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The Effects of Triploidy Towards Growth Performance, Survival Rate, Gender Ratio and Sex Differentiation on Banana Shrimp, *Penaeus merguiensis* (De Man, 1888) Postlarvae Compared to Diploid Siblings

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Abstract: Study was done to show the effect of triploidy on growth performance, survival rate and gender ratio on *Penaeus merguiensis* compared to diploid sibling otherwise identifying the sex external characteristic of the postlarval. Cold shocks (10°C, 15°C and 20°C) with control (28°C) and three different time of exposures (10, 15 and 20 min)were introduced to each treatments. The fertilization rate was significantly different (P <0.05) among treatments and the hatching rate was no significantly different found (P >0.05). Larvae got poor survival rate when reached mysis stage and there was no significantly different (P >0.05) among each treatments. Triploidy were identified in 15°C treatment for all time of exposures and other treatments produced diploid. 15°C treatment and control were reared until postlarval 50 and the survival rate were significantly different (P <0.05). Male shrimps were differentiated by present of petasma and female by appearance of sharp ridges thelycum (srt). For the gender ratio, it was significantly higher differences (P <0.05) where 15°C treatment produced 94% males compared to control that produced 85% females. For growth performance of total length and body weight, there were significantly higher difference (P <0.05) among treatments which 15°C treatment identified gives faster growth towards the treated larvae compared to control. For the relationsips of total length and body weight, there were significantly different at 0.01(2-tailed) between control and triploid treatments, even though have a quite similar proportional for each treatments. The Specific growth rate obtained were also identified significantly different (P <0.05) between control and the 15°C treatment.

Key words: Gender ratio · Growth performance · *Penaeus merguiensis* · Sex external characteristic · Survival rate

INTRODUCTION

All over the world, Penaeid shrimp was known by several entrepreneurs to be the important culture species because of it higher growth rate and tolerance to varies environment [1]. Banana shrimp, *Penaeus merguiensis* is one of the most widely culture species found in Malaysia. *P. merguiensis* was known as an important candidate to be culture wisely because of it exported value [2]. To help meet demand, advantages of increasing sterility are being exploited during commercial growing of triploidy *P. merguiensis* to obtain suitable size for commercialize shrimp. Triploidy induction was recognized as one of the method that can be induced in crustacean either by using chemical or physical treatment to produce bigger size of shrimp. According to [3], temperature, pressure and chemical shock can be used, but temperature is preferred, because it is safe and inexpensive also easy to control. Triploidy is now widely used to get faster growth than diploids [4]. From the previous study done, shows that

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first Polar Body (PB1) triploids receive both maternal chromosome sets plus a single third set from the paternal parent. Reproductively sterile shrimp are also of commercial value as a way to protect elite genotypes from unlicensed breeding [5]. Study done on the effect of monosex cultured in red claw crayfish, *Cherax quadricarinatus*, found that men typically being larger than females and possessing prominent claws with a distinctive red patch. Apart from rapid growth, higher yield and lower FCR, a higher proportion of harvested animals are of larger size and of higher value, in male only populations as compared to female-only or mixed-sex populations [6]. According to [7] reproductive sterility is a common expected feature of triploid animals, but the situation is not the same for different species as reported by [7] and [8] where there found that triploid males shrimps exhibits testicular development similar to that of diploid, including an active spermatogenetic process. According to [9] their also observed sperm in the van deferens of triploid male but it is not real sperms just spermatids. Study done by [10] found that, sex ratio in prawn and shrimp are consistently 1:1 in research and commercial population with no evidence on influence on sex development. Triploid males generally show more gonad development than females maybe due to fact that triploidy does not interfere with many mitotic divisions required in testes [11]. According to [12], there are no published reports on experimental manipulations of this type for penaeids and their sex determination mechanism remains unknown. The aim of these study are to identify the effects of triploidy towards growth performance, survival rate and gender ratio of postlarvae (PL) compared to diploid siblings and to differentiate between male and female PL shrimp form the sex characteristics. Recently, female penaeid shrimp are of commercial value as they grow significantly faster than their male counterparts [13] therefore, this study is aim to produce bigger size of male (PL) shrimp by using cold shock treatment for triploidy induction.

MATERIALS AND METHODS

Experiment was conducted at the Institute of Tropical Aquaculture, Universiti Malaysia Terengganu. One out of 10 of wild female broodstock of *P. merguiensis* at stages four (ripen stage) was used for experiment. Raw sea water was pump from nearby coastal water to the sedimentation tank. The water quality parameter for the study were maintained at 25-30 ppt of salinity, more than 6 mg. L^{-1} for dissolve oxygen, 6.5-8.7 for pH and 28°C temperature. Female broodstock were stocked in 360 L of spawned tank and waiting for it to spawning. Spawning tank with capacity 80 gals, rounded in shape and black in color were put inside the dark room and wait for the gravid female to spawning. Uneaten foods were siphoned out from the tanks daily. An exchange of 30% water was given everyday using 1 ìm filtered seawater. After about 3 hours waiting started from 2200 hrs, the gravid females started to spawn. After spawning, the animal was removed from the tank. The eggs were cleaned using modified sieve 60µm mesh size and induced to triploid using cold shock at 10°C, 15°C and 20°C. Protocol induced triploid by [13] on the Pacific white shrimp, *L. vannamei* was applied with some modification. Triploidy were induced by temperature shocking eggs after fertilization before the second polar body extruded. The ignition of eggs release is record as time zero. At 6 min after spawning, the water in the drum is well mix. Eggs were removed from the drum and being place in the buckets containing a sieve (60µm mesh size) that could be removed to the rearing tank. The time of application of the shock was determined after earlier microscopic observation of the extrusion of polar bodies. They are expelled at 8 and 15 min after spawning and 10 minute time after spawning was chosen. Survivals of fertilized eggs were verified at the blastula-gastula stage by the microscopic observation of 20 eggs for each treatment. Each of the treatments have three replicates of 10, 15 and 20 min duration times. The PL was fed with *Artemia.* sp and commercial pellet microencapsulated diets (MEDs) according to the shrimp stages. After reached PL50, the larvae were harvested by drain all the water in tank to the collected sieve using pipe tube. The number of harvest PL is estimate from a single water basin of known volume from which animals within were individually count. This basin serves as a constant where visual comparisons are made with the rest of the harvest in similar basins. Male were determined by present of male reproductive organ which are male gonopore complex (mg) and petasma and the female differentiated by present is sharp ridges thelycum (srt) at fifth walking leg and all the PL of the shrimp were observed under dissecting microscope and by Advance microscope to find the PL sexes. Karyo video Test 3.1 was used to determined the real chromosome number. For each treatment, chromosome numbers were counted in three cells at metaphase. Chromosome at metaphase stage was examined under the microscope and was photographed by using Advance microscopes *Nikon eclipse* 80i (*Nikon*, Japan). For the data analysis, Two-way ANOVA analysis of variance for the homogeneity was used for fertilization

rate and hatching rate analysis. Survival and growth of control between the triploid exposed shrimp were analyzed using Two-way ANOVA analysis of variance with Post Hoc Test and Turkey to see the differences among the treatments. Relationship between the body weight (BW) and total length (TL) were analyzed using correlation (2- tailed).The results were present as mean ± SD. Chi- square Goodness of fit-test for nonparametric test was used to analysis the sex ratio of the PL treated whether there is a significant difference between the expected frequencies and the observed frequencies. Percentage survival in replicate tanks were used to analyze survival and individual PL (BW) were used to assess it specific growth rate (SGR).

RESULT

It was identified that the fertilization rate was significantly different (P= 0.006; P <0.05) among treatments with control at 20 min. time exposures have a higher fertilization rate, 85.00 ± 7.21% and 20°C 15 min. give the lowest fertilization rate, 23.54±4.51% (Figure 1). Meanwhile, for the hatching rate there was no significantly difference (p= 0.160; P>0.05) among treatments, which found highest in control, 58.96 ± 7.64% and also in 15°C 20 min. treatments, 49.17± 3.21% (Figure 2). Larvae reared from the fertilized egg until mysis 3 take about 10 days to undergo PL1. The survival rate were very poor and not significantly different (p= 0.196 ; P >0.05) among treatments which higher in control, 41.45 ± 2.29% and were show higher in 15°C 10 min. about 26.94± 5.60% (Figure 3). Triploidy were identified in 15°C for all time of exposures by using Video Karyo Test 3.1 and other treatments were produced diploid. 15°C treatment and control of PL1 larvae were reared until PL50. It was identified that the survival rate among these treatments were significantly different (p= 0.021; P <0.05), which control 39.6 ± 4.53% and treatment 15°C about 28.48 ± 2.69% (Figure 4) For the gender ratio, it was significantly higher differences (p= 0.001; P <0.05), where 94.29 ± 2.53% produced male in 15°C cold shock treatment compared with control that produced 14.29 ± 2.89% of male (Figure 5). Otherwise, growth performance for TL, there was significantly difference (p= 0.000; P <0.05), among control and 15°C treatment (Figure 6) same as for BW, (p= 0.000; P <0.05) which show 15°C treatment give faster and superior growth to the shrimp larvae (Figure 7). For the relationship of TL and BW, it was significant different at 0.01(2-tailed) among control and triploid treatments, even though it was a quite similar proportional

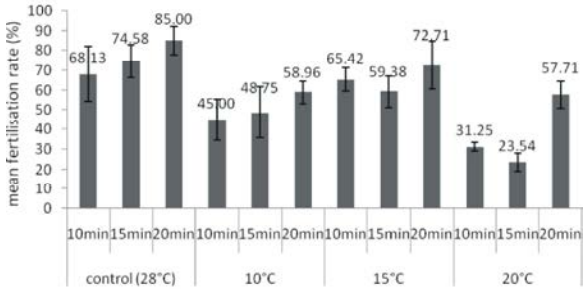

Fig. 1: Mean fertilization rate between different cold shock treatments (n=160).

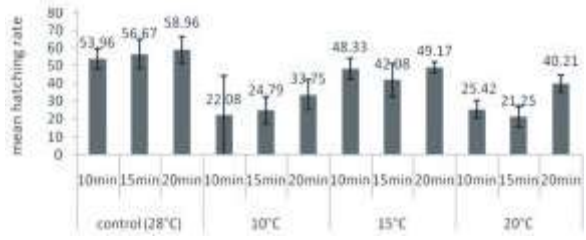

Fig. 2: Mean hatching rate between different cold shock treatments (n=160).

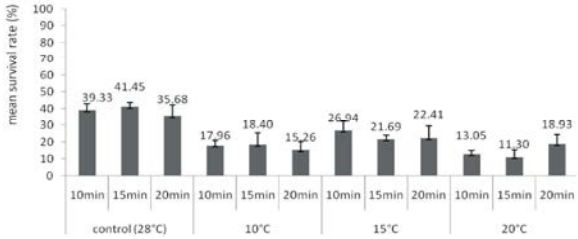

Fig. 3: Mean survival rate from Nauplii 1 stage till mysis 3 stage between different cold shock treatments (n=160).

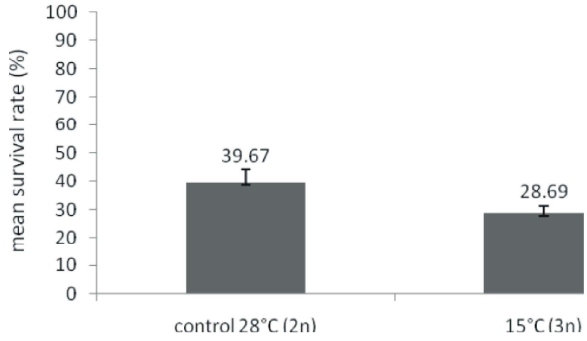

Fig. 4: Mean survival rate between diploid (control) and triploid (15°C) siblings (n=70).

for each of treatment, control R^2 = 0.968 and for 15°C treatment was R^2 = 0.987 (Figure 8). For the SGR among treatment, it was significantly different (p= 0.001; P <0.05), where in control 0.0014 ± 0.00031% g. day^{-1} and in 15°C

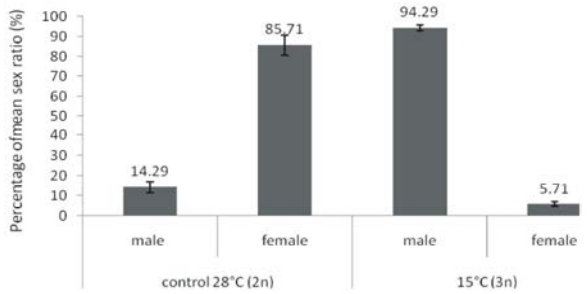

Fig. 5: Mean sex ratio percentage produced from diploid (control) and triploid (15°C) siblings (n=70)

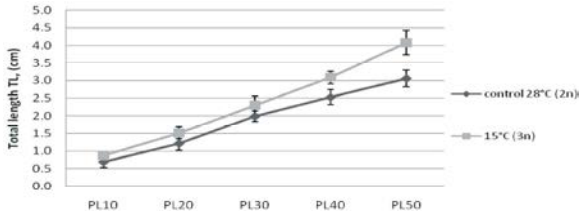

Fig. 6: Mean growth performance of total length from postlarvae1 stage till postlarvae 50 stages between diploid (control) and triploid (15°C) siblings (n=100).

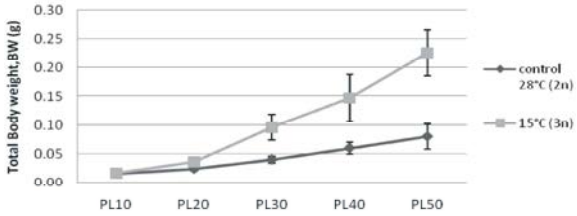

Fig. 7: Mean growth performance of body weight from PL10 till PL50 stages between diploid (control) and triploid (15°C) siblings (n=100).

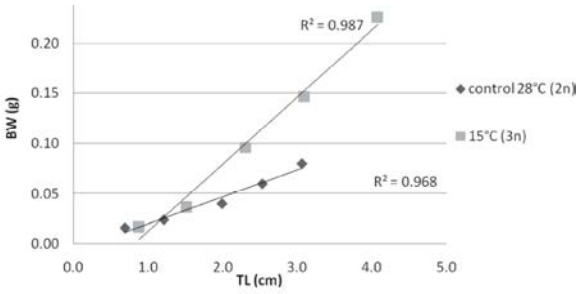

Fig. 8: Total length and Body weight relationship from PL10 till PL50 stages between diploid (control) and triploid (15°C) siblings (n=100).

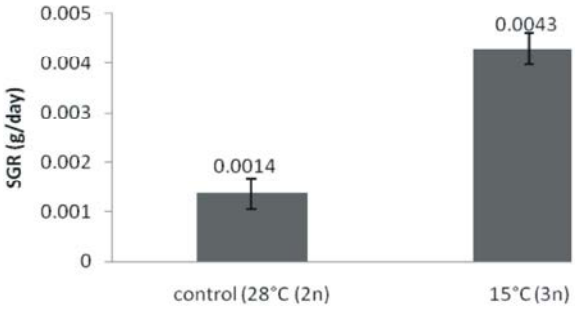

Fig. 9: Specific growth rate from PL10 till PL50 stages between diploid (control) and triploid (15°C) siblings (n=100).

treatment 0.0043 ± 0.00032% g.day^{-1}(Figure 9). For the sex characteristic differentiation, male were differentiated from female with the present of petasma on the first swimming legs meanwhile for the female, distinguished by the appearance of sharp ridges thelycum (srt) (Figure 10 (a); (b); (c), 11(a); 11(b), 12(a); 12(b)).

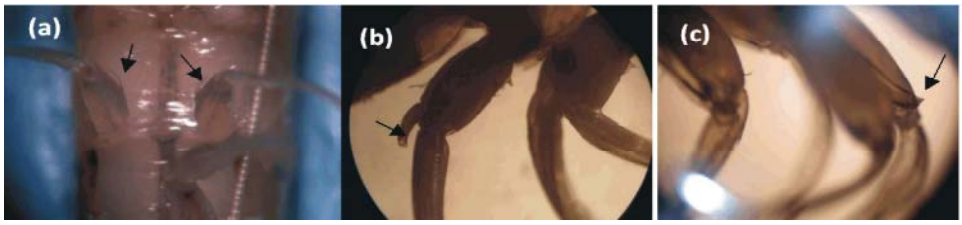

Fig. 10: Males were differentiated from female by the appeared of Petasma (P) on the first swimming leg of Postlarvae 50 shrimp using dissecting microscopes (a) and advance microscopes for (b) and advance microscope for (c) (10x).

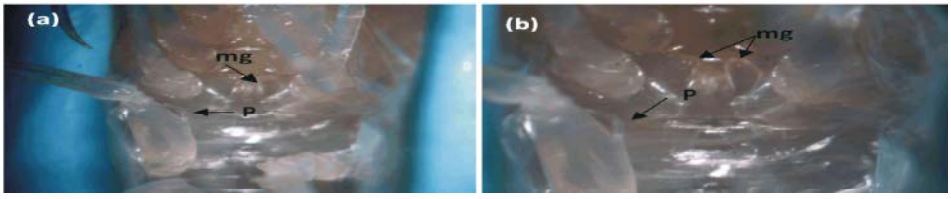

Fig. 11: (a) Control males; (b) Treated males with the appearance of petasma (P) at the first swimming leg and male gonophores (mg) at the fifth walking legs using dissecting microscope (10x).

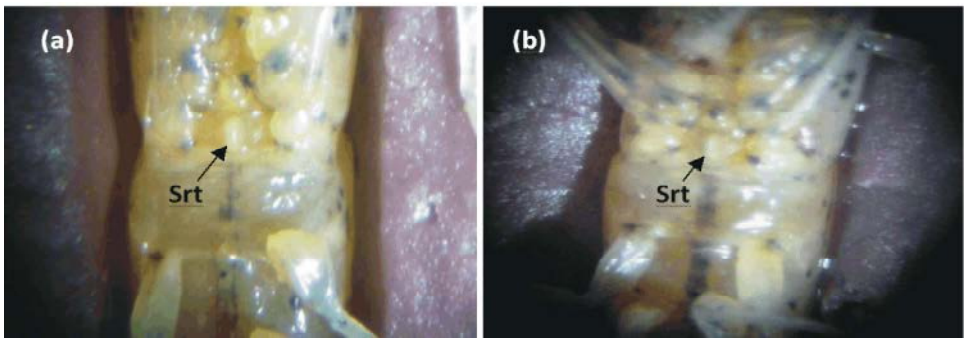

Fig. 12: (a) Control females; (b) Treated females were differentiated with male by the appeared of sharp ridges thelycum (Srt) using dissecting microscopes (10x).

DISCUSSION

From results of the fertilization rate and hatching rate it was shown that *P. merguiensis* have a very low survival rate as compared to other marine shrimp such as Black Tiger shrimp, *P. monodon* cultured in captivity. When it reach mysis 3 stages about 10 days from nauplii 1 stage, the survival rate was very poor around 11% to 41% which found higher in control. According to [14], temperature had the greatest effect on survival, with both linear and quadratic terms required to describe the curvilinear response. This maybe one of the factors gives the lower survival rate to the larvae reared. There were also a difference identified on growth performance on total length and body weight between the diploid and the triploid siblings which the triploid PL possessed higher growth performance for TL and the BW. The higher differences from the SGR value between this two treatment also shows that triploid is growth faster than it diploid siblings. The results obtained from present study was same as in the earlier study done on *M. chinensis* by [9] to produced triploid and from the results showed that the triploid larvae also showed better growth compared to it diploids.

However, the survival rate of PL50 of the treated shrimp was very poor because of it abnormalities containing (3n) number of chromosomes make the shrimp become weak on it immunity system and easier to die. High mortality was observed during the rearing of this PL shrimp start from it hatch until reach PL50. According to [5] on triploid Kuruma shrimp, *Marsupenaeus japonicus* treated with 6-DMAP during it larvae stages, there was a high mortality occur to the shrimp until the numbers of shrimp is not enough for statistical analysis and the results obtained by using chemical shock 6-DMAP produced 32 females out of 34 numbers of PL shrimp which is 94.11% females being produced. However, it was differ within this study that produced 94.29% males shrimp by inducing cold shock treatment to the PL shrimp. According to [12] and [15] the presence of males in the progeny from the mating, suggested the female sex could not be homogametic (WW) but was heterogametic (WZ) and the males are homogametic (ZZ) that will produce either (ZZZ) sterile males progeny or (WWZ), (WZZ) of sterile females progeny. Referred to [16], normally females with the (WZ) system (heterogamete female) are simpler and easy to construct a genetic breeding system for all-males organisms. [17] found that if the male were homogametic, triploid progeny would have the genotype (WWZ), (WZZ) or (ZZZ), but the sex of these potential genotypes has not been determined. From the results of the study, inducing triploid using cold shock treatment can lead to the higher rate of sterile males produced. However, this result cannot be extrapolated to all decapods as sex determination mechanisms can vary considerably within crustacean groups [12]. Another study done by [18] shows that cold shock treatment between 5 °C and 10 °C final temperature (when spawned at 27°C to 28°C) is suitable for inducing triploid in *L. vannamei, F. merguiensis, M. japonicus* and *M. chinensis*. However, from this study, the optimum parameter at 15 °C was identified as the optimum parameter to induce triploidy in *P.merguiensis* shrimp. This could be due to the environmental circumstances that differ between places or country that affected the physical parameter. From the results achieved, it was showed that the treated organisms of the triploidy PL shrimp growth larger and faster compared to it diploid siblings.

CONCLUSION

From the study shows that triploidy can give a faster growth as compared with it diploid siblings of banana

shrimp, *P. merguiensis* .Therefore, this triploid induction has a potential to improved harvested yield which can produce biggest male when culture through captivity. Temperatures at 15°C for durations times 10, 15 and 20 min. was identified as the best parameter to induce triploid in *P. merguiensis* which give 94% monosex of male shrimp. However, the survivals of the triploidy larvae still the biggest problems which produced low survival rate at the end of the study period. Future study can be done to improve the survival rate of the triploidy shrimp PL in the future.

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