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Field evaluation of culture performance of triploid *Penaeus monodon* with its diploid counterpart

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Abstract

The success in triploid (3n) cold-shock induction of the black tiger shrimp *Penaeus monodon* led to the field trial of the 3n shrimp performance, compared to that of the diploid (2n) shrimp. The first three trials were performed in 90-ton round canvas ponds at the stocking density of 25-40 individuals/m^2. The average body weights (BW) of the 3n shrimp were significantly higher than that of the 2n control in all the three trials. The last two trials were performed in two phases: the first phase was rearing in the 90-ton round canvas ponds for one month and the second phase in earthen ponds for another 3-4 months. The final average BWs of the 3n shrimp in both phases were significantly higher than that of the 2n shrimp, while their average survival rate was not different. These promising results encourage wider-scale tests in the future.

Key words: Biofloc, field trial, *Penaeus monodon*, triploid shrimp

Introduction

The black tiger shrimp, *Penaeus monodon* Fabricius (1798), is endemic to tropical countries, including Vietnam, Indonesia, India and Thailand. Cultivation of this shrimp species in Thailand has been successfully practiced for more than five decades with satisfactory results. However, since 1989, the industry has been hard-hit by several problems, especially from disease outbreaks and slow growth (Flegel, 1997). During these periods, majority of Thai farmers have switched to rear

the exotic Pacific whiteleg shrimp, *Litopenaeus vannamei*, which has been very well standardized for its culture performance (Castillo-Juárez H, Campos-Montes GR, Caballero-Zamora A, & Montaldo HH, 2015).

Triploidy induction was applied to *P. monodon* with the aim to improve growth rate and to skew the sex ratio towards faster-growing females (Pongtippatee et al., 2012; Sellars, Arce, & Hertzler, 2012a; Sellars, Wood, Murphy, McCulloch, & Preston, 2012b; Wood, Coman, Foote, & Sellars, 2011). Using cold-shock induction, Pongtippatee et al. (2012) had found that the triploid (3n) *P. monodon* grew significantly faster than the diploid (2n) shrimp. With this background, in the present study, comparison of culture performance between 2n and 3n *P. monodon* was determined in field trials in order to investigate specific advantage of commercial production of the 3n shrimp.

Materials and Methods

Triploid *P. monodon* seed was produced by preventing the extrusion of the second polar bodies, using cold-shock method as described previously (Pongtippatee et al., 2012). Briefly, 10 *P. monodon* broodstock with stage-4 ovarian maturation were placed in spawning tanks containing 200 L of 28 ^{o}C seawater. At 8 min after the beginning of spawning activity alarmed by the spawning detection device (Mueangdee et al., 2013), the broodstock were removed. Approximately half of the fertilized eggs were gently collected and placed in a 1L-beaker containing 8 ^{o}C seawater for 10 min and then poured into 200L of 28 ^{o}C seawater. This group comprised eggs that would develop to 3n seed. Another half of the eggs were treated identically with an exception that the seawater temperature was maintained at 28 ^{o}C throughout; these eggs would develop to 2n seed. The eggs in both groups were gently aerated and allowed to hatch out as nauplii at 10-12 h later. The nauplii were further reared to zoea, mysis and PL at stage 15 (PL15) with the provision of live *Chaetoceros* spp. and *Artemia* in sequence. At PL15, both types of seed were separately reared in canvas ponds.

Five trials were carried out (Table 1); trials 1-3 were the culture in canvas ponds throughout from PL15 to marketable size (for 4-5 months), and trials 4 and 5 were the culture in the canvas pond (for 1 month) before transferring to and rearing in earthen ponds to marketable size (for another 3-4 months). The canvas ponds were round ponds with 12m diameter and 1m high, and filled up with 15ppt seawater to the depth of 80 cm (approximately 90 ton of water). The earthen ponds were 0.5hectare rectangular ponds with approx. 20ppt seawater at the depth of 120 cm. Trials 4 and 5 simulated current commercial culture practice to prevent the diseases commonly found in the field, especially Acute Hepatopancreatic Necrosis Disease and infection by *Enterocytozoon hepatopenaei* (Biju et al., 2016; Soto-Rodriguez, Gomez-Gil, Lozano-Olvera, Betancourt-Lozano, & Morales-Covarrubias, 2015; Sritunyalucksana, Sanguanrut, Salachan, Thitamadee, & Flegel, 2014; Tourtip *et al*., 2009; Tran et al., 2013).

Table 1: Five field trials of diploid and triploid *Penaeus monodon* culture

Trial No.	Rearing system
1	Rearing in 90m^3 round canvas pond for 4.5 months at stocking density of 30 PLs/m^2
2	Rearing in 90m^3 round canvas pond for 5.0 months at stocking density of 25 PLs/m^2
3	Rearing in 90m^3 round canvas pond for 4.0 months at stocking density of 40 PLs/m^2
4	Rearing in 90m^3 round canvas pond for 1 month, at stocking density of 600 PLs/m^2 and then transferred to earthen pond at 30 individuals/m^2, rearing for another 4 months
5	Rearing in 90m^3 round canvas pond for 1 month, at stocking density of 2,000 PLs/m^2 and then transferred to earthen pond at 20 individuals/m^2, rearing for another 3.5 months

In the trials 1-3, the stocking density was 25-40 PLs/m^2, which was similar to that of the commercial earthen pond. The water in the ponds was aerated, with all necessary parameters of water quality being monitored to ensure optimum conditions for the shrimp [total ammonia nitrogen (TAN) and total nitrite at <0.5 ppm; alkalinity at 120-150 ppm; pH at 8.0-8.5, dissolved oxygen at >4 ppm; and salinity at 15-20 ppt]. Water exchange was performed whenever TAN or nitrite level was >0.5 ppm. The shrimp were provided with commercial feed pellets at the rate of 3-4% biomass/day, divided into 6-8 meals a day. To reduce the risk of AHPND, as well as to reduce water exchange rate, the shrimp were reared under biofloc technology, using molasses as carbon source (Avnimelech, 2012).

In the trial 4 and 5, the stocking density in the canvas pond was 600 and 2,000 PLs/m^2, respectively. The rearing method was the same as in trials 1-3. In the earthen pond phase, the stocking density was 20-30 individuals/m^2. Biofloc technology was again employed to reduce the risk of AHPND. In addition, the shrimp were also co-cultured with 50-g red tilapia (*Oreochromis* sp.) at the stocking density of 0.1 fish/m^2. The co-culture method has been tried in many shrimp farms and claimed to be a simple way to reduce the risk of the disease (Cadiz, Traifalgar, Sanares, Andrino-Felarca, & Corre, 2016; Withyachumnarnkul, Gerdmusic, Jutipongraksa, Pradeep, & Chaiyapechara, 2014).

In all the trials, when the shrimp reached 5-10 g in size, 20 of them were randomly checked for their triploidy or diploidy status by hemocyte isolation and tested by fluorescence activating cell sorting (FACS) analysis as described earlier (Pongtippatee *et al*., 2012). At the final harvest, survival and average body weight (BW) of the cultured shrimp were determined. The survival rate was determined from the estimated number of the shrimp at

harvest, compared to the stocking number; and the average BW was calculated from individual BWs of 100 shrimp randomly sampled from the pond. An analysis of variance (ANOVA) and Tukey test were performed to determine significant difference among groups.

Results and Discussion

The FACS analysis confirmed that at least 80% of the triploid-induced shrimp had three sets of chromosome, while the control shrimp had two sets. The finding supported that the induction was successful and gave us confidence that the comparison between the 2n and 3n shrimp was valid.

In the trials 1-3, the average BWs of the shrimp at harvest were significantly higher in the 3n shrimp, in both sexes, compared to their 2n counterparts (Fig. 1). The 3n shrimp was about 1.3-1.9x time larger than that of the 2n shrimp. In all the trials, the average BWs of the female was slightly higher than the male, which is usually the case for *P. monodon* (Hansford, 1991; Hansford & Hewitt, 1994).

For trials 4-5, the average BWs of the 3n shrimp was significantly higher than that of the 2n shrimp after the 1-month canvas pond culture (Fig. 2). In trial 4, the 3n shrimp BW (0.70 ± 0.03 g) was significantly ($p<0.01$) higher than that of the 2n shrimp (0.32 ± 0.02 g); likewise, in trial 5, 3n shrimp BW (0.29 ± 0.04 g) was also significantly ($p<0.05$) higher than that of the 2n shrimp (0.15 ± 0.04 g). As expected, the average BW of the shrimp in trial 4, with 600 individuals/m^2 stocking density was higher than that of trial 5 with 2,000 individuals/m^2 stocking density. At the end of the earthen pond culture, the average BWs of the 3n shrimp were again significantly higher (1.2-1.4x times) than that of the 2n shrimp. The average daily growth of the 3n shrimp was higher than that of the 2n shrimp in both trials.

At the time of shrimp harvest, the red tilapia grew up to 300-500 g estimated visually, and without noticeable mortality. No attempt was done on the determinations of the growth and survival rate of the fish as the practice has been done routinely in the farm under studied.

The average survival rate for the 2n and 3n shrimp from PL15 to marketable size of the five trials was 64.6 ± 8.6 % for the 2n and 71.7 ± 11.8 % for the 3n shrimp, in which they were not statistically different. With better growth and comparable survival rate between the 2n and 3n *P. monodon*, it seems that the 3n shrimp could be a commercially viable product. In the past, several aquatic animals have been successfully induced for polyploidy conditions, however, a few have become successful in commercial distribution. The triploid oysters are probably the most commercially successful being due to their increase in the growth rate, while triploid salmonids do not perform well in field trials (Hulata, 2001). In triploid marine shrimps, Sellars et al. (2010) have provided a good review on the topics. The Chinese marine shrimp, *Fenneropenaeus chinensis*, can be successfully induced as triploid animals, which grow faster than the diploid ones; however, the set-back is that the increase in their growth rate occurred mainly during their adult stage, which was due to the fact that the triploid animals were sterile and thus their feed intake was directed to support metabolism related to growth rate, not the reproductive function. Triploid *L. vannamei* did not survive well, and the Japanese marine shrimp, *F. japonicus*, grew at the same rate as their diploid counterparts; therefore, the triploid status of both shrimp species may not be flourished commercially.

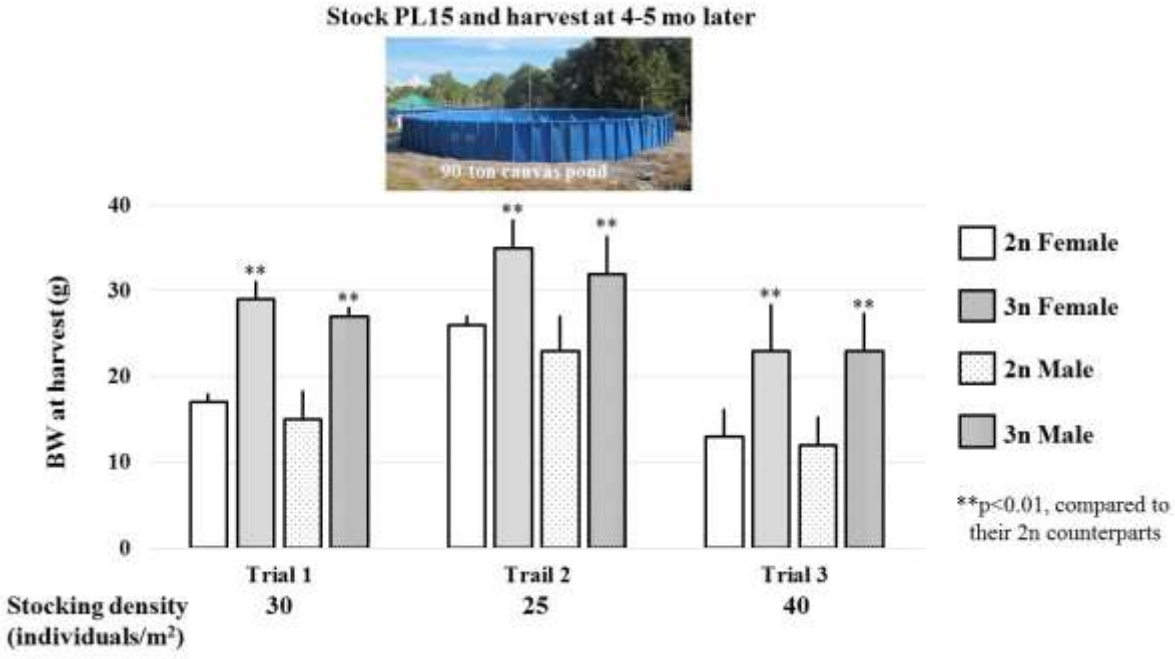

Figure 1: Average body weight (BW) of diploid (2n) and triploid (3n) *Penaeus monodon* in trials 1-3 at the end of 4-5 months of rearing in 90-ton round canvas ponds

Although all the trials reported herein suggest that 3n *P. monodon* outperformed the 2n shrimp in terms of growth rate, it may require more tests in the earthen pond to prove with confidence. In order to have more field tests, the seed should be generated with a better method than what was described herein, which requires a close and careful maneuver by an operator. It is also labor-intensive and prone to human errors. A device that automatically controls the temperature of the seawater at the defined periods and durations in sequence needs to be developed.

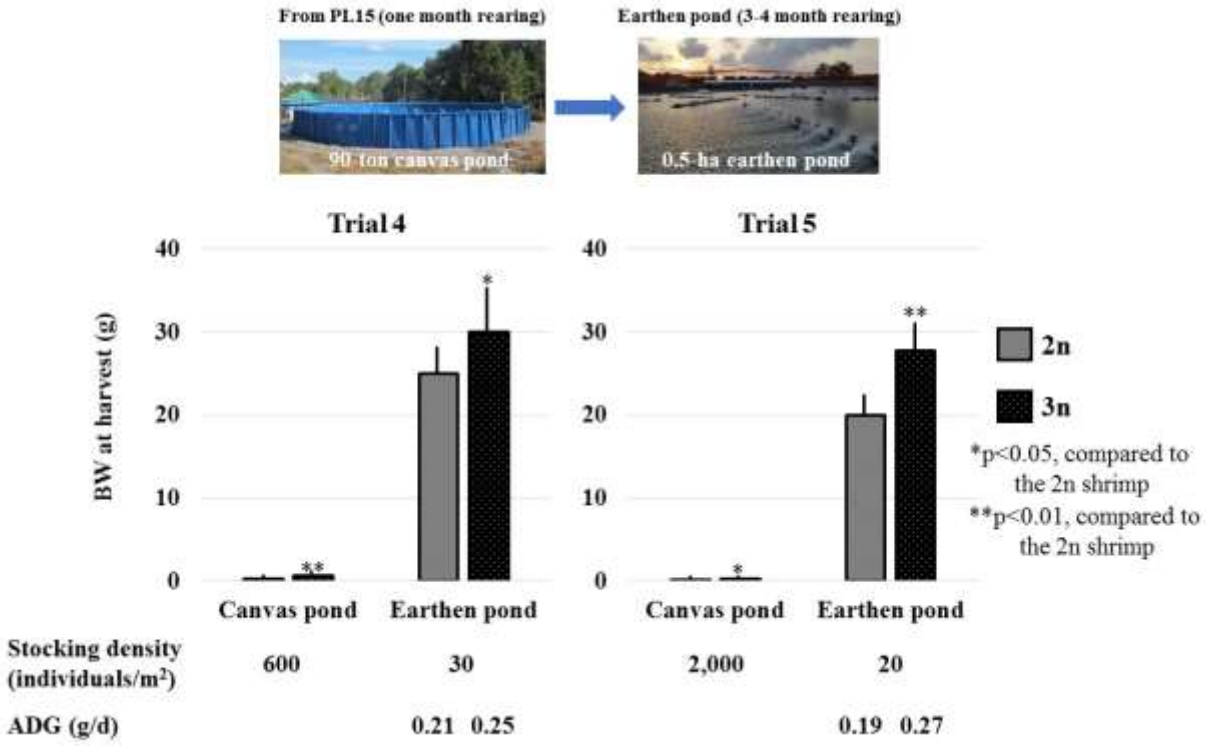

Figure 2: Average final body weight (BW) of diploid (2n) and triploid (3n) *Penaeus monodon* in trials 4 and 5 at the end of rearing in 90-ton round canvas ponds and in earthen ponds. ADG, average daily growth

At present, farming of *L. vannamei* is spreading worldwide and *P. monodon* farming has shared only a small fraction of the shrimp farming industry. It may be difficult to promote *P. monodon* farming even employing fast-growing 3n *P. monodon* seed. However, the advantage of *P. monodon* farming is that the price of *P. monodon* in certain niche markets is higher than that of *L. vannamei*, especially at the size of 30 g and above. In addition, a small group of farmers still prefers *P. monodon* farming because of its low investment on farming preparation, compared to that required for *L. vannamei* farming. With high supply of *L. vannamei* worldwide, its price is fluctuating and at times too low to make profit. This constraint has led to methods to increase the production of shrimp per ton of rearing water, mainly by increasing stocking density. The attempt has also increased pond preparation cost, e.g. for polyethylene lining, increasing aeration units, thorough water filtration, chemical treatments and mineral supplementation.

Final note, farming of triploid (3n) *P. monodon* is unlikely to be a revolutionary alternative to replace the ordinary diploid (2n) *P. monodon* farming, as the slight increase in growth rate of the 3n over the 2n shrimp is not considered as a big advantage. However, the authority who operate *P. monodon* selective breeding program may find the advantage of preventing unauthorized people from rearing the broodstock from the 3n PLs that have been generated through the breeding program for high-quality traits, e.g., fast growth and disease-resistance, as the 3n shrimp would be sterile.

Conclusions

This study suggests that triploid (3n) *P. monodon* grew faster than their diploid (2n) counterparts in field trials, which were tested under comparable conditions. The 3n and 2n shrimp also had comparable survival rate. It is therefore possible to grow 3n *P. monodon* commercially.

The only obstacle is how to generate the 3n shrimp in a large commercial scale. The method of the 3n induction at present is the manual one, which is not feasible; an automatic triploid inducer is needed for its mass production.

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References

Avnimelech Y (2012). Biofloc Technology - A Practical Guide Book (2nd ed). Baton Rouge, Louisiana: World Aquaculture Society.

Biju N, Sathiyaraj G, Raj M, Shanmugam V, Baskaran B, Govindan U, Kumaresan G, Kasthuriraju KK, Chellamma TSRY (2016). High prevalence of *Enterocytozoon hepatopenaei* in shrimps *Penaeus monodon* and *Litopenaeus vannamei* sampled from slow growth ponds in India. Dis. Aquat. Org. 120: 225-230.

Cadiz RE, Traifalgar RFM, Sanares RC, Andrino-Felarca KGS, Corre JrVL (2016). Comparative efficacies of tilapia green water and biofloc technology (BFT) in suppressing population growth of green Vibrios and *Vibrio parahaemolyticus* in the intensive tank culture of *Penaeus vannamei*. Aquac. Aquar. Conserv. Legis. 9: 195-203.

Castillo-Juárez H, Campos-Montes GR, Caballero-Zamora A, Montaldo HH (2015) Genetic improvement of Pacific white shrimp [*Penaeus* (*Litopenaeus*) *vannamei*]: perspectives for genomic selection. Front. Genet. 6:93. doi: 10.3389/fgene.2015.00093

Flegel TW (1997). Special topic review: major viral diseases of the black tiger prawn (*Penaeus monodon*) in Thailand. J. Microbiol. 13: 433–442.

Hansford SW (1991). More females in prawn ponds could cut production costs. Aust. Fish. 50 (10): 32–33.

Hansford SW, Hewitt DR (1994). Growth and nutrient digestibility by male and female *Penaeus monodon*: evidence of sexual dimorphism. Aquaculture. 125: 147–154.

Hulata G (2001) Genetic manipulations in aquaculture: a review of stock improvement by classical and modern technologies. Genetica 111, 155–173.

Mueangdee N, Pongtippatee P, Promkun P, Bootterb M, Chuaymung U, Sunyaprark W, Phrommoon J, Kanjanasopa W, Withyachumnarnkul B (2013). Spawning detection device for the black tiger shrimp *Penaeus monodon* broodstock. Aquaculture. 380–383: 21–22.

Pongtippatee P, Laburee K, Thaweethamsewee P, Hiranphan R, Asuvapongpatana S, Weerachatyanukul W, Srisawat T, Withyachumnarnkul B (2012). Triploid *Penaeus monodon*: Sex ratio and growth rate. Aquaculture. 356-357: 7-12.

Sellars MJ, Li F, Preston NP, Xiang J (2010) Penaeid shrimp polyploidy: global status and future direction. Aquaculture 310, 1–7.

Sellars MJ, Arce SM, Hertzler PL (2012a). Triploidy induction in the Pacific white shrimp *Litopenaeus vannamei*: An assessment of induction agents and parameters, embryo viability, and early larval survival. Mar. Biotechnol. 14: 740–751.

Sellars MJ, Wood A, Murphy B, McCulloch RM, Preston NP (2012b). Triploid black tiger shrimp (*Penaeus monodon*) performance from egg to harvest age. Aquaculture. 324–325: 242–249.

Soto-Rodriguez SA, Gomez-Gil B, Lozano-Olvera R, Betancourt-Lozano M, Morales-Covarrubias MS (2015). Field and experimental evidence of *Vibrio parahaemolyticus* as the causative agent of acute hepatopancreatic necrosis disease of cultured shrimp (*Litopenaeus vannamei*) in Northwestern Mexico. Appl. Environ. Microbiol. 81: 1689-1699.

Sritunyalucksana K, Sanguanrut P, Salachan PV, Thitamadee S, Flegel TW (2014). Urgent appeal to control spread of the shrimp microsporidian parasite *Enterocytozoon hepatopenaei* (EHP). Retrieved from https://enaca.org/publications/health/disease-cards/urgent-appeal-to-control-ehp-3.pdf

Tourtip S, Wongtripop S, Stentiford GD, Bateman KS, Sriurairatana S, Chavadej J, Sritunyalucksana K, Withyachumnarnkul W (2009). *Enterocytozoon hepatopenaei* sp. nov. (Microsporida: Enterocytozoonidae), a parasite of the black tiger shrimp *Penaeus monodon* (Decapoda: Penaeidae): Fine structure and phylogenetic relationships. J. Invertebr. Pathol. 102: 21–29.

Tran L, Nunan L, Redman RM, Mohney LL, Pantoja CR, Fitzsimmons K, Lightner DV (2013). Determination of the infectious nature of the agent of acute hepatopnacreatic necrosis syndrome affecting penaeid shrimp. Dis. Aquat. Org. 105: 45-55.

Withyachumnarnkul B, Gerdmusic C, Jutipongraksa T, Pradeep PJ, Chaiyapechara S (2014). Experience on *Penaeus monodon*/red tilapia co-culture using biofloc technique. In CL Browdy, J Hargreaves, T Hoang, Y. Avnimelech (Eds.), Proceedings of Biofloc Technology and Shrimp Diseases Workshop (pp. 143-150). Copper Hill, Virginia: The Aquaculture Engineering Society.

Wood AT, Coman GJ, Foote AR, Sellars MJ (2011). Triploid induction of black tiger shrimp, *Penaeus monodon* (Fabricius) using cold shock. Aquac. Res. 42: 1741–1744.