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The Effect of Ages on Reproductive Performances of Banana Shrimp, *Penaeus indicus*

Pengaruh Umur Terhadap Performa Reproduksi Induk Udang Putih, Penaeus Indicus

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ABSTRACT

Age and weight were the main target on domesticated shrimp due to affect on their reproductive performances. The objective of the present study was to know the effect of broodstock ages on reproductive performances of P. *indicus*. Two series experiments were conducted in the Hatchery Unit of MCBA Jepara by using the 1st generation (G-1) domesticated broodstocks at two differences ages such as 11 months (U-11) and 14 months (U-14). All maturation and spawning process were carried out under separated room-indoor system. Larva rearing and seed productions were conducted into 10 m^{-3} concrete tanks based on shrimp seed production protocols being developed on MCBA Jepara. Results found that the difference of broodstock ages significantly (*p<0.05*) affects on daily spawning rate, nauplii productions, spawning frequencies, and larvae survival, were (18.78±4,6 vs 8.85±3,69); (47.000±2.400 vs 27.421±8.555 N spawner-); (1.67 for 9 days vs 1.79 for 22 days) and (26.81±7,30% v 37.27s ± 1.95%), respectively. From the experiments it can be concluded that 11 months ages brooders were suggested for mass seed production purposed. However, further research of less than ages mentioned needs to take into account.

ABSTRAK

Umur dan berat menjadi target dalam proses domestikasi induk oleh karena berpengaruh terhadap performa reproduksi. Penelitian bertujuan untuk mengetahui pengaruh umur terhadap performa reproduksi induk *P. indicus.* Dua seri penelitian telah dilaksanakan di Unit Pembenihan BBPBAP Jepara menggunakan induk hasil domestikasi generasi-1 (G-1) di tambak pada umur 11 bulan (U-11) dan 14 bulan (U-14). Proses maturasi dan pemijahan dilaksanakan dalam ruangan terpisah dan tertutup. Pemeliharaan dan produksi benih menggunakan bak semen kapasitas 10 m^{-3} sesuai dengan protokol sistem produksi benih udang penaeid di BBPBAP Jepara. Hasil penelitian menunjukkan bahwa perbedaan umur (U-11 dan U-14) berpengaruh secara signifikan (*p<0.05*) terhadap laju pemijahan harian (18,78±4,6 %h^{-} vs 8,85±3,69 %h^{-}), produksi nauplius (47.000±2.400 N $induk^{-}$ vs 27.421±8.555 N $induk^{-}$) dan frekuensi pemijahan (1,67 selama 9 hari vs 1,79 selama 22 hari) serta sintasan benih (26,81±7,30% v

37,27s ± 1,95%). Hasil dari penelitian ini disimpulkan bahwa untuk kepentingan produksi benih secara massal maka induk poduktif yang digunakan berumur 11 bulan. Performa reproduksi induk kurang dari umur tersebut perlu dikaji lebih lanjut.

Kata Kunci	*Domestikasi, Penaeus indicus, Performa reproduksi, Umur*
Keywords	*Domestication, Penaeus indicus, Reproductive performance, Ages*
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INTRODUCTION

Domestication of white shrimp, P. indicus at BBPBAP Jepara since 2000 as an effort to diversify shrimp cultivation commodities in Indonesia. This species is one of six species (P. vannamei, P. monodon, P. Chinensis, P. Japonicus, P. merguiensis and P. indicus) that contribute to global production of cultivated shrimp (Vance & Rothlisberg, 2020). However, production began to decline after 2006 due to disease and the shift of farmers to P. vannamei and P. monodon shrimp (Vance & Rothlisberg, 2020). As a local species, it is certainly more adaptive to the aquatic environment in Indonesia, and is even reported to be resistant to high temperatures (Hoang et al., 2020) and has a wide salinity tolerance (Yousif, Kumar, and Ali A 2003; Sajeela et al., 2019).

Selection of cultivated species that are resistant to global climate change is a current concern. Supported by the availability of pond land, development at the level of simple (traditional) technology will have a significant impact on national shrimp production. Another important thing is that it has high genetic variation (Regunathan, 2008), lower feed protein requirements (30%-35%) and is proven to be more profitable compared to higher protein levels (Panigrahi et al., 2020; Lalramchhani et al. , 2020; Estante-Superio et al., 2022).

Domesticated products are intended for genetic improvement of a species as well as controlling the production system and the health status of the seeds produced. In addition, domestication is seen as an ecological and economic approach for the continuity of the shrimp farming industry (Regunathan, 2008). In aquaculture, the essence of domestication is total control of the life cycle of an organism including its hatchery activities (Liao and Huang 2000).

Parent weight and age are targets in the domestication process because they influence reproductive performance (Coman and Crocos 2003; Racotta et al., 2003). In general, large parents tend to have better reproductive performance than small parents (Hoang et al., 2002). However, studies on P. merguiensis shrimp showed that 13-month-old broodstock with the same weight as 10-month-olds had lower reproductive performance and seed survival (Hoang et al., 2002). The same thing was reported in P. semiculcatus shrimp, that older parents (12-14 months) produced fewer eggs than younger parents (Coman & Crocos, 2003). Experience in India reports the same trend that P. indicus (natural) with a brood weight of 20-25g produces more eggs (eggs/gram brood weight) compared to larger brood sizes (Anand et al., 2019). The effect of age on the reproductive performance of domesticated F. indicus broodstock is limited so this test needs to be carried out.

METHODS

Test Material

Generation-1 (G-1) parent production was carried out at NSBC ponds, BBPBAP Jepara in 2020-2021. The seeds used come from natural parent spawning (Prigi, East Java) and are declared SPF (specific pathogen free) for WSSV, IMNV, IHHNV, TSV. The weight and length of the parent were 43.0 ± 10.2 g respectively; 16.02±1.05 cm (female) and 28.8±5.4g; 14.0±0.79 cm (male). Nauplius production per broodstock averages 80,000 with seed survival (<Pl-10) less than 25%. The general principles of maintenance and production of G-1 broodstock include individual selection, removal and thinning of the population as well as sorting males and females to reach brood size.

Test Implementation

Testing of the reproductive performance of G-1 broodstock from two different age groups, namely 11 months (U-11) and 14 months (U-14) was carried out at the Shrimp Hatchery Unit, BBPBAP Jepara in January - June 2021. Determination of broodstock age was calculated from the nauplius stage until it becomes a parent and produces another nauplius. The selected broodstock are adapted for 5-7 days in a maturation tank and kept separately between males and females. The weight of the broodstock used for testing aged 11 and 14 months was relatively the same and ranged from 38-44 g (females) and 21-25 g (males).

After an adaptation phase of 5-7 days, eye ablation continues to stimulate gonad maturation. Male and female parents are kept together in a tank measuring 5x6x0.8 m with a ratio of 1:1. During maintenance, they are given fresh food in the form of Nereis sp. worms, squid and oysters at 25-30% biomass and maturation food (50% protein content) at 1-2% biomass. The salinity and temperature of the media were relatively the same throughout the test and ranged from 29-30 ppt and 28-29 oC. Meanwhile, in the spawning and production media for nauplius, the water temperature reaches 29.0-30.5 oC. Water changes are carried out in the morning and evening as much as 100-200% per day before feeding.

Observations of gonad development are carried out in the afternoon and usually start on the third or fourth day after ablation. The parents who are ready to spawn are transferred to the spawning tank using a fiber tub (active volume 800 L). Separate spawning room from broodstock maturation tank and dark conditions. The mother who has released the egg is transferred back to the maturation tank the next day. To optimize the hatching of eggs into nauplius, the water temperature was increased to 30 ± 1 oC using a 100 watt automatic heater and provided with light using a lamp installed above the fiber tub.

The nauplius produced (24 hours or more after hatching) are ready to be harvested and then reared in a seed production tank (capacity 10 m3) in an indoor room. Media water uses water that has been sterilized and distributed into larval rearing tanks with a water height of up to 70 cm. Add water up to 100 cm before the stage changes to post larvae. Fresh water is used to reduce salt levels and also as a moulting stimulator. When the nauplius changes stage to zoea (usually in the afternoon), it is immediately given natural Skeletonema costatum food of 20,000-30,000 cells mL-. Artificial feed is given in the form of micro encapsulated diet (FRIPPAK) with the type and amount according to the larval-seed stage, namely 0.75 – 2 ppm for feeding six times a day. Artemia is given from the post larvae stage until harvest in the morning and evening. Water parameters such as dissolved oxygen, pH, temperature and salinity were relatively the same throughout the test and each ranged from 5.16 to 5.91 ppm; 8.0-8.4; 28.9-31.2 oC; and 25-30 ppt. Seed harvesting is done after Pl-8 or more.

Test data and statistical analysis

The test data includes the reproductive performance of the broodstock, namely: latency period (length of time required to spawn after ablation, h), spawning rate (number of broodstock that spawn each day, % h-), and broodtail nauplius production and seed survival (%). Statistical analysis (one-way ANOVA) of reproductive performance and survival of seeds from different parental ages with IBM SPPS (version 25) at the 95% confidence level or (*p<0.05*).

RESULT AND DISCUSSION

Parent reproductive performance G-1

PThe reproductive performance of P. indicus parents of different ages is presented in Table 1

Table 1. Reproductive performance of P. indicus parents at the age of 11 (U-11) and 14 (U-14) months.

Reproductive performance	11 months old (U-11)	14 months old (U-14)
Parent weight (g)	39,53±2,50[a]	39,95±2,76[a]
Latency period (h)	4	5
Mijah frequency (kali)	1,69	1,79
Mijah period (h)	9	22
Percentage of broodstock (%/h)	18,78±4,6[a]	8,95±3,69[b]
Nauplius production (N/ekor)	47,000±2.400[a]	27,421±8.55[b]

Mean ± SD followed by different letters on the same line indicates that they are significantly different (P<0.05).

The weight of the parents used is relatively the same even though they are of different ages, namely 36-44 g (females) and 20-26 g (males). Such condition of the parent has been reported by (W. Emmerson, 1980), that the parent is >39 grams, the condition factor and weight are starting to decline. The latency period is reached on the fourth and fifth days post-ablation, faster than the non-ablation parent, which reaches seven days (Nur et al., 2022; in-press). This period is classified as normal as a result of domestication. In fact, the natural maternal latency period has been reported to reach 7-10 days (Anand et al., 2019). The spawning frequency of U-11 and U-14 broodstock was 1.69 (for 9 days) and 1.79 (for 22 days), respectively. This parameter is very important for the operational efficiency and effectiveness of the hatchery unit. At this stage the need for fresh feed is greater and the price is relatively expensive. The maturation feed (pellets) used cannot be fully relied on to replace the function of fresh feed in the maturation process. The spawning frequency (1.69–1.79) reflected that the U-11 parent had a shorter spawning interval compared to the U-14 parent. Vazquez Boucard et al., (2004) reported that indicus parents can spawn 3.9 times a month or at intervals of 2-6 days. The results of this study showed that 69% of broodstock (U-11) spawned twice during the nine day spawning period. This is faster than the U-14 parent which reached 79% within 22 days.

There were significant differences in the spawning performance of P. indicus broodstock of different ages. The daily percentage of broodstock spawning at U-11 was higher (18.78±4.6 % h-) and showed a significant difference (p<0.05) compared to U-14 (8.95±3.69 % h-). The same thing happened in nauplius production, namely 47,000 ± 2,400 vs. 27,421 ± 8,555 parent-nauplius.

Compared with the performance of the previous parent (natural parent), the production of G-1 parent nauplius experienced a significant decrease from 80,000 parent nauplius to 27,000-47,000 parent nauplius. This can be explained and is possibly related to the source of nutrition during the broodstock production process in the pond. Pellet feed dominates the nutrient source and the use of fresh feed is very minimal and only in the last few months of maintenance. Use of fresh food such as squid, oysters and worms, Nereis sp. more dominant after shrimp are reared in hatchery units. On the other hand, this type of feed is very necessary in the maturation process, especially because it contains protein, fat (fatty acids) and steroid hormones and minerals (Coman et al., 2011; Hoa et al., 2009; Phoonsamran et al., 2017). Lipid compounds are stored in the hepatopancreas and function for the formation of vitelline (the most important part of lipids for maturation), the development of oocytes and embryos as well as a source of food (energy) for the nauplius (Vazquez Boucard et al., 2004; Auttarat et al., 2006).

The reduced productivity of domesticated broodstock compared to natural broodstock is a common phenomenon in the domestication of several types of shrimp. In tiger prawns, P. monodon, the incidence has been observed and it is suspected that vitelline from the follicle cells to the oocyte is slow and inhibits the maturation process compared to the natural parent (Urtgam et al., 2015). Another study stated that the results of biochemical analysis of eggs and nauplius between natural parents and domesticated parents of P. indicus contained significant differences such as long chain fatty acids (HUFA), especially EPA (Eicosapentaenoic acid), DHA (Docosahexaenoic acid) and ARA (Arachidonic acid). AC ID). Likewise, the glucose, cartonoid and triacylglycerol contents are higher in the natural parent (Regunathan, 2008).

Efforts have been made to improve the reproductive performance of domesticated broodstock so that it is economically viable, such as: improving nutrition from the initial rearing phase (Keys & Crocos, 2006) to the maturation phase (Santander-Avanceña et al., 2021); introduction of specific nutrients such as omega-3 fatty acids (Coman et al., 2011, 2013), selection systems (Norman-López et al., 2016; Ren et al., 2020), identification of genes involved in lipid metabolism during maturation (Rotllant et al., 2015) as well as improvements in the prematurity stage such as the application of biofloc technology (Braga et al., 2018; Cardona et al., 2016; El-Sayed, 2021; Emerenciano et al., 2013, 2014; Magaña-Gallegos et al., 2018, 2021). Currently, P. vannamei is a domesticated product that has changed the global shrimp industry and dominates cultured shrimp production (Wyban, 2009). Improving reproductive performance requires a comprehensive approach starting from aspects of nutrition, rearing environment and other biological characteristics. This is what causes domestication and breeding activities to require a relatively long time and greater costs. The development of selection technology based on genome information (genomic selection) is a new approach to the genetic improvement system of a species (Zenger et al., 2019) and the implications for P. indicus shrimp or other local shrimp species are expected to be realized soon.

Seed Survival
The survival of P. indicus seeds of different ages is presented in Fig 1

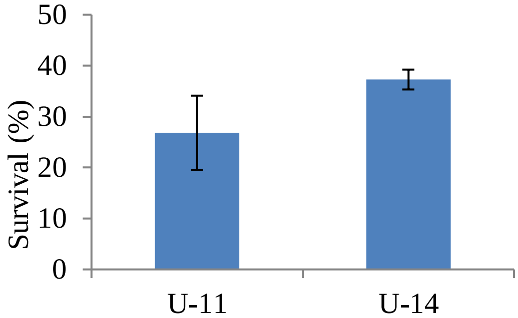

Figure 1. Survival of P. indicus shrimp seeds showed a difference ($p<0.05$) between the ages of 11 months (U-11; n=9) and 14 months (U-14; *n=12*).

Apart from the performance of the parents, the performance of the larvae (change of stage) and seeds (survival) is an important part of observation in each rearing. The change from the nauplius stage to zoea generally takes place in the afternoon. The transition to the next stage such as mysis and post larvae takes place normally, namely around 3-4 days.

Experience in the last two years shows that survival of P. indicus shrimp seeds is relatively low (<30%). Cannibalism is considered to be the trigger for low survival and begins to occur in the early post larvae stages. The habit of the seeds inhabiting the bottom or sticking to the walls of the tank means that the shrimp's territory is limited. The same thing has been reported by (Anand et al., 2019), but the technical explanation is different. It was reported that the synchronization of molting from the zoea stage to mysis-1 (91.5% vs 8.5%) and mysis-3 to PL-1 (30-40%) was considered to be the trigger for low seed survival.

Efforts have been made to increase seed survival, such as using the probiotic Bacillus sp both in water media and for Artemia nauplius and increasing survival by around 11-17% (Ziaei-Nejad et al., 2006). Another test result was by adding prebiotics in the form of inulin and combining it with SELCO to enrich Artemia before being given to post larvae (Hoseinifar et al., 2010; 2015). However, testing on a mass scale needs to be carried out. The application of the synbiotic system has been carried out in mass-scale P. indicus seed production, but it has not had an effect on increasing survival but the effect is more significant on the development of intestinal villi and goblet cells. (Nur et al 2021, *unpublised*).

Food and environmental factors play a role in seed survival. (Kumlu, 1998) reported that a combination of Tetrachelmis chuii and Skeletonema costatum with a density of 60-70 cells/µL up to mysis-3, followed by administration of Artemia nauplius (5 mL-) resulted in the best survival, development and growth from the zoea stage up to Pl-1 (Kumlu & Jones, 1995). However, in mass-scale seed production systems, there are difficulties in providing two types of natural feed at once and the potential for greater contamination.

The larvae's preference for other types of diatoms is Thalassiosira weissflogii and is combined with rotifers and Artemia at the mysis stage (W. Emmerson, 1980). However, the energy value of rotifers is lower so it is not recommended for mass production systems for indicus shrimp (W. D. Emmerson, 1984). Furthermore, a study of environmental aspects reported that the best salinity and temperature for rearing larvae was 20-25 ppt and 28°C (Kumlu & Jones, 1995). This is relevant to the results of the challenge test (without acclimatization; 96 hours) that P. indicus larvae survived well at a salinity of 5-20 ppt (Karan et al., 2020). However, rearing P. indicus shrimp larvae in tropical areas can tolerate temperatures up to 35oC (Hoang et al., 2020). Generally, seed maintenance and production is carried out at temperatures >30% and salinity >25 ppt, so further study of these two parameters is needed at lower levels. Another thing that is considered important is more frequent feeding considering the fairly high level of cannibalism in this species.

In Figure 1 above, it is known that U-14 parents produced higher seed survival (37.27 ± 1.95%) and were significantly different ($p<0.05$) compared to U-11 parents (26.81 ± 7, 30%). The influence of the differences cannot yet be explained with certainty. However, parent nutrition (such as the hepatopancreas and ovaries) is an important indicator for further study. Parental nutrition and its influence on the biochemical composition of eggs and nauplius have been studied (Regunathan, 2008), but information on seed survival rates is still limited.

CONCLUSION

The results of the study concluded that differences in the age of domesticated P. indicus parents had an effect on the reproductive performance of the parents and the survival of the seeds produced. 11 month old broodstock (U-11) has better reproductive performance and can be used for mass seed production activities, although the survival of the seeds produced is lower than 14 month old broodstock (U-14).

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