Repeated Spawning Performance of Female Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man) in a Recirculating Water System

Padet Hongmanee, Akkarasiri Sangsawang, Sukkrit Nimitrkul, Sahabhop Dokkaew, Satid Chatchaiphan, Wara Taparhudee, Orapin Jintasathaporn, Uthairat Na-Nakorn and Rueangchay Yoonpund*

ABSTRACT

Repeated spawning of female giant freshwater prawn (*Macrobrachium rosenbergii* de Man) is a promising strategy to enhance broodstock utilization and seed production efficiency. However, concerns persist regarding its potential impacts on reproductive performance and larval quality. This study evaluated the effects of repeated spawning in individually reared females maintained in floating perforated boxes under a single-pair mating system, with in vivo egg incubation over a 180-day period. Only 33.33% of the initial broodstock completed three spawning cycles. Although the realized larval yield (number of hatching larvae per female) remained stable across spawnings, the relative larval yield (number of larvae per gram of female body weight) significantly declined ($p<0.05$), despite consistently high hatching rates (88.40–89.89%). The spawning interval increased with each successive spawning (35.42–42.33 days). While post-larval weight remained unaffected ($p\geq0.05$), post-larval quality deteriorated with repeated spawning, as evidenced by reduced survival rates, prolonged larval development duration, and decreased stress resistance (shorter time to 50% mortality after acute freshwater exposure), despite comparable weight per 100 post-larvae across spawning events. These findings highlight the trade-offs between maximizing broodstock use and maintaining seed quality, emphasizing the need for improved broodstock management strategies in repeated spawning systems.

Keywords: Giant freshwater prawn, Multiple spawning, Post-larval quality, Repeated spawning

INTRODUCTION

The giant freshwater prawn (*Macrobrachium rosenbergii* de Man) is an economically valuable species in global freshwater aquaculture, with a total production of 337,449.44 tonnes in 2022 (FAO, 2024). Commercial farming of this species was first established in Thailand in 1977 (Kongkeo and Davy, 2010; New, 2010; Na-Nakorn and Jintasathaporn, 2012). However, recent production statistics rank Thailand third in annual production, following China and Bangladesh (FAO, 2024). In Thailand, prawn seed production relies heavily on local broodstock, with berried females typically collected from grow-out ponds for post-larval production (Kongkeo and Davy, 2010; New, 2010). A major limitation of this practice is that females are used only once for spawning before being discarded. Exploring the feasibility of repeated spawning, where females are reused for multiple reproductive cycles, could significantly enhance post-larval production efficiency. Moreover, with the increase adoption of genetically improved broodstock and sex-reversed females for the production of all-male post-larvae, the need for effective repeated spawning strategies in female broodstock management has become even more critical. However, studies on repeated spawning in *M. rosenbergii are* limited.

Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand
*Corresponding author. E-mail address: ffisrvy@ku.ac.th
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The reproductive performance of *M. rosenbergii* females has been studied from various perspectives, including spawning cycles and developmental stages (Damrongphol *et al*., 1991), general spawning characteristics (Damrongphol *et al*., 1991; Habashy, 2013), variation in reproductive performance among broodstock from different regions (Nhan *et al*., 2009; Kumar, 2023), and the effects of nutrition—such as fatty acid compositions (Cavalli *et al*., 1999) and vitamin supplementation (Cavalli *et al*., 2003)—on reproductive success. Cavalli *et al*. (2001) reported no significant decline in fecundity over five consecutive spawns, suggesting that *M. rosenbergii* females can sustain multiple spawning cycles.

Despite this, findings from other decapod crustaceans indicate that repeated spawning can negatively impact reproductive performances. For example, a decline in brood quality has been observed in the marine swimming crab (*Portunus trituberculatus*) (Wu *et al*., 2010), while reductions in fecundity, lipid concentration, and energy content of eggs have been reported in the small freshwater caridean shrimp (*Neocaridina davidi*) (Tropea and Greco, 2015). Dietary lipid content and feeding frequency has been found to influence reproductive cycling and spawn intervals in crustaceans (Jiang *et al*., 2020). Given these conflicting findings and the fact that the broodstock used in Cavalli *et al*. (2001) originated from a different population than Thai local stock, it remains uncertain whether repeated spawning in Thai *M. rosenbergii* females may compromise reproductive performance.

This study aims to evaluate the potential of reusing female *M. rosenbergii* for repeated spawning under controlled indoor recirculating water conditions. By isolating females from males and monitoring their reproductive performance over six months, this study seeks to determine whether individual females can sustain multiple spawning events without compromising post-larval quality. Additionally, the mortality rate of broodstock under these conditions will be assessed. The findings of this study would contribute to optimizing broodstock management strategies for enhancing *M. rosenbergii* hatchery production efficiency.

MATERIALS AND METHODS

Experimental animals

Approximately 100 mature males and 100 berried female giant freshwater prawns, each carrying grey to black eggs from the first harvest (five months of age), were purchased from a commercial farm in Bang Phae District, Ratchaburi Province, Central Thailand, to serve as experimental broodstocks. To ensure biosecurity, randomly selected males and females were tested for *M. rosenbergii* nodavirus (MrNV) and extra small virus (XSV) using reverse transcriptase polymerase chain reaction (RT-PCR). All tested individuals were confirmed to be free of MrNV and XSV.

Each berried female was then individually stocked in a 20-L black plastic container filled with 15 L of 15 ppt saline water, with continuous aeration. A total of 72 females successfully spawned within 24 h and were designated as the experimental female broodstock. The average initial weight and total length of the females after egg hatching were 21.59±3.24 g and 12.27±0.88 cm, respectively. Male broodstock used in this study were relatively uniform in size and exhibited the blue claw morphotype, with an initial weight of 25.21±2.64 g and a total length of 14.89±0.63 cm.

Experimental set-up

An experimental hatchery system was established in an area of around 55 m^2. The hatchery frame was enclosed with a transparent plastic sheet to stabilize air temperature and minimize external disturbances throughout the experimental period. Two rectangular fiberglass tanks (5,000 L and 3,000 L) were used to rear broodstock. A 2.5 cm diameter polyethylene pipe connected the two tanks, with each end fitted with a 1.2 cm mesh plastic screen to prevent direct mixing of tanks contents.

A closed recirculating water system was maintained throughout the experiment at a flow rate of 0.3 $L \cdot min^{-1}$. No water exchange was performed, except for the addition of dechlorinated freshwater to compensate for evaporation and maintain water level. Tank bottoms were cleaned daily by siphoning to remove waste and uneaten feed.

Experimental female were individually housed in perforated plastic boxes measuring 25×40×25 cm^3, with 2 cm lateral mesh openings. Each box was covered with a polyethylene net of 1.5 cm mesh and suspended at a depth of 50 cm from the water surface. A total of 45 rearing boxes were placed in the 5,000 L tank, and the remaining boxes were housed in the 3,000 L tank.

Male prawns were communally stocked at a density of 10 males·m^{-2} in the same tanks as the females, resulting in 50 males in the 5,000 L and 30 males in the 3,000 L tank. In total, 72 females, and 80 males were used in the experiment. All broodstock were fed a commercial sinking pellet containing 38% crude protein at a feeding rate of 3% of body weight, administered twice daily at 9:00 a.m. and 4:00 p.m.

Breeding methods

Breeding was initiated when females reached gonadal maturation stage 5, as described by Chang and Shih (1995) which is characterized by a slightly reddish ovary extending from behind the eyes to the first abdominal segment. A stage 5 female in the pre-molting stage was paired with a randomly selected, intermolt male from the same tank. Following successful mating, the female laid fertilized eggs and carried them on the pleopods (swimming legs) until the eggs turned grey-black, at which point the male was removed. Each successfully mated male was used only once and was not returned to the rearing tank to avoid repeated paring.

Berried females were individually transferred to 20 L black plastic containers filled with 15 L of 15 ppt saline water and supplied with continuous aeration. Two days after egg hatching, females were recorded and re-stocked into their individual containers for potential subsequent spawning. This breeding process was repeated up to three times per female.

Larval nursing

Within 24 h of hatching, larvae from each spawning female were randomly sampled and stocked at a density of 80 larvae·L^{-1} (Suwannatot, 2003) into three replicates of 20-L black plastic containers, each filled with 15 L of 15 ppt saline water. Continuous aeration was provided throughout the nursing period. For the first 10 days, the larvae were fed chilled, newly hatched *Artemia* nauplii every three hours, with the amount adjusted to ensure adequate availability. Feeding was discontinued after 8:00 p.m. and resumed the following morning. No water exchange was performed during the first 10 days. Thereafter, 50% of the water volume was exchanged every two days, following removal of waste from the container bottom by siphoning.

Water quality monitoring

Water quality in the broodstock rearing tanks was monitored weekly by measuring key parameters: dissolved oxygen (DO), temperature, pH, alkalinity, total ammonia nitrogen (TAN), and nitrite (NO_2^-). These same parameters were measured every four days in the larval nursing containers. All measurements were taken at 09:00 a.m. DO and pH were measured using a YSI, Model 63; temperature was recorded using a YSI, Model 550A. Alkalinity was determined using the titration method (AHPA *et al*., 1998), TAN was analyzed by the Indophenol Blue method (Grasshoff, 1976), and nitrite (NO_2^-) concentrations were measured using the diazotization method (Grasshoff, 1976).

Data collection

Spawning interval (days)

The time between the completion of one spawning (hatching) and the subsequent spawning was recorded for each female, allowing for three consecutive spawning intervals.

Number of hatched larvae, larval yield, percent hatched larvae

To estimate the number of newly hatched larvae, subsamples were collected three times from each nursing container using a 5 mL micropipette. The larvae in each sample were counted, and the average number per mL was used to estimate the total larval yield (realized larval yield). The relative larval yield was then calculated as the number of larvae produced per gram of female body weight.

To calculate the hatching rate (%), the number of the eggs originally carried by each female was estimated as the sum of the number of hatched larvae per female and the number of eggs remaining attached to the females' abdomen after spawning. To count the unhatched eggs, they were gently removed with a soft toothbrush while the female was immersed in 3 cm of water in a 20×28×4.5 cm^3 aluminum tray. All detached eggs were then counted and recorded. The larval hatching rate (%) was then calculated using the following formula:

Larval hatching rate (%) = [Number of hatched larvae / (Number of hatched larvae + Number of un-hatching eggs)] × 100.

Post-larval weight (mg·100 PL^{-1})

From each nursing container, 100 post-larvae were randomly sampled, gently blotted dry with tissue paper, and weighed to the nearest 0.01 mg. This procedure was repeated three times, and the mean value was reported.

Post-larval survival (%) and larval development period (days)

PL survival was evaluated for each spawning event by recording the number of larvae that successfully developed into the post-larval stage. Survival was calculated as:

PL survival (%) = (Number of post-larvae / Number of initial hatched larvae) × 100

The larval development period was recorded as the number of days from hatching to the post-larval stage for each of the three consecutive spawnings.

Post-larval quality (stress test)

A stress test on post-larvae was conducted following the protocols described by Tackaert *et al*. (1989), Fegan (1992) and Samocha *et al*. (1998). Post-larvae originally reared in 15 ppt saline water were immediately exposed to freshwater (0 ppt). Sixty post-larvae were randomly sampled from each nursing container and divided into three replicates, with 20 post-larvae per replicate. The tolerance test was performed in a 500 mL glass container with a continuous aeration. Mortality was recorded every five minutes, and the test was terminated when 50% cumulative mortality was reached.

Statistical analysis

All data were first analyzed for normality and homogeneity of variance. Datasets were tested using one-way analysis of variances (ANOVA), with each spawning event considered as a treatment and the number of spawned females as replications. Subsequent comparisons among means were performed using Duncan's New Multiple Range Test. All tests were considered significant at $p<0.05$. Data analysis was performed using SPSS Statistics 25.0 software (IBM, USA).

RESULTS

Survivals and spawning successes of female broodstock

Female broodstock survival declined with an increasing number of spawning. A total of 62 females (86.11% of the initial stock) survived after the first spawning event. This number decreased to 57 (79.17%) and 49 (68.06%) females after the second and third spawning events, respectively, over a six-month period.

The success of spawning declined over three consecutive spawnings, from 82.26% in the

first spawning to 56.14% and 48.98% in the second and third spawnings, respectively, based on the total number of surviving females at each time point. Overall, only 24 females (33.33%) of *M. rosenbergii* completed three consecutive spawnings during the 6-month experimental period. At the start of the experiment, their average initial body weight and total length were 21.59±3.24 g and 12.27±0.88 cm, respectively. Significant growth ($p<0.05$) in both body weight and length was observed across the three spawning events (Table 1).

Significant effects of repeated spawning ($p<0.05$) were observed in five out of eight measured traits. The spawning interval increased (Table 1), although high variation was noted within each spawning batch: 27–46 days for the first, 28–48 days for the second, and 30–52 days for the third spawning event. The average realized larval yield did not differ significantly across the three consecutive spawnings, ranging from $1.24±0.13×10^4$ to $1.28±0.20×10^4$ ind·female^{-1}. However, when normalized by female weight, the relative larval yield significantly declined with successive spawnings.

Repeated spawning had no significant effect on hatching rates, which remained between 88.40–89.89% ($p≥0.05$), or on post-larval size, as the weight per 100 PL ranged from 49.61±1.87 to 50.03±1.82 mg. However, PL quality appeared to decline in successive spawnings, as survival rates significantly decreased from 27.65±2.05% in the first spawning to 22.92±2.71% and 20.88±2.52% in the second and third spawns, respectively. Additionally, larval development duration significantly increased with each subsequent spawn, from 26.67±1.21 days in the first spawn to 28.56±1.28, and 29.02±1.54 days for the second and third spawnings, respectively.

Post-larval (PL) quality

Post-larval quality was evaluated through a salinity stress test, where PLs were subjected to a sudden drop in salinity from 15 ppt to 0 ppt. The time to reach 50% mortality significantly decreased across repeated spawning ($p<0.05$). The recorded times were 51.83±1.69 min (range: 48.30–54.20 min) in the first spawn, 51.14±1.97 min (48.30–55.00 min) in the second, and 49.76±2.08 min (44.20–53.30 min) in the third.

Water quality in broodstock rearing tanks

Water quality in the rearing tank for giant freshwater prawn broodstock, maintained under a closed water recirculating system, was regularly

Table 1. Size of female broodstock and reproductive performance across three consecutive spawnings of *Macrobrachium rosenbergii* during a six-month experimental period.

Parameters	Spawning event			p-value
	1st	2nd	3rd	
Body weight (g)	23.87±3.39^a	25.70±3.24^b	27.67±3.47^b	0.001
Length (cm)	13.27±0.99^a	13.97±0.92^b	14.96±1.25^c	0.000
Spawning interval (days)	35.42±5.04^a	38.71±5.43^b	42.33±5.40^c	0.000
Realized larval yield (ind·female^{-1})	$1.24±0.13×10^4$	$1.27±0.16×10^4$	$1.28±0.20×10^4$	0.586
Relative larval yield (ind·g female^{-1})	521.50±37^a	494.90±23.06^b	463.58±35.16^c	0.000
Larval hatching rate (%)	89.89±2.79	88.40±2.71	88.83±2.53	0.148
Post-larval survival rate (%)	27.65±2.05^a	22.92±2.71^b	20.88±2.52^c	0.000
Weight per 100 PL (mg)	49.65±1.74	49.61±1.87	50.03±1.82	0.673
Larval development period (days)	26.67±1.21^a	28.56 ±1.28^b	29.02±1.54^b	0.000
Stress test of PL (min)	51.83±1.69^a	51.14±1.97^a	49.76±2.08^b	0.001

Note: Mean±SD in the same row superscripted with different lowercase letters are significantly different ($p<0.05$); ind. = individual; PL = Post-larvae; Stress test of PL = time until 50% mortality after immediately exposed to freshwater.

monitored every seven days over a six-month period. Results showed that most water quality parameters remained within optimal ranges as recommended by ACFS (2022). The only exception was total ammonia nitrogen, which occasionally exceeded the maximum recommended values of 0.5 mg·L^{-1}; however, the average concentration remained within acceptable limits (Appendix Table 1).

Water quality during larval nursing until post-larvae

All water quality parameters in the 20 L plastic nursing containers were measured every four days throughout the larval development periods until the post-larvae. The results showed no significant differences ($p \geq 0.05$) in water temperature, dissolved oxygen, pH, alkalinity, total ammonia nitrogen, or nitrite nitrogen among three spawning batches at each nursing period (i.e., on days 4, 8, 12, 16, 20, and 24) (Appendix tables 2–7). Most of the measured parameters remained within the optimal range recommended by the Department of Fisheries (DOF, 2019).

Although alkalinity remained relatively stable throughout the nursing period, the average values were slightly lower than the optimum level of 100 mg·L^{-1} as $CaCO_3$. The only exception was total ammonia nitrogen, where average values (0.695±0.05–0.898±0.07 mg·L^{-1}) (Appendix tables 6 and 7) exceeded 0.5 mg·L^{-1} from day 20 onward until the end of the nursing period.

DISCUSSION

Feasibility of repeated spawning of Macrobrachium rosenbergii under solitary hatchery rearing conditions

The present study confirmed the feasibility of using *M. rosenbergii* females for repeated spawning under hatchery conditions, corroborating earlier findings such as those of Cavalli *et al*. (2001). Our results show that females could spawn up to three times within the experimental period, reaffirming that this species is capable of multiple spawnings under captivity without the need for hormonal induction or eyestalk ablation.

Broodstock survival and spawning performance

Unlike Cavalli *et al*. (2001), who reported a high survival rate of 83.3% by rearing females in isolated compartments, our study found comparatively lower survival despite individual rearing. The lower survival in our study may be attributable to differences in rearing conditions, such as ambient water temperature, feed composition (38% crude protein vs 44% in Cavalli *et al*., 2001), and possibly handling stress.

In terms of reproductive performance, the maximum number of consecutive spawnings (three) was slightly lower than the five reported by Cavalli *et al*. (2001). A key difference lies in the method of egg incubation: our study employed *in vivo* hatching, while Cavalli *et al*. (2001) used *in vitro* techniques, which may promote faster egg development and reduce spawning intervals. Additionally, differences in feed quality or environmental conditions, including ambient temperature fluctuations and nutritional composition, may have influenced these outcomes. Jiang *et al*. (2020) demonstrated that dietary lipid content and feeding frequency can significantly influence reproductive cycling and spawning intervals in crustaceans.

Importantly, somatic growth was not inhibited by repeated spawning, suggesting that females allocated energy to both growth and reproduction concurrently. This observation is consistent with the findings of Cavalli *et al*. (2001), who estimated that molting in females is allocated 40% for growth and 60% for reproduction.

Realized and relative larval yield

Hatching success remained consistent across all spawnings, indicating robust maternal capacity and consistent egg quality. However, relative larval yield declined in successive spawnings, even though the realized larval number remained stable. This discrepancy can be explained by the confounding effect of increasing female body size, as fecundity generally scales with body mass. Similar observations have been reported in *N. davidi* (Tropea and Greco, 2015).

Cavalli *et al*. (2001) reported stable fecundity across spawnings, but their larval production per female was significantly higher—nearly fourfold—compared to our findings. This difference may be attributed to feed nutritional quality, particularly protein and lipid content, affecting both fecundity and egg quality. In their study, the number of larvae per gram female increased with successive spawns, while ours declined, further emphasizing the impact of diet.

To mitigate the adverse effects of repeated spawning, broodstock nutrition should be prioritized. Key dietary components include protein (40–45%), lipids (8–10%), and carbohydrate (25–35%) (reviewed by Ibrahim, 2023). Particular attention should be given to the provision of essential fatty acids such as linoleic acid (18:2n-6), n-3 HUFA (Cavalli *et al*., 1999; Harikrishnan *et al*., 2019), and arachidonic acid (20:4n-6) (Kangpanich *et al*., 2016). Moreover, it is advisable to allow broodstock a recovery period between spawning cycles to restore physiological condition.

Post-larval survival, period of larval development to post-larvae, and stress test results

A major contribution of this study lies in the evaluation of post-larval performance, which was not examined by Cavalli *et al*. (2001). Although hatching success remained high across spawnings, larval survival to the post-larvae declined significantly with successive spawnings. In addition, the duration of larval development was prolonged, and post-larval vigor appeared to decrease in later spawnings. These patterns suggest a reduction in larval quality despite stable hatching rates. While maternal nutrition is widely recognized as a key factor influencing larval quality, our findings align with those of Tropea and Greco (2015), who demonstrated that *N. davidi* larvae exhibited higher lipid content than the eggs, indicating that exogenous feeding after yolk absorption plays a significant role in larval development. Therefore, the reduced survival and prolonged development observed in our study are more likely attributable to factors such as maternal fatigue or stress-induced impairments in egg provisioning—potentially affecting initial yolk quality—rather than to nutritional limitations alone.

A similar pattern has been observed in marine species such as *Penaeus monodon* and *Litopenaeus vannamei*, where multiple spawnings have led to "reproductive exhaustion," a phenomenon where females become progressively less productive after multiple spawnings (Palacios *et al*., 2000). Moreover, forcing multiple spawns (e.g., via eyestalk ablation) in penaeid shrimp causes a pronounced depletion of hepatopancreatic lipid reserves and a consequent drop in egg quality and larval survival (Boucard *et al*., 2004). In our study, the decline in larval performance despite adequate hatch rates suggests early signs of reproductive senescence or subtle changes in egg quality not detectable through egg count or hatchability alone.

Water quality

Water quality in holding broodstock tanks remained within acceptable ranges throughout the study (ACFS, 2022). Although total ammonia nitrogen (TAN) occasionally exceeded recommended levels, average values remained below the threshold. Slightly lower alkalinity during nursing and elevated TAN levels on days 20 and 24 (0.695–0.898 $mg \cdot L^{-1}$) may be attributed to *Artemia* excretion and organic waste accumulation, even with 50% water exchange every two days. Nitrite concentrations consistently remained within safe levels, i.e., below 2 $mg \cdot L^{-1}$ NO_2^--N as recommended by Mallasen and Valenti (2006). According to the results, all water quality parameters show no significant differences during larval development period across the three consecutive spawnings, this ensures that they have no effect on the declining in post-larval survivals, prolonged periods of post-larval development and post-larval quality across spawning three times.

CONCLUSIONS

This study confirms that repeated spawning in *M. rosenbergii* is a viable strategy under hatchery conditions, yielding consistent larval production across spawnings. Although realized larval yield and hatching success remained unaffected, relative larval yield decreased, and most notably, larval survival to post-larvae declined in later spawnings. These findings indicate that while repeated spawning

remains feasible, close attention should be paid to larval quality in successive cycles. The inclusion of post-larval performance as an evaluation criterion adds new insights to the understanding of reproductive output in this species and highlights the importance of comprehensive broodstock management to maintain larval quality across multiple spawnings.

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