Domestication of black tiger shrimp (*Penaeus monodon*) in recirculation systems in Vietnam

Nguyen Duy Hoa

Promoter: **Prof. dr. Patrick Sorgeloos**
Department of Animal Production, Faculty of Bioscience Engineering, Ghent University, Belgium.

Local promoter: **Dr. Nguyen Van Hao**
Research Institute for Aquaculture No.2, Ho Chi Minh City, Vietnam.

Dean: **Prof. dr. ir. Guido Van Huylenbroeck**

Rector: **Prof. dr. Paul Van Cauwenberge**

Examination Committee and Reading Committee (*):

Prof. dr. ir. Herman Van Langenhove, Chairman, *Department of Organic Chemistry, Faculty of Bioscience Engineering, Ghent University, Belgium.* Herman.VanLangenhove@UGent.be

Prof. dr. Guy Smagghe, Secretary, *Department of Crop Protection, Faculty of Bioscience Engineering, Gent University, Belgium.* Guy.Smagghe@UGent.be

Prof. dr. Patrick Sorgeloos, Promoter, *Department of Animal Production, Faculty of Bioscience Engineering, Ghent University, Belgium.* Patrick.sorgeloos@UGent.be

Dr. Nguyen Van Hao, **Local VLIR promoter**, *Research Institute for Aquaculture No. 2, Ho Chi Minh City, Vietnam.* Haoria2@hcm.vnn.vn

Prof. dr. ir. Peter Bossier, *Department of Animal Production, Faculty of Bioscience Engineering, Ghent University, Belgium.* Peter.bossier@ugent.be

***Prof. dr. Geert Janssens**, *Department of Animal Nutrition, Genetics, Breeding and Ethology, Faculty of Veterinary Medicine, Ghent University, Belgium.* Geert.janssens@UGent.be

***Prof. dr. Lewis LeVay**, *University of Wales, Bangor, England.* L.levay@bangor.ac.uk

***Dr. Roeland Wouters,** *INVE Technologies N.V., Dendermonde, Belgium.* R.wouters@inve.be

Nguyen Duy Hoa

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
ARA	Arachidonic acid
CSIRO	Commonwealth Scientific and Industrial Research Organization
DHA	Docosahexaenoic acid
DW	Dry weight
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl esters
FAO	Food and agriculture organization
Fo:	Wild origin generation
F1:	First domesticated generation
F2:	Second domesticated generation
HUFA	Highly unsaturated fatty acid
ICP-PhD	International Course Program-PhD
PCR	Polymerase chain reaction
PGs	Prostaglandins
PUFA	Poly-unsaturated fatty acid
SEAFDEC	Southeast Asian Fisheries Development Center
SPF	Specific-pathogen-free
VASEP	The Vietnam Association of Seafood Exporters and Producers
VLIR	Vlaamse Interuniversitaire Raad (Flemish Inter-University Council)

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CHAPTER I

GENERAL INTRODUCTION

Chapter I

General introduction

The black tiger shrimp, *Penaeus monodon* Fabricius (1798), is one of the largest penaeid shrimps in the world, reaching 270 mm in body length (Motoh, 1985), and is of considerable commercial importance in international markets. *P. monodon* is indigenous to Vietnam and has contributed significantly to the development of the aquaculture sector and hence the economy, with a national production between 350,000-400,000 tons a year, recently. According to VASEP (Phi et al., 2009), shrimp farming contributes around 40% in value of the total fisheries export turn-over of the country. In 2007, the national shrimp production of approximately 350,000 tons, was estimated to comprise of 270,000 tons of *P. monodon* and 80,000 tons of white shrimp, *Litopenaeus vannamei* (Merican, 2008). According to Jeff Jie-Cheng Chuang, Vice president of Uni-President Vietnam, the annual nationwide demand for postlarvae is around 35 billion (Merican, 2008).

To date, shrimp hatcheries in Vietnam have been able to supply the demands of the commercial shrimp farms, but prevalence of diseases and the low quality of the postlarvae have resulted in huge damage to both the hatchery and grow-out sector. Disease outbreaks, especially White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV), Taura Syndrome Virus (TSV), Monodon BaculoVirus (MBV), etc. and to a lesser extent, luminescent bacterial infections have resulted in a declining global production in recent years for shrimp farmers in Asia and in the America and this leads to an almost inevitable ‘boom-and-bust’ cycle of the shrimp farming industry

(Flegel et al., 2004). Another factor contributing to the unstable development of the shrimp farming industry is the reliance on wild shrimp broodstock for the hatchery industry due to fluctuations in supply and declining natural stocks. The fishing of wild broodstock poses serious problems to the natural resource and from an ecological perspective, the fishing of wild stocks moreover is unacceptable in the long term. The use of wild broodstock shrimp also poses serious risks for vertical disease transmission from the breeders to the offspring, resulting in disease outbreaks and for this reason, viral diseases must be controlled at the broodstock level itself (Craig, 1998; Yano, 2000; Arce, 2005; Coman et al., 2005).

Successful domestication of *Penaeus monodon* will ensure sufficient quantities of good quality broodstock and consequently reliable offspring production. The development of reliable domesticated stocks to produce high quality and SPF seed is therefore imperative to a long-term sustainable development of *P. monodon* aquaculture. With the goal of developing a successful domestication technique to produce SPF domesticated *P. monodon* stock, this thesis aimed to: i) develop a sand-based recirculation system for the indoor domestication of *P. monodon*, ii) develop a protocol for each rearing phase of the domestication process in the developed recirculation system; iii) develop suitable broodstock diets and feeding regimes to improve reproductive performance, more specifically fecundity and egg hatching success, of the domesticated *P. monodon* stock; and iv) evaluate the grow-out performance of the offspring, produced from the domesticated stocks, at a commercial farm environment. The overall goal of this study was to contribute to the sustainable development of the *P. monodon* farming sector in Vietnam through development of techniques that could lead to the commercialization of domesticated breeder stocks.

The thesis outline can be summarized as follows:

Chapter I (General introduction) draws an outline covering the main points of this thesis.

Chapter II (Literature review) gives an overview of the development of *P. monodon* domestication techniques during the last decades, focusing on techniques how to close the life cycle of *P. monodon* in captivity, especially, nutritional and environmental conditions and other zootechniques applied during the domestication process. Environmental and seasonal factors, substrate, nutritional requirements, genetic and male effects, as well as endocrinology, which are all known to affect the quality of domesticated *P. monodon* broodstocks, are being discussed in this review. Knowledge on reproductive biology and maturation of penaeid shrimp are also reviewed in order to better understand the requirements for maturation and reproduction of penaeid shrimp. Knowledge on shrimp nutrition, especially on *P. monodon* and recent studies on broodstock nutrition of *P. monodon* investigating the specific role of arachidonic acid in maturation, spawning and reproductive performance are also reviewed and discussed in view of quality improvement of domesticated *P. monodon* stocks.

Chapter III (Rearing system) reports on the development of a sand-based recirculation system through different trials on water exchange regimes, rearing water salinity, stocking density and diets for the grow-out phase of *P. monodon*.

Chapter IV (Domestication progress) developed a protocol for each phase of the domestication process from eggs to breeders. The developed techniques focussed on aspects of the rearing environment and diets for the 3 different rearing phases,

including the grow-out phase (from 1 to 40g), the prematuration phase (from 40 to 80g), and the maturation phase (from 80 to larger than 100g). Performance was evaluated in terms of growth, survival, spawning and reproductive performance.

Chapter V (Broodstock nutrition) consists of two parts:

Section I:

A fresh-food maturation diet with an adequate HUFA composition for broodstock nutrition studies in black tiger shrimp Penaeus monodon (Fabricius, 1798). This section reports on the development of a fresh-food maturation diet for improving the reproduction of *P. monodon* and which could serve as a basic fresh-food maturation diet for *P. monodon* maturation or as a control diet for further studies on artificial maturation diets. The formulated fresh-food mixture was constructed based on the ratios of ARA/EPA, DHA/EPA and ω3/ω6 fatty acids in mature ovaries of wild *P. monodon*.

Section II:

Spawning performance and offspring quality of domesticated black tiger shrimp Penaeus monodon fed a semi-moist maturation pellet. This section describes the development of a feeding regime for optimising maturation and reproduction of *P. monodon* in captivity by testing different substitution levels of the fresh-food maturation diet (developed in section I) with a recently developed semi-moist maturation diet (BREED-S FRESH®) (INVE Aquaculture N.V., Belgium).

In Chapter VI (Grow-out performance) grow-out performance of domesticated *P. monodon* postlarvae at different commercial shrimp farms was evaluated and compared with non-domesticated *P. monodon* stocks.

Chapter VII (General discussion) reiterates and discusses the overall results of the experiments and the progress in domestication of *P. monodon*. Based on the discussion, the general conclusions are drawn and prospective research topics are proposed.

Chapter VIII (References) contains all the bibliographic citations mentioned in this thesis.

CHAPTER II

LITERATURE REVIEW

Chapter II

Domestication techniques for black tiger shrimp *Penaeus monodon* (Fabricius, 1798): a literature review.

Nguyen Duy Hoa[1,3], Mathieu Wille[3], Roeland Wouters[2], Nguyen Van Hao[1], Patrick Sorgeloos[3].

Research Institute for Aquaculture No.2, Ho Chi Minh City, Vietnam[1].

INVE Technologies N.V., Dendermonde, Belgium[2].

Laboratory of Aquaculture & Artemia Reference Center, Ghent university, Belgium[3].

Abstract

Closing the life cycle of *P. monodon* in indoor rearing systems has recently been successful with several generations being produced in research programs. However, domesticated *P. monodon* stocks have not yet been commercialized mainly due to poor reproductive performance, especially low natural mating, low fecundity and poor egg hatching success. This paper summarizes present knowledge and current rearing techniques used for domestication of *P. monodon* for production of specific-pathogen-free domesticated stocks. Environmental and seasonal parameters, tank substrate, nutritional requirements, genetic and male effects, and endocrinological factors are all known to affect the quality of domesticated *P. monodon* broodstock and are therefore being discussed in more detail. A number of selected *P. monodon* domestication programs, especially those using indoor rearing systems, are reviewed. Overall rearing techniques, nutritional and environmental conditions and other zootechniques applied during the domestication process are discussed. Knowledge on reproductive biology and maturation of penaeid shrimp is also discussed to elucidate the factors affecting maturation and reproduction of penaeid shrimp. Knowledge on shrimp nutrition, with special focus on *P. monodon* and recent studies on broodstock nutrition of *P. monodon* investigating the specific roles of arachidonic acid in maturation, spawning and reproductive performance of *P. monodon* are also reviewed and discussed.

Keywords: domestication, P. monodon.

1. Introduction

Worldwide expansion of the shrimp farming industry was attributed mostly the success in the production of high quality nauplii through controlled maturation and

reproduction (Browdy, 1992). Several reviews on maturation of penaeid shrimp have been published in the 1980's and 1990's (Chamberlain, 1985; Primavera, 1985; Woo, 1988; Bray and Lawrence, 1992; Browdy, 1992; Benzie, 1997; Browdy, 1998). However, all these reviews indicated that the majority of the studies at that time (from the 1970's to the 1990's) focused on short-term maturation and breeding technology, rather than full domestication (closing the life cycle from egg to breeder in captivity). This was especially the case for black tiger shrimp (*P. monodon*). Also in recent years, a number of reviews on maturation and breeding technology of penaeid shrimp have been published, for example a review on shrimp endocrinology (Huberman, 2000), a review on cultivation of specific pathogen free (SPF) penaeid shrimp broodstock in closed recirculation systems (Yano, 2000) and a review on broodstock shrimp nutrition (Wouters et al., 2001) as well as a review on shrimp larval quality in relation to broodstock condition (Racotta et al., 2003).

Domestication of penaeid shrimp has first succeeded with white shrimp *L. vannamei* and was subsequently widely developed and commercialized during the 1990's, spreading from the West into Asia since the 2000's. In contrast, while domestication of *P. monodon* had also started early in the 1970's (Aquacop, 1979; Primavera, 1983; Withyachumnarnkul, 1998), these domestication programs typically started with pond-reared animals (not eggs or larvae), using outdoor ponds without proper biosecurity and therefore, although some generations of *P. monodon* have been produced, these programs ultimately proved not successful and were hence not commercialized. This might be the reason why no review is found on domestication technology for *P. monodon*. Full domestication of *P. monodon* from egg to breeder in biosecure closed recirculation tanks in indoor rearing system to produce SPF shrimp only started in the mid 1990's with several generations of domesticated black tiger

shrimp being produced during the 2000's (Coman et al, 2005; 2006; 2007b,c). Domestication of white shrimp (*L. vannamei*) has changed the face of aquaculture since the early 1990's. World shrimp farming production using domesticated *L. vannamei* expanded from only 10% of total shrimp production in 1998 to 75% of total world shrimp production in 2006 (Wyban, 2007). Unlike white shrimp *L. vannamei*, growing up of *P. monodon* in indoor rearing systems to a size suitable to serve as breeders was not as easy as it might seem; also triggering maturation and optimizing reproduction of *P. monodon* breeders in captivity proved very difficult. Therefore, while specific-pathogen-free or disease-resistant domesticated stocks of white shrimp *L. vannamei* have been widely developed and commercialized, the domestication of *P. monodon* is still largely in an experimental stage due to poor performance of domesticated broodstocks, especially in terms of egg fecundity and hatching success (Coman et al., 2005; 2006; 2007b).

In the current paper, selected studies on domestication of *P. monodon* are reviewed and rearing, maturation and breeding techniques discussed in order to draft recommendations for successful domestication of *P. monodon* in captivity as well as for improvement of the quality of domesticated *P. monodon* stocks. The discussion focuses on aspects of shrimp reproductive biology, tank substrate, environmental conditions, nutrition, endocrinology, male and genetic effects, as well as the comparison between wild and domesticated *P. monodon* in order to formulate possible solutions for solving the bottlenecks in domestication of *P. monodon*.

2. Domestication technique for black tiger shrimp *Penaeus monodon*

Broadly speaking, domestication can be defined as that condition wherein the breeding, care and feeding of an organism are more or less controlled by man (Hale,

1969). From an agricultural viewpoint, true domestication means: (i) the individual is valued and kept with a specific purpose; (ii) its breeding is subject to human control; (iii) its behaviour is different from that of the wild ancestor; (iv) its morphology (including size and coloration) exhibits variation never seen in the wild; and (v) some of which would never survive without human protection (Balon, 1995). In aquaculture, domestication is regarded as the acclimatization of an organism to captive conditions with the two key points being rapid growth and a potential for induced spawning in captivity (Hassin et al., 1997). In this paper, domestication is regarded as the full control of the life cycle of the organism from egg to breeder which is capable to produce offspring through generations in captivity.

Since the 1970's several programs have attempted to produce domesticated *P. monodon* breeders whether or not genetically improved and/or specific pathogen free (SPF). Several research institutes and private companies have been involved, such as Aquacop in Tahiti (French Polynesia), the Southeast Asian Fisheries Development Center (SEAFDEC) in the Philippines, National Center for Genetic Engineering and Biotechnology in Thailand, Department of Fisheries of Malaysia, a commercial program by a French group at Aqualma from Madagascar, a research program by Commonwealth Scientific and Industrial Research Organization (CSIRO) in Australia, and a commercial project by Moana Technologies Inc, which was founded in Belgium, but operates from Hawaii. The following table provides an overview of published and non-published information of these *P. monodon* domestication programs:

Table 1. Overview of *P. monodon* domestication programs

Organization/References	Domestication characteristics
Aquacop in Tahiti (French Polynesia) /Aquacop, 1975; 1977; 1979; 1983.	Research program by French scientists. This program was successfully producing non-SPF captive stocks in earthen ponds and concrete tanks up to the third generation. Survival and broodstock performance was good.
SEAFDEC in the Philippines / Primavera and Gabasa, 1981; Primavera, 1983; 1985.	Research program by Philippino and Japanese scientists. This program produced non-SPF captive broodstock in earthen ponds. Survival and broodstock performance was very low.
National Center for Genetic Engineering and Biotechnology in Thailand / Withyachumnarnkul, 1998.	This research program attempted to produce SPF broodstock; It failed due to the limited biosecurity in earthen ponds. Survival and broodstock quality was very low.
Aqualma in Madagascar / Groumellec, 2008.	This program is carried out by a French group since 1999. It is a commercial program to produce SPF domesticated stocks with high genetic variation, intended to support *P. monodon* farming in Madagascar.
Department of Fisheries of Malaysia / Subramaniam et al., 2006.	This program reported to produce genetically improved SPF broodstock by family selection. However, results have not been commercialized yet in the international markets.
CSIRO in Australia / Coman et al., 2005; 2006; 2007b; 2007c; Preston, 2009.	This research program is carried out by Australian scientists. The complete SPF domestication program has been reported in 2005. Recently attempts have been made towards genetic improvement through a selective breeding program.
MOANA Technologies Inc / (Personal communication)	This commercial project produced SPF genetically selected stocks with a diverse gene pool of SPF parent stocks. Since 2008, Moana genetically selected SPF stocks have been commercialized in India, Thailand and Vietnam.

Closing the life cycle of *P. monodon* was first succeeded at Tahiti (French Polynesia) during the 1970's where three generations of domesticated *P. monodon* stocks were produced (Aquacop, 1977; 1979). This program started with 9 females and 4 males of wild origin, which were kept in 12-m^3 tanks with sand substrate. A F1 generation was obtained in November 1975 and an F2 generation in August 1976. Postlarvae were reared in 700-m^2 earthen ponds at a stocking density of 30-60 shrimp per m^2 until they reached 1-2 g size; they were then transferred into 2,500-m^2 earthen ponds at a stocking density of 5-30 shrimps per m^2 or to concrete tanks of 800 to 1,200m^2 at a density of 50 shrimps per m^2 where they reached commercial size (15-22 g mean weight). At harvest a few hundreds were stocked in earthen ponds at a density of 2-3 shrimps per m^2 until they reached maturation size. Daily renewal of the water varied around 10% of the total volume and various artificial pellets and fresh feeds were given to the shrimp. The breeders were then transferred into a 400-m^2 tank with a sand bottom where flow-through water was injected through perforated plastic pipes which allowed a self-cleaning action and avoided reduction of the substrate. Density was 2-4 animals per m^2 with a water depth of 2m. In this tank, animals were selected by divers and the largest and healthiest were stocked in 12-m^2 circular maturation tanks with 80 cm of water. Coral sand was used as tank substrate. 200 to 300 % of the total volume was exchanged daily through perforated plastic pipes imbedded in the sand. The water temperature fluctuated between 25-29^oC, salinity was 34-35$g.L^{-1}$ and pH was stable at 8.2. The natural light was reduced 60-90% by shading. The maturation diet consisted of fresh troca flesh and a squid-containing pellet with 60% protein given at 15% of the biomass. This domestication technology produced domesticated breeders with a fecundity varying between 60,000 and 600,000 eggs, averaging 75,000 eggs for females of 45g and 300,000 eggs for females of 140g.

Viability of the eggs was variable; some spawns were totally or partially unfertilized despite the presence of spermmatophores in the thelycum. The small size of the males was suspected to be responsible for the variation in fertilization success. The broodstock diet made from squid-meal with 60% protein gave the best output.

Another intensive research program on *P. monodon* broodstock rearing during the 70's and 80's was carried out at the Southeast Asian Fisheries Development Center (SEAFDEC) (Primavera and Gabasa, 1981; Primavera, 1983). This program produced *P. monodon* broodstock in earthen ponds. Over 1 year was needed to produce broodstocks of 80-100g. The maturation of the broodstock was then carried out in a recirculation system, but mortality of the animals in the recirculation system was very high, reaching up to 5.6% per week for males and 9.2% per week for females. The broodstock diet given to the shrimp included artificial pellets at 2% (dry weight) and fresh foods (frozen tahong, live annelids, squid, and cow liver) at 5% (wet weight) of the biomass. Broodstock shrimp produced by this technology resulted in 100,000 to 600,000 eggs per spawn with an average of 200,000, while hatching success was only between 30.5%-37.5%.

In the 1990's a research program was carried out in Thailand to close the life cycle of *P. monodon* (Withyachumnarnkul et al., 1998). Pond-reared animals were used as starting material. The program selected the 1% top-sized shrimp from commercial farms weighing around 50-70g. These shrimp were screened for specific-pathogen-free (SPF) status. The SPF shrimp was stocked in a 1,600-m^2 earthen pond at a stocking density of 4-8 animals per m^2 for 8 months until the females reached 150-180g and males reached 80-110g. Survival of the shrimp after 8 months was only 30%. Although the shrimp produced 300,000 eggs per spawn, the hatching success was only around 30%.

Another program to develop SPF black tiger domesticated stock under commercial scale started in the late 1990's in Madagascar (Groumellec, 2008). All the broodstock shrimp were sourced locally in Madagascar and the facilities were designed according to high biosecurity standards. All founding populations passed through a primary and secondary quarantine phase. All OIE (Organization of International Epizootics) listed pathogens were tested and the selected population was certified free from these pathogens. More than 10,000 wild individuals were collected along the West Coast of Madagascar from 1999 to 2002. The resulting population was established from 198 selected individuals, representing more than 95% of the genetic variability of the base population. From there, the breeding program was designed to keep as much as possible the genetic variability within this domesticated population (effective population > 300 at each generation). After the first five generations, the estimated gain in growth was estimated to be 15% per generation, integrating domestication selection, mild directional selection for growth and improvements in rearing methodology.

The Malaysian program on SPF black tiger shrimp started in 2001 and this program produced 3 generations of SPF *P. monodon* broodstock in 2006 (Subramaniam et al., 2006). The program in Malaysia started with wild collected shrimp, which passed through a primary quarantine and were screened for multiple viral pathogens including White Spot Syndrome Virus (WSSV), Gill-Associated Virus (GAV), Taura Syndrome Virus (TSV), Monodon Baculo Virus (MBV) and Hepatopancreatic Parvo Virus (HPV). The SPF stocks were then transferred to a secondary quarantine area, consisting of maturation and larval/postlarval rearing units to produce F1 progeny. Postlarvae of 15 days (PL15) were stocked in outdoor, plastic lined, broodstock grow-out ponds for mass selection and were raised to adult size by maintaining tight bio-

security conditions. For family selection, PL28 were stocked in cages till they were 2g. These 2g shrimps were tagged and released into grow-out ponds. All grow-out broodstock were screened for multiple viruses at two-month intervals until maturity. This technology produced animals of 80-100g within 9-12 months. These domesticated *P. monodon* gave on average 200,000 nauplii per spawn. Interestingly, the percentage of good spawns per day of these domesticated animals was improved from an average 1.8% per day in the wild stock to 3.3% per day in F1 and F2 stocks, while weight gain increased 7.3% in F3 as compared to F2 animals and close to 13% in F2 as compared to F1 animals.

A program to fully close the life cycle of *P. monodon* from egg to breeder in biosecure, indoor rearing systems to produce SPF broodstocks was started by the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) in the late 1990's (Coman et al., 2005; 2006; 2007b). The program from CSIRO (Coman et al., 2005; 2006; 2007b) similarly started from wild animals, which passed primary and secondary quarantines for selection of SPF broodstock to produce clean progeny for closing the life cycle of the shrimp. The SPF 1-g shrimp were stocked in $10m^3$ sand-based recirculation systems at 10 animals per m^2, which was reduced to 4.5 animals per m^2 after 5 months and then to 3.5 animals per m^2 from the 8^{th} month. The program considered the first 8 months of the life cycle as grow-out phase and from 8 months to 11.5 months as maturation phase, after which the animals could then be used for hatchery work. In the grow-out phase the animals were given a diet consisting of 20% squid, 5% bivalve and 70% high protein pellets, while the maturation diet was composed of 30% squid, 20% bivalve, 5% marine worm (polychaete) and the remaining pellets. The animals were reared in water of 27.3 ± 0.9^oC, salinity of 29 ± $3g.L^{-1}$, alkalinity was 80-140$mg.L^{-1}$, while ammonia NH_4^+ <

0.7mg.L^{-1} and nitrite NO_2-N < 5mg.L^{-1}. This technology resulted in very good survival (up to 80%) of the shrimp after 11 months in the indoor sand-based recirculation system. However, the fecundity and egg hatching success of the animals were much lower as compared to other programs. The 11-month old females of 117g produced only 129,780 ± 17,700 eggs per spawn with a hatching success averaging 31.6%. Recently, domestication of *P. monodon* in biosecure, controlled environments has led to progress in the selective breeding of *P. monodon* in Australia, where selectively bred families had higher nauplii production and higher production yield (Preston, 2009).

Since 2000, the Moana project has been able to create a domesticated *P. monodon* shrimp line and set up a SPF production program through successful selective breeding of the species (Suzy Horemans, manager Moana Vietnam, personal communication). Today, Moana has already a 7^{th} generation grand-parent stock which is SPF for all important diseases. This genetically selected SPF stock has been commercialized in India, Thailand, and Vietnam and the grow-out performance of this stock indicates an improved growth. According to Suzy Horemans, trials in Vietnam produced 30g animals in approximately 130 days rearing for Moana postlarvae versus 160 days for wild postlarvae, while this was 120 days versus 140 days in trials in India, respectively. The trials in Thailand with Moana postlarvae were similar as in Vietnam, obtaining 30g shrimps after 130 days rearing.

In conclusion of the various research programs on domestication of *P. monodon* some remarkable similarities can be drawn:

1) Strict screening for viruses, passing the shrimp through a primary and secondary quarantine, as well as application of strict hygiene and biosecurity measures are necessary for good growth and survival of the SPF shrimp.

2) Closing the life cycle of *P. monodon* is possible when starting with SPF stocks and applying good biosecure conditions. Indoor closed sand-based recirculation systems are suitable for maintaining high biosecurity as well as to provide stable and good environmental conditions (dissolved oxygen, ammonia, nitrite, etc.) for grow-out and maturation rearing of *P. monodon*.
3) The different phases in the life cycle of *P. monodon* should be respected and the rearing conditions and diet adjusted accordingly in order to provide a suitable rearing environment for each stage.
4) High protein pellets and a diversified fresh-food mixture provide a suitable diet for the grow-out phase of *P. monodon* during domestication in captivity. A stable pH at 8.2 and high salinity (34-35 $g.L^{-1}$) together with an alkalinity controlled between 80 to 140 $mg.L^{-1}$ through periodical addition of sodium bicarbonate to the rearing tanks provides good environmental conditions. In addition light intensity in the maturation tanks should be reduced up to 90% of the ambient daylight through shading for successful maturation and spawning of *P. monodon*.
5) Although high protein pellets and a diversified fresh-food mixture were adequate for grow-out of *P. monodon* from egg to breeder of 100g, low performance of the domesticated broodstock in terms of natural mating, fecundity and hatching success still remains a bottleneck for the commercialization of domesticated *P. monodon* stocks. More broodstock nutrition studies are therefore needed to improve the quality of the domesticated broodstock.
6) Domestication in conjunction with selective breeding resulted in considerable improvement of growth of domesticated *P. monodon* through succesive

generations (7.3-13% in the Malaysian program and 15% in the program in Madagascar).

It remains unclear why reproductive performance of domesticated *P. monodon* stocks was lower as compared to their wild counterparts, especially in terms of natural mating success, fecundity and hatching success, and why domestication of *P. monodon* was more difficult than for *L. vannamei*. In order to help explain this and formulate ways to improve domestication techniques in the future, in the following sections, several factors that affect maturation and reproduction of penaeid shrimp in general, and *P. monodon* in particular, are discussed in more detail:

2.1 Environmental conditions

Several studies cited by Chamberlain (1985) indicated the importance of environmental and seasonal factors in shrimp maturation and reproduction. In the marine environment, penaeid shrimp often migrates to species-specific depth zones (Perez-Farfante, 1969). Therefore, parameters such as water hydrostatic pressure, light intensity and spectrum related to depth could affect the reproductive biology of penaeid shrimp. Hydrostatic pressure has been documented to affect the behaviour of planktonic decapod larvae, but rarely affecting benthic adults (Hardy & Bainbridge, 1951; Knight-Jones & Morgan, 1966). It was also noted that some penaeid shrimp can spawn naturally without eyestalk ablation in shallow water conditions or in race-way systems. Therefore it seems unlikely that hydrostatic pressure plays an important role in reproduction. Light intensity and light spectrum are considered two factors related to depth that affects penaeid reproduction. These two factors are related to cloud cover and diurnal and seasonal variation in illumination and to variations caused by dissolved and suspended matter and may account for fluctuations in spawning of wild shrimp (Chamberlain, 1985). Considerable evidence suggests sensitivity of adult

decapods to both light intensity and spectrum (Herman, 1962; Segal, 1970; Goldsmith, 1972). Studies by Chamberlain and Lawrence (1981) and Wurts and Stickney (1984) on *Litopenaeus vannamei* and *Penaeus stylirostris* shrimp indicated positive effects of blue or green light with intensity less than 12 μWcm^{-2} on maturation and spawning. Rearing water used for domestication of *P. monodon* is usually clear because of sand filtration. High light intensity inhibits maturation in penaeid shrimp and is stressful to the broodstock, therefore a dark environment created by the reduction of light intensity is essential for *P. monodon, L. vannamei* and *P. japonicus* broodstocks and even within the same species the optimum light intensity for maturation tank might slightly differ for each population caused by differences in depth and turbidity conditions (Yano, 2000). According to Primavera (1983, 1985) reduced light intensity by covering the tanks speeded up the maturation process and prevented stress on black tiger breeders. Emmerson (1980) indicated the black coloured tanks resulted in two times more spawns as compared to bright colour tanks.

Seasonal peaks in reproduction are characteristic of penaeids, even in tropical species which may exhibit some degree of reproductive activity throughout the year (Lindner & Anderson, 1956; Subrahmanyam, 1965; Rao, 1968; Pillay & Nair, 1971; Badawi, 1975). Annual spawning peaks, which are synchronized within a species and often variable among species, are probably entrained primarily to photoperiod and/or temperature (Giese, 1959). However, in tropical areas where annual variations in photoperiod and temperature are minimal, salinity may serve as the seasonal reproductive cue; particularly if pronounced wet and dry seasons exist (Pillay & Nair, 1971). Aragón (2007) studied the relationship between water temperature and spawning season of *Penaeus stylirostris* in a lagoon in California and found a

spawning peak from May to August when the temperature of the surface-water was higher and less variable. Adult *P. monodon* migrate to the deep sea for maturation and spawning where environmental parameters such as salinity, pH, temperature, ammonia and nitrite are known to be very stable and/or low, which reduces stress as well as provide a quite environment for inducing maturation and spawning. Similar environmental conditions should be provided to captive breeders. According to Ruangpanit et al. (1984), *P. monodon* caught from their spawning grounds in the Indian Ocean where salinity was 33g.L^{-1} resulted in better maturation and egg fertilisation as compared to shrimp caught from Songkhla lake where the salinity was 22-28g.L^{-1}. High salinity and pH similar to open ocean must be maintained for maturation; however, lower salinity (15-25 g.L^{-1}) seems best to stimulate growth in the grow-out phase (Yano, 2000; Queensland Department of Primary Industries and Fisheries, 2006). Maintaining pH stable at around 8.2 by periodically adding sodium bicarbonate into the tanks is considered good for maturation, spawning and hatching success (Primavera, 1985). An environment with stable water temperature, low concentrations of ammonia and nitrite, etc. therefore should be created for successful domestication of *P. monodon.* Closed systems with application of recirculation technology are considered to meet these requirements.

2.2 Substrate and stress

Adult penaeid shrimp migrate from coastal areas to deeper sea for maturation and for natural mating and spawning. Specific depths and substrate types are preferred as spawning grounds, which largely differ from species to species. The shrimp *Penaeus duorarum* prefers depths of 26-60m and a sandy mud bottom containing more than 50% fine sediment (<50µm) with a high organic content (Garcia and L'home, 1980), while *P. monodon* inhabits depths of up to 160m, where white sand was found most

suitable for this species (Motoh, 1985). Most successful domestication programs for *P. monodon* used sand as substrate for the rearing tanks. Survival of the shrimp in those programs using sand substrate was very high (Aquacop, 1979; Coman et al., 2005). Similar results were obtained when artificial substrate such as Aquamat® was added (Arnold et al., 2006). Shrimp broodstock need a quiet environment, without excess food, noise, or human disturbance (Yano, 2000). Therefore, maturation tank rooms should be isolated completely from other rooms. Maturation of shrimp is also strongly affected by stocking density because high stocking density causes poor water quality, e.g. high ammonia levels, and high levels of stress due to the behavioural interaction of the shrimp (Yano, 1993).

2.3 Nutrition

In contrast to white shrimp *L. vannamei,* only recently progress has been made to unravel the nutritional requirements of *P. monodon*. A high protein level of 40-50%, a lipid level of 4-11%, 1% cholesterol and 0.5-1% n-3 high polyunsaturated fatty acids in the diet are thought to maintain optimum growth (Shiau, 2008). Higher lipid levels (7-14%) might be required in broodstock diets (Meunpol, 2005b) as ovarian lipid content progressively increased through maturation stages and lipids of maturing females accumulate in the hepantoceas is known to be transferred to the ripening ovaries and finally to the eggs (Millamena et al., 1993). For broodstock diets of *P. monodon* a protein level of at least 50% may be required (Marsden et al., 1997). In some domestication programs the broodstock pellet for *P. monodon* even contained up to 60% protein (Aquacop, 1979). High dietary protein levels normally support growth rather than maturation. The high protein requirement of *P. monodon* breeders is therefore species specific or it may indicate a specific requirement for specific amino acids or other associated nutrients (vitamins, carotenoids, etc.) needed for the

maturation and reproduction. The high protein requirement of *P. monodon* was not taken into account in several of the early domestication attempts of *P. monodon.* This could be a reason for the high mortality and low growth of the species in these studies. In commercial grow-out ponds, *P. monodon* can grow and develop well on grow-out pellets containing only 36-40% protein; however in these, besides the pellets provided by the farmer, the animals feed on other feed sources such as algae, bacterial substrates or other organisms available in the pond. In contrast these extra protein sources are not or only in very limited amounts available in indoor rearing systems, especially in clear water systems. Therefore, high protein diets should be provided to *P. monodon* in indoor rearing system. From the early studies, only the domestication program of Aquacop used a pellet feed with high protein level (60%). Results of this program were the best in the 1970's. The more recent domestication program at CSIRO also fed the shrimp a high protein pellet (55%). This resulted in a very good growth and survival after 11 months (up to 80% survival and the animals reached 120g within 11 months). However, these research programs still encountered poor performance of the domesticated broodstocks in terms of fecundity and egg hatching success. The large fraction of grow-out pellets in the diet (70% until month 8 and 45% during the maturation phase from month 8 to month 11) together with the incompletely shading of the maturation tanks were however suspected to be responsible for the low fecundity and egg hatching results in the CSIRO program.

Improper nutrition is known to prevent shrimp gonad maturation (Aquacop, 1977) and perhaps even affect larval viability. According to several studies cited by Chamberlain (1985), the chief component of crustacean egg yolk is a high-density, lipoglycoprotein, termed crustacean lipovitellin, which contains 27-35% lipid, a small carbohydrate component, and no protein-bound phosphorus (Zagalsky, 1976). The

lipid component consists largely of phospholipids (usually containing high levels of medium-length, monounsaturated fatty acids), a carotenoid, and small quantities of cholesterol and triglycerides. The source of extra-ovarian lipovitellin, which is found only in the hemolymph of females with maturing oocytes, is generally assumed to be the hepatopancreas, although it has been found there only in barely detectable quantities (Fyffe and O'Connor, 1974). Chamberlain and Lawrence (1983) found that the hepatopancreas of *Penaeus setiferus* and *Penaeus aztecus* stores a relatively minor proportion of the nutrients needed for ovarian development; therefore, most of the nutrients are drawn from immediate food intake.

Kanazawa (1985) reviewed fatty acid requirements of penaeid shrimp and emphasized the importance of highly unsaturated fatty acids (HUFA) in increasing weight gain of shrimp. The nutritive value of lipids for prawn and shrimp is related to the type and content of essential fatty acids (EFA) such as ω3-HUFA. The review pointed out the inferior quality of soybean oil (rich in 18:3ω3), possibly due to low levels of ω3-HUFA such as 20:5ω3 and 22:6ω3. The fatty acid composition of the lipids has also been shown to affect the reproductive performance of shrimp (for a review see Harrison, 1990; Wouters et al., 2001). In an experiment carried out by Millamena et al. (1985) with 4 distinct diets, the diet with higher PUFA content and ω3/ω6 ratio and a fatty acid profile closely resembling that of maturing ovaries of wild *P. monodon*, gave higher reproductive performance (number of spawns, fecundity, nauplii production and hatching rate) compared to the other diets. The characteristically high levels of PUFA in mature shrimp ovaries and presence in spawned eggs are indicative of their metabolic and physiological importance in penaeid shrimp reproduction (Millamena et al., 1993).

It has been proposed that prostaglandins (PGs) play an important role in egg production and spawning of freshwater snails and bivalves (Kunigelis and Saleudin, 1986; Matsutani and Nomura, 1987; Osada and Nomura, 1990). Some researchers (Spaziani et al., 1993, 1995; Sagi et al., 1995) reported that PGs are related to vitellogenesis and spawning in decapod crustaceans. Tahara and Yano (2004) reported that ovarian prostaglandins and arachidonic acid (ARA) are involved in ovarian maturation of kuruma prawn (*Marsupenaeus japonicus*). ARA and EPA are essential components of cell membranes and are precursors for 2-series and 3-series PGs, respectively, while DHA is known to be important for development of the central nervous system in crustaceans (Xu et al., 1994).

Diets which have been found suitable for reproduction of shrimp in captivity were all composed entirely or partially of a diversified mixture of natural food organisms. Sometimes the combination with a dry pellet feed produced better reproductive results (Chamberlain, 1985). Of all natural food organisms used, marine worms and squid are the most successful for maturation and spawning of penaeid shrimp (Yano, 2000; Wouters et al., 2001; Meunpol, 2005b). Why these natural organisms promote maturation and spawning of penaeid shrimp is not fully understood. Several studies, supported by analytical data of lipid classes and fatty acids of these organisms, indicate they are a rich source of ω3-HUFA and PUFA fatty acids, especially EPA, DHA and ARA (Primavara, 1985; Marsden et al., 1992, Wouters et al., 2001; Racotta et al., 2003). It has been proven that marine worms (polychaetes) are critical to successful nauplii production in *L. vannamei* (Browdy, 1992) through female ovarian promotion (Bray and Lawrence, 1992). Polychaetes are not only a source of HUFA and contain high levels of ARA (Meunpol, 2005a) but also possibly a source of other hormonally active compounds (Lytle et al., 1990). Recently, another study (Huang et

al., 2008) indicated ARA content of eggs was highly correlated with fecundity and egg production. It is also believed that EPA competes with the synthesis of enzymes that produce prostaglandins from ARA; resulting in EPA having a modulating influence over the quantity and efficacy of ARA-derived prostaglandins (Furuita et al., 2003). The important roles of ARA, EPA and DHA therefore should be taken into account for the formulation of broodstock shrimp diets to improve maturation and reproductive performance, especially fecundity and egg hatching success are still the bottlenecks to the commercialization of domesticated *P. monodon* breeders. It is generally accepted that some vitamins are also important for shrimp maturation but knowledge of optimum amounts and the role of these vitamins in shrimp maturation is still fragmentary. Formulated diets containing pollack liver oil (12.8% EPA) with a high amount of vitamin E were effective in inducing and accelerating maturation and spawning and increasing larvae production in *P. monodon* held in tanks (Yano et al., 1996).

2.4 Male effects and genetic considerations

A study on maturation of male *P. monodon* shrimp was carried out by Makinouchi and Hirata (1995). These authors indicated relatively higher natural mating when using wild males (66.7%) as compared to pond-reared males (32.4%). Hatching success of the eggs was also higher when using wild males (18.5%) than when using pond-reared males (6.6%). According to Parnes et al. (2007), low natural mating of penaeid shrimp in captivity was due to the absence of sufficient ready-to-mate males. Reduction in reproductive performance of captive male shrimp results from a number of reasons, including stocking animals in captivity for a long period of time (Chamberlain et al., 1983; Leung-Trujillo & Lawrence, 1987) and/or inappropriate maturation diets (Meunpol, 2005b). In addition, age and size of the male shrimp could

also be a factor affected hatching success. Jiang et al., (2009) found a positive correlation for gonad weight, spermatophore weight, sperm count, and percentage of normal sperm to body weight and age of *P. monodon* male shrimp.

Genetic effects such as a decrease in broodstock quality due to inbreeding have been considered one of the main reasons for inferior performance of pond-reared penaeid shrimp (Sbordoni et al., 1986). Inbreeding is an important consideration when starting up a selective breeding program and one of the first reasons to natural resource preservation. Inbreeding is also one of the factors reducing fitness and production traits in population (Keller and Waller, 2002; Kim et al., 2007). Chow and Sandifer (1991) indicated that different results from different shrimp hatcheries were due to different genetic sources of shrimp broodstock used in the hatcheries. According to Coman et al. (2005), there were significant variations in the growth and reproductive performance among families, ages, and environmental conditions (tank/raceway systems) in the Australian domesticated *P. monodon* stocks. Goyard et al. (2008) suggested that cross breeding between different lines of domesticated stocks is the easiest way for genetic improvement. Recently, studies on genetics indicated a very good heredity on reproductive traits for penaeid shrimp and this could open a good outlook to detect reproductive genes for marker assisted selection in future shrimp selective breeding programs. A number of these studies are summarized as below:

- Arcos et al. (2004) found very high heredity in white shrimp *L. vannamei* for vitellin content of oocytes (h^2=0.47) and number of days from ablation to first spawning (h^2=0.54).
- Arcos et al. (2005) found a very high heredity in white shrimp *L. vannamei* for oocyte diameter (h^2=0.57) and oocyte maturation (h^2=0.71).

- Macbeth et al. (2007) found a very high heredity in *P. monodon* for days from ablation to first spawning (h^2=0.47), number of eggs (h^2=0.41) and nauplii production (h^2=0.27).

In shrimp hatchery settings, it is well known that a large percentage of the females never spawns or spawns only once, whereas a smaller percentage of the females is able to spawn multiple times (Bray et al., 1990; Wyban and Sweeney, 1991; Cavalli et al., 1997; Palacios et al., 1999). Ibarra et al. (2005) suggested that selection for high-performance broodstock shrimp using the trait of multiple spawning along with traits of egg quality could be an important strategy to improve nauplii production.

2.5 Endocrinology

Sources of hormones involved in penaeid reproduction are the medulla terminalis X organ of the optic ganglia, sinus gland, brain and thoracic ganglion, androgenic gland, ovary, and Y organ. Eyestalk-ablation removes the complex of X organ and sinus gland and eliminates the hormone GIH (Gonadal Inhibiting Hormone) and therefore speeds up the maturation of penaeid shrimp. Desai and Achutthankutty (1997) found a regeneration phenomenon of the eyes after eyestalk-ablation in *P. monodon* and this process gradually increased GIH levels again. This could explain why lower fecundity is often observed for later spawns as compared to the first spawns. According to Primavera (1985), ovarian development of penaeid shrimp might already start earlier during the life cycle in an estuarine environment; however, full maturation and spawning only occurs in deeper sea. Therefore, Primavera (1985) suspected that the migration from shallow water to deeper sea with variations in environmental parameters could reduce GIH in penaeid shrimp. Studies attempting to administer hormones through the food to penaeid shrimp didn’t seem to be effective. This could

probably be attributed to the peptide nature of the hormone, which makes it easily hydrolyzed by enzymes. Penaeid shrimp are very sensitive to stress and therefore repeated administrations of different hormones through injection into the hemolymph of penaeid shrimp is still in experimental stages. Apart from the type of hormone, also the time and the methodology to inject the hormones should be taken into account. With respect to the molting cycle, shrimp prove very sensitive to hormone injection just before or after molting because the shell is still soft and animals are easily injured during this time; therefore, injection of hormones should wait until the new shell has become hardened (normally 4 days after molting). Recently, studies on hormone application in shrimp indicated Methyl Farnesoate (MF) and Serotonin (5-HT) resulted in positive effects when injected into penaeid shrimp. Alfaro et al. (2008) administered Juvenile Hormone (HJ III) and MF to white shrimp *L. vannamei* and found that MF significantly improved ($P<0.05$) sperm quantity with a lower ratio of abnormal sperm, while HJ III treatment was comparable to the control treatment ($P>0.05$). Serotonin administered at 50µg/g body weight improved ovarian maturation, spawning ratio, hatching success and nauplii production of black tiger shrimp (Wonprasert et al., 2006).

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CHAPTER III

REARING SYSTEM

Chapter III

Effect of water exchange, salinity regime, stocking density and diets on growth and survival of domesticated black tiger shrimp *Penaeus monodon* (Fabricius, 1798) reared in sand-based recirculating systems

Nguyen Duy Hoa[1,3], Greg John Coman[4], Mathieu Wille[3], Roeland Wouters[2], Nguyen Quoc Hung[1], Vu Thanh[1], Tran Kim Dong[1], Nguyen Van Hao[1], Patrick Sorgeloos[3].

Research Institute for Aquaculture No.2, Ho Chi Minh City, Vietnam[1].

INVE Technologies N.V., Dendermonde, Belgium[2].

Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium[3].

Commonwealth Scientific and Industrial Research Organisation, Food Futures Flagship, Division of Marine and Atmospheric Research [4]

Abstract

The effect of water exchange, salinity regime, stocking density and diets on growth and survival of domesticated *Penaeus monodon* juveniles was evaluated in two independent experiments. In the first experiment, 1g-domesticated *P. monodon* juveniles were stocked at 10 animals per m^2 in 1-m^3 tanks to test two feeding regimes (mono diet of high protein pellets and a combination diet of high protein pellets and a fresh-food mixture) and two rearing systems (a sand-based recirculation system with low water exchange; and a sand-based system with high rates of flow-through water). In the second experiment, performance of the animals was assessed when reared under 2 different water salinities and 2 stocking density treatments.

Results of the two experiments indicated that the sand-based recirculation system with a water exchange regime at a rate of 5-10% a day and a combination diet of high protein pellets (55%) and a fresh-food mixture consisting of 75% squid, 15% oyster and 10% blood cockle were suitable for the indoor rearing of the grow-out phase of *P. monodon*. Water salinity of 20-23g.L^{-1} and low stocking density of 10 animals per m^2 resulted in the best growth of the shrimp. Survival of the *P. monodon* in the grow-out phase was significantly higher ($P<0.05$) at low salinity of 20-23g.L^{-1} as compared to high salinity of 32-33 g.L^{-1} irrespective of stocking density.

Keywords: recirculation, domestication, diet, shrimp

1. Introduction

The black tiger shrimp (*P. monodon*) is one of the most suitable and important indigenous shrimp species for cultivation in Vietnam (Trong & Hoa, 2007) and elsewhere in the Asia-Pacific region. A major constraint to the sustainability of the

black tiger shrimp farming industry has been the difficulty in closing the life cycle of this species from egg to breeders in indoor (biosecure) rearing systems to allow production of specific-pathogen-free domesticated stocks. Using high quality rearing systems and providing optimal nutrition are considered to be the most important aspects for successful maturation and domestication of penaeid shrimp (Chamberlain, 1985; Primavera, 1985; Woo, 1988; Yano, 2000). Recirculation systems suitable for short-term (around 3 months) maturation of penaeid shrimp in captivity have been around since the 70's (Aquacop, 1975; Beard and Wickins, 1980; Primavera, 1983; Menavesta, et al., 1991). However, the development of suitable sand-based static-water/recirculating or flow-through water systems for rearing shrimp from postlarval or juvenile ages through to broodstock ages (i.e. at least 12 months) has been a key component in efforts to close the life cycle of *P. monodon* in Australia over the past 10 years (Coman et al., 2005). Considerable research efforts have been focused on improving the reproductive performance of the *P. monodon* broodstock produced in these systems, especially fecundities and egg hatching success (Coman et al., 2005; 2006; 2007b); and improvements in reproductive performance are now being realised due to dietary and environmental improvements and via generational advancements of the stocks (Coman et al., 2007c). However, there is still significant scope to further improve diets and refine environmental conditions (e.g. salinity) to improve the quality of the domesticated *P. monodon* broodstock produced in these systems; and to develop a standardized protocol for each phase of the domestication process in these indoor recirculation systems.

In the natural environment, juvenile *P. monodon* inhabit brackish water areas as nursery grounds (for a period up to 4 months) whereas sub-adults inhabit estuarine or inner littoral areas before migrating to deeper coastal waters, while adults inhabit

offshore areas up to a depth of about 160m (Motoh, 1985). Consequently, juvenile shrimp would be exposed to very different environmental conditions as compared to later stages. In these natural environments, *P. monodon* would encounter and feed on a diversity of organisms. Logically, such a diversity of food components would provide complete nutrition for the shrimp and provide for high health and fast growth as compared to shrimp fed on only a single component. Several studies on broodstock nutrition have confirmed the advantages of nutritional diversity on reproductive outputs of penaeid shrimp (Primavera et al., 1979; Chamberlain & Lawrence, 1981; Bray et al., 1990). However, similar studies of food diversity have not been conducted for the juvenile stages of *P. monodon*.

The present study evaluated the effects of several variables, including water-exchange regime; salinity regimes and stocking density, and food component diversity in the diets on performance of *P. monodon* juveniles during the initial grow out phase, when reared in indoor sand-based recirculation tank systems. The study aimed to identify the most suitable protocol for the initial phases of rearing for domesticating *P. monodon* in indoor recirculation tank systems.

2. Material and methods

2.1 System design

A recirculating system was designed with agricultural (AG) pipes and sand substrate. Water is recirculated on the basis of airlift mechanism (Fig. 1). “The sand substrate system” was based on the system detailed in Crocos and Coman (1997). The system consisted of a combination of slotted and un-slotted coils of AG pipes on the bottom of the tank. The slotted and un-slotted pipes were coiled in parallel, but with each starting from the opposite side of the tank. The two coils were joined in the middle of

the tank by a central polyvinyl chloride (PVC) joiner. On the outside of the tank, the slotted pipe was connected to a “pusher” standpipe, and the un-slotted coil to a puller standpipe.

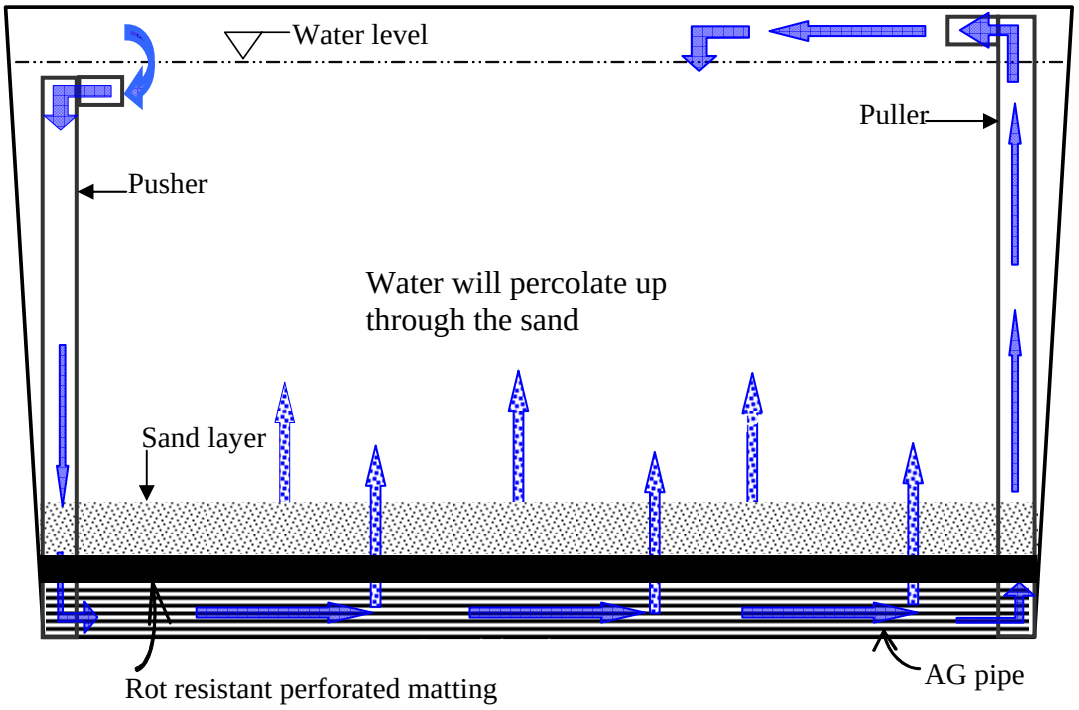

Figure 1. Design of sand-based recirculation tank

The puller “pulls” water from the centre of the coils and puts it back into the tank via the un-slotted pipe. The pusher “pushes” the rearing water from the above of sand layer through the coils and towards the central joiner via the slotted pipe. The functioning of the systems of AG coils and the standpipes is to circulate aerated seawater beneath the sand. Aerated water from the slotted pipe percolates up through the sand as it moves towards the centre of the tank, keeping the sand oxygenated.

For the sand-based system with high rates of flow-through water, a piece of 20-cm PVC pipe (34-mm diameter) was used as the out-flow stand pipe at the center of the tank that connected with a PVC pipe outside of the tank allowing a means to control tank water height within the system as indicated in the Fig. 2.

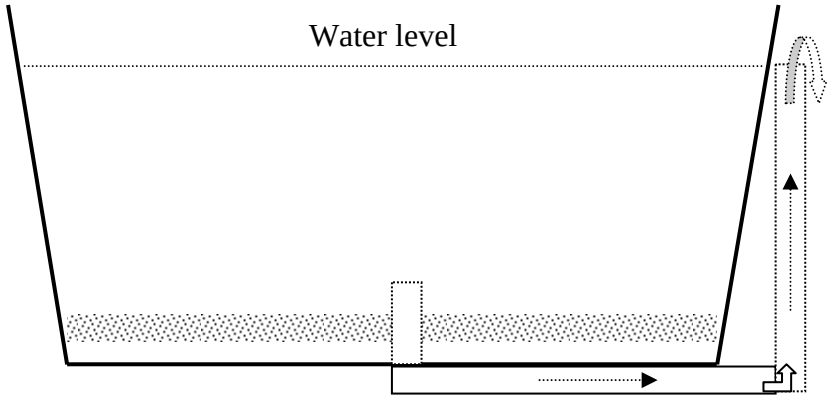

Figure 2. Design of Sand-based system with flow-through

2.2 Experimental design

2.2.1 Experiment 1

12 circular 1-m^3 tanks were installed as sand-based systems as described above. Two rearing systems were used in the experiment, with 6 tanks allocated for sand-based recirculation system with low water exchange at 5-10% a day (system R) to compensate the water lost due to daily siphoning of uneaten feed, and the other 6 tanks allocated for sand-based system with top-down flow-through water at 200% a day (system F). For each rearing system, 3 tanks were allocated for a mono diet of high protein commercial pellets (Lucky Star, Taiwan Hung Kuo Industrial Co., Ltd; 55 % protein) (diet L) and the other three tanks were allocated for a combination diet (diet C) of 50% high protein commercial pellets and 50% fresh-food mixture [75% squid (*Photololigo* sp.), 15% oyster (*Crassostrea* sp.) and 10% blood cockle (*Anadara granosa*)]. Therefore, a 2x2-factorial completely randomized design involving 4 treatments with 3 replicates per treatment was carried out. Treatment 1 included 3 tanks for system R and shrimp fed with 100% diet L (RL), Treatment 2, included 3 tanks for system R and shrimp fed with diet C (RC), Treatment 3, included system F and shrimp fed with 100% diet L (FL) and Treatment 4 included system F and shrimp fed with diet C (FC).

Domesticated *P. monodon* juveniles of 1g in average body weight were randomly stocked into the experimental tanks at 10 animals per m^2. Shrimp were fed ad-libitum 6 times a day (7:00, 10:00, 13:00, 17:00, 20:00, and 23:00). Uneaten feed were siphoned out two times a day (at 6:00 and 16:00). Full strength seawater (32-33g.L^{-1}) was used to rear the animals and water temperature was maintained between 28 to 30.5 ^oC during the experiment. The 12 tanks were covered by transparent plastic sheets during the whole period of the experiment. The experiment ran for 126 days.

2.2.2 Experiment 2

Due to their performance in experiment 1, diet C and the low water exchange recirculation system were selected to use for experiment 2. However, water recirculation rate was adjusted from 280% a day in experiment 1 to 400% a day in experiment 2.

20 circular 1-m^3 tanks were installed as sand-based recirculation systems as above. Two salinity regimes were applied to rear the animals, with 10 tanks at 20-23g.L^{-1} and the other 10 tanks at 32-33g.L^{-1}. For each salinity regime, 5 tanks were allocated for low stocking density at 10 animals per m^2 and the other 5 tanks were allocated for high stocking density at 20 animals per m^2. Therefore, a 2x2 factorial experiment in a completely randomized design involving 4 treatments with 5 tanks as 5 replicates per treatment was carried out. Treatment 1 was low salinity water and low stocking density (LSLD); Treatment 2 was low salinity and high stocking density (LSHD); Treatment 3 was high salinity water and low stocking density (HSLD); and Treatment 4 was high salinity water and high stocking density (HSHD). Again as in experiment 1, domesticated *P. monodon* juveniles of 1g in average body weight were randomly stocked into the experimental tanks and all feeding and husbandry management followed the protocols described in experiment 1. The experiment ran for 67 days.

2.2.3 Performance parameters

At the end of the experiments, all shrimp from each tank were harvested and individually blot-dried with a towel and weighed (wet weight) to obtain a final mean harvest weight; with each tank being considered as an experimental unit (i.e. treatment replicate). Shrimp growth was expressed as the mean (± SE) wet weight of the shrimp within each treatment at harvest. Mortalities of shrimp in the tanks were monitored daily, and the final numbers of shrimp remaining in each tank at the end of the experiments was used as the measure of survival. Growth and survival data were analysed by two-way ANOVA. Where interactions were found between main effects, the data were reanalysed at each level of the main effects using the student T-test. Where no interaction was found, significant differences were identified for the main effects, and treatment means were compared using Tukeys pairwise comparison. Dissolved oxygen, pH, alkalinity, ammonia and nitrite were measured once a week in experiment 1, and ammonia and nitrite were measured biweekly in experiment 2.

3. Results

3.1 Experiment 1

3.1.1 Water quality

Dissolved oxygen mostly remained between 6-7mg.L^{-1} in low water exchange recirculation system (R) for both dietary treatments (RL and RC), while it fluctuated between 4-6 mg.L^{-1} in flow-through water system (F) for both dietary treatments (FL and FC). The pH was very similar between R and F rearing systems, ranging from 7.3 to 8.0, but was more stable in the R system than in the F system (Fig. 3b). Alkalinity declined over time in both rearing systems, gradually decreasing from 100mg.L^{-1} at

the beginning of the experiment to 60mg.L^{-1} at the end of the experiment. However, in the R system the decline was more rapid, with alkalinities remaining high between 80-100mg.L^{-1} during the first 6 weeks of the experiment, but then decreasing down to 70 and then 60mg.L^{-1} after this period. In the F systems, alkalinities remained high between 80-100mg.L^{-1} during the first three months, before starting to decrease down to 70mg.L^{-1} (Fig. 3a).

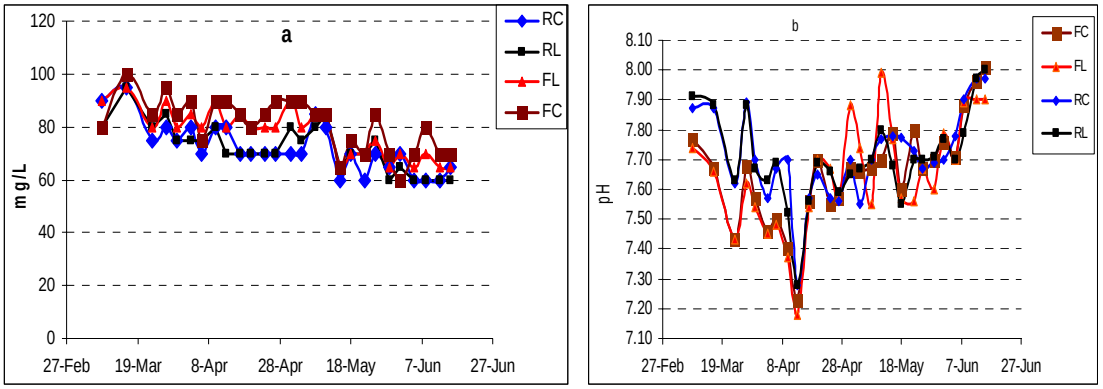

Figure 3. Alkalinity (a) and pH (b) values in low water exchange recirculation systems and in flow-through water systems. Experiment 1.

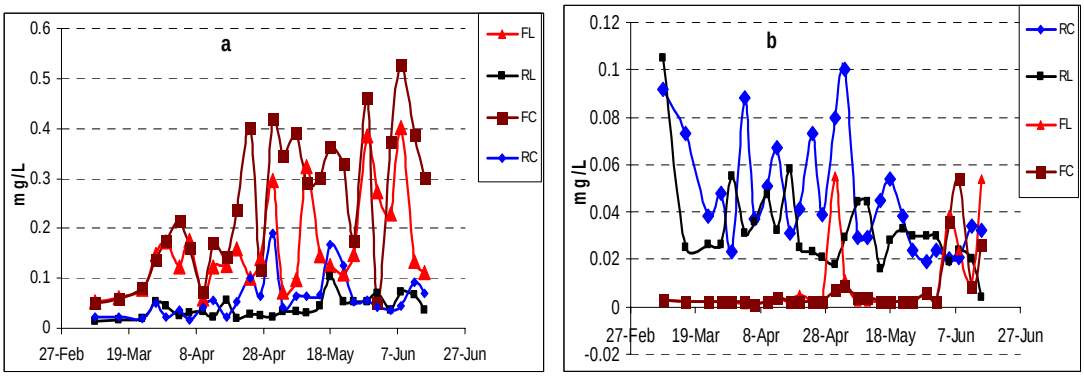

Figure 4. Total ammonia (a) and nitrite (b) concentration in low water exchange recirculation systems and in flow-through water systems. Experiment 1.

Trends in ammonia and nitrite concentrations differed between R and F systems. The total ammonia concentration fluctuated highly between 0.1-0.4mg.L^{-1} in the F system,

while it was very stable below 0.1mg.L^{-1} in the R system. In addition, ammonia concentrations were much more stable in diet L treatment than in diet C treatment (Fig. 4a). In contrast, trends in nitrite concentration were opposite to ammonia, with much lower nitrite concentrations in the F system (<0.02mg.L^{-1}) as compared to the R system (fluctuating between 0.02 and 0.08mg.L^{-1} but mostly below 0.06mg.L^{-1}) (Fig. 4b). All these ammonia and nitrite concentrations remained within the acceptable range for *P. monodon* (Chen et al., 1990; Chien, 1992).

3.1.2 Survival and growth of the shrimp

Analysis of variance indicated significant differences (P<0.01) for mean weight of the shrimp between rearing systems and between dietary treatments. Rearing systems also had a significant effect (P<0.01) on survival of the shrimp, while dietary treatments did not have any effect (P>0.05) on survival. The rearing system x diet interaction term was not significant (P>0.05) for growth or survival parameters, indicating the effect of rearing system and diet was acting independently on shrimp performance (Table 1).

Growth was significantly greater (P<0.05) in the low water exchange recirculation system, with mean weight of the shrimp averaging 36.31 ± 1.13g across dietary treatments as compared to 27.96 ± 1.53g across dietary treatments for the flow-through water system. The mean weight of the shrimp was also significantly greater (P<0.05) when fed on diet C (34.40 ± 1.80g) as compared to diet L (29.87 ± 2.30g) when averaged across rearing system treatments (Table 2). Shrimp survival was significantly greater (P<0.05) in low water exchange recirculation systems (80 ± 4.47%) as compared to flow-through water system (55 ± 4.28%). Dietary treatments had no significant effect (P>0.05) on survival, averaging 68.33 ± 4.77% for diet C and 66.67 ± 8.82% for diet L (Table 2).

The growth of the 1-g juvenile shrimp, over a period of 126 days, averaged 0.29 $g.day^{-1}$, 0.27$g.day^{-1}$, 0.24 $g.day^{-1}$ and 0.19 $g.day^{-1}$ respectively for the treatments of low-water exchange recirculation system and diet C (RC); low water exchange recirculation system and diet L (RL); flow-through water system and diet C (FC); and flow-through water system and diet L (FL).

Table 1. Mean squares, significance levels and percentage of variation explained by analysis of variance of the average weight and survival of the shrimp after 126 days reared in low water exchange recirculation system or in flow-through water system, and shrimp fed with 100% Lucky Star pellets (L) or combination diet (C). Experiment 1.

Source		Df	Mean square	% MS[a]
Mean weight	Rearing system	1	209.00	73.32**
	Diet	1	61.38	21.53**
	Rearing system x Diet	1	9.97	3.50
	Error	8	4.71	1.65
	R^2	0.88		
Survival	Rearing system	1	1875	84.91**
	Diet	1	8.33	0.38
	Rearing system x Diet	1	208.33	9.43
	Error	8	116.67	5.28
	R^2	0.69		

[a]: Based on total mean squares of all effects

*: Significant at $P < 0.05$, **: Significant at $P < 0.01$, ***: Significant at $P < 0.0001$

Table 2. Mean (±SE) average weight and survival of *P. monodon* reared from 1g for 126 days in low water exchange recirculation system (R) or in flow-through system (F), and shrimp fed on combination diet (C) or 100% Lucky Star pellets (L). Experiment 1.

Measure	Diet	Rearing system		Means for dietary treatments
		System R	System F	
Mean weight (g)	Diet C	37.66 (2.10)	31.14 (1.10)	**34.40 (1.80)^{A}**
	Diet L	34.96 (0.45)	24.79 (0.67)	**29.87 (2.30)^{B}**
	Means for rearing system	**36.31 (1.13)^{a}**	**27.96 (1.53)^{b}**	
Survival (%)	Diet C	76.67 (3.33)	60 (5.77)	**68.33 (4.77)^{A}**
	Diet L	83.33 (8.82)	50 (5.77)	**66.67 (8.82)^{A}**
	Means for rearing system	**80 (4.47)^{a}**	**55 (4.28)^{b}**	

Values for each rearing system-diet combination are means (±SE) of three replicate tanks.
System treatments with different superscripts (lower case) are significantly different ($P<0.05$)
Dietary treatments with different superscripts (upper case) are significantly different ($P<0.05$)

3.2 Experiment 2

3.2.1 Water quality

The ammonia and nitrite concentrations in experiment 2 followed a similar trend to that found for treatment RC in experiment 1. However, the water recirculation rate was adjusted from 280% a day in experiment 1 to 400% a day in experiment 2 and therefore most of the measured values for both ammonia and nitrite concentrations in experiment 2 were below limits of detection. Highest concentrations of ammonia and nitrite of 0.06mg.L^{-1} and 0.04mg.L^{-1} (respectively) were detected in the high salinity and high stocking density treatment (HSHD).

3.2.2 Growth and survival of the shrimp

Analysis of variance (Two-way ANOVA) found a significant interaction ($P<0.05$) between stocking density and salinity for average final weight; indicating the two factors were not acting independently on growth. However, Two-way ANOVA found no significant interaction between stocking density and salinity for shrimp survival; indicating the two factors were acting independently on survival (Table 3).

Table 3. Mean squares, significance levels and percentage of variation explained by analysis of variance of the average weight and survival of the shrimp after 67 days reared at low salinity water (20-23g.L^{-1}) or high salinity water (32-33g.L^{-1}) with low stocking density (10 animals per m^2) or high stocking density (20 animals per m^2). Experiment 2.

Source		Df	Mean square	% MS[a]
Average weight	Salinity	1	13.93	34.58*
	Stocking density	1	6.62	16.45
	Stocking density x Salinity	1	17.28	42.90*
	Error	16	2.45	6.07
	R^2	0.49		
Survival	Salinity	1	1805	78.97**
	Stocking density	1	125	5.47
	Stocking density x Salinity	1	245	10.72
	Error	16	110.625	4.84
	R^2	0.55		

[a]: Based on total mean squares of all effects

*: Significant at $P < 0.05$, **: Significant at $P < 0.01$, ***: Significant at $P < 0.0001$

Consequently, growth data were reanalysed separately for each main effect using the Student's T-test. At lower density, average weight of the shrimp was significantly higher ($P<0.05$) in the lower salinity (19.98 ± 0.77g) than the higher salinity tanks (16.45 ± 0.49g). However, at the higher density, no significant difference was found between salinity treatments (Table 4).

Survivals were significantly greater (P<0.05) in the low salinity tanks (95 ± 1.83%) than the high salinity tanks (76 ± 4.52%) when averaged across both density regimes. Density treatments had no significant effect on survival (P>0.05), averaging 83 ± 5.59% and 88 ± 3.35% for low and high density treatments respectively (Table 4).

Table 4. Mean (±SE) average weight and survival of *P. monodon* reared from 1g for 67 days at low salinity (20-23g.L^{-1}) or high salinity (32-33g.L^{-1}) with low stocking density (10 animals per m^2) and high stocking density (20 animals per m^2). Experiment 2.

Measure	Density regimes	Salinity regimes		Means for density treatments
		Low salinity	High salinity	
Average weight (g)	Low density	19.98 (0.77)^{a}	16.45 (0.49)^{b}	**18.22 (0.73)**
	High density	16.97 (0.65)^{A}	17.16 (0.84)^{A}	**17.07 (0.50)**
	Means for salinity regimes	**18.48 (0.69)**	**16.81 (0.47)**	
Survival (%)	Low density	96 (2.54)	70 (7.07)	**83 (5.59)^{a}**
	High density	94 (2.92)	82 (4.90)	**88 (3.35)^{a}**
	Means for salinity regimes	**95 (1.83)^{a}**	**76 (4.52)^{b}**	

Values for each stocking density-salinity combination are means (±SE) of five replicate tanks.
Salinity treatments with different superscripts (lower case) are significantly different (P<0.05).
Combined density-salinity treatments with different superscripts (lower case for combined treatments at low density or upper case for combined treatments at high density) are significantly different (P<0.05).
Density treatments with different superscripts (lower case) are significantly different (P<0.05).
There was no interaction effect between salinity x stocking density on survival therefore significant differences within the treatments of each experimental factor for survival were not indicated. Only the main effect of each experimental factor for survival (salinity and stocking density) was noted.

In term of growth per day (g), the shrimp grew at 0.28 $g.day^{-1}$, 0.23$g.day^{-1}$, 0.24 $g.day^{-1}$ and 0.24 $g.day^{-1}$ respectively in low salinity and low density (LSLD); low salinity and high density (LSHD); high salinity and low density (HSLD); and high salinity and high density (HSHD) treatments.

3. Discussion

This study demonstrated that 1g-domesticated *P. monodon* juveniles could be cultured to around 35g with high survival of around 80% in approximately 120 days in indoor sand-based recirculation systems with low water exchange (5-10%/day) when fed on a mono diet of high protein pellets (55% protein) or a combination diet of 50% high protein pellets plus 50% fresh-food mixture (75% squid, 15% oyster and 10% blood cockle). The results indicated that growth and survival of shrimp differed between rearing systems, with faster growth and higher survival achieved when using sand-based recirculation with low water exchange at 5-10% a day, as compared to flow-through water system at 200% a day.

Our findings are in good agreement with Tseng et al. (1998) who scored the highest growth (0.28$g.day^{-1}$) and survival (89 ± 6%) at a stocking density of 40 animals per m^2, with *P.monodon* juveniles growing from 7.8 g to 23.3 g within 56 days. In our experiments in sand-based recirculation systems, we obtained growths of 0.27-0.29 $g.day^{-1}$ at average survivals of 80% for treatments RL and RC after 126 days in experiment 1, and growth of 0.28$g.day^{-1}$ with survival of 96% in the LSLD treatment after 67 days in experiment 2. The growth of *P. monodon* juveniles in our recirculation system was much better than that obtained in former studies using pellets and natural food where the shrimps achieved growths of 0.11-0.14$g.day^{-1}$ at a

stocking density of 27 animals per m^2 (Hansford and Hewitt, 1994) and 0.14-0.17g.day^{-1} at a stocking density of 15 animals per m^2 (Allan et al., 1995).

The present study also indicated that the growth of shrimp during the grow-out phase (juvenile stage) was better when shrimp were fed a combination diet (diet C) of high protein pellets plus a fresh-food mixture, rather than when fed only high protein pellets (diet L). Also at adult stages, several previous studies have indicated that a combination of artificial pellets and a fresh-food mixture yielded the best reproduction outputs (Primavera et al., 1979; Chamberlain & Lawrence, 1981; Bray et al., 1990). A combination of both fresh-food mixture and artificial pellets yielded the best results possibly because its broader range of nutrients ensured the dietary requirements of the shrimp.

Similar to the finding of the Queensland Department of Primary Industries and Fisheries (2006) who reported an optimal salinity for the grow-out phase of *P. monodon* at 15-25 g.L^{-1}, our best growth and highest survival was also scored at 20-23 g.L^{-1}. At higher salinities animals apparently have to spend more energy for osmotic pressure regulation resulting in lower growths; furthermore spending more energy for osmotic pressure regulation could also cause stress, which can accumulate over time, and result in lower survival and growth.

In the present study, shrimp survival was different between rearing systems, with higher survivals obtained in the low water exchange recirculation system as compared to flow-through water system. Courtland (1999) indicated that improved water conditions in closed recirculation systems reduced stress in broodstock shrimp and decreased broodstock mortality from 0.5% per night in a flow-through system to 0.1% in a recirculation system. In another study, Millamena et al. (1991) also found recirculation systems to be better than flow-through systems in terms of reproductive

traits for *P. monodon.* In another study in Thailand, a recirculation system designed in earthen ponds for rearing postlarval stage 15 of *P. monodon* for 60 days resulted in an average survival of 56% when practiced at a water exchange of 10% either one or twice a week (Kaweekityota, et al., 2007), while survival of the shrimp in sand-based recirculation system obtained in the present experiments is much higher than in the recirculation system designed in earthen ponds in Thailand.

In terms of water quality, the low water exchange recirculation system resulted in the most stable and best water quality. The airlift mechanism produced sufficient dissolved oxygen levels for the low water exchange recirculation system, while the system with flow-through water at a rate of 200% a day resulted in continuous renewal with fresh seawater from the reservoir tank. It should be noted that new seawater entering the flow-through tanks from the reservoir tank was not aerated before flowing into the tanks, and so contained lower dissolved oxygen levels than the water in the rearing tanks.

The sand substrate used in the tank systems acted as an internal bio-filter (Coman et al., 2005) producing positive effects on water quality by reducing the ammonia concentrations in the low water exchange recirculation systems. As a result lower ammonia concentrations but higher nitrite concentrations were recorded under the low water exchange recirculation system (system R) as compared to the flow-through water system (system F). Apparently, bacteria converted most ammonia in the system R into nitrite. The process to convert nitrite into nitrate was however only speeded up when increasing the water recirculation rate from 280% a day in experiment 1 to 400% a day in experiment 2 and therefore most of the nitrite was converted to nitrate in experiment 2. However, metabolizing bacteria in the bio-filter produce carbon dioxide (CO_2) which gradually acidified the rearing water over time and in addition,

the nitrifying process also consumes bicarbonate (HCO_3) to convert ammonium into nitrate (Eding et al., 2005); as a result, the alkalinity levels dropped sooner (after 6 weeks) in system R. Alkalinity was only going down after three months in system F. The reason for this was the high flow-through rate at 200% a day, which did not allow nitrifying bacteria to develop effectively on the sand substrate initially. Possibly the sand substrate in the flow-through water system started to function as an internal bio-filter after 3 months. In this regard Primavera (1985) and Coman et al. (2005) suggested to periodically add sodium bi-carbonate ($NaHCO_3$) to the recirculation tank, therefore it could be interesting to test a recirculation system at lower flow-through rates for future research work in stead of the high rates (200% a day) used in the present study.

4. Conclusions

A sand-based recirculation system with a water exchange rate of 5-10% a day and a combination diet of high protein pellets (55% protein) and fresh-food mixture (75% squid, 15% oyster, and 10% blood cockle) provide suitable conditions for the indoor rearing of the grow-out phase (juvenile stage) of *P. monodon*. In this study, a water salinity of 20-23 $g.L^{-1}$ with low stocking density of 10 animals per m^2 produced the best growth during the grow-out phase of *P. monodon*. Survival of the *P. monodon* in the grow-out phase was significantly higher ($P<0.05$) at low salinity of 20-23 $g.L^{-1}$ as compared to high salinity of 32-33 $g.L^{-1}$ irrespective of stocking density.

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CHAPTER IV

DOMESTICATION PROGRESS

Chapter IV

Progress in the domestication of black tiger shrimp *Penaeus monodon* (Fabricius, 1798) in a sand-based recirculation system in Vietnam

Nguyen Duy Hoa[1,3], Mathieu Wille[3], Roeland Wouters[2], Vu Thanh[1], Nguyen Quoc Hung[1], Tran Kim Dong[1], Nguyen Van Hao[1], Patrick Sorgeloos[3].

Research Institute for Aquaculture No.2, Ho Chi Minh city, Vietnam[1].

INVE Technologies N.V., Dendermonde, Belgium[2].

Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium[3].

Abstract

Growth, survival, spawning and reproductive performance of black tiger shrimp (*Penaeus monodon*) were evaluated during a domestication study from 2007 to early 2009. The shrimp were domesticated in sand-based recirculation tanks in bio-secure facilities. The rearing cycle was divided in 3 phases according to the weight of the female shrimp (phase 1 from 1 to 40g, phase 2 from 40 to 80g, and phase 3 from 80 to larger than 100g). Stocking densities were 10 to 13 animals per m^2 for phase 1 (grow-out phase), then reduced to 4 to 6 animals per m^2 for phase 2 (pre-maturation phase) and finally 2 to 3 animals per m^2 for phase 3 (maturation phase). High protein (55%) grow-out pellets and a diversified fresh-food mixture (squid, oyster, and blood cockle) were given in the first two phases of the domestication process while a semi-moist maturation diet (BREED-S Fresh®) (INVE Aquaculture N.V., Belgium) and a maturation fresh-food mixture (squid, marine worm, oyster, and pork liver) were given to the shrimp in phase 3. Water with a salinity of 20-23 $g.L^{-1}$ was used to rear the animals in phase 1 while full strength seawater (32-35 $g.L^{-1}$) was applied for phase 2 and 3.

At the end of grow-out phase, the female shrimp obtained an average survival of 81.02 ± 2.91% with an average growth of 0.336$g.day^{-1}$ in around 4 months rearing. The prematuration phase resulted in lower survival, averaging 65.95 ± 10.24% with an average female growth of 0.352 ± 0.03$g.day^{-1}$ in around 4 months rearing. Survival in the maturation phase was very high, averaging 98.56 ± 2.04% with an average female growth of 0.356 ± 0.015$g.day^{-1}$ and approximately 2 months to rear the female animals from 80 to 100g. Spawning and reproductive performance of the 11-month old F2-domesticated broodstock animals revealed high fecundity and egg hatching success (309,630 ± 120,685 eggs/spawning and 75.03 ± 1.91% hatching success).

Maturation frequency (100%), spawning success (80%), number of spawns per female (3.64 ± 1.06), and success of metamorphosis of nauplii into zoea (96.28 ± 0.85%) were very high for these F2-domesticated animals.

Key words: domestication, shrimp, *P. monodon*

1. Introduction

Penaeid shrimp farming has been practiced in Vietnam since the 1960's and has rapidly developed since the 2000's with a national production reaching over 300,000 tons a year (376,700 tons in 2007; FAO, 2007). The shrimp farming industry in Vietnam contributes around 40% of the total fisheries export turnover (Phi et al., 2009). Black tiger shrimp (*Penaeus monodon*), an indigenous species, is the most important farmed shrimp species in Vietnam (Trong and Hoa, 2007).Until now, shrimp hatcheries in Vietnam have been able to supply the demands of commercial shrimp farms, but the prevalence of diseases has resulted in huge losses for both the hatchery and grow-out sector. Disease outbreaks, especially White Spot Syndrome Virus (WSSV), Yellow Head Virus (YHV), Taura Syndrome Virus (TSV), Monodon Baculovirus (MBV), Hepatopancreatic Parvovirus (HPV) and, to a lesser extent, luminescent bacterial infections have resulted in a declining global production in recent years and this has led to an almost inevitable 'boom-and-bust' cycle of the shrimp farming industry (Flegel and Alday-Sanz, 1998; Flegel et al., 2004). Another factor contributing to the unstable development of the shrimp farming industry is the reliance on wild broodstock for the shrimp hatchery industry, i.e. supply fluctuates a lot from season to season and natural stocks are declining. From an ecological perspective, this practice moreover is unacceptable in the long term. As long as domestication of black tiger shrimp can not fill in the commercial needs for postlarvae, black tiger shrimp farming industry continue to rely on wild breeders.

Wild broodstock shrimp are also a known source of several viral pathogens that have caused significant losses in production due to vertical transmission of viral diseases to the offspring (Gjedrem and Fimland, 1995; Flegel et al., 1997; Currie, 1998) and for this reason, viral diseases must be controlled at the broodstock level itself (Browdy, 1998; Yano, 2000; Simon and Briggs, 2003; Coman et al., 2005).

Therefore, developing techniques for successful domestication of black tiger shrimp to produce specific-pathogen-free domesticated broodstocks is a necessary strategy for sustainable shrimp farming in Vietnam. The current study aims to develop a protocol for closing the life cycle of *P. monodon* in indoor rearing systems in order to produce captive *P. monodon* breeders that are capable to spawn in captivity through several generations.

2. Material and methods

2.1 Source of shrimp and domestication protocol

Wild black tiger shrimp breeders (F0-generation) were sourced from Rach Goc sea, Ca Mau, Vietnam and screened for viral diseases which are highly pathogenic to *P. monodon* (WWSV, YHV, HPV, and MBV). The SPF breeders were then transferred to primary quarantine facilities and reared in separate couples for one month. A second screening for viruses was applied at the end of primary quarantine to select SPF shrimp breeders for maturation rearing in secondary quarantine facilities in the next three months. Thereafter the SPF broodstock shrimp were eyestalk-ablated for the production of SPF-postlarvae (F1-animals). The SPF F1-postlarvae were reared in 3-m^3 composite tanks using conventional rearing techniques until they reached 1g. Their SPF status was checked before stocking these animals in biosecure sand-based recirculation tanks to produce SPF domesticated F1 breeders. The F1 breeders were

then used for breeding to produce F2-domesticated stocks. The rearing cycle of *P. monodon* in the bio-secure sand-based recirculation tanks was split up into three phases as indicated in the following diagram:

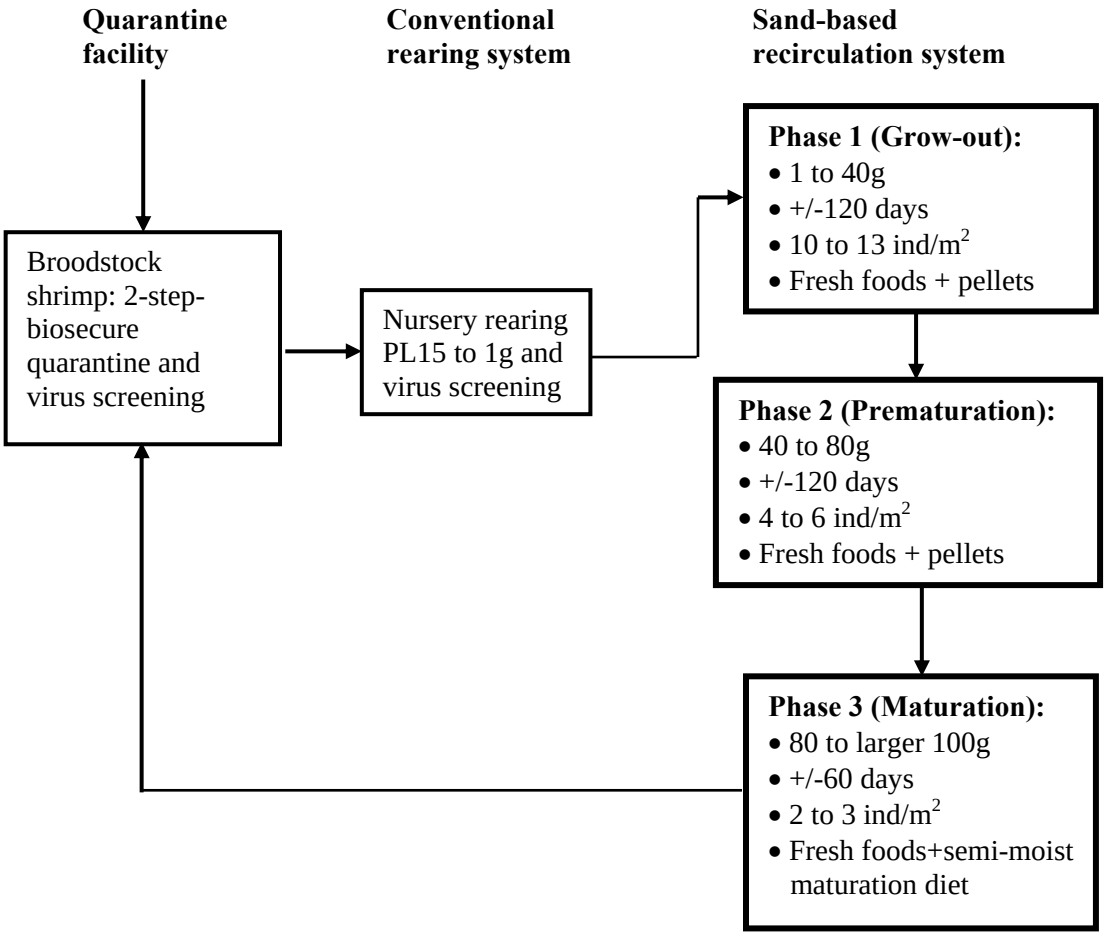

Diagram 1. Procedure for closing the life cycle of *P. monodon* in recirculation tanks

In this protocol, stocking densities were 10 to 13 shrimps per m^2 for the 1st phase, 4 to 6 per m^2 during the 2nd phase and 2 to 3 per m^2 for maturation rearing (phase 3).

High protein pellets (Lucky Star, Taiwan Hung Kuo Industrial Co., Ltd; 55% protein), fresh-food ingredients (squid, oyster, marine worm, blood cockle, and pork liver) and semi-moist maturation pellets (Breed-S Fresh®, INVE Aquaculture N.V., Belgium) were fed to the shrimp in different proportions (on dry weight basic) depending on the rearing phase as described in the following table (Table 1).

Table 1. Diet regimes in the different rearing phases

Phase 1 – Grow-out (1 to 40g)	Phase 2 - Prematuration (40 to 80g)	Phase 3 – Maturation (80 to ≥100g)
80% high protein pellets plus 20% fresh-food mixture (75% squid, 15% oyster, and 10% blood cockle)	50% high protein pellets plus 50% fresh-food mixture (75% squid, 15% oyster, and 10% blood cockle)	60% semi-moist maturation pellets plus 40% fresh-food mixture (37% squid, 27% oyster, 17% marine worm and 19% pork liver)

2.2 Rearing conditions

In phase 1 and 2, the shrimp were reared in circular 10-m³ fiberglass sand-based recirculation tanks (3.6m in diameter and 0.9m water depth). For phase 3 (from 80g to larger than 100g), the animals were reared in 4-m³ (2.35m in diameter and 0.9m water depth) and 10-m³ (3.6m in diameter and 0.9m water depth) fiberglass sand-based recirculation tanks. A sand-based water recirculation system with airlift mechanism was applied to provide sufficient dissolved oxygen for maintaining the substrate in an aerobic condition. Seawater from Vung Tau beach with salinity between 32-35 $g.L^{-1}$ was pumped into 100-m³ settling tanks, treated with $KMnO_4$ at 1-2 $mg.L^{-1}$ (depending on turbidity) and heavily aerated for at least 24 hours. Then the clear seawater was run through a high pressure crude-sand filter for at least 6 hours before being pumped into the reservoirs of the domestication buildings. In the domestication buildings the seawater was treated with calcium hypochlorite at $30mg.L^{-1}$ for at least 12 hours and continuously pumped over a fine-sand filter, finally chlorine residues were neutralized with sodium thiosulphate ($Na_2S_2O_3$). Treated seawater was then passed through 1µm-filter bags and 0.5µm cartridge filters before being supplied to the rearing tanks. Seawater was mixed with tap-water to produce water of 20-23 $g.L^{-1}$ salinity for rearing the animals in the first phase then full-strength seawater of 32-35 $g.L^{-1}$ was

used to rear the animals in phases 2 and 3. Tanks were maintained under a low water exchange regime with approximately 5% of the tank volume exchanged per day to compensate for water lost by siphoning. A high recirculation rate (approximate 400% a day) was applied to provide high dissolved oxygen levels (6-7.3 $mg.L^{-1}$) for the sand-substrate that acts as an internal bio-filter and this way maintained low total ammonia and nitrite concentrations (average NH_4-N and NO_2-N levels were 0.109 ± 0.126 $mg.L^{-1}$, and 0.057 ± 0.085 $mg.L^{-1}$, respectively for the first two phases, while these were 0.076 ± 0.033 $mg.L^{-1}$ and 0.021 ± 0.015 $mg.L^{-1}$, respectively during the final maturation phase). Alkalinity was maintained between 80 to 110$mg.L^{-1}$ through periodic addition of sodium bi-carbonate ($NaHCO_3$). The pH values fluctuated in the range of 7.8 to 8.3. Water temperature ranged between 28 and 30.5 °C for most of the months, but dropped to 26.5-28°C during December and January. However, 14 thermostatic heaters were used to maintain temperature at 29^oC during maturation rearing and hatching of the eggs. The roof of the bio-secure domestication buildings were partially painted black to reduce ambient light, but the tanks were not covered during the first two phases of the domestication process; however, during the last phase (from 80g to 100g up) the tanks were completely covered with black plastic sheets to induce maturation of the broodstock shrimp.

2.3 Growth and survival measurement

All shrimp were individually weighed at the end of each phase and the average weight was recorded for each tank. Growth of the shrimp was calculated in terms of weight gain per day for males and females separately. The final average values for growth and survival in each rearing phase were then averaged from the averages of the different rearing trials.

2.4 Spawning and reproductive performance

Females were eye-tagged with numbered plastic tags for individual monitoring and tail-tagged with different colors of soft wool for follow-up of molting events. During the two months of maturation rearing, molting and mortality were recorded daily. Molted females were examined to check natural mating. If natural mating had not taken place, artificial insemination was applied on the morning after molting. Then, eyestalk-ablation was applied on day-4 after molting and ablated females were returned to the maturation tank. Daily observation of ovarian maturation was done using a torch light. Ready-to-spawn females (ovarian stage-IV, Tan-Fermin & Pudadera, 1989) were transferred to individual 800-l tanks for spawning and gentle aeration was supplied to each spawning tank. Spawned females were returned to the maturation tanks the next morning for re-maturation. Approximately 6 h after spawning, eggs were drained from the spawning tank into a 45-l bucket and gently homogenized by increasing the aeration. Six samples of 1ml were taken with a pipette at different depths in the water column and the eggs counted for estimation of the egg production. The eggs were then washed with clean seawater (1-2 minutes), rinsed with a formalin solution (300 $mg.L^{-1}$ for 30 seconds), treated with povidone-iodine solution (20 $mg.L^{-1}$ for 30 seconds) and finally washed again with clean seawater (1-2 minutes) before stocking in the hatching tanks. Upon hatching, the number of nauplii was estimated in the same way as the eggs. The nauplii were treated with a formalin solution (300$mg.L^{-1}$ for 30 seconds). Four 1-l containers were arranged and provided with slight aeration for rearing 50-100 nauplii to the zoea stage for estimation of metamorphosis success. The spawning and reproductive performances of the animals were evaluated during a period of 5 weeks (two molting cycles). Reproductive performance of the animals was evaluated by natural mating success (% of the molted

females carrying a spermatophore on their thelycum), % maturation (% of the surviving ablated females that developed stage-IV ovaries), average weight of the females at spawning, % spawning (% of the mature animals that spawned), number of spawns per female, egg fecundity (number of eggs per spawning), hatching success (% of eggs hatching into nauplii; spawns with zero hatching were not included in the calculation), and success of metamorphosis (% of nauplii that metamorphosed into zoea, determined within 10 to 11 hours after the first zoeae appeared).

3. Results

3.1 Survival and growth of the shrimp

Table 2, 3 and 4 presents shrimp survival and growth data in phase 1, 2 and 3. Average survival of the F1 & F2 animals at the end of phase 1 was 81.02 ± 2.91%, while this was lower in phase 2, averaging 65.95 ± 10.24%. Survival in phase 3 was very high, averaging 98.56 ± 2.04%. An average growth of the females calculated from different batches of phase 1 was 0.336g.day^{-1} or approximately 119 days to rear the females from 1 to 40g. Similarly, the average growth of the females was 0.352g.day^{-1} in phase 2; while duration to rear the animals from 40 to 80g in phase 2 was approximate 114 days. In phase 3, average growth of the female shrimp was 0.356 ± 0.015 g.day^{-1} or approximate 56 days to rear the female animals from 80 to 100g.

Table 2. Grow-out performance of shrimp in phase 1 (1 to 40g)

Parameters	**2007**	**2008-1**	**2008-2**
Number of tanks	3	4	4
Tank volume (m^3)	10	10	10
Stocking density (ind/tank)	100	120	130
Initial average weight (g/ind)	1.430 ± 0.060	0.965 ± 0.049	1.143 ± 0.167
Duration of culture (days)	117	191	120
Harvest weight (g/ind)	38.26 ± 4.49	62.35 ± 5.90	37.62 ± 4.38
Female harvest weight (g/ind)	42.19 ± 2.13	67.32 ± 13.40	38.78 ± 10.01
Male harvest weight (g/ind)	33.71 ± 1.41	56.96 ± 11.23	35.62 ± 8.05
Growth (g/day)	0.314	0.321	0.304
Overall average growth (g/day)		0.313 ± 0.0085	
Male growth (g/day)	0.275	0.293	0.287
Average male growth (g/day)		0.285^a ± 0.009	
Female growth (g/day)	0.348	0.347	0.313
Average female growth (g/day)		0.336^b ± 0.019	
Survival (%)	84.33 ± 10.50	79.89 ± 9.15	78.84 ± 16.31
Average survival (%)		81.02 ± 2.91	

Different superscript letters denote significant differences between male and female shrimps ($P<0.05$)

Table 3. Performance of shrimp in phase 2 of prematuration rearing (from 40 to 80g)

Parameters	**2007-1**	**2007-2**	**2008-1**	**2008-2**
Number of tanks	5	5	12	6
Tank volume (m^3)	10	10	4	10
Stocking density (ind/tank)	55	40	15	58.8
Initial average weight (g/ind)	54.96 ± 3.54	41.17 ± 7.47	38.99 ± 3.07	39.39 ± 3.42
Initial female weight (g/ind)	57.25 ± 3.78	47.00 ± 4.24	41.50 ± 9.94	41.04 ± 3.04
Initial male weight (g/ind)	52.67 ± 0.85	35.35 ± 4.76	36.50 ± 7.52	37.73 ± 3.28
Duration of culture (days)	78	107	95	127
Harvest weight (g/ind)	74.7 ± 8.61	71.6 ± 15.51	68.8 ± 11.84	76.1 ± 12.42
Female harvest weight (g/ind)	81.78 ± 3.72	84.36 ± 9.19	78.34 ± 16.95	87.15 ± 5.19
Male harvest weight (g/ind)	68.65 ± 6.72	58.98 ± 7.36	59.71 ± 10.81	65.07 ± 4.49
Overall growth (g/day)	0.253	0.285	0.314	0.289
Average overall growth(g/day)	0.285 ± 0.025			
Male growth (g/day)	0.175	0.221	0.244	0.220
Average male growth (g/day)	0.215^{a} ± 0.029			
Female growth (g/day)	0.314	0.349	0.387	0.360
Average female growth (g/day)	0.352^{b} ± 0.03			
Survival (%)	66.9 ± 12.68	53.5 ± 10.69	64.9 ± 11.10	78.5 ± 2.01
Overall average survival (%)	65.95 ± 10.24			

Different superscript letters denote significant differences between male and female shrimps ($P<0.05$)

Table 4. Performance of shrimp in phase 3 of maturation rearing (from 80 to 100g up)

Parameters	2008-1 (F2-animals)	2008-2 (F2-animals)
Number of tanks	12	12
Tank volume (m^3)	4	4
Stocking density (inds/tank)	8	10
Sex ratio (male: female)	4 : 4	6 : 4
Initial female weight (g/ind)	85.29 ± 3.26	95.88 ± 9.56
Initial male weight (g/ind)	64.55 ± 0.99	Not weighted
Duration of culture (days)	60	70
Female harvest weight (g/ind)	107.40 ± 8.23	120.20 ± 12.16
Natural mating (%)	22.22	17.95
Male harvest weight (g/ind)	79.03 ± 1.86	Not weighted
Male growth (g/day)	0.241	n/a
Female growth (g/day)	0.368	0.347
Average female growth (g/day)	0.356 ± 0.015	
Survival (%)	97.11 ± 5.48	100
Average survival (%)	98.56 ± 2.04	

3.2 Spawning and reproductive performance of the shrimp

Sixteen 11-month old F2-domesticated females were eyestalk ablated for evaluation of spawning and reproductive performance during October to December 2008. The results of spawning and reproductive performance of these F2-domesticated breeders are presented in Table 5.

Table 5. Spawning and reproductive performance of domesticated black tiger shrimp

Parameters	Value
Sex ratio (male : female)	1 : 1
Age at spawning (months)	11
Average weight at spawning (g)	106.85 ± 5.01
Number of ablated females (inds)	16
Female survival after ablation (%)	93.75
Percentage maturation (%)	100
Spawning success (%)	80
Number of spawns/female	3.64 ± 1.06
Egg fecundity range (Number of eggs/spawning)	60,000-617,000
Average egg fecundity (Number of eggs/spawning)	309,630 ± 120,685
Egg hatching success (%)	75.03 ± 1.91
Metamorphosis into zoea (%)	96.28 ± 0.85

The number of eggs per spawning varied from 60,000 to 617,000 eggs per spawn. In general, spawning and reproductive performance results indicated high fecundity and egg hatching (309,630 ± 120,685 eggs/spawning and 75.03 ± 1.91% hatching success). Other reproductive parameters for these F2-domesticated animals also presented high values: 100% for maturation success, 80% for spawning success, 3.64 ± 1.06 spawns per female and 96.28 ± 0.85% for success of metamorphosis (Table 5). However, natural mating was very low for these domesticated animals. Data were not recorded systematically during the spawning performance experiment, but results

from the maturation phase (Table 4) indicated a natural mating success of only 17.95-22.22%.

Interestingly, there were 46% of the spawns (21 spawns of total 46 spawns) producing high egg fecundity (higher than the average fecundity of 309,630 eggs/spawn) which contributed 60% of the total egg production (8,601,000 eggs of the total 14,243,000 eggs).

4. Discussion

4.1 Survival and growth performance

Our results demonstrated that *P. monodon* can be domesticated in indoor tank systems within a 12-month production cycle. Our domestication technique for black tiger shrimp in Vietnam uses a sand-based recirculation technology adapted from the technology developed by the Australian Commonwealth Scientific and Industrial Research Organisation (CSIRO) as described by Coman et al. (2005), which we however modified by splitting up the rearing cycle into 3 phases in function of differences in the life cycle of the black tiger shrimp (Motoh, 1985). According to Motoh (1985), the life cycle of *P. monodon* can be divided into 6 stages, namely embryo (12 hours), larvae (20 days), juvenile (15 days), adolescent (4 months), sub-adult (4 months) and adult (10 months). Juveniles inhabit brackish water areas as nursery grounds, whereas sub-adults move to a variety of estuarine or inner littoral areas before migrating to deeper water, while adults inhabit offshore areas up to a depth of 160m. The general approach of our black tiger domestication program is similar to the domestication program of CSIRO as described in Coman et al. (2005), i.e. using a sand-based recirculation system, similar stocking densities and food (diversified fresh-food mixture and high protein pellets). However, the CSIRO

domestication protocol was only considering 2 phases with the first 8-months as grow-out phase and from 8 to 11.5 months as maturation phase. Our protocol considered 3 different rearing phases, i.e. the first 4 months (from 1 to 40g, adolescent stage) as grow-out phase, the next 4 months (from 40 to 80g, sub-adult stage) as pre-maturation phase and from 8 to 12 months onwards as maturation and spawning phase. Also, the protocol we applied in the 2nd phase had a lower inclusion of grow-out pellets but higher inclusion of fresh-food mixture as compared to the CSIRO protocol. A totally dark environment together with fresh-food maturation diet and semi-moist maturation pellets (BREED-S Fresh®) (INVE Aquaculture N.V., Belgium) were provided in the last phase of our domestication program while high inclusion of grow-out pellets (70%) is still offered to the shrimp until 8 months in the CSIRO's program.

In our program, shrimp growth was only slightly lower compared to the CSIRO program, with an average weight of 107.40 ± 8.23g for females and 79.03 ± 1.86 for males at 11 months, while in the CSIRO program this was 117.1 ± 5.8g for females and 87.9 ± 7.6g for males at 11 months. The higher inclusion of high-protein pellets (55% protein) used until month 8 in the CSIRO program and genetic differences between both stocks could have contributed to these differences. According to Coman et al. (2005), there were significant variations in growth and reproductive performance among families, ages, and environmental conditions (tank/raceway systems) in the Australian domesticated stocks. For comparison, in an SPF broodstock program in Malaysia (Subramaniam et al., 2006), using a totally different protocol, the average weight of the animals was considerably lower, averaging 80-100g for F1 and F2 females at 9-12 months.

Grow-out from 1 to 40g at a lower salinity of 20-23g.L^{-1} in the present study resulted in very high shrimp survival (81.02 ± 2.91%). In a separate experiment as part of our domestication program (data not shown) it was found that growth and survival from 1g to 20g were better at a salinity of 20g.L^{-1} than at 32g.L^{-1}. According to the Queensland Department of Primary Industries and Fisheries (2006) optimal salinity for the grow-out phase of black tiger shrimp is 15-25g.L^{-1}. At higher salinity the animals have to spend more energy for osmotic regulation resulting in a lower growth. Using optimal salinity has probably contributed to the observed high survival. Also the growth of the three batches of F1 and F2 animals in the recirculation system was very good (0.314, 0.304, 0.321g/day) and much higher than the average growths obtained for pond-reared animals (ranging between 0.17 and 0.28g/day with an average of 0.198 ± 0.03g/day; data of 23 shrimp ponds provided by shrimp farmers in the Mekong Delta, Vietnam). The low stocking density (10-13 animals/m^2) and the high protein pellets together with a diversified fresh-food diet (squid, oyster, and blood cockle) probably accounted for the better shrimp growth in the indoor sand-based recirculation tanks as compared to the pond-reared animals. The latter are usually fed only commercial shrimp pellets with lower protein content (36-40% protein) and are reared at higher stocking densities (25-35 animals/m^2).

Shrimp survival was much lower in phase 2 as compared to phase 1 and phase 3 (Table 2, 3, 4). It is difficult to speculate what was the exact cause. Diet should not be the reason for the lower survival in phase 2, cause growth in this phase remained high. It is possible that light regime and water salinity had an impact on survival. In phase 2, animals were reared in similar culture systems, but the water salinity and light regime were different from phase 1 and phase 3, respectively. Water salinity in phase 2 was similar to the one used in phase 3 but higher than in phase 1. Maybe the salinity

should be more gradually increased from brackish water to full strength seawater as happens in nature when the animals migrate from the coastal area to the sea. With respect to light, the animals in phase 3 were reared in total darkness (covered tanks) while the animals in phase 2 were reared in un-covered tanks. Higher light intensities may be suitable for small animals (phase 1), while it is possible that this may have stressed the larger animals in phase 2. The light regime may not only be important to trigger maturation, but, if not optimal, may also cause stress that ultimately may affect the shrimp survival. Our observations indicated that even the torch light used to observe ovarian development sometimes disturbed the females, resulting in a quick resorbtion of their developed ovaries back to stage 0 within the next day. It therefore seems that completely covering the tanks in phase 3 provided the quiet and stress-free environment necessary for optimal survival and maturation of the animals. Primavera (1983, 1985) concluded that covering the rearing tanks minimizes disturbance to black tiger broodstock shrimp. Further observations during our domestication program indicated that black tiger shrimp prefer to burrow in the sand layer when they get larger in size, which may again suggest that larger animals are more sensitive to environmental disturbance than smaller ones. Therefore, it may be advantageous to cover the tanks already in phase 2 to reduce the light intensity in stead of using the same light regime as in phase 1. The Queensland Department of Primary Industries and Fisheries (2006) reported that wild *P. monodon* matures at an age of 5 to 12 months and at a body weight of 35-50g for males and 68-75g for females; therefore, it might be worthwhile to consider providing semi-moist maturation pellets and covering the tanks at a size of 50-60g already instead of waiting until they reach a weight of 80g as we did in our protocol.

4.2 Spawning and reproductive performance

The current study indicated natural mating of the domesticated animals was very low (Table 4). Several studies indicated that natural mating of penaeid shrimp in captivity is very low (Aquacop, 1983; Primavera, 1979; 1985; Makinouchi and Hirata, 1995; Parnes et al., 2007). According to Parnes et al. (2007), the reason for the low natural mating of penaeid shrimp in recirculating maturation systems is due to the absence of sufficient ready-to-mate males. The inferior quality of domesticated males, the sometimes synchronous molting of males and females, and the sex ratio could be factors for the absence of sufficient ready-to-mate males that limit successful natural mating of shrimp in captivity. Notably, the synchronization of molting between males and females in some cases results in shortage of hard-shelled males for mating with molted females and therefore the sex ratio should be biased towards males.

Only a very limited number of black tiger shrimp domestication studies have been reported in literature. As mentioned above, our domestication protocol most closely resembled the techniques developed by CSIRO (Coman et al., 2005). Although breeder weight in the latter study was higher, the breeders in our study produced more than double the number of eggs (309,630 eggs per spawning, resulted in approximately 200,000 nauplii) compared to the CSIRO study (only 114,000-125,000 eggs per spawning) (Coman et al., 2005). Genetic differences between both breeding stocks and different maturation diets might account for this. Reproductive performance of the shrimp in the current study was very similar to the results obtained in a domestication program in Malaysia. Using a totally different rearing protocol, reproductive output in the latter study also averaged 200,000 nauplii per spawn (Subramaniam et al. 2006).

Interestingly, there were 6 females that spawned multiple times over two molting cycles (5 to 6 times). Around 46% of the total spawns contributed approximately 60% of the total egg production. In shrimp hatchery settings, it is well known that a large percentage of females never spawns or spawns only once, whereas a smaller percentage of the females is able to spawn multiple times (Bray et al., 1990; Wyban and Sweeney, 1991; Cavalli et al., 1997; Palacios et al., 1999; Aros et al., 2003). Ibarra et al. (2005) suggested that selection for high-performance broodstock shrimp using the trait of multiple spawning capacities along with traits of egg quality could be an important strategy to improve nauplii production.

5. Conclusions

The progress in domestication of black tiger shrimp obtained in our program shows a promising outlook for mass production of SPF domesticated black tiger shrimp. However, further improvements in husbandry and feeding regimes are necessary - especially with respect to stress reduction and improvement of maturation diets - to ensure a better and more stable performance of the domesticated breeders. In addition, before starting a proper breeding program, the characteristics and traits of different founder stocks from different geographical origin should be investigated. Future work could also include selective breeding to produce domesticated stocks with improved reproductive performance.

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CHAPTER V

BROODSTOCK NUTRITION

Chapter V Section I

A fresh-food maturation diet with an adequate HUFA composition for broodstock nutrition studies in black tiger shrimp *Penaeus monodon* (Fabricius, 1798)

Nguyen Duy Hoa[1,3], Roeland Wouters[2], Mathieu Wille[3], Vu Thanh[1], Tran Kim Dong[1], Nguyen Van Hao[1], Patrick Sorgeloos[3].

Research Institute for Aquaculture No.2, Ho Chi Minh City, Vietnam[1].

INVE Technologies N.V., Dendermonde, Belgium[2].

Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium[3].

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Abstract

Two fresh-food maturation diets were tested on wild *P. monodon* broodstock during a period of one month in primary quarantine and three months in secondary quarantine; diet A was composed of 70.30% squid (*Photololigo* sp.), 7.66% marine worm (polychaetes), 7.94% oyster (*Crassostrea* sp.), and 14.10% pork liver and diet B was composed of 37.39% squid, 16.50% marine worm, 27.14% oyster, and 18.98% pork liver; all on dry weight basis. The formulation of diet B was to resemble the ratios of ARA/EPA, DHA/EPA, and ω3/ω6 fatty acids of mature ovaries of wild *P. monodon*. At the start of primary quarantine, the average weight of the shrimps allocated to the two diets was not significantly different (129.71 ± 2.96g for females and 87.88 ± 2.49g for males in the group of diet A and 131.74 ± 1.75g for females and 88.95 ± 2.05g for males in the group of diet B).

After secondary quarantine, the growth (% weight gain) of the female shrimps receiving diet B was significantly greater ($P<0.05$) than the female shrimps fed diet A (24.44^{b} ± 4.98% compared to 12.89^{a} ± 3.24%, respectively). Shrimp fed diet B performed better than shrimp fed diet A in terms of spawning frequency (85% versus 57%), and fecundity ($458{,}796^{b}$ ± 35,658 and $245{,}718^{a}$ ± 34,736 eggs/spawn, respectively), but number of spawns, hatching success, fertilization success and metamorphosis success of the nauplii into zoea did not differ between the treatments ($P>0.05$). The success of diet B in terms of spawning frequency, fecundity, fertilization success and hatching success indicates the importance of the ARA/EPA and DHA/EPA ratios in broodstock nutrition of black tiger shrimp. This study also confirmed the success of natural mating of *P. monodon* in small tanks (1.25 m^2

bottom area). The 2-step biosecure quarantine procedure was applicable for producing SPF shrimp.

Keywords: shrimp, diet, maturation, reproductive performance

1. Introduction

Typical feeding practices for maturation of broodstock shrimp still rely on a wide range of unprocessed or frozen marine animals, including squid, various mollusks (mussels, clam, oyster…), fish roe, marine worm (polychaetes), and crustaceans (shrimp, crab, enriched *Artemia* biomass). Even beef or pork liver is frequently used in Vietnamese shrimp hatcheries. Recently however, crustaceans have been excluded from shrimp maturation regimes due to the risk of disease transmission. A review of the literature indicated frequent use of squid, marine worms, and mollusks as crucial dietary components due to their positive effects on Penaeid shrimp reproduction and due to their high levels of polyunsaturated fatty acids, especially arachidonic acid (ARA, 20:4ω6), eicosapentaenoic acid (EPA, 20:5ω3) and docosahexaenoic acid (DHA, 22:6ω3) (Primavera, 1983; Millamena et al., 1985; Menavesta et al., 1993; Cavalli et al., 1997; Browdy, 1998; Wouters et al., 2001; Coman et al., 2007a). In practice, the combination of these fresh-food items differs largely from hatchery to hatchery, between or even within countries. Furthermore, no information was found concerning a reference fresh-food feeding regime which could serve as a control diet for shrimp broodstock nutrition studies. A questionnaire filled-in by several shrimp hatcheries near Vung Tau City, Vietnam revealed that fresh-food feeding regimes for broodstock shrimps often consisted of around 75% of squid meat supplemented with 25% of a combination of oyster (or blood cockle), marine worm (polychaetes) and pork or beef liver.

In the present study, two experimental diets (diets A and B) were designed with the above-mentioned fresh-food ingredients but in different proportions (Table 1). Diet B was formulated with the approach of having a more balanced inclusion of fresh-food ingredients and moreover closely resembling ARA/EPA and DHA/EPA ratios of mature ovaries of wild *P. monodon* (Marsden et al., 1992). Diet A was based on formulas commonly practiced in several shrimp hatcheries near Vung Tau City, Vietnam.

Table 1. Proportion of the two fresh-food maturation diets

Ingredient	Diet A	Diet B
	% of wet weight (% dry weight)	% of wet weight (% dry weight)
Oyster	8 (7.94)	29 (27.14)
Pork liver	8 (14.10)	11 (18.98)
Squid	76 (70.30)	42 (37.39)
Marine worm	8 (7.66)	18 (16.50)
Total	100	100

2. Material and methods

This study was conducted at the coastal National Breeding Center for Southern Marine Aquaculture of Research Institute for Aquaculture No.2, located near Vung Tau City, Vietnam.

2.1 Broodstock shrimp

Wild shrimp breeders were collected from Rach Goc sea in the Mekong Delta of Ca Mau province, transferred to a biosecure building and stocked individually in separate tanks for screening of viruses (WSSV, YHV, MBV, and HPV). The specific-

pathogen-free (SPF) breeders were then transferred to and reared in a primary quarantine facility during one month. A second screening of viruses was applied at the end of primary quarantine to select SPF shrimp breeders for maturation in a secondary quarantine facility for another three months. After two months in secondary quarantine, the shrimp were eyestalk-ablated for evaluation of spawning and reproductive performance.

2.2 Fresh-food maturation diets

Fresh-food, including squid and oyster were obtained from fishermen at Vung Tau sea and pork liver was obtained from the supermarket at Vung Tau City in quantities sufficient for the duration of the trial and stored in a freezer of -25 ^{o}C. Marine worms (live organism) were bought daily alive through a middle man in Vung Tau City. Samples of the fresh-food ingredients were sent to the Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium for analyses of proximate composition, fatty acid profile (FAME) and lipid classes. The formulation of the diets was done using special feed formulation software at INVE Technologies N.V., Belgium. Two diets with different combinations of fresh-food items were formulated and fed to the shrimp during one month in primary quarantine and three months in secondary quarantine.

Diet A: a common combination of fresh-food items (squid, marine worm, oyster, and pork liver) practiced by several shrimp hatcheries near Vung Tau City, with 76% of squid and an equal inclusion of 8% of oyster, marine worm and pork liver (in wet weight).

Diet B: diet B was formulated to closely resemble ARA/EPA and DHA/EPA ratios reported for mature ovaries of wild black tiger shrimp (Table 2) (Marsden et al., 1992).

The two diets were fed to the shrimp at a ratio of approximately 15% of the shrimp body-weight (live weight) 4 times a day (at 6:00, 14:00, 18:00 and 23:00). Fresh-food was washed and soaked in freshwater for 30 minutes to eliminate marine parasites before feeding to the shrimp. The daily fresh-food ration was weighed daily according to the proportion of each ingredient, however the spawners were offered each component separately to avoid prefential consumption.

Table 2. Proximate composition, fatty acid profile and ratios of specific fatty acids of the diets and of mature ovaries of *P. monodon*

Nutrient	Diet A	Diet B	Mature ovaries of *P. monodon* (Marsden et al., 1992)
Crude protein (mg/g DW)	655.326	640.269	
Crude fiber (mg/g DW)	8.999	8.591	
Crude fat (mg/g DW)	140.581	187.832	
20:4ω6 (ARA) (mg/g DW)	4.599	5.850	
20:5ω3 (EPA) (mg/g DW)	6.363	9.361	
22:6ω3 (DHA) (mg/g DW)	14.657	10.881	
ω3 HUFA (mg/g DW)	19.923	20.183	
ω6 PUFA (mg/g DW)	4.16	5.832	
ARA/EPA ratio	0.723	0.625	0.624
DHA/EPA ratio	2.30	1.16	1.16
ω3/ω6 ratio	4.79	3.46	3.00

2.3 Experimental design

Primary quarantine (1 month):

After screening a total of 67 shrimps for viruses, 54 SPF shrimps (30 females and 24 males) were selected for primary quarantine. The SPF shrimps were allocated to thirty one-cubic-meter tanks with flow-through of disinfected seawater at a rate of 200% a day (Fig. 1). At the start of the primary quarantine, the average weight of the shrimp allocated to each diet was not significantly different (129.71 ± 2.96g for females and 87.88 ± 2.49g for males in the group of diet A and 131.74 ± 1.75g for females and 88.95 ± 2.05g for males in the group of diet B). Each dietary treatment consisted of 15 tanks (12 couples stocked in 12 tanks and the other 3 single females stocked in the other three tanks), divided into three replicate blocks (each block consisted of 4 couples and one single female) as indicated in Fig. 1.

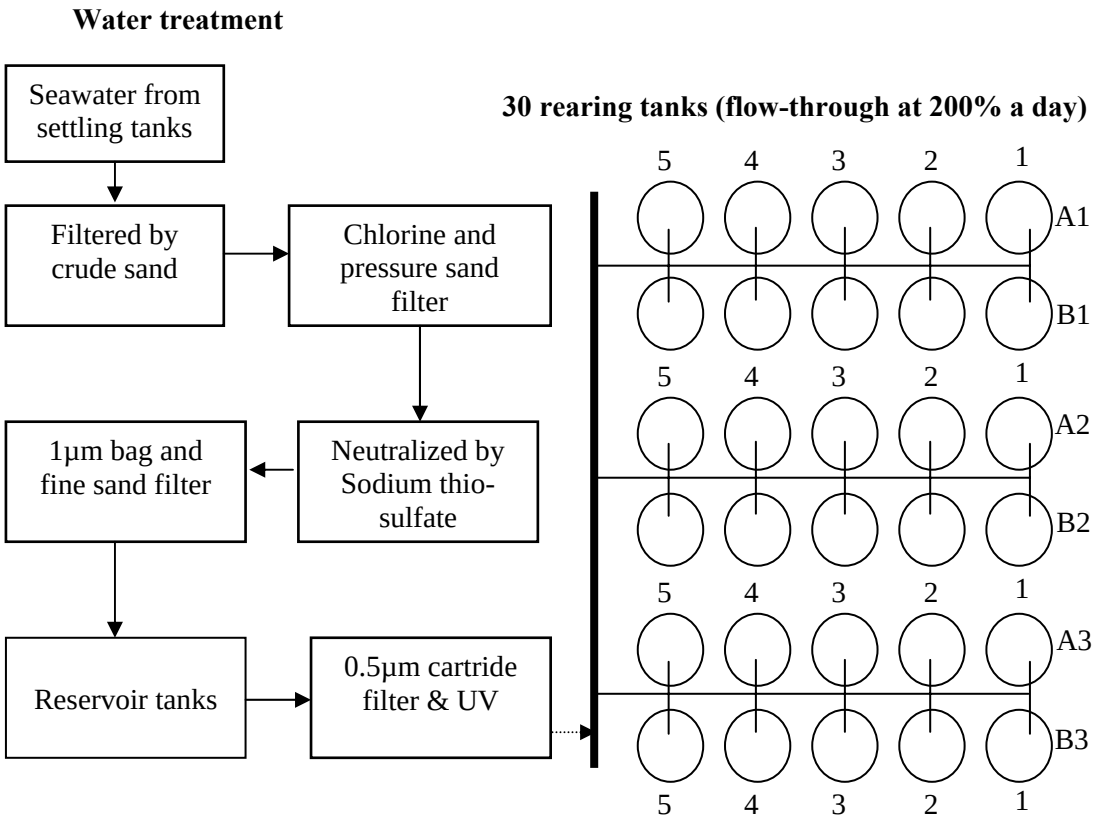

Figure 1. Schematic diagram of the water treatment and primary quarantine system

Secondary quarantine (3 months):

At the end of primary quarantine, the screening of viruses resulted in 52 SPF shrimps (30 females and 22 males). Two animals were infected with MBV. The SPF shrimp were transferred to the secondary quarantine facility and reared in six 5-m^3 recirculation tanks with sand substrate at a recirculation rate of 400% a day by an airlift mechanism while 10% of the tank water was replaced daily with newly-treated seawater. Black plastic curtains were installed in-between maturation tanks to create a dark environment for inducing maturation.

The shrimps from each treatment group in the primary quarantine were allocated to the corresponding dietary treatment in the 5-m^3 tanks (2.5m diameter) with 3 replicates for each diet. Each tank was stocked with 5 females and 3 to 4 males. After 2 months in secondary quarantine, eyestalk-ablation was applied to the molted females on the fourth day after molting and artificial insemination was applied to those females that were not naturally mated. Good spermatophores from selected males were squeezed out and inserted into the thelycum of un-mated females of the corresponding treatment using a smooth blade of grass. Insemination was performed by a skilled hatchery technician.

2.4 Evaluated parameters

Water quality:

- Temperature, salinity, pH, and dissolved oxygen were measured daily.
- Ammonia, nitrite, alkalinity, and nitrate were measured weekly.

Growth of the animals:

- After two months in secondary quarantine, only the females were weighted for the evaluation of the growth.

Growth (%) = (final weight – initial weight)*100/ (initial weight)

Maturation of the animals:

- Animals were eye tagged with plastic tags and tail tagged with colored soft wool for observation of molting and ovarian development of each female.
- A torch light was used to observe and record the maturation stages of each female. Females were examined for ovarian maturation daily with ripe females (ready to spawn) recorded as stage-4 ovaries (Tan-Fermin & Pudadera, 1989)

Natural mating:

- The presence of spermatophores on the thelycum (natural mating) was only checked 4 days after molting in order to reduce handling stress.
- Natural mating was observed during primary quarantine and during the first 2 months of secondary quarantine.

Natural mating (%) = (naturally mated molts/total molts) x 100

Spawning of the animals:

- After 2 months of secondary quarantine, females that were not naturally mated were artificial inseminated on day-2 and eyestalk ablated on day-4 after molting.
- Ripe females were transferred to 800 L (1.25m^2 bottom area) circular spawning tanks with moderate aeration and a water temperature of 28-29^oC, and allowed to spawn. The females were returned to their maturation tanks early the following morning to re-mature for the next spawning.

Fecundity (eggs per spawning):

- Six hours after spawning, the eggs were siphoned from the spawning tanks into 20-l bucket (concentrator) using a plastic tube and eggs were homogenously distributed through air-stone aeration.

- Six samples of 1ml were taken to count the number of eggs and the average value of the 6 samples was used to calculate fecundity.

Fertilization of the eggs:

- Shortly after spawning, 3 samples of 100 newly-spawned eggs were taken for microscopic observation.
- 4 or 8 cell-stage embryo was the indicator for distinguishing the fertilized and un-fertilized eggs. Un-divided eggs or eggs with an asymmetrical pattern were classified as un-fertilized eggs.

Hatching success and spawning frequency:

- Four samples of 50 newly-spawned fertilized eggs were transferred to a Petri-dish at room temperature to evaluate the hatching success. 50% of the seawater was replaced 4 times a day using needle and syringe during incubation.
- The spawning frequency (%) is expressed as the percentage of the ablated animals that spawned.

Metamorphosis success:

- Four replicates of 50 nauplii were placed in a 1-litre container to rear the nauplii to zoea1. Gentle aeration was provided with air-stones.
- The number of zoea1 stage larvae was counted 10 hours after the first zoea1 appeared, for estimation of the metamorphosis success.

3. Data treatment

Each tank was considered as one experimental unit. Statistical analysis was performed on the mean values of all experimental units within a replicate. Each dietary treatment counted three replicates. One-way-ANOVA and Student T-test were used to compare

the mean values obtained from the two dietary treatments (using Excel and Statistica 6.0 software).

4. Results

4.1 Water quality

Daily temperature fluctuated in the range of 28.5-30.5^{o}C. Dissolved oxygen fluctuated between 5 and 6 mg.L^{-1} in the primary quarantine system and 7 to 8 mg.L^{-1} in the secondary quarantine system. In the primary quarantine system, total ammonia was always below 0.01 mg.L^{-1}while recorded nitrite values were 0.03–0.27 mg.L^{-1}. In the secondary quarantine system, total ammonia and nitrite concentrations were mostly below the detection limit. The highest total ammonia concentration measured in the secondary quarantine system was 0.006 mg.L^{-1}. Salinity was between 30 and 34 g/.L^{-1} and pH between 7.6 and 8.3 during the whole period of primary and secondary quarantines.

4.2 Survival

At the end of primary quarantine, survival of the spawners was 100% for both diets. At the end of secondary quarantine, survival of the spawners was 96.15% and 92.30% for diet A and diet B, respectively.

4.3 Growth

At the end of primary quarantine, growth (expressed in % weight gain) of the shrimp fed diet B was significantly ($P<0.05$) different from the shrimp fed diet A. After two months in secondary quarantine growth of females was also significantly higher ($P< 0.05$) in dietary treatment B (Table 3).

Table 3. Growth (%) of the female shrimp after primary and secondary quarantine

	Growth of the shrimp (% weight gain)	
	Diet A	Diet B
After secondary quarantine	$12.89^{a} \pm 3.24$	$24.44^{b} \pm 4.98$

Different superscripted letters were significant different at P < 0.05

4.4 Natural mating

After 1 month in primary quarantine there were 16 molts from 24 females (the other six females which were reared separately were not taken into account due to the absence of males) of which 7 molted females had spermatophores on their thelycum. This corresponds to a natural mating rate of 44%. There were 3 spermatophore-carrying females from 7 molted females in diet A, corresponding to 42.86%, and 4 spermatophore-carrying females from 9 molted females in diet B, corresponding to 44.44%. During the first 2 months in secondary quarantine there were 54 molts from 30 females of which 35 females had been mated naturally, corresponding to a mating success of 65%. Of the 35 mated females, 16 mated females from in total 25 molts were coming from treatment diet A and 19 mated females from in total 29 molts were from treatment diet B, corresponding to a natural mating of 64% and 66%, respectively. There were no significant differences (P>0.05) for natural mating success during secondary quarantine between the two dietary treatments.

4.5 Reproductive performance

14 females from group A and 13 females from group B were eyestalk-ablated for evaluation of diet effects on reproductive performance (Table 4). In total, 8 females from diet A produced 16 spawns and 11 females from diet B produced 24 spawns, corresponding to a spawning frequency of 57% and 85% or 2.00 and 2.18 spawns per

female for diet A and diet B, respectively. Shrimp fed diet B exhibited a higher spawning frequency and significantly higher number of eggs per spawn than shrimp fed diet A ($P < 0.05$). Fertilization success, hatching success and metamorphosis success of nauplii into zoea were not significantly different ($P > 0.05$) among the dietary treatments.

Table 4. Reproductive performance of the shrimp fed two fresh-food maturation diets

Parameters	Diet A	Diet B
Natural mating during secondary quarantine (%)	63.89 ± 12.73^{a}	65.56 ± 5.09^{a}
Spawning frequency (%)	57	85
Spawns/Female	2.00	2.18
Eggs per spawning (egg)	$245{,}718^{a} \pm 34{,}736$ (n=16)	$458{,}796^{b} \pm 35{,}658$ (n=24)
Fertilization success (%)	$91.66^{a} \pm 2.51$ (n=11)	$92.79^{a} \pm 3.63$ (n=23)
Hatching success (%)	$83.78^{a} \pm 3.03$ (n=11)	$81.37^{a} \pm 9.75$ (n=23)
Metamorphosis success (%)	$87.50^{a} \pm 4.13$ (n=11)	$93.92^{a} \pm 3.62$ (n=23)

Different superscripted letters were significant different at $P < 0.05$

4.6 SPF postlarvae production

Part of the nauplli produced from the experiment was reared in 1-m^3 tanks in a biosecure facility and screened for SPF status (WSSV, YHV, MBV, and HPV). We applied the larval rearing protocol for Vietnam shrimp hatcheries as developed by NACA (Network for Aquaculture Centers in Asia-Pacific), SUMA (Support of Brackish Water and Marine Aquaculture funded by Denmark), and the former Vietnam Ministry of Fisheries (NACA et al., 2005). Four batches of postlarvae stage PL15 were obtained from different spawns of both dietary treatments. PCR analysis as well as histological tests revealed they were SPF. The results proved that the

applied 2-step biosecure quarantine procedure was effective for SPF-shrimp production.

5. Discussion

Courtship and mating behavior had been described for *P. monodon* (Primavera, 1979) with the important finding that mating requires a certain minimum water volume and depth. However, observation of natural mating of wild black tiger shrimp from our experiment indicated a good natural mating ratio of 44% in small tanks (1.25 square-meter bottom tanks: stocked with one couple in each tank) during primary quarantine or a natural mating ratio of 65% in large tanks (5 square-meter bottom tanks: stocked with 5 females and 3-4 males in each tank) during secondary quarantine. According to our observations, the actual mating only takes a very short time and the success of mating seems more related to the presence of hard-shelled males of good quality (sufficient ready-to-mate males). Water volume requirements in our opinion seem more related to provide sufficient space for the females to shed the old exoskeleton during the molting process. Of course, large tanks, which are normally stocked with higher numbers of males, do have more males available for mating than small tanks, which could explain the earlier observations. The present study nevertheless showed the possibility to obtain natural mating of black tiger shrimp in small tanks, offering opportunities for research on *P. monodon* in multiple, replicated small tanks, which could prove a very useful tool e.g. to study sperm quality in male shrimp.

Kanazawa (1985) reviewed fatty acid requirements of Penaeid shrimp and emphasized the necessity of ω3-highly unsaturated fatty acids (HUFA) in increasing weight gain of shrimp. The nutritive value of lipids for prawn and shrimp is related to the types and content of essential fatty acids (EFA) such as ω3-HUFA. The review

pointed out the inferior quality of soybean oil (rich in 18:3ω3), possibly due to low levels of ω3-HUFA such as 20:5ω3 and 22:6ω3. Natural marine organisms normally consist of lipids rich in ω3-HUFA (Primavera, 1985); therefore, a diversified mix of natural food organism is generally recommended for Penaeid broodstock shrimp (Naessens et al., 1997). Chamberlain and Lawrence (1981) reported that a squid diet outperformed shrimp, clam or worms when fed separately. However, a combination of feeds yielded the best reproductive performance, outperforming any of the diets fed separately. Bray et al. (1990) reported that diets with multiple fresh components including squid, blood worms, shrimp and brine shrimp performed better than solely squid. The two diets in our experiment had almost the same protein levels (65.5% versus 64.0%) and fiber contents (9.0% versus 8.6%) for diet A and diet B, respectively but diet A consisted mainly of squid (70.30%) while diet B had a more balanced inclusion of the same fresh-food ingredients (namely squid, marine worm, oyster and pork liver). Diet B resulted in a slightly increased ω3-HUFA level, especially in 20:5ω3 which could have contributed to the better growth of shrimp observed with this diet. The composition of some natural feeds used in Penaeid shrimp maturation in Australia was reported by Marsden et al. (1992). While squid had the highest level of essential amino acids, green mussels had the highest level of long-chain fatty acids and the highest ω3/ω6 ratio. Our analysis showed that squid had higher levels of DHA than EPA and ARA, while oyster had higher levels of EPA than ARA and DHA. Marine worms and pork liver had higher levels of ARA than EPA and DHA. Therefore, the better balanced inclusion of the different fresh-food ingredients in diet B tends to result in a more balanced fatty acid profile.

The fatty acid composition of the lipids has also been shown to affect the reproductive performance of shrimp (for a review see Harrison, 1990; Wouters et al., 2001). In an

experiment carried out by Millamena et al. (1985) with 4 distinct diets, the diet with higher PUFA content and ω3/ω6 ratio and a fatty acid profile closely resembling that of maturing ovaries of wild *P. monodon*, gave higher reproductive performance (in terms of number of spawns, fecundity, egg and nauplii production and egg hatching success) compared to the other diets.

It has been proposed that prostaglandins (PGs) play an important role in egg production and spawning of freshwater snails and bivalves (Kunigelis and Saleudin, 1986; Matsutani and Nomura, 1987; Osada and Nomura, 1990). Some researchers (Spaziani et al., 1993, 1995; Sagi et al., 1995) reported that PGs are related to vitellogenesis and spawning in decapod crustaceans. Tahara and Yano (2004) reported that ovarian prostaglandins (PGs) and arachidonic acid (ARA) are involved in ovarian maturation of kuruma prawn (*Marsupenaeus japonicus*). ARA and EPA are essential components of cell membranes and are precursors for 2-series and 3-series PGs, respectively, while DHA is known to be important for development of the central nervous system in crustaceans (Xu et al., 1994). It has been proven that marine worms (polychaetes) are critical to successful nauplii production in *L. vannamei* (Browdy, 1992) through female ovarian promotion (Bray and Lawrence, 1992). Polychaetes are not only a source of HUFA but also possibly a source of other hormonally active compounds (Lytle et al., 1990). Using liquid chromatography-mass spectrometry analysis, the atokous polychaetes were found to contain several types of prostaglandins, of which PGF2α was the most abundant at a concentration of 0.2μg/g wet weight of the polychaete. Injection of an ethanol extract from the polychaete *Perinereis nuntia*, which was further purified by fast performance liquid chromatography, into domesticated black tiger shrimp resulted in an induction of ovarian maturation and spawning in the eyestalk-unablated animals (Poltana, 2005).

In vitro incubation of *P. monodon* previtellogenesis oocytes with PGE_2 extracted from polychaetes and with synthetic PGE_2 showed that PGE_2 had an effect on oocyte maturation especially during the late maturation and ovulation (Meunpol et al., 2005a). In another report, Meunpol et al. (2005b) tested a diet constructed to mimic the HUFA profile of polychaetes (high ARA level and moderate DHA and EPA levels) and fed it to farmed male black tiger shrimp. The diet resulted in reproductive output comparable to what can be expected for wild males.

In our experiment, diet B was formulated to closely resemble ARA/EPA and DHA/EPA ratios of maturing ovaries of the species (Marsden et al., 1992). Compared to diet A, diet B had a similar level of ω3-HUFA, but relatively more EPA and ARA. Compared to diet A, diet B resulted in a higher spawning frequency and significantly higher egg fecundity ($P<0.05$). Our analytical results showed that oysters contained higher levels of EPA than squid, marine worm and pork liver; while pork liver, oyster and marine worm contained higher levels of ARA than squid. Therefore, the higher inclusion of oyster and marine worm (polychaetes) in diet B resulted in higher EPA and ARA levels than in diet A and this may have contributed to the higher spawning frequency and significantly higher egg production of the shrimp fed diet B. However, the results revealed no significant differences ($P>0.05$) for fertilization success, hatching success, and metamorphosis success of the nauplii into zoea between the two dietary treatments. In other words, egg and nauplii quality were comparable between the two dietary treatments. The higher DHA level in diet A may have contributed to a similar egg and larval quality as in diet B despite the fact that spawning frequency and egg fecundity were lower in diet A. The present study seems to be in agreement with previous studies on the roles of ARA, EPA and DHA in reproduction (see above). As precursors of prostaglandins, ARA and EPA may be more involved in spawning and

egg or nauplii production while DHA plays a more prominent role in egg and larval quality.

A fresh-food diet including crab, squid, clam, and beef liver was fed to female wild black tiger shrimp of 65-120g in an experiment carried out by Uddin et al. (2005) and the results indicated a fecundity of 170,300 (70,000-375,000) eggs per spawn with an average fertilization success of 77.26% and a hatching success of 83.28%. In another study, wild black tiger broodstock shrimp with an average weight of 90g gave a fecundity of 140,340 eggs per spawning with an average hatching success of 36.4% when fed a diet consisting of 30% squid, 15% bivalves, 5% polychaete and 50% pellet (Coman et al., 2006). Wild female shrimp with an average weight of 101.70 ± 4.76g to 125.27 ± 9.97g fed frozen squid and formulated maturation pellets resulted in 3826 eggs g^{-1} female with an average hatching success of 38.4% when applying a black light regime and 3994 eggs g^{-1} female with an average hatching success of 87.4% using a natural light regime and 6357 eggs g^{-1} female with an average hatching success of 61.8% when using a green light regime (Primavera and Caballero, 1992). Female shrimp in our experiment weighed 146.43 ± 4.20g and 163.94 ± 6.56g in the diet A and diet B treatment, respectively. They produced on average 245,717 ± 34,736 and 458,796 ± 35,658 eggs per spawning with a spawning frequency of 57% and 85%, fertilization success of 91.66 ± 2.51% and 92.79 ± 3.63%, and hatching success of 83.78 ± 3.03% and 81.37 ± 9.57% for diet A and diet B, respectively. When comparing these results with previous studies, diet B proves successful in terms of spawning frequency, fecundity, fertilization success and hatching success, indicating that the dietary ARA/EPA and DHA/EPA ratios may be important factors in broodstock nutrition and domestication, especially since low fecundity is often observed in domesticated black tiger broodstock.

6. Conclusions

A diversified fresh-food mixture of squid, oyster, marine worm, and pork liver formulated to have the similar ARA/EPA and DHA/EPA ratios to that of mature *P. monodon* ovaries enhanced significantly the growth and fecundity of the shrimp. This study also confirms that black tiger shrimp are able to mate naturally in small tanks with a bottom area of 1.25 square meters. In this respect, the quality of the males and the availability of hard-shelled males seem to be much more important factors for successful natural mating of black tiger shrimp than the tank volume or space aspect. Minimum space requirements should however be respected to sustain the normal molting process of the females.

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Chapter V Section II

Spawning performance and offspring quality of domesticated black tiger shrimp *Penaeus monodon* fed a semi-moist maturation pellet

Nguyen Duy Hoa[1,3], Roeland Wouters[2], Mathieu Wille[3], Vu Thanh[1], Tran Kim Dong[1], Nguyen Van Hao[1], Patrick Sorgeloos[3].

Research Institute for Aquaculture No.2, Ho Chi Minh City, Vietnam[1].

INVE Technologies N.V., Dendermonde, Belgium[2].

Laboratory of Aquaculture & Artemia Reference Center, Ghent university, Belgium[3].

Abstract

In two independent experiments, spawning performance and offspring quality of 11 and 13-month old F2-domesticated black tiger shrimp (*Penaeus monodon*) fed different feeding regimes was evaluated. A soft, semi-moist maturation diet (BREED-S FRESH®, INVE Aquaculture N.V., Belgium) was used for partial replacement of a fresh-food diet. In experiment 1, the shrimp were fed three feeding regimes: 100% fresh-food mixture (100FF), 40% semi-moist maturation pellets plus 60% fresh-food mixture (40SP), and 60% semi-moist maturation pellets plus 40% fresh-food mixture (60SP). The fresh-food mixture was composed of squid, oyster, marine worm and pork liver and was formulated to resemble the ARA/EPA, DHA/EPA, and ω3/ω6 fatty acid ratios of mature ovaries of wild black tiger shrimp. In experiment 2, treatments 100FF and 60SP were repeated but marine worms were omitted from the fresh-food mixture to reduce the risk of disease transmission. The experimental feeding started approximately 2 months before eyestalk ablation, and was continued 5 weeks post-ablation during which the evaluation parameters were monitored.

The present study demonstrated that up to 60% of the fresh-food mixture could be replaced with commercial semi-moist pellets without loss in spawning performance of the domesticated *P. monodon*: spawns per female and fecundity were very similar ($P>0.05$) between the dietary treatments in both experiments. Interestingly, feeding the semi-moist pellets to the broodstock resulted in improved egg and larval quality. In both experiments a significantly higher hatching success ($P<0.05$) was obtained in the treatment 60SP than in the treatment 100FF. In experiment 1, a significantly higher metamorphosis success ($P<0.05$) was also obtained in treatment 60SP than in treatments 100FF and 40SP. This positive effect on offspring quality persisted during

the hatchery cycle, resulting in a higher survival at stage postlarvae 15 in treatment 60SP.

Keywords: semi-moist pellet, maturation, domestication, spawning performance

1. Introduction

Domestication of black tiger shrimp (*P. monodon*), i.e. the use of pond-reared broodstock, has been practiced for more than two decades (Aquacop, 1979; Primavera, 1983; Withyachumnarnkul et al., 1998). However, closing the rearing cycle of *P. monodon* from egg to breeders in indoor tank systems has only started around 1997 (Coman et al., 2005). Moreover, low fecundity and poor egg hatching success (Coman et al., 2005; 2006; 2007b) have remained serious bottlenecks for commercializing of domesticated black tiger broodstock shrimp to the hatchery industry.

In the majority of the successful attempts, the maturation, spawning and reproduction of penaeid shrimp in captivity was accomplished through manipulation of nutrition, temperature, salinity, pH, light and density (Aquacop, 1979; Chamberlain, 1985; Primavera, 1985;). In particular, the role of nutrition in the reproduction process has been demonstrated for a variety of commercially important penaeid species (Kanazawa., 1985; Leger et al., 1989; Wouters et al., 2001; Racotta et al., 2003; Huang et al., 2008). One of the findings that has lead to practical feeding regimes - that are now being used world-wide – is that mixed diets of different natural fresh-frozen food items or combinations of fresh-frozen foods and dried formulated feed are generally better performing than single food feeding protocols (Arnstein & Beard, 1975; Beard et al., 1977; Santiago, 1977; Aquacop, 1979; Brown et al., 1979; Primavera et al., 1979; Beard and Wicklins, 1980; Emmerson, 1980; Kelemec &

Smith, 1980; Lawrence et al., 1980; Chamberlain & Lawrence, 1981; Alfaro & Lozano, 1993; Samuel et al., 1999; Wouters et al., 2002). Even though dried formulated broodstock feeds can not completely replace fresh food items, they are gradually gaining popularity in the shrimp hatchery business. Compared to fresh or fresh-frozen food, formulated feeds offer the following advantages: biosecurity, availability, consistent nutritional value and ease of use. In addition, essential nutrients, active substances (e.g. hormones) and therapeutics can easily be added.

The domestication of *P. monodon* shrimp is still in an experimental stage, while domesticated stocks of *L. vannamei* –among other species- have been commercialized for several years. One of the issues that required special attention in the domestication of *L. vannamei* was the weaning of breeders to artificial diets. Also with *P. monodon* broodstock, the limited ingestion of dry diets is a significant problem. The present study, therefore, investigated the potential of a newly formulated and commercially available semi-moist pellet (BREED-S FRESH® developed by INVE Aquaculture N.V., Belgium) to replace a typical broodstock diet based on diversified fresh-food components. The formulated semi-moist pellet has a very soft texture to promote pellet ingestion by the animals.

2. Material and methods

2.1 Broodstock shrimp and experimental design

9-month old F2-generation domesticated animals were recruited for the first experiment (experiment 1). The shrimp, with an average weight of 85.29 ± 3.26g for females and 64.56 ± 0.99g for males, were allocated to the experiment at a stocking density of 4 females and 4 males per 5-cubic-meter recirculation tank. The average weight of broodstock shrimp allocated to each feeding regime was not significantly

different (P >0.05) amongst females or amongst males. The shrimp were fed three different feeding regimes, including 100% fresh-food mixture (100FF), 40% semi-moist pellets plus 60% fresh-food mixture (40SP), and 60% semi-moist pellets plus 40% fresh-food mixture (60SP) for a period of 60 days, with 4 replicate tanks per dietary treatment. After this period, the 11-month old females (average weight 107.40 ± 8.23g) were eyestalk ablated for evaluation of spawning performance and offspring quality. In second experiment (experiment 2), 10.5-month old F2-generation domesticated animals (average weight of 95.88 ± 9.56g for females and 70-80g for males) were allocated to the tanks at a stocking density of 6 males and 4 females per 5-m^3 recirculation tank. The average weights of females was not significantly different (P>0.05) between dietary treatments. The males were randomly allocated to the experiment and were not weighed before or during maturation feeding to reduce as much stress as possible. Mating occurred by natural mating or by artificial insemination (Hoa et al., 2009). The shrimp were fed two feeding regimes during 70 days pre-ablation: 100% fresh-food mixture (100FF), and 60% semi-moist pellets plus 40% fresh-food mixture (60SP), with 6 tank replicates per dietary treatment. After this period, the 13-month age females - having an average weight of 120.20 ± 12.16g - were eyestalk ablated for evaluation of spawning performance and offspring quality.

2.2 Experimental diets

The fresh-food diet in experiment 1 was formulated to resemble the ARA/EPA, DHA/EPA and ω3/ω6 fatty acid ratios of mature ovaries of wild *P. monodon* (Marsden et al., 1992) and were composed of 37.39% squid, 27.14% oyster, 16.50% marine worm and 18.98% pork liver (Hoa et al., 2009), while the fresh-food mixture in experiment 2 was composed of 75 % squid, 16% oyster, and 9% pork liver, all on

dry weight basis (Table 4 & 5). Marine worms were excluded from experiment 2 to reduce the risk of disease transmission (Vijayan et al., 2005). Squid and oyster were obtained from fishermen at the Vung Tau Sea and pork liver was obtained from the supermarket at Vung Tau City in quantities sufficient for the duration of the trials and stored in a freezer at -25^{o}C. Marine worms (live) were bought daily through a middle man in Vung Tau City. The experimental semi-moist pellets (SP) with 60% protein, 15% lipid (on dry matter) and 28% moisture was imported from INVE Thailand Ltd. and stored in a freezer at -25^{o}C for the whole duration of each experiment. The composition of the semi-moist pellets and the fresh-food mixture is described in Table 1, 2 and 3. Meanwhile, the pellet became commercially available in the year 2009 under the name BREED-S FRESH®.

A preliminary experiment was set up to determine the ingestion rate (% dry matter ingested per day per wet weight shrimp biomass) of semi-moist pellet and the fresh foods by the broodstock. The minimum and maximum registered ingestion rates were then used to adjust the feeding rate. Finally, a feeding rate of 3 to 4% was applied during the experiments.

Table 1. Composition of the experimental semi-moist pellet

Ingredient group	Concentration (% of dry diet)
Fish meal	20.00
Squid meal	15.00
Marine protein blend *	23.27
Wheat gluten	10.00
Rice starch	9.50
Mineral mix	5.78
Soy lecithin	4.61
Fish oil	5.56
Kelp	2.00
Amino acids *	1.50
Vitamin mix*	1.36
Yeast	1.13
Astaxanthin	0.27
Antioxidants *	0.02
Sum	100

(*: proprietary blend INVE Technologies N.V.)

Table 2. Proximate composition, fatty acid and vitamin of the semi-moist BREED-S FRESH® pellet.

Composition	Concentration on DM
Protein (%)	60.1
Lipid (%)	15.5
EPA ($mg.g^{-1}$ DW)	10
DHA ($mg.g^{-1}$ DW)	15
ARA ($mg.g^{-1}$ DW)	4
n-3 HUFA ($mg.g^{-1}$ DW)	28
n-3 ($mg.g^{-1}$ DW)	29
n-6 ($mg.g^{-1}$ DW)	24
Vitamin A (IU/Kg)	22,600
Vitamin D3 (IU/Kg)	7,960
Vitamin E ($mg.Kg^{-1}$)	508
Vitamin C (mg/Kg^{-1})	2992

Table 3. Proximate and fatty acid composition of the fresh-food mixture in experiment 1 & 2

Compostion	Experiment 1	Experiment 2
Protein (%)	64.27	73.10
Lipid (%)	22.23	14.80
EPA ($mg.g^{-1}$ DW)	9.36	8.14
DHA ($mg.g^{-1}$ DW)	10.88	16.28
ARA ($mg.g^{-1}$ DW)	5.85	4.10
n-3 HUFA ($mg.g^{-1}$ DW)	21.88	25.36
n- 3 ($mg.g^{-1}$ DW)	23.24	26.14
n-6 ($mg.g^{-1}$ DW)	14.23	8.86

Table 4. Feeding regimes (% of dry matter) used in experiment 1

Ingredient	100FF	40SP	60SP
Squid	37.39	22.43	14.96
Oyster	27.14	16.28	10.86
Polychaetes	16.50	9.90	6.60
Pork liver	18.98	11.39	7.59
BREED-S FRESH® pellet	0	40	60

Table 5. Feeding regimes (% of dry matter) used in experiment 2

Ingredient	100FF	60SP
Squid	75.75	30.3
Oyster	15.25	6.1
Pork liver	9	3.6
BREED-S FRESH® pellet	0	60

Note: FF stands for fresh food; SP stands for semi-moist pellet BREED-S FRESH®

2.3 Spawning and reproductive assessment

Females were eye-tagged by numbered plastic rings for individual observation and tail-tagged using different colored soft-wool for molting observation. During two months of maturation rearing, molts and mortality were recorded daily and on day 4 after molting females were examined for the evaluation of natural mating. At the end of maturation rearing (60 days in the experiment 1 and 70 days in the experiment 2), female shrimp were again weighed. After this period, if natural mating did not take place, artificial insemination was applied in the next morning of the molting. Eyestalk-ablation was applied on day 4 after molting and ablated-females were returned to the respective maturation tank for daily observation of ovarian maturation. A torch light was used to observe maturation stages of the shrimp and ready-to-spawn females (ovarian stage-IV, Tan-Fermin & Pudadera, 1989) were individually transferred to 800-l tanks and allowed to spawn. Gentle aeration was supplied to each tank. Spawned females were returned to the maturation tanks early in the morning for re-maturation. Approximately 6 hours after spawning, eggs were harvested from the spawning tank into a 45-l bucket and evenly distributed by increasing aeration. Six samples of 1-ml were taken with a pipette at different depths in the water column and the eggs counted for estimation of fecundity. The eggs were then washed with clean treated seawater (1-2 minutes), rinsed with a formalin solution (300 $mg.L^{-1}$ for 30 seconds), treated with povidone-iodine solution (20 $mg.L^{-1}$ for 30 seconds) and finally washed again with clean seawater (1-2 minutes) before stocking in the hatching tanks. The number of nauplii was estimated in the same way as for the eggs. They were also treated with formalin in a similar way. Four 1-l containers were arranged and provided with slight aeration for rearing 50-100 nauplii to the zoea stage for estimation of the metamorphosis success. The spawning and reproductive

performance of the animals was evaluated during a period of 5 weeks over two molting cycles of the ablated animals.

Spawning performance and offspring quality of the animals was evaluated by means of natural mating success (% of the molted females containing a spermatophore in their thelycum), average weight of the females at spawning, number of spawns per female, fecundity (number of eggs per spawning), hatching success (% of eggs hatching into nauplii; spawns with zero hatching were not included in the calculation), and metamorphosis success (% of nauplii that metamorphosed into zoea, counted 10 hours after the first zoeae appeared).

Part of the nauplii, which were produced from the spawns of the three dietary treatments, was reared separately in 1-m^3 and 3-m^3 tanks at stocking density 150 nauplii L^{-1}. The larval rearing protocol of NACA, SUMA and the former Vietnam Ministry of Fisheries (NACA et al., 2005) was applied. Nauplii from treatment 100FF were reared in 14 tanks of 1m^3 and 1 tank of 3m^3, nauplii from treatment 40SP were reared in 11 tanks of 1m^3 and 1 tank of 3m^3, and nauplii from treatment 60SP were reared in 7 tanks of 1m^3 and 3 tanks of 3m^3, until stage postlarvae 15 (PL15). Survival of the nauplii (%) until postlarve 15 was recorded in all tanks of each dietary treatment.

2.4 Water quality measurement

Temperature, salinity, pH, and dissolved oxygen were measured daily. Ammonia, nitrite, alkalinity, and nitrate were measured bi-weekly.

2.5 Data treatment

Each tank was considered as one experimental unit. Statistical analysis was performed on the mean values of the replicates. Each dietary treatment counted 4 replicates in

experiment 1 and 6 replicates in experiment 2. For larval rearing to postlarvae 15, each tank was also considered as one experimental unit. One-way-ANOVA and Student T-test were used to compare mean values.

3. Results

3.1 Water quality

Daily temperature fluctuated in the range of 28.5-30.5 ^{o}C. Dissolved oxygen was always between 7 to 8 mg.L^{-1}. The average ammonia and nitrite concentrations measured during the experiments were 0.076 ± 0.033 mg.L^{-1} and 0.021 ± 0.014 mg.L^{-1}, respectively. Salinity was between 32 and 35 g.L^{-1}, and pH between 7.6 and 8.3 during the whole period of the experiments. Alkalinity was measured in the range of 80-100 mg.L^{-1} during the whole period of the experiments.

3.2 Spawning performance and offspring quality

A low natural mating incidence was recorded for domesticated black tiger shrimp in both experiments. The observation during two months of maturation rearing indicated a natural mating success of 22.22% in experiment 1 (16 of the 72 moltings) and 17.95% in experiment 2 (14 of the 78 moltings). As such, artificial insemination was applied to fertilize fully mature females that were not mated naturally. On the other hand, egg fecundity results were high and hatching rates were very high, in both experiments. In experiment 1, average fecundity was 291,346 ± 31,972, 277,647 ± 71,736, and 306,636 ± 18,865 for the treatment 100FF, 40SP and 60SP, respectively. Experiment 2 resulted in lower fecundity than that of experiment 1, namely 265,949 ± 56,819 and 273,888 ± 37,008 eggs per spawn for treatment 100FF and 60SP, respectively (Table. 6 & 7).

Table 6. Spawning and reproductive performance of shrimp fed different diets in experiment 1

Parameters	100FF	40SP	60SP
Number of females	16	16	16
Initial female weight (g)	85.67 ± 3.43[a]	84.96 ± 3.79[a]	85.24 ± 3.55[a]
Number of males	16	16	16
Initial male weight (g)	63.91 ± 1.42[a]	65.15 ± 0.45[a]	64.61 ± 0.59[a]
Survival (%)	96.87	100	96.87
Female weight at spawning (g)	114.49 ± 6.64[a]	100.86 ± 7.39[b]	106.85 ± 5.01[ab]
Numbers of spawns/female	3.58 ± 0.50[a]	3.31 ± 0.54[a]	3.64 ± 1.06[a]
Fecundity (egg/spawning)	291,346 ± 31,972[a]	277,647 ± 71,736[a]	306,636 ± 18,865[a]
Hatching success (%)[*]	62.55 ± 5.74[a]	66.68 ± 6.01[ab]	75.03 ± 1.91[b]
Metamorphosis success (%)	93.11 ± 1.73[a]	93.63 ± 2.06[a]	96.28 ± 0.85[b]

[*]Unhatched spawns were not included in the calculation

Different superscripted letters were significantly different at $P < 0.05$

The effect of fresh-food replacement with 60% semi-moist pellets was similar in both experiments (Table. 6 & 7). A replacement of up to 60% of the fresh-food mixture with this artificial pellet did not affect ($P>0.05$) any of the spawning performance evaluation parameters. Hatching success, on the other hand, was significantly higher ($P<0.05$) in treatment 60SP than in treatment 100FF in both experiments. In experiment 1, a significant increase in metamorphosis success ($P<0.05$) in treatment 60SP was observed compared to that in treatments 100FF and 40SP, and finally also an increased percentage of larvae survived up to stage PL15 in large larviculture tanks stocked with offspring from treatment 60SP (Fig. 1).

Table 7. Spawning and reproductive performance of shrimp in experiment 2

Parameters	100FF	60SP
Number of females	24	24
Initial female weight (g)	95.93 ± 9.45[a]	95.83 ± 10.57[a]
Number of males	36	36
Initial male weight (g)	70-80	70-80
Survival (%)	100	100
Female weight at spawning (g)	119.63 ± 13.79[a]	120.77 ± 11.60[a]
Numbers of spawns/female	3.08 ± 1.16[a]	3.33 ± 1.22[a]
Fecundity (egg/spawning)	265,949 ± 56,819[a]	273,888 ± 37,008[a]
Hatching success (%)*	66.07 ± 7.56[a]	77.95 ± 4.76[b]
Metamorphosis success (%)	92.35 ± 1.53[a]	93.60 ± 1.63[a]

*Unhatched spawns were not included in the calculation

Different superscripted letters were significantly different at $P < 0.05$.

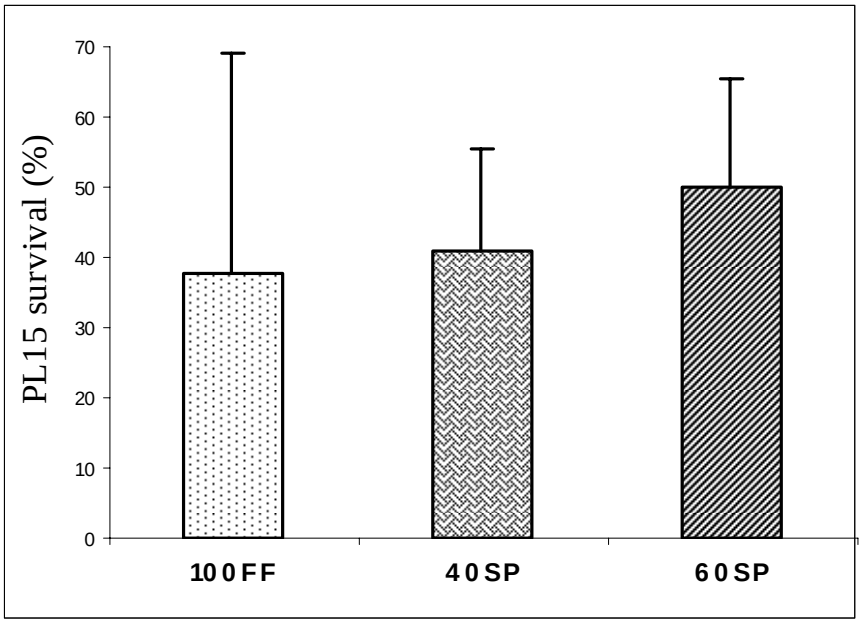

Fig. 1 Survival at postlarval stage PL15 of *Penaeus monodon* offspring originating from treatments 100FF, 40SP and 60SP of maturation experiment 1.

4. Discussion

It has been shown that mixed diets of different natural fresh-food ingredients or combinations of fresh-food ingredients and dried formulated feed yield better spawning performance than a single food feeding regime (Beard et al., 1977; Primavera et al., 1979; Emmerson, 1980; Lawrence et al., 1980). Several studies have demonstrated the effect of fresh-food replacement with dry diets on broodstock spawning and reproductive performance. Applying a fresh-food replacement level of 40 to 50% with a dry pellet (BREED-*Shrimp*® dry pellets) Verstraete et al. (1995) and Coutteau et al. (1998) reported a good reproductive output with white shrimp *L. vannamei* and *L. stylirostris*. Other authors were able to replace 50 to 88% of fresh-food mixtures with experimental formulated dry diets for several species (*L. vannamei* and *L. stylirostris* by Galgani & Aquacop, 1989; *L. vannamei* by Wouters et al., 2002; *Pleoticus muelleri by* Diáz and Fenucci, 2004); however, high fresh-food replacement with dry feeds has not been successful with *P. monodon* broodstock.

One of the bottlenecks often encountered with dry diets is that broodstock shrimp prefer fresh food items over dry diets due to the better palatability of fresh organisms. In a survey by Wouters et al. (2000) it was found that most hatchery operators encountered problems with applying dry artificial feeds at a rate higher than 1% of the wet shrimp biomass on a daily basis. The approach by Marsden et al. (1997) to use a highly palatable moist pellet for *P. monodon* broodstock did result in good spawning results at a 100% fresh-food replacement level. However, due to the very high moisture content (80% water) and the high inclusion of minced mussel and calf liver into the artificial diet, this type of diet was too expensive for commercialization and cannot be stored over a prolonged period even when kept refrigerated. The development of the BREED-S FRESH® pellet used in the current study, aimed at

increasing the softness of the pellet by allowing between 25 to 30% moist in the pellet in combination with adequate mold-inhibitors. The positive effect of the obtained palatability was demonstrated by the high pellet ingestion rates (3 to 4% dry weight matter per wet weight biomass per day) in the current trials and in preliminary trials with domesticated *P. monodon* broodstock. This high ingestion rate was an important factor in the successful substitution of fresh food at a level of 40% and 60%. Replacement levels above 60% has not been tested, however it does not seem unlikely that the pellet could be used at higher levels (70 to 100%) because at the 60% substitution level the hatching success was higher than in the control treatment in both experiments.

According to Xu et al. (1994), ARA (22:4n-6) and EPA (20:5n-3) are essential components of cell membranes and are precursors for 2-series and 3-series prostaglandins in crustaceans. Tahara and Yano (2004) reported that ovarian prostaglandins and ARA are involved in ovarian maturation of Kuruma prawn (*Marsupenaeus japonicus*). In another study with pond-reared *P. monodon* (Huang et al., 2008), egg ARA content was positively correlated with fecundity and egg production (r^2 = 0.6109 and 0.9876, respectively) while DHA (22:6n-3) and DHA/EPA ratio were negatively correlated with fecundity of *P. monodon* broodstock (r^2 = 0.5362 and 0.8702, respectively). EPA was also found to improve fecundity of *P. chinensis* (Xu et al., 1994). In another study by our team with wild *P. monodon* (Hoa et al., 2009), it was shown that a diet with high EPA and ARA levels significantly improved fecundity. It is also believed that EPA competes with the synthesis of enzymes that produce prostaglandins from ARA; resulting in EPA having a modulating influence over the quantity and efficacy of ARA-derived prostaglandins (Furuita et al., 2003). Although DHA exhibited a negative correlation with fecundity

of broodstock (Xu et al., 1994; Huang et al., 2008), it is believed that DHA may play some other roles in early embryogenesis (Xu et al., 1994; Wouters et al., 2001) related to the development of the central nervous system in crustaceans and to egg hatchability. As broodstock shrimp used in experiment 2 were 2 months older and about 10 to 15 grams heavier than the animals in experiment 1, it was expected to observe a higher fecundity of females used in experiment 2. On the contrary, fecundity was lower in experiment 2 than in experiment 1 (Table 6 & 7). Omitting marine worms in the fresh-food mixture in favor of squid in experiment 2 resulted in higher dietary DHA levels, but lower EPA and ARA levels as compared to experiment 1 (Table 3). These differences could partially explain the reduced fecundity and increased hatching success observed in experiment 2. However, in both experiments, the hatching success was significantly higher ($P<0.05$) in treatment 60SP than in treatment 100FF, indicating that BREED-S FRESH® pellets promoted hatching success better than the fresh-food mixture.

Maturation and reproductive performance of shrimp is strongly affected by the nutritional composition of the diet. Chamberlain and Lawrence (1981) reported that a squid diet outperformed shrimp, clam or worms when fed separately. In a previous study (Hoa et al., 2009), we demonstrated that a different mixture of fresh-food items resulted in different spawning performance. Similarly, we can expect that the change in fresh-food mixture in experiment 2 of the current study has had an effect. Our analytical results indicated that squid contains higher DHA level than marine worm, oyster, and pork liver. The main component in the fresh-food mixture of the experiment 2 was squid, resulting in higher DHA levels than in the control diet in experiment 1 (Table 3) which may explain why the metamorphosis success in the treatment 60SP of the experiment 2 was comparable ($P>0.05$) to that of the treatment

100FF, while in experiment 1 it was significantly higher ($P<0.05$) in treatment 60SP than in 100FF. Most probably, the observed good quality of the offspring with artificial semi-moist pellet is the result of a more balanced nutrient formulation compared to fresh food, in particular with respect to carotenoids, vitamins, minerals and highly-unsaturated fatty acids provided as was reported in several publications (reviewed by Wouters et al., 2001), For example, vitamin E has been shown to improve the percentage of normal sperm and the rate of ovarian maturation in *L. setiferus* (Chamberlain, 1988). Cahu et al. (1991, 1995) also indicated an increase in hatching percentage when increasing dietary α-tocopherol levels from 40 to 350 mg kg^{-1}. Ascorbic acid (vitamin C) levels in *F. indicus* eggs were also affected by dietary vitamin levels, and high hatching success of *F. indicus* eggs was related to high ascorbic acid levels in the eggs (Cahu et al., 1995).

The present study revealed a low natural mating success of F2-generation domesticated animals both in 11-month and 13-month age shrimp (22.22% and 17.95%, respectively). Parnes et al. (2007) observed low natural mating of penaeid shrimp in maturation recirculation systems and attributed this to the absence of sufficient ready-to-mate males. Makinouchi and Hirata (1995) reported higher natural mating success when using wild black tiger males (66.7%) than when using pond-reared males (32.4%). Reduction in reproductive performance of captive male shrimp results from a number of reasons, including stocking animals in captivity for a long period of time (Chamberlain et al., 1983; Leung-Trujillo & Lawrence, 1987) and/or inappropriate maturation diets (Meunpol, 2005b). In addition, age and size of the male shrimp could also be a factor affected the egg hatching success. Jiang et al., (2009) found a positive correlation between black tiger male size (body weight and age) and gonad weight, spermatophore weight, sperm count, and percentage of normal sperm.

In the present study, more males were stocked in experiment 2 than in experiment 1, yet natural mating was almost similar in both experiments. On the other hand, it was easier to select good spermatophores for artificial insemination in experiment 2. Possibly, the use of older and heavier males in experiment 2 also contributed to the slightly higher hatching success in experiment 2. These findings suggest that more research is needed to better understand the importance of male quality in domestication programs.

5. Conclusions and recommendations

This study with domesticated black tiger shrimp concludes that the tested semi-moist BREED-S FRESH® pellet yielded higher hatching success and comparable spawning performance (female shrimp growth, numbers of spawn per female, and fecundity) compared to a control treatment consisting of 100% fresh-food mixture, when applied at a 60% fresh-food replacement level. The results also call for more attention to male size, age and density in future research work.

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CHAPTER VI

GROW-OUT PERFORMANCE

Chapter VI

Grow-out performance of domesticated *Penaeus monodon* (Fabricius, 1798)

Hoa, N.D.[1,2], Wille, M.[2], Thanh, V.[1], Hao, N.V.[1], Sorgeloos, P.[2].

Research Institute for Aquaculture No.2, Ho Chi Minh City, Vietnam[1].

Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium[2].

Abstract

Grow-out performance of domesticated *P. monodon* stock was evaluated in an intensive farming trial in three different farms in Vung Tau and Soc Trang provinces, Vietnam. Preliminary results on farming this domesticated *P. monodon* stock indicated good grow-out performance in terms of growth and harvest yield. The yield of domesticated animals at harvest varied between 4.23 and 6.64 tons per ha in the Vung Tau farms and almost 5 tons per ha in the farm in Soc Trang, which was significantly higher ($P<0.05$) than for non-domesticated stock, averaging 4.4 tons per ha. Growth of domesticated *P. monodon* stock was also significantly higher ($P<0.05$) than for the non-domesticated stock, resulting in a shorter culture period to produce 20-gram animals (105 days for the domesticated stock in stead of 120 days for the non-domesticated stock). Survival and feed conversion rate (FCR) were comparable between the two stocks. A negative aspect of the domesticated stock was the observation of non-uniform body colour of the shrimp upon harvest. This is one of the aspects that need to be addressed in future genetic improvement programs.

Keywords: domestication, grow-out, *P. monodon*

1. Introduction

High performance lines of specific-pathogen-free (SPF) shrimp have been developed for Pacific white shrimp *L. vannamei* (Wyban, 2007). Production of black tiger shrimp *P. monodon* is however still largely based on wild stocks. *P. monodon* shrimp throughout the Asia Pacific region are infected with multiple viral diseases such as White Spot Syndrome Virus (WSSV), Monodon Baculovirus (MBV), Yellow Head Virus (YHV), Gill-Associated Virus (GAV), Hepatopancreatic Parvo-like Virus

(HPV), etc. Vietnamese shrimp farmers have experienced recurrent epidemics of WSSV, MBV and YHV since 1994 and have faced heavy economic losses due to slow growth and heavy mortalities. From 2004, the Vietnamese government therefore provided support for aquaculture research institutes to develop domestication techniques for *P. monodon*. The life cycle of *P. monodon* was then successfully closed at the Research Institute for Aquaculture No.2 (RIA2) in 2007 with more than 300 couples of SPF *P. monodon* breeders and 7 millions of domesticated postlarvae produced at the end of 2008 and early 2009. In the current study, part of these SPF domesticated postlarvae were used in a grow-out trial at different farms following an intensive farming model in Vung Tau and Soc Trang provinces, Vietnam.

2. Material and methods

15 day old SPF domesticated *P. monodon* postlarvae (PL15) were reared to PL24 stage and screened for a number of viruses (WSSV, YHV, GAV, MBV, and HPV) to confirm SPF status before stocking in a grow-out trial at three farms using an intensive farming model. In Vung Tau, one pond at Mr. Hung's farm (Vung Tau 1 farm) and 2 ponds at Mr. Tuan's farm (Vung Tau 2 farm) were stocked with F2 domesticated postlarvae (PL24), while in Soc Trang, at Mr. Huy's farm (Soc Trang farm), 4 ponds were stocked with F3 domesticated postlarvae (PL24) and 5 ponds were stocked with non-domesticated postlarvae (PL24) for comparison. The shrimp were stocked at a density of 26 animals at the Vung Tau 1 farm and 31-32 animals per m^2 at the other farms (Vung Tau 2 and Soc Trang farms).

Grobest commercial shrimp pellets (minimum 45% protein) were given to the shrimp at the Vung Tau 1 and 2 farms and Uni-president commercial shrimp pellets (38-44% protein) were given to the shrimp at the Soc Trang farm. All the ponds at the farm in

Soc Trang were around 0.6 ha in size, while the pond at the Vung Tau 1 farm was 0.4 ha and the two ponds at the Vung Tau 2 farm were respectively 0.4 ha and 0.17 ha. Water depth was approximately 1.5m in all ponds. Water salinity was around 15-18g.L^{-1} at the Soc Trang farm and 20-25g.L^{-1} at the Vung Tau farms. An intensive farming model was applied with paddle wheels installed in the experimental ponds.

At harvest, average size of the animals (number of animals per kg), growth (g.day^{-1}), survival (%), feed conversion ratio – FCR (amount of feed used per kg shrimp produced), and yield (tons per ha) were calculated.

3. Results

Grow-out performance of the shrimp varied from farm to farm, depending on the local conditions and the experience of the farmer. Results at the Vung Tau 1 farm were the best, resulting in the highest growth (0.24 g.day^{-1}), obtaining 31g-shrimp after a culture period of 130 days with a yield of 6.18 tons per ha, a low FCR of 1.29 and good survival of 74% (Table 1). Results at the Vung Tau 2 farm differed between the two ponds with good yield (6.64 tons per ha) and good growth (0.21 g.day^{-1}), and good survival after a culture period of 120 days (81%) in the 0.17 ha pond, while the other pond obtained lower growth (0.17 g.day^{-1}), smaller sized animals (51 shrimp per kg), and lower yield (4.23 tons per ha) and survival (67%) (Table 1). Results from the farm in Soc Trang indicated no significant differences ($P>0.05$) in terms of harvest size, survival and feed conversion rate between domesticated and non-domesticated *P. monodon* stocks, while a significantly different ($P<0.05$) growth and harvest yield were observed between the two stocks (Table 2).

Table 1. Grow-out performance of F2-domesticated *P. monodon* at the Vung Tau farms

Parameters	Pond 1(Vung Tau 1)	Pond 2 (Vung Tau 2)	Pond 3 (Vung Tau 2)
Pond area (ha)	0.4	0.17	0.4
Stocking density (PL/m^2)	26	32	32
Culture period (days)	130	120	115
Size (number of animals per kg)	32	39	51
Growth (g.day^{-1})	0.24	0.21	0.17
Survival (%)	74	81	67
Feed conversion rate – FCR	1.29	1.73	1.59
Yield (ton/ha)	6.18	6.64	4.23

Table 2. Grow-out performance of F3-domesticated and non-domesticated *P. monodon* at the Soc Trang farm

Parameters	Domesticated postlarvae	Non-domesticated postlarvae
Number of ponds	4	5
Pond area (ha)	0.6	0.6
Stocking density (PL/m^2)	31	31
Culture period (days)	105	120
Size (number of animals per kg)	48.5 ± 2.08^a	50.8 ± 2.95^a
Growth (g.day^{-1})	0.197 ± 0.008^a	0.165 ± 0.009^b
Survival (%)	75.75 ± 7.37^a	72.40 ± 6.43^a
Feed conversion rate - FCR	1.47 ± 0.146^a	1.55 ± 0.205^a
Yield (ton/ha)	4.94 ± 0.219^a	4.37 ± 0.184^b

Different superscript letters between columns denote significant differences ($P<0.05$)

Applying the same farming techniques at the Soc Trang farm (same pond-area, stocking density, feed, etc.), growth of the domesticated stock (0.197 g.day-1) was much higher than for the non-domesticated stock (0.165 g.day^{-1}). The yield obtained for the domesticated animals was also much higher as compared to the non-domesticated animals (4.94 and 4.37 tons/ha, respectively).

4. Discussion

Results from the grow-out trial with domesticated *P. monodon* at different farms indicated a better yield and higher growth ($g.day^{-1}$) when using domesticated stock as compared to non-domesticated *P. monodon* stock. However, observations at harvest showed a phenomenon of non-uniform body colour with the domesticated stock. Uniformity in colour is a very important aspect of product quality, which plays a big role in price setting. From discussion with some scientists and experienced shrimp farmers at the Research Institute for Aquaculture No.2 in Vietnam it was speculated that diet and environmental condition may be responsible for this phenomenon. However, at the same farm in Soc Trang with the same farming practices and the same diet, the non-domesticated stock displayed almost uniform body coloration. Owing to the lower fecundity of the domesticated breeders, the domesticated postlarvae originated from several domesticated females during the hatchery phase. Very likely this is also an important factor contributing to the non-uniform body colour in the domesticated stock at harvest. In contrast, the non-domesticated postlarvae were produced from only a few wild females (with high fecundity). Genetic improvement in the next step of the domestication program should address this problem.

The growth and harvest yield of the domesticated animals varied from farm to farm due to differences in farmer's experience and farming techniques at the different farms. Even within the same farm, differences in environmental conditions between ponds or differences in experience of the workers assigned to individual ponds, resulted in considerable differences in performance. However, at the same farm and when using the same farming techniques (Soc Trang farm), the growth and harvest yield were consistently higher when stocking domesticated *P. monodon* postlarvae

than when using non-domesticated stock, indicating not only differences due to farming techniques but also differences due to stock source. Using domesticated *P. monodon* stock reduced the grow-out period to produce a 20-gram animals to 105 days in stead of 120 days for the non-domesticated stock and significantly increased the yield to almost 5 tons per ha when stocking domesticated stock as compared to non-domesticated stock, which averaged only 4.4 tons per ha.

The growth of the domesticated *P. monodon* stock in the present study was comparable or better than values reported in some previous studies using non-domesticated *P. monodon* stock. For comparison, a few grow-out performance data in different farming systems are presented in Table 3 (modified from Tseng *et al*.,1998).

Table 3. Average growth and survival of *P. monodon* shrimp at different stocking density (SD), initial body weight (BW_o) and culture duration.

Reporter	Growth ($g.day^{-1}$)	Survival (%)	SD ($inds/m^2$)	BW_o (g)	Duration (days)
Bombeo-Tuburan et al. (1993)[a]	0.20-0.36	76.0-92.3	0.4	0.8	90
Hansford and Hewitt (1994)[b]	0.11-0.14	-	27	8.5-8.7	69
Allan et al. (1995)[c]	0.14-0.17	88.2-92.7	15	2.0-7.5	71
Tseng et al. (1998)[b]	0.25-0.30	80-100	40	7.5-8.0	56
Tseng et al. (1998)[b]	0.23	72.4-79.3	80	5.4-5.5	56
Tseng et al. (1998)[b]	0.13-0.19	60.3	160	5.4-5.9	56

[a]Shrimp cultured in extensive open ponds, using natural food.

[b]Shrimp cultured in small tanks, using pelleted feed.

[c]Shrimp cultured in small tanks, using pelleted and natural food.

In these studies, growth varied greatly between 0.11 and 0.36 $g.day^{-1}$. In the current study, growth of the domesticated *Penaeus mondon* stock was 0.17 $g.day^{-1}$ and 0.21 $g.day^{-1}$ for the two ponds at theVung Tau 2 farm, 0.24 $g.day^{-1}$ for the pond at the

Vung Tau 1 farm, and 0.197 ± 0.008 g.day^{-1} or 0.165 ± 0.008 g.day^{-1} for domesticated and non-domesticated stocks, respectively at the Soc Trang farm.

5. Conclusions

The domesticated *P. monodon* stock resulted in better growth, higher harvest yield and shorter grow-out period than non-domesticated *P. monodon* stock when grown out under intensive farming conditions at commercial shrimp farms.

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CHAPTER VII

GENERAL DISCUSSION

Chapter VII

General discussion

In Chapter II, current knowledge on domestication techniques for *P. monodon* was reviewed. Since the 1970's, several attempts have been undertaken to develop domesticated *P. monodon* stocks. Most of the studies during the 70's to the 90's; however, failed as they were confronted with low survival of the domesticated *P. monodon* stocks. Most of these early programs domesticated the shrimp in outdoor earthen ponds, which didn't allow proper biosecurity (Aquacop, 1979; Primavera, 1983; Withyachumnarnkul, 1998). It was not until the 2000's that some *P. monodon* domestication programs (for example in Australia and in Hawaii), that were able to sustain good survival of the SPF domesticated *P. monodon* stocks, clearly proved that indoor sand-based raceway systems or sand-based recirculation systems with strict biosecurity measures can be successful. However, the domesticated *P. monodon* stocks produced in these systems have not yet been commercialized, primarily due to poor performance in terms of egg fecundity and egg hatching success (Coman et al., 2005; 2006; 2007b). Thus, there is still significant scope to further improve broodstock nutrition and refine rearing conditions to improve the quality of the domesticated *P. monodon* stocks produced in these recirculation systems.

For that reason, a first step in our research consisted of the develepment of an indoor closed rearing system for domestication of *Penaeus monodon*.

Although closed systems are continuously gaining popularity in aquaculture, open systems are still most common. Commercial aquaculture of fish or shellfish in open-

system facilities using earthen ponds contains serious risks due to eutrophication of coastal water with phosphorous and nitrogen originating from wastewater of industrial and domestic origin. Unfavourable environmental conditions under eutrophic conditions may reduce growth or reproductive output, and impair health in fish or shellfish in open-system facilities. Furthermore, physiological stress under unfavourable environmental conditions may lead to immune suppression. This immune suppression will become lethal, either directly or indirectly, as a result of disease with an increased opportunity for spreading of infectious diseases. Indoor closed system facilities are effective to avoid unfavourable environmental conditions and diminish disease risk under these conditions. For this reason, current research programs to develop technology for the production of specific pathogen free (SPF) broodstock therefore focus on indoor closed recirculation systems which allow for proper control of pathogenic viruses, bacteria or parasites (Yano, 2000; Otoshi et al., 2003).

Since 2005 through mid 2006, our domestication program for *P. monodon* used an advanced recirculation system, which was adapted from recirculation systems in marine fish hatcheries in the Western hemisphere, with internal and external biofilters, and advanced protein skimmers. However, in these, the internal biofilter consisted of coral stones and coarse sand. Coarse sand is not a suitable substrate for *P. monodon* as it causes injury to the pleopods when the shrimp try to burrow in the sand. Water was also recirculated using pumps, pushing the rearing water into the small slotted pipes in the tank bottom beneath the coral stones and sand layer, in this way creating a strong water current percolating up (via small holes in the slotted pipes) through the coral stone and sand layers, resulting in disturbances to the shrimp. Water recirculation using pumps also heated up the rearing water in the tanks up to 31.5 ^{o}C during the

day, which again caused stress to the animals. Therefore, although water quality (in terms of ammonia, nitrite and dissolved oxygen levels) was very good in this system, the system was stressing the animals over time. Moreover, also the commercial pellets with low protein level (38-40%) fed to shrimp during this time resulted in poor growth and weak animals. A high incidence of cannibalism and "molt-associated mortality", a phenomenon which is believed to be related to limited energy reserves of the shrimp so that they can not successfully complete molting, resulted in low survival. The start of this program therefore appeared more difficult than expected as high mortality was experienced in the first months of ongrowing the juvenile *P. monodon* shrimp. After discussion with different shrimp experts and water recirculation specialists, and close observation of the animals, several new approaches were evaluated and adjustments to the rearing systems and feeding schedule were made, which eventually led to the development of a suitable sand-based recirculation system by mid 2006. This modified system offered a unique set-up for domestication and for further testing of the effect of different biotic and abiotic parameters in *P. monodon* shrimp domestication.

In Chapter III, using the developed sand-based recirculation system, a number of experiments were carried out to test different water exchange regimes, salinity regimes, stocking densities and diets in order to optimize the rearing techniques during the initial grow-out phase of *P. monodon*. Results of these experiments in small tanks ($1m^3$) and in large tanks ($10m^3$) indicated that the animals obtained a very good growth during the first 4 months of the grow-out phase (approximate 40g within 120 days starting from 1g animals) when reared in the sand-based recirculation system with a low water exchange regime at a rate of 5-10% a day and when fed a combination diet of 50% high protein pellets (55% protein) and 50% fresh-food

mixture (75% squid, 15% oyster, and 10% blood cockle). The low water exchange regime (5-10% per day) for this system also significantly improved survival of the shrimp in the initial grow-out phase. Low salinity regimes of 20-23g.L^{-1} and low stocking density of 10 animals per m^2 resulted in the best growth of the animals during the initial grow-out phase. Applying low salinity (20-23g.L^{-1}) during the initial grow-out phase also significantly increased survival of the shrimp as compared to full strength seawater (32-33g.L^{-1}) irrespective of stocking density.

Recirculation systems generally result in better and more stable water quality as compared to flow-through systems or earthen ponds with discontinuous water renewal. Therefore they promote better growth and survival (Tseng, et al., 1998; Courtland, 1999), and better maturation and reproduction (Chamberlain & Lawrence, 1981; Bray et al., 1990; Manesveta et al., 1991; Millamena et al., 1991; Yano, 2000; Timmons and Ebeling, 2007). They also allow tight biosecurity for SPF shrimp programs (Yano, 2000; Otoshi, 2003; Coman et al., 2005; 2006; 2007b;c). The sand layer applied in the recirculation system not only acted as an internal bio-filter (Coman et al., 2005) to improve water quality but also served as substrate for the shrimp to settle on and burrow into (Strasser and Felder, 1999), mimicking the natural spawning grounds. This behaviour is very specific for penaeid shrimp (Chamberlain, 1985) and definitely helps the animals to get away from environmental disturbances when reared in captivity. It seems *P. monodon* is especially sensitive in this respect and requires a quite environment, without excess food, noise, or other human activities in order to attain full maturation (Menasveta et al., 1991; Yano, 2000). The particle size of the sand also seems to be very crucial. *P. monodon* prefers fine sand to burrow in, however it is easier to oxygenate coarser sand, and therefore selection of the sand as substrate for shrimp in indoor recirculation systems is a trade off between

these two factors. Furthermore, the sand substrate plays an important role in water filtration, producing very clean and transparent water for the shrimp breeders. In nature penaeid shrimp mature in deeper oceanic waters; therefore, water used for maturation tanks must be clean and of very high quality, like that in the open ocean. Seawater should come from open ocean areas and then pass through a particle filter such as a fine-sand bed filter. Larger and less compact particle sand beds remove only the largest suspended materials allowing smaller materials to pass, causing turbidity and finally stressing the animals.

Water in the recirculation tanks with low water exchange regime is continuously recirculated and aerated, producing high and stable dissolved oxygen concentrations, which favour normal growth of the shrimp as well as the development of beneficial aerobic bacteria (especially *Nitrosomonas* sp. and *Nitrobacter* sp.) in the internal bio filter (sand-substrate). Consequently, higher survival and better growth was obtained in the low water exchange recirculation systems than in the flow-through water systems. However, metabolic activity of the bacteria in the internal bio-filter of the recirculation systems also produce carbon dioxide (CO_2) which gradually acidified the rearing water over time; as a result, the alkalinity levels also dropped over time. In this regard Primavera (1985) and Coman et al. (2005) suggested periodically adding sodium bi-carbonate ($NaHCO_3$) to the recirculation tanks. Water recirculation rates are very important to maintain adequate dissolved oxygen levels for the aerobic ammonia-oxidizing and nitrite-oxidizing bacteria in the sand substrate and should be at least 200% to 600% of the tank volume a day (Yano, 2000). The water recirculation rate of in the sand-based recirculation system developed in this study ranged from 280% to 400% a day.

With respect to salinity, low salinity water (20-23g.L^{-1}) probably resembles the natural coastal and estuarine habitat of juvenile, and therefore better growth and survival of the shrimp during this stage was obtained at this salinity compared to full strength seawater (32-33g.L^{-1}). At higher salinities animals have to spend more energy for osmotic pressure regulation resulting in lower growths; furthermore spending more energy for osmotic pressure regulation could also cause stress, which can accumulate over time, and result in lower survival and growth (Queensland Department of Primary Industries and Fisheries, 2006). Reduced growths associated with increased salinity may also be related to feed consumption (Villarreal et al., 2003). Although no direct data were obtained in the current study with respect to food intake, it was evident from visual observations that the juvenile shrimp reared in full strength seawater consumed less feed than when reared at lower salinity.

Together with the development of the sand-based recirculation system, a modified feeding regime consisting of a combination of high protein pellets (55% crude protein) and a high quality, diversified fresh-food mixture was applied from mid 2006 onwards. This feeding regime resulted in a much higher growth and produced more healthy animals compared to previous periods. Consequently, higher survival and significantly less “molt associated mortality” and cannibalism was observed compared to before. It seems likely that the more diversified diet fulfilled the nutritional requirements of the animals much better.

In Chapter IV, a protocol, covering all the rearing phases, was developed for complete domestication of *P. monodon* in indoor recirculation systems. This protocol was modifying from the protocol of the CSIRO program. However in our study, the total rearing cycle was split up into three phases (juvenile, sub-adult and adult stages) according to the life cycle of the *P. monodon* (Motoh, 1985) in stead of

only two phases in the CSIRO program (as described in Chapter IV). The juvenile stage (first 4 months) was considered as the grow-out phase where the animals prefer conditions related to the coastal habitat where they live in nature. Lower salinity brackish water and a more diversified diet similar to what is found in the coastal feeding grounds seem to be more suitable for this phase. In the sub-adult stage (month 4 to 8) where the animals migrate to areas of higher salinity a fresh-food diet should be available for the shrimp, while in the adult stage (from 8 months onwards) where animals in nature move to deeper depths of the open sea, white sand-substrate, a quiet and low light environment, and high quality maturation feeds should be provided to induce maturation. The rearing protocol accordingly applied a water salinity of 20-23g.L^{-1} to rear the animals in the initial grow-out phase up to 40g (approximate 4 months starting from 1g animals or 5 months when starting with PL15), while full strength seawater of 32-35g.L^{-1} was applied in the following two phases to rear the animals from 40 to 80g (approximately 4 months) and 80 to 110g (approximate 70 days). The animals were fed on a combination diet of 80% high protein pellets (55% protein) and 20% fresh-food mixture consisting of 75% squid, 15% oyster and 10% blood cockle in the grow-out phase, while the proportion of the fresh-food mixture was increased to 50% in the prematuration phase or sub-adult stage. In the maturation phase or adult stage, the high protein grow-out pellet used in the juvenile and sub-adult stages was replaced with a high quality semi-moist maturation pellet and totally dark environment was created. A very strict screening for specific viruses was applied to recruit in-coming animals for the domestication program.

The developed protocol demonstrated that the life cycle of *P. monodon* from postlarvae to animals of around 110g could successfully be closed within 11-12 months and that this domestication protocol could be applied commercially to provide

SPF domesticated *P. monodon* breeders to the hatchery industry. Furthermore, this domestication scheme could potentially be optimised by recruiting a new batch of animals twice year with a 4-month interval and starting to harvest breeders 1 month earlier. In this way, the suggested domestication cycle could not only produce shrimp seed for the two main shrimp farming seasons in the South of Vietnam (the first shrimp farming season lasts from February to June and the second farming season lasts from June to November), but could also reduce the facilities needed for the domestication process. Normally the production of postlarvae requires around two months from the arrival of the breeders at the hatchery until PL15 stage; therefore, the first harvest of domesticated breeders should start in December for the hatcheries to be able to provide postlarvae to the shrimp farmers in February. For the second farming season, harvest of breeders should start in April of the next year to provide the broodstock for the shrimp hatcheries to produce postlarvae for a second crop in June.

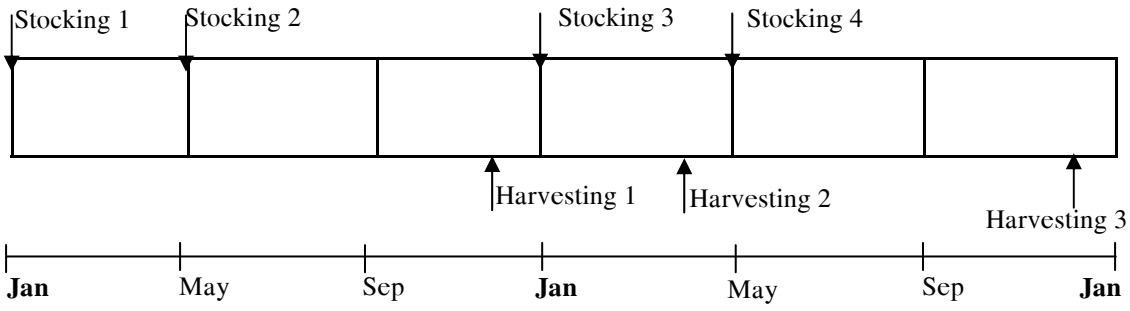

Diagram 1. Suggested domestication cycle to produce SPF domesticated *P. monodon*

Although our domestication protocol produced after 11 months domesticated animals that were 10 grams smaller (107g) than in the CSIRO program (117g), the fecundity and egg hatching success of the animals produced in our domestication program were almost double than that of the animals produced in the CSIRO program as reported in

Coman et al. (2005; 2006; 2007a,b). Higher inclusion of a quality fresh-food mixture, which was applied earlier in the sub-adult stage and the application of high quality semi-moist maturation pellets (Breed-S Fresh®) (INVE, Aquaculture N.V., Belgium) used in the maturation stage in our domestication program may have improved the quality of the domesticated animals in terms of fecundity and egg hatching success, as compared to the domesticated *P. monodon* in the CSIRO program, which were fed mainly commercial pellets during the first 8 months of the domestication process and still received 45% pellets during the maturation phase (Coman et al., 2005). Survival of the shrimp in the CSIRO program until 11 months was as high as in our program; however, it rapidly dropped to around 40% (Coman et al., 2005) after 11 months, which may indicate the condition of the animals was impaired to some extent.

The much lower survival of the shrimp during the second rearing phase (approximate 66% on average) as compared to the other two phases in our domestication program could point out suboptimal environmental conditions or diet in this phase. The Queensland Department of Primary Industries and Fisheries (2006) reported that wild *P. monodon* mature at an age of 5 to 12 months and at a body weight of 35-50g for males and 68-75g for females; therefore, it might be beneficial to consider providing maturation diets and covering the tanks at a size of 50-60g already instead of waiting until they reach a weight of 80g as we did in our program. Further modifications of the rearing protocol are therefore still required to better meet the requirements of the animals for each phase in their development. In our opinion, the first phase could be prolonged to 50g in stead of 40g, while in the second phase (50g to 80g) the tanks could be covered already but shrimp should still be fed a high percentage of fresh-food mixture to promote good growth and quality; however, the high protein grow-out pellets applied in this phase should be replaced by the semi-moist maturation pellet

already, in a lower inclusion level as compared to the maturation phase. A feeding regime consisting of 60% fresh-food mixture plus 40% semi-moist maturation pellet during the second rearing phase should be tested, while for the maturation phase a feeding regime of 40% fresh-food mixture and 60% semi-moist maturation pellets, similar to what was used in the current study, could be applied.

In Chapter V, broodstock nutritional requirements were discussed and maturation diets as well as feeding regimes were developed for domesticated *P. monodon* broodstock.

The lipid content of diets has long been known to have a large effect on reproduction in marine fish and crustaceans. One class of lipids, the highly unsaturated fatty acids (HUFA's), especially arachidonic acid (ARA), eicosapentanoic acid (EPA), and docosahexaenoic acid (DHA) has been shown to be particularly important for reproduction in many shrimp species, and accordingly, has received considerable research attention (for reviews see Wouters at el, 2001; Racotta et al., 2003). Recently, research on aquatic broodstock nutrition has recognised the importance in the ratios of ARA/EPA and DHA/EPA in maturation diets. Several studies evaluating the biochemical composition of the mature ovaries, egg and nauplii of wild penaeid broodstock have reported ratios of EPA to DHA ranging from 0.5 to 1.0, and ratios of ARA to EPA ranging from 0.3 to 0.6 (Crocos et al., 1992; Marsden et al., 1992; D'souza and Kelly, 2000). A study by Bransden et al. (2004) found that the dietary ARA level can alter tissue ARA/EPA and prostaglandin production in larval stripped trumpeter (*Latris lineate*). Accordingly, both the concentration of ARA in the diet, and the ARA/EPA ratio, will influence reproductive performance and subsequent embryonic and larval quality in aquatic species. Mazzora et al. (2003) found that dietary ARA/EPA ratios of 0.22 resulted in higher egg fertilization, embryo

development, hatching, and larval quality than a diet with an ARA/EPA ratio of only 0.05. Japanese flounder (*Paralichthys olivaceus*) fed on a diet containing ARA/EPA ratios of 0.71 showed higher egg production, egg quality, hatching and larval quality, than broodstock fed on diets with lower (0.12) or higher (1.49) ratios (Furuita et al., 2003). While there seem clear benefits of increased levels of dietary ARA for reproduction, negative effects of overdoses of ARA have also been reported (Sargent et al., 1999; Glencross & Smith, 2001; Furuita et al., 2003; Tveiten et al., 2004). Notably, higher ratios of ARA/EPA and DHA/EPA are required for reproduction in tropical, as compared to temperate, species (Emata et al., 2003; Ogata et al., 2004). Regarding types of broodstock diets, penaeid shrimp broodstock rearing still depends to a large extent on fresh or frozen natural foods, sometimes supplemented with artificial pellets. So far no one has successfully applied 100% dry artificial diets both in research and in commercial applications. Almost every attempt to completely replace the fresh-food mixture with artificial dry diets resulted in a decrease in ovarian maturation, a reduced number of spawns, and inferior larval quality (Wouters et al., 2000). However, partial replacement of fresh foods with artificial diets often gives better reproductive results. In commercial maturation facilities, the use of artificial maturation diets is common, but only at low level. One of the bottlenecks often encountered with dry diets is that broodstock shrimp prefer fresh food items over dry diets due to the better palatability of fresh organisms and therefore recent research has switched to an approach of using artificial diets with soft texture, called “semi-moist diets” for shrimp broodstock in stead of using dry artificial diets (Marsden et al., 1997; Wouters et al., 2000; 2001).

Our domestication program successfully developed a fresh-food mixture for *P. monodon* broodstock based on the ratios of DHA/EPA, ARA/EPA and ω3/ω6 fatty

acids in the mature ovaries of wild *P. monodon* (Marsden et al., 1992) (Section I of Chapter V). This fresh-food mixture significantly improved the growth and fecundity of the animals, supporting good performance of both the sub-adult stage (prematuration stage) and the adult stage (maturation stage). In the maturation phase, a substitution of this fresh-food mixture by a semi-moist maturation diet (BREED-S FRESH®) (INVE Aquaculture N.V., Belgium) was tested to determine a suitable feeding regime for domesticated *P. monodon* broodstock (Section II of Chapter V). The replacement of the fresh-food mixture by up to 60% of the semi-moist maturation pellets did not result in any negative affects on maturation and reproductive performance. Moreover, the semi-moist pellet resulted in better egg hatching success and higher offspring survival to the PL15 stage. Further testing should attempt higher replacement levels (70-100%) as this would significantly improve biosecurity for production of SPF shrimp. Modifications of the ARA/EPA and DHA/EPA ratios or the quantity of ARA in the maturation diet of *P. monodon* should also be further tested to improve the reproductive performance of *P. monodon* broodstocks.

In Chapter VI, domesticated *P. monodon* postlarvae were ongrown at three different farms in intensive farming trials. The grow-out performance of these domesticated postlarvae was evaluated.

Successful domestication of *P. monodon* in our program resulted in more than 300 SPF domesticated *P. monodon* breeders and 7 million domesticated postlarvae were produced from the experiments in 2008 and early 2009. At the moment, the developed domestication protocol is being applied at the coastal National Breeding Center for Southern Marine Aquaculture in Vung Tau City in an up-scaling program aiming to produce up to 1,000 couples of domesticated *Penaeus mondon* breeders a year.

Preliminary results on the grow-out performance of the domesticated *P. monodon* postlarvae at three farms using an intensive farming model (Chapter VI) indicated higher yields at harvest (approximate 5 tons per ha on an average) and better growth than when stocking non-domesticated *P. monodon* stock. Domestication programs offer good scope for genetic improvement (for example in terms of growth) through selective breeding. In a domestication program for *P. monodon* in Malaysia, a 13% improvement in weight gain was observed in F2 as compared to F1 animals over a culture period of 105 days and a 7.3% further weight gain improvement in F3 as compared to F2 animals (Subramaniam et al., 2006). One problem that was identified when using domesticated stocks related to the non-uniform body colour of the shrimp at harvest. More work on genetic improvements is therefore required before commercialization of these domesticated *P. monodon* stocks.

Over the last few years, *P. monodon* farming has stagnated and has been substituted with Pacific white shrimp (*Litopenaeus vannamei*) to meet the increasing demand on international markets. The major factor compromising the further development and sustainability of *P. monodon* farming has been the lack of a reliable supply of *P. monodon* breeders and high quality seed. The consistent availability of SPF domesticated stocks would greatly aid sustainable development of the *P. monodon* farming industry. Successful domestication programs together with efforts on genetic improvement could give a tremendous boost to the local shrimp industry in the coming years.

General conclusions

This thesis, for the first time, has documented a protocol for domestication of *P. monodon* in indoor recirculation systems in Vietnam, successfully closing the rearing cycle from egg to breeder, which are capable to produce viable offspring through several generations. Also this research documented the successful replacement of up to 60% of a fresh-food maturation diet with a commercial semi-moist diet for *P. monodon* at a commercial scale shrimp hatchery. Furthermore, the following conclusions can be drawn from this PhD study:

1) A sand-based recirculation system and a 2-step biosecure, quarantine procedure is applicable for domestication and production of SPF domesticated *P. monodon* stocks.
2) The life cycle of *P. monodon* from egg to breeder can be successfully closed in indoor recirculation systems within 11-12 months.
3) A fresh-food mixture of squid, oyster, blood cockle, marine worm and pork liver constructed to resemble the ratios of ARA/EPA, DHA/EPA and ω3/ω6 fatty acids in mature ovaries of wild *P. monodon* significantly improved growth and fecundity of *P. monodon*.
4) The recently developed, commercial semi-moist maturation diet (BREED-S FRESH®, INVE Aquaculture N.V., Belgium) successfully replaced up to 60% of the fresh-food mixture in the feeding regime for *P. monodon* maturation.
5) Postlarvae originating from domesticated *P. monodon* stock demonstrated better growth than postlarvae from non-domesticated *P. monodon* stock, resulting in a shorter culture period and higher yield at harvest for domesticated *P. monodon* stock.

Suggestions for further research

To further improve the quality of domesticated *P. monodon* stocks, future research could focus on the following aspects:

1) Suggestion to further refine the rearing environment and diets in order to optimize the different rearing phases in the domestication protocol:
    - Phase 1 (grow-out phase or juvenile stage) should be prolonged until 50g in stead of 40g in the current protocol. Shrimp should be fed 60% high protein pellets plus 40% fresh-food mixture (75% squid, 15% oyster, and 10% blood cockle) and reared at low water salinity (20-25 $g.L^{-1}$).
    - Phase 2 (pre-maturation phase or sub-adult stage) should start from 50g in stead of 40g in the current protocol. Shrimp should be fed 40% semi-moist maturation pellets (in stead of high protein grow-out pellets applied in the current protocol) plus 60% fresh-food mixture and reared at full-strength salinity seawater. The tanks should already be covered at this stage in stead of the high light intensity that was applied in the current protocol.
    - Phase 3 (maturation phase or adult stage): the current protocol could be followed; however, a higher replacement level of 70 to 100% of the fresh-food mixture by the semi-moist maturation pellet could be tested to assure more biosecurity for SPF shrimp production. In addition, the effects of size and age on male quality as well as the effect of sex ratio should be studied.

2) Sustainable domestication and genetic improvement to improve quality of *P. monodon* stocks kept in captivity:

   - Monitor fecundity and larval quality while the domestication is ongoing to allow obtaining animals for easy reproduction under captivity (e.g. multiple spawning).
   - Perform the process of domestication with animals originating from different spawning areas in order to maintain sufficient amount of genetic diversity allowing later on performing genetic improvement by selection.
   - In a first step, there is an urgent need to develop good biosecurity protocols in order to improve survival in the domestication process. In a later step, genetic improvement could be more important in order to produce *P. monodon* stocks that are more adapted to the local rearing conditions and are more resistant to diseases.

CHAPTER VIII

REFERENCES

Chapter VIII

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SUMMARY

Developing techniques for producing SPF domesticated *P. monodon* stocks is very necessary to keep the farming industry of this species sustainable and to protect natural resources of wild *P. monodon* from over-fishing. Knowledge obtained through the 1970's to the 2000's, which was reviewed in **Chapter II**, indicated several factors may be important for successful domestication of *P. monodon*. Indoor sand-based recirculation systems with strict biosecurity are vital to obtain good survival in SPF *P. monodon* domestication programs. Light conditions, water salinity, stocking density and diets at the different stages of development should also be taken into account for successful domestication of *P. monodon*. Reducing stress to the animals by shading the tanks and maintaining low stocking densities, providing high quality maturation diets, and creating good and stable water quality conditions are important factors to improve reproductive quality of domesticated *P. monodon* breeders.

Chapter III reports on the development of a rearing system for *P. monodon* domestication. In this study different trials on the water exchange regime, rearing water salinity, stocking density and diets for grow-out culture of the juvenile stage from 1 to 40g animals were performed. The sand-based recirculation system with low water exchange at 5-10% a day and a combination diet of 50% high protein (55% protein) pellets and 50% fresh-food mixture consisting of 75% squid, 15% oyster and 10% blood cockle proved suitable for the indoor rearing of the grow-out phase of *P. monodon*. Low water salinity of 20-23g.L^{-1} and a low stocking density of 10 animals per m^2 resulted in the best growth of the shrimp in this initial grow-out phase. Survival of the *Penaeus monodon* in the grow-out phase was also significantly higher

at low water salinity of 20-23g.L^{-1} than at high water salinity of 32-33 g.L^{-1} irrespective of the stocking density.

In Chapter IV a protocol was developed for the different rearing phases in the domestication process in order to close the life cycle of *P. monodon* from eggs to breeders in the indoor rearing system. Nauplii were conventionally reared to 1g animals before being stocked in the sand-based recirculation system. In the first grow-out phase (juvenile stage), 1-g domesticated *P. monodon* were stocked at 10-13 animals per m^2 in the sand-based recirculation system with low water salinity of 20-23 g.L^{-1}, and fed with a combination diet of 80% high protein (55% protein) pellets and 20% fresh-food mixture. In the prematuration phase (sub-adult stage), stocking density was reduced to 4-6 animals per m^2 and water salinity increased to full-strength seawater salinity of 32-35 g.L^{-1}. The animals were then fed a combination diet of 50% high protein pellets plus 50% fresh-food mixture. In the maturation phase (adult stage), stocking density was further decreased to 2-4 animals per m^2 with the same water salinity as in the prematuration phase and shrimp were fed a combination diet of high quality semi-moist maturation pellets (BREED-S Fresh®, INVE Aquaculture N.V., Belgium) and a fresh-food mixture. The fresh-food mixture used in the first two rearing phases consisted of 75% squid, 15% oyster, and 10% blood cockle, while in the maturation phase it was composed out of squid, oyster, marine worm and pork liver in proportions to resemble the ratios of ARA/EPA, DHA/EPA and ω3/ω6 fatty acids of the mature ovaries of wild *P. monodon*. A totally dark environment was created by shading the tanks to rear the animals in the maturation phase, while a high light intensity was applied in the first two phases of the domestication process. This domestication protocol enabled to close the rearing cycle of *P. monodon* from

postlarvae to 110g female and 80g male breeders in the indoor sand-based recirculation system within 11-12 months. Survival of the animals was very good in the grow-out and maturation phases, averaging 81% and 96%; respectively, while it was a little bit lower in the prematuration phase, averaging 66%. SPF domesticated *P. monodon* breeders produced this way demonstrated good maturation and reproductive performance with 94-98% maturation success, 88-90% spawning success, an average egg production of 250,000-300,000 eggs per spawning and a hatching success of 65-75%, resulting in approximately 200,000 nauplii per spawning.

Chapter V reports on the development of a fresh-food maturation diet and a feeding regime to improve the quality of the domesticated *P. monodon* broodstock. In Section I of Chapter V a fresh-food formula for maturation and reproduction of *P. monodon* broodstock was developed. The fresh-food mixture consisted of squid, oyster, marine worm and pork liver, formulated to resemble the ratios of ARA/EPA, DHA/EPA and ω3/ω6 fatty acids of the mature ovaries of wild *P. monodon*. The formula significantly improved growth and egg fecundity of *P. monodon*. The developed fresh-food mixture could be used as a basic fresh-food maturation diet for maturation of *Penaeus monodon* in captivity and may serve as a control diet for further studies on the development of artificial maturation diets. In Section II of Chapter V, 3 different feeding regimes for maturation of domesticated *P. monodon* broodstock were tested. The 3 feeding regimes were based on the level of substitution of the fresh-food mixture by a recently developed semi-moist maturation diet (BREED-S Fresh®, INVE Aquaculture N.V., Belgium), and included 100% fresh-food mixture (100FF), 60% fresh-food mixture plus 40% semi-moist maturation pellet (40SP), and 40% fresh-food mixture plus 60% semi-moist maturation pellet (60SP). The replacement of

up to 60% of the fresh-food mixture with the semi-moist maturation pellets (60SP) did not produce any negative effects on maturation and reproductive performance of the domesticated *P. monodon*. Interestingly, the semi-moist maturation pellets significantly improved egg hatching success and resulted in higher survival of the larvae up to PL15 stage.

In Chapter VI the grow-out performance of domesticated *P. monodon* at commercially intensive shrimp farms was evaluated. Preliminary results on grow-out performance indicated that domesticated *P. monodon* stock demonstrated faster growth and higher yield at harvest than non-domesticated *P. monodon* stocks, resulting in a 15-day shorter culture period for domesticated *P. mondon* stocks to obtain animals of 20g as compared to non-domesticated animals. However, a phenomenon of non-uniform body colour at harvest of the domesticated *P. monodon* requires further attention. Genetic improvement of the stocks may help to overcome this problem in the future and to produce domesticated *P. monodon* stocks that are better adapted to the local rearing environments and more resistant to diseases.

This is the first time SPF domesticated *P. monodon* stocks have been successfully produced in the indoor rearing systems in Vietnam. The domesticated *P. monodon* stocks produced under the protocol developed in this thesis showed good maturation and good reproductive performance suitable for commercial application in shrimp hatcheries. However, further studies are still required to further optimise the domestication protocol, especially to standardize each phase of the domestication process. It was suggested to prolong the grow-out phase until the animals reach 50g in stead of 40g as in the current protocol. High protein grow-out pellets applied in the prematuration phase should be replaced with semi-moist maturation pellets and the

tanks should already be shaded when the animals reach a size of 50-60g in stead of waiting until 80g as in the current protocol. For the maturation phase, improving the rearing environment to further reduce stress and improving male quality to ensure the availability of sufficient numbers of good quality hard-shelled males for natural mating or artificial insemination are timely. Further studies on the dietary ratios of ARA/EPA and DHA/EPA should be carried out to further optimize maturation diets for *P. monodon*.

The knowledge obtained through this thesis work with respect to zootechnical and nutritional aspects of *P. monodon* broodstock rearing, more specifically the development of an indoor sand-based recirculation system, the formulation of a fresh-food maturation diet, and the application of a semi-moist maturation pellet for domesticated *P. monodon* stocks should contribute towards the development of genetic improvement and selective breeding programs for domesticated *P. monodon* stocks in future research work.

SAMENVATTING

De ontwikkeling van technieken voor de productie van SPF gedomesticeerde *P. monodon* garnalen is erg nodig om de kweek van deze soort duurzaam te houden en de natuurlijke bronnen te beschermen van overbevissing. Kennis opgedaan tijdens de jaren 1970 en 2000, die besproken werd in **Hoofdstuk II**, brachten verschillende factoren naar voor die belangrijk kunnen zijn voor de succesvolle domesticatie van *P. monodon*. Indoor-recirculatiesystemen met zandsubstraat met strikte bioveiligheid zijn van groot belang voor het bekomen van goede overleving in domesticatieprogramma's voor SPF *P. monodon*. Lichtcondities, saliniteit van het water, stockeringsdensiteit en voeders voor de verschillende ontwikkelingsstadia moeten ook bekeken worden voor de succesvolle domesticatie van *P. monodon*. Het verminderen van stress voor de dieren door de tanks te verduisteren, een lage densiteit aan te houden, hoogwaardige voeders te voorzien en goede en stabiele waterkwaliteit te behouden zijn belangrijke factoren om de reproductieve kwaliteit van gedomesticeerde *P. monodon* ouderdieren te verbeteren.

Hoofdstuk III beschrijft de ontwikkeling van een kweeksysteem voor domesticatie van *P. monodon*. In deze studie werden verschillende testen uitgevoerd op het waterverversingsregime, saliniteit van het kweekwater, de stockeringsdensiteit en de voeders voor de vetmestfase van het juvenielstadium voor dieren van 1 tot 40g. Het recirculatiesysteem met zandsubstraat met een lage verversingsgraad van 5-10% per dag en een combinatiedieet van 50% korrels met hoog eiwitgehalte (55% eiwit) en een versvoer-mengsel bestaande uit 75% inktvis, 15% oester en 10% bloedkokkel bleek geschikt voor het binnenshuis kweken van de vetmestfase van *P. monodon*. Een

lage watersaliniteit van 20-23 $g.L^{-1}$ en een lage stockeringsdensiteit van 10 dieren per m^2 resulteerde in de beste groeisnelheid van de dieren in deze initiële vetmestfase. Overleving van de *P. monodon* in de vetmestfase was ook significant hoger bij lage watersaliniteit van 20-23 $g.L^{-1}$ dan bij hoge saliniteit van 32-33 $g.L^{-1}$, onafhankelijk van de stockeringsdensiteit.

In **Hoofdstuk IV** werd een protocol ontwikkeld voor de verschillende fasen in het kweekproces om de levenscyclus van P. monodon vanaf eieren tot ouderdier te sluiten in het indoor kweeksysteem. Nauplii werden conventioneel opgekweekt tot dieren van 1g vooraleer ze gestockeerd werden in het recirculatiesysteem met zandsubstraat. In de eerste vetmestfase (juveniel stadium) werden de 1g-dieren gestockeerd aan 10-13 dieren mer m^2 in het recirculatiesysteem, in een lage watersaliniteit van 20-23 $g.L^{-1}$ en gevoederd met een combinatiedieet van 80% korrels met hoog eiwitgehalte (55% eiwit) en 20% versvoer-mengsel. In de prematuratiefase (subadult stadium) werd de stockeringsdensiteit verminderd tot 4-6 dieren per m^2 en de watersaliniteit verhoogt tot zeewatersaliniteit van 32-35 $g.L^{-1}$. De dieren werden dan gevoed met een combinatie van 50% korrels met hoog eiwitgehalte plus 50% versvoer. In de maturatiefase (adult stadium) werd de stockeringsdensiteit verder verminderd tot 2-4 dieren per m^2, in dezelfde saliniteit als in de prematuratiefase en de dieren werden gevoed met een combinatie van semi-moist maturatie korrels van hoge kwaliteit (BREED-S Fresh®, INVE Aquaculture N.V., België) en een versvoer-mengsel. Het versvoer gebruikt tijdens de eerste twee kweekfasen bestond uit 75% inktvis, 15% oester en 10% bloedkokkel, terwijl het in de maturatiefase samengesteld werd uit inktvis, oester, borstelwormen en varkenslever in een dusdanige verhouding om de verhoudingen van ARA/EPA, DHA/EPA en ω3/ω6 vetzuren van de mature ovaria

van wilde *P. monodon* te benaderen. In de maturatiefase werd een donkere omgeving gecreëerd door de tanks af te dekken, terwijl een hoge lichtintensiteit toegepast werd tijdens de eerste twee fases van het domesticatieproces. Dit domesticatieprotocol liet toe om de kweekcyclus van *P. monodon* van postlarve tot wijfjes van 110g en mannetjes van 80g te sluiten in het indoor recirculatiesysteem binnen een periode van 11-12 maanden. Overleving van de dieren was zeer goed tijdens de vetmest- en maturatiefase, gemmiddeld 81% en 96% respectievelijk, terwijl het iets lager was in de prematuratiefase, gemiddeld 66%. De aldus bekomen ouderdieren vertoonden goede maturatie- en reproductieve eigenschappen met 94-98% maturatiesucces , 88-90% eiaflegsucces, en een gemiddelde eiproductie van 250,000-300,000 eieren per afleg en een ontluikingspercentage van 65-75% hetgeen resulteerde in ongeveer 200,000 nauplii per afleg.

Hoofdstuk V beschrijft de ontwikkeling van een versvoer-maturatiedieet en een voederregime om de kwaliteit van de gedomesticeerde *P. monodon* ouderdieren te verbeteren. In deel I van hoofdstuk V werd een versvoer-dieet voor de maturatie en reproductie van *P. monodon* ouderdieren ontwikkeld. Het versvoer-mengsel bestond uit inktvis, oester, borstelwormen en varkenslever in een verhouding die de verhouding van ARA/EPA, DHA/EPA en ω3/ω6 vetzuren van de mature ovaria van wilde *P. monodon* nabootste. Deze formule verbeterde significant de groei en eifecunditeit van *P. monodon*. Het ontwikkelde versvoer-mengsel kan gebruikt worden als een basis versvoer-maturatiedieet voor *P. monodon* in gevangenschap en kan ook dienen als controlevoer in verdere studies naar de ontwikkeling van artificiële maturatievoeders. In deel II van hoofdstuk V, werden 3 verschillende voederregimes voor de maturatie van gedomesticeerde *P. monodon* uitgetest. De 3 voederregimes

waren gebaseerd op het substitutieniveau van het versvoer-mengsel met een recent ontwikkeld semi-moist maturatievoeder (BREED-S Fresh®, INVE Aquaculture, N.V., België), en bestonden uit 100% versvoer (100FF), 60% versvoer plus 40% semi-moist maturatiekorrels (40 SP), en 40% versvoer plus 60% semi-moist korrels (60SP). De vervanging tot 60% van het versvoer door semi-moist maturatiekorrels (60SP) gaf geen negatieve effecten op maturatie of reproductieve eigenschappen van de gedomesticeerde *P. monodon*. De semi-moist maturatiekorrels verbeterden echter significant het ontluikingspercentage van de eieren en resulteerde in hogere overleving van de larven tot het PL15 stadium.

In Hoofdstuk VI werden de vetmest-eigenschappen van de gedomesticeerde *P. monodon* gevalueerd in een aantal commerciële intensieve garnaalkwekerijen. De voorlopige resultaten toonden aan dat de gedomesticeerde *P. monodon* een hogere groeisnelheid en een hogere opbrengst bij oogst opleverden in vergelijking met niet-gedomesticeerde dieren, hetgeen resulteerde in een 15-dagen kortere kweekperiode om dieren van 20g te bekomen. Er werd echter een fenomeen van niet-uniformiteit van de lichaamskleur bij oogst vastgesteld bij de gedomesticeerde dieren. Dit vraagt verder onderzoek. Genetische verbetering van de dieren kan dit probleem mogelijks verbeteren in de toekomst en laat mogelijks ook toe om dieren te produceren die beter aangepast zijn aan de lokale kweekomstandigheden en beter bestand zijn tegen ziektes.

Dit is de eerste keer dat er in Vietnam succesvol SPF gedomesticeerde *P. monodon* dieren geproduceerd werden. De gedomesticeerde *P. monodon* dieren die volgens de ontwikkelde technieken werden geproduceerd, vertoonden een goede maturatie en goede voortplantingseigenschappen, geschikt voor commerciële toepassing in

broedhuizen. Verdere studie is echter nodig om het domesticatieprotocol verder te optimaliseren, in het bijzonder om elke fase van het domesticatieproces the standardiseren. Er werd voorgesteld om de vetmestfase te verlengen tot wanneer de dieren 50g bereiken in plaats van 40g in het huidige protocol. Vetmestkorrels met een hoog eiwitgehalte toegepast tijdens de prematuratiefase dienen vervangen te worden door semi-moist maturatiekorrels en de tanks moeten reeds verduisterd worden vanaf een gewicht van 50-60g in plaats van te wachten tot 80g in het huidige protocol. Voor de maturatiefase is het nodig om de kweekomgeving te verbeteren om stress verder te verminderen en de kwaliteit van de mannetjes te verbeteren om de beschikbaarheid van voldoende aantallen mannetjes van goede kwaliteit en met harde schaal te verzekeren voor natuurlijke of artificiële bevruchting. Verdere onderzoek is ook nodig naar de optimale verhoudingen van ARA/EPA en DHA/EPA in het voer om het maturatiedieet verder te optimaliseren.

De kennis ontwikkeld doorheen deze PhD studie met betrekking tot zootechnische en nutritionele aspecten van de kweek van *P. monodon* ouderdieren, meer spececifiek de ontwikkeling van een indoor recirculatiesysteem met zandsubstraat, de formulatie van een versvoer-maturatiedieet en de toepassing van een semi-moist maturatiekorrel voor de domesticatie van *P. monodon* zou moeten bijdragen tot de ontwikkeling van genetische verbetering en selectieve kweekprogramma's voor gedomesticeerde *P. monodon* in toekomsting onderzoekswerk.

CURRICULUM VITAE

Laboratory of Aquaculture & Artemia Reference Center

Ghent University

Rozier 44

9000 Gent

Belgium

Personal information

Full name: Nguyen Duy Hoa

First name: Hoa

Family name: Nguyen Duy

Date of birth: June 30, 1970

Place of birth: Quang Tri, Vietnam

Nationality: Vietnamese

Family status: Married

Children: 2

Home address: 58/19A, Zone 4, Tan Chanh Hiep, Dist. 12, Ho Chi Minh City, Vietnam

Main office: Research Institute for Aquaculture No.2

116 Nguyen Dinh Chieu Street, District 1, Ho Chi Minh City, Vietnam

Working office: National Breeding Center for Southern Marine Aquaculture

167 Thuy Van, Thang Tam ward, Vung Tau city, Vietnam

Email: hoaria2@yahoo.com

Education

2005 – Present:	Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium Registered for PhD-research in Biological Science (Aquaculture) Thesis: "Domestication of black tiger shrimp (*Penaeus monodon*) in recirculation systems in Vietnam"
2001-2003 :	Laboratory of Aquaculture & Artemia Reference Center, Ghent University, Belgium Master of Science in Aquaculture Thesis: "Detection of bacteria in the digestive tract of *Artemia salina* using immunohistochemical methods
1989-1994	Faculty of Fisheries. Nong Lam University, Ho Chi Minh City, Vietnam Bachelor in Aquaculture Thesis: "Artificial propagation of sand goby fish *Oxyeleotris marmoratus*"

Professional history

11/2003 to present	Research work on "Domestication of the black tiger shrimp (*Penaeus monodon*) and production of specific-pathogen-free shrimp"
01/1999 – 10/2001	Key staff in the project "Artificial propagation of Asian seabass (*Lates calcarifer*)"

	Involved in the project "Artificial propagation of grouper fish"
01/1997 – 12/1998	Technical assistant and secretary for the international project "Rural Extension for Aquaculture in the Mekong Delta" funded by DANIDA and the Mekong River Commission, coordinated by the Research Institute for Aquaculture No2, Ho Chi Minh city, Vietnam.
11/1994 – 12/1996	Staff of the Division of Scientific Information and International Cooperation – Research Institute for Aquaculture No.2, Ho Chi Minh City, Vietnam
06/1994 – 11/1994	Research staff in the Outreach-program of the Faculty of Fisheries, Nong Lam University, Vietnam cooperated with the Asian Institute of Technology, Thailand

Training and workshops

06/1999-07/1999	2–month training course on marine fish hatcheries at the Southeast Asian Fisheries Development Center (SEAFDEC), the Philippines
August, 2007	Symposium of the Asian-Pacific Chapter of the World Aquaculture Society, Ha Noi, Vietnam
May, 2009	Symposium for Sustainable Aquaculture. University of Science, Völklingen, Germany.
September, 2009	Larvi'09, Fifth Fish and Shellfish Larviculture Symposium., Ghent University, Belgium.

Appointments

06/1994 – 11/1994	Head of Experimental Fish Hatchery, Faculty of Fisheries, Nong Lam University, Ho Chi Minh City, Vietnam
2006-2007	Head of Aquaculture Technology Unit, Division of Fish Experimental Biology, Research Institute for Aquaculture No.2, Ho Chi Minh City, Vietnam
2008 to present	Head of Crustacean Section & Vice-director of National Breeding Center for Southern Marine Aquaculture, Research Institute for Aquaculture No.2, Ho Chi Minh City, Vietnam

Publications

National publications

1. Tuan, N., **Hoa, N.D.,** Thanh, N.H., 2002. Artificial propagation of Asian seabass fish (*Lates calcarifer*). Journal of Mekong Fisheries. Vietnam Agriculture Publisher, 139-146.
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6. **Hoa, N.D**., Wouters, R., Wille, M., Thanh, V., Dong, Tr. K., Hao, N.V., Sorgeloos, P., 2009. Spawning performance and offspring quality of domesticated black tiger shrimp *Penaeus monodon* fed a semi-moist maturation pellet. *In*: Hendry, C.I., Stappen, G.V., Wille, M., Sorgeloos, P. (Eds.), Larvi'09 – 5th Fish and Shellfish Larviculture Symposium, Ghent University, Belgium. European Aquaculture Society. Special Publication Vol. 38, 80-83.
7. **Hoa, N.D**., Wouters, R., Wille, M., Thanh, V., Dong, Tr. K., Hao, N.V., Sorgeloos, P., 2009. A fresh-food maturation diet with an adequate HUFA composition for broodstock nutrition studies in black tiger shrimp *Penaeus monodon* (Fabricius, 1798). Aquaculture 297 (1-4), 116-121.