Re-maturation of wild banana shrimp *(Fenneropenaeus merguiensis)* broodstock in tank

Damar Suwoyo[1], *Abidin* Nur[1*], Suryati[1], *Lisa* Ruliaty[1] and *Mohamad* Soleh[1]

[1]Research Center of Fishery, National Research and Innovation Agency, Jl. Raya Bogor KM 17 Cibonong, Kab. Bogor, Indonesia.

Abstract. The amount of post spawning of *F. merguiensis* broodstocks were subjected to further culture for three months period and fed various diets under recirculation system. The aim of the study was to evaluate their reproductive performances and seed production parameters. The broodstocks were fed a fresh diet consisting of chopped squid and *Nereis* sp, combined with a maturation diet (Skretting-Crude Protein: 59%). After the three-month culture period, a total of 17 pairs of broodstocks (53.53 ± 7.0 g for females and 28 ± 2.39 g for males) were selected, ablated, and reared for another ten days. Reproductive performance of these broodstocks and the post-larvae produced were observed. Broodstock's performance in terms of egg and nauplii production was comparable to that naturally matured (wild) brooders. The average nauplii production was 250,000 individuals, with a latency period starting on the third day after ablation, and 77% of the broodstocks spawned within the given period. Furthermore, larval survival was high and reaching up to 40% until Pl-10. In conclusion, banana shrimp broodstocks can be maintained for certain period while maintaining their reproductive performance, if the diets and environmental parameters are kept within favorable conditions.

1 Introduction

The banana shrimp *(F. merguiensis)* is an important fisheries species that is found in both tropical and subtropical waters throughout the Indo-West Pacific area [1], [2]. Furthermore, it has high-value species, including one of the six that contributed to world aquaculture production [3]. Due to increased demand worldwide, an effort to reduce reliance on wild broodstock for seed production purpose must be considered. Domestication of these species has been carried out in Indonesia since 2018 and has yielded promising results for continued aquaculture development of the species [4], [5]. One of the most important results was the shrimp can be bred successfully in hatchery for seed production purpose.

The first chain in shrimp seed production systems is broodstock maintenance. Feeding and water media control are critical variables in the optimal generation of eggs and nauplii during maintenance. The amount and type of feed utilized influence the reproductive process

* Corresponding author: abidinnoer2@gmail.com

and the quality of the producing seeds [6]. During the maturation phase, the weight of the ovary grows 4-9 times in one week, and sufficient nutrients are necessary for the development of the yolk into the pre-feeding larval stage [7]. Lipids are the key nutrients engaged in the biochemical processes during maturation [8] and are kept in the oocytes to enrich the development of succeeding larvae.

Various parameters influencing egg quality and quantity, including broodstock age and size, broodstock source, genetic variation, and nutrition [9]. Polychaete worms, mollusks, and crustaceans are common broodstock diets. However, the nutritional value varies depending on the season and the type of feed. To match the availability of fresh feed in their natural habitat, maturation feed should contain highly digestible protein, necessary fatty acids, cholesterol, and chemo-attractants [10]. A trial on male *P. merguiensis* broodstock over six weeks of maintenance found that squid meal was chosen above other types of feed such as polychaetes and oysters [11].

To address the shortcomings of using fresh or frozen feed, artificial feed for shrimp broodstock has been developed as an alternative feed source. This sort of feed has several advantages, including guaranteed availability, more stable nutrient content, practical use, extended storage capabilities, and the option to add certain needed nutrients [7]. According to research on *P. vannamei*, a combination of fresh feed and maturation feed resulted in decreased post-ablation mortality in broodstock. In *P. stylirostris*, replacing up to 50% of fresh meal with artificial feed resulted in comparable reproductive responses and larvae quality as using exclusively fresh feed [12]. Extended culture periods of the wild broodstocks was the earlier information required for the seasonal abundance of these species. The application of two types of diet during extended culture period of post spawned *F. merguiensis* broodstock was the main area of the current research and aimed to evaluate their reproductive performances and post larvae produced.

2 Material and Method

2.1 Broadstock source

Wild catch broodstock was obtained from Pangandaran water, West Java, and was previously spawned at that location. The nauplii, along with their broodstock, were then used for seed production activities and broodstock maintenance testing at Shrimp Hatchery Unit, BBPBAP Jepara, Central Java, Indonesia.

2.2 Shrimp culture and experiment design

Amount of 30 males and 80 females broodstock were separately maintained in cement tanks measuring 5.6 x 1.5 x 0.8 m (with a water depth of 60 cm). The maintenance lasted for three months using a recirculating system. Another tank was used to cultivate sea grave (*Caulerpa. lentillifera*) with an initial stocking of approximately 20 kg and used as a biofilter. The use of seaweed was based on its effectiveness in absorbing ammonia [13] and nitrate [14]. The water circulation was achieved using an aquarium water-pump (flow rate of 2,800 L/h) directed to the broodstock tanks (male and female). From both tanks, at the outlet section (equipped with a black mesh net filter), the water was connected to the biofilter tank. The water flow rate was adjusted in such a way that the water in the broodstock tanks was completely replaced 300% per day. A complete water change in the broodstock tanks was conducted once a week. Leftover feed, post-molting shrimp shells, and other waste were removed daily.

Feed used were squid, *Nereis sp*., and maturation diet (Skretting, Protein: 59%, lipid: 11%, fiber: 0.3%, and ash: 11.5%) provided in the morning, afternoon, and evening, respectively. The percentage of feed usage ranged from 20% (fresh feed) and 1-2% (maturation diet) of the shrimp biomass.

After three months culture period, 17 pairs of broodstock were selected and used for reproductive performance testing. The average weight of female and male broodstock were 53.53 ± 7.0 g and 28 ± 2.39 g, respectively. Eyestalk ablation was performed on the female broodstock, and they were subsequently reared in an indoor tank measuring 2 x 3 x 1.3 m for 10 days. The effective water depth was 50 cm, and the water was circulated through a flow-through system after passing through a series cartridge filter. The photoperiod lasted for 10 hours of light (55 lux by using a 40-watt neon lamp placed 120 cm above the tank) and 14 hours of dark condition. Dissolved oxygen, temperature, salinity, and pH were recorded of 5.5-6.2 ppm, 30-32 oC, 31-33 ppt and 7.8-8.2, respectively.

Selection of gonadal maturation was conducted in the late afternoon and aid by water resist-LED rechargeable headlight. Mature brooders were transferred to a fiberglass tank (with a capacity of 1 m^3). The eggs and nauplii were maintained in that tank before being moved to the larval rearing tank.

The larvae culture was reared in cement tanks (with a capacity of 3 m^3) in an indoor setting, with two tanks used. Treated seawater was provide and fill up to the larvae rearing tank (around 60-70% of the culture volume) one day before the nauplii stocked. The nauplii, aged approximately 30 hours, were stocked at a density of 100 individuals/L. Shortly after metamorphosing into zoea (during the night), they were provided with *Skeletonema costatum* (twice a day) until the mysis-3 stage. Artificial feed was given six times a day at a dosage of 0.5-1.5 ppm (zoea-post larvae). Upon reaching the post-larval (PL) stage, Artemia feed was introduced at a dosage of 1-2.5 individuals/mL until PL-10 (harvesting). Water quality for such parameters of dissolved oxygen, temperature, salinity, pH, and total ammonia nitrogen (TAN) were 5.7-6.5 ppm, 29-31 ºC, 28-30 ppt, 7.8-8.4 and 0.020-0.069 ppm, respectively. At the end of the rearing period, 25-30 samples of the seed were taken for analysis to detect the presence of specific pathogens by using Polymerase Chain Reaction (PCR) test, especially *White Spot Syndrome Virus* (WSSV), *Infectious Hypodermal Hematopoietic Necrosis Virus* (IHHNV), and *Infectious Myonecrosis Virus* (IMNV).

2.3 Data collection

Data collection for reproductive performance includes the latency period, number of broodstock spawning post-ablation, number of eggs and nauplii, seed survival rate, as well as several water quality parameters (temperature, salinity, dissolved oxygen, and pH). The quantification of nauplii is performed volumetrically, while the seed survival rate is calculated at the end of the rearing period.

3 Result and Discussion

3.1 Reproductive performances

After 10 days culture period, it was found that three shrimps died and presumably due to cannibalism after moulted. Around 71% (10 pcs) of the broodstock survivor successfully spawned and produced nauplii (Table1).

Table 1. Reproductive performance of *P. merguiensis* brooder for ten days maturation period.

Parameters	Value
Number of females (pcs)	17
Number of males (pcs)	17
Survival rate (%)	82.35
Number of spawners (pcs)	10
Latency period (days)	3
Fertilization rate (%)	90
Hatching rate (%)	90
Egg diameter (μm)	2.48-2.49
Average number of eggs/females	250.000
Average number of nauplii/females	200.000

3.2 Post larvae production

It can produce 240.000 PL-10 from 600.000 nauplii, or a 40% survival rate. Post larvae from that population tested negative for WSSV, IHHNV, and IMNV. Furthermore, the number of spin rostrum is around 5 and the midgut ratio is greater than 4. Aside from that, the larvae were consistent in size, vivid in color, active, and phototaxis.

3.3 Discussions

Water quality in the broodstock rearing tanks and seed production media is within a range that supports shrimp cultivation. Specifically, in the case of re-maturation activities, improving the quality of the media with sea grapes *(C. lentillifera)* also helps save water usage and sterilization materials through a recirculation system. The nutrient-rich metabolites produced by shrimp can be effectively utilized by sea grapes as a biofilter before being reused by the cultured shrimp broodstock. The nutrient sources commonly used generally have high protein levels (such as squid, *Nereis sp*. worms, and maturation pellets), which also contribute to the potential build up of inorganic nitrogen waste. This factor supports the growth of cultivated sea grapes biomass. Besides nutrient availability, media salinity also supports sea grapes growth (31-32 ppt). Salinity levels below 30 ppt result in slow growth, and salinity levels below 25 ppt can cause mortality[15]. The contribution of sea grapes is not only limited to improving media quality but may also have a correlation with the immune response of the cultured broodstock. Our findings from the previous experiments demonstrated that cohabitation of white shrimp *(F. merguiensis)* with sea grapes *(C. lentillifera)* can enhance immune parameters such as total hemocyte count and phagocytic activity. Further study on shrimp *P. vannamei* co-culture with *C. lentillifera* shown that not only reduced the nitrogen compound in the media but also improve growth and survival of these shrimp[16][17].

Thinning is done through partial harvesting to prevent excessive development of seaweed during the experiment. An experiment using sea grapes *(C. lentillifera)* to grow tiger shrimp, it was able to absorb up to 50% of ammonia[18]. The shrimp density and amount of seaweed utilized, however, were not mentioned. *C. lentillifera,* in addition to being excellent at lowering ammonia, is also effective at absorbing nitrate when compared to the seaweed species *Gracilaria lichenoides* [14]. It has been observed that in seaweed media, the water is clear and plankton levels are low. Copepods, on the other hand, flourish and provide an extra source of nourishment for cultured broodstock. This study, however, did not contain observations on the types and populations.

According to current research, the maintenance duration of banana shrimp broodstock can be extended up to three months while maintaining strong reproductive performance and a broodstock survival rate of more than 80%. This method is a good strategy for dealing with the seasonal availability of broodstock supplies.

The reproductive performance of the broodstock samples reveals the efficacy of fresh feed treatment, maturation feed, and water management support via recirculation. With an average nauplius generation of 250,000 individuals, broodstock productivity approaches 77%. Fertilization (90%) and egg hatching rates (90%) are regarded as high and serve as early markers of good post-spawning broodstock management. The use of both fresh feed and artificial maturation feed is required and their effects for various shrimp species has been summarized (Table 2).

Table 2. Effect of fresh diet substitution with artificial diet on reproductive performances of shrimp broodstocks[6].

Artificial diets (%)	Substitution effects	Species
50	High survival rate, consistent on spawning and fecundity	*Litopenaeus stylirostris* *L. vannamei*
50	More frequent of spawning, fertility and fecundity increased	*L. vannamei*
50-100	Higher spawning frequency	*L. schmitti*
60	Length of zoea and fecundity were lower	*L. stylirostris*
88-100	Higher spawning frequency	*L. stylirostris* *L. vannamei* *Fennerepenaeus indicus*
100	No significant different	*L. chinensis*
100	Low fecundity and hatching rate	*F. indicus*
100	Higher spawning frequency and larval survival	*P. monodon*

Both types of feed used contribute to the reproductive performance of the broodstock. Polychaete worms like *Nereis sp.* can enhance the reproductive system of shrimp due to their high nutritional content, such as arachidonic acid (ARA) and reproductive hormones[10]. ARA is one of essential fatty acid and plays a crucial role in the reproductive system of shrimp broodstock [19], [20]. Squid are not only rich in cholesterol but also contain numerous steroid hormones that play a role in the vitellogenesis process [19]. Further, the maturation feed used not only contains essential macro and micronutrients but also has a low fiber content of 0.3%, resulting in higher feed digestibility. However, the percentage of this feed used still low compared to the above mentioned on Table 2.

The next test results were the seed production using two separate semen tanks, each with a capacity of 3 m^3. The number of produced seeds (Pl-10) was 240,000 individuals, with a survival rate reaching 40%. The transition stages from nauplius to Pl-10 proceeded normally, and the seeds appeared uniform and active. Health status observations using PCR showed negative results for WSSV, IHHNV and IMNV. This achievement is equivalent to our previous finding by using nauplius from matured gonad broodstock maintained in indoor tanks (with a capacity of 12 m^3) with an average survival rate of 36% (ranging from 25% to 50%) over six rearing cycles.

4 Conclussion

The post-spawned maintenance of white shrimp *(F. merguiensis)* broodstock for a relatively long period can be achieved with good reproductive performance. Providing high-quality feed and improving the environmental conditions of the rearing medium are essential factors during the broodstock maintenance for an extended period.

References

1. W. Wang, S. Yang, C. Wang, L. Shi, H. Guo, S. Chan, Fish Shellfish Immunol **71** (2017)
2. W.C. Liu, S.H. Zhou, B. Balasubramanian, F.Y. Zeng, C.B. Sun, H.Y. Pang, Fish Shellfish Immunol **104** (2020)
3. D.J. Vance, P.C. Rothlisberg, *The biology and ecology of the banana prawns: Penaeus merguiensis de Man and P. indicus H. Milne Edwards*, in Advances in Marine Biology, Academic Press (2020)
4. P.A. Wiradana, A. Nur, M.D. Sani, M. Affandi, T.W.C. Putranto, Ecol. Environ. Conserv **26** (2020)
5. A. Nur, A. Yudhistira, L. Ruliaty, JMAI (2022)
6. R. Wouters, P. Lavens, J. Nieto, P. Sorgeloos, Aquaculture **202** (2001)
7. R. Wouters, B. Zambrano, M. Espin, Aquac (2002)
8. G. Cuzon, G. Gaxiola, C. Rosas, Reprod. Biol (2008)
9. I. S. Racotta, E. Palacios, A. M. Ibarra, Aquaculture (2003)
10. N. Chimsung, Songklanakarin J. Science and Technology **36** (2014)
11. A. J. Memon et al, J. Anim. Vet. Adv **11** (2012)
12. P. Coutteau, E. Dencece, D. Pham, E. Pinon, Y. Balcazar, *Mixed maturation diets improve shrimp broodstock perfomance*, Global aquaculture Advocate, June (2001)

13. K. Hamano, I. Tsutsui, P. Srisapoome, *Co-culture of black tiger shrimp (Penaeus monodon) and sea grape (Caulerpa lentillifera)*. Research Highlights, JICA project (2021)
14. H. Liu, F. Wang, Q. Wang, S. Dong, X. Tian, J. Appl. Phycol **28** (2016)
15. M.D.S. Rabia, Environ. Sci **4** (2016)
16. K. Van Ly, D.K. Murungu, D.P. Nguyen, N.A.T. Nguyen, Fishes **6** (2021)
17. N.T.N. Anh, F.A. Shayo, N. Nevejan, N. Van Hoa, J. Appl. Phycol **33** (2021)
18. K. Hamano, I. Tsutsui, P. Srisapoome, *The use of seaweed for improving water quality in brackish water shrimp farming ponds and effects on shrimp health*, JIRCAS Work. Rep., no. 44 (2006)
19. N.D. Hoa, R. Wouters, M. Wille, V. Thanh, T.K. Dong, Aquaculture (2009)
20. G.J. Coman, S.J. Arnold, M. Barclay, D.M. Smith, Aquac. Nutr **17** (2011)