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RESEARCH ARTICLE

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Comparison of ovarian maturation and spawning after unilateral eyestalk ablation of wild-caught and pond-reared *Penaeus monodon*

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Abstract

The present study compares the efficiency of ovarian maturation and spawning success between wild-caught and pond-reared *Penaeus monodon* females after unilateral eyestalk ablation. The earliest spawning time after eyestalk ablation was 5.9 days in wild-caught females, which is significantly shorter than the spawning time in pond-reared females (10.5 days). Both wild-caught and pond-reared females repeatedly spawned after eyestalk ablation. On average, each wild-caught female spawned 2.94 times while each pond-reared female spawned only 1.09 times. The spawning induction rate, egg hatching rate, and the number of eggs per spawning were significantly greater in wild-caught females than in pond-reared females. However, the egg size was not significantly different between wild-caught and pond-reared females. Four shrimp sizes (60, 80, 100 and 120 (± 1.0) g) were tested in this study and body weight significantly affected ovarian induction in pond-reared females but not in wild-caught females. Within the same body-weight class, the egg number per spawn in wild-caught females was significantly greater than that in pond-reared females. The egg production per spawn of the pond-reared females in the 120-g size group was two times higher than that in the pond-reared females in the 80-g size group. In conclusion, the fecundity of wild-caught *P. monodon* females is significantly higher than that of pond-reared *P. monodon* females. In breeding pond-reared *P. monodon*, the recommended minimum body weight of females is over 80 g, and the desirable body weight is over 100 g.

Additional key words: ovary maturation; body weight; wild-caught shrimp; pond-reared shrimp; eyestalk ablation; aquaculture.

Abbreviations used: BW (body weight); EST (earliest spawning time); GIH (gonad inhibiting hormone); SIR (spawning induction rate).

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Introduction

In most shrimp hatcheries, larval shrimp production mainly relies on wild-caught broodstock (Palacios *et al.*, 1999; Hall *et al.*, 2003; Peixoto *et al.*, 2003), but this practice constrains production efficiency as the availability of wild broodstock is seasonal. Coman *et al.* (2006) suggested that domestication of major shrimp species would bring overall economic benefits to the shrimp aquaculture industry in the long term. *Penaeus monodon* is an important species for shrimp aquaculture in the Asian-Pacific region. As the market demand for shrimp is continuously increasing, breeding and rearing of *P. monodon* at a large scale are desired. Hatchery production of shrimp seedlings requires a reliable source of gravid female shrimp. In the past, the female *P. monodon* has been normally caught from the wild prior to the spawning season. However, the source of a breeding female is unreliable in most commercial hatcheries due to seasonal variation and access limitation to broodstocks. Furthermore, the wild-caught females are likely to carry virus, which may increase the risk of failure in shrimp culture and breeding (Yang *et al.*, 2011). To overcome the unreliability of wild broodstock supply and increase the production efficiency of *P. monodon*, the use of domesticated pathogen-free broodstock is necessary.

Current literature in shrimp reproduction suggests that some reproductive parameters such as spawning rate, spawning number after eyestalk ablation, and spawning frequency in tank-reared *P. monodon* females are similar to wild-caught females (Menasveta *et al.*, 1993, 1994; Hansford & Marsden, 1995; Coman *et al.*, 2005). However, the reproductive parameters such as hatching rate and larval survival of tank-reared *P. monodon* broodstocks (<40%) are generally lower than those in wild broodstocks (Primavera & Posadas, 1981; Primavera & Caballero, 1992; Hansford & Marsden, 1995).

In shrimp, the sinus gland complex inside the eyestalk (also known as the X-organ) can secrete gonad inhibiting hormone (GIH) to postpone the maturation process and the time of ovulation. Eyestalk ablation can partially destroy the X-organ and reduce the level of GIH in hemolymph to induce ovarian maturation. Due to ease of operation, eyestalk ablation is commonly used to induce ovarian maturation in many species of shrimp such as *Penaeus semisulcatus* (Browdy & Samocha, 1985), *Macrobrachium acanthurus* (Cristiane & Lídia, 2010), *P. monodon* (Shailender *et al.*, 2013b; Nagur *et al.*, 2014), and *M. rosenbergii* (Shailender *et al.*, 2013a). In this study, the technique of the eyestalk ablation was used to compare the reproductive performance of *P. monodon* between wild and pond reared females.

The aim of this study was to identify the suitable source and size of brood shrimp that can be used for seedling production in a hatchery. The ovarian maturation and spawning performance parameters were used to compare the response of wild-caught and artificially-reared female *P. monodon* to eyestalk ablation at different body weights. This study can provide a practical guide for brood shrimp selection in artificial breeding of *P. monodon* in a hatchery.

Material and methods

The experiment was conducted in South China Sea Fisheries Research Institute, R&D Center, Sanya City, China (109.50E, 18.25N), in indoor cement ponds including acclimation ponds (40 m^2), maturation ponds (10 m^2), and spawning ponds (1 m^2). Seawater was filtered through sand filters (80 cm thick). During the experimental period, water quality was maintained at temperature 29 ± 0.5°C, salinity 32‰ and pH 8.1. The experiments were conducted in accordance with the guidelines and approval of the Ethics Committee of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences (2012A409).

A total of 80 wild females and 40 wild males from the Sanya coastal area and 80 females and 40 males from ponds were used in this study. According to body weight (BW), the 80 females were assigned to four size groups (20 females/group) including 60, 80, 100 and 120 (SE ± 1.0) g. The wild-caught and pond-reared shrimp were separately acclimated in ponds (80 cm deep) with filtered seawater. The animals were acclimated for 5 days before the experiment was started. During acclimation, all brood shrimp were daily fed with mussel, squid, clam, and crab for three times (08:00, 16:00 and 20:00 h) at a daily ration of 10% BW, and the uneaten food and feces were siphoned out daily. The water was aerated continuously with a blower, and the daily water exchange rate was set at 50% of the pond volume.

Ablation was operated following the method by Primavera (1978). In each weight group, the eyestalk of five females were unilaterally ablated in three replicates. A total of 60 wild-caught females and 60 pond-reared females were used for eyestalk ablation. After ablations, females were restocked into the maturation ponds according to body size in triplicate. The sex ratio of shrimp in each maturation pond was maintained at 2:1 (female: male). The maturation ponds were covered with a shade cloth to maintain the light intensity at 10 lx. The experimental shrimp were daily fed with live nereid worms at daily ration of 10% BW for three times at 08:00, 16:00 and 20:00 h. Two days after ablation, all females were checked daily at 19:00 h for ovarian maturation by shining a light beam from an underwater flashlight through the abdomen. Unlike wild females, pond-reared females usually spawn after reaching stage III of ovarian development (Jiang *et al.*, 2009). Shrimp at ovarian stage III were individually placed in the spawning ponds. In the next morning, completely or partially spawned females were transferred to other maturation ponds according to body size for re-maturation. Un-spawned females were kept in the spawning ponds for another 1-2 nights.

After spawning, ten l00 mL aliquots were randomly collected from the spawning pond for egg counting. The number of eggs and nauplii (*N*) in the aliquots were determined on a dissecting microscope. The number of females with ovaries at stage III or above, eggs, nauplii, egg diameter, dead females and spawning number after eyestalk ablation were all recorded for each size group.

The following equations were used to calculate the parameters for reproductive performance:

$$\text{Spawning rate (\%)} = \\ = \text{Females with stage III ovaries} \times 100/ \\ \text{Total number of ablated females}$$

Hatching rate (%) = Number of nauplii ×100/ Total number of eggs

Spawning mortality (%) = Dead females ×100/ Ablated females

Spawning induction rate (SIR, %) = = Induced females×100/ Ablated females

Earliest spawning time (EST) was the earliest time (day) of first spawn after eyestalk ablation.

Data presented in this study were expressed as mean ± SE. Two-way ANOVA (by SPSS 18.0) were conducted to test the effects of BW and female type (wild *vs* pond reared) on shrimp reproduction performance. The least-significant difference (LSD) comparison was used when a significant main effect was detected at $p<0.05$.

Results

The fecundity of wild-caught *P. monodon* females was significantly higher than that of pond-reared *P. monodon* (Table 1). Throughout the 30-day experiment, the total number of spawns was 116 times in wild-caught females and 54 times in pond-reared females. The total number of nauplii was 63.9 million from wild-caught and 13.8 million from pond-reared females. The average number of spawns per female was 2.94 times in wild-caught shrimp and 1.09 times in pond-reared shrimp. The average nauplii production was 1.76 million per female in wild-caught shrimp and 0.26 million in pond-reared shrimp.

The accumulated mortalities of both wild-caught and pond-reared females after eyestalk ablation were not significantly different ($p>0.05$, Table 2). In wild-caught shrimps, the female mortality in the 60 g, 80 g or 120 g class was significantly higher than that in the 100 g class ($p<0.05$). In pond-reared shrimps, a lower mortality was observed in the 80 g class than in other classes ($p<0.05$, Table 2).

Table 1. Number of spawning and nauplii production in wild-caught and pond-reared *P. monodon*

Body-weight (g)	Spawns		Nauplii production ($\times 10^6$)	
	Wild	Pond	Wild	Pond
60 ± 1.0	2	0	0.1	0
80 ± 1.0	34	15	14.9	2.2
100 ± 1.0	40	20	22.3	5.1
120 ± 1.0	40	19	26.6	6.3
Total	116	54	63.9	13.8
Average*	2.94	1.09	1.76	0.26

*Average = Total /Spawning females, $n = 3$.

Eyestalk ablation successfully induced maturation of both wild-caught and pond-reared females. The average spawning induction rate (SIR) was 66.3% (15.4%-91.6%) in wild-caught shrimp and 45.4% (0%-70.0%) in pond-reared shrimp (Table 2). Within the wild-caught group, the SIRs were not significantly affected by BW, except for the 60 g shrimps ($p>0.05$). However, BW significantly affected the SIR of pond-reared females ($p<0.05$). The SIR of females in the 100 g or 120 g class was significantly higher than that in the 80 g class (Table 2, $p<0.05$), but there was no successful maturation induction in the 60 g class.

The hatching rate was 83.4% (80.6-86.0%) in wild-caught females and 47.2% (46.6-48.3%) in pond-reared females (Table 2), but the hatching rates in wild-caught and pond-reared females were not significantly affected by BW ($p>0.05$). The hatching rate in wild-caught females was significantly higher than that in pond-reared females ($p<0.05$, Table 2).

After eyestalk ablation, the earliest spawning time (EST) was significantly different between size groups ($p<0.05$, Table 3). The average EST of the wild-caught

Table 2. Mortality and spawning induction and hatching rates of wild caught and pond reared *P. monodon* after eyestalk ablation

Body-weight (g)	Spawning induction rate (%)		Hatching rate (%)		Mortality (%)	
	Wild	Pond	Wild	Pond	Wild	Pond
60 ± 1.0	$15.4±7.8^{Aa}$	$0±0^{Ba}$	$56.8±5.6^{Aa}$	$0±0^{Ba}$	$13.3±6.6^{Ab}$	$13.3±6.6^{Ab}$
80 ± 1.0	$78.3±11.6^{Ab}$	$50.0±5.7^{Bb}$	$80.6±2.9^{Ab}$	$46.7±1.6^{Bb}$	$13.3±6.6^{Ab}$	$6.6±6.6^{Aa}$
100 ± 1.0	$80.0±11.5^{Ab}$	$61.6±7.2^{Bc}$	$83.3±4.4^{Ab}$	$48.3±6.0^{Bb}$	$6.6±6.6^{Aa}$	$13.3±6.6^{Ab}$
120 ± 1.0	$91.6±8.3^{Ab}$	$70.0±8.6^{Bc}$	$86.0±2.0^{Ab}$	$46.6±2.8^{Bb}$	$13.3±6.6^{Ab}$	$13.3±6.6^{Ab}$

Data in the same row with different capital letters and data in the same group with different small letters were significantly different ($p<0.05$, $n = 3$).

Table 3. The duration of first spawning after ablation, egg number/spawn and egg diameter of wild and pond reared *P. monodon*

Body weight (g)	Earliest spawning time (days)		Egg number (×10^5) /spawn		Egg diameter (µm)	
	Wild	Pond	Wild	Pond	Wild	Pond
60 ± 1.0	6.9± 0.5^{Aa}	0 ± 0	2.22±0.11^{Aa}	0 ± 0 ^{Ba}	289.4±5.2^{Aa}	0 ± 0
80 ± 1.0	6.3 ± 0.3^{Aa}	10.0 ± 0.5^{Ba}	5.50±0.50^{Ab}	3.33±0.57^{Bb}	291.6±8.3^{Aa}	283.3±8.3^{Aa}
100 ± 1.0	5.7 ± 0.5^{Aa}	10.3 ± 0.6^{Ba}	6.33±0.57^{Ab}	5.30±0.28^{Bc}	287.5±7.2^{Aa}	275.0±14.0^{Aa}
120 ± 1.0	6.0 ± 0.6^{Aa}	10.3 ± 0.3^{Ba}	8.16±0.6^{Ac}	6.83±0.60^{Bc}	275.0±14.4^{Aa}	279.1±4.1^{Aa}

Data in the same row with different capital letters and data in the same group with different small letters were significantly different (p<0.05, n = 3).

and pond-reared females was 5.9 d (5.7-6.9 d) and 10.5 d (10.0-11.6 d), respectively (Table 3). The EST was significantly shorter in wild-caught females than in pond-reared ones within the same BW class. No significant differences of EST between BW groups were observed in wild-caught shrimps or in pond-reared shrimps (p>0.05, Table 3).

Table 3 shows that fecundity ranged from 220,000 to 816,000 eggs in wild-caught females and from 333,000 to 683,000 eggs in pond-reared females (except for the 60 g group). Egg production per spawn in wild-caught female was significantly different between BW classes (p<0.05). Egg production increased with the increase of BW in both wild-caught and pond-reared females. Egg production per spawn in wild-caught females was significantly higher than that in pond-reared females within the same BW class (p<0.05). The egg production per spawn in the pond-reared females in the 120-g group was two times higher than that in the pond-reared females in the 80-g group (Table 3). Fig. 1 indicates that the relationship between egg number (y) per spawn and BW (x) could be expressed in a linear equation: $y = 0.0943x - 2.9343$ (R^2=0.9341) in wild-caught females and $y = 0.1119x - 6.3262$ ($R^2 = 0.9646$) in pond-reared females.

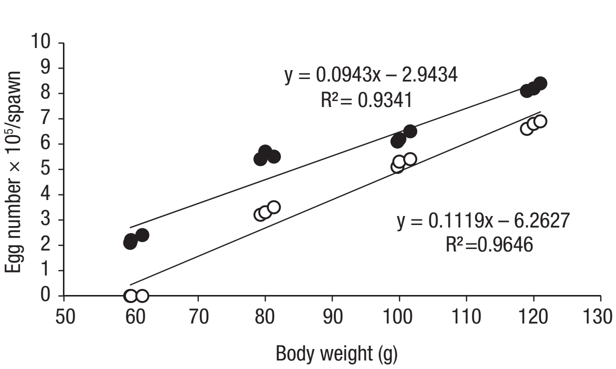

Figure 1. Relationship between body weight and egg number/spawn in wild-caught (black circles) and pond-reared (blank circles) *P. monodon* (n = 3).

The egg diameter was not significantly affected by the body weight of both wild-caught females and pond-reared females (p>0.05, Table 3). The average egg diameter produced by wild-caught females was 285.9 µm (275.0-291.6 µm), and the average diameter of eggs produced by pond-reared females was 279.1 µm (275.0-283.3 µm).

Discussion

In shrimp hatcheries, recent research has focused on overcoming the reliance of seed supply on a wild-caught broodstock. In the past decades, a series of studies were conducted to compare the reproductive performance of wild-caught broodstocks and pond-reared broodstocks. Existing results indicate that wild-caught shrimp such as pink shrimp *Farfantepenaeus duorarum* (Emerenciano *et al.*, 2012), tiger prawn *Penaeus esculentus* (Keys & Crocos, 2006), and Carpas shrimp *Farfantepenaeus paulensis* (Peixoto *et al.*, 2008) show better reproductive performance than domesticated shrimp. In *P. monodon*, Menasveta *et al.* (1994) compared the effects of source and size of females on ovarian maturation, but in their work, the size of females was limited to two similar size groups (>120 g, or <110 g BW). Although larger females have advantage in reproductive performance such as in *P. monodon* (Menasevta *et al.*, 1994), it is difficult to obtain larger individuals in an artificial rearing condition (Jiang *et al.*, 2013). In the present study, to verify the possibility of using artificially-reared females in a medium size to perform artificial breeding, the size classes in test shrimps were further expanded into four groups (60, 80, 100 and 120 g). The fecundity of wild *P. monodon* females was significantly higher than that of pond-reared ones, but the reproductive performance of pond-reared shrimps was acceptable when the BW of females was over 80 g.

In the present study, the rate of ovarian maturation by eyestalk ablation was significantly greater in wild-caught females than in pond-reared ones within the same BW group, and the lowest maturation was observed in the pond-reared female with BW of 60 ±1.0 g. Although body size significantly affected the rate of induced ovarian maturation in pond-reared *P. monodon*, it did not affect ovarian maturation in wild-caught *P. monodon*. Primavera (1978) indicated that the degree of shrimp maturation was related to female size. Chotipuntu *et al*. (2013) found that the BW at first maturation in wild *P. monodon* females was about 78 g, whereas Huang *et al*. (2013) reported that the BW of sexually matured pond-reared *P. monodon* females was over 75 g. Similarly, the present study showed that ovarian maturation occurred when the BW of pond-reared females reached 80 g, and the maturation rate increased with the increase of BW in pond-reared females. This may suggest that the minimum requirement of the BW of pond-reared females is 80 g for normal ovarian maturation, and larger BW may be advantageous for ovarian maturation in pond-reared *P. monodon* females.

The latency period between the time of eyestalk ablation and first spawn is directly related to the capacity for multiple spawns in shrimp (Palacios *et al.*, 1999; Arcos *et al.*, 2003; Emerenciano *et al.*, 2012). In the present study, the time of first spawn after eyestalk ablation was significantly shorter in wild-caught females (5.9 days) than in pond-reared ones (10.5 days). Similar trends are observed in other shrimp species such as *Penaeus paulensis* (Cavalli *et al.*, 1997), *Litopenaeus vannamei* (Palacios *et al.*, 1999) and *Farfantepenaeus duorarum* (Emerenciano *et al.*, 2012), where wild-caught females start spawning earlier and have a higher spawning frequency than pond-reared females. Generally, pond-rearing conditions offer a limited variety of food items to shrimp, and food supply is mainly based on commercial pellets and a limited choice of fresh or frozen food such as squids, mussels and worms. Emerenciano *et al.* (2012) suggested that the cause of early spawning in wild shrimps may be due to a better nutritional supply to females in the wild.

In the present study, the wild-caught *P. monodon* female achieved significantly higher number of eggs per spawn than pond-reared females within a similar BW. The increase of egg production is concomitant with the increase of BW, and larger females produced more eggs in both wild-caught and pond-reared shrimp. Arnold *et al.* (2013) found that the total number of eggs per spawn was about 25% lower in artificially-reared *P. monodon* females (413,000 eggs) than in wild-caught females (552,000 eggs). Similarly, wild-caught *F. duorarum* can produce significantly higher number of eggs (Emerenciano *et al.*, 2012). The same trend was also observed in species such as *Penaeus esculentus* (Keys & Crocos, 2006) and *Farfantepenaeus paulensis* (Peixoto *et al.*, 2008).

In summary, the fecundity of wild-caught *P. monodon* females is significantly higher than that of pond-reared *P. monodon* females. In breeding pond-reared *P. monodon*, the recommended minimum body weight of females is over 80 g, and the desirable body weight is over 100 g.

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