Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 4, Issue, 10, pp.059-064, October, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

MASCULINE EFFECT OF ANDROGEN HORMONE, 17A-METHYLTESTERONE ON TIGER SHRIMP, *Penaeus monodon* (FABRICIUS, 1798) POSTLARVAE

***Ikhwanuddin, M.[1], Talpur, A. D.[1], Zulizah, O.[1], Hidayah, A. M.[1] and Shamsuddin, A. A.[2] and Abol-Munafi, A. B.[3]**

[1]Institute of Tropical Aquaculture, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia
[2]Institute of Marine Biotechnology, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia
[3]Faculty of Fisheries and Aqua-Industries, Universiti Malaysia Terengganu, Kuala Terengganu, Terengganu, Malaysia

ARTICLE INFO

Article History:
Received 4th July, 2012
Received in revised form
15thAugust, 2012
Accepted 26th September, 2012
Published online 30th October, 2012

Key words:
17α-Methyltesterone,
Penaeus monodon, Postlarvae,
sex ratio, Population.

ABSTRACT

The study has been conducted to determine the effect of the androgen hormone, 17α-Methyltesterone (MT) on survival rate, growth rate and the sex ratio of tiger shrimp, *Penaeus monodon* (Fabricius,1978) Postlarvae (PL). Five different doses of MT 200, 400, 600, 800, 1000 mg/kg feed were incorporated in the commercial diet of *P. monodon* PL for 50 days from PL_3 till PL_{53} and a control without MT. The highest survival rate 68.0% ± 4.6 of shrimp PL was archived in control treatment compared to the MT treatment doses. The highest dose of MT1000 mg/kg feed produced the highest body weight (BW) of 0.26g ± 0.1 and TL of 31.99 ± 3.8. Highest concentration of MT 1000 mg/kg feed did produce highest 76.6% male population among survivors was significantly different among the treatments. The study concluded that MT beside with the production of male population of *P. monodon* PL could also act as a growth promoter with the increased hormone dose but it adversely decreased survival rate.

INTRODUCTION

The monosex culture technology has become common practice in finfish and crustaceans (Beardmore *et al*., 2001; Devlin and Nagahama, 2002; Gomelsky, 2003) and attempts have been made to apply this aqua-technology to crustacean culture (Tayamen & Shelton, 1978; Macintosh *et al*., 1985; Lee & Donaldson, 2001; Curtis & Jones, 1995; Sagi *et al*., 1997), since male and female crustaceans display differential in terms of growth rates, behavior patterns and husbandry needs. The growth of fish/shellfish in aquaculture system may affected by a wide variety of factors including the gender, sexual maturity and age of the animals (Hartnoll, 1982; Aiken & Waddy, 1992). In marketing, these problems are arising because of the wide range of sizes within a population at harvest of commercial production of the culture. Solution for these problems is the use of monosex culture populations to obtain the uniform size and weight; at the same time as either, the production of all female or male population should reduce the magnitude of size variation and contribute to higher yields. The monosex culture has become biotechnological efficient for producing of all male populations, especially in countries where economically valuable crustaceans constitute an important source of income.

***Corresponding author:** ikhwanuddin@umt.edu.my

The growth of females is hampered by a lower yield (Sagi & Aflalo, 2005). One of the most common techniques for producing monosex populations is steroid induced sex inversion (Hunter & Donalsdon, 1983) where using of sex hormones to induce sex reversal either directly (Guerrero 1975) or indirectly by breeding sex reversed individuals (Jensen & Shelton, 1979). Sex reversal using hormone incorporated pelleted diets has been success in many fish species and crustaceans. The dietary administration of hormones to crustacean is likely the most practical approach to affect sex ratio on a commercial scale. The tiger shrimp, *P. monodon* strongly influences the marketable yield and the growth of this animal particularly affected by a factor of gender and on maturation females can frequently breed in culture atmosphere. By preventing of breeding activity in the culture, such a management strategy probably results in the diverting of energy from reproduction to growth and thereby contributes to sustainable aquaculture. A number of crustacean species exhibit bimodal growth patterns, in which males exhibit superior growth to females or vice versa (Hartnoll, 1982). Thus, this study were design to determine the effect of different concentration of androgen hormone 17 α – methyltestosterone on the sex ratio for the production of all males, the survival rate, and the growth rates of *P. monodon* PL. The monosex culture technology has become common practice in finfish and crustaceans (Beardmore *et al*., 2001; Devlin and Nagahama, 2002; Gomelsky, 2003) and attempts have been made to apply this aqua-technology to crustacean culture (Tayamen & Shelton, 1978; Macintosh *et al*., 1985; Lee & Donaldson, 2001; Curtis & Jones, 1995; Sagi *et al*., 1997), since male and female crustaceans display differential in terms of growth rates, behavior patterns and husbandry needs. The growth of fish/shellfish in aquaculture system may affected by a wide variety of factors including the gender, sexual maturity and age of the animals (Hartnoll, 1982; Aiken & Waddy, 1992). In marketing, these problems are arising because of the wide range of sizes within a population at harvest of commercial production of the culture. Solution for these problems is the use of monosex culture populations to obtain the uniform size and weight; at the same time as either, the production of all female or male population should reduce the magnitude of size variation and contribute to higher yields. The monosex culture has become biotechnological efficient for producing of all male populations, especially in countries where economically valuable crustaceans constitute an important source of income. The growth of females is hampered by a lower yield (Sagi & Aflalo, 2005). One of the most common techniques for producing monosex populations is steroid induced sex inversion (Hunter & Donalsdon, 1983) where using of sex hormones to induce sex reversal either directly (Guerrero 1975) or indirectly by breeding sex reversed individuals (Jensen & Shelton, 1979). Sex reversal using hormone incorporated pelleted diets has been success in many fish species and crustaceans. The dietary administration of hormones to crustacean is likely the most practical approach to affect sex ratio on a commercial scale.

The tiger shrimp, *P. monodon* strongly influences the marketable yield and the growth of this animal particularly affected by a factor of gender and on maturation females can frequently breed in culture atmosphere. By preventing of breeding activity in the culture, such a management strategy probably results in the diverting of energy from reproduction to growth and thereby contributes to sustainable aquaculture. A number of crustacean species exhibit bimodal growth patterns, in which males exhibit superior growth to females or vice versa (Hartnoll, 1982). Thus, this study were design to determine the effect of different concentration of androgen hormone 17 α – methyltestosterone on the sex ratio for the production of all males, the survival rate, and the growth rates of *P. monodon* PL.

Stocking of larvae

P. monodon PL_3 were randomly stocked at a density of 10PL per liter into 10L experimental aquaria with black background. All the aquaria tanks were placed in the water bath system provided with submersible water heaters and aeration. All tanks were covered with black netting in order to avoid direct sunlight during the day time.

Diet for Post larvae

PL were fed a commercial diet (Higashimaru Co., Ltd, Japan; 49% protein and 20% crude ash) containing five concentrations of androgen hormone 17α-Methyltestosterone (MT) including 200, 400, 600, 800 and 1000 mg/kg feed and was added according to a procedure described by Guerrero (1975). The desired quantity of MT was dissolved in 20 ml of 95% ethanol equivalent to 80% of the weight of feed. Ethanol and MT mixed first then the diet was added to it, diet was shielded to avoid light contact and placed under a vented laboratory hood. After 30 minutes, most of the ethanol in the trays evaporated. Then, diets were transferred onto aluminum foil trays, spread out in a thin layer in order to complete the evaporation of the remaining ethanol. The process was terminated when no alcohol could be detected by smell. The diets then placed into plastic containers and stored in a freezer until feeding to PL. The control diet prepared similar way but no MT was added. All experimental treatments with different diet concentrations were replicated three times. The replicate controls without supplemented of MT. The PL were fed for 50 days with 4 times a day at 08, 12, 16 and 20 h.

Data collection

Total length (TL) (mm) and the wet body weight (BW) (g) of randomly sampled 30 PL from each treatment including control were collected. TL was measured using vernier calipers and BW with digital electronic analytical balance (Sartorius, USA). The specific growth rate (SGR) was calculated using the following formula;

$$\text{Specific growth rate (SGR)} = \frac{\text{ln final body weight (g) of PL} - \text{ln initial body weight (g) of PL} \times 100}{\text{No. of culture period days}}$$

The survival of *P. monodon* PL was counted at the end of the experiment using following formula;

$$\text{Survival rate \%} = \frac{\text{Total PL harvested}}{\text{Number of PL stocked}} \times 100$$

Shrimp PL were sexually differentiated as described by Perez Farfante (1988), Treece and Yates (1988) and Dall *et al*. (1990). Sex differentiation was based on the following sex structures; (i) the development of the appendix masculine at the second pair of pleopods, (ii) the development of the gonophores complex at the fifth pereiopods and (iii) the pair of oblique sharp ridges on the anterior sternite XIV of the female thelycum. The structures were phenotypically examined under a dissecting microscope to note the present or absent of these sex structures for sex ratio determination.

Statistical analyses of data

Data was analysed via one – way ANOVA using SPSS software version 16 for windows to determine differences among treatments and Post hoc followed by Turkey's test to determine significant different at p<0.05 among treatments.

RESULTS

Water quality parameter

No adverse effect on water quality was observed during the experiment trials, which is expected to support adequate growth of shrimp larvae. Temperature was maintained between 28.5- 30.3 ^{o}C, salinity ranged 29.3-30.1 ppt, pH 7.8-8.0, and dissolved oxygen (DO) remained within range of 6.2-6.4 mg/L within the treated groups. In control temperature, salinity, pH and DO was 30.0 ^{o}C, 29.7 ppt, 7.9 and 6.4 mg/L.

No significance differences found among water parameters between different concentrations of hormone ($p>0.05$).

Biological measurement

Initial mean BW of random shrimp PL was measured to 0.03g ± 0.0 with the initial mean TL of 3.4 mm ± 0.6 before stocking. An increase in the BW of shrimp PL was MT concentrations dependant. The highest final BW 0.26 ± 0.08 (g) was achieved in group treated with 1000 mg/kg feed Figure 1. The final BW was significantly different ($p<0.05$) among the different treatments.

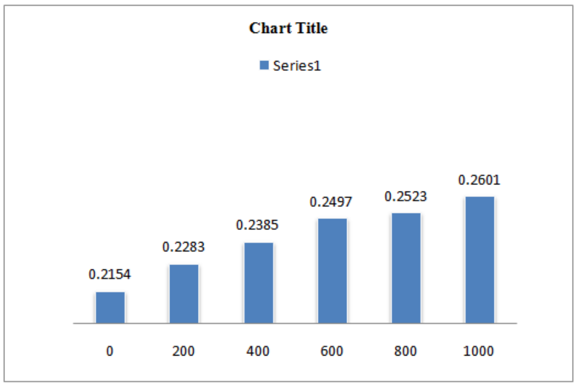


Fig. 1. Mean final BW (g) of *P. monodon* PL fed with control (without hormone) andwithdifferent MT hormone concentration. Error bars denote 95% confidence (P=0.05).

Like BW, the Length of shrimp PL was dose response. Highest TL 31.99 mm ± 3.8 was observed with those PL treated with 1000 mg/kg feed of MT followed 31.83 mm ± 4.9 and 31.82 mm ± 4.0 treated with 600 and 800 mg/kg feed of MT respectively Figure 2. TL was significantly differences among treatments ($p< 0.05$).

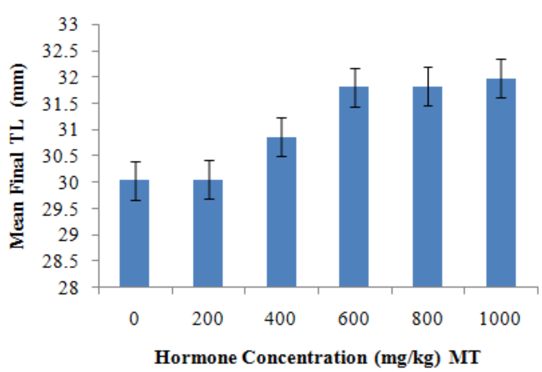


Fig. 2. Mean final TL (mm) of *P. monodon* PL for control (withouthormone) and with different MT hormone concentration. Error bars denote 95% confidence (P=0.05).

Specific growth rate (SGR)

Highest dose 1000 mg/kg feed did produce higher specific growth rate (SGR) 4.33 ± 0.12 g and the lowest SGR 3.96 ± 0.03 g was recorded from the control treatment Figure 3. However, there was no significant difference ($p>0.05$) found between the control and MT doses for SGR.

Survival rate

The significantly highest survival 68.00 ± 4.58% was observed in control treatment and the lowest survival rate 25.67 ± 5.85% in treatment MT 1000 mg/kg feed . Among the treated groups the highest survival 43.33 ± 8.96% was observed in treated group MT 400 mg/kg feed. MT concentration doses 200, 600, 800 and 1000 mg/kg feed had produced relatively low survival Figure 4. Survival rate among different treatments was significant differences ($p<0.05$).

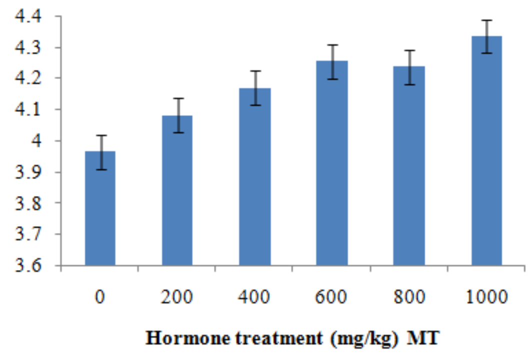


Fig. 3. The Mean SGR (%)of shrimp PL fed without hormone (control) and with different MT hormone concentration mg/kg feed. Error bars denote 95% confidence (P=0.05).

Survival rate

The significantly highest survival 68.00 ± 4.58% was observed in control treatment and the lowest survival rate 25.67 ± 5.85% in treatment MT 1000 mg/kg feed . Among the treated groups the highest survival 43.33 ± 8.96% was observed in treated group MT 400 mg/kg feed. MT concentration doses 200, 600, 800 and 1000 mg/kg feed had produced relatively low survival Figure 4. Survival rate among different treatments was significant differences ($p<0.05$).

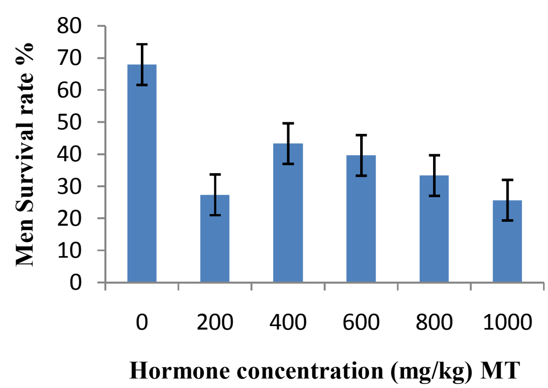
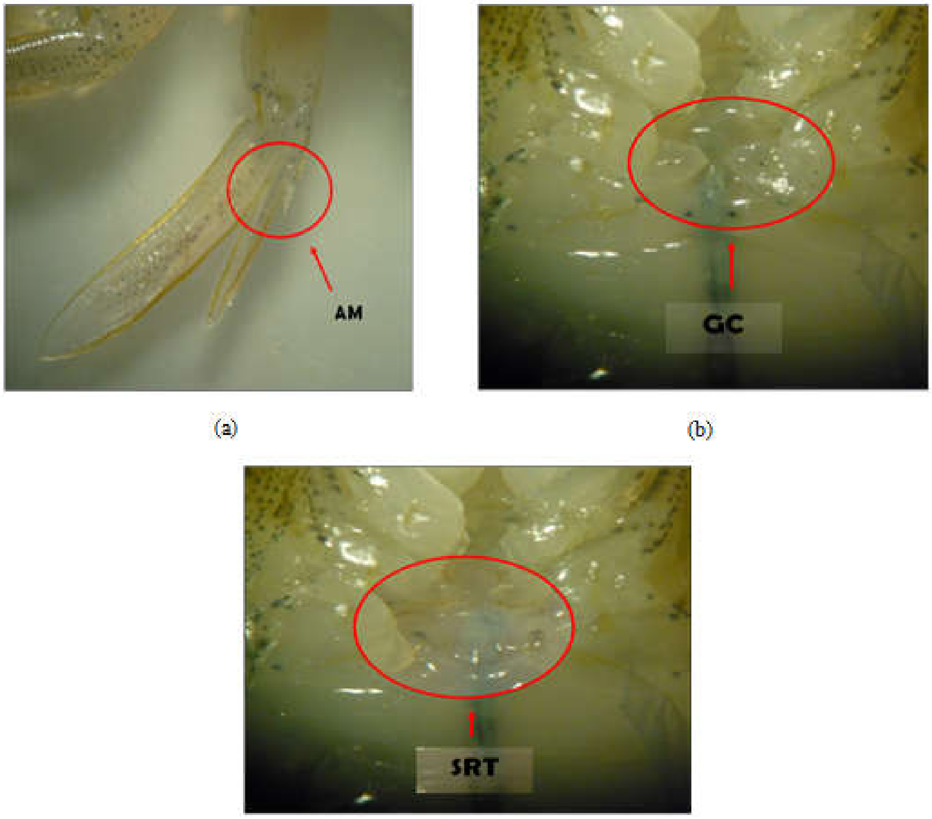


Fig. 4. The Mean survival rate (%) of PL *P. monodon* fed without hormone (control) and with different MT hormone concentration mg/kg.Error bars denote 95% confidence (P=0.05).

Sex determination

The external sex differentiation of male *P. monodon* PL was observed based on appendix masculine (AM) at the second pair of pleopods (Figure 5(a)) and gonophores complex (GC) at the fifth pereiopods (Figure 5(b)). The female *P. monodon* PL sex differentiation was identified from anterior part of sternite XIV that characterizes the female thelycum (SRT) (Figure 5(c).



(a) (b)

(c)

Fig. 5. Gender differentiation of *P.monodon* PL into male (a – b) and female (c). (a) Appendix Masculine (AM), (b) Gonophore Complex (GC), (c) Sharp Ridges in Thelycum (SRT)

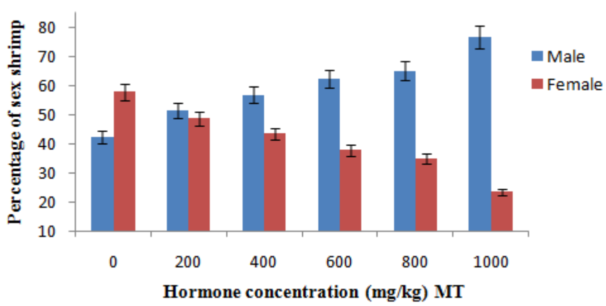


Figure 6. Percentage of sex ratio (male : female) of *P. monodon* PL for control (without hormone)and different MT concentration mg/kg. Error bars denote 95% confidence (P=0.05)

DISCUSSION

Water quality parameters such as temperature, salinity, pH, and DO have a major impact on the health and performance of larval batches. Study by Clay and McNevin (2005) reported that poor water quality could lead to poor growth, low survival, late molting or staging, increased fouling and deformities. The water quality parameters during the present study were within standard aquaculture range throughout the culture period (Boyd & Zimmermann, 2000). Therefore, it can be said that MT added feed did not produce any adverse effect on water quality, therefore, had no adverse influence on growth, survival and sex reversal of *P. monodon* in the present study. Numbers of experiments have demonstrated the effect of MT as a growth promoter and being an effective agent for sex reversal in fish (Baghel *et al*., 2004; Macintosh *et al.*, 1985). Based on result of the present study, the increased growth in all treatments in both BW and TL was observed compared to Sthe control treatment. Although the SGR did not show significant difference with each treatment but the size of the shrimp increased with the increase in hormone concentration. The MT dose 200 mg/kg feed and 400 mg/kg feed did show less effect this might be the dose was not enough to active the growth. Another possible explanation for the differing effects on growth rate was related to findings by Macintosh and Little (1995) who pointed out that any condition that adversely affects food consumption might decrease treatment efficacy. The MT concentration 600 mg/kg feed to 1000 mg/kg feed was comparatively more effective.

The researchers have reported that the growth rate of fish treated with androgens appeared to be faster than that of most fish treated with estrogen (Tayamen & Shelton, 1978) in particular have shown considerable success in inducement of sex reversal and as a growth promoter (Antiporda, 1986). The present study demonstrated that the growth rate in every treatment with MT did increase in both BW and TL together. The hypothesis of the present study supported from earlier research where the BW and TL was related together (Antiporda, 1986; Baghel *et al*., 2004; Ohs *et al*., 2006). Similarly, BW and TL were related together in present study as well. Number of factors may have caused the survival rate of *P. monodon* PL during the present study where at the end of the experiment, survival rate was achieved between 25 - 68%. Mortality of the PL during the experimental period had an important consideration could affected the sex ratio. The result of the present study suggests that the concentration of MT may have a more pronounced effect on the survival rather than on the growth of shrimp. However, the dose response evaluation was complicated because of low survival. Higher the MT dose (1000 mg/kg feed) lowers the survival. It was observed that MT dose 400 mg/kg feed was more suitable as survival promoter in the present study but on other hand, it was less effective on growth and TL. The dose efficacy resulted variation in survival the cause was known. Untreated control did produce highest survival over the treated groups, which reflect that MT doses have adverse effect on survival to some extent. Study by Olmstead and LeBlanc (2000) reported that the reduced survival rate in the juvenile could be due to the role of hormone as antiecdysteroids in fact the exogenous steroids act as moult inhibiting agents in crustaceans. It can be explained that MT hormone doses might have affected the moulting process and resulted in low survivals. Sex determination and differentiation is particularly interesting subject matters in crustacean (Garza-Torres *et al*., 2009). The gender determination or affected differentially the development of female and male shrimp were appeared depend on the different environmental conditions starting from PL_1. It appears that penaeids possess a stable genetic sex determination system (Campos-Ramos *et al*., 2006). However, gender differentiation took place at about the same age independent of size during development under rearing conditions, after PL reach a threshold size. During the present study after 50 days, sexes were observed in shrimp PL by the present or the absent of the appendix masculine, the male copulatory organ which is particulary located at the endopodite of the second pair of pleopods and presence of gonopohores complex (Nagamine *et al*., 1980). In previous study, external differentiation between female and male shrimp takes place during the second month after PL transformation (Charniaux-Cotton & Payen, 1985; Yin *et al*., 1986; Nakamura *et al*., 1992; Li & Xiang, 2002). In the present study, the morphological external structures of the male gonopores and female were described and used to determine sex. Females started developing a pair of oblique, sharp ridges in the thelycum that is characteristic of *P. monodon*. The differentiation of external sexual characteristics took place between the PL age of 50 – 60 days period after hatching based on threshold body weight of 0.150 – 0.200 g, and a length of 20 – 25 mm. The results of the present were in accordance with previously mentioned studies. In the present study, sex ratio with androgen hormone treatment showed that the administration of MT can be an effective method to alter sex ratio of *P. monodon* during PL growth. The androgen treatment used was successful in producing large numbers sex reversed males. The androgen treatment with MT at 1000 mg/kg feed for 50 days, was very effective in the present study. Other investigators have reported sex reversal of freshwater prawn *M. rosenbergii* at dose rates less than 125 mg MT/kg feed (Antiporda, 1986; Baghel *et al*., 2004; Ohs *et al*., 2006).

However, results from present study were inconsistent and did not achieve the all male by sex reversal through MT induced in feed. Thus, it is necessary to identify the optimal dose of MT for consistent, successful sex ratio and sex reversal in shrimp is to be investigated. During the present study using the hormone concentration at MT 400 – 1000 mg/kg feed did show the increasing number of male sex ratio, but no any dose of MT did produce 100% male of *P.monodon* PL. It can be explained that the administration doses of MT tested during the present study might be insufficient for crustacean standards under the circumstances. Another possible explanation for failure to produce 100% male was that the amount of hormone given for 50 days might not enough as the effect of the hormone dose time is dependent (Macintosh *et al*., 1985). In other study on Mozambique tilapias by Varadaraj *et al.* (1994) shows that factors that affect sex reversal include type of hormone, duration of treatment, water quality and temperature, stocking density, age and length, quality of feed and feeding rate. In the present study, only PL_3 were used and it could be assumed the designated dose of MT was not active to reverse the sex to all male population. It can be conclude that the androgen hormone (MT) could be an effective hormone for the production of all male postlarvae of *P. monodon* but more dosages of hormone and long period of treatment should be investigated. Nevertheless, in the present study sex reversal of *P. monodon* PL has been achieved with MT but not at maximum extent. Further, the hormone can also act as a growth promoter in *P. monodon* PL on one hand and the survival rate decreased when hormone level increased on other hand.

Acknowledgement

This study was partially supported by Ministry of Science, Technology and Innovation (MOSTI), (Science Fund), Government of Malaysia under grant Vot. No. 52044.

REFERENCES

Aiken, D.E. and Waddy, S.L. 1992. The growth process in crayfish. *Aquaculture*, 6, 335-381.

Antiporda, J.L. 1986. Preliminary studies on the effects of methyltestosterone on *Macrobrachium rosenbergii* juverniles. *FAO*.

Baghel. D. S., Lakra. W. S. and Rao G. P. S. 2004. Altered sex ratio in giant freshwater prawn *Macrobrachium rosenbergii* (de man) using hormone bioencapsulated live *Artemia* feed. *Aquaculture,* 35, 943 – 947.

Boyd, C. and Zimmermann, S. 2000. Grow out systems – water quality and soil management. In: New, M.B., Valenti, W.S (Eds.), Freshwater Prawn culture. Blackwell Publishing, London, pp.221 – 238.

Campos-Ramos, R., Garza-Torres, R., Guerrero-Tortolero D.A., Maeda-Martinez, A.M. and Obregon-Barboza H. 2006. Environmental sex determination, external sex differentiation and structure of the androgenic gland in the Pacific white shrimp *Litopenaeus vannamei* (Boone). *Aquaculture Research,* 37, 1583 – 1593.

Charniaux-Cotton H. and Payen G. 1985. Sexual differentiation. In: The Biology of Crustacea, vol. 9, Integuments, Pigments, and Hormonal Processes (ed. by D.E.Bliss & L.H.Mantel), pp. 217–299. Academic Press, New York, USA.

Clay, J., and McNevin, A.A. 2005. Farm level issues in Aquaculture Certification: Shrimp. *FAO*.

Curtis, M.C. and Jones, C.M. 1995. Observations on monosex culture of redclaw crayfish *Cherax quadrinatus* von Martens (Decapoda: Parastacidae) in earthen ponds. *Journal of the World Aquaculture Society,* 26, 154-159.

Devlin, R.H. and Nagahama, Y. 2002. Sex determination and sex differentiation in fish: an overview of genetic, physiological, andenvironmental influences. *Aquaculture,* 208, 191–364.

Dall, W., Hill, B.J., Rothlisberg, P.C. and Staples, D.J. 1990. The biology of the Penaeidae. *Adv. Mar. Biol.,* 27, 1489.

Garza-Torres R., Campos-Ramos R. and Maeda-Martínez A.M. 2009. Organogenesis and subsequent development of the genital organs in female and male Pacific white shrimp *Penaeus (Litopenaeus) vannamei*. *Aquaculture,* 296, 136 – 142.

Gomelsky, B. 2003. Chromosome set manipulation and sex control incommon carp: a review. Aquat. Living Resour. 16, 408–415.

Guerrero, R.D. 1975. Use of androgen for the production of all male Tilapia aurea (Steindachner). Transactions of the American Fisheries Society 104, 342–348.

Hartnoll, R.G. 1982. Growth. In: Bliss, D.E. (Ed.). *The Biology of Crustacea* 34, Academic Press, New York 111-196.

Hunter, G.A. and Donaldson, E.M. 1983. Hormonal sex control and its application to fish culture. In: W.S. Hoar, D.J. Randall, and E.M. Donaldson (Editors), Fish Physiology, Reproduction, Behaviour, and Fertility Control, Vol. IX-B. Academic Press, New York, pp. 223-303.

Jensen, G.L. and Shelton, W.L. 1979. Effects of estrogen on Tilapia aurea: Implications for production of monosex gametic male tilapia. Aquaculture 16, 233–242.

Lee, C.C. and Donaldson E.M. 2001. Reproductive biotechnology in finfish aquaculture. Proceedings of workshop, 320pp. Honolulu, Hawaii, October 4 – 7, Elsevier, Armsterdam.

Li, F. and Xiang, J. 2002. Study on early sex differentiation of *Penaeus chinensis*. Studia Marina Sinica/Haiyang Kexue Jikan 44, 101–105.

Macintosh, D.J., Varghese T.J. and Rao, G.P.S. 1985. Hormonal sex reversal of wild spawned tilapia in India. *Journal of Fish Biology* 26, 87 – 94.

MacIntosh, D.J. and Little, D.C. 1995. Nile tilapia (*Oreochromis niloticus*). In: N.R. Bromage and R.J. Roberts (Editors), Broodstock Management and Egg and Larval Quality, Chapter 12. Blackwell Scientific Ltd., Cambridge, Massachusetts, USA, pp. 277-320.

Nagamine C., Knight A.W., Maggenti A. and Paxman G. 1980. Effects of androgenic gland ablation on male primary and secondary sexual characteristics in the Malaysian prawn, *Macrobrachium rosenbergii* (de Man) (Decapoda, Palaemonidae), with first evidence of induced feminization in a nonhermaphroditic decapod. General and Comparative Endocrinology 41, 423–441.

Nakamura K., Matsuzaki N. and Yonekura K.I. 1992. Organogenesis of genital organs and androgenic gland in the kuruma prawn. Nippon Suisan Gakkaishi 58, 2261–2267.

Ohs, C. L., D'Abramo. L. R. and Kelly. A. M. 2006. Effect of dietary administration of methyltestosterone on the Sex Ratio of Postlarval Freshwater Prawn, *Macrobrachium rosenbergii*, during the nursery stage of culture. *World Aquaculture* 37, 328 – 333.

Olmstead, A.W., and LeBlanc G.A. 2000. Effect of endocrine active chemicals on the development of sex characteristics of *Daphnia magna*. *Toxicology* 19; 2107 – 2113.

Pérez Farfante I. 1988. Illustrated key to penaeoid shrimps of commerce in the Americas. NOAA Technical Report NMFS 64.

Sagi, A. and Aflalo, E.D. 2005. The androgenic gland and monosex culture of freshwater prawn *Macrobrachium rosenbergii* (De Man): a biotechnological perspective. *Aquaculture Research*. 36: 231-237.

Sagi, A., Milstein, A., Eran, Y., Joseph, D., Khalaila, L., Abdu, U., Harpaz, S. and Karplus, I. 1997. Culture of the Australian red-claw (*Cherax quadricarinatus*) in Israel. II. Second grow out season of over wintered populations. *Israel Journal of Aquaculture – Bamidgeh*. 49, 222-229.

Tayamen M.M. and Shelton W.L. 1978. Inducement of sex reversal in *Sarotherodon niloticus* (Linnaeus). *Aquaculture* 14, 349 – 354.

Treece G.D. and Yates M.E. 1988. Laboratory manual for the culture of penaeid shrimp larvae. Marine Advisory Service, Sea Grant College Program, Texas A&M University.

Varadaraj, K., Kumari, S.S. and Pandian, T.J. 1994. Comparison of conditions for hormonal sex reversal of *Mozambique tilapias*. *Prog. Fish. Cult*., 56, 81-90.

Yin Z.F., Song W.B., Ma, L. and Yu J.P. 1986. Studies on the development and differentiation of external genital organs of *P. orientalis*. Transactions of Oceanology and Limnology/Haiyang Huzhao Tongbao, Qingdao 4, 56–61.

****