groups ( $P > 0.05$ ). Survival rates within size class were compared for different treatment groups based on numbers of tags per organism. The number of tags injected per organism (1 tag or 2 tags) did not affect survival rates significantly in the *1g* class or in the *2g* class at tagging ( $\chi^2(1) = 0.0, P = 0.94$  and  $\chi^2(1) = 17.7, P = 0.12$  respectively) or survival rates after the growth period ( $\chi^2(1) = 0.3, P = 0.61$  and  $\chi^2(1) = 0.1, P = 0.71$  respectively). On average, survival rates were higher for the *2g* class than for the *1g* class regardless of whether 1 or 2 tag(s) had been injected initially.

### ***Growth rate***

For the *1g* class (Table 1), tagged and control group's mean harvest body weights were not significantly different ( $F_{3,256} = 1.7, P = 0.17$ ). For the *2g* class, significant differences in harvest body weight were found between groups ( $F_{3,291} = 7.2, P < 0.0001$ ).

### ***Tag retention rate***

Different treatment groups (*1g* and *2g* classes) had no significant effect on tag retention rate after tagging ( $\chi^2(2) = 1.1, P = 0.58$  and  $\chi^2(2) = 1.5, P = 0.46$  respectively). Tag retention rates were initially high (97.8 - 99.1%) after tagging, following which tag retention rate was 100% to the end of the 70 day growth period in hapas.

## **3.2. Experiment 2**

### ***Survival rate***

Data from Pond B (Table 2) showed that the survival rate (78.1%) was high and tag retention was very high 99.8% (1,247/1,250) with 98.9% (1,236/1,250) surviving GFP having retained both tags. Only a very small number of GFP were found to have lost both tags (i.e. “two tags lost”) in Pond B (3 out of 1,250 or 0.24% of surviving GFP). For Pond A it was assumed that all 436 untagged GFP harvested belonged to the control group. Using an estimate based on rate of tag losses in Pond B, there was an assumed error rate of approximately 8 control GFP in Pond A controls that were actually tagged GFP that had lost both tags. Pond A showed a lower survival rate compared with Pond B (48.6% vs. 78.1%). This was likely due to the presence of predators (tilapia, catfish and snakehead walking catfish) in this pond. The tagged group in Pond A however, showed a significantly higher survival rate than did the control group (49.5% vs. 43.6%;  $\chi^2(1) = 11.8, P < 0.001$ ).

### ***Growth rate***

As for survival rate, data from Pond A showed that the tagged group (two and one tag retained GFP) had significantly higher mean body weight than did controls ( $40.9 \pm 0.67$  g vs.  $36.3 \pm 1.50$  g;  $t_{616} = 2.8, P = 0.005$ ). The largest GFP harvested from Ponds A and D weighed 170 g and 110 g respectively, and both had retained two tags.

### ***Tag retention rate***

The observed tag retention rate in Pond B was 99.8% (Table 2) suggesting that only a very small percentage of tag(s) had been lost. While tag retention rates were still quite high, it was lower in the large size group than in the other two groups (97.2, 99.4 and 99.1% for large, medium and small groups, respectively;  $\chi^2(2) = 25.1, P < 0.0001$ ).

### ***Tag readability rate***

In Experiments 1 and 2, tag readability rate was 100% with all tags were clear even though some tags had fragmented and were assumed to be 100% correctly scored. The assumption of 100% correct tag scoring was tested in Experiment 3.

## **3.3. Experiment 3**

Correct tag scoring rates by different observers (with different experience levels), body sizes and colours are summarized in Tables 3, 4 and 5. Significant differences in correct tags scoring rate were found between observers, and between experience levels of observers. The most accurate observers belonged to the most experienced group (Advanced observers) (Table 3). Tag readability was affected not only by observer experience but also by size and/or tag colour. Tables 4 shows that GFP body size had a significant effect on tag readability with the largest size class showing the poorest correct tag scoring rate (91% compared with 95.4% and 93.9%). Significant differences were also found between relative readability of the colours used with pink and red tags showing the lowest rates of correct tag scoring, typically resulting from a failure to discriminate between these two colours (Table 5).

#### **4. Discussion**

Most negative impacts of VIE tagging on survival and/or performance have been shown to be a function of the size of organisms when tagged (Pillai *et al.*, 2009; Soula *et al.*, 2007; Godin *et al.*, 1996). Tagged organisms of relatively smaller size often show lower survival rates (Brown *et al.*, 2003) and their size also can limit the volume of VIE that can be injected. In turn, this can directly affect tag retention and tag readability rates (small tag(s) may become obscured by muscle and/or pigmented exoskeleton as tagged organisms grow). (2003). Brown *et al.* (2003) found that 70 days after tagging, the post-larvae had better tag visibility than the larva. Tagging results can also be affected by tag position (Brennan *et al.*, 2007) and the number of tags used per organism (Kneib and Huggler, 2001; Woods and James, 2003; Woods and Martin-Smith, 2004). Use of multiple tags per organism, compared with use of only a single tag, may produce more negative outcomes for tagged organisms (Gosselin *et al.*, 2007).

In the current study, GFP tagged at different size classes; in different body positions; and using different numbers of tags were tested simultaneously with no systematic differences observed between tagged group and control group even though some differences were observed in survival and growth among tagged groups in the 2g class (Experiment 1). The control group however, did not show the fastest growth rate or highest survival rate. Our results therefore suggest that VIE tags do not negatively affect GFP growth rate. This finding is supported by results from the family selection experiment (Experiment 2) where GFP with 2 tags showed higher survival rates and mean body weight than did the control group.

Survival rates at tagging were high in all tagged groups (over 90%), however, higher survival rates were found for larger (2g class) than the smaller (1g class) GFP by the end of the growth period. Survival rates of tagged GFP in the current study compare favourably with those reported for juvenile freshwater crayfish (Jerry *et al.*, 2001). Survival rates however, using the same VIE tags were higher for some other crustacean species notably in studies of juvenile blue crab (Davis *et al.*, 2004), white shrimp (Godin *et al.*, 1996) and lobster (Woods and James, 2003). While VIE tags are known for high tag retention rates of up to 100% (Godin *et al.*, 1996; Woods and James, 2003; Pillai *et al.*, 2007; Astorga *et al.*, 2005), previous studies have only employed either short-term experiments where little weight gain was achieved and organisms were still of relatively small size at completion of the experiments and had soft and translucent exoskeletons or were conducted in clear-water-laboratory-culture conditions with little possibility for algal and/or sediment staining of the exoskeleton. It is notable however, that size of animals rather than time is the major factor affecting VIE retention and readability (Davis *et al.*, 2004). In the current study, conditions were very different with the experiments conducted under pond culture conditions. After approximately 100 days under field-based farming conditions, tag retention rates were still very high at 99.8% (Table 2, data on Pond B) at a mean harvest weight of approximately 40 g. This confirms that VIE tag loss was negligible even under field-based conditions.

In Experiment 1 in the current study, we recognised that the 1g class retained tiny marks at harvest that could be clearly identified in 20 g size GFP but that potentially may produce lower readability rate and correct tag scoring when GFP grow to a mature size of 50-150 g. This is because 1g juvenile GFP are too small in size to allow an adequate volume of VIE to be injected. Moreover, survival rate in the 1g class was lower than the 2g class after the growth period. For these reasons, a 2g size class is suggested as the most suitable size for tagging juvenile GFP. Use of a single tag or two tag(s) and at different tag positions (first, sixth segment or both places) did not appear to produce any negative outcomes (declines in survivals or growth rates) in GFP. If however, more tags are required for a larger number of families to be tagged, then this would need to be tested first before being applied.

The tagging method tested in the current study had been employed in a family selection stock improvement program for GFP at RIA2 where tags must be able to be scored correctly in



mature organisms. Female GFP had to be tagged in the anterior abdominal somite to avoid potential for tags to be concealed later when they became gravid (Kneib and Huggler, 2001). This practice needs to be implemented at the tagging stage for all organisms because sex cannot be determined at time of tagging. For this reason, only the first and sixth segments were chosen as tagging sites to ensure that tags could be assessed easily in mature females. Other studies (i.e. (Brown *et al.*, 2003)) have suggested that other body segments can also provide potential tagging sites increasing the capacity of the method.

Previous studies have also shown that tag readability can be biased by observer experience (Claverie and Smith, 2007; Gosselin *et al.*, 2007). In the current study, we found that VIE tag readability could be biased depending on the relative expertise of individual observers. More experienced observers produced higher correct tag scoring rates that can reach up to 97% in mature-sized GFP (up to 90 g). Less experienced observers can however, effectively improve their tag scoring skills after a short period of training. Tag scoring efficiency is also significantly affected by GFP size with smaller organisms having higher correct tag scorings. We also recognize that tags placed in the first segment where there is more transparent muscle typically are easier to be scored than tags placed in the sixth segment. The VIE tag colour can also affect the rate of correct tag scoring with pink being incorrectly scored more commonly than other colours. This finding agrees well with results reported by Pillai *et al.* (2007) who also evaluated VIE tags in juvenile GFP. We suggest that pink should be omitted for future studies to increase the reliability of red. Approximately 0.4% of cases where no VIE tag was implanted (defined as non-tag positions) were incorrectly scored as one of the five used colour tags. This may have resulted from muscle pigmentation in some adult GFP.

One potential limitation of using VIE as a tagging technique in family selection programs is the tag code capacity. When organisms can be tagged at four potential body locations with two tags (as seen in Experiment 2), using five to six colours this combination produces 150-216 potential tag codes respectively. The VIE tag manufacturer (NMT) offers ten different colours so theoretically 600 unique tag codes can be created. This number of tag codes is more than enough for use in most programs where 50-200 families are commonly produced. The VIE tags are therefore not only a very cost effective tagging method but tagging speed is also an advantage. In Experiment 2, over 6,700 juvenile GFP were tagged with two tags per organism and tagging

speed was 100-120 organisms per person per hour. The major disadvantage of the VIE tagging system is the inability of individual identification as is available with the Coded Wire Tags (CWT) or Visible Implant Elastomer (VI-Alpha) tagging systems. This problem can be overcome however, if a secondary tagging system is used at harvest. The PIT tags have been tested successfully in mature-sized GFP (Caceci *et al.*, 1999) or much cheaper, but not less effective bird-leg-bands ([www.achughes.com](http://www.achughes.com)) can be placed around the prawn eye stalk. While PIT tags require a high capital investment for tags and the tag reader, tags can be reused many times. Bird-leg-bands on the contrary are very low cost, are easy to apply, and can be scored externally and so provide an ideal option. Use of a secondary tagging system will not only allow each individual's breeding value to be predicted but the relationship of individuals over generations can be recorded to provide pedigree data with only limited extra cost and labour requirements.

## **5. Conclusions**

The present study has demonstrated that the VIE tagging system can be a valuable tool for use in crustacean family-based stock improvement programs. The VIE tags can be used to tag juvenile GFP with no negative effects evident on growth or survival rate. The VIE tagging system also shows very high tag retention rates, high tag readability with high correct scoring rates through to mature size. The system is also economical for tagging a large number of organisms in a short time period. When different colours, tag positions and multiple tags are employed, a relatively large number of families can be tagged effectively.

## **Acknowledgements**

The authors thank Vu, T. N., Ky, T. L. and Nga, T. K. N. for their valuable technical assistance in both the laboratory and field trials. This work was supported by the Ministry of Agriculture and Rural Development (MARD) in Vietnam and the WorldFish Center in Malaysia through the "Family-based selective breeding program on giant freshwater prawn in Vietnam". The Australian government based AusAID program providing Hung Dinh an ALA award to undertake PhD research at QUT University. We also thank two anonymous reviewers comments have greatly improved this manuscript.

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## Tables and figures

Table 1: Survival and tag retention rates of tagged groups on two different weight classes

Weight classes	Tagged groups	7 days after tagging		70 days growth period in hapas		
		Survival	Retention	Bw	Survival	Retention
		(%)	(%)	(g)	(%)	(%)
1g class	Group 1 (n=208)	96.2 <sup>(a)</sup>	99.0 <sup>(a)</sup>	8.67±0.299 <sup>(a)</sup>	69.0 <sup>(a, A)</sup>	100 <sup>(a)</sup>
	Group 2 (n=353)	90.1 <sup>(a)</sup>	97.8 <sup>(a)</sup>	7.89±0.356 <sup>(a)</sup>	57.0 <sup>(a, B)</sup>	100 <sup>(a)</sup>
	Group 3 (n=256)	92.2 <sup>(a)</sup>	97.9 <sup>(a)</sup>	8.98±0.436 <sup>(a)</sup>	66.0 <sup>(a, A)</sup>	100 <sup>(a)</sup>
	Control (n=245)	93.9 <sup>(a)</sup>	-	8.95±0.390 <sup>(a)</sup>	68.0 <sup>(a, A)</sup>	-
2g class	Group 1 (n=321)	96.0 <sup>(a)</sup>	98.1 <sup>(a)</sup>	15.18±0.615 <sup>(a)</sup>	64.0 <sup>(c, A)</sup>	100 <sup>(a)</sup>
	Group 2 (n=305)	98.0 <sup>(a)</sup>	99.0 <sup>(a)</sup>	11.64±0.581 <sup>(b)</sup>	84.0 <sup>(a, A)</sup>	100 <sup>(a)</sup>
	Group 3 (n=336)	94.9 <sup>(a)</sup>	99.1 <sup>(a)</sup>	13.76±0.566 <sup>(ab)</sup>	72.0 <sup>(b, A)</sup>	100 <sup>(a)</sup>
	Control (n=330)	96.1 <sup>(a)</sup>	-	14.73±0.638 <sup>(a)</sup>	75.0 <sup>(b, A)</sup>	-

*Values in each column and within weight class not sharing the same lower case superscript are significantly different ( $P < 0.05$ )*

*Values of survival after 70 days growth period within group across 2 weight classes not sharing the same upper case superscript are significantly different ( $P < 0.05$ )*

Table 2: Tagging results from family selection stock improvement program at RIA2

Ponds		Pond A (3,500 m <sup>2</sup> )( <i>N</i> )		Pond B (800 m <sup>2</sup> )( <i>N</i> )		
At tagging	Tagged	5,193		1,600		
	Control	1,000		0		
	Total	6,193		1,600		
At harvest		<i>N</i>	Body weight (g)	<i>N</i>	Body weight (g)	
	2 tags	2,535	40.5 ± 0.66	1,236	30.6 ± 0.67	
	1 tag	37	68.0 ± 6.04	11	36.3 ± 5.94	
	0 tag/control	436 <sup>(*)</sup>	36.3 ± 1.50	3	34.9 ± 3.2	
	Total	3,008	40.2 ± 0.60	1,250	30.7 ± 0.67	
	Survival (%)					
			48.6		78.1	
		Tagged group	49.5		77.9	
		Control group	43.6		-	

(\*): It was unable to distinguish between control and tagged organisms if they had lost both tags



Table 3: Correct tag scoring rates by different observers

Observer codes	Experience level <sup>(*)</sup>	By observer		By experienced level	
		Correct rate (%)	Statistic	Correct rate (%)	Statistic
1	Advanced	97.0 <sup>(a)</sup>	$\chi^2(4) = 13.4,$ $P = 0.010$	94.7 <sup>(a)</sup>	$\chi^2(2) = 7.8,$ $P = 0.021$
4	Advanced	94.7 <sup>(ab)</sup>			
5	Advanced	93.8 <sup>(ab)</sup>			
2	Moderate	94.7 <sup>(ab)</sup>		94.7 <sup>(a)</sup>	
3	Basic	90.3 <sup>(b)</sup>		90.3 <sup>(b)</sup>	

Values in each column not sharing the same lower case superscript are significantly different ( $P < 0.05$ )

Table 4: Correct tag scoring rates with different GFP body size groups

Body size groups	Correct rate (%)	Statistic
Small (n = 117)	95.4 <sup>(a)</sup>	$\chi^2(2) = 7.4, P = 0.025$
Medium (n = 112)	93.9 <sup>(ab)</sup>	
Large (n = 71)	91.0 <sup>(b)</sup>	

Values in each column not sharing the same lower case superscript are significantly different ( $P < 0.05$ )

Table 5: Correct tag scoring rates with different VIE tag colours

VIE tag colours	Correct rate (%)	Statistic
Non-tag	99.6 <sup>(a)</sup>	$\chi^2(5) = 177.8, P < 0.0001$
Red	95.3 <sup>(c)</sup>	
Orange	98.3 <sup>(b)</sup>	
Blue	98.5 <sup>(b)</sup>	
Yellow	98.7 <sup>(ab)</sup>	
Pink	91.1 <sup>(c)</sup>	

Values in each column not sharing the same lower case superscript are significantly different ( $P < 0.05$ )

**CHAPTER 3. Quantitative genetic parameter estimates for body and carcass traits in a cultured stock of giant freshwater prawn (*Macrobrachium rosenbergii*) selected for fast growth**

- Submitted to Aquaculture (in review).

## **Preface to Chapter 3**

This study was initiated to estimate the heritabilities and correlations between body and carcass weight traits in a cultured stock of giant freshwater prawn selected for growth rate in Vietnam. The data set consisted of 18,387 body and 1,730 carcass records, as well as full pedigree information collected over four generations. Statistical models were tested in several scenarios before one was chosen for estimating variances, covariances, heritabilities and correlations. The current study was the first study that incorporates male GFP morphotype and female GFP reproductive status in formal analyses. Genetic parameters were not only estimated for the mixed-sex population but also for the female and the male sub-populations. Our results confirm that selection for high growth rate based on breeding values estimated by fitting an animal model to the data can significantly improve mean body and carcass weight in GFP.

**Quantitative genetic parameter estimates for body and carcass traits in a cultured stock of giant freshwater prawn (*Macrobrachium rosenbergii*) selected for fast growth**

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## **Abstract**

The aim of the current study was to estimate the heritability and correlations between body and carcass weight traits in a cultured stock of giant freshwater prawn (GFP) (*Macrobrachium rosenbergii*) selected for growth rate in Vietnam. The data set consisted of 18,387 body and 1,730 carcass records, as well as full pedigree information collected over four generations. Variance and covariance components were estimated by restricted maximum likelihood fitting a multi-trait animal model. Across generations, estimates of heritability for body and carcass weight traits were moderate and ranged from 0.14 to 0.19 and 0.17 to 0.21, respectively. Body trait heritabilities estimated for females were significantly higher than for males whereas carcass weight trait heritabilities estimated for females and males were not significantly different ( $P > 0.05$ ). Maternal and common environmental effects for body traits accounted for 4 to 5% of the total variance and were greater in females (7 to 10%) than in males (4 to 5%). Genetic correlations among body traits were generally high in both sexes. Genetic correlations between body and carcass weight traits were also high in the mixed sexes. Our results confirm therefore that selection for high growth rate based on breeding values estimated by fitting an animal model to the data can significantly improve mean body and carcass weight in GFP.

**Keywords:** *Macrobrachium rosenbergii*, body and carcass weight traits, heritability, correlations.

## **1. Introduction**

The giant freshwater prawn (GFP, *Macrobrachium rosenbergii*) is one of the most important crustaceans in the inland aquaculture sector of many tropical and subtropical countries. In the last decade there has been an expansion of GFP farming in many Asian countries, with an average annual rate of increase of 48% between 1999 and 2001 (New, 2005). China dominates GFP production and contributes approximately 29% to total world output, followed by India, Thailand, Bangladesh and Taiwan. Vietnam is not currently one of the major producers of GFP, but production is increasing (New *et al.*, 2008). GFP has become a favoured aquaculture species in Vietnam because growth rate is relatively high which allows market size to be reached within 6 to 8 months. GFP grows well on a relatively low protein, low cost diet and attracts a high market price and demand is high in both domestic and export markets. Recent efforts have been directed at increasing the profitability of GFP culture by developing more sustainable culture practices for the species (Kutty, 2005) and producing of all-male stocks applying neo-female technology (Rungsin *et al.*, 2006). All-male culture of GFP produces greater returns than mixed or all-female culture (Nair *et al.*, 2006). Even though there is capacity to expand GFP production, production efficiency is currently low because there are no improved culture strains available and farmed stocks are vulnerable to disease (Thanh, 2009). The current situation highlights the need for implementation of a systematic stock improvement program for GFP, aimed at improving economically important traits in this species.

The benefits derived from selective breeding have been demonstrated repeatedly in livestock and in some fish species (Gjedrem, 2000), but systematic artificial selection has rarely been applied to shrimps. Key elements of any selective breeding program include the definition of clear breeding objectives and availability of estimates of relevant phenotypic and genetic parameters (e.g. heritability estimates and correlations among traits). Growth rate has shown moderate to high heritability in a number of penaeid species that should result in a reasonable rate of response to selection of 10 to 20% per generation (Gjedrem, 2005) and theoretically this could double growth rate over 5 to 10 generations. Consequently, improving growth rate has been identified as the most important trait in the breeding objective in cultured shrimp species. Currently, there is a paucity of peer-reviewed literature documenting selective breeding programs in commercially important crustaceans. There have, however, been a few recent studies in crustaceans, that have either estimated heritability for weight or for multiple traits including growth rate. Species examined include: redclaw crayfish (*C. quadricarinatus*) (McPhee *et al.*,

2004; Jones *et al.*, 2000), pacific white shrimp (*P. vannamei*) (Gitterle, Rye *et al.*, 2005; Juárez *et al.*, 2007; Argue *et al.*, 2002; Arcos *et al.*, 2004; Pérez-Rostro and Ibarra, 2003a; 2003b; Pérez-Rostro *et al.*, 1999), black tiger shrimp (*P. monodon*) (Benzie *et al.*, 1997; Macbeth *et al.*, 2007; Kenway *et al.*, 2006; Coman *et al.*, 2010b), kuruma prawn (*P. japonicus*) (Hetzl *et al.*, 2000) and giant freshwater prawn (*M. rosenbergii*) (Malecha *et al.*, 1984; Kitcharoen *et al.*, 2011; Thanh, 2009; Luan *et al.*, 2012). Several studies have also estimated heritability of weight at different ages (Coman *et al.*, 2010b; Kitcharoen *et al.*, 2011; McPhee *et al.*, 2004; Kenway *et al.*, 2006) or between sexes (Argue *et al.*, 2002; Pérez-Rostro *et al.*, 1999; Kitcharoen *et al.*, 2011).

In the present study we analyzed a four generation data set from a fully pedigreed selective breeding program for GFP in Vietnam. We estimated phenotypic and genetic parameters for body and carcass weight traits. Because GFP is sexually dimorphic, we report all estimates for mixed sexes as well as for females and males separately.

## **2. Materials and methods**

### **2.1. The founder population**

In 2007, three GFP cultured strains (two native Vietnamese, Dong Nai and Mekong, and an exotic strain from Malaysia) were sampled and taken to the National Breeding Center for Southern Freshwater Aquaculture (NABRECSOFA), under the Research Institute for Aquaculture No. 2 in Vietnam (RIA2). Brood stock for the two native strains were collected from two geographically independent natural drainage basins in Vietnam the Mekong River and the Dong Nai River (Thanh *et al.*, 2009) as juveniles and sub-adults (5 to 10 g individuals). The Mekong River has a very large drainage basin so brood stock were collected from the two largest streams namely the Tien and Hau Rivers. Wild GFP were collected at different localities (upstream, middle and the lower sections of both rivers) from sites with different ecological conditions (freshwater and brackish water) in a number of different provinces (An Giang, Dong Thap, Soc Trang, Ben Tre and Tien Giang provinces) and at different times of the year. To maximize diversity, brood stock from the Dong Nai River were collected in the same way as for the Mekong strain. The Malaysian strain (juveniles) was obtained from Malaysia, and provided by the WorldFish Center. The three stocks were kept separately in mesh hapas (100 m<sup>2</sup>) at a stocking density of two individuals per m<sup>2</sup> in a 5,000 m<sup>2</sup> earthen pond. Air supply systems were

installed in each hapa and operated at night (9 pm to 6 am) to maintain adequate dissolved oxygen concentrations. Two months before the spawning season, healthy adult females and males were chosen as brood stock for each strain and held in separate hapas for conditioning. Brood stock were fed twice daily, with a 40% crude protein commercial prawn pellet in the morning, and chopped beef liver, squid or flesh of marine fish in the afternoon.

## **2.2. Mating design and family production**

To generate the F0 generation (year 2008), a complete 3 x 3 diallel cross resulting in 9 crosses was carried out in order to establish a synthetic base population for selection (Table 1). The target was to produce 108 full-sib families from 9 crosses. In the F1 (year 2009), F2 (year 2010) and F3 (year 2011) generations, matings were made between genetically unrelated brood stock (to minimize inbreeding) to produce full-sib and (paternal) half-sib families.

In order to shorten the mating time to produce full-sib and half-sib families, from the F1 generation, a GIFT approach (WorldFishCenter, 2004) was applied where 5 to 8 healthy females from two families were stocked with a single male from another family in each 4m<sup>2</sup> mesh mating hapa submerged to a depth of 0.8 m in an earthen pond. All mating hapas were checked weekly, and if at least one female from either family had mated successfully (berried female) the male would then be removed. In this way, paternal half-sibs were produced. If one or more females were mated but they came from the same family, all other females from that family were then removed; females from a second family remained with the male until the end of the mating period to produce half-sib families. When males died during the mating period, another male from the same family was added. If more than a single female from a family mated successfully, one was used to produce a full-sib family and the others were used as backup in case larval rearing failed in the target family.

All berried females at each checking time were removed as a batch into a larger (30 m<sup>2</sup>) mesh hapa at a stocking density of 1 individual per m<sup>2</sup> to avoid unnecessary disturbance from checking activities. Berried females were checked 10 days after being restocked into hapas and at one or two day intervals after the first check. As all individuals were tagged (elastomer tags), we could trace their mating history. When eggs changed from orange to dark grey, berried females were ready to spawn. Ripe berried females were then transferred to the hatchery for spawning



where they were disinfected using a 1 minute exposure to 1 ppm iodine. They were then stocked individually into a 70 l circular plastic container with a water column of 60 cm. Berried females were kept at 28 to 32°C, water salinity at 12 ‰ (diluted seawater) with aeration provided via an air-stone. Spawning usually occurred at night (between 10 pm and 2 am) and newly hatched nauplii from each family were collected the next morning. Nauplii from each family were disinfected with 5 ppm formalin for 1 minute and families placed separately into larval rearing tanks. At the start of the experiment, the target was to produce 108 families (12 families from each of the nine crosses). Only 81 families were produced however, with the number of families ranging from 6 to 13 in each of the 9 crosses (Table 1). The success rate for mating was 75%.

### **2.3. Larvae rearing**

For the F0 to F2 generations, larvae from each family were reared separately in 70 l circular plastic containers while larvae from each family in the F3 generation were reared separately in 1 m<sup>3</sup> fiberglass tanks. Stocking densities used were 40 and 30 nauplii per liter for circular plastic containers and fiberglass tanks, respectively. We employed an open clearwater larval culture system (Phuong *et al.*, 2006) with addition of probiotics. This is currently the most popular larval rearing system used in the Mekong delta in Vietnam. Larvae were fed only with newly hatched brine shrimp nauplii (3 times per day) for the first ten days followed by a combination of brine shrimp nauplii and egg custard (chicken egg, high calcium milk powder, shrimp, squid flesh and fish oil) (Thanh *et al.*, 2009). For the first ten days, no water exchanges were conducted, following which, 20 to 50% of the water was exchanged every two days or when necessary. Control of temperature during larval rearing was a very important factor. Water temperature should ideally be maintained between 30 to 34°C and fluctuations should not exceed 4°C during any one day. Post-larvae (PL) were normally observed after 20 to 30 days in larval rearing tanks and metamorphosis into the PL stage occurred after 25 to 40 days.

### **2.4. Juvenile rearing and tagging**

Postlarvae (PL) from each family were reared separately in 1 m<sup>3</sup> fiberglass tanks for two weeks at a stocking density of 1,000 PLs per m<sup>3</sup>. They were fed with a commercial prawn pellet (available for *P. monodon*) at starting feed size. Constant aeration was applied and waste matter was removed everyday via a siphon. A mesh net was supplied to provide substrate for PLs to

hide, in order to reduce cannibalism. After 2 weeks, families were transferred separately into a fine mesh hapa of 4 m<sup>2</sup> submerged into an earthen pond at a stocking density of 150 individuals per m<sup>2</sup>. Hapas were supplied with air from 9 pm to 6 am and PLs were fed with a 40% crude protein commercial prawn pellet (manufactured by Uni-President Co. in Vietnam). Hapas were cleaned every two weeks to ensure good water flow. PLs were kept in hapas for six to eight weeks until they reached a suitable size for tagging (around 2 g). All juveniles in each family were tagged as a batch using visible implant elastomer (VIE) tags as described by Hung *et al.* (2012) . Two tags of 5 to 6 different colors were applied to individual prawns to maintain pedigree records. After tagging, juveniles from each family were kept in a 1m<sup>3</sup> fiberglass tank with constant aeration and fed with pellets for 3 days to acclimate. After tags were verified, 60 to 120 juveniles chosen randomly from each family were released into two common earthen ponds of 3,500 m<sup>2</sup> and a single 800 m<sup>2</sup> pond for grow-out.

## **2.5. Pond grow-out**

Grow-out stocking density was set at 2 individuals per m<sup>2</sup>. No aeration was required and environmental factors were checked frequently. Water was exchanged at least twice a month via gravity flow or when required by pumping. Juveniles in grow-out ponds were fed with commercial prawn pellet containing 35% crude protein at stocking that was reduced to 30% for the last 4 grow-out weeks. At harvest, all individuals were harvested using a cast-net and were weighed and measured. Later, after data analysis, individuals with the highest breeding values were chosen as brood stock (the Selection line) for the next generation. The Control group in each generation consisted of individuals that had a mean (or as close as possible to the mean) EBV in that generation.

## **2.6. Trait measurement**

### ***2.6.1. Male morphotype and female reproductive status records***

Five adult morphotypes for males and three individual reproductive status classes for females were recorded.

For males, three basic morphotypes that have been documented by Kuris *et al* (1987) and include: blue claw males (BC), orange claw (OC) and small (SM) males. BC males are generally

the largest individuals having very long claws that are deep blue. BC males are dominant, territorial males but grow relatively slowly. OC males are also large and have long claws (but shorter than BC males) and are usually orange in color. OC males are not territorial, have poorer mating success and show higher growth rates. SM males are small and have short claws that are generally not pigmented and are translucent. SM males are subordinate, non territorial and only mate with females using an opportunistic reproductive behavior. In addition to the three basic male morphotypes, males that had lost their chelipeds (No claw - NC) (Hulata *et al.*, 1990) and individuals that appeared senescent (Old blue claw - OBC) (Sagi and Ra'anan, 1988) were also scored. Hulata *et al* (1990) reported that NC males were mainly BC males that had underwent cheliped autotomy. We also observed NC males that resulted from fighting with other males or that occurred due to netting or handling during harvest or measurement. In nature, NC males can regenerate new orange claw(s) and will continue to grow. OBC males are believed to result from BC males, they have a senescent appearance and their bodies are covered with algae.

Female GFP can be classified according to their reproductive status. Ra'Anan *et al* (1991) classified female GFP into four groups namely: (a) pre-reproductive females, with narrow brood chambers and no visible gonads; and (b) ripening females, with narrow brood chambers but with visible gonads (Sagi and Ra'anan, 1985). The first two female groups are immature as males never attached spermatophores to them. Mature females are either (c) ovigerous with enlarged brood chambers bearing eggs or (d) post-ovigerous, with an enlarged brood chamber but with no visible gonads. Female GFP in this study were harvested and recorded at the mature stage so groups (a) and (b) as described above were not present. For mature females, Ra'anan *et al* (1991) classified them into two groups while the (c) group he called berried female (BF) that are females with an egg mass suspended under the abdomen and the later (d) group were called spawned females (SF) that are females that had already spawned. Apart from the two mature female groups described, we also found post-ovigerous females, with an enlarged brood chamber and having visible gonads that we called, mature ovary females (MOF). This female GFP classification system was also applied by Thanh *et al* (2009) and Aflolo *et al* (2012a). One of the reasons for classifying female GFP according to their reproductive status comes from the fact that their body weights are affected by reproductive status. Part of the variation in weight of sexually mature females may be attributed to the presence of eggs in ovigerous females and this can comprise up to 10 to 15% of their total weight (Ra'Anan *et al.*, 1991). At spawning, berried

females release eggs and become spawned females and as a consequence, experience a body weight loss.

### **2.6.2. Body and carcass weight trait records**

Six body traits were measured on each individual at harvest. These included: body weight (BW), body length (BL), cephalothorax length (CL), abdominal length (AL), cephalothorax width (CW) and abdominal width (AW). Estimation of carcass weight traits required slaughter, so all prawns harvested from the 800 m<sup>2</sup> pond in generation 2 were slaughtered. Three carcass weight traits including abdominal weight (AWT), skeleton-off weight (SOW), telson-off weight (TOW) were recorded. Details about the measurement of these traits are presented in Table 2 and Figure 1. In addition to body and carcass weight trait records, tag code, sex and culture pond were also recorded at harvest.

## **2.7. Statistical analysis**

### **2.7.1. Statistic model testing**

Statistical analyses were carried out on a data set consisting of 18,387 records collected over four generations of selection. All traits were evaluated for normality before undertaking further analyses and raw data were transformed where appropriate. Exploratory analyses using a general linear model (GLM, in SAS 9.1 Institute, Inc, 1997) were undertaken. Differences in body weight among morphotypes in males produced a skewed distribution for body weight. Hence, body weight transformation to natural logarithms, square root, cubic root and box-cox were carried out. The square root transformation gave the best results in terms of restoring normality and was selected for all body weight analyses. Other body and carcass weight traits approximated normal distributions and so data did not require transformation. A summary of all fixed effects and covariates is given in Table 3. Statistical significance of effects were assessed based on Wald tests using a mixed model using ASReml (Gilmour *et al.*, 2009).

After all significant fixed effects were identified; models were fitted according to several scenarios. Models 1 and 2 included either 5 male morphotypes or the 3 basic (BC, OC and SM) male morphotypes, respectively. These models also included the 3 female reproductive statuses fitted as fixed effects. Both model 1 and 2 included dam as a random effect. To assess the

importance of fitting dam as a random effect, a third model was fitted (model 3) that was as for model 2 but with the dam effect removed. We calculated the differences between the log likelihoods of the two models (2 and 3) for all traits. The criterion used was that if twice the log likelihood difference was greater than 3.8, then the difference would be statistically significant ( $P < 0.05$ ). Similarly, to assess the importance of fitting male morphotype and female reproductive status, a fourth model was fitted (model 4), that was as for model 2 but with male morphotype and female reproductive status removed. The results from fitting the different models are shown in Table 4. In addition to treating male morphotype and female reproductive status as fixed effects (models 1 to 4) these features can also be treated as traits. This later approach enabled exploration of genetic variation for male morphotype and female reproductive status.

Variance and covariance components for additive genetic effects, common full-sib effects and residual effects for body and carcass traits were estimated using a restricted maximum likelihood (REML) method with the ASreml software (Gilmour *et al.*, 2009).

In matrix notation the mixed model can be written as:

$$\mathbf{y} = \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{a} + \mathbf{W}\mathbf{c} + \mathbf{e} \quad [1]$$

where  $\mathbf{y}$  is the vector of observations for body or carcass weight traits,  $\mathbf{b}$  is the vector of the fixed effects including generation (spawning seasons, 1 to 4), line (selection or control), sex (female or male), pond (two grow-out ponds used per generation), male morphotype was fitted as an effect in males, whereas female reproductive status was fitted as an effect in females, the second order interaction between generation and line and interaction between pond and generation. A linear covariate (number of days from stock to harvest) was fitted within sex, ponds and generation subclasses. Vector  $\mathbf{a}$  is the random animal additive genetic effects  $\sim (0, \mathbf{A}\sigma_a^2)$  where  $\mathbf{A}$  is the additive genetic (numerator) relationship matrix among the animals,  $\mathbf{c}$  is the vector of dam effects (or maternal and common environmental effects in addition to additive genetics)  $\sim (0, \mathbf{I}\sigma_c^2)$  and  $\mathbf{e}$  is the vector of residual effects  $\sim (0, \mathbf{I}\sigma_e^2)$ . The dam component ( $\sigma_D^2$ ) is most likely a combination of maternal and common environment effects (thus,  $\sigma_D^2 = \sigma_{M+CE}^2$ , referred to as  $\sigma_C^2$ ) caused by the separate rearing of full-sib families until individuals reached a suitable size for physical tagging.  $\mathbf{X}$ ,  $\mathbf{Z}$  and  $\mathbf{W}$  are incidence matrices relating observations to fixed effects, additive genetic effect of the individual animal and common full-sib effect included in the model,

respectively. Under the model [1],  $\text{var}(\mathbf{a}) = \mathbf{G} = \mathbf{A}\sigma_a^2$ . The remaining effects are assumed to be distributed as  $\text{var}(\mathbf{e}) = \mathbf{R} = \mathbf{I}\sigma_e^2$ ,  $\text{var}(\mathbf{c}) = \mathbf{W} = \mathbf{I}\sigma_c^2$ , where  $\mathbf{I}$  is an identity matrix with ones on the diagonal. The expectations of all random effects are zero,  $\text{cov}(\mathbf{a}, \mathbf{e}) = 0$  and  $\text{cov}(\mathbf{a}, \mathbf{c}) = 0$  and thus  $\text{var}(\mathbf{y}) = \mathbf{ZGZ}'\sigma_a^2 + \mathbf{WI}\sigma_c^2\mathbf{W}' + \mathbf{R}$ .

The mixed model equation for the best linear unbiased estimator (BLUE) of estimable functions of  $\mathbf{b}$  and the best linear unbiased prediction of  $\mathbf{a}$  and  $\mathbf{c}$  are:

$$\begin{bmatrix} \hat{b} \\ \hat{a} \\ \hat{c} \end{bmatrix} = \begin{bmatrix} \mathbf{X}'\mathbf{X} & \mathbf{XZ} & \mathbf{X}'\mathbf{W} \\ \mathbf{Z}'\mathbf{X} & \mathbf{Z}'\mathbf{Z} + \mathbf{A}^{-1}\alpha_1 & \mathbf{Z}'\mathbf{W} \\ \mathbf{W}'\mathbf{X} & \mathbf{W}'\mathbf{Z} & \mathbf{W}'\mathbf{W} + \mathbf{I}\alpha_2 \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{X}'\mathbf{y} \\ \mathbf{Z}'\mathbf{y} \\ \mathbf{W}'\mathbf{y} \end{bmatrix} \quad [2]$$

where  $\alpha_1 = \sigma_e^2 / \sigma_a^2$  and  $\alpha_2 = \sigma_e^2 / \sigma_c^2$ .

### 2.7.2. Heritabilities, phenotypic and genetic correlations

Heritabilities were estimated from a single trait model (Equation 2). Phenotypic and genetic correlations were obtained from a series of bi- and tri-variate analyses, involving body weight (recorded in all animals) to avoid selection bias (Kennedy, 1990). The pedigree included all animals, back to the base population to minimize possible biases in the estimation of genetic parameters.

We conducted two separate analyses to obtain genetic parameters. The first analysis was conducted on the full data set (mixed sexes) where measurements from females and males were considered together, and the model included all significant fixed and random effects as described above. In the second analysis, female and male measurements were treated as different traits in order to examine whether there were differences associated with sex. In the second analysis, the model was basically as described above, except that the effect of sex was removed. Genetic correlations between expression of body traits in females and males were estimated via genetic relationships ( $\mathbf{A}$ ) in the full pedigree.

Heritability for body and carcass traits were calculated as  $h^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2}$  and the maternal and common environmental effect as  $c^2 = \frac{\hat{\sigma}_c^2}{\hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2}$  where  $\sigma_a^2$  is the additive genetic variance, the maternal and common environmental variance ( $\sigma_c^2$ ) and the residual variance ( $\sigma_e^2$ ). Genetic and phenotypic correlations among traits were calculated as the covariance divided by the product of the standard deviations of traits:  $r = \frac{\sigma_{12}}{\sqrt{\sigma_1^2} \sqrt{\sigma_2^2}}$  where  $\sigma_{12}$  was the estimated additive genetic or phenotypic covariance between the two traits, and  $\sigma_1^2$  and  $\sigma_2^2$  are the additive genetic or phenotypic variances of traits 1 and 2, respectively.

To our knowledge, in the literature there are no reports of formal tests for significant differences in genetic parameter estimates between sexes or environments. We used the z-score as an approximate method for assessing whether heritability, maternal and common environmental effects and correlation estimates were significantly different from each other, or from zero, and whether genetic correlations between sexes were significantly different from one. The formula to calculate z-scores used was as follows:

$$z = \frac{x_i - x_j}{(\sigma_i^2 + \sigma_j^2)^{0.5}}$$

where  $x_i$  and  $x_j$  are the estimates of heritability, maternal and common environmental effects, or genetic correlations for the two traits (or the two sexes), and  $\sigma_i$  and  $\sigma_j$  are their respective standard errors. Both  $x_j$  and  $\sigma_j$  were set to zero or one when we tested whether an estimate was significantly different from zero or one, respectively. Resulting z-scores were then tested against a large sample normal distribution. The z-score test is generally equivalent to the weighted least squares approach used by Ponzoni (1975).

### 3. Results

#### 3.1. Descriptive statistics

Table 2 shows the number of observations, simple mean, standard deviation and coefficient of variation values for body and carcass weight traits for females, males and mixed sexes. The mean body weight of males was 92.9% greater than for females. The mean for other body traits and carcass weight traits of males were 3.0 to 32.9% greater than for females. The coefficient of variation for body weight was high, whereas for other body traits and for carcass weight traits they were lower and ranged from 17.2 to 43.8%. For all traits, males had coefficient of variations twice as large as that of females. The mixed sexes had coefficient of variation estimates that were within the range of the female and the male estimates but that were closer to those of males.

### **3.2. Heritability estimates using different models**

Tables 3 shows the significance of fixed effects in the model where: sex, interaction between pond and generation, interaction between generation and line, male morphotype fitted in males, female reproductive status fitted in females were significant ( $P < 0.01$  to  $P < 0.001$ ) for all traits. The linear covariate (number of days from stock to harvest) fitted within sex, ponds and generation subclasses was also significant ( $P < 0.001$ ) for all body and carcass weight traits.

Results of testing the 4 models are presented in Table 4 and shows that regardless of whether male morphotypes were classified into 5 (model 1) or 3 (model 2) classes heritability estimates were not significantly different. Heritability estimates for body traits were however significantly higher if either dam was excluded from random effects (model 3) or male morphotype and female reproductive status were excluded from fixed effects (model 4) in the statistical model. Heritability estimates for carcass weight traits were higher using either model 3 or model 4 but differences were not significant (except AWT heritability estimated in model 3). Comparisons of twice the differences between log-likelihoods of model 2 and model 3 indicated that when dam was included this significantly improved the model (significantly higher log-likelihoods achieved in model 2 than with model 3) for all traits except for SOW.

### **3.3. Least square means for female reproductive status and male morphotype**

Table 5 contains least square means (LSMs) for all body and carcass weight traits. For females, the three classifications related to reproductive status (BF, MOF and SF) showed small differences in proportions but significant different in LSM body weights. The five male



classifications related to individual morphotype were unbalanced in terms of proportions in each class. More than a half (54%) of the male population were Orange claw (OC) males, approximately 17% were Blue claw (BC) males and Small (SM) males, and the remaining approximately 12% comprised combined No claw (NC) males and Old blue claw (OBC) males. The SM male LSM for body weight was only approximately 9 to 13% that of the other male classes. The large differences in LSM body weight among male morphotypes affects the distribution so body weight required transformation before analysis. A similar phenomenon was evident for LSMs for other body and carcass weight traits where non-normal distributions were evident especially among male morphotype groups but were much smaller than for body weight and hence these data did not require transformation. Interestingly, BC and OBC males showed significantly higher body weight than did OC males but OC males possessed significantly higher abdominal weight than did OBC males but non-significant difference was observed for abdominal weight with BC males. This indicates that BC males and, in particular OBC males possess significantly heavier and larger (e.g. for BC males) heads (cephalothoraxes) than OC males that are in a fast-growth phase. NC males showed significantly smaller body weight than OBC or OC males, but were very similar for other body traits (BL, CL, AL, CW and AW). Carcass weights (AWT, SOW and TOW) for NC males however, were closer to those of OC males than for OBC males. This suggests that NC males are more likely come from OC males than OBC males even though in theory NC males can result from any male morphotype. In females, BF showed significantly heavier body weight than MOF and SF mainly contributed by abdominal weight (AWT). But, when the exoskeleton was removed together with an egg mass present in BF females, the edible components (SOW and/or TOW) were significantly lower than that for MOF and non-significantly different from SF. This indicates that presence of eggs has a significant influence on female body weight.

### **3.4. Heritability estimates by sex**

Heritability estimates using model 2 for mixed sexes (Table 6) for all traits were moderate and ranged from 0.14 to 0.21. The moderate heritability estimate for body weight ( $0.14 \pm 0.028$ ) indicates that there is potential to improve growth rate in GFP via artificial selection. Maternal and common environment effects however, accounted for only a small proportion of the total variance in the mixed sexes and ranged from 4 to 5% for body traits and a higher proportion of 8

to 11% for carcass weight traits. When female and male expressions were treated as different characteristics, all body traits showed significantly higher heritability estimates in females than in males (Table 6) but heritability estimates for carcass weight traits were not significantly different ( $P > 0.05$ ). Maternal and common environment effect ( $c^2$ ) estimates were also significantly higher in females than in males for all body traits but not for carcass weight traits.

When treating male morphotype and female reproductive status as traits, the heritability estimates were low and negligible  $0.085 \pm 0.032$  and  $0.007 \pm 0.005$ , respectively. Heritability estimates for male morphotype and female reproductive status suggest that it may be possible to change the relative proportions of male morphotypes by selection but not the relative proportions of female reproductive status. In addition, OC males show a higher carcass ratio (AWT/BW) than BC or OBC males. Furthermore, because OC males are in growth phase, they may be the most desirable morphotype for production.

### **3.5. Correlations between body traits in the two sexes**

Phenotypic and genetic correlations among the various body traits are presented in Table 6. In general, genetic correlations by sex follow the same pattern with correlations between body traits all positive and high and approaching unity. No significant differences were observed in females (0.93 to 0.99) compared with males (0.96 to 0.99). Very high genetic correlations among body traits suggest that they are likely to be controlled by the same set of genes and hence can be improved simultaneously in a selection program. Consistently with genetic correlations, phenotypic correlations among body traits were also high in females (0.76 to 0.95) and males (0.76 to 0.94) and were not significantly different between the sexes.

Genetic correlations between body traits and carcass weight traits for mixed sexes were also high (0.90 to 0.99, data not shown). Carcass weights or the saleable and edible flesh component also showed high genetic correlations with body weight (0.96 to 0.99, data not shown) and this result indicates that harvest body weight can be an effective selection criterion for improving both growth rate and carcass weight in a breeding program. All phenotypic correlations between body and carcass weight traits were generally consistent with genetic correlations.

## 4. Discussion

### 4.1. Sexual dimorphism and phenotypic variation

Sexual dimorphism for growth traits has been reported in many shrimp species including *P. vannamei* (Pérez-Rostro and Ibarra, 2003a; Argue *et al.*, 2002) and *M. rosenbergii* (Malecha *et al.*, 1984; Kitcharoen *et al.*, 2011; Luan *et al.*, 2012). Sexual dimorphism in cultured white shrimp (*P. vannamei*) occurs at body weights of 10 to 17 g, where females are significantly larger and heavier than males for most body traits including body weight (4.8%) and total length (1.2%) (Pérez-Rostro and Ibarra, 2003a). By contrast, GFP (*M. rosenbergii*) males generally reach significantly larger size and heavier body weight (approximately 93% in the current study) compared with females.

The genetic variation observed for body traits (e.g. body weight) and carcass weight traits (e.g. abdominal weight) in the GFP population examined here indicates that there is a good potential for this population to continue to respond to selection in future generations. The coefficient of variation for body weight estimated here (67.9%) for GFP is higher than that reported for the freshwater crayfish (*C. destructor*) (42%) (Jerry *et al.*, 2002) or pacific white shrimp (*P. vannamei*) (15 to 29%) (Gitterle, Rye *et al.*, 2005) or pacific blue shrimp (*P. stylirostris*) (19%) (Lester, 1983) or in giant freshwater prawn (*M. rosenbergii*) (38.3 to 48.4% in different generations) (Luan *et al.*, 2012). Compared with some fish species, the coefficient of variation for body weight in GFP was also higher than for GIFT tilapia (25.1% reported by Nguyen *et al.* (2010), 25 to 34% reported by Maluwa and Gjerde (2006) and 48 to 60% reported by Ponzoni *et al.* (2005)) or for striped catfish (calculated at 40.0 and 24.1% in G2 and G3 generations, respectively) (Sang, 2010). Low genetic variation in a GFP population subjected to selection for growth in study by Luan *et al.* (2012) may have resulted from low genetic diversity in two (Zhejiang and Guangxi) out of three (Zhejiang, Guangxi and Burma) founder strains used to produce the selected line that also had long history of domestication. In contrast, a study by Thanh (2009) assessed variation at six microsatellites to show that there was no significant difference in levels of genetic diversity between the F1 generation and the wild founding population. The same population was used as the founding stock in the current study. The high level of genetic variation evident in the population used in the current study may in part, result

from the relatively large amount of genetic diversity that had been accumulated in the founder generation and the way that the stock has been managed subsequently.

#### **4.2. Model testing**

Male GFP morphotypes have been reported to result from social dominance effects (Karplus *et al.*, 1991) in the male population. While three basic male morphotypes namely BC, OC and SM have been well documented, the other two (NC and OBC) morphotypes recorded here are less well recognized and may not result from the same process. The majority of NC males are believed to result from fighting with other males in the pond or in the temporary container tank during harvesting or result from handling, factors that are unlikely to be related to social dominance. Classifying males as OBC vs. BC may also cause problems based on how to separate one from another for on amount of algae attached or senescent appearance. A comparison between models 1 and 2 shows minor differences in variance and heritability estimates across traits. This may be due to the fact that the two morphotypes (NC and OBC) jointly only accounted for approximately 12% of the male population, and so their effect was diluted in the overall data set. Although models 3 and 4 produced similar heritability estimates that are significantly higher than those estimated using model 2, the higher outcomes result from different reasons. The nested mating design in the current study enabled us to partition the common full-sib effect from the additive genetic effect. Hence, when dam was excluded from the model (model 3) the common full-sib effect is confounded with the additive genetic effect. On the other hand, when male morphotype and (or) female reproductive status were excluded from the model (model 4) this can confound part of the environmental variance with additive genetic affect. Both will bias estimates upwards as we observed here with different and higher heritability estimates achieved for body traits using model 3 or model 4 compared with model 2. GFP male morphotype and female reproductive status have been recognized in previous studies (Thanh *et al.*, 2009; Aflalo *et al.*, 2012a), but they have never been integrated into formal quantitative genetic analyses. However, as male morphotype and female reproductive status both showed significant effects on the model, their inclusion in the model is warranted when estimating genetic parameters in GFP in order to avoid biasing the estimates.

### 4.3. Heritabilities and maternal and common environment effects

GFP is known to be not only sexually dimorphic for growth rate but heritability estimates for growth traits are also known to differ significantly between the sexes. Malecha *et al.* (1984) reported that heritability for growth rate in GFP females was  $0.35 \pm 0.15$  while that for males was not significantly different from zero. Kitcharoen *et al.* (2011) also reported that heritability for body weight in GFP at sixth months of age (under bulk rearing conditions) was  $0.33 \pm 0.14$  for females but only  $0.03 \pm 0.04$  for males. Other body traits including total length, cephalothorax length and claw length showed similar trends with heritability estimates for females moderate to high while for males, they were very low and close to zero. The studies by Malecha *et al.* (1984) and Kitcharoen *et al.* (2011) were based however, on only a small number of families and included data from only a single generation with limited pedigree information. Due to the limited sample sizes available in both studies, (Kitcharoen's study only employed 8 sires and 16 dams). It is not surprising therefore, that all estimates of heritability in the study by Kitcharoen *et al.* (2011) were not significantly different from zero. In addition, both studies were conducted under laboratory (rather than field conditions) where experimental animals showed only very small weight gains across the experimental period. Male morphotype and female reproductive status were not recorded or included in the model, and may have influenced their estimates. After six months of grow-out, GFP in Kitcharoen's study had reached only approximately 10 g, which is much less over the same time than frame in pond culture. Genetic parameters are best estimated under actual culture conditions and where possible on individuals that have reached market size. Recent large scale study using a similar design to the current study of GFP by Luan *et al.* (2012) showed that across five generations, the body weight heritability estimate for females ( $0.137 \pm 0.024$ ) was significantly higher than for males ( $0.033 \pm 0.016$ ) even though differences in some generations were non-significant. In the current study, heritability estimates for all body traits were moderate to high (0.29 to 0.39) for females and significantly higher than for males (0.02 to 0.09) and all were significantly different from zero ( $P < 0.05$ ) (except for AL and AW heritability estimates in males). In contrast, heritability estimates for all carcass weight traits were moderate to high for both females (0.37 to 0.41) and males (0.16 to 0.23) and were not significantly different between the sexes. Results here suggest therefore, that selection to improve growth rate in GFP could be more effectively when applied to females rather than to males. Ignoring males totally in the selection program however, can reduce the effectiveness of the

program and potentially result in smaller overall genetic gains. In a mixed sexes GFP population, Luan *et al.* (2012) reported body weight heritability estimated across five generations ( $0.056 \pm 0.014$ ) that was lower than that recorded in the current study ( $0.14 \pm 0.028$ ) even though neither male morphotype nor female reproductive status were fitted in the model. This suggests that Luan's reported heritability estimates would be lower if the same model used here was fitted to their data.

In *P. vannamei*, Argue *et al.* (2002) reported a higher heritability for growth rate in females than for males both in pond culture and in raceway culture. In tilapia, even though body weight of males is commonly 7 to 23% higher than that for females, estimates of heritabilities between the sexes were not significantly different for body weight or for other body traits (e.g. heritability for body weight was 0.18 and 0.20 in females and males, respectively) (Nguyen *et al.*, 2007). In Pacific white shrimp (*P. Vannamei*), Pérez-Rostro *et al.* (1999) have however, reported no evidence for significant differences between the sexes for heritability estimates even though sexual dimorphism for growth rate was evident. The estimate of heritability for body weight in GFP in the current study is lower however, than that reported for some aquatic species notably; pacific white shrimp (*P. vannamei*) (Argue *et al.*, 2002; Juárez *et al.*, 2007; Gitterle, Rye *et al.*, 2005), black tiger prawn (*P. monodon*) (Macbeth *et al.*, 2007; Kenway *et al.*, 2006; Coman *et al.*, 2010b), kuruma prawn (*P. japonicus*) (Hetzl *et al.*, 2000), redclaw crayfish (*C. quadricarinatus*) (McPhee *et al.*, 2004; Jones *et al.*, 2000), GIFT tilapia (Nguyen *et al.*, 2007) and striped catfish (*P. hypophthalmus*) (Sang, 2010) but higher than earlier reports for the same species (*M. rosenbergii*) (Malecha *et al.*, 1984; Kitcharoen *et al.*, 2011; Luan *et al.*, 2012). Heritability estimates for other body traits and carcass weight traits in GFP here were also lower than estimates reported recently for GIFT tilapia (Nguyen *et al.*, 2007) and striped catfish (Sang, 2010).

Maternal and common environmental effects ( $c^2$ ) due to rearing of full-sibs in hapas before tagging are often present early during the life cycle of aquatic organisms, but any effects may not necessarily be carried over to the rest of the life cycle. The  $c^2$  estimates in the mixed sexes data here ranged from 4 to 5% and from 8 to 11% of the total variation for body traits and carcass weight traits, respectively. This is similar to the range reported in GIFT tilapia (Nguyen, Ponzoni, Abu-Bakar *et al.*, 2010) (4 to 8%). This range is lower however, than some maternal and

common environmental effect estimates reported in other species, for example in Striped catfish (Sang, 2010) (14 to 19%). Potential strategies for reducing  $c^2$  in effective breeding programs include: shortening the spawning and nursing periods for full-sib families by upgrading hatchery capacity and improving nursing techniques, tagging the animals at an earlier age or using molecular techniques for posterior assignment of parents that enables common family rearing at a very early stage. This will minimize differences in the length of nursing and grow-out time for full-sib families. Maternal and common environment effects can have a large impact on heritability estimates for body traits, but neglecting the common environment effect would have resulted in overestimation of heritability for this trait. As an illustration, we estimated heritabilities without including the common environmental effect and found that estimated heritability for all body traits increased.

#### **4.4. Genetic correlations**

For body traits, we found positive and high (almost unity) genetic correlations between trait expressions in females and males. However, heritability estimates for females were significantly greater than for males. The additive genetic variances for body traits were generally similar between the sexes, but smaller for males. The greatest difference was in the phenotypic variance (indicative of a greater environmental variance) which was considerably greater for males than for females. Hence, the much lower heritability estimates for males despite the fact that the very high genetic correlations between trait expression in the two sexes suggest that the same set of gene control body traits in both. High genetic correlations between body trait expressions in the two sexes observed here for GFP is similar to findings in Pacific white shrimp by Pérez-Rostro *et al.* (1999) and in GIFT tilapia by Nguyen *et al.* (2007) who reported genetic correlations between sexes for body traits to be very high (0.91 to 0.96). We also observed very high genetic correlations between abdominal weight, which is the most economically important trait to improve with other morphometric body traits ( $r_g = 0.97 - 0.99$ ) in the mixed sexes analysis. This outcome suggests that body traits were closely genetically correlated and are largely controlled essentially by the same set of genes. The strength of the genetic correlations estimated here suggest that any one of the traits tested could be used, on its own or simultaneously, to predict abdominal weight without a requirement for sacrificing individuals. This result is similar to that reported for Pacific white shrimp (*P. vannamei*) (Pérez-Rostro *et al.*, 1999) where abdominal

weight showed very high genetic correlations with body weight, total length, cephalothorax weight, cephalothorax width and abdominal length in both sexes. Selection to improve any of these traits therefore, would likely produce correlated responses in the other traits. For GFP, several products are available in the market including whole body (Head On Shell On – HOSO), abdomen only (Headless Shell On - HLSO), head-off and skeleton-off (Peeled Tail On – PTO) and head-off, skeleton-off and telson-off (Peeled & Undeveined - PUD). All can be classified as carcass weight traits and if growth rate was improved via selection then all correlated traits are likely to show genetic gains.

## **5. Conclusions**

Estimates of heritability for body traits in GFP here were moderate. This result suggests that body weight and other related traits can respond to selection based on estimated breeding values. Genetic correlations among the various body traits and between body traits and carcass weight traits were generally high, and this was consistent for both sexes. Social structure in adult male GFP remains a problem because individual growth rate in males is affected by social rank. Currently, we still know little about the genetic vs. environmental factors that determine how morphotype is expressed in male GFP. More information about this would help with designing more effective genetic improvement programs for this valuable and challenging cultured species.

## **Acknowledgements**

The authors thank Vu, T. N., Ky, T. L. and Nga, T. K. N. for their valuable technical assistance in both the laboratory and field trials, and Dr. Alex Safari (WorldFish Center) for assistance with some aspects of the final data analysis. This work was supported by the Ministry of Agriculture and Rural Development (MARD) in Vietnam and the WorldFish Center in Malaysia through the “Family-based selective breeding program on giant freshwater prawn in Vietnam”. The Australian government AusAID program provided Hung Dinh with an ALA award to undertake his PhD research at Queensland University of Technology (QUT).

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## Figures and tables

### Tables

Table 1: Diallel cross design between Dong Nai strain (DN), Mekong strain (MK) and Malaysian strain (MY) to generate the F0 synthetic base population and number of successful full-sib families produced

Complete diallel cross design		Female parent strain		
		MK	DN	MY
Male parent strain	MK	MK x MK (13)	MK x DN (6)	MK x MY (10)
	DN	DN x MK (10)	DN x DN (10)	DN x MY (6)
	MY	MY x MK (10)	MY x DN (8)	MY x MY (7)

Table 2: Basic statistics for body and carcass weight traits

Traits	Sex	Records (number)	Mean	Standard deviation	Coefficient variation (%)
BW	Female	10,014	26.7	8.8	32.9
	Male	8,373	51.5	37.3	72.5
	All	18,387	38.0	25.8	67.9
BL	Female	10,014	8.5	0.9	11.1
	Male	8,373	9.7	2.3	24.5
	All	18,387	9.0	1.8	20.2
CL	Female	10,014	3.5	0.5	13.8
	Male	8,373	4.2	1.2	28.6
	All	18,387	3.8	1.0	25.4
AL	Female	10,014	5.0	0.5	10.0
	Male	8,373	5.5	1.2	21.5
	All	18,387	5.2	0.9	17.2
CW	Female	10,014	2.1	0.3	13.4
	Male	8,373	2.6	0.7	28.8
	All	18,387	2.3	0.6	25.1
AW	Female	10,014	1.7	0.2	13.5
	Male	8,373	1.7	0.4	25.7
	All	18,387	1.7	0.3	20.3
AWT	Female	1,050	13.6	3.2	23.9
	Male	680	16.6	8.6	51.9
	All	1,730	14.8	6.2	41.7
SOW	Female	1,050	10.5	2.3	22.2
	Male	680	14.0	7.3	52.0
	All	1,730	11.9	5.2	43.7
TOW	Female	1,050	10.2	2.3	22.3
	Male	680	13.4	7.0	52.4
	All	1,730	11.5	5.0	43.8

**Note:** BW: body weight (g, total live body weight at harvest); BL: body length (cm, distance from eye orbit to tip of telson); CL: cephalothorax length (cm, distance from eye orbit to the hind margin of the carapace); AL: abdominal length (cm, distance from the hind margin of the carapace to tip of telson); CW: cephalothorax width (cm, the greatest width of the carapace); AW: abdominal width (cm, width of second abdominal segment); AWT: abdominal weight (g, weight of the abdomen); SOW: skeleton-off weight (g, weight of the abdomen after removing exoskeleton); TOW: telson-off weight (g, weight of the abdomen after removing exoskeleton and telson)

Table 3: Test of fixed effects and linear covariate using F-values

Sources of variation	Body traits						Carcass weight traits		
	BW <sup>0.5</sup>	BL	CL	AL	CW	AW	AWT	SOW	TOW
Sex	61.0***	268.1***	237.8***	227.8***	224.4***	11.3***	190.8***	224.8***	210.2***
Pond*Generation	10.1***	7.9***	7.0***	7.6***	10.4***	8.8***			
Generation*Line	8.1***	5.2**	6.7***	5.4**	6.1***	7.7***	15.7***	12.3***	14.4**
	11,470.5**	12,147.0**	18,204.0*	9,988.4**	11,536.7*	9,227.6**			
Mtype(male)	*	*	**	*	**	*	836.6***	847.8***	820.7***
Ftype(female)	92.2***	17.8***	18.6***	12.8***	31.4***	487.0***	72.5***	7.0**	6.4**
Pond*Generation*Sex*Day	482.0***	394.3***	462.0***	274.5***	458.4***	391.6***	13.3***	11.7***	9.1***

**Note:** \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$

*Mtype(male): male morphotype fitted in male; Ftype(female): female reproductive status fitted in female; Day: Grow-out days (day, days from stock to harvest)*

*Trait notations used in this table were described in Table 2*



Table 4: Estimates of variance components, heritabilities ( $h^2$ ) and maternal and common environmental effects of body traits ( $c^2$ ) using different models

Traits	Model	$V_A$	$V_C$	$V_E$	$h^2 \pm se$	$c^2 \pm se$
BW <sup>(0.5)</sup>	1	0.144	0.040	0.749	0.15±0.029	0.04±0.009
	2	0.136	0.043	0.768	0.14±0.028	0.05±0.009
	3	0.339		0.670	0.34±0.027 <sup>(***)</sup>	
	4	0.732	0.151	1.524	0.30±0.042 <sup>(**)</sup>	0.06±0.011
BL	1	0.169	0.031	0.611	0.21±0.034	0.04±0.009
	2	0.153	0.035	0.637	0.19±0.032	0.04±0.009
	3	0.330		0.550	0.38±0.028 <sup>(***)</sup>	
	4	0.740	0.126	1.292	0.34±0.042 <sup>(**)</sup>	0.06±0.010
CL	1	0.033	0.008	0.172	0.15±0.029	0.04±0.008
	2	0.031	0.009	0.178	0.14±0.028	0.04±0.008
	3	0.073		0.157	0.32±0.026 <sup>(***)</sup>	
	4	0.162	0.032	0.348	0.30±0.042 <sup>(**)</sup>	0.06±0.011
AL	1	0.038	0.010	0.196	0.16±0.030	0.04±0.008
	2	0.035	0.011	0.202	0.14±0.028	0.04±0.009
	3	0.086		0.177	0.33±0.026 <sup>(***)</sup>	
	4	0.172	0.034	0.372	0.30±0.041 <sup>(**)</sup>	0.06±0.010
CW	1	0.014	0.003	0.065	0.17±0.031	0.04±0.009
	2	0.013	0.004	0.067	0.16±0.030	0.05±0.009
	3	0.031		0.058	0.35±0.027 <sup>(***)</sup>	
	4	0.057	0.014	0.137	0.27±0.041 <sup>(*)</sup>	0.07±0.011
AW	1	0.005	0.002	0.027	0.16±0.030	0.05±0.009
	2	0.005	0.002	0.028	0.15±0.029	0.05±0.009
	3	0.013		0.024	0.35±0.026 <sup>(***)</sup>	
	4	0.021	0.005	0.052	0.27±0.040 <sup>(*)</sup>	0.07±0.011
AWT	1	2.992	1.147	8.779	0.23±0.126	0.09±0.054
	2	2.165	1.408	9.411	0.17±0.113	0.11±0.052
	3	5.939		7.550	0.44±0.071 <sup>(*)</sup>	
	4	4.780	6.860	18.322	0.16±0.145	0.23±0.074
SOW	1	2.535	0.499	5.919	0.28±0.124	0.06±0.050
	2	1.922	0.714	6.450	0.21±0.113	0.08±0.049
	3	3.811		5.521	0.41±0.068	
	4	4.166	3.990	12.210	0.20±0.150	0.2±0.0730
TOW	1	2.116	0.545	5.919	0.25±0.121	0.06±0.050
	2	1.512	0.763	6.416	0.17±0.109	0.09±0.049
	3	3.503		5.439	0.39±0.067	
	4	3.246	4.019	11.956	0.17±0.146	0.21±0.073

**Note:** \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$

Heritability comparisons for each trait were conducted between other models and model 2

$V_A$ : additive genetic variance,  $V_C$ : maternal and common environmental variance, and  $V_E$ : environmental variance

Trait notations used in this table were described in Table 2

Table 5: Least squares means for male morphotype and female reproductive status

Sex	Female			Male				
Categories	BF	MOF	SF	BC	NC	OBC	OC	SM
<i>Body traits</i>								
<i>n</i>	3,701	2,800	3,513	1,441	391	665	4,515	1,361
<i>%</i>	37.0%	28.0%	35.0%	17.2%	4.7%	7.9%	53.9%	16.3%
BW	27.3 <sup>(e)</sup>	24.8 <sup>(f)</sup>	23.0 <sup>(g)</sup>	77.5 <sup>(a)</sup>	54.1 <sup>(d)</sup>	70.6 <sup>(b)</sup>	58.5 <sup>(c)</sup>	7.2 <sup>(h)</sup>
BL	8.5 <sup>(c)</sup>	8.4 <sup>(d)</sup>	8.3 <sup>(d)</sup>	11.1 <sup>(a)</sup>	10.6 <sup>(b)</sup>	10.5 <sup>(b)</sup>	10.5 <sup>(b)</sup>	5.9 <sup>(e)</sup>
CL	3.5 <sup>(c)</sup>	3.4 <sup>(d)</sup>	3.4 <sup>(d)</sup>	5.0 <sup>(a)</sup>	4.7 <sup>(b)</sup>	4.7 <sup>(b)</sup>	4.6 <sup>(b)</sup>	2.4 <sup>(e)</sup>
AL	5.1 <sup>(c)</sup>	5.0 <sup>(d)</sup>	5.0 <sup>(d)</sup>	6.1 <sup>(a)</sup>	5.9 <sup>(b)</sup>	5.8 <sup>(b)</sup>	5.9 <sup>(b)</sup>	3.5 <sup>(e)</sup>
CW	2.1 <sup>(e)</sup>	2.1 <sup>(d)</sup>	2.1 <sup>(e)</sup>	3.0 <sup>(a)</sup>	2.8 <sup>(c)</sup>	2.9 <sup>(b)</sup>	2.8 <sup>(c)</sup>	1.4 <sup>(f)</sup>
AW	1.7 <sup>(c)</sup>	1.6 <sup>(d)</sup>	1.6 <sup>(e)</sup>	2.0 <sup>(a)</sup>	1.9 <sup>(b)</sup>	1.8 <sup>(b)</sup>	1.9 <sup>(b)</sup>	1.0 <sup>(f)</sup>
<i>Carcass weight traits</i>								
<i>n</i>	264	350	436	51	25	30	402	172
<i>%</i>	25.2%	33.3%	41.5%	7.5%	3.7%	4.4%	59.1%	25.3%
AWT	15.2 <sup>(b)</sup>	12.9 <sup>(c)</sup>	11.8 <sup>(d)</sup>	18.9 <sup>(a)</sup>	18.4 <sup>(a)</sup>	12.3 <sup>(cd)</sup>	20.4 <sup>(a)</sup>	4.6 <sup>(e)</sup>
SOW	9.8 <sup>(d)</sup>	10.7 <sup>(c)</sup>	9.8 <sup>(d)</sup>	15.7 <sup>(b)</sup>	15.9 <sup>(ab)</sup>	9.4 <sup>(cd)</sup>	17.3 <sup>(a)</sup>	4.0 <sup>(e)</sup>
TOW	9.4 <sup>(d)</sup>	10.3 <sup>(c)</sup>	9.5 <sup>(d)</sup>	15.1 <sup>(b)</sup>	15.4 <sup>(ab)</sup>	9.1 <sup>(cd)</sup>	16.6 <sup>(a)</sup>	3.8 <sup>(e)</sup>

**Note:** Least square means (estimates using model 1) not sharing the same upper case superscript are significantly different ( $P < 0.01$ )

BF: berried female; MOF: mature ovaries female; SF: spawned female; BC: blue claw male; NC: no claw male; OBC: old blue claw male; OC: orange claw male and SM: small male  
Trait notations used in this table were described in Table 2

Table 6: Additive genetic variance ( $\sigma^2_A$ ), phenotypic variance ( $\sigma^2_P$ ), heritability ( $h^2$ ) and common environmental effect ( $c^2$ ) estimates for females and males

Traits		Sex	N	$V_A$	$V_P$	$h^2 \pm se$	$c^2 \pm se$
Body traits	BW <sup>(0.5)</sup>	Female	10,014	0.165	0.426	0.39±0.054 <sup>(a)</sup>	0.10±0.017 <sup>(a)</sup>
		Male	7,982	0.124	1.597	0.08±0.025 <sup>(b)</sup>	0.05±0.011 <sup>(b)</sup>
	BL	Female	10,014	0.187	0.403	0.38±0.053 <sup>(a)</sup>	0.10±0.017 <sup>(a)</sup>
		Male	7,982	0.068	1.224	0.06±0.022 <sup>(b)</sup>	0.05±0.010 <sup>(b)</sup>
	CL	Female	10,014	0.041	0.115	0.36±0.051 <sup>(a)</sup>	0.07±0.015 <sup>(a)</sup>
		Male	7,982	0.021	0.345	0.06±0.022 <sup>(b)</sup>	0.04±0.010 <sup>(b)</sup>
	AL	Female	10,014	0.049	0.164	0.30±0.049 <sup>(a)</sup>	0.09±0.016 <sup>(a)</sup>
		Male	7,982	0.007	0.349	0.02±0.014 <sup>(b)</sup>	0.05±0.009 <sup>(b)</sup>
	CW	Female	10,014	0.014	0.046	0.31±0.050 <sup>(a)</sup>	0.09±0.016 <sup>(a)</sup>
		Male	7,982	0.012	0.129	0.09±0.027 <sup>(b)</sup>	0.05±0.011 <sup>(b)</sup>
	AW	Female	10,014	0.008	0.029	0.29±0.049 <sup>(a)</sup>	0.10±0.016 <sup>(a)</sup>
		Male	7,982	0.001	0.043	0.03±0.017 <sup>(b)</sup>	0.05±0.010 <sup>(b)</sup>
Carcass weight traits	AWT	Female	1,050	3.055	8.145	0.37±0.172	0.09±0.071
		Male	655	3.391	21.400	0.16±0.163	0.19±0.085
	SOW	Female	1,050	2.092	5.111	0.41±0.175	0.08±0.071
		Male	655	3.599	15.830	0.23±0.166	0.14±0.081
	TOW	Female	1,050	1.997	4.842	0.41±0.175	0.07±0.071
		Male	655	3.188	15.237	0.21±0.161	0.13±0.079

**Note:** Heritability and common environmental effect estimate for each trait between sexes using model 2 not sharing the same upper case superscript are significantly different ( $P < 0.05$ ).

Trait notations used in this table were described in Table 2

Table 7: Phenotypic (above) and genetic correlations (below the diagonal) between body traits for females and males

Sex	Female						Male					
Traits	BW <sup>(0.5)</sup>	BL	CL	AL	CW	AW	BW <sup>(0.5)</sup>	BL	CL	AL	CW	AW
BW <sup>(0.5)</sup>		0.95±0.01	0.90±0.01	0.88±0.01	0.90±0.01	0.86±0.01		0.94±0.01	0.92±0.01	0.85±0.01	0.94±0.01	0.86±0.01
BL	0.99±0.01		0.93±0.01	0.95±0.01	0.87±0.01	0.84±0.01	0.99±0.01		0.94±0.01	0.93±0.01	0.93±0.01	0.88±0.01
CL	0.99±0.01	0.99±0.01		0.76±0.01	0.85±0.01	0.79±0.01	0.99±0.01	0.99±0.01		0.76±0.01	0.90±0.01	0.84±0.01
AL	0.99±0.01	0.99±0.01	0.97±0.01		0.81±0.01	0.78±0.01	0.99±0.03	0.99±0.01	0.99±0.03		0.84±0.01	0.82±0.01
CW	0.99±0.01	0.93±0.02	0.99±0.01	0.97±0.01		0.84±0.01	0.98±0.01	0.98±0.02	0.99±0.01	0.99±0.06		0.89±0.01
AW	0.99±0.01	0.99±0.01	0.98±0.01	0.99±0.01	0.93±0.02		0.97±0.02	0.96±0.07	0.99±0.02	0.99±0.03	0.98±0.02	

*Note: Trait notations used in this table were described in Table 2*

### Figures

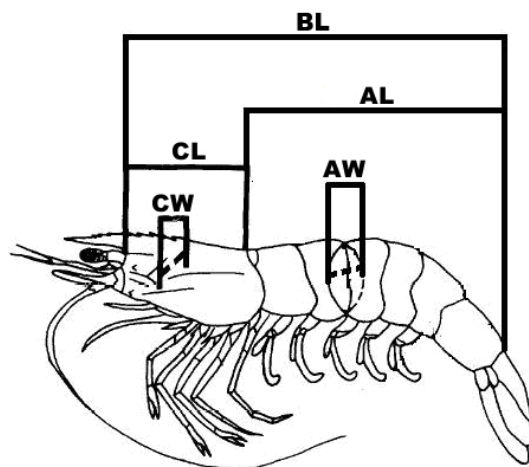


Figure 1: Body traits measurement

*(Trait abbreviations and definitions were described in Table 2)*

**CHAPTER 4. Genetic response to combined family selection for  
improved mean body weight in giant freshwater prawn  
(*Macrobrachium rosenbergii*) in Vietnam**

- Submitted to Aquaculture (in review).

## **Preface to Chapter 4**

In the current study we estimate the genetic responses in body and carcass weight traits achieved in a giant freshwater prawn (GFP) (*Macrobrachium rosenbergii*) population selected for high growth in Vietnam over three selection generations. Selection responses were estimated using three methods: (i) comparing the least squares means (LSMs) for the Selection line and the Control group in the same generation; (ii) comparing the estimated breeding values (EBVs) between the progeny of the Selection line and the Control group in the same generation, and (iii) comparing EBVs of the progeny of the Selection line in two consecutive generations. For all traits examined, responses were expressed in actual units, in percentages, which refers to actual units in relation to the LSMs of the Control groups, and in genetic standard deviation units.

**Genetic response to combined family selection for improved mean body weight in giant freshwater prawn (*Macrobrachium rosenbergii*) in Vietnam**

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## **Abstract**

The aim of the current study was to estimate the genetic changes in body and carcass weight traits achieved in a giant freshwater prawn (GFP) (*Macrobrachium rosenbergii*) population selected for high growth in Vietnam. The data set consisted of 18,387 individual body and 1,730 carcass weight records, as well as full pedigree information collected over four generations. Average selection response (per generation) in body weight at harvest (transformed to square root) estimated as the difference between the Selection line and the Control group was 7.4% calculated from least squares mean (LSMs), 7.0% from estimated breeding values (EBVs) and 4.4% calculated from EBVs between two consecutive generations. Favourable correlated selection responses (estimated from LSMs) were found for other body traits including: total length, cephalothorax length, abdominal length, cephalothorax width, abdominal width (12.1%, 14.5%, 10.4%, 15.5% and 13.3% over three selection generations, respectively). Data in the second generation of selection showed positive correlated responses for carcass weight traits including: abdominal weight, skeleton-off weight and telson-off weight of 8.8%, 8.6% and 8.8%, respectively. Results from the current study indicate that growth rate responds well to the application of combined (between and within) family selection and correlated carcass weight traits can also be improved in parallel using this approach.

**Keywords:** *M. rosenbergii*, body traits, direct response, correlated responses



## 1. Introduction

Giant freshwater prawn (GFP, *Macrobrachium rosenbergii*) is an important freshwater culture species in Southeast Asia and elsewhere. In particular, GFP is now one of the most economically important crustacean species in the freshwater aquaculture sector in Vietnam where it is favoured because it is indigenous and performs well under a variety of local farming systems (Phuong *et al.*, 2006). Production costs are relatively low since this species does not require formulated feeds that contain high animal protein content. In Vietnam, GFP is mainly produced for domestic consumption and it is relatively high priced all year round. It is also recognised as possessing excellent potential for export. GFP aquaculture is considered to be a sustainable practice, because it requires less water exchange and lower feed inputs compared with most other economically important local freshwater species in Vietnam including striped catfish (*Pangasianodon hypophthalmus*). Hence, GFP aquaculture is more sustainable because GFP are produced at relatively low biomass per unit area (as compared with striped catfish which is farmed much more intensively). Thus pollution from GFP culture is negligible. In recent years, many low-lying provinces in the Mekong Delta of Vietnam have expanded the scale of GFP production to improve the livelihoods of poor farmers.

A major constraint on expansion of GFP farming in Vietnam however, has been a lack of quality seed (PLs) and to date all cultured stocks farmed there are unimproved. In addition, current breeding practices used in hatcheries are not optimal for production of high quality seed. Problems include: the low numbers of breeders used in hatcheries, long term use of breeders and replacement of brood stock with individuals usually sourced from ponds on the same farm. These factors have contributed to a decline in culture performance that has been observed at a number of sites over the country. Combined effects of the factors outlined above increase inbreeding rates and are likely to result in inbreeding depression over time. New and Valenti (2000) observed that sourcing brood stock from grow-out ponds rather than from the wild often resulted in high levels of inbreeding accumulated over time and this was believed to be a major factor for declines in GFP growth performance in Thailand. In addition, the common practice of sourcing female brood stock based on their readiness to spawn can also affect performance negatively overtime as the practice exerts an indirect negative effect on mean weight at harvest (Mather and de Bruyn, 2003). The authors comment further that although genetic attributes of GFP culture stocks world wide are unknown, many factors are in play that suggest that levels of genetic variation in GFP culture stocks may be low and

declining. Therefore, the current status of GFP aquaculture highlights the need to initiate systematic stock improvement programs for the species to improve economically important traits.

Two large, successful family selection breeding programs have been implemented in fish by AKVAFORSK in Norway for salmonids and the WorldFish Center (formerly ICLARM) for Nile tilapia. Evaluation of the genetic gains achieved in breeding programs for salmonids show that growth rate was improved by 13% and 14.4% per generation in rainbow trout and Atlantic salmon, respectively (Gjerde, 1986). Improved Atlantic salmon grew 83.9% faster after six generations of selection compared with a wild reference stock and this resulted in a reduction in time to reach sexual maturity (8% per generation) (Gjerde and Korsvoll, 1999). The Genetic Improvement of Farmed Tilapia (GIFT) program was initiated in the late 1980s and results show that after seven generations of selection in Malaysia, the improved strain had achieved a genetic gain of 10 to 15% per generation and an cumulative increase of 104% in growth rate compared with the base population (Ponzoni *et al.*, 2011). More recently, a family selection breeding program was initiated for striped catfish *P. hypophthalmus* (Sang, 2010) in Vietnam to improve growth rate and fillet yield which has produced a selection response of 4.7 to 12.4% in growth rate. In crustaceans, recent stock improvement programs have selected for improved growth rates in redclaw crayfish (*Cherax quadricarinatus*) (McPhee *et al.*, 2004), freshwater crayfish (yabby) (*Cherax destructor*) (Jerry *et al.*, 2005) and in some marine shrimp species notably, kuruma prawn (*Penaeus japonicus*) (Hetzl *et al.*, 2000; Preston *et al.*, 2004), pacific white shrimp (*Litopenaeus vanamei*) (De Donato *et al.*, 2005; Argue *et al.*, 2002), pacific blue shrimp (*Penaeus stylirostris*) (Goyard *et al.*, 2002), and black tiger shrimp (*Penaeus monodon*) (Benzie *et al.*, 1997; Kenway *et al.*, 2006). Other programs in shrimps have targeted disease resistance traits in *P. monodon* (Kenway *et al.*, 2006). Reported selection responses differed among species and between studies on the same species, and generally ranged from 4 to 23% improvement per generation. Selection for harvest weight has also resulted in favourable correlated responses in other body traits including total length and abdominal width (Pérez-Rostro and Ibarra, 2003a) and some carcass weight traits (Sang, 2010; Nguyen, Ponzoni, Abu-Bakar *et al.*, 2010) because of favourable genetic correlations.

GFP is often marketed by body weight or carcass weight in several product forms including: Head On Shell On (HOSO), Headless Shell On (HLSO), Peeled Tail On (PTO) or

Peeled and Undeined (PUD). Hence, body weight and carcass weights are economically important traits that appear worthy of selection in a genetic improvement program. Genetic parameters including genetic variation, heritability and genetic correlations among body and carcass weight traits in GFP have been reported by Malecha *et al.* (1984), Kitcharoen *et al.* (2010), Luan *et al.* (2012) and Hung *et al.* (in review-b). Results reported by Hung *et al.* (in review-b) showed that body weight, other body traits, as well as carcass weight traits had moderate heritabilities. Thus preliminary data on GFP suggests that growth rate and carcass weight traits may respond positively to selection. The aim of the current study was to estimate direct and correlated selection responses to combined within and between family selection over three generations in a synthetic GFP cultured strain in Vietnam.

## **2. Materials and methods**

### **2.1. Production of the synthetic base population**

Founder populations included approximately 200 wild GFP pairs collected from two rivers in Vietnam (referred to here as the Dong Nai and Mekong strains) and an exotic culture strain sourced from Malaysia (Malaysia strain). These three strains were assembled in 2007. To generate the synthetic base population (F0) for the selection program, a complete 3 x 3 diallel cross resulting in a total of 9 crosses was performed in 2008. Eighty-one full-sib families were produced. All individuals were tagged and the population thus created was subject to combined family and individual selection. Approximately 4 to 10 males and females with the best estimated breeding values (EBVs) in each family were chosen from 69 families among the 9 crosses and maintained as brood stock to produce the first generation (F1). Individual EBVs were estimated using the Best Linear Unbiased Prediction (BLUP) method, fitting a mixed animal model. The genetic composition of the synthetic base population consisted of near equal contributions from each of the three founder strains: Mekong (MK), Dong Nai (DN) and Malaysia (MY) (34.2%, 32.8% and 32.0%, respectively).

### **2.2. Production of families**

In subsequent generations: F1 (year 2009), F2 (year 2010) and F3 (year 2011) mating was made among genetically unrelated brood stock based on their EBVs and their relationship to other animals in the pedigree. Two populations (Selection line and Control group) were produced and communally reared each generation. The Selection line was selected for highest EBVs for harvest body weight with restrictions imposed on the mating of relatives. By

contrast, the Control group consisted of individuals chosen each generation that possessed EBVs as close as possible to the population mean in each generation. The Control group was not maintained as a line, it was re-created each generation and mated at the same time as the Selection line. The family production process was the same in the Selection line and in the Control group. To produce the Control group, as many as 17 to 42 full-sib families were produced each generation for the estimation of selection responses. The number of sires and dams that successfully produced progeny in each line and generation is given in Table 1.

Mating was usually conducted in March and its duration was limited to 30 days to minimize age difference among families. Full-sib and (parental) half-sibs were produced from matings between genetically unrelated individuals to minimise inbreeding. In addition, breeders in each generation were selected from as many families as possible, and a limited number of individuals were selected from each family to enable equal contribution of each family to the next generation to maintain genetic diversity. Females were initially checked 10 days after a successful mating, followed by subsequent checks at two day intervals. Berried females were transferred to the hatchery for spawning when eggs had changed colour to dark grey. Each female was kept separately in a 70 l plastic container so that larvae could be collected independently for each family. Larvae from each family were reared separately in an open, clear water system (Phuong *et al.*, 2006) with addition of probiotics. Nursing water temperature ranged between 30 and 34°C, and postlarvae (PL) were normally observed after 20 to 30 days in larval rearing tanks and metamorphosed to the PL stage after 25 to 40 days. PLs from each family were reared separately in fibreglass tanks and then in mesh net hapas until they reached a size suitable for tagging as juveniles at around 2 g. At this stage, 60 to 120 juveniles from each family were tagged as a batch using visible implant elastomer (VIE) tags as described by Hung *et al.*(2012). After tagging, juveniles were released into two earthen ponds of 3,500 m<sup>2</sup> and a single 800 m<sup>2</sup> pond for grow-out.

### **2.3. Grow-out and harvest**

Grow-out occurred over approximately a 16 to 18 week period at a stocking density of two individuals per m<sup>2</sup>. No aeration was required and environmental factors were checked frequently. Water was exchanged at least twice a month by gravity or when necessary by pumping. Juveniles in grow-out ponds were fed a commercial formulated pellet feed that contained a crude protein content of 35% at stocking. This was reduced to 30% for the last 4 weeks of grow-out.

Individuals were harvested using a cast-net and were weighed and measured individually. After data analysis, individuals with the highest EBVs were chosen as parents of the next generation of the Selection line. Body traits were measured on each individual at harvest including: body weight (BW, g), body length (BL, cm), cephalothorax length (CL, cm), abdominal length (AL, cm), cephalothorax width (CW, cm) and abdominal width (AW, cm). Estimation of carcass weight traits required slaughter, so all individuals were harvested from the 800 m<sup>2</sup> pond were slaughtered. Three carcass weight traits were recorded: abdominal weight (AWT, g), skeleton-off weight (SOW, g), telson-off weight (TOW, g). Detailed descriptions of trait measurements are provided in Hung *et al.* (in review-b). In addition to body and carcass weight traits records, tag code, sex, female reproductive status and male morphotype of each individual were also recorded at harvest.

## 2.4. Statistical analysis

### 2.4.1. Statistical model

Exploratory analyses using a general linear model (GLM) were initially performed to examine characteristics of the data, data distributions. Due to large differences in body weight among male morphotypes, data were skewed and hence were subjected to square root transformation for analysis. Other body and carcass weight traits approximated normal distributions so the data did not require transformation. Estimated breeding values (EBVs) and least squares means (LSMs) were obtained from a standard mixed model. In matrix notation the model is written as:

$$y = Xb + Za + Wc + e$$

Where:

- $y$  is the vector of observations for body and carcass weight traits.
- $a$  is the vector of the random animal additive genetic effects.
- $b$  is the vector of the fixed effects that included sex (female or male), generation (or spawning seasons, 1 to 4), line (the term 'line' is here used in a broad sense that includes the Selection line and the Control group), ponds (two ponds each generation), male morphotype was fitted as an effect in males, whereas female reproductive status was fitted as an effect in females, pond nested within generation and the second order interaction between generation and line. A linear covariate (number of days from stocking to harvest) was fitted within sex, pond and generation subclasses.
- $c$  is the vector of dam effects or maternal and common environmental effects.

-  $X$ ,  $Z$  and  $W$  are incidence matrices relating observations to fixed effects, additive genetic effect of the individual animal and common full-sib effect included in the model, respectively. Statistical significance of fixed and covariate effects on body and carcass weight traits were assessed based on Wald test fitting a mixed model as described above using the computer program ASReml (Gilmour *et al.*, 2009). Two and three-way interactions among the main effects were also examined and removed from the model if they failed to show significance in the traits of interest.

#### **2.4.2. Estimation of selection response**

Selection responses were estimated using three methods: (i) comparing the least squares means (LSMs) for the Selection line and the Control group in the same generation; (ii) comparing the estimated breeding values (EBVs) between the progeny of the Selection line and the Control group in the same generation, and (iii) comparing EBVs of the progeny of the Selection line in two consecutive generations. In all cases, the same statistical models as described above were applied to compute LSMs and EBVs. For all traits examined, responses were expressed in actual units, in percentages, which refers to actual units in relation to the LSMs of the Control groups, and in genetic standard deviation units. As earlier stated, body weight required square root transformation to normalise residuals. James (2007) shows that square root transformation of data reduces the percentage response to approximately half that of untransformed data. For this reason, in the current study, we doubled the percentage genetic responses estimated from the square root of body weight in our results. Cumulative genetic response (in percentage) over generations was calculated using the following formula:  $P_c = \prod_{i=1}^n (1 + p_i) - 1$  where  $P_c$  is the total genetic response (%);  $p_i$  is the genetic response (%) for the  $i^{th}$  generation;  $i$  is the generation ( $i = 1, 2, 3$ ). The formula used here accounts for the fact that as generations progress, means change if there is genetic gain. Hence, the percentage in each generation is calculated relative to a different mean. Average genetic response (% per generation) was calculated as:  $P_a = P_c / n$  where  $P_c$  is the cumulative genetic response over  $n$  generations.

### **3. Results**

#### **3.1. Test of fixed effects**

Table 2 shows the significance of the fixed effects included in the model. Sex, pond nested within generation, interaction between generation and line, male morphotype fitted in males, female reproductive status fitted in females, were significant ( $P < 0.01$  to  $P < 0.001$ ) for all traits. The linear covariate (number of days from stocking to harvest) was fitted within sex, pond and generation subclasses and it was significant ( $P < 0.001$ ) for all body and carcass weight traits.

### **3.2. Direct selection responses**

Direct selection responses for body weight estimated as difference in LSMs and EBVs and expressed in three different ways are presented in Table 3. Substantial selection responses were recorded that ranged from 1.2 to 9.2% (significantly different from zero) each generation. Cumulative selection response for body weight was 22.2, 21.1 and 13.3% over three selection generations or average 7.4, 7.0 and 4.4% per generation estimated by methods (i), (ii) and (iii), respectively. In general, the F2 showed over twice the selection response compared with the F1 generation whereas F2 and F3 responses were similar. There was a good agreement between methods (i) and (ii) which showed higher estimates than method (iii).

### **3.3. Correlated selection responses**

Table 4 shows that there were correlated selection responses for body (other than body weight) and carcass weight traits in every selection generation. The correlated selection responses show a similar pattern to the direct responses presented in Table 3.

Body traits (TL, CL, AL, CW and AW) showed positive correlated selection responses with cumulative selection responses ranging from 10.4 to 15.5%, from 10.5 to 14.2% and from 6.7 to 9.8% over three selection generations using methods (i), (ii) and (iii), respectively. Overall, Tables 3 and 4 show good consistency in selection responses between method (i) (measured as the difference in LSMs between the Selection line and the Control group in the same generation) and method (ii) (measured as the difference in EBVs between the Selection line and the Control group in the same generation). Regardless of the method used to estimate responses, selection responses increased significantly from the F1 to F2, then decreased slightly in the F3, likely due to lower selection intensity than previous generations.

Data from the F2 generation retrieved from 403 and 1,327 carcass weight records in the Control group and the Selection line, respectively, showed positive correlated responses. Estimates using method (i) showed that correlated selection responses for carcass weight traits were high and ranged from 8.6 to 8.8%. Tables 3 and 4 also show that in the F2 generation mean body weight (BW) increased 8.86% whereas mean abdominal weight (AWT) increased by 8.82% (both estimates using method (i)), meaning that the relative increase in body weight was similar for the abdomen and for the cephalothorax.

#### **4. Discussion**

Results presented here confirm that mean body weight for GFP can be improved using combined within and between family selection. Cumulative selection responses for body weight ranged from 13.3 to 22.2% depending on the method employed for estimation. Selection response was lower in the F1 than in the F2 and F3 generations and this may have resulted from the lower selection intensity applied in the first generation. There were 89, 96 and 144 families in the Selection line in the F1, F2 and F3 generations, respectively. In addition, there were approximately 34, 48 and 39 individuals per family used for selection in the F1, F2 and F3 generations, respectively. The lower number of families and lower number of individuals in each family in the F1 relative to that available in the F2 and F3 (coupled with the fact that the same number of potential breeders were selected in each generation) most likely resulted in a higher selection intensity in the F2 and F3 than in the F1.

A comparison of the results for different methods used to estimate selection responses show that there is good agreement between methods (i) and (ii) which computed the differences (in LSMs and EBVs, respectively) between the Selection line and the Control group in the same generation. However, both methods differ (higher response estimates) from method (iii) that computed differences (in EBVs) in the Selection line between consecutive generations. Results presented here are different to those reported for Nile tilapia by Ponzoni *et al.* (2005) where the authors used the same methods of estimation and reported very good agreement among them. In the same species, Rezk *et al.* (2009) reported that response estimated from method (iii) was higher than from method (ii). Our results were in agreement with a study by Maluwa and Gjerde (2007) in tilapia (*Oreochromis shiranus*) selected for growth rate for two generations. These authors reported a similar response with methods (i) and (ii), which were higher than the estimate using method (iii). In our study, the lower genetic response estimates for body weight from method (iii) compared with estimates from



method (ii) resulted from the fact that mean breeding values for body weight in the Control group parents that produced progeny successfully were lower than mean breeding values in the previous generation. This resulted in smaller differences in two consecutive generations (method (iii)) than between the Selection line and the Control group in the same generation (method (ii)). In a study of the same species (*M. rosenbergii*) by Luan *et al.* (2012), there is a large difference between responses estimated from LSMs and from EBVs. There is also difference between estimates from within and between generations even though mean breeding values of the control group parents were reported to be approximate the selection population means of the previous generations. A similar trend was also present in this study for other body and carcass weight traits where the correlated responses estimated from LSMs (method i) were much higher than the response estimated as differences within generations (method ii) that in turn, were higher than estimates from differences between generations (method iii). Ponzoni *et al.* (2005) suggested of using alternative approaches of estimating the selection responses to achieve a better interpretation of the results. The differences in selection response estimates between studies may be in part due to the method of establishing the Control group. In our study with GFP, the Control group was re-created in each generation of the selection program, whereas a separate control line was maintained in the GIFT program in Malaysia (Ponzoni *et al.*, 2011).

The selection response for growth rate in GFP achieved here compares favourably with results reported in pacific white shrimp *P. vannamei* where a mass selection approach was employed (De Donato *et al.*, 2005) (14.5% after 11 generations). The authors however, reported a much higher selection response later when they applied family selection (15% per generation) to the same population. Selection responses were lower in pacific blue shrimp *P. stylirostris* (Goyard *et al.*, 2002) (average 4% per generation), giant freshwater prawn *M. rosenbergii* (Luan *et al.*, 2012) (6.2 to 26.2% over five generations) and in striped catfish *P. hypophthalmus* (Sang, 2010) (4.7 to 12.4% in different populations and rearing environments). Selection responses comparable with those in the current study have been reported for kuruma prawn *P. japonicus* (Hetzl *et al.*, 2000). They reported a positive response of 8.3% after a single generation in a two-way selection experiment, and 9.3 to 14% gain compared with a non-selected (wild population) line after one generation of mass selection following three generations where individuals were reared under controlled conditions in tanks (Preston *et al.*, 2004). Even though realized heritability was moderate ( $h^2 = 0.17$ ) and the selection intensity reported by Hetzel *et al.* (2000) was low, a moderate

selection response was obtained in *P. japonicus*, possibly resulting from a large phenotypic variation in the target population. In GIFT tilapia, Ponzoni *et al.* (2005) reported a selection response of 8.4 to 11.4% for growth rate estimated using a variety of methods in a family selection program. Higher selection responses for growth rate (15% per generation after three generations of within family selection) have been reported however, in the freshwater crayfish (yabby) *C. destructor* (Jerry *et al.*, 2005), and in pacific white shrimp *P. vannamei* (Argue *et al.*, 2002) (21.2 to 25.0% for growth rate). The relatively high selection response achieved for yabby may have been the result of large genetic variation in the study population. In GFP here, we expected a moderate response to direct selection for growth rate because of high genetic variation in the population and a moderate heritability ( $h^2 = 0.14 \pm 0.028$ ) (Hung *et al.*, in review-b). Our results together with those reported in the literature show that substantial genetic improvement can be achieved in well-designed selective breeding programs for farmed aquaculture species.

Positive outcomes for selection to improve growth rate can also potentially produce favourable correlated genetic responses in other economically important traits. Pacific white shrimp (*P. vannamei*) that were subject to mass selection to improve growth rate also showed positive correlated responses for survival rate (28.8% higher), and for FCR (18.8% lower) (De Donato *et al.*, 2005). A similar result was achieved in the same species by Perez-Rostro and Ibarra (2003a). It was noticeable however, that survival rate during the larvae to post-larvae period declined by 2 to 3% although this difference was not significant (Argue *et al.*, 2002). The authors also found that a population selected for resistance to Taura Syndrome Virus (18.4%) showed a negative correlated effect on growth rate (- 4.6%). In contrast, several studies have reported positive genetic correlations between growth rate and disease resistance to bacterial or fungal infections in fish (e.g. (Gjedrem *et al.*, 1991; Standal and Gjerde, 1987; Nilsson, 1992) and in marine shrimp (e.g. *P. vannamei*, Gitterle *et al.*, (2005)). Positive estimates for genetic correlations between growth rate and survival rate have been reported in a number of aquaculture species and they are usually moderate in magnitude, indicating that selection for fast growth rate is also likely to result in improved survival rates. A favourable correlated response between body weight and fillet weight (4.5 to 12%) has also been reported in striped catfish by Sang (2010), but correlated responses were not found for fillet yield, fillet fat or fillet colour as these traits showed very low heritabilities, and only low to moderate genetic correlations with body weight. In GIFT tilapia, Nguyen *et al.* (2010) found a positive correlated response for fillet weight but not for fillet yield even though fillet

weight and fillet yield showed a positive genetic correlation. Nguyen *et al.* (2010) concluded that indirect selection for increased fillet yield in a selection program to improve growth rate was not an effective approach for GIFT tilapia.

The correlated responses in BL, CL and AL in the current study showing that selection for body weight resulted in longer animal (BL, 12.2%) but the cephalothorax (CL, 14.5%) was increased relatively more than the abdomen (AL, 10.4%). Similarly, the cephalothorax width (CW, 15.5%) also increased relatively more than the abdomen width (AL, 13.3%). These suggest that whereas volume of the animal increased, the cephalothorax's volume increased relatively more than the abdomen's volume. The correlated responses in AL and AW however, were not similar, suggest that selection for body weight resulted not only longer but also wider (or deeper) animal. In other word, body dimensions did not increase proportionally and it is likely having a potential, albeit at a slow rate, to change body shape of the animal as predicted from selection index theory by Nguyen *et al.* (2007). Data in the generation 2 however, show that selection response for body weight and abdominal weight are very similar (approximately 8.8%). This outcomes suggests a linear increase in the body weight and carcass weight and potential sign of unchanged carcass ratio (AWT/BW) over selection for body weight.

The significant positive selection response observed for GFP in the current study in part, may result from the large amount of genetic variation assembled in the base population that was established from the best performing individuals in a full 3 x 3 diallel cross combined with use of a family mating strategy based on pedigree information. After three generations of selection, the phenotypic variation estimated in the mixed sex remained high (67.9%) (Hung *et al.*, in review-b). A number of studies in aquatic species that have failed to achieve positive selection responses have identified a number of potentially contributing factors: a narrow genetic base, small effective population size and rapid accumulation of inbreeding as seen in common carp (Moav and Wohlfarth, 1976) and in tilapia (Hulata *et al.*, 1986; Teichert-Coddington and Smitherman, 1988; Huang and Liao, 1990). Positive correlated responses for traits other than body weight were found here, and may be expected because these traits showed high, positive genetic correlations with body weight (Hung *et al.*, in review-b).

## 5. Conclusions

The results of the current study demonstrate that combined within and between family selection can improve body weight in GFP by approximately 20.0% over three generations of selection. Positive correlated responses in other body and carcass weight traits were also detected. In the future, during the course of selection to improve growth rate in GFP, carcass ratio traits should be monitored to avoid any undesirable correlated response.

## Acknowledgements

The authors thank Vu, T. N., Ky, T. L. and Nga, T. K. N. for their valuable technical assistance in both the laboratory and field trials, and Dr. Alex Safari (WorldFish Center) for some assistance with data analysis. This work was supported by the Ministry of Agriculture and Rural Development (MARD) in Vietnam and the WorldFish Center in Malaysia through the “Family-based selective breeding program on giant freshwater prawn in Vietnam”. The Australian government AusAID program provided Hung Dinh with an ALA award to undertake his PhD research at Queensland University of Technology (QUT).

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## Tables and figures

Table 1: Data structure: number of sires, dams and progeny, by generation and line or group

Generation	Line or group	Sires	Dams	Progeny
G0 (2008)	Base population	81	81	1,870
G1 (2009)	Selection	76	89	3,060
	Control	17	17	711
G2 (2010)	Selection	60	96	4,646
	Control	20	20	1,256
G3 (2011)	Selection	65	144	5,520
	Control	42	42	1,324
Total		361	489	18,387

Table 2: Animal mixed model used to analyze body and carcass weight traits

Sources of variation	Fixed effects					Covariate	Random effects	
	S	P(G)	G*L	Mtype (M)	Ftype (F)	Age(G,P,S)	Animal	Dam
<i>Body traits</i>								
BW <sup>(0.5)</sup>	***	***	***	**	***	***	√	√
BL	***	***	**	***	***	***	√	√
CL	***	***	***	***	***	***	√	√
AL	***	***	**	***	***	***	√	√
CW	***	***	***	***	***	***	√	√
AW	***	***	***	***	***	***	√	√
<i>Carcass weight traits</i>								
AWT	***		***	***	***	***	√	√
SOW	***		***	***	**	***	√	√
TOW	***		***	***	**	***	√	√

**Note:** \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$

S: Sex; P: Pond; G: Generation; L: Line; Mtype: Male morphotype; Ftype: Female reproductive status; M: Male; F: Female; Age: Number of days from stocking to harvest  
 BW: body weight (g, total live body weight at harvest); BL: body length (cm, distance from eye orbit to tip of telson); CL: cephalothorax length (cm, distance from eye orbit to the hind margin of the carapace); AL: abdominal length (cm, distance from the hind margin of the carapace to tip of telson); CW: cephalothorax width (cm, the greatest width of the carapace); AW: abdominal width (cm, width of second abdominal segment); AWT: abdominal weight (g, weight of the abdomen); SOW: skeleton-off weight (g, weight of the abdomen after removing skeleton); TOW: telson-off weight (g, weight of the abdomen after removing skeleton and telson)



Table 3: Direct selection response for body weight (BW) estimated by different methods and expressed in different ways

Methods	Generation(year)	Selection response BW <sup>0.5</sup>		
		Actual units (g <sup>0.5</sup> )	Percentage (%)	Genetic STD unit (actual/ $\sigma_A$ )
Method i	F1 (2009)	0.21	4.02	0.58
	F2 (2010)	0.48	8.86	1.31
	F3 (2011)	0.44	7.91	1.19
	Cumulative		22.20	
Method ii	F1 (2009)	0.10	3.66	0.26
	F2 (2010)	0.25	9.17	0.68
	F3 (2011)	0.19	7.04	0.53
	Cumulative		21.13	
Method iii	F1 (2009)	0.03	1.21	0.09
	F2 (2010)	0.18	6.54	0.48
	F3 (2011)	0.14	5.09	0.38
	Cumulative		13.31	

**Note:** Actual units are the difference in mean EBVs: percentage refers to actual units, in relation to the least squares means of the Control group: genetic standard deviation equals square root of the additive genetic variance

Method (i) was calculated as the difference in LSMs between the Selection line and the Control group in each generation.

Method (ii) was calculated as the difference in EBVs between the Selection line and the Control group in each generation.

Method (iii) was calculated as the difference in EBVs of the Selection line between two consecutive generations.

Table 4: Correlated selection responses for body and carcass weight traits estimated by different methods and expressed in different ways

Traits	Methods	Generation (year)	Predicted selection response		
			Actual units	Percentage (%)	Genetic STD unit (actual/ $\sigma_A$ )
BL	Method i	F1 (2009)	0.18	2.45	0.46
		F2 (2010)	0.46	5.46	1.19
		F3 (2011)	0.37	4.12	0.94
		Cumulative		12.16	
	Method ii	F1 (2009)	0.13	2.99	0.32
		F2 (2010)	0.25	5.79	0.63
		F3 (2011)	0.19	4.35	0.50
		Cumulative		13.69	
	Method iii	F1 (2009)	0.05	1.10	0.12
		F2 (2010)	0.20	4.60	0.50
		F3 (2011)	0.15	3.42	0.39
		Cumulative		9.36	
CL	Method i	F1 (2009)	0.09	2.60	0.51
		F2 (2010)	0.22	6.31	1.27
		F3 (2011)	0.19	4.97	1.07
		Cumulative		14.50	
	Method ii	F1 (2009)	0.04	3.24	0.23
		F2 (2010)	0.10	5.85	0.59
		F3 (2011)	0.09	4.82	0.52
		Cumulative		13.55	
	Method iii	F1 (2009)	0.02	0.97	0.10
		F2 (2010)	0.08	4.45	0.45
		F3 (2011)	0.07	3.59	0.39
		Cumulative		9.25	
AL	Method i	F1 (2009)	0.09	1.83	0.48
		F2 (2010)	0.24	4.81	1.28
		F3 (2011)	0.18	3.48	0.95
		Cumulative		10.44	

CW	Method ii	F1 (2009)	0.06	2.43	0.32
		F2 (2010)	0.11	4.59	0.61
		F3 (2011)	0.08	3.17	0.43
		Cumulative		10.52	
	Method iii	F1 (2009)	0.01	0.57	0.08
		F2 (2010)	0.09	3.52	0.47
		F3 (2011)	0.06	2.51	0.34
		Cumulative		6.73	
	Method i	F1 (2009)	0.07	3.23	0.59
		F2 (2010)	0.14	6.55	1.25
		F3 (2011)	0.11	4.96	0.99
		Cumulative		15.45	
	Method ii	F1 (2009)	0.03	2.44	0.22
		F2 (2010)	0.07	6.24	0.59
		F3 (2011)	0.06	4.93	0.49
		Cumulative		14.20	
Method iii	F1 (2009)	0.01	1.16	0.11	
	F2 (2010)	0.05	4.86	0.46	
	F3 (2011)	0.04	3.54	0.35	
	Cumulative		9.86		
AW	Method i	F1 (2009)	0.03	1.81	0.39
		F2 (2010)	0.09	5.57	1.25
		F3 (2011)	0.09	5.40	1.28
		Cumulative		13.29	
	Method ii	F1 (2009)	0.02	2.39	0.26
		F2 (2010)	0.04	5.37	0.60
		F3 (2011)	0.04	4.46	0.53
		Cumulative		12.70	
	Method iii	F1 (2009)	0.00	0.62	0.07
		F2 (2010)	0.03	4.40	0.49
		F3 (2011)	0.02	2.82	0.33
		Cumulative		8.01	
AWT	Method i	F2 (2010)	1.10	8.82	0.75
	Method ii	F2 (2010)	0.33	5.30	0.22
	Method iii	F2 (2010)	0.13	2.02	0.09

SOW	Method i	F2 (2010)	0.86	8.64	0.62
	Method ii	F2 (2010)	0.36	7.18	0.26
	Method iii	F2 (2010)	0.12	2.35	0.08
TOW	Method i	F2 (2010)	0.84	8.77	0.68
	Method ii	F2 (2010)	0.28	5.85	0.23
	Method iii	F2 (2010)	0.10	2.00	0.08

*Note: Actual units are the difference in mean EBVs: percentage refers to actual units, in relation to the least squares means of the Control group: genetic standard deviation equals square root of the additive genetic variance*

*Trait notations used in this table were described in Table 2*

**CHAPTER 5. Quantitative genetic parameters for body traits at  
different ages in a cultured stock of giant freshwater prawn  
(*Macrobrachium rosenbergii*) selected for fast growth**

- In preparation – to be submitted to Aquaculture Research.

## **Preface to Chapter 5**

In the current study we used data from F3 generation to estimate the heritabilities and correlations between body traits at two ages: weeks 10 and 18 and correlation of body traits between ages. We also tested the hypothesis that selection for body weight at pre-market age (week 10 after stocking) can achieve a similar selection response to the current practice of applying selection at market age (week 18). The primary focus therefore, of the current study was to evaluate potential for selecting individuals at a younger age than market age to increase the rate of improvement in the GFP stock improvement program in Vietnam.

**Quantitative genetic parameters for body traits at different ages in a cultured stock of giant freshwater prawn (*Macrobrachium rosenbergii*) selected for fast growth**

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## **Abstract**

The aim of the current study was to estimate heritabilities and correlations for body traits at different ages (weeks 10 and 18 after stocking) in a giant freshwater prawn (*Macrobrachium rosenbergii*) population selected for fast growth rate in Vietnam. The data set consisted of 4,650 body records (2,432 and 2,218 records collected at weeks 10 and 18, respectively) in the full pedigree comprising a total of 18,387 records. Variance and covariance components were estimated using restricted maximum likelihood fitting a multi-trait animal model. Estimates of heritability for body traits (body weight, body length, cephalothorax length, abdominal length, cephalothorax width and abdominal width) were moderate and ranged from 0.06 to 0.11 and 0.11 to 0.22 at weeks 10 and 18, respectively. Body trait heritabilities estimated at week 10 were not significantly lower than at week 18. Genetic correlations between body traits within age and genetic correlations for body traits between ages were generally high. Our results suggest that selection for high growth rate in GFP can be undertaken successfully before full market size has been reached.

**Keywords:** *Macrobrachium rosenbergii*, heritability at different ages, correlations between ages.



## 1. Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) or GFP is one of the most important crustaceans in the inland aquaculture sector in Vietnam. Three major production systems are practiced in Vietnam; namely integrated rice-prawn culture, and alternative rice-prawn culture types 1 and 2 (Phuong *et al.*, 2006). Recently, the rice-prawn culture type 1 model has become the most widely practiced system because it shows high productivity and good economic returns. In this model, post-larvae (PL) are usually stocked between April and May at a stocking density of 10 to 12 PLs per m<sup>2</sup> and adults harvested in December prior to the winter-spring rice crop. This model was developed with the aim to utilize favorable conditions in flooded areas across the Mekong delta in Vietnam. Culture ponds used in this model are a rice field surrounded by a fine mesh fence supported by bamboo poles pitched around field dykes. Ponds are usually large ranging from 1 to 5 ha in size, most commonly 3 and 4 ha in size. Water depth in GFP ponds depends on the flood level so it will vary among years and between months within a year. Generally, water depth ranges from approximately 1.5 m at PL stocking to as much as 7 m at the peak of the flood season and decreases to approximately 2.0 m when adult GFP are ready for harvest. Water depth is usually from 3 to 5 m for almost 4 months across the main culture period. In this production model, while natural food plays an important role, supplementary feeding with commercial pellets is also required because of the large pond biomass. GFP has become a favoured aquaculture species in Vietnam because growth rate is relatively high and the product is attractive to both domestic and export markets. Even though capacity exists to expand GFP production, production efficiency is currently low because there are no improved culture strains available to the industry and performance of farmed stocks has declined with regard to growth rate and disease tolerance (Thanh, 2009). To address this issue, a systematic stock improvement program for GFP was initiated at a breeding station belong to the Institute for Aquaculture Research No.2 (RIA2). The central aim of this program was to improve economically important traits in GFP for the local industry.

The original program at RIA2 employed selection on harvest body weight at approximately 18 weeks from stocking (close to commercial harvest time). Selection was aimed at minimizing genotype by age interaction, and maximizing the genetic gain obtained in commercial production. A number of studies have reported that genetic correlations for body weight at different ages can be high (close to unity) including in tilapia (*O. niloticus*)

(Rutten *et al.*, 2004) and common carp (*C. carpio*) (Ninh, Ponzoni, Nguyen, Woolliams, Taggart *et al.*, 2011). It is often recommended however, that selection should be performed at post-market age in order to include additional traits for example disease tolerance or reproductive condition in the selection index. Genetic correlations between body weight and disease tolerance reported in some shrimp species have been however, either low (Gitterle, Rye *et al.*, 2005) or negative (Argue *et al.*, 2002; Kenway *et al.*, 2006). Similarly, genetic correlations between body weight at different ages and reproductive traits in *P. monodon* were also low (both positive or negative) (Macbeth *et al.*, 2007). These results suggest however, that growth rate may not necessarily be combined successfully with disease tolerance and reproductive conditions in a single selection program. Moreover, genetic correlations between market and post-market body weight have also reported to be low in *P. monodon* (Coman *et al.*, 2010b) and in *P. vannamei* (Argue *et al.*, 2000). These results suggest therefore, that selection at post-market age may not produce the expected benefit from modeling. It was also suggested that if the genetic correlation between market and pre-market weight is high, selection at younger ages can reduce the costs of a breeding program in terms of feed, facilities and labor required to raise animals to a larger size. Selection at pre-market size can also have additional benefits include shortening the selection interval so that more selection generations can be practiced over the same time frame. Higher survival rates at younger ages can also allow higher selection intensity to be applied and hence potentially increase the rate of genetic gain. In a study of rainbow trout (*Oncorhynchus mykiss*), Su *et al.* (2002) reported that genetic correlations between body weight at younger ages were smaller than those at older ages. This phenomenon has also been reported in *P. monodon* (Coman *et al.*, 2010b; Kenway *et al.*, 2006) and in *L. vannamei* (Pérez-Rostro and Ibarra, 2003b) where genetic correlations between pre-market and market weight were low (0.30) to medium (0.56 to 0.77). Thus results in other aquatic species suggest that although animals can be selected for pre-market weight, they should not be selected at very young ages (or when body weight is very low) in order to achieve a reasonable genetic correlation with final market weight.

In the current study we tested the hypothesis that selection for body weight at pre-market age (week 10 after stocking) can achieve a similar selection response to the current practice of applying selection at market age (week 18). Estimates of heritability, phenotypic and genetic correlations for body traits at two ages are presented. The primary focus therefore, of the current study was to evaluate potential for selecting individuals at a younger age than

market age to increase the rate of improvement in the GFP stock improvement program in Vietnam.

## **2. Materials and methods**

### **2.1. Population and trait measurement**

A detailed description of the population, selection procedures and management of experimental animals used here are given in Hung *et al.* (in review-b). In brief, the founder population consisted of three strains collected from two geographically independent natural drainage basins in Vietnam the Mekong River (MK strain), the Dong Nai River (DN strain) and a third strain imported from Malaysia (the Malaysian strain, ML) acquired from the WorldFish Center. To generate the F0 generation, a complete 3 x 3 diallel cross involving 9 crosses was carried out in order to establish a diverse synthetic base population for selection. In later generations (F1, F2, F3), mating was made between genetically unrelated brood stock to produce full-sib and (paternal) half-sib families. Larvae from each family were reared separately in 70 l circular plastic containers (F0 to F2) and in 1 m<sup>3</sup> fiberglass tanks (F3). We employed an open clearwater larval culture system (Phuong *et al.*, 2006) with addition of probiotics. Larvae were fed only with newly hatched brine shrimp nauplii (3 times per day) for the first ten days followed later by a combination of brine shrimp nauplii and egg custard (chicken egg, high calcium milk powder, shrimp, squid flesh and fish oil) (Thanh *et al.*, 2009). Post-larvae (PL) were normally observed after 20 to 30 days in larval rearing tanks that metamorphosed into the PL stage after 25 to 40 days. Post-larvae from each family were reared separately in 1 m<sup>3</sup> fiberglass tanks for two weeks at a stocking density of 1,000 PLs per m<sup>3</sup>. They were fed with a commercial prawn pellet (available for *P. monodon*) at starting feed size. After 2 weeks, families were transferred into separate fine mesh hapas of 4 m<sup>2</sup> submerged into an earthen pond at a stocking density of 150 individuals per m<sup>2</sup>. Hapas were supplied with air from 9 pm to 6 am and PLs were fed with a 40% crude protein commercial prawn pellet (manufactured by Uni-President Co., Vietnam). PLs were kept in hapas for six to eight weeks until they reached a suitable size for tagging (around 2 g). All juveniles in each family were tagged as a batch using visible implant elastomer (VIE) tags as described by Hung *et al.* (2012). Two tags of 5 to 6 different colors were applied to individual prawns to maintain pedigree records. After tagging, juveniles from each family were kept in a 1m<sup>3</sup> fiberglass tank supplied with constant aeration and fed with pellets for 3 days to acclimate. After tags were verified, 120 juveniles chosen randomly from each family were released into

two common earthen ponds (ponds 1 and 2) of 3,500 m<sup>2</sup> for grow-out. Grow-out stocking density was set at 2 individuals per m<sup>2</sup>. No aeration was required and environmental factors were checked regularly. Water was exchanged at least twice a month via gravity flow or by pumping when required. Juveniles in grow-out ponds were fed with a commercial prawn pellet containing 35% crude protein at stocking that was reduced to 30% for the last 4 grow-out weeks. Grow-out time was set for 18 weeks (market age) so that total culture period was 26 to 28 weeks from PL to harvest as is the case in commercial production. At harvest, all individuals were harvested using a cast-net and were weighed and measured. At this age, animals had reached market size (mean body weight at harvest is over 50 g) when genetic evaluation was conducted and selection of brood stock was practiced. The Selection line was selected for highest breeding values (EBVs) for body weight. The Control group was developed by choosing individuals with EBVs as close to the population mean as possible. This practice was adhered to as closely as possible in all generations. Furthermore, in the F3 generation, we conducted an additional experiment to examine heritabilities and correlations between different ages. Surplus offspring produced from the mainstream selection program were also tagged and stocked communally in two additional ponds. They were the progeny of 91 sires and 170 dams. Representatives of all families were available in both ponds. Standard grow-out and management practices as described above were employed until harvest. In this experiment, we harvested prawns at week 10 from pond 1 while harvest occurred at week 18 in pond 2. A total of 4,650 records included 2,432 and 2,218 records in pond 1 and pond 2, respectively constitute the current dataset for estimating heritabilities and correlations at two different ages.

At harvest, five adult morphotypes for males: blue claw males (BC), orange claw males (OC), small males (SM), Old blue claw males and No claw males and three individual reproductive status classes for females: Berried females (BF), Spawned females (SF) and Mature ovary females were recorded. Details of male morphotype and female reproductive status are described in Hung *et al.* (in review-b). Apart from male morphotype and female reproductive status, six body traits were also measured on each individual at harvest. These included: body weight (BW), body length (BL), cephalothorax length (CL), abdominal length (AL), cephalothorax width (CW) and abdominal width (AW). Details about trait abbreviations and measurements are presented in Table 1 and also in Hung *et al.* (in review-b). In addition to body trait records, tag code, sex and pond were recorded at harvest.

## **2.2. Statistical analysis**

### 2.2.1. *Statistic model*

Statistical analyses were carried out on a data set consisting of 4,650 pedigree records collected in the F3 generation. All traits were evaluated for normality before further analyses were conducted and raw data were transformed where appropriate. Exploratory analyses using a general linear model (GLM, in SAS 9.1 Institute, Inc, 1997) were undertaken. Differences in body weight among morphotypes in males produced a skewed distribution. Hence, this character was transformed to natural logarithms, square root, cubic root and box-cox. Square root transformation gave the best results in terms of restoring normality and was selected for use with body weight analyses. Other body traits approximated normal distributions and so data did not require transformation. A summary of all fixed effects and covariates is given in Table 3. Statistical significance of effects were assessed based on Wald tests using a mixed model in ASReml (Gilmour *et al.*, 2009).

Variance and covariance components for additive genetics, common full-sibs and residual effects for body and carcass traits were estimated using a restricted maximum likelihood (REML) method using ASreml software (Gilmour *et al.*, 2009). The mathematical expression for the model is as follows:

$$y_{ijklmn} = \mu + S_i + L_j + Mtype_k(M) + Ftype_l(F) + Age_{ijklmn}(S,P) + a_m + d_n + e_{ijklmn} \quad [1]$$

where  $y_{ijklmn}$  is the measurements of body trait at harvest,  $\mu$  is a constant,  $S_i$  is the fixed effect of sex (male or female),  $L_j$  is line (selection or control),  $Mtype_k$  is male morphotype ( $k = 1, 2, 3$ ) that is fitted in males (M),  $Ftype_l$  is female reproductive status ( $l = 1, 2, 3$ ) that is fitted in females (F),  $P_m$  is pond ( $m = 1,2$ ),  $Age_{ijklmn}$  is the age at harvest (nested within sex and pond subclasses),  $a_m$  is the random animal additive genetic effects,  $d_n$  is the dam effects (or maternal and common environmental effects in addition to additive genetics),  $e_{ijklmn}$  is the random residual effects.

### 2.2.2. *Heritabilities, phenotypic and genetic correlations*

Heritabilities were estimated from a single trait model (Equation 1). Phenotypic and genetic correlations were obtained from a series of bi- and tri-variate analyses, involving body weight (recorded in all animals) to avoid selection bias (Kennedy, 1990). The pedigree included all animals, traced back to the base population to minimize possible bias when estimating genetic parameters. Heritability for body traits were calculated as

$$h^2 = \frac{\hat{\sigma}_a^2}{\hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2} \text{ and the maternal and common environmental effect as } c^2 = \frac{\hat{\sigma}_c^2}{\hat{\sigma}_a^2 + \hat{\sigma}_c^2 + \hat{\sigma}_e^2}$$

where  $\sigma_a^2$  is the additive genetic variance, the maternal and common environmental variance ( $\sigma_c^2$ ) and the residual variance ( $\sigma_e^2$ ). Genetic and phenotypic correlations among traits were calculated as the covariance divided by the product of the standard deviations of traits:

$$r = \frac{\sigma_{12}}{\sqrt{\sigma_1^2} \sqrt{\sigma_2^2}} \text{ where } \sigma_{12} \text{ was the estimated additive genetic or phenotypic covariance}$$

between the two traits, and  $\sigma_1^2$  and  $\sigma_2^2$  are the additive genetic or phenotypic variances of traits 1 and 2, respectively.

To our knowledge, in the literature there are no reports of formal tests for testing for significant differences in genetic parameter estimates between sexes, environments or ages. We used the z-score as an approximate method for assessing whether heritability, maternal and common environmental effects and correlation estimates were significantly different from each other, or from zero, and whether genetic correlations for body traits between ages were significantly different from one. The formula to calculate z-scores used was as follows:

$$z = \frac{x_i - x_j}{(\sigma_i^2 + \sigma_j^2)^{0.5}}$$

where  $x_i$  and  $x_j$  are the estimates of heritability, maternal and common environmental effects, or genetic correlations for the two traits (or the two ages), and  $\sigma_i$  and  $\sigma_j$  are their respective standard errors. Both  $x_j$  and  $\sigma_j$  were set to zero or one when we tested if an estimate was significantly different from zero or one, respectively. Resulting z-scores were then tested against a large sample normal distribution. The z-score test is generally equivalent to the weighted least squares approach used by Ponzoni (1975).

### 3. Results

Table 1 shows the number of observations, simple mean, standard deviation and coefficient of variation values for body traits in the two different age classes. Mean body weight increased 64.6% from week 10 to week 18. Means of other body traits also increased but at a much lower marginal from 7.5 to 22.7% for the same period. The coefficient of variation for body weight was high (48%), while it was much lower for other body traits and ranged from 10.2 to 18.7%. For all traits, a slight decrease was evident in the coefficient of

variation from week 10 to week 18. A difference in the proportion of male morphotype and female reproductive status classifications was evident between weeks 10 and 18 (Table 2). For females, the three classifications related to reproductive status (MOF, BF and SF) showed small differences in proportions at both weeks 10 and 18 while the three male morphotype classifications related to individual morphotype (SM, OC and BC) were significantly unbalance at both ages. Proportions of SM males were small at both ages suggesting that most male GFP had reached a relatively large size after 10 weeks in grow-out ponds but they still present in the male population at the market age. A shift from OC males to BC males occurred from week 10 to week 18. At week 10, when the male population was in growth phase, most males were OC (75.7%) while at market age (week 18), most were BC males (58%). This result suggests that over the grow-out time, the proportion of OC males increased to a peak and then decreased, by contrast, BC males increased and reached a peak at harvest.

Significance of fixed effects in the model are presented in Table 3 where: sex, line, male morphotype fitted in males and female reproductive status fitted in females, respectively were significant for most body traits. The linear covariate (number of days from stock to harvest) fitted within sex, pond subclasses was also significant ( $P < 0.001$ ) for most traits measured at the two different age classes.

Heritability estimates at weeks 10 and 18 for all traits were moderate (Table 4) and ranged from 0.06 to 0.11 and 0.11 to 0.22 in weeks 10 and 18, respectively. Moderate heritability estimate for body weight at week 18 ( $0.15 \pm 0.07$ ) indicates that there is potential to improve growth rate in GFP via artificial selection. Maternal and common environmental effects however, accounted for only a small proportion of the total variance and ranged from 0 to 3% for all traits. All body traits showed higher, but not significant heritability estimates at week 18 compared with week 10 except for abdominal width (AW).

Phenotypic and genetic correlations among the various body traits examined within age are presented in Table 5. In general, genetic correlations at weeks 10 and 18 were similar with correlations between body traits all positive and high, approaching unity. No significant differences were observed at week 10 (0.65 to 0.99) compared with week 18 (0.89 to 0.98). Very high genetic correlations among body traits at both ages suggest that body traits are likely to be controlled by the same set of genes and hence can be improved simultaneously in a selection program. Consistent with results for genetic correlations, phenotypic correlations among body traits at week 10 (0.54 to 0.88) and week 18 (0.79 to 0.94) were also high

although slightly lower than reported for genetic correlations and were not significantly different between the two age classes. Genetic correlations for all traits in the two different age classes were also high and approached unity (Table 6) although all estimates had associated relatively high standard errors. The genetic correlation for body weight at weeks 10 and 18 was high ( $0.97 \pm 0.19$ ). All genetic correlations for body traits between the two different age classes were in general, consistent with common environmental correlations.

#### **4. Discussion**

The present study focused on estimation of genetic parameters for body and carcass traits at two different ages (weeks 10 and 18 after stocking). We found that genetic correlations between body traits were all positive and high (almost unity) at both pre-market (week 10) and market age (week 18). High genetic correlations between traits in the two GFP age classes observed here, are similar to earlier reports in *Lipopenaeus vanamei* (Pérez-Rostro and Ibarra, 2003b) and in the same species (*M. rosenbergii*) (Kitcharoen *et al.*, 2011). Hung *et al.* (in review-b) also reported very high genetic correlations between body traits in both sexes of *M. rosenbergii* at market age. These outcomes indicate that for GFP at least, the body traits examined at different ages or in different sexes are closely correlated genetically and so are probably controlled by the same set of genes. Selection to improve any single trait would therefore, likely produce correlated responses in the other traits examined. Genetic correlation between pre-market and market body weight was close to unity in the current study and was significantly higher than that reported for *P. monodon* (Coman *et al.*, 2010b; Kenway *et al.*, 2006). The lower genetic correlation for weight by age in *P. monodon* may result from the fact that there was only a single pre-market weight recorded (weight at tagging) when individuals were approximately 4 times less than mean market weight. No other intermediate pre-market body weights were measured in either study. In a study of rainbow trout (*Oncorhynchus mykiss*) (Su *et al.*, 2002), where body weight was measured eight times at 28 day intervals during the first year, genetic correlations between body weights taken at younger ages compared with body weight at 1 year old, increased from 0.24 to 0.93 for day 168 to day 336, respectively. The genetic correlation only exceeded 0.7 after the fourth measurement at day 280. Results suggest that genetic correlations for body weight by age in some species, can depend not only on stage of development but also the time interval between weight measurements. Genetic correlations tend to be low to intermediate during the early growth phase but increase as animals approach market weight. Maternal and common environmental



effects due to rearing of full-sibs separately before tagging are often present during the early life cycle of aquatic organisms, but any effects may not necessarily be carried over to the rest of the life cycle. As individuals increase in body weight many fold from tagging to market size, any possible effect of early life rearing is likely to reduce significantly over time. This outcome suggests that selection applied at very young age when compensatory or differential growth among families due to maternal and common environmental effect is still taking place is likely to result in only poor or even an absence of a correlated response at later ages. In the current study, we were only able to perform a single pre-market measurement of body traits. Although genetic correlations observed were high for most traits examined, where possible additional measurements over a shorter measurement interval should be trialed to potentially achieve better estimates between market and pre-market body traits. Genetic correlation for body weight at weeks 10 and 18 was high in the current study suggesting that pre-market body weight can provide an effective selection criterion for improving market body weight in a breeding program.

Genetic variation observed for body traits (e.g. body weight) in the F3 population indicates that there is still very large scope for the population to continue to respond positively to selection in future generations. Body weight heritability estimate in the F3 generation at market age here is similar to the estimate reported earlier across four generations in the same population (Hung et al., in review-b). In both studies, our heritability estimates for body weight were somewhat higher than earlier reports for the same species (*M. rosenbergii*) (Malecha *et al.*, 1984; Kitcharoen *et al.*, 2011; Luan *et al.*, 2012). Kitcharoen *et al.* (2011) reported heritability estimates for body weight to be  $0.11 \pm 0.08$  at 5 months vs.  $0.07 \pm 0.04$  and  $0.20 \pm 0.71$  (both were not significantly different from zero) at 6 months of age for bulk and individually reared prawns, respectively. Their study was based however, on only a small number of families and included data from only a single generation that was based on limited pedigree information. In addition, their study was conducted under laboratory (rather than field conditions) where experimental animals showed only a relatively small weight gain over the experimental period. The increase in heritability estimates from pre-market to market for body weight for GFP are similar to results reported in *P. vannamei* (Pérez-Rostro and Ibarra, 2003b; Pérez-Rostro *et al.*, 1999) but contrast results in *P. monodon* (Kenway et al., 2006; Coman et al., 2010b). Body weight heritability in the F3 generation at market age ( $0.15 \pm 0.07$ ) in the current study was lower than that reported for some aquatic species notably in pacific white shrimp (*P. vannamei*) (Argue et al., 2002; Juárez et al., 2007; Gitterle, Rye et al., 2005),

black tiger prawn (*P. monodon*) (Macbeth et al., 2007; Kenway et al., 2006; Coman et al., 2010b), kuruma prawn (*P. japonicus*) (Hetzl et al., 2000), redclaw crayfish (*C. quadricarinatus*) (McPhee et al., 2004; Jones et al., 2000), GIFT tilapia (Nguyen et al., 2007) and striped catfish (*P. hypophthalmus*) (Sang, 2010). But note however, that heritability estimates reported for some shrimp species were sometime overestimated because maternal and common environmental effects were not included in the statistical models used to analyze body traits.

Male morphotype and female reproductive status, were for the first time, integrated into a single formal quantitative genetic analyses of GFP in our studies, e.g. (Hung et al., in review-b). Male GFP morphotypes are considered to result from social dominance effects (Karplus et al., 1991) in the male population. In the present study, we fitted only three male morphotypes (BC, OC and SM) into the statistical models tested, although two additional morphotypes (NC and OBC) were also recorded. Data on NC and OBC morphotypes were excluded from the analyses due to the subjective nature of recording the two morphotypes and their relatively low frequency in the male population. The NC and OBC male morphotypes jointly only accounted for approximately 9% of the total male population (data not shown). Moreover, comparing models based on fitting either three (BC, OC and SM) or fitting five (BC, OC, SM, NC and OBC) male morphotypes resulted in only minor differences (Hung et al., in review-b) in variance and heritability estimates among traits. Female reproductive status in GFP has also been recorded in previous studies (Thanh et al., 2009; Aflalo et al., 2012a; Hung et al., in review-b). Consistent with our earlier studies, female reproductive status also showed significant effects on the model. We therefore argue that both male morphotype and female reproductive status should be included in statistical models in future studies of GFP when estimating genetic parameters in order to avoid biasing estimates.

Survival rate observed at week 10 (pond 1) was not significantly higher than at week 18 (pond 2). Survival rate however, often vary from pond to pond even when they are exposed to a identical protocol. Here survival was stable between week 10 and week 18 potentially the result of good managements obtained and/or a high survival rate in GFP after 10 weeks. The coefficient of variation for body weight estimated for the F3 generation here was lower than that estimated across four generations in the same population in an earlier study (48% compared with 67.9%) (Hung et al., in review-b). This suggests that management during the grow out phase had been improved over the course of the breeding program. The

coefficient of variation for body weight estimated for GFP here (48.0%) is however, higher than that reported for a freshwater crayfish (*C. destructor*) (42%) (Jerry *et al.*, 2002), pacific white shrimp (*P. vannamei*) (15 to 29%) (Gitterle, Rye *et al.*, 2005) or pacific blue shrimp (*P. stylirostris*) (19%) (Lester, 1983) but accorded with an earlier estimate in GFP (*M. rosenbergii*) (38.3 to 48.4% in different generations) (Luan *et al.*, 2012). Notably, the coefficient of variation for body weight stabilized from week 10 to week 18 in the F3 generation, likely because of the relatively low stocking density and good environmental conditions experienced in the study. Favorable culture conditions may have resulted in lower competition for food and space among individuals than commonly occurs during commercial production. Compared with some fish species, the coefficient of variation for body weight in the F3 generation was higher than for GIFT tilapia reported by Nguyen *et al.* (2010) and Maluwa and Gjerde (2006) (25.1% and 25 to 34%, respectively) but lower than results reported by Ponzoni *et al.* (2005) (48 to 60%) also in tilapia. Our results were similar to estimates reported for striped catfish (40.0 % - F2 generation) (Sang, 2010).

In summary, our findings indicate that there was no genotype by age interaction for body traits in the GFP population studied here. Differences in heritability estimates for body traits at the two different ages resulted essentially from scaling effects. There was however, no re-ranking effect since the genetic correlations between homologous traits expressions were close to unity. In both cases, selection index calculations using the genetic parameters estimated here indicate that they would have very little impact on accuracy of selection or underlying genetic component if selection was practiced at either week 10 or week 18. We conclude therefore, that a high correlated response in market body weight can be achieved for GFP when selection is practiced on pre-market body weight at week 10 rather than later at harvest (week 18).

## **5. Conclusions**

Estimates of heritability for body weight in GFP here were moderate at both pre-market and market ages. High genetic correlation between pre-market and market body weight in GFP suggests that body weight will respond positively to selection when selection is applied at a pre-market body weight. Genetic correlations among body traits examined here were generally high, and were consistent for both age classes suggesting that multiple body traits can be selected simultaneously in selection programs in GFP.

## Acknowledgements

The authors thank Vu, T. N., Ky, T. L. and Nga, T. K. N. for their valuable technical assistance in both the laboratory and field trials, Drs. Raul Ponzoni and Alex Safari (WorldFish Center) for assistance with some aspects of the data analysis. This work was supported by the Ministry of Agriculture and Rural Development (MARD) in Vietnam and the WorldFish Center in Malaysia through the “Family-based selective breeding program on giant freshwater prawn in Vietnam”. The Australian government AusAID program provided Hung Dinh with an ALA award to undertake his PhD research at Queensland University of Technology (QUT).

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## Figures and tables

### Tables

Table 1: Number of records (n), overall mean, standard deviation (SD) and the coefficient of variation (CV, %) for body traits by age

<i>Trait</i>	<i>Age</i>	<i>n</i>	<i>Mean</i>	<i>SD</i>	<i>CV (%)</i>
BW	Week 10	2,432	30.8	14.8	48.0
	Week 18	2,218	50.7	24.4	48.1
BL	Week 10	2,432	9.0	1.3	13.9
	Week 18	2,218	10.1	1.3	12.8
CL	Week 10	2,432	3.7	0.7	18.0
	Week 18	2,218	4.4	0.7	16.9
AL	Week 10	2,432	5.3	0.7	12.4
	Week 18	2,218	5.7	0.6	10.2
CW	Week 10	2,432	2.2	0.4	18.7
	Week 18	2,218	2.7	0.5	17.2
AW	Week 10	2,432	1.7	0.2	11.6
	Week 18	2,218	2.0	0.2	11.3

**Note:** *BW*: body weight (g, total live body weight at harvest); *BL*: body length (cm, distance from eye orbit to tip of telson); *CL*: cephalothorax length (cm, distance from eye orbit to the hind margin of the carapace); *AL*: abdominal length (cm, distance from the hind margin of the carapace to tip of telson); *CW*: cephalothorax width (cm, the greatest width of the carapace); *AW*: abdominal width (cm, width of second abdominal segment)

Table 2: Proportion (%) of male morphotype and female reproductive classifications at two different ages

Sex	Female				Male			
	MOF	BF	SF	SUM	SM	OC	BC	SUM
Week 10	29.5	30.0	40.5	100.0	4.7	75.7	19.6	100.0
Week 18	33.3	32.3	34.4	100.0	3.0	39.0	58.0	100.0

**Note:** *MOF*: mature ovary females; *BF*: berried females; *SF*: spawned females; *SM*: small males; *OC*: orange claw male; *BC*: blue claw males

Table 3: Significance of fixed effects in the statistical model for body traits by age

<i>Traits</i>	<i>Sqrt(BW)</i>		<i>BL</i>		<i>CL</i>		<i>AL</i>		<i>CW</i>		<i>AW</i>	
	<i>Week 10</i>	<i>Week 18</i>	<i>Week 10</i>	<i>Week 18</i>	<i>Week 10</i>	<i>Week 18</i>	<i>Week 10</i>	<i>Week 18</i>	<i>Week 10</i>	<i>Week 18</i>	<i>Week 10</i>	<i>Week 18</i>
Sex	1.9	8.5**	12.7***	5.6**	4.8*	6.2**	14.7***	4.0*	5.3*	10.0***	7.0**	0.5
Line	37.8***	49.3***	30.1***	46.1***	26.6***	48.7***	25.5***	37.2***	26.7***	38.2***	33.0***	46.3***
Mtype(M)	476.5***	201.8***	571.9***	272.6***	386.2***	277.3***	452.9***	215.9***	501.2***	246.7***	395.0***	221.5***
Ftype(F)	13.7***	11.8***	14.0***	4.0*	7.1***	6.7**	13.8***	1.2	0.7	7.0***	71.1***	136.8***
Age(Sex,Pond)	3.0	7.2**	4.3*	5.3**	4.4*	5.1*	3.6*	4.4*	2.9	7.2***	4.1*	2.5

**Note:** \*:  $P < 0.05$ ; \*\*:  $P < 0.01$ ; \*\*\*:  $P < 0.001$ ;

*Mtype*: male morphotype; *Ftype*: female reproductive status; *M*: male; *F*: female; *Age*: Grow-out days (days from stock to harvest)

Trait notations used in this table were described in Table 1.

Table 4: Variance components, heritability ( $h^2$ ) and maternal common environmental effect ( $c^2$ ) with their standard errors ( $\pm se$ ) for body traits by age

<i>Trait</i>	<i>Age</i>	$V_a$	$V_c$	$V_e$	$h^2 \pm se$	$c^2 \pm se$
Sqrt(BW)	Week 10	0.0533	0.0000	0.4519	0.11±0.02	0.00±0.00
	Week 18	0.1201	0.0176	0.6514	0.15±0.07	0.02±0.03
BL	Week 10	0.0492	0.0006	0.4436	0.10±0.04	0.00±0.07
	Week 18	0.0878	0.0105	0.4505	0.16±0.07	0.02±0.03
CL	Week 10	0.0091	0.0000	0.1366	0.06±0.01	0.00±0.00
	Week 18	0.0310	0.0007	0.1230	0.20±0.08	0.00±0.03
AL	Week 10	0.0138	0.0064	0.1660	0.07±0.04	0.03±0.07
	Week 18	0.0164	0.0041	0.1314	0.11±0.07	0.03±0.03
CW	Week 10	0.0057	0.0007	0.0476	0.10±0.04	0.01±0.07
	Week 18	0.0122	0.0000	0.0535	0.19±0.03	0.00±0.00
AW	Week 10	0.0023	0.0007	0.0203	0.10±0.04	0.03±0.07
	Week 18	0.0063	0.0000	0.0219	0.22±0.04*	0.00±0.00

**Note:** \*:  $P < 0.05$

$V_a$ : additive genetic variance,  $V_c$ : maternal and common environmental variance, and  $V_e$ : environmental variance

Trait notations used in this table were described in Table 1

Table 5: Phenotypic (above) and genetic correlations (below the diagonal) with their standard errors ( $\pm se$ ) for body traits by age

<i>Ages</i>	<i>Week 10</i>						<i>Week 18</i>					
<i>Body traits</i>	<i>Sqrt(BW)</i>	<i>BL</i>	<i>CL</i>	<i>AL</i>	<i>CW</i>	<i>AW</i>	<i>Sqrt(BW)</i>	<i>BL</i>	<i>CL</i>	<i>AL</i>	<i>CW</i>	<i>AW</i>
Sqrt(BW)		0.67±0.04	0.77±0.01	0.68±0.01	0.77±0.01	0.68±0.01		0.93±0.01	0.90±0.01	0.86±0.01	0.89±0.01	0.80±0.01
BL	0.99±0.32		0.87±0.02	0.88±0.01	0.77±0.01	0.73±0.01	0.98±0.02		0.94±0.01	0.94±0.01	0.89±0.01	0.83±0.01
CL	0.92±0.08	0.97±0.04		0.54±0.03	0.70±0.01	0.67±0.01	0.98±0.02	0.98±0.02		0.79±0.01	0.86±0.01	0.79±0.01
AL	0.99±0.27	0.99±0.09	0.99±0.04		0.64±0.01	0.61±0.01	0.88±0.10	0.97±0.02	0.92±0.06		0.81±0.01	0.86±0.01
CW	0.92±0.08	0.72±0.03	0.99±0.02	0.65±0.15		0.76±0.01	0.98±0.02	0.97±0.04	0.97±0.04	0.90±0.08		0.82±0.01
AW	0.99±0.27	0.86±0.13	0.87±0.11	0.86±0.02	0.83±0.12		0.95±0.04	0.98±0.04	0.92±0.05	0.97±0.04	0.89±0.07	

**Note:** Trait notations used in this table were described in Table 1

Table 6: Genetic ( $r_g$ ) and common environmental ( $r_c$ ) correlations ( $\pm se$ ) between the expression of for body traits at weeks 10 and 18

<i>Trait</i>	$r_g$	$r_c$
Sqrt(BW)	0.97±0.19	0.99±0.03
BL	0.98±0.26	0.94±0.03
CL	0.93±0.22	0.97±0.03
AL	0.98±0.25	0.81±0.03
CW	0.98±0.30	0.85±0.03
AW	0.99±0.26	0.96±0.03

**Note:** Trait notations used in this table were described in Table 1



## CHAPTER 6: General Discussion and Conclusion

Sustainable production of farmed giant freshwater prawn in Vietnam can be achieved in the long term when: an improved stock is available to the local culture industry, management of the hatchery system is optimised for an improved strain, artificial feeds are available matched to the nutritional requirements of the improved strains and pond management systems are optimal. The current study sought to address some of these important issues and was focused on developing efficient protocols to produce a culture strain of GFP in Vietnam with improved growth performance.

*Development of protocols for producing families and tagging of juveniles for use in a family selection program:* To produce families for a selection program we employed an open clear-water larval culture system (Phuong *et al.*, 2006) with addition of probiotics. This is considered currently to be the most sustainable approach and is widely practiced in Vietnam. In order to produce full-sib and half-sib families over a short time period in order to narrow the age difference between families, a GIFT approach (WorldFish Center, 2004) was applied where several healthy females from two families were mated to a single male from another family. Matings were restricted between genetically unrelated families in order to minimize inbreeding. Results showed that the current protocol can be applied successfully in GFP and that we could produce as many as 100 to 200 families each generation. A fundamental requirement for an efficient family selection approach is availability of a reliable system of individual family identification. Here we required a tagging method that was suitable for a species that replaces its exoskeleton (moult) at regular intervals during the growth cycle. Tag retention has been a problem in the past in many farmed crustacean species because external tags are often lost when individuals moult and this has inhibited application of modern stock improvement technologies, in particular family selection. The VIE tag system we developed and applied to GFP in the current project was highly successful. Results showed that juvenile GFP at 2g were of suitable size for VIE tags with no negative effects evident for growth or survival. Tag retention rates were above 97.8% in all experiments and tag readability rates were 100% with a correct assignment rate of 95% through to mature animal size of up to 170g. Development of an efficient tagging method will allow the stock improvement program for this line to be continued over future generations and potentially, can be applied in GFP stock improvement programs elsewhere.

*Establishment of a synthetic base population with high levels of genetic diversity for genetic selection:* The founder population for this study consisted of a synthetic line that was developed from two local wild Vietnamese GFP strains and an introduced culture line from Malaysia to ensure a broad genetic base that would allow long-term genetic gains from the breeding program to be maintained over time. The local strains were wild caught individuals collected from different localities; ecological conditions and times while the Malaysian stock was produced from wild caught brood stock from the National Prawn Fry Production and Research Center, Department of Fisheries, Malaysia. To generate the synthetic base population, a complete 3 x 3 diallel cross including 9 crosses was carried out followed by a mild intensity combined selection approach to ensure that as many as possible families had representation in the synthetic population. Results showed that the genetic variation in this population was high, the phenotypic coefficient of variation after three generations of selection was 68% allowing this population to be improved continuously in future generations including when breeding objectives are extended.

*Estimation of heritabilities and phenotypic and genotypic correlations for traits of economic importance:* While genetic parameters have been estimated for GFP culture stocks in some earlier studies (Malecha *et al.*, 1984; Kitcharoen *et al.*, 2011; Luan *et al.*, 2012), problems have been identified with the data sets generated and/or statistical models used (Hung *et al.*, in review-b) and hence, previous genetic parameter estimates may show some bias. GFP is a very unusual and unique aquatic species. Basically, GFP is the only farmed aquatic species where well-defined impacts from social factors affect male growth phenotypes (Karplus *et al.*, 1989). Different male morphotypes have a significant effect on mean body weight and consequently unless this factor is accounted for in the model adopted they will tend to skew the distribution for body weight. Body weight and morphotype in GFP must be considered and treated differently from that practiced in other aquaculture species. Here, we trialled four different models where either male morphotype and female reproductive status were treated as fixed effects or as traits before the most appropriate model was chosen. Results show that across generations, estimates of heritability for body and carcass weight traits were moderate and ranged from 0.14 to 0.19 and 0.17 to 0.21, respectively. Body trait heritabilities estimated for females were significantly higher than for males whereas carcass weight trait heritabilities estimated for females and males were not significantly different ( $P > 0.05$ ). Genetic correlations among body traits were generally high in both sexes. Our results confirm

therefore that selection for high growth rate based on breeding values estimated by fitting an animal model to the data can significantly improve mean body and carcass weight in GFP.

*Measurement of direct and correlated genetic responses in specific traits of economic importance:* The current study is the first large-scale breeding program targeted at stock improvement of GFP. We estimated genetic responses using three different methods: (i) comparing the least squares means (LSMs) for the Selection line and the Control group in the same generation; (ii) comparing the estimated breeding values (EBVs) between the progeny of the Selection line and the Control group in the same generation, and (iii) comparing EBVs of the progeny of the Selection line in two consecutive generations. Results showed that we achieved an average of ~ 7% positive response to selection (genetic gain) each generation for growth rate. The outcome of the breeding program therefore, is that a fast growth line is now available for the industry in Vietnam that should improve profitability for farmers who culture this species.

*Estimation of heritabilities and phenotypic and genotypic correlations for traits of economic importance at different ages:* Selection is usually practiced at market size and age but if this procedure could be performed at a younger age it potentially can increase the selection response by increasing the selection intensity and shortening the selection interval. Results showed that estimates of heritability for body traits were moderate and ranged from 0.06 to 0.11 and 0.11 to 0.22 at weeks 10 and 18, respectively. Body trait heritabilities estimated at week 10 were not significantly different from week 18. Genetic correlations between body traits within age and genetic correlations for body traits between ages were generally high. Our results suggest that selection for high growth rate in GFP can be undertaken successfully before full market size has been reached (at week 10).

Overall we believe that knowledge gained in the current study about stock improvement of GFP will also be integral in supporting future development and implementation of breeding programs not only for *M. rosenbergii*, but also for other economically important prawn and shrimp species (e.g. black tiger shrimp and white shrimp).

## **6.1. Base population and management of genetic diversity in well-designed selective breeding programs**

Base populations used in selection programs require high levels of genetic diversity in order to secure long-term genetic gains (Falconer and Mackay, 1996). The stock improvement program for Atlantic salmon initiated by AKVAFORSK in Norway at the beginning of the 1970s is still ongoing today, and established a variable base population by collecting wild fertilized eggs from 40 Norwegian and a single Swedish river to generate in total, 442 full-sib families (Gjedrem, 2012). Brood stock collected from each river were then crossed to produce a synthetic line with levels of genetic variation much higher than was available from any single river. While wild genetic diversity from the different river populations were represented equally in the brood stock used to produce the base population, this changed significantly after two to three generations of selection. Analysis showed that, genetic material from the Namsen River dominated the first year-class, with additional diversity contributed from five other river populations, while genetic material from a mixed population representing several wild river populations dominated the second year-class (55%) in addition to genetic material from seven other pure river populations. Genetic material from a mixed population from the Nidelv and Gaula Rivers dominated the third year-class (90%), in combination with genetic material from two other rivers, and, finally, all genetic material in the last year-class originated from a mixed hatchery population (the Mowi breed) (Thodesen and Gjedrem, 2006). Genetic variation remained consistently high however, across all year-classes because genetic variation among individuals within populations was much more extensive than genetic variation among populations. The GIFT stock improvement program for Nile tilapia (Ponzoni *et al.*, 2011) was initiated in the late 1980s by ICLARM. Founder populations included eight tilapia strains with four farmed stocks included from the Philippines (denoted as Israel, Singapore, Taiwan and Thailand strains) and four wild stocks imported from across the natural range in Africa (Egypt, Ghana, Kenya and Senegal). Each founding, unimproved stock was evaluated initially in the Philippines, under a wide range of farming systems and agro-climatic conditions to identify divergent lines that were productive in local farming conditions. A complete diallel cross was then conducted for the eight strains (8 x 8 cross) to establish a genetically-diverse synthetic base population for selection to develop an improved culture line (Bentsen *et al.*, 1998; Eknath *et al.*, 2007). The two examples outlined above provide excellent examples of successful genetic improvement programs for farmed aquatic species, highlighting the importance of basing any stock

improvement program on an initial broad genetic base. In our study, we applied a similar approach but we started with fewer founding strains. The breeding program for GFP here combined genetic diversity from two local Vietnamese and a single exotic cultured strain (from Malaysia) to secure high genetic variation levels in the founding stock. This approach potentially, will secure the utility of this strain for a long-term selection program in Vietnam, because while artificial selection will always reduce total genetic variation levels as non-favoured allelic forms of genes are removed from the synthetic gene pool over time via selection, if initial levels of genetic variation are very high, loss of alleles will only have a limited impact on the inbreeding rate. If the selection program for GFP however, is continued over many generations into the future, it may be good practice to consider refreshing the synthetic gene pool with new genetic variation periodically from external stocks, as is practiced in the Norwegian Atlantic salmon breed improvement program.

## **6.2. Breeding goals and selection strategy for GFP**

While a relatively wide range of quantitative traits have been targeted for improvement in cultured aquatic species including: body weight, age at sexual maturation, fat percentage, flesh colour and disease resistance (in rainbow trout (Rye and Lillevik, 1990), coho salmon, chinook salmon (Winkelman and Peterson, 1994) and Atlantic salmon (Rye and Refstie, 1995; Rye and Lillevik, 1990; Rye and Gjerde, 1996)), in Asia to date, the focus in most species has been to improve growth traits. Growth rate has been improved successfully in silver barb (Anon, 2002; Hussain *et al.*, 2002), rohu carp (Anon, 2002), blunt snout bream (Li and Cai, 2003), Nile tilapia (Ponzoni *et al.*, 2011; Ponzoni *et al.*, 2008) and striped catfish (Sang, 2010) with the most common selection methods employed, being individual (or mass selection) and family selection. While individual selection is generally considered to be more efficient for traits with high heritability (e.g. growth traits), it is less reliable for traits showing low heritability. For such traits or traits that are difficult to measure (e.g. flesh quality, age at sexual maturity and survival rate), family selection combined with pedigree matings is more often effective.

In a breeding program reported in Russia for common carp (*Cyprinus carpio*), mass selection was used to improve growth rate and cold tolerance (Kirpichnikov *et al.*, 1974). The program crossed a cold tolerant strain of Amur wild carp with a fast growing Galician carp strain and applied individual selection over five generations. While efficiency of growth rate did not show any improvement, survival over winter increased from 30% to 77%. In another

mass selection program conducted in carp also in Russia, growth rate was improved by 0.5% to 1.4% per generation (Kirpichnikov *et al.*, 1993) and the first selective breeding program on carp in Israel also focused on improving growth rate across five generations (Moav and Wohlfarth, 1973) while a third program attempted to change body shape (height/length ratio) in a single generation of selection (Ankorion. *et al.*, 1992). In Atlantic salmon (*Salmo salar*), the breeding program in Norway was designed initially to improve relative growth performance following which the breeding objectives were broadened by gradually including additional economically important traits including age at sexual maturation (1981: 1<sup>st</sup> generation), resistance to the disease, furunculosis (1993: 4<sup>th</sup> generation), resistance to infectious salmon anaemia, ISA (1994: 4<sup>th</sup> generation), fillet colour (1994: 4<sup>th</sup> generation), fat content (1995: 4<sup>th</sup> generation), fat distribution (1995: 4<sup>th</sup> generation), growth in freshwater (2001: 7<sup>th</sup> generation), body shape (2001: 7<sup>th</sup> generation) and most recently, resistance to infectious pancreatic necrosis, IPN (2001: 7<sup>th</sup> generation) (Gjedrem, 2000). Because only body weight at harvest and body shape traits can be recorded in live individuals until the next generation of families have been produced, certain traits including disease resistance and carcass quality traits, require that relatives of the breeding candidates (e.g. full-sibs and half-sibs) be sacrificed to obtain the relevant data. The national selective breeding program on Atlantic salmon used therefore, a combined (between and within) family selection strategy to improve simultaneously all targeted traits included in the original breeding objectives. In tilapia, the initial breeding goal for the GIFT program was to develop more productive stocks of tilapia by selection for high growth rate but later also incorporated some additional economically important traits (e.g. disease resistance and late maturation) (Pullin *et al.*, 1991). In recent generations, breeding goals have been expanded with the addition of selection for fatty acid profiles (Nguyen, Ponzoni, Yee *et al.*, 2010) and flesh quality (e.g. protein, fat and moisture content, pH and colour) (Nguyen, Ponzoni, Hamzah *et al.*, 2010). Results of breeding programs for Atlantic salmon and tilapia described here and for other fish species indicate in general, that most breeding programs for aquatic species usually start by focussing attention on improvement of growth rate and progress, later to include additional favourable traits, in particular, traits that show favourable correlations with growth rate.

For GFP to date, the breeding program in Vietnam has focused only on improving growth rate. Additional traits including reproductive quality, sex ratio, proportion of edible to non-edible meat (carcass ratio) and meat quality (e.g. protein, fat and moisture contents) have been considered as future target traits to be added to the breeding objectives. A few studies of

*M. rosenbergii* have indicated that individual reproductive quality is associated with either the brood stock resources used (Phuong and Bui, 2006; Nhan *et al.*, 2009) or the technologies apply in culture (Nhan *et al.*, 2010). The authors argued that brood stock should be sourced from local, pond-cultured stocks that requires a conditioning period to be productive. Phuong and Bui (2006) suggest further, that breeders should also have reached at least 20 g in size and preferably size should exceed 35 g to achieve optimum reproductive outcomes. Results in the second selection generation in our study showed that the selection response for both body weight and abdominal weight were approximately 8.6% (Hung *et al.*, in review-a), indicating that the increase in both body weight and carcass weight were linear over time. Carcass ratio traits (e.g. abdominal weight / body weight, skeleton-off weight / body weight and telson-off weight / body weight) however, will need to be monitored continuously in future generations of selection. In order to integrate any new trait into the selection program, genetic parameters including heritability estimates and correlations of each trait with body weight will need to be investigated. It is important however, that the number of traits to be added to the breeding objectives in the GFP selective breeding program in Vietnam be restricted to keep the breeding program focussed and the cost of the program in balance with the increases in productivity achieved.

### **6.3. Genetic parameters in GFP**

Genetic parameter estimates in the current study indicate that additive genetic variation exists for all body and carcass traits in GFP that were investigated. The most important function of heritability estimates is their predictive role and as an expression of the reliability of the phenotypic value as a guide to individual and family breeding values (Falconer and Mackay, 1996). The magnitude of the heritability estimate for a particular trait under consideration for improvement is very important because it will largely determine the best breeding strategy to be employed to produce maximum genetic gains.

Sexual dimorphism for growth rate is an economically important trait in GFP culture because males grow significantly faster and reach larger body size compared with females. As an example of the relative difference in growth rate between GFP sexes, data presented here show that mean body weight for males was 93% larger than for females in the same culture population. Previous studies have also reported that heritability estimates for body weight in GFP differ significantly between the sexes. Malecha *et al.*, (1984) reported that heritability estimates for mean growth rate for females in his study was 0.35 ( $\pm$  0.15) while that for males

were not significantly different from zero. Kitcharoen *et al.* (2011) reported heritability estimates for growth rate at sixth months of age in GFP to be 0.33 ( $\pm$  0.14) for females but only 0.03 ( $\pm$  0.04) for males. Recently in a study over five generations, Luan *et al.* (2012) reported the mean heritability for growth rate in female GFP was 0.137 ( $\pm$  0.024) was significantly higher than for males (0.033  $\pm$  0.016). So all studies of GFP growth rate conducted to date, are in general agreement that heritability estimates for mean body weight in females are in the medium to high range while they are very low (not significantly different from zero) for males. Near zero heritability estimates for growth rate in male GFP would suggest that applying selection in males to improve growth rate is not likely to be productive and that selection would better be focussed only on the female phenotype. Results presented here however, show that even while heritability estimates for body traits were moderate to high ( $h^2 = 0.29 - 0.39$ ) for females and that they were significantly higher than estimates in males ( $h^2 = 0.02 - 0.09$ ), all were significantly greater than zero in both sexes ( $P < 0.05$ ) with the exception of AL and AW estimates in males. In addition, heritability estimates for all carcass weight traits examined in both GFP sexes here were moderate to high (females: 0.37 - 0.41; males: 0.16 - 0.23), and were not significantly different between the sexes. Our results suggest therefore, that even though selection to improve growth rate in GFP is more effectively applied to females, when male phenotype is ignored totally, this can reduce the effectiveness of the selection program and potentially may result in smaller overall genetic gains. A potential explanation for the difference between heritability estimates between males and females in earlier studies by Malecha *et al.* (1984), Kitcharoen *et al.* (2011) and those here, is that both of the earlier studies were based on only small data sets and had been conducted under laboratory not in real production conditions where growth rate over the experimental period employed was less than 20% of that achieved in our study. So in the previous studies very few if any, individuals had reached breeding size at the end of their experiments. In addition, Malecha *et al.* (1984) and Kitcharoen *et al.* (2011) and more recently Luan *et al.* (2012), failed to record and fit male morphotype and female reproductive status in their statistical models and both fixed factors have been shown to be important factors to consider in models for GFP to avoid upward biasing heritability estimates (Hung *et al.*, in review-b).

Genetic correlations between abdominal weight (one of the most economically important production traits in GFP) with other morphometric body traits assessed in the study here, were also very high ( $r_g = 0.97 - 0.99$ ). This result suggests that all body traits assessed



in the study here, were genetically closely correlated and most likely they are controlled essentially by the same set of genes. Selection to improve any one of these traits therefore, would likely produce correlated positive responses in the other traits indirectly without a need for their inclusion when breeding values are estimated. In parallel, genetic correlations for body traits at two different ages (weeks 10 and 18) in the current study were also high ( $r_g = 0.93 - 0.99$ ) suggesting that selection for fast growth rate in GFP can be undertaken successfully before full market size has been reached (at week 10 rather than week 18). Employing this option can shorten the selection interval and reduce the overall time and running costs for selective breeding programs in this species.

#### **6.4. Selection response in GFP**

Genetic improvement programs in a number of aquatic species have demonstrated conclusively that stock productivity can be improved significantly via selection (Gjedrem, 2000; Hulata, 2001). In Atlantic salmon for example, studies of offspring from the 5<sup>th</sup> selection generation suggested a mean selection response of 14% per generation for growth and a correlated improvement of 4 – 5% per generation for feed utilization (FCR) (Thodesen *et al.*, 1999). Farmed Atlantic salmon in Norway today grow twice as fast as their wild counterparts and require 25% less feed. As a result the Norwegian salmon farming industry has reduced its feed costs by more than US\$ 230 million per year. While selection response can be estimated using different statistical methods (Bolivar and Newkirk, 2002; Gjedrem and Baranski, 2009; Gjedrem, 2005; Gall *et al.*, 1993), in general all methods produce similar outcomes when estimated on the same data set (Maluwa and Gjerde, 2007; Ponzoni *et al.*, 2005; Rezk *et al.*, 2009). According to Gjedrem and Baranski (2009), employing a control population is an appropriate method to use when breeding programs are only implemented for 2 or 3 generations of selection, while they consider average breeding values or repeated mating approaches as better benchmarks for estimating genetic gain in longer-term breeding programs. Applying a genetic trend analysis to the data however, using mixed model methods is also a good alternative when complete pedigree information is available over multiple generations and genetic ties are continuously produced between generations.

The selection response for body weight in the current study, estimated as the difference between least square means (LSMs) or estimated breeding values (EBVs) of the Selection line vs. the Control group, was approximately 7% per generation. In addition, favourable correlated selection responses were found for other body traits measured after

three selection generations and ranged from 10 to 16%. Data in the second generation of selection also showed positive correlated responses of approximately 9% for carcass weight. Together these results indicate that growth rate in GFP responds well to application of combined (between and within) family selection and that important carcass weight traits will be improved in parallel using this approach.

## **6.5. Future directions for GFP stock improvement in Vietnam**

GFP was domesticated more than 40 years ago in Hawaii and Thailand (Malecha *et al.*, 1984; Chareontawee *et al.*, 2007), but the GFP culture industry everywhere still currently relies on farming essentially wild/unimproved stocks. While GFP postlarvae have been produced in hatcheries for culture for several decades in Vietnam, mass seed production at a commercial scale has only been achieved recently, and brood stock are still mainly sourced from the wild or directly from culture ponds without any attention paid to their relative genetic quality (Phuong and Bui, 2006; Phuong *et al.*, 2006). These practices are inefficient and ultimately hamper expansion and development of the local industry. The selective breeding program for GFP in Vietnam initiated here is an initial step towards long-term more sustainable production of GFP in the country. While there is a long way to go to confirm the productivity advantages to farmers of farming the improved line developed here because the strain will require rigorous testing under real farming conditions and in different production environments, regardless it is still an important step for GFP culture in Vietnam and constitutes the transition from farming essentially ‘wild’ organisms to farming improved breeds.

### **6.5.1. Genetic (DNA) tags in stock improvement programs**

A major constraint on applying family selection in breeding programs in aquatic animals is that larvae, fry and juveniles are often too small to be tagged at, or soon after birth and so families need to be maintained separately until individuals are large enough to be physically tagged. As a consequence, early communal rearing of families produces another source of variation referred to as common environmental (e.g. hapa or tank) effects for full-sib families. When applying selecting for growth rate, impacts of common environmental effects can be largely accounted for by developing and applying robust genetic markers that are used later for a posterior parentage assignment (Herbinger *et al.*, 1999)

DNA fingerprinting has been used to develop genetic tags in commercial breeding programs (Estoup *et al.*, 1998; Fishback *et al.*, 2002; Vandeputte *et al.*, 2011; Vandeputte *et al.*, 2004). Parentage testing and pedigree assignment using genetic rather than physical tags has four main advantages: (i) a large number of families can be tested without requiring large numbers of tanks and ponds; (ii) it reduces the effect common to full-sibs; (iii) it can shorten the generation interval; and (iv) it can minimise interaction between the selection and production environments. Consequently, genetic tagging has potential to increase overall genetic gains in stock improvement programs of aquatic species (Ninh, Ponzoni, Nguyen, Woolliams, McAndrew *et al.*, 2011). Both experimental and theoretical studies show that 8 - 14 microsatellite markers are required to assign progeny to parents successfully, with a high degree of accuracy (90 to 99%) in aquatic animal species (Estoup *et al.*, 1998; Fishback *et al.*, 2002; Vandeputte *et al.*, 2011; Ninh, Ponzoni, Nguyen, Woolliams, McAndrew *et al.*, 2011; Ninh, 2009; Vandeputte *et al.*, 2004).

A posterior assignment of offspring to parents allows families to be pooled from incubation, thus enabling communal early rearing (CER) after hatching to reduce significantly or even eliminate common environmental effects and to increase genetic gains as compared with the conventional approach of separate early rearing of individual families (SER) until juveniles have reached a suitable size to be physically tagged (Ninh, Ponzoni, Nguyen, Woolliams, McAndrew *et al.*, 2011). It has also been reported that fish reared using the CER scheme grew faster than reared with SER (Ninh, 2009) and CER allowed a shorter generation interval than was possible with SER. Early communal rearing reduces the need for using hapas, that are not considered a favourable growing environment for many aquatic species in particular, giant freshwater prawn. The superiority of CER over SER has been demonstrated in many species when molecular parentage assignment has been applied in practical selective breeding programs for aquaculture species. While the costs of genotyping still remain relatively high, it is likely to decline significantly with the recent development of DNA chips (small solid surfaces that contain DNA sequences from many different gene loci attached to a glass plate at fixed locations). Individual sequences attached to the chip act as probes that can be used to produce a multi-locus genotype for each individual that is screened, so potentially thousands of mutational sites (SNPs) can be genotyped in an individual in the same run. For the purpose of parentage assignment and pedigree verification, SNPs are likely in time to replace microsatellite markers for application as genetic markers in aquaculture species due to their lower error rates. Studies have shown however, that due to their lower individual

information content, two to eight times more SNP markers are required than microsatellite loci to obtain a similar level of parental assignment power (Weller *et al.*, 2006; Hauser *et al.*, 2011). Genetic tagging however is unlikely to ever completely replace physical tagging because animals still require a physical tag so they can be identified externally for breeding and evaluation purpose.

### **6.5.2. Production of an all-male stock using the improved GFP strain**

Sexual dimorphism for growth traits has been reported in a number of cultured shrimp species including *L. vannamei* (Pérez-Rostro and Ibarra, 2003a; Argue *et al.*, 2002) and *M. rosenbergii* (Malecha *et al.*, 1984; Kitcharoen *et al.*, 2011; Luan *et al.*, 2012). Sexual dimorphism of cultured white shrimp (*L. vannamei*) occurs at body weights ranging from 10 to 17 g, where females reach significantly larger sizes (body length, 1.2%) and heavier weights (body weight, 4.8%) than males (Pérez-Rostro and Ibarra, 2003a). Male GFP generally reach significantly larger size and heavier body weight (approximately 93% in the current study) compared with females. As males grow faster and reach larger body sizes, culturing all-male GFP cohorts can result in significantly higher yields over a shorter culture period than that achieved from mix-sex culture or all-female culture cohorts (Sagi *et al.*, 1986; Cohen *et al.*, 1988). To this end, attempts have been made to sex juvenile GFP manually at early developmental stages to allow separation of the sexes for grow-out. Where this has been trialed experimentally, culture of all-male cohorts has shown better yields and associated improved financial returns (Sagi *et al.*, 1986). Some biotechnological approaches have also been trialed recently to produce all-male GFP culture lines, of which neo-female technology appears to offer the greatest promise (Aflalo *et al.*, 2006; Aflalo *et al.*, 2012b; Sagi and D. Aflalo, 2005). Aflalo *et al.* (2012b) suggested recently a combined approach, with selective breeding used to produce a fast growing line combined with neo-female technology to produce fast-growth, all-male cohorts for culture. In the current study, GFP growth rate showed a cumulative improvement of approximately 21%, so potentially if neo-female technology was applied to the improved strain here, the all-male stock that would result should have growth advantages resulting from both selection and sex-related (all male) phenotypes. This development could significantly increase returns to farmers.

### **6.5.3. Dissemination of the improved GFP stock in Vietnam**

While the estimated genetic response over three generations of selection was approximately 21% (Hung *et al.*, in review-a) in the current study, such productivity gain is unlikely to impact farmer livelihoods in Vietnam unless the progeny from the improved strain reaches the production system in a state that allows them to prosper over the grow-out period until they reach market weight. Achieving genetic gains in a breed improvement program can often be easier than achieving effective multiplication and dissemination of the resulting improved strain. For stock improvement, breeders simply have to control reproduction and mating in the brood stock in strategic ways while once a new breed is available, farmers need to be convinced that it is worth their time and effort to farm the new strain and often they need training in how to maximise benefits from farming it. In addition, simply assuming that availability of an improved strain will result in hatchery managers multiplying and disseminating the strain effectively is simplistic. Two models have been described for aquaculture seed production and distribution referred to as; ‘centralized’ or ‘decentralized’ systems. A centralized model (Figure 3a) is considered suitable for culture species where a small number of very large hatcheries are established in strategic locations that supply fry to a large number of farmers, usually within a somewhat restricted area. Centralised hatcheries however, usually fail in time, to effectively deliver seed to farms in more remote areas because of the difficulty and cost of seed transport. In contrast, a decentralized system (Figure 3b) contains many smaller hatcheries (even including ‘backyard’ hatcheries) located over a much larger geographical range within relatively easy reach, of most farms. Effective dissemination of an improved GFP breed to farmers in Vietnam will require therefore, careful analysis of the local industry and its needs, and development of a plan that specifies the number of hatcheries required and their geographical locations to effectively service all potential farmers.

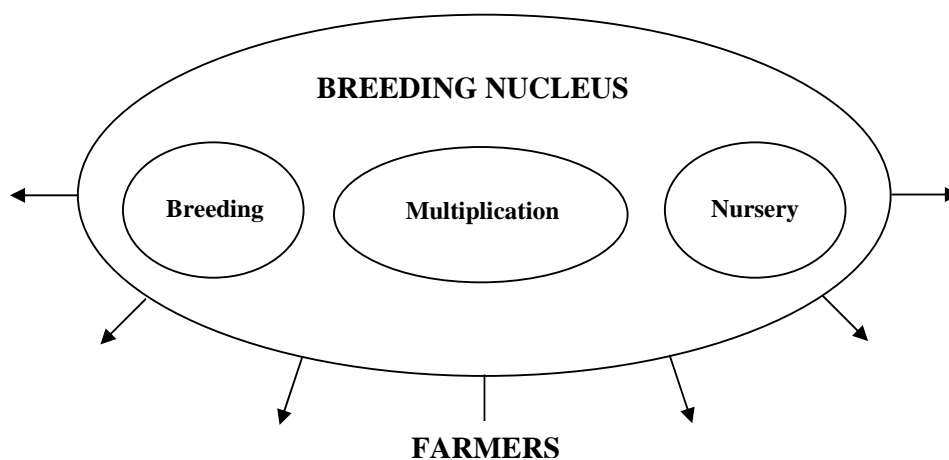


Figure 3a: Fully centralized model

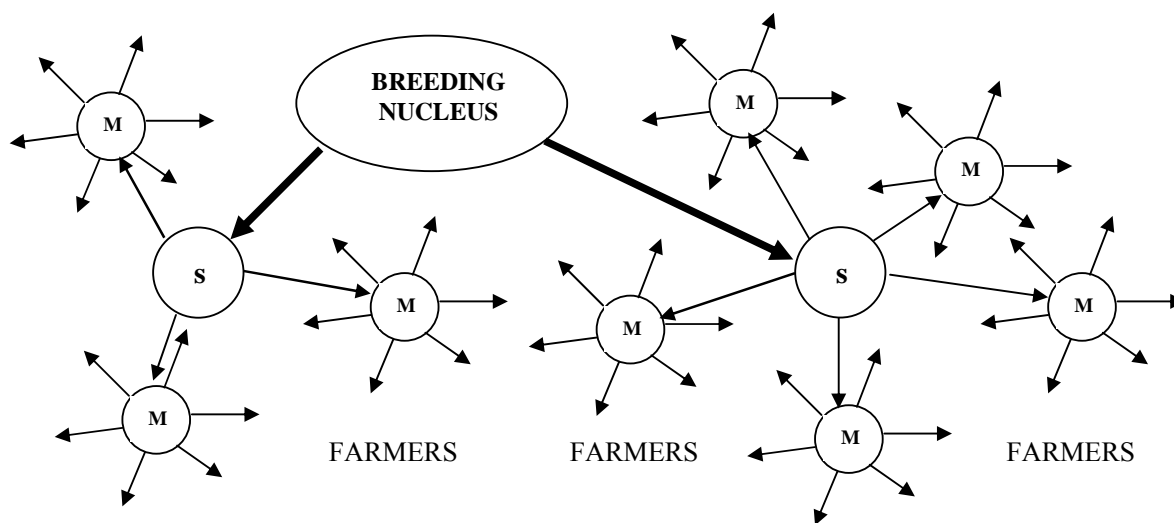


Figure 3b: Decentralized model (S = additional satellite nuclei to extend the reach of the improved stock; M = multiplier); the arrows from M indicate fry going to farmers

Regardless of the dissemination model adopted, genetic considerations must be factored into the plan that include when brood stock need to be replaced, the number of breeders that are used (to maintain healthy effective population sizes -  $Ne$ ), protocols required to minimize inbreeding and periodical (and preferably frequent) introductions of new brood stock from the improved line or from hatcheries with a reputation for maintaining brood stock quality over time.

In Vietnam, the major area used for GFP production is the Mekong Delta, thus this area will require an effective dissemination system to allow ‘take-up’ of the Vietnamese improved strain developed there. In the Mekong Delta, local transportation is not an obstacle and improved seed can be delivered to farms within a reasonable time by both trucks and/or boats. In this respect, both models of dissemination for the improved stock could be applied. Currently, a decentralized hatchery model however appears to be the better option because GFP hatcheries in the Mekong Delta while numerous, generally remain small-scale.

## 6.6. Conclusions

The current study is the first large-scale genetic stock improvement program for giant freshwater prawn in Vietnam and potentially, anywhere around the world. Results have shown that many full- and (paternal) half-sib families can be produced for GFP in a relatively short period of time, and VIE tags are an effective tagging system for juveniles of the target species that allows a family selection approach to be used to improve culture traits. The

synthetic base population developed here showed moderate heritability estimates for all body and carcass traits examined and heritability estimates significantly higher for females than for males. While heritability estimates were much higher in females than in males for the traits examined, the data show that selection should be practiced on both sexes to maximise overall genetic gains. Genetic correlations between body traits and carcass traits were high with all approaching unity, suggesting that the traits examined are likely to be controlled by the same set of genes. This result indicates that selection focussed on improving body weight would likely improve the other traits examined here, in parallel. In addition, genetic correlations between body traits taken at two times during grow-out (weeks 10 and 18) were high, suggesting that selection to improve growth rate is effective at a pre-harvest age, and if practiced early during grow-out (week 10 rather than week 18), this can speed up the breeding program and reduce overall costs.

The current project is also the first genetic improvement study of GFP that has included male morphotype and female reproductive status as fixed effects in the formal statistical models used to estimate genetic parameters. Both male morphotype and female reproductive status when included as fixed effects contributed significant variation to the analysis and hence should be fitted in models in future studies of GFP in order to avoid biasing estimates. The study potentially, may also provide a model for how culture stocks of some economically important penaeid shrimp species in Vietnam, for example *P. monodon* and *L. vannamei* could be improved via family-based selection in the future. As a final comment, we believe that the GFP culture industry in Vietnam can benefit significantly from the stock improvement program conducted here and with development and implementation of an effective dissemination strategy, farmers should see outcomes in the form of a more productive culture line that will provide better financial returns in the near future.

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## Appendices

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