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# Reproductive Aspects of SPF *Penaeus monodon* Grown in Closed Culture Captivity

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**Abstract**

Reproductive performance of *P. monodon* in terms of viable spermatophore, ovarian development and spawning characteristics was examined. The specific pathogen free (SPF) larvae were obtained from the SPF wild caught broodstock spawned in captivity. The SPF post-larvae were reared to mature entirely in the disinfected medium in circulation system. It was found that male shrimp matured far earlier than female shrimp. Maturation of male shrimp was determined by appearing of the ampoules observed between the fifth pereiopod and the first pleopod. The first maturation of male was observed within 6 months at an average body weight of 11 ± 2 g (n = 6). Sperm quantity and quality were examined in shrimp of 12 months old with an average body weight of 48 ± 3 g (n = 5). The average sperm count per spermatophore was 29.7 ± 16.5×$10^6$ with viability of 79.0 ± 11.1 %. Maturation of female shrimp was determined by appearing of the gravid ovaries of stage 3 - 4 observed on dorsal part of abdominal segments. The first maturation of female was found within 16 months with an average body weight of 78 ± 4 g (n = 2). The other group of 12 month-old female shrimp was manipulated to mature using eyestalk ablation and artificial insemination techniques. Spawning characteristics of the ablated shrimp found to vary among individuals. A number of ablated female exhibited multiple spawning up to 3 consecutive times with a single insemination. Numbers of egg and percentages of hatch apparently decreased in the later spawning in the group of shrimp spawned 3 consecutive times with single insemination ($p > 0.05$). Egg counts in each spawning varied from 79,000 to 260,000 showed no particular pattern of relationship with body weight. Hatching feasibility varied from nil to 85 %. This study demonstrated the promising potential in producing specific pathogen free broodstock in tropical climate environment. However, further research is needed to enhance growth performance and survival rate. The quality of spawn in terms of viable spermatophore and ovarian development is yet to be improved.

**Keywords:** Broodstock, maturation, multiple spawning, *Penaeus monodon*, specific pathogen free

## Introduction

Among the important aquaculture species of Thailand, *Penaeus monodon* is considered the most economically beneficial species for growing. However, the outbreak of the epidemic shrimp diseases together with the deterioration of the culture grounds have caused *P. monodon* productions significantly dropping 93 % from 198×$10^3$ metric ton in 2003 to 14×$10^3$ metric ton in 2007 [1]. Nevertheless, the aquaculture scientists still put an optimistic view in *P. monodon* aquaculture industry as the physical and biological characteristics of this species comprise many excellent aquaculture aspects. Many efforts have been putting to bring *P. monodon* back to its own industry which includes genetic improvement by means of breeding programs. However, the

breeding programs would not be possible without the disease-free domesticated populations which are only obtained from captive culture. Successful domestication of this species will ensure sufficient quantities of good quality broodstock and consequently reliable offspring production. The development of reliable specific pathogen free seed broodstock to produce high quality seed is required for a long-term sustainable development of shrimp industry.

Domestication of penaeid shrimp has first succeeded with the white shrimp *P. vannamei* (*Litopenaeus vannamei* in some publications) and commercialized during the 1990's while domestication of *P. monodon* had been started as early as 1970's [2]. Not until 2000's when full domestication of *P. monodon* from egg to brooders in closed circulation tanks had been reported [3]. However, unlike white shrimp, bringing up of *P. monodon* in indoor rearing system to mature stage is not easy comparing to white shrimp as the biological characteristics of the two species are divergent such as growth rate, feeding behaviors etc. In addition, induction of maturation of domesticated *P. monodon* was apparently even more difficult. Domestication of this species is still largely in an experimental stage due to poor performance of domesticated broodstock, especially in terms of sperm quality, egg fecundity and hatching success.

This study was conducted to investigate the reproductive characteristics of SPF *P. monodon* reared in captivity in the enclosed system under the environmental conditions of tropical climate of southern Thailand.

## Materials and methods

### Initial broodstock

The experiments were carried out at the Shrimp Quarantine Center, Walailak University, Nakhon Si Thammarat in southern Thailand. The initial broodstock were collected from the Andaman Sea, the adjacent waters of the southern Thailand. Shrimp were kept in quarantine when swimmerets were taken for examination of viral contamination. Seven specific viral pathogens including White Spot Syndrome virus (WSSV), Monodon Baculovirus (MBV), Yellow Head virus (YHV), Taura Syndrome virus (TSV), Infectious Hypodermal and Hematopoietic Necrosis virus (IHHNV), Hepatopancreactic parvo-like virus (HPV) and Gill-associated virus (GAV) were examined using Real Time PCR as described by Yan *et al*. [4]. Shrimp that are free from the 7 viral pathogens are so called "specific pathogen free shrimp (SPF shrimp)" in this study. The SPF female shrimp with the gravid ovaries of stage 3 - 4 were used as the starting-up broodstock. The shrimp were allowed to spawn individually in the spawning tanks in the disinfected seawater.

### Larval rearing protocol

Within 5 - 7 h followed spawning, the eggs were collected and rinsed with disinfected seawater and hatched in the disinfected vessels. The larvae were reared in 500 l plastic tanks in the temperature controlled hatchery. Larvae were fed with a mixture of high density of Chaetoceros diatom culture and the commercial encapsulated diets. The diatom was obtained from the culture in the sterilized media. The post-larvae were fed with the instar-I *Artimia nauplii*. The nauplii were hatched from the disinfected Artemia cyst and rinsed with the infected seawater prior to feeding the larvae.

### Recirculation system

Water in this study was reused keeping in enclosed reservoirs. Seawater was collected from a shore few kilometers from the experimental station. Salinity was adjusted for shrimp's optimal growth at 20 - 25 ppt [5] and maturation condition at 30 - 32 ppt [6] using either ground freshwater or concentrated seawater from salt pan. The recycling systems comprised a series of treatment including sedimentation units, mechanical filters, activated carbon filters, biological filters, seaweed cultured units, and finally water disinfection units. The disinfection was performed using sodium hypochlorite powder and UV radiation. Water quality in terms of pH, alkalinity and hardness was regularly adjusted using $NaHCO_3$, $CaCO_3$ and $MgSO_4$, respectively.

### Grow-out protocol

The grow-out units were located in the enclosed building divided in 5 sections, each section comprised of 8 concrete tanks of 12 $m^3$ in volume. Shrimp at post-larva 15 with an average total length of 1.29 ± 0.20 cm were subjected to viral contagious examinations before stocking in the rearing tanks. The SPF shrimp were transferred

to the grow-out units at an initial density of 500 shrimps/$m^2$. Shrimp were exclusively fed with commercial diets 4 times a day. The shrimp of different class sizes were fed with diets of different protein contents recommended by the manufacturer. Through rearing period, growth rates were frequently measured. The shrimp were graded and restocked at appropriate densities. The shrimp at 3 - 10 months old were stocked at a density of 60 shrimps/$m^2$. The exchange of water was done daily at a ration of 15 %. Water quality was monitored in terms of oxygen concentration, pH, alkalinity, and total ammonia, nitrite, calcium and magnesium concentrations (**Table 1**).

**Broodstock conditioning protocol**

After rearing for 10 months in the grow-out units, the healthy male and female shrimp were selected and transferred to rear in the broodstock units. The broodstock units were located in the enclosed building comprises of 8 concrete tanks of 5 $m^3$ in volume. The brooders were stocked at a density of 3 shrimps/$m^2$. Water used in this unit was recycled, disinfected and adjusted to shrimp's optimal salinity. The shrimp were fed with squid and cockle meat and occasionally with live polychate-sandworms. Water quality was monitored in terms of oxygen concentration, pH, alkalinity, and total ammonia, nitrite, calcium and magnesium concentrations (**Table 1**).

**Examination of male maturity**

First maturation in male was regularly investigated. Spermatophores were determined by observing on the external view of ampoule between the fifth pereiopod and the first pleopod. To investigate sperm quality and quantity, spermatophores were obtained by dissecting the ventral body wall between the fifth pereiopod and the first pleopod. The spermatophores were weighed, dissected and gently squeezed out with forceps. Sperm quality was assessed by sperm count to yield the percentages of normal and abnormal sperm. Sperm quantity was performed by counting number of sperm in spermatophores using hemocytometer as described by Leung-Trujillo and Lawrence [7]. Prior to examination, the spermatophores were homogenized in a calcium-free saline solution (composition per 1 L solution: 21.63 g NaCl, 1.12 g KCl, 0.53 g $H_3BO_3$, 0.19 g NaOH, and 4.93 g $MgSO_4.7H_2O$, and pH adjusted to 7.4 with 1 N HCl). Sperm with a spherical body and an elongate spike was considered normal [8]. Abnormal sperms were distinguished from normal by malformed body or by a bent, short, or missing spike [9].

**Examination of female maturity**

Maturation of female shrimp was determined by appearing of the gravid ovaries of stage 3 - 4 observed from outside the body on dorsal part of abdominal segments. The maturation was examined from 2 groups of shrimp. Shrimp of the first group were allowed to mature naturally. Shrimp of the second group of 12 months of age were subjected to unilateral ablation to induce maturation. The ablated females were allowed to molt. Artificial insemination was performed by extruding spermatophores from males of the same age. The extruded spermatophores were then inserted into the cavities underneath thelycums of female shrimp. Insemination was only done to female at the stage of post molt when the shell remained in semi-rigid stage. Following insemination, females were transferred to the maturation ponds and stocked at a density of 3 shrimps/$m^2$. The ovarian development was monitored. The change of water was done daily at a ration of 20 %. Water quality was monitored in terms of oxygen concentration, pH, alkalinity, and total ammonia, nitrite, calcium and magnesium concentrations (**Table 1**).

**Table 1** Physicochemical properties of the water in of *P. monodon* culture ponds.

| Pond | Water temp. (°C) | Salinity (ppt) | DO (mg/L) | pH | Alkalinity (Mg $CaCO_3$/L) | Total Ammonia (mg $NH_3$/L) | Nitrite (mg $NO_2$/L) | Calcium (mg/L) | Magnesium (mg/L) |
|---|---|---|---|---|---|---|---|---|---|
| Grow-out | 26.9-28.7 | 20-25 | 5.23-6.55 | 7.8-8.2 | 120-160 | 0.02-0.10 | 0.05-0.10 | 300-500 | 1200-1500 |
| Broodstock | 26.9-29.4 | 30-32 | 5.12-6.43 | 7.6-8.3 | 120-160 | 0.02-0.10 | 0.05-0.10 | 300-500 | 1200-1500 |

**Spawning protocol**

The female broodstock with ripe ovaries of stage 3 - 4 [10] were transferred to spawn in the spawning tanks. Following spawning, eggs were collected, rinsed with disinfected seawater and transferred to the hatching containers. Numbers of egg were counted. The eggs were allowed to hatch for 24 h. The numbers of viable nauplius were counted and used as the percentage of hatch. Length and weight of the spawners were measured.

**Statistical analysis**

Where necessary, data were subjected to one-way analysis of variance (ANOVA). Comparison of means was performed using Duncan's new Multiple Range Test (MRT). A significance of 5 % was used.

## Results

**Maturation of male**

Within 6 months in grow-out condition where shrimp were fed with commercial diets, spermatophores were observed in 6 male shrimp (from unknown number of male population) with an average body weight of 11 ± 2 g and total length of 10.5 ± 0.6 cm. Spermatophore was examined in shrimp of 12 months old (**Table 2**). A total of 5 shrimp with an average body weight of 48 ± 3 g and total length of 15.8 ± 0.1 cm were used. Spermatophore quality in terms of spermatophore weight, sperm count and viable sperm were obtained at 25.7 ± 9.1 mg, 29.7 ± $16.5\times10^6$/spermatophore and 79.0 ± 11.1 %, respectively.

**Maturation of female**

Two groups of shrimp were used in this study. A total of 20 shrimps in the first group were reared until the gravid ovaries were clearly observed. Only 12 females of the first group survived when first maturation was observed. The first maturation with ovaries of stage 3 - 4 was observed in 2 female shrimps at 16 months old with an average body weight of 78 ± 4 g and total length of 19.5 ± 0.7 cm. The rest of females of the first group (10 shrimps) with an average body weight of 86 ± 15 g and total length of 20.3 ± 1.2 cm were then subjected to artificial insemination following eye stalk ablation. All shrimp matured to spawn when full ovarian development was observed within 7 - 29 days after ablation. In the second trials, 10 females were reared for 12 months. There was no natural maturation observed through the period of rearing. Only 3 shrimps survived at an average body weight of 86 ± 2 g and a total length of 20.4 ± 0.7 cm. Shrimp were subjected to artificial insemination and unilateral ablation. Shrimp's ovaries matured to spawn within 9 - 18 days.

**Fecundity**

Fertility of the females subjected to ablation (13 shrimps) was examined in term of fecundity. The relative fecundity is the ratio of the numbers of egg to one milligram of body weight. Some shrimp showed to spawn up to 3 consecutive times with a single insemination. However, only eggs of the first spawned were taken into account for fecundity examination. It was found that the fecundities varied among individuals. The average fecundities (eggs/mg body weight) of shrimp of 12 months old (1.55 ± 0.26) and 16 months old (2.15 ± 0.74) were significantly different ($p < 0.05$) (**Table 3**). Numbers of eggs varied from 79,000 - 260,000 shown not to relate with the body weight of 62 - 115 g (**Figure 1**).

**Table 2** Spermatophore quality of 12 month-old *P. monodon.*

| Reproductive measure | Average values (n = 5) |
|---|---|
| Body weight (g) | 48 ± 3 |
| Total length (cm) | 15.8 ± 0.5 |
| Spermatophore weight (mg) | 25.7 ± 9.1 |
| Sperm count ($10^6$) / spermatophore | 29.7 ± 16.5 |
| Viable sperm (%) | 79.0 ± 11.1 |

**Table 3** Fecundity of individual eyestalk ablated *P. monodon* of first spawning. Values in each column with different letters are significantly different ($p < 0.05$).

| Individual code No. | Age (month) | Body weight (g) | Total length (cm) | No. of egg (×1,000) | Fecundity (eggs/mg body weight) |
|---|---|---|---|---|---|
| Q13/1 | 16 | 82 | 20.0 | 98 | 1.20 |
| Q13/2 | 16 | 75 | 19.5 | 220 | 2.93 |
| Q13/4 | 16 | 70 | 19.0 | 260 | 3.57 |
| Q13/7 | 16 | 88 | 21.0 | 220 | 2.50 |
| Q14/2 | 16 | 115 | 23.0 | 200 | 1.74 |
| Q14/5 | 16 | 62 | 19.0 | 79 | 1.27 |
| Q15/1 | 16 | 91 | 20.0 | 213 | 2.34 |
| Q15/2 | 16 | 92 | 20.5 | 180 | 1.96 |
| Q15/4 | 16 | 90 | 20.0 | 210 | 2.33 |
| Q15/5 | 16 | 95 | 21.0 | 158 | 1.66 |
| **Mean** | | **86** | **20.3** | **184** | **2.15** [a] |
| **SD** | | **15** | **1.2** | **57** | **0.74** |
| Q20/2 | 12 | 87 | 20.5 | 109 | 1.25 |
| Q20/3 | 12 | 84 | 19.7 | 140 | 1.67 |
| Q21/3 | 12 | 87 | 21.0 | 150 | 1.72 |
| **Mean** | | **86** | **20.4** | **133** | **1.55** [b] |
| **SD** | | **2** | **0.7** | **21** | **0.26** |

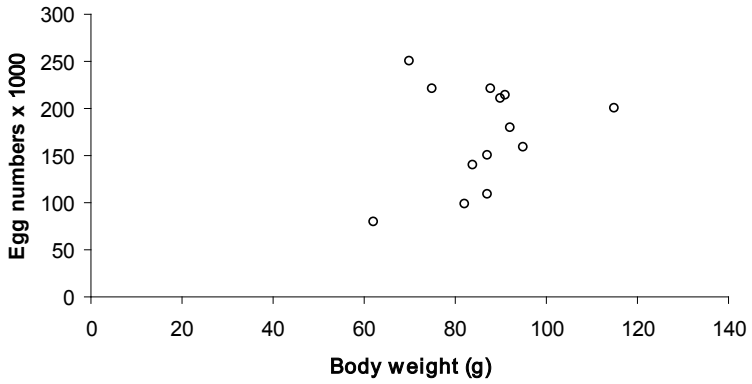


**Figure 1** Relation between body weight and numbers of spawned egg of the ablated *P. monodon* reared in captivity.

**Insemination and fertilization**

Multiple spawns with a single insemination were observed in this study. Course of spawning was categorized into 3 groups. Female of the first group spawn only once following insemination (**Table 4**). The second group spawned twice with a single insemination (**Table 5**). The last group spawned up to three times with a single insemination (**Table 6**). When the feasibility of spawn was examined, it was found that numbers of egg and percentages of hatch obtained in each spawn varied among individuals both within and between groups. In the group of double spawn, numbers of egg obtained from the first and second spawns were not different ($p > 0.05$). As well as in the group of triple spawn, numbers of egg obtained from the first, second and third spawn were not different ($p > 0.05$).With multiple spawning, each spawn occurred in 3 - 5 days followed the previous spawn.

**Table 4** Single spawning of individual *P. monodon* subjected to single insemination.

| **Individual code No.** | **Number of egg** (×1,000) | **Hatch** (%) |
|---|---|---|
| Q13/1 | 98 | 21 |
| Q14/2 | 200 | 85 |
| Q14/2* | 320 | 20 |
| Q14/5 | 79 | 37 |
| Q15/1 | 213 | 30 |
| Q20/2 | 109 | 33 |
| Q20/3 | 140 | 18 |
| Q21/3 | 150 | 50 |
| Q21/3* | 129 | 38 |
| **Mean** | **160** | **37** |
| **SD** | **75** | **21** |

* These shrimp were inseminated twice, each insemination performed single spawning.

**Table 5** Double spawning of individual *P. monodon* subjected to single insemination. Values in each row with same letter are not significantly different ($p > 0.05$).

| | **1st spawn** | | **Time** | **2nd spawn** | |
|---|---|---|---|---|---|
| **Individual code No.** | **Egg** (×1,000) | **Hatch** (%) | **interval** (day) | **Egg** (×1,000) | **Hatch** (%) |
| Q13/2 | 220 | 0 | 4 | 280 | 0 |
| Q13/2* | 269 | 7 | 3 | 184 | 10 |
| Q13/4 | 260 | 0 | 4 | 140 | 0 |
| Q13/7 | 220 | 19 | 3 | 200 | 0 |
| Q15/2 | 180 | 11 | 4 | 160 | 40 |
| Q15/4 | 210 | 10 | 4 | 250 | 40 |
| Q15/5 | 158 | 25 | 5 | 79 | 24 |
| **Mean** | **206**[a] | **13** | **4** | **166**[a] | **21** |
| **SD** | **39** | **10** | **1** | **64** | **20** |

* This shrimp was inseminated twice, each insemination performed double spawning.

**Table 6** Triple spawning of individual *P. monodon* subjected to single insemination. Values in each row with same letter are not significantly different ($p > 0.05$).

| | 1st spawn | | Time | 2nd spawn | | Time | 3rd spawn | |
|---|---|---|---|---|---|---|---|---|
| **Individual code No.** | **Egg** (×1,000) | **Hatch** (%) | **interval** (day) | **Egg** (×1,000) | **Hatch** (%) | **interval** (day) | **Egg** (×1,000) | **Hatch** (%) |
| Q13/4* | 250 | 0 | 3 | 200 | 0 | 3 | 180 | 0 |
| Q13/7* | 221 | 45 | 3 | 210 | 18 | 3 | 160 | 0 |
| Q15/2* | 187 | 20 | 3 | 230 | 35 | 3 | 158 | 51 |
| Q15/2** | 139 | 15 | 3 | 145 | 35 | 3 | 70 | 10 |
| Q15/4* | 220 | 10 | 4 | 249 | 40 | 4 | 239 | 0 |
| **Mean** | **203**[a] | **18** | **3** | **207**[a] | **26** | **3** | **161**[a] | **12** |
| **SD** | **42** | **17** | **0** | **39** | **17** | **0** | **61** | **22** |

* These shrimp were inseminated more than once but performed triple spawning at second insemination.
** This shrimp was inseminated more than once but performed triple spawning at third insemination.

**Table 7** Accumulated percent hatch of individual in single, double and triple spawning. Values in each row with different letters are significantly different ($p < 0.05$).

| **Individual No.** | **Single spawning Hatch (%)** | **Double spawning Hatch (%)** | **Triple spawning Hatch (%)** |
|---|---|---|---|
| 1 | 21 | 0 | 0 |
| 2 | 85 | 8 | 21 |
| 3 | 20 | 0 | 35 |
| 4 | 37 | 10 | 20 |
| 5 | 30 | 26 | 17 |
| 6 | 33 | 25 | |
| 7 | 18 | 25 | |
| 8 | 50 | | |
| 9 | 38 | | |
| **mean** | **37**[a] | **13**[b] | **19**[b] |
| **SD** | **21** | **12** | **13** |

The hatching feasibility of multiple spawning was also determined in term of accumulated percent hatch (**Table 7**). It was found that percent hatch of single spawned eggs (37 ± 21) was higher than those of double (13 ± 12) and triple spawning (19 ± 12) ($p < 0.05$).

**Discussion**

In this study, the first maturation of male shrimp was observed within 6 months with an average body weight of 11 ± 2 g. Jiang *et al.* [11] have also found the first maturation of *P. monodon* within 5 months when reared in earthen ponds. However, body weight of the shrimp (21.7 g) was apparently larger than that found in this study. This figure suggested that first maturation of male shrimp was likely related with age rather than size of shrimp. Ogle [12] stated that it was still unclear whether maturation of male shrimp was age- or size-related. However, Ceballos-Vázquez *et al*. [9] reported significant relationship between quality of spermatophore and body weight in *Litopenaeus vannamei* of 6 - 10 months old. Practically male shrimp are not used as brooder until they reach 10 - 12 months old when sperm are viable for fertilization. Quality and quantity of sperm were

investigated in this study in shrimp of 12 months old. It was found that spermatophore weight and sperm count were relatively smaller than that reported by Jiang *et al*. [11] in shrimp with similar size when reared in earthen ponds. However, the viable sperm of shrimp in this study was greater than that reported by Jiang *et al.* [11]. Significance in differences of sperm quality is resulted from various factors. Culture conditions especially food availability and quality have been reported to play a significant role in sperm quality of penaeid shrimp [5,13,14].

Reproductive performance in female *P. monodon* was obtained from a total 15 observed shrimps. Two shrimps were found to spawn naturally while spawning of the rest 13 shrimps obtained by manipulation. In natural spawning, first maturation was not observed until 16 months of rearing. This maturation was 12 months longer than that reported by Primavera [15]. Information on first maturation of domesticated *P. monodon* is scarce as most of the studies manipulated shrimp before they exhibited natural maturation. In other species, Ceballos-Vázquez *et al.* [16] reported the maturation of 6 months old *Litopenaeus vannamei* when reared in the tidal ponds. Retard in maturation of shrimp in this study possibly resulted from the captive conditions and handling stress. Reproductive development of shrimp in captivity where pathogen contamination is controlled is apparently restrained because the types of food source are limited compared to wild environment or earthen ponds where natural food is available. Marsden *et al*. [17] stated that wild caught brooders produced better quality eggs than those matured in captivity.

Ovarian development of the ablated shrimp to fully ripe was obtained in a period of time which is comparatively similar to those of shrimp reared in earthen ponds reported by Coman *et al*. [18]. All shrimp spawned with relatively low fecundities. However, the fecundities were similar to the domesticated shrimp reported by Coman *et al*. [18]. This suggests that captive environment of the system employed in this study is in acceptable conditions for *P. monodon* broodstock compared to earthen pond environment.

Most of manipulated shrimp exhibited multiple spawning up to 3 consecutive times, with a single insemination. This suggests that male germ cells in the thelycum receptacle of females are feasible for multiply fertilization, resulting in multiple spawn. A number of shrimp seed producers in Thailand has also observed multiple spawning of wild caught *P. monodon* when kept in captivity without male shrimp. It is likely that unless the thelycum receptacle is discarded through molt cycle [19], course of fertilization will continue for a period of time. Anderson *et al*. [20] stated that potential multiple spawning of wild ridgeback prawn (*Sicyonia ingentis*) occurred without interruption of molt or mating. Their studies in laboratory also demonstrated multiple spawning within a single prolonged molt cycle. In this study, course of multiple spawning occurred within 3 - 5 days following previous spawns. This indicates that the oocytes in a single ovary may not develop at the same times and the different batches of eggs will take 3 - 5 days to develop from one stage to another. This statement is well supported by the study of Anderson *et al*. [20]. They found that ovarian development of multiple spawning in female *S. ingentis* through vitellogenesis required approximately 4 days to pass through the most advanced oocyte stages when the cortical specializations are fully formed until next spawning. Palacios *et al*. [21] also stated that the capacity for multiple spawning could be related to metabolism of energetic lipids that are accumulated in the hepatopancreas. However, a certain captive condition may not sufficiently support deposition of nutrition in the oocytes to produce viable offspring as this study found that percentages of hatch declined in the later spawning.

## Conclusions

This study demonstrated the promising potential in producing specific pathogen free broodstock in tropical climate environment. The shrimp were found not to perform well in terms of growth rates. However, maturation was obtained without manipulation. Shrimp of 12 months old showed to provide feasible eggs and sperm. Most of manipulated female exhibited multiple spawning with a single artificial insemination, and feasible offspring was obtained. However, further research studies are needed to enhance growth performance and survival rate. The quality of spawn in terms of viable spermatophore and ovarian development is yet to be improved.

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**References**

[1] Department of Fisheries. *Fisheries Statistic of Thailand in 2007*. Department of Research and Analysis, Information Center (*in Thai*) 2007.

[2] Aquacop. Penaeid reared broodstock: Closing the cycle of *Penaeus momodon*, *P. stylirostris* and *P. vannamei*. *Proceedings of the World Mariculture Society* 1979; **10**, 445-52.

[3] GJ Coman, PJ Crocos, SJ Arnold, SJ Key and NP Preston. Growth, survival and reproductive performance of domesticated Australian stock of the giant tiger prawn, *Penaeus monodon*, reared in tanks and raceways. *J. World Aquaculture Soc.* 2005; **36**, 464-79.

[4] D Yan, KFJ Tang and DV Lightner. Development of a real-time PCR assay for detection of monodon baculovirus (MBV) in penaeid shrimp. *J. Invertebr. Pathol.* 2009; **102**, 97-100.

[5] ND Hoa, R Wouters, M Wille, V Thanh, TK Dong, NV Hao and P Sorgeloos. A fresh-food maturation diet with an adequate HUFA composition for broodstock nutrition studies in black tiger shrimp *Penaeus monodon* (Fabricius, 1798). *Aquaculture* 2009; **297**, 116-21.

[6] AG Hillier. Artificial conditions influencing the maturation and spawning of sub-adult *Penaeus monodon* (Fabricius). *Aquaculture* 1984; **36**, 179-84.

[7] JR Leung-Trujillo and AL Lawrence. Observation on the decline in sperm quality of *Penaeus setiferus* under laboratory conditions. *Aquaculture* 1987; **65**, 363-70.

[8] P Talbot, D Howard, J Leung-Trujillo, TW Lee, WY Li, H Ro and AL Lawrence. Characterization of male reproductive tract degenerative syndrome in captive penaeid shrimp (*Penaeus setiferus*). *Aquaculture* 1989; **78**, 365-77.

[9] BP Ceballos-Vázquez, C Rosas and IS Racotta. Sperm quality in relation to age and weight of white shrimp *Litopenaeus vannamei*. *Aquaculture* 2003; **228**, 141-51.

[10] IG Anderson. *Prawn Anatomy, Histology and Life History Biology*. *In*: Invertebrate in Aquaculture, University of Sydney, Australia, 1989, p. 89-100.

[11] SG Jiang, JH Huang, FL Zhou, X Chen, QB Yang, WG Wen and ZM Ma. Observations of reproductive development and maturation of male *Penaeus monodon* reared in tidal and earthen ponds. *Aquaculture* 2009; **292**, 121-8.

[12] JT Ogle. A review of current (1992) state of our knowledge concerning reproduction in open thelycum penaeid shrimp with emphasis on *Litopenaeus vannamei*. *Invertebr. Reprod. Dev.* 1992; **22**, 267-74.

[13] Y Goimier, C Pascual, A Sánchez, G Gaxiola, A Sánchez and C Rosas. Relation between reproductive, physiological, and immunological condition of *Litopenaeus setiferus* pre-adult males fed different dietary protein levels (Crustacea; Penaeidae). *Anim. Reprod. Sci.* 2006; **92**. 193-208.

[14] R Wouters, P Lavens, J Nieto and P Sorgeloos. Penaeid shrimp broodstock nutrition: An updated review on research and development. *Aquaculture* 2001; **202**, 1-21.

[15] JH Primavera. Induced maturation and spawning in five-month-old *Penaeus monodon* Fabricius by eyestalk ablation. *Aquaculture* 1978; **13**, 355-9.

[16] BP Ceballos-Vázquez, E Palacios, J Aguilar-Villavicencio and IS Racotta. Gonadal development in male and female domesticated whiteleg shrimp, *Litopenaeus vannamei*, in relation to age and weight. *Aquaculture* 2010; **308**, 116-23.

[17] G Marsden, P Mather and N Richardson. Captivity, ablation and starvation of the prawn *Penaeus monodon* affects protein and lipid content in ovary and hepatopancreas tissues. *Aquaculture* 2007; **271**, 507-15.

[18] GJ Coman, SJ Arnold, TR Callaghan and NP Preston. Effect of two maturation diet combinations on reproductive performance of domesticated *Penaeus monodon*. *Aquaculture* 2007; **263**, 75-83.

[19] RT Bauer and LJ Min. Spermatophores and plug substance of the marine shrimp *Trachypenaeus similes* (Crustacean:

Decapoda: Penaeidae): Formation in male reproductive tract and disposition in the inseminated female. *Biol. Bull*. 1993; **185**, 174-85.

[20] SL Anderson, WH Clark Jr and ES Chang. Multiple spawning and molt synchrony in a free spawning shrimp (*Sicyonia ingentis*: Penaeoidea). *Biol. Bull.* 1985; **168**, 377-94.

[21] E Palacios, AM Ibarra and IS Racotta. Tissue biochemical composition in relation to multiple spawning in wild and pond-reared *Penaeus vannamei* broodstock. *Aquaculture* 2000; **185**, 353-71.