MINISTRY OF EDUCATION AND TRAINING
NHA TRANG UNIVERSITY

NGUYEN HUU HUNG

RESEARCH ON GENETIC DIVERSITY AND GENETIC PARAMETERS ACCORDING TO GROWTH TRAITS FOR TIGER SHRIMP (*Penaeus monodon* Fabricius, 1798) BREEDING

Major: Aquaculture
Major code: 9620301

SUMMARY OF DOTORAL THESIS

KHANH HOA - 2020

The doctoral thesis was completed at Nha Trang University

Supervisor:

1. Dr. Nguyen Van Hao
2. Prof. Dr. Lai Van Hung

Reviewer 1: Dr Nguyen Minh Thanh

Reviewer 2: Dr Tran Thi Thuy Ha

Reviewer 3: Dr Dang Thuy Binh

The thesis is protected at the national evaluation council meeting at Nha Trang University at the time of day 19 month 9 year 2020

The thesis can be found at: National Library; Library of Nha Trang University

INTRODUCTION

The demand for seeds for commercial farming of tiger shrimp is about 30 billion/year (Directorate of Fisheries, 2017). However, the majority of broodstock supplied to hatcheries must be imported from abroad and exploited naturally. In order to meet the demand for tiger shrimp for the 2018 crop season, 30,000 black tiger shrimp are needed. The source of broodstock largely depends on natural exploitation. Naturally caught broodstock from the wild bring many consequences, of which the most important thing is to spread causative pathogens from the wild into the farming models. In practice, the current demand for tiger prawn of farmers is fully met by domestic production, but the quality of shrimp seed is always a problem. Black tiger shrimp are not strictly controlled on pathogens as well as quality, leading to huge losses for shrimp farmers. Black tiger shrimp is identified as one of the two main shrimp farming species in our country, the demand for seeds is increasing in quantity and quality, so the active development of high quality prawn broodstock through breeding selection in Vietnam is very necessary.

In Vietnam, studies of tiger shrimp before 2000 focused on biology, reproduction and seed production. From 2000 to the present, the studies of prawn cultivating have been done mainly to indoor re-cycle system farming technology. Although there have been a number of tiger shrimp breeding programs conducted in the world but for reasons related to technology security, these projects are not published and are not transferred.

From the above analysis, it is necessary to conduct a research program on breeding selection for high quality seed for this shrimp in Vietnam. Student research topic "Research on genetic diversity and genetic parameters according to growth traits for tiger shrimp breeding" is carried out in accordance with Decision No. 1081 / QD-DHNT of the Rector of Nha Trang University, November 23rd, 2015 under the Doctoral program, specialized in Aquaculture.

The research topic of PhD student is one of the important contents of the state-level project "Application of quantitative genetics and molecular genetics to create initial materials for tiger shrimp breeding according to growth traits" under the Project on development and application of biotechnology in Agriculture and Fisheries to the Ministry of Agriculture and Rural Development by 2020 by the Research Institute for Aquaculture II.

Objectives of the thesis:

Identifying genetic diversity and basic genetic parameters as a basis for creating a high-quality genetic variation of tiger shrimp for initial breeding of tiger shrimp according to growth traits.

To achieve this goal, the thesis has implemented the following contents:

1. Evaluate of genetic variation of initial tiger shrimp populations by Microsatellite technique.

2. Evaluate the growth of shrimp lines by hybrid method as a basis for initial material formation for the breeding program.

3. Estimate some basic genetic parameters on tiger shrimp populations have been established.

The significance of the thesis:

- Scientific significance:

This programe provide a scientific basis for assessing genetic variation of tiger shrimp populations to create base breeding populations with high genetic diversity by evaluating shrimp lines by hybrid hybrids and other Estimated results on some genetic parameters (evaluation of genetic and environmental interactions, heritability, selective efficacy).

The results confirmed the scientific basis of the theory and practice of breeding populations that really contributed to improving the genetic quality of black tiger shrimp populations in Vietnam. Research results are an important foundation for further research on this subject.

- Practical significance:

The research results have created a selective product of tiger prawn for the first generation (G_1) with good genetic parameters. This is the original source of materials for generations to choose from. Shrimp breeding is disease-free and grows faster than shrimp seed produced from wild broodstock. When commercialized, will provide the market with fast growth, no pathogen. This helps to solve the two biggest limitations of the current quality of tiger shrimp as disease and slow growth rate which slow the industry from development in recent years.

New points of the thesis:

The results of the thesis are novel in genetic numbers published following a systematic and complete way of basic genetic parameters of black tiger shrimp in Vietnam. This is a major breakthrough that opens up opportunities and conditions for further and longer breeding selections on this subject.

CHAPTER I. LITERATURE REVIEW

1.1. Achievements of breeding programs for aquaculture species in the country and around the world.

1.1.1 The breeding programs on aquaculture species

Breeding selection programme initially started on Atlantic salmon (*Salmo solar*) from 1971 in Norway (Gjedrem *et al.* 1991, Gjedrem 2005). Quantitative genetics and statistic algorithms have been applied in breeding selection on rainbow trout (*Oncorhynchus mykiss*) in Norway, Poland and Danmark (Gjedrem 2005), cod fish in Norway (Kolstad *et al.* 2006), and American catfish (Dunham and Argue 1998), tilapia (Eknath *et al.* 1993), Indian rohu, Pacific oyster, Japanese tiger prawn (Hetzel *et al.* 2000), Whiteleg shrimp (Argue *et al.* 2002); (De Donato *et al.* 2005, De Donato *et al.* 2008), Crawfish (Jerry *et al.* 2005). The initial findings showed that the efficency of selection is high, increasing about 4 – 15% per generation.

In Vietnam, programes for breeding selection in aquaculture have been done on some common species such as silver carp (*hypophthalmichthys molitrix)*, Vietnam silver carp (*H. harmandi*) (An and Thien 1987), common carp (*Cyprinus carpio*) (Trong 1983), GIFT tilapia *(O. niloticus)* (Dan and Little 2000), Freshwater prawn (Thanh and et al., 2009), whiteleg shrimp (Ninh *et al.* 2017), tiger prawn (Nguyen Van Hao and et al., 2015).

1.1.2. Geographical diversity of the initial material collection to form the breeding population

Selective programe on Atlantic salmon (*S. salar*) collected wild fish from fourty different rivers in Norway (Gjedrem *et al.* 1991). GIFT tilapia (Genetically Improved Farmed Tilapia) (*O. niloticus*) was formulated from eight populations (Bentsen *et al.* 1998, Eknath *et al.* 2007). Red tilapia was collected from seven different population in all over South America. Foundation populations of tiger prawn from Moana company was collected wildly from different areas in Asea.

In Vietnam, researches for freshwater prawn (*M. rosenbergii*), initial materials was selected from 3 lines from Me Kong, Dong Nai, Malaysia) (Thanh *et al.* 2009). Breeding selection for common carp was formed from 3 lines of Vietnam, Hungary and Indonesia (Ninh *et al.* 2011). Breeding selection was come from seven imported pounder

populations (Tran The Muu, 2015).

1.1.3. The evaluation the initial flow of materials for breeding programs

There are two methods to form an initial population for breeding. The first method is to collect shrimp from a variety of lines, then to randomly mix, unlimited between lines, in the first generation. This is the method used to form the Atlantic salmon breeding population in Norway (Gjedrem *et al.* 1991). The second method is hybridization between lines, then select with low intensity in the first generation selection. This method is used to create populations of zebra tilapia (*O. niloticus*) under the GIFT program (Bentsen *et al.* 1998).

1.1.4 Bio-security and disease free maintenance in shrimp breeding selection

Ensuring biosecurity and disease free are a prerequisite for breeding research and have been applied to The Hawaii - US Institute of Oceanography Breeding Program (OI) on white shrimp, the program Research on the cultivation of prawn-free broodstock in Malaysia, the program of processing prawn from CSIRO - Australia, the tiger prawn breeding program of Moana Company - Hawaii.

1.1.5. The marker technique for breeding program

Fluorescent color has been applied to mark success on worms (Butt *et al.* 2009), eels (Imbert *et al.* 2007), amphibians (Heemeyer *et al.* 2007), crustaceans (Zeeh and Wood 2009), fish (Doupé *et al.* 2003, Woods and Martin-Smith 2004, Astorga *et al.* 2005, Jensen *et al.* 2008), on a number of different shrimp species (Godin *et al.* 1996), crayfish (Mazlum 2007), lobster (Uglem *et al.* 1996, Frisch *et al.* 2006) and giant freshwater shrimp (Hung *et al.* 2012).

1.1.6 The evaluation of genetic and environmental interaction

The evaluation of G × E interaction studies has been published a lot in tilapia (Uraiwan *et al.* 1995, Maluwa *et al.* 2006, Bentsen *et al.* 2012, Khaw *et al.* 2012, Trọng *et al.* 2013), salmon species (Atlantic salmon, rainbow trout, etc.) (Winkelman and Peterson 1994, Fishback *et al.* 2002, Kause *et al.* 2003, Kolstad *et al.* 2006, Pierce *et al.* 2008), carp (*Cyprinus carpio*) (Ponzoni *et al.* 2005, Wang *et al.* 2007, Ponzoni *et al.* 2008), bass family (*Lates calcarifer*) (Saillant *et al.* 2006, Dupont-Nivet *et al.* 2008, Domingos *et al.* 2013), oysters (*Pinctada maxima*) (Kvingedal *et al.* 2007, Swan *et al.* 2007).

1.1.7. Heritability and genetic correlation

Heritability for different traits in shrimp has been published by the authors: Wong and McAndrew 1990, Benzie *et al.* 1997, Hetzel *et al.* 2000, Argue *et al.* 2002, Goyard *et al.* 2002, Gitterle *et al.* 2005a, Gitterle *et al.* 2005b.

1.1.8. The selective effect

Selective effects on aquatic subjects are usually quite high, up to 10 to 20% per generation as in Atlantic salmon (*Salmo salar*) (Gjedrem 2000, Quinton *et al.* 2005), coho salmon (*Oncorhynchus kisutch*) (Hershberger *et al.* 1990, Neira *et al.* 2004), American catfish (*Ictalurus punctatus*) (Dunham 2007), tilapia (*Oreochromis niloticus*) (Bentsen *et al.* 1998, Ponzoni *et al.* 2005, Eknath *et al.* 2007, Trọng *et al.* 2013), Indian carp (*Labeo rohita*) (Mahapatra *et al.* 2006) and catfish (*Pangasianodon hypophthalmus*) (Nguyễn Văn Sáng *et al.* 2012). Unlike fish studies, there are very few crustacean breeding programs and mainly focus on some marine shrimp species (Hetzel *et al.* 2000, Argue *et al.* 2002, Goyard *et al.* 2002, Preston *et al.* 2004, De Donato *et al.* 2005, Gitterle *et al.* 2005, Gitterle *et al.* 2006), freshwater crayfish (Jones and Ruscoe 2000, Jerry *et al.* 2005), giant freshwater shrimp (*Macrobrachium rosenbergii*) (Luan *et al.* 2012, Hung *et al.* 2014, Hung and Nguyen 2014, Luan *et al.* 2014, Luan *et al.* 2015).

1.2. The domestication and completion of closed life cycle of objects selected in artificial conditions in the country and in the world

Worldwide, shrimp farming began early in the mid-1970s, including whiteleg shrimp (*Liptopenaeus vannamei*) and Western blue shrimp (*Liptopenaeus stylirostris*) (Cuzon *et al.* 2004), tiger shrimp (Aquacop 1977, Primavera and Gabasa Jr 1981, Primavera 1998, Subramaniam *et al.* 2006). In Vietnam, although many domestic research programs on broodstock have been published, closing the life cycle of black tiger shrimp from eggs to broodstock and creating generations of home-grown shrimp in domestic conditions. For the first time successfully and initially small-scale commercialization, it is the program "Close up the life cycle and produce disease-free black tiger shrimp and tiger shrimp" (Hoa *et al.* 2009).

1.3. Microsatellite techniques in assessing genetic variation of breeding materials in aquaculture

Today, molecular markers, mainly AFLP and microsatellite, have been widely

used to evaluate genetic diversity between natural populations and domesticated pants for some economically valuable aquatic species such as salmon (Skaala *et al.* 2004), common carp (Kohlmann *et al.* 2005), tilapia (Romana-Eguia *et al.* 2004); (Xu and Thornalley 2001, Cruz *et al.* 2004, Dixon *et al.* 2008).

1.4. Application of modern methods (MAS – Marker Assisted Selection; GWS – Genome Wide Selection) in breeding program.

Breeding based on molecular markers linked to a trait of interest called marker-assisted selection (MAS) is a process that uses molecular markers to indirectly select one or more genetic important traits, this is a method widely supported by the world because this technique shortens the breeding time and can get results after three selective generations (Tanksley and Nelson, 1996). Breeding genetic selection based on whole-genome has been extensively researched and developed for animal husbandry (Solberg *et al*., 2008. In aquaculture, a recent review of MAS in fish breeding, Sonesson (2003) reported that MAS will be valuable for traits that are difficult to record and observe such as disease resistance, fillet quality, feed efficiency and maturity.

CHAPTER II. RESEARCH METHOD

2.1. Object, time, place of study and research scope

2.1.1. Research subjects

The research object is black tiger shrimp (*Penaeus monodon* Fabricius, 1798).

2.1.2. Research time

The duration of the study is from 2013 to 2018, in which the time for data analysis is from 2012 to 2015.

2.1.3. Research location

- National Center for Seafood Breeding - Institute of Aquaculture Research II. Implementing the main contents of the thesis.

- National Center for Central Seafood Breeding - Research Institute for Aquaculture III. Implementation of the evaluation of genetic-environmental interaction.

- Bac Lieu Experimental Research Centre - Aquaculture Research Institute II. Implementation of the evaluation of genotype-environment interaction (G x E).

- Dang Lam private enterprise (Vung Tau). Implementation of the evaluation of genotype-environment interaction (G x E).

- Southern Center for Fisheries Environmental and Disease Monitoring - Research Institute for Aquaculture II. Implement content of screening tests of common viral pathogens.

2.1.4. The research scope

Research is limited to sources of material collected from the ocean through suppliers. The studies were conducted in the South Central region and Mekong Delta. The study of genetic diversity limited to the use of microsatelite tools and genetic parameters is estimated for two generations G_0 and G_1.

2.2. Research materials

The research material is broodstock of tiger shrimp collected from Thailand, Singapore and Vietnam with a total of 460 females and 376 male shrimps.

2.2.1. Research materials for genetic variation evaluation by microsatellite

The pool pattern was randomly collected from four broodstock broodstock with a total of 137 samples analyzed.

The primers used for the study was from Sigma, a total of 15 microsattlite primers were used to evaluate the genetic diversity of the four original tiger shrimp.

2.2.2. The research material was used to evaluate the flow by mixed hybridization and estimate some basic genetic parameters

- A total of 69 shrimp families from 16 hybrids created G_0 generation with the number of 200 individuals/family, the average size of 2g generated from the original four shrimp materials of different origins.

- A total of 76 families from first generation (G_1) with 7,412 shrimp at the average size of 2 - 3g.

2.3. Research Methods

2.3.1. Method of assessing genetic variation by microsatellite

Research methods include steps: sample preparation, DNA extraction, microsatellite amplification by PCR technique, Agarose gel electrophoresis, allen analysis.

2.3.2. Method of evaluating the flow by mixed hybrid method

2.3.2.1. Maturation methods, broodstock reproduction, larval rearing

The methods of use includes: isolation culturing, broodstock maturation, sperm insertation, maternal eye cutting, maturation shrimp inspection, reproduction, egg collection, egg washing and incubation, larval rearing from Nauplius to PL15 postlarvae, nursing from PL15 post-larval stage to mark size.

2.3.2.2 Growth method of shrimp families of generations G_0 and G_1

- The G_0 generation cultured in four locations, including: Khanh Hoa province, Bac Lieu province, Vung Tau province and biosecurity tanks.

- The G_1 generartion cultured in two locations, including: Khanh Hoa province and biosecurity tanks.

The process of growth adopts the published process and is currently being applied in localities.

2.3.2.3. Individual marking method

Fluorescent colors (VIE) is used to mark shrimp individuals according to the manufacturer's instructions (Northwest Marine Technology Company).

2.4. Experimental layout

2.4.1. Experimental arrangement of selected primers used to evaluate genetic variation by microsatelite

Surveying 29 primer pairs on 28 random shrimp DNA samples to select 15 primer pairs using genetic diversity analysis of four primary broodstock.

2.4.2. Arrangement of full hybrid combinations of 4 shrimp populations

Families of G_0 generation were created from the combination of all hybrids of 4 shrimp groups (4 x 4 = 16 hybrids) including 4 inbred hybrids (AA, TT, NN and GG) and 12 cross-bred hybrids (AT, AN, AG, TA, TN, TG, NA, NT, NG, GA, GT, GN).

2.4.3. Hybrid layout of generation G_0 to formulate G_1 generation

Generation G_1 families are bred based on Index value and traceability of pedigree to avoid inbreeding and the presence of three Index groups with different ratios.

2.4.4. Growth arrangement to evaluate genetic and environmental interaction

Growth culture evaluated genetic and environmental interactions conducted in earthen ponds in South Central (Khanh Hoa), Southwest (Bac Lieu) and Dong Nam Bo (Vung Tau) and biosecurity (Trung National Center for Southern Breeding Seafood)

2.5. Methods of data analysis and processing

2.5.1. Methods of analyzing and processing data evaluate genetic diversity

Using GenAlEx software (Peakall and Smouse 2006) Genepop 4.2 to calculate allele frequencies, observed heterozygotes (H0), expected heterozygotes (He), and genetic differences (Fst), inbreeding coefficient (F_{IS}), test deviation from the Hardy-Weinberg Equilibrium.

2.5.2. Methods of analyzing and processing data for assessing growth of different shrimp lines by mixed hybrid method

Before communal stocking, each families was randomly scaled 30 individuals to record body weight when starting to place into biosafety tanks and ponds. After the culture period, about 80 days, all the shrimp was harvested in the tanks and ponds. Collected data include body weight (g, error of 0.1g), gender (male/female), culture tank and tagging signals (to retrieve families).

- Least square mean (LSM) estimate

The linear model selected after screening all fixed and covariance effects was formulated:

$$Weight_{ijkl} = \mu + hybrid_i + sex_j + age + tagging\ weight + tank_k + (hybrid \times tank)_l + residue_{ijkl}$$

2.5.3. Methods of data collection and statistic analysis for basic genetic descriptic parameters

Descriptive statistics was affected by sex (different weight between male and female) and broodstock (male or female broodstock) being analysed by software R 3.2.1 (Core 2015). The variance components are estimated by ASReml 3.0 (Gilmour *et al.* 2009).

- *Genetic and environment interaction for two culture environment*

The genetic correlation of the trait of harvest weight between two culture environments is estimated by the formula

$$\boldsymbol{r_g = \frac{\sigma_{12}}{\sqrt{\sigma_1^2} \times \sqrt{\sigma_2^2}}},$$

Where $\boldsymbol{\sigma_{12}}$ is covariance of cumulative genetic effects của of harvest weight between two environments, $\boldsymbol{\sigma_1^2}$ and $\boldsymbol{\sigma_2^2}$ are variance of cumulative genetic effects of harvest weight from environment 1 and 2 (Falconer and Mackay 1996).

- *Methods of estimating heritability*

Heritabilities for weight traits was estimated as formula:

$$\boldsymbol{h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_C^2 + \sigma_{TN}^2 + \sigma_E^2}}$$

And common invironmental effects

$$\boldsymbol{c^2 = \frac{\sigma_C^2}{\sigma_A^2 + \sigma_C^2 + \sigma_{TN}^2 + \sigma_E^2}}.$$

Heritabilities for survival rate (alive = 1; dead = 0) were estimated as formula.

$$h^2 = \frac{\sigma_A^2}{\sigma_A^2 + \sigma_E^2 \times \frac{\pi^2}{3}}$$

with σ_E^2 was valued as 1 (Gilmour *et al.* 2009).

- *Non-consecutive selection efficiency*: $R_1 = i \times h^2 \times \sigma_P$

- *Family selection efficiency*: $R_2 = i \times r \times \sigma_P$

CHAPTER III. RESEARCH RESULTS AND DISCUSSION

3.1. Evaluation of genetic variation in black tiger shrimp by microsatellite markers

3.1.1. Results of microsatellite selection for genetic variation

Based on the survey results, 29 microsatellites with good amplification products were selected. There are 29 pairs of primers being use to run polymorphic analysis on 28 samples of random shrimp DNA. And then, PCR performance and the number of alleles of each pair of primers to screen were evaluated and then the microsatellite for best performance were selected. The results of a total of 15 microsatellite markers were used to analyze and evaluate the genetic variation of the four shrimp populations as Indian, Pacific, Gia Hoa and Domestic: W2, W3, W4, W9, W10, P1, P2, P4, P5, P7, L1, L3, L4, N1, N2.

3.1.2. Genetic diversity of 15 microsatellite on original materials of four groups of black tiger shrimp broodstock

Results of the survey of genetic diversity of 15 microsatellite species in the four groups of tiger prawn in the beginning showed that only three microsatellite species (P2, P4 and P5) are distributed in balance with HWE, the remaining microsatellite deviated from HWE. This is compatible with observed heterozygous results in the study from 0.176 to 0.557, an average of 0.407 lower than the expected heterozygous (0.490 - 0.738), an average of 0.633. The inbreeding value (F_{IS}) is relatively high, averaging 0.357 ± 0.19. A F_{IS}-value of over 0.125 may indicate a state of genetic degradation due to inbreeding in the population. The above parameters show some limitations on the general genetic diversity of four broodstock broodstock used as initial materials.

3.1.3. Genetic diversity of four populations of original tiger shrimp

Survey results of genetic diversity within each population showed that the observed heterozygous genotype index (Ho) in this study has an average value of 0.41 lower than all of the microsatellite coefficients compared to the coefficient. Expected heterozygous (He) is 0.63. Among the four analytical sample groups, the average PIC (polymorphic information) of Gia Hoa population is the lowest of 0.515; Three shrimp populations are equivalent to 0.554. Microsatellites analyzed on domestic shrimp population distributed in accordance with Hardy-Weinberg equilibrium. The inbreeding value of F_{IS} for domestic shrimp is the lowest (0.316) compared to the three groups of

shrimp originating from the Indian Ocean, Pacific and Gia Hoa (0.374; 0.398; 0.338 respectively). The genetic diversity values of the three populations of Indian Ocean, Pacific and domestic shrimp are relatively equal and high, equivalent to 0.645. The cultured shrimp has the lowest genetic diversity index of 0.599 and has large variation in the population (0.398 - 0.710). There are significant genetic differences with the remaining shrimp populations.

3.2. Evaluation of growth of black tiger shrimp populations

3.2.1. Disease screening on the original broodstock stocks

Results of screening with different PCR techniques for 4 common dangerous viruses showed that the rate of disease-free shrimp was 48.0% for female shrimp and 54.5% for male shrimp.

3.2.2. Families production and hybrid families

The results of hybridation of 4 shrimp groups have created 69 families (58 full-sib families and 11 half-sib families) out of 16 hybrid combinations.

The genetic contribution ratio of the Inland group (N) is the highest and the lowest is the Gia Hoa group (28.7% and 20.4% respectively), the results are shown in Table 3.1. In theory, the rate of contribution of genetic material for each herd of balanced shrimp, 25%, is the best.

Table 3.1. Contribition ratio of original shrimp population materials G_0

Shrimp populations	**Indian (A)**		**Gia Hoa (G)**		**Domestic (N)**		**Pacific (T)**	
Sex	Female	Male	Female	Male	Female	Male	Female	Male
Number	19	10	6	16	17	14	13	13
Contribution ratio in each hybrid (%)	34.5	18.9	10.9	30.2	30.9	26.4	23.6	24.5
Total (Male and female)	29 (26.9%)		22 (20.4%)		31 (28.7%)		26 (24.1%)	

3.2.3. Rearing result of generation G0 families

3.2.3.1 Rearing from nauplius to 15th postlarvae (PL15)

Rearing result from Nauplius to 15th postlarvae was presented on Table 3.2:

Table 3.2. Rearing result from nauplius to 15th postlarvae

No	Hybrids	Number of families	Survival rate (%)
1	T × T	6	63.3 ± 6.3^{a}
2	T × N	4	57 ± 10.9^{a}
3	T × G	4	59 ± 6.6^{a}
4	T × A	4	60.5 ± 13.9^{a}
5	N × N	6	51 ± 4.3^{a}
6	N × G	6	49 ± 13.3^{a}
7	N × T	5	55.6 ± 8.3^{a}
8	N × A	4	52.5 ± 10.3^{a}
9	G × G	1	50^{a}
10	G × N	2	60 ± 8.49^{a}
11	G × T	2	49 ± 1.41^{a}
12	G × A	2	44 ± 2.83^{b}
13	A × A	7	49.1 ± 23.8^{a}
14	A × T	6	46.7 ± 22.97^{a}
15	A × N	5	60 ± 3.16^{a}
16	A × G	5	54.4 ± 5.6^{a}

Values in the same column with different letter symbols are significantly different (P<0,05). A: Indian shrimp; T: Pacific shrimp; G: Gia hoa shrimp; N: Domestic shrimp.

3.2.3.2. Shrimp development from 15th postlarvae (PL15) to tagging size

Shrimp after 30 days of rearing from PL15, the average of shrimp families achieved weight and length of 0.4 g and 2.8 cm respectively. 60-day-old shrimp, the average weight reached 2.0 ± 0.5 g and length 4.5 ± 0.4 cm.

3.2.4. Growth of farmed shrimp in biosafety tanks

3.2.4.1. Descriptive statistics and affecting factors on G0 shrimp growth

After being marked and stocked for 80 days in a bio-secure tank, body weight data was measured from 1,803 individuals originating from a full 69 families with an average weight of 26.1g. The proportion of female shrimp (52.6%) is higher than that of male shrimp (47.4%). Female shrimp with higher harvest weight (P <0.05) than male shrimp.

Table 3.3: Descriptive statistics for shrimp body weight traits

Shrimp group	Number of individuals (*N*)	Mean body weight (g)	Variable coefficient (*CV* - %)
Whole populations	1803	26.1 ± 0.14	22.2
Female	949	27.8 ± 0.19 a	21.4
Male	854	24.2 ± 0.17 b	20.5

Determining the fixed and covariate elements in the linear model, the result has 5 single elements and a two-way correlation has a significant effect.

3.2.4.2. Shrimp growth among hybrid combinations

The results of the growth comparison between inbred hybrids and cross-bred hybrids indicate quite significant differences. Other hybrids give higher growth results.

Table 3.4. Results of analysis of growth data of shrimp in hybrid groups

Order	Hybrids	Number of families	Number of shrimps (n)	Body weight (g)	Survival rate (%)
1	T × G	4	123	$31,6^{e} \pm 0,43$	$87,85^{c} \pm 1,42$
2	G × G	1	54	$30,1^{de} \pm 0,77$	$77,14^{bc} \pm 8,08$
3	G × N	2	103	$28,4^{cde} \pm 0,46$	$85,71^{bc} \pm 24,74$
4	N × G	6	157	$27,5^{bcd} \pm 0,41$	$74,76^{bc} \pm 14,27$
5	G × A	2	55	$27,1^{abcd} \pm 0,66$	$78,57^{bc} \pm 22,22$
6	A × G	5	139	$26,9^{abcd} \pm 0,40$	$78,28^{bc} \pm 20,36$
7	G × T	2	46	$26,7^{abcd} \pm 0,77$	$43,80^{a} \pm 8,08$
8	N × N	6	147	$25,4^{abc} \pm 0,41$	$70,00^{abc} \pm 16,92$
9	T × N	4	88	$25,2^{abc} \pm 0,54$	$62,85^{abc} \pm 14,19$
10	A × T	6	155	$25,0^{abc} \pm 0,40$	$88,57^{c} \pm 5,34$
11	A × A	7	172	$24,9^{abc} \pm 0,37$	$81,90^{bc} \pm 24,08$
12	T × A	4	110	$24,8^{abc} \pm 0,45$	$78,57^{bc} \pm 9,75$
13	N × T	5	117	$24,8^{abc} \pm 0,43$	$66,85^{abc} \pm 9,16$
14	T × T	6	122	$24,6^{abc} \pm 0,50$	$58,09^{ab} \pm 18,57$
15	A × N	5	139	$23,9^{ab} \pm 0,42$	$75,42^{bc} \pm 24,71$
16	N × A	4	89	$23,3^{a} \pm 0,51$	$63,57^{abc} \pm 14,63$

In this study, the impact on the growth of offspring is due to the origin of

broodstock. The influence of broodstock and or prawn factors is unclear.

3.3. Estimation of some basic genetic parameters

3.3.1. Evaluation of genetic and environmental interaction (G × E)

3.3.1.1. Descriptive statistics and influencing factors

Table 3.5. Breeding results grow in different environments

Culturing location	Factors	Shrimp number (*N*)	Mean	Variable coefficient (*CV*-%)
Biosecure tanks	Culturing duration (80 days)			
	Population weight (g)	1816	$26.1 \pm 0.14^{(b)}$	22.1
	Female weight (g)	957	$27.8 \pm 0.19^{(a)}$	21.4
	Male weight (g)	859	$24.2 \pm 0.17^{(b)}$	20.5
Khanh Hoa	Culturing duration (94 days)			
	Population weight (g)	4316	$29.3 \pm 0.08^{(c)}$	17.4
	Female weight (g)	2229	$30.8 \pm 0.11^{(a)}$	17.0
	Male weight (g)	2087	$27.8 \pm 0.10^{(b)}$	16.0
Bac lieu	Culturing duration (95 days)			
	Population weight (g)	4979	$25.0 \pm 0.08^{(a)}$	23.2
	Female weight (g)	2531	$26.2 \pm 0.12^{(a)}$	23.2
	Male weight (g)	2448	$23.8 \pm 0.10^{(b)}$	21.9
Vung Tau	Culturing duration (100 days)			
	Population weight (g)	3458	$26.1 \pm 0.13^{(b)}$	28.7
	Female weight (g)	1697	$27.4 \pm 0.20^{(a)}$	29.5
	Male weight (g)	1761	$24.8 \pm 0.16^{(b)}$	26.7

Values for comparing mean weights between female and male at same location presented by different letters showed significant difference (P<0,05).

Determining the fixed and covariate elements in the linear model, the result has 5 single elements and a two-way correlation has a significant effect and is included in the mathematical model.

3.3.1.2. Correlation between genotype and environment (G × E)

The analytical results show that G × E correlation is positively correlated (> 0) and lies from low to high from 0.29 to 0.85. There is a high genetic correlation between culture in biosecurity tank and Khanh Hoa culture site (0.70), Khanh Hoa and Bac Lieu (0.74); The correlation with the two sites of Bac Lieu and Vung Tau is 0.42 and 0.29 respectively.

Table 3.6. Correlation of genotype (r_g) of body mass traits in different culture environments for G_0 vaf G_1

Culturing locations	**G_0 generation**				**G_1 generation**
	Biosecurity tanks	**Khanh Hoa**	**Bac Lieu**	**Vung Tau**	**Khanh Hoa**
Biosecurity tanks		0,70 ± 0,09	0,42 ± 0,13	0,29 ± 0,15	0,75 ± 0,09
Khanh Hoa			0,74 ± 0,08	0,51 ± 0,12	
Bac Lieu				0,85 ± 0,05	

3.3.2. Heritability

The results of formulating families, culturing and evaluating the growth of G_1 generation of shrimp families are presented in Table 3.7 and 3.8.

Table 3.7. The number of full-sib and half-sib families in G_1 generation

Family type	**Total families**	**Number of shrimp**	**Male × Female**	**Full-sibs families**	**Half-sibs families**
Half-sibs families	50	5155	1 × 2	6	12
			1 × 3	3	9
			1 × 4	3	12
			1 × 5	2	10
			1 × 7	1	7
Full-sibs families	26	2257	1 × 1		
Total	**76**	**7412**			

Table 3.8. Quantity and weight of shrimps at harvesting time in two ponds in Khanh Hoa and in biosecure tanks

Environments	Batch	Sex	Quantity	Weight at harvest time (g)				
				Mean weight (g/shrimp)	*Standard deviation (SD)*	*Variable coefficient (%)*	*Min (g/shrimp)*	*Max(g/shrimp)*
Khanh Hoa (pond)	1	Female	303	23.26	7.90	33.97	5.77	53.59
		Male	256	20.94	7.10	33.93	7.52	50.34
	2	Female	938	20.99	6.24	29.72	5.72	42.65
		Male	794	19.51	5.13	26.28	5.88	37.33
	3	Female	559	29.81	7.66	25.70	9.03	58.71
		Male	451	26.53	7.18	27.08	8.04	54.41
The total number of shrimp harvested at Khanh Hoa: 3301 individuals								
Biosecure tanks	1	Female	672	22.70	7.17	31.82	6.25	49.60
		Male	646	20.53	6.24	30.41	6.55	42.55
	2	Female	1009	24.26	8.24	33.96	7.06	56.40
		Male	914	21.64	6.50	30.06	7.16	42.83
	3	Female	461	24.13	7.29	30.20	7.20	46.60
		Male	409	21.95	6.78	30.88	5.90	42.60
The total number of shrimp harvested at biosecure tanks: 4.111 individuals								

The total harvested shrimp was 7412 of which 3301 were from pond culture and 4111 from tank culture. The average weight of cultured shrimp in ponds ranged from 19.51 ± 5.13 to 29.81 ± 7.66 g/shrimp, and shrimp in tanks with weight from 20.53 ± 6.24 to 24.26 ± 8.24 g/shrimp. The coefficient of variation in cultured shrimp weight is high, from 25.70 to 33.97% for shrimp cultured in ponds and 30.06 to 33.96 g/shrimp for tank shrimp.

- *Estimating heritability*

For the G_1 shrimp weight trait at harvest, the estimate of the variance components (σ_A^2, σ_C^2 và σ_P^2), the heritability factor (h^2) and the effect of common environmental effect (c^2) for the two culture environments in Khanh Hoa and the biosecure tanks are shown in Table 3.9.

Table 3.9. The variance components (σ_A^2, σ_C^2, σ_{TN}^2, σ_E^2,và σ_P^2) and heritabilities (h^2) and common environmental effects (c^2) on shrimp weight of G_1 generation (±SE)

Environments	σ_A^2	σ_C^2	σ_{TN}^2	σ_E^2	σ_P^2	h^2	c^2
Khanh Hoa	37.47	12.11	4.68	8.47	62.73	0.60± 0.17	0.19± 0.09
Biosecure tanks	35.54	10.64	2.76	14.53	63.47	0.56± 0.15	0.17± 0.09

Heritability for weight traits is estimated at a high level, shrimp in ponds is 0.60 ± 0.17 and in tanks is 0.56 ± 0.15. The impact of the environment was also large, shrimp ponds in Khanh Hoa were 0.19 ± 0.09 and cultured in the biosecure tanks was 0.17 ± 0.09. The genetic correlation of shrimp weight when harvested between two culture ponds and tanks was estimated at 0.75 ± 0.09, indicating a moderate environmental interaction.

For the trait of survival and estimation of heritability for the two culture environments in Khanh Hoa and biosecure tanks is presented in Table 3.10. Heritability was estimated to be quite good and comparable between the two environments, for shrimp farming in Khanh Hoa was 0.18 ± 0.02 and in biosecure tank was 0.19 ± 0.02. For both environments, the genetic correlation between survival and harvest weight is positive and relatively low, with 0.40 ± 0.08 in Khanh Hoa and 0.29 ± 0.08 in biosecure tanks.

Table 3.10. Heritability (h^2) of survival and genetic correlation (r_g) between individual survival rate and shrimp harvest weight in Khanh Hoa and biosecure tanks

Traits	h^2	r_g	
		Weight in Khanh Hoa	Weight in Biosecure tanks
Traits in Khanh Hoa (%)	0.18 ± 0.02	0.40 ± 0.08	
Traits in biosecure tanks (%)	0.19 ± 0.02		0.29 ± 0.08

The correlation between survival rate and estimated breeding value (EBV) of survival rate in each environment and between Khanh Hoa and biosecure tanks is shown in Table 3.11. The correlation between survival rate and EBV of survival rate is high, for Khanh Hoa is 0.80 and for biosecure tanks is 0.89. Between the two environments,

the correlation between survival rate and EBV survival rate is a positive correlation, ranging from 0.37 to 0.55.

Table 3.11. Correlation between survival rate and estimated breeding value (EBV) of survival rate in Khanh Hoa and biosecure tanks

Traits	Survival rate in biosecure tanks	EBV survival in Khánh Hòa	EBV survival in biosecure tanks
Survival rate in Khánh Hòa (%)	0.46	0.80	0.37
Survival rate in biosecure tanks (%)		0.43	0.89
EBV survival in Khánh Hòa			0.55

3.3.3. Selection efficiency after a generation of growth traits

3.3.3.1. Selection efficiency

For population G_0, the efficiency in non-consecutive selection (R_1) is 0.9g, corresponding to 3.3%. For G_1 population, selective efficiency R_1 is 4.3 g (19.2%). When selecting families, R_2 is similar to that of R_1, giving G_0 of 0.9 g (3.3%) and giving G_1 of 4.1 g (18.1%).

Table 3.12. Selection rate, selective intensity, heritability, standard deviation of the weight trait and selection efficiency of two populations of tiger shrimp G_0 and G_1

Generation	**R**	***p* (%)**	***i***	***h²***	**σ_P**	***R* (g)**	**Weight (g)**	**Gain (%)**
G_0	R_1	80.4	0.35	0.43	5.7	0.9	26.1	3.3
	R_2	80.4	0.35	0.43	5.7	0.9	26.1	3.3
G_1	R_1	34.1	1.06	0.56	7.3	4.3	22.6	19.2
	R_2	34.1	1.06	0.53	7.3	4.1	22.6	18.1

p = selection rate, i = selection intensity (Falconer and Mackay 1996), h^2 = heritability, r = selection efficiency = $\sqrt{\frac{1}{2}h^2}$, σ_P = standard deviation of harvest weight, R_1 = non-consecutive selection efficiency and R_2 = family selection efficiency.

CHAPTER IV. CONCLUSION AND RECOMMENDATION

4.1. Conclusion

1. The some limitations on the general genetic diversity of four broodstock sources used as initial materials. The results of heterozygous observations averaged 0.407 (0.176 to 0.557) is lower than expected heterozygosity (0.490 - 0.738), an average of 0.633. The inbreeding index (FIS) is relatively high, averaging 0.357 ± 0.19.

2. Within the scope of the study, the genetic material of the Domestic population is relatively good. Domestic shrimp population have distribution in compliance with HWE equilibrium compared to the remaining shrimp populations. The F_{IS} inbreeding value of the Domestic group was the lowest (0.316) compared to the three populations of Indian, Pacific, and Gia Hoa (0.374; 0.398 and 0.338). The genetic diversity values of the three populations of Indian, Pacific, and Inland shrimp are relatively equal and high, equivalent to 0.645.

3. The average PIC index (polymorphic information) of the cultured shrimp is the lowest of 0.515 compared to the remaining shrimp (~ 0.554). The cultured shrimp has the lowest genetic diversity index of 0.599 and has large variation in the population (0.398 - 0.710). There is a genetic difference of the cultured shrimps with three remaining populations ($P \leq 0.001$). The difference between pairs of shrimp (Indian, Pacific), (Indian Ocean, Inland) and (Pacific, Inland) is not significant.

4. High variability in phenotype of body mass traits in both male and female shrimp (about 21.3%) allows the prediction of tiger shrimp populations in this study to have potential to improve quality in further breeding selection.

5. The impact on growth of offspring is due to the origin of broodstock, the influence of broodstock and / or prawn factor is unclear. The results of the growth comparison between inbred hybrids and other crossbred hybrids show quite significant differences, in which other hybrid hybrids produce higher growth results. In inbred hybrids, the hybrid combination of Gia Hoa shrimp (G x G) produces the best growth results. For hybrids of wild-caught shrimp populations, the domestic herd cross-bred (N x N) represents the dominant phenotype of growth traits compared to the remaining two groups of shrimp (A x A and T x T).

6. Most of the hybrids that yield the best growth in phenotype are inbred crosses. Out of the six crosses that yield the best results (except for in-line GG), all are of the Gia Hoa (G) line with the other three. Off-line crosses between wild-caught shrimp (eg, NA, NT, TA, etc.) often show lower growth.

7. All genetic correlations of stem body traits harvested in different culture media were positive, inconsistent and range from low to high (rg from 0.29 to 0.85). (> 0), which allows mild GxE interactions to be predicted.

8. Genetic correlation (r_g) of shrimp weight when harvested between two culture environments in Khanh Hoa and biosecure tanks (G_1 generation) is estimated to be 0.75 ± 0.09. Besides, the interaction of G x E (generation G_0) is low (rg = 0.42) of weight trait between biosecure tanks and ponds in Bac Lieu, where is the main farming region requiring to maintain and further enhance the genetic diversity of this material source in order to better adapt to different types of environments and farming models.

9. Genetic correlation between trait of survival rate and harvest weight ranged from 0.29 ± 0.08 (biosecure tanks) to 0.40 ± 0.08 (Khanh Hoa), indicating that selection was improved. Growth will not negatively affect the survival rate of farmed shrimp. The genetic correlation between EBV survival rate and survival rate in each environment was high (0.80 for Khanh Hoa and 0.89 for biosecure tanks). Between the two environments, the correlation between survival rate and EBV survival rate is a positive and relatively low correlation ranging from 0.37 to 0.55 due to the nature of the two culture environments and the care regime is very different.

10. The heritability of shrimp weight is estimated at a high level, for shrimp farming in Khanh Hoa is 0.60 ± 0.17 and for shrimp in the biosafety tank is 0.56 ± 0.15. The effect of the environment was also quite good, for Khanh Hoa was 0.19 ± 0.09 and for the biosecure tanks was 0.17 ± 0.09. For the individual survival rate, the estimate of heritability for farmed shrimp in Khanh Hoa was 0.18 ± 0.02 and the biosecure tanks was 0.19 ± 0.02.

11. The selective effect for population G_0 in the case of non-consecutive selection (R_1) is 0.9 g, corresponding to 3.3%. For G_1 population, R1 is 4.3 g (19.2%). When selecting families, R_2 is similar to that of R_1, giving G_0 of 0.9 g (3.3%) and giving G_1 of 4.1 g (18.1%).

4.2. Idea suggestion

1. Results of evaluation of genetic diversity in the whole population and each of broodstock as initial materials for the breeding process suggest that it is necessary to collect and supplement the diverse shrimp stocks. especially paying attention to populations of naturally derived shrimp.

2. Continuing researches to reduce the impact of environmental factors (c^2) such as well implementing the complete hybrid structure (hierarchical hybrid structure) is needed, reducing family production time, shrimp tagging size (i.e. reducing the duration of a separate family nursing time). The procedure for techniques of care and nutrition in the pre- and mature stages is complete, improving the rearing and rearing system in different farming environments to contribute to increasing the accuracy of the heritability estimation.

3. The use molecular genetics will be continued to support genetic numbers in seed selection to accelerate selective efficiency.