

## Effect of Testosterone Hormone on Performance of Male Broodstock of Black Tiger Shrimp *Penaeus monodon* Fabricius, 1798

G. Maheswarudu<sup>a\*</sup>, U. Rajkumar<sup>b</sup>, Miriam Paul Sreeram<sup>c</sup>, M.S. Chakravarty<sup>d</sup>, C.K. Sajejev<sup>e</sup>

<sup>a</sup> Central Marine Fisheries Research Institute, Kochi, India-682018

<sup>b</sup> Directorate of Poultry Research, Rajendra Nagar, Hyderabad, India-530030

<sup>c</sup> PIM Unit, ICAR, Krishi Bhawan, New Delhi, India -110 001

<sup>d</sup> Department of Marine Living Resources, Andhra University, Visakhapatnam, India -530 003

<sup>e</sup> MRC of Central Marine Fisheries Research Institute, Mumbai, India-400 061

### Article history:

Received: 24 December, 2014

Accepted: 27 December, 2014

Available online: 07 April, 2015

### Keywords:

Testosterone hormone, *Penaeus monodon*, mating success, moulting period, Y-organ and Androgenic gland

### Corresponding Author:

Maheswarudu G.\*

Principal Scientist & Head

Email: maheswarudu ( at ) yahoo ( dot ) com

Rajkumar U.

Principal Scientist

Sreeram M.P.

Scientist

Chakravarty M.S.

Professor

Sajejev C.K.

Assistant Chief Technical Officer

### Abstract

In domestication and development of captive broodstock of tiger shrimp *Penaeus monodon* mating failures due to poor performance of male brood stock. To overcome this drawback the effect of the testosterone hormone on the performance of male broodstock was evaluated by injecting the hormone at the rate of 5 µg/g. body weight at 5

days interval in test group against control group for a period of 120 days. In both groups male and female were stocked at 1:1 ratio, each animal was tagged and marked, and were fed with clam and squid meat. Mating success, spermatophore structure and duration of moult cycle were observed and compared between two groups. In test group; mating success/animal in females was significantly ( $P < 0.05$ ) enhanced ( $65 \pm 17.46\%$  versus  $47.4 \pm 19.49\%$ ); spermatophores were solid and intact than those of soft with fluid material in control group; mean moulting period/ animal of males significantly ( $P < 0.01$ ) reduced ( $20.85 \pm 2.37$  days versus  $26.2 \pm 4.25$  days). Histological study revealed that the effect of the hormone on Androgenic gland and Y-organ was significant with the hypertrophy of cell and accumulation of secretory globules, respectively. Thus the present study suggests that mating success can be enhanced in tiger shrimp domestication programme through inducing males by hormone for achieving selective breeding between desired/ targeted families/groups.

### Citation:

Maheswarudu G., Rajkumar U., Sreeram M.P., Chakravarty M.S., Sajejev C.K. 2015. Effect of Testosterone Hormone on Performance of Male Broodstock of Black Tiger Shrimp *Penaeus monodon* Fabricius, 1798. The Journal of Veterinary Science. *Photon* 116, 446-456.

### All Rights Reserved with Photon.

Photon Ignitor: ISJN17846372D767707042015

## 1. Introduction

### 1.1 Importance of captive broodstock development

Tiger shrimp *Penaeus monodon* has been the principal species contributing significantly to shrimp production all along the Indian coast until nineties. Unfortunately shrimp culture industry has faced setback during 1995-96 due to outbreak of dreaded white spot syndrome virus and since then the industry has been confronting with same quiet frequently. Of late, the commercial hatcheries are depending on wild gravid females due to failure of

brood stock management system in captivity as well as non-response of wild females to eyestalk ablation. Inconsistent availability of gravid females from wild is the main reason to inadequate production of seed at appropriate time. Availability of limited number of gravid females from wild and the high cost (Miriam Paul Sreeram et al., 2004) also compelling the hatchery sector to use pathogen-carrier gravid females and the culture sector to stock pathogen carrier seed that was

produced from those gravid females leading to vertical transmission and subsequent disease manifestation under stress conditions (Mohan et al., 1997; Tsai et al., 1997). Hence domestication of the tiger shrimp is essential to produce specific pathogen free brood stock/disease resistant brood stock in captivity to revive the industry to its previous position by arresting disease manifestation (Sakthivel and Ramamurthy, 2003; Maheswarudu, 2007, 2014).

### 1.2 Background information

Central Marine Fisheries Research Institute, India, has developed protocol for the captive broodstock development and reared *P. monodon* broodstock up to three generations successfully for the first time in India (Maheswarudu, 2007, 2014). As domestication programme has been advancing to successive generations, poor performance of male broodstock is the major bottleneck for further advancement of captive broodstock development programme. The incidence of natural mating is very less in animals reared under captive conditions. In domestication programme of tiger shrimp when mating is failed between targeted groups/families artificial insemination is resorted to overcome the situation (Maheswarudu et al., 2000). But, artificial insemination is possible when male of desired group is able to produce sperm/spermatophore. Hence enhancement of the reproductive performance by inducing spermatogenesis of the males is necessary for the success of the domestication programme to achieve targeted goals of selective breeding (Maheswarudu, 2014). The presence and effect of the vertebrate steroid hormone (Progesterone, estradiol and testosterone) on crustaceans has been reported by several authors (Sarojini, 1963; Kulkarni et al., 1979; 1984; Burns et al., 1984; Yano, 1985; 1987; Ollivier et al., 1986; Quackenbush and Keily, 1986; Tim Verslycke et al., 2002). The effect of progesterone and 17-hydroxy progesterone in ovarian development and vitellogenesis was studied by Kulkarni et al. (1979); Tsukimura and Kamemoto (1988). Though the presence of steroid hormone and its effect in female shrimp has been well documented, the studies in male reproduction, hormonal metabolic pathways and its effects are limited.

### 1.3 Concept of the study

In domestication programme of tiger shrimp, F<sub>3</sub> generation males were in efficient to produce spermatophore while resorting to artificial insemination to achieve successive inbreeding. Then a thought has emerged to induce spermatogenesis in males and a comprehensive search has given idea of deployment of testosterone hormone for inducing spermatogenesis in males. When F<sub>3</sub> generation males were injected with

testosterone hormone at 10 µg/g. body weight males were able to produce spermatophore but mortality was observed. Since the present study was designed to evaluate the effect of testosterone hormone in males of *P. monodon* for inducing the maturation, sperm development and sex libido by injecting hormone at a rate of 5 µg/g. body weight.

## 2. Materials and Methods

### 2.1 Objective of the study

The objective of the study is to induce the maturation and sperm development in male broodstock of tiger shrimp by injecting testosterone hormone periodically and to enhance the subsequent natural mating, which will help to achieve selective breeding between targeted groups/families in domestication programme.

### 2.2 Experimental design and broodstock maintenance

The study was conducted at Mariculture laboratories, Visakhapatnam Regional Centre of Central Marine Fisheries Research Institute (17° 41'12.54"N; 83° 13' 6.53"E) during October, 2002-February, 2003. About forty live broodstock of *P. monodon* (20 males and 20 females) were collected from the trawl net operation in the Bay of Bengal off Visakhapatnam and transported to the lab in 50 l jerry cans by providing aeration (battery operated aerator). The shrimp were acclimatized in the laboratory for one week prior to experimentation. The animals were randomly distributed into four groups, five males and five females in each group-one control and three- experimental. Each animal of every group was tagged with ring tag at the base of eyestalk. Each animal of every group was also marked by cutting uropod tip; four animals were marked by cutting tip of each uropod and fifth animal was marked without cutting any uropod, to record the moulting time of each animal from the moulted exoskeleton. These groups were stocked separately in 5 t capacity round fibreglass tanks, which were provided with a sand bed filter for recirculation of water and to simulate the natural habitat for the shrimp (Maheswarudu et al., 1996). The shrimp were fed with known quantity of clam meat and squid at 1:1 ratio daily in the evening (17.00 hrs) and the left over feed (clam meat and squid) was recorded on the succeeding day in the morning (09.00 hrs) to record feed consumption per day. Every day in the morning hours all the fecal matter was siphoned out from each tank and about 40% water was exchanged. If any moulted exoskeletons were found in the tank, they were collected. Sex of moulted shrimp was recorded. While observing moulted exoskeleton, if female moulted, moulted female with the ring tag was caught with soft cloth to avoid injuries to the animal and observed thelycum for spermatophore

deposition. Spermatophore from the moulted exoskeleton of each female was taken out and used for observation on sperm development as well as to confirm the mating success during the previous moulting period.

### 2.3 Water parameters

Temperature (twice), salinity and pH (once) were recorded daily in each tank. Ammonia was estimated at weekly intervals in all the tanks with a standard protocol using spectrophotometer.

### 2.4 Hormone injection

The males of the experimental group were injected with testosterone hormone (Testosterone Cypionate intramuscular injection, Sun Pharmaceuticals) @ 5 µg/g. body weight at 5 days interval for a period of 120 days. Hormone was dissolved in distilled water at the concentration 1µl =25 µg. Dissolved testosterone hormone in required quantity based on the weight of the animal was injected in to abdominal muscle to each male, in the middle of lateral region between first and second abdominal segments, by using micro syringe. Males of control group were injected with crustacean ringer solution. Females of both groups were neither eyestalk ablated nor the experimental tanks were covered with black cloth to reduce light penetration; shrimp diet was restricted to clam meat and squid meat avoiding littoral oligochaete *Pontodrilus bermudensis*; in view of avoiding induced maturation and subsequent spawning, as sperm will be released from the spermatophore that was deposited in the thelyca of females during spawning (Radhakrishnan et al., 2000; Maheswarudu and Vineetha, 2013).

### 2.5 Sperm development

While injecting males of both the groups were examined for the presence/development of the spermatophore at the base of fifth walking leg. The moulted thelycum of each female was examined, spermatheca was collected and further examined under microscope.

### 2.6 Data analysis

The data on number of moults under gone, duration of moult cycle, survival rate and number of days survived was compared between control and test groups of males by employing T-test. Similarly all the parameters including mating success was compared between test and control groups of females. Feed consumption for pooled sexes was compared between control and test groups. The experimental period was divided into four spells, each of 30 days; I (1-30 days), II (31 - 60 days), III (61-90 days), IV (91-120 days) and data on mating success and duration of moulting period was correlated accordingly to assess the effect of the hormone over a period of experimentation.

## 2.7 Histology

### 2.7.1 Objective of the study

Since testosterone hormone induced reproductive performance of males by enhancing mating success and also induced moulting by reducing duration of moult cycle histological study was conducted; to confirm the action of hormone on Androgenic gland, responsible for sperm development; and on Y- organ that is responsible for moulting.

Histological study was carried out on the target organs namely Y-organ and Androgenic gland on which the hormone was thought of acting. About 10 live broodstock of *P. monodon* male, intermoult staged (Size range 193 mm TL to 205 mm TL), were collected and acclimatized in the lab prior to experimentation as described above. All these animals were maintained in 5 t capacity fibre glass tanks under diffused aeration, and by feeding with clam meat and squid meat during the experimentation. Two animals were sacrificed and Y- organ and Androgenic gland were collected and fixed in Bouin's fluid for control at the beginning. Remaining 8 animals were injected with testosterone hormone at the rate 5 µg/g. body weight and re introduced in the 5t capacity fibre glass tank. Injected animals were sacrificed at different intervals for collection and fixation of Y-organ and Androgenic gland; two after 6 hrs, two after 12 hrs, two after 24 hrs and two after 48 hrs. The Y-organ is located between the mandibular and posterior dorso lateral muscle of the prebranchial chamber and the Androgenic gland among the muscle of the coxopodite of the periopod attached to the subterminal region of the vas deference, just behind the terminal ampule (Vijayan et al., 1993). The Y-organ along with the branchiostegites and the Androgenic gland were removed from the specimens of the control group in the beginning and from the experimental groups at 6 hrs, 12 hrs, 24hrs, 48hrs after injecting testosterone hormone. The Y-organ and the Androgenic glands were immediately fixed in alcoholic Bouins fluid for 24hrs and processed for histological studies.

The tissues were post chromated after fixation in Scot's tap water for 24 hrs, dehydrated through graded alcohols (70%, 90%, 95% and 100%) cleared in xylene, in filtered in liquid wax at 60° C for two hours and were embedded in wax. Sections 8-10 µm thickness were cut and stained with Heidenhain's Azan (Pearse, 1968).

## 3. Results

### 3.1 Water parameters

Details of water parameters such as temperature, salinity, pH and ammonia in the experimental tanks during the 120 days experimental period are given in table, 1.

The water parameters did not differ between control and test tanks, both the groups were similar in both control and test tanks. Water temperature, salinity, pH and ammonia maintained in the experimental tanks were at  $26.81 \pm 1.18^\circ \text{C}$ ,  $27.12 \pm 1.37$  ppt,  $8.05 \pm 0.05$  and  $0.00085 \pm 0.00019$  mg/L respectively. The ammonia concentration was very less below the normal values. The recirculation system with sand bed filter in the tanks was very effective in maintaining the water quality and hence low levels of ammonia.

### 3.2 Reproductive Performance

#### 3.2.1 Male

Details of biological parameters of males and females of both control and test groups of *P. monodon* are given in table 2. There was no significant difference between the control and test group males as the mean total length, mean weight, survival rate at the end of the experiment, number of days survived/ animal, number of moults but differed significantly, duration of moult cycle ( $P < 0.01$ ) which was decreased in test group. The duration of the moult cycle between control and test groups was wider during third and fourth part of the experiment than that of first and second (Fig.1). Presences of spermatophores were more comparatively in test males. Complete solid intact spermatophores were observed in the thelyca of test group animals where as fluidly, liquid and soft spermatophores in thelyca of control group.

#### 3.2.2 Female

The number of days survived/ animal, number of moults undergone/animal, duration of moult cycle and number of matings/animal did not differed significantly, whereas mating success/ animal (%) has differed significantly ( $P < 0.05$ ) between control and test groups of females and it is higher in test groups, particularly during the first and second quarter of the experiment than that of the subsequent period (Fig. 2).

#### 3.2.3 Feed consumption for both sexes

Feed consumption for both sexes of each control and test group is presented in table 2. Though feed consumption was high in test group (6.65% of biomass) than that of control group (6.32% of biomass) it is not statistically significant.

### 3.3 Histology

#### 3.3.1 Androgenic gland

Androgenic gland of the untreated shrimp was observed to be normal without any abnormalities whereas that of the shrimp injected with testosterone hormone there was increased glandular secretions starting from 6 hrs of experimentation. There was accumulation of glandular secretion in the cell showing the deep red colour. The glandular cell size was increased with hypertrophied nucleus.

The gland lumen was also loaded with deep red glandular accumulation showing high secretory activities. At 48 hrs the secretory activity was reduced (Fig.3).

#### 3.3.2 Y- organ

The Y-organ of the control did not show any sign of secretions. At 6hrs of experimentation deep red granules made their appearance and increased granules were observed at 12 hrs, 24 hrs and 48 hrs. More activity was noticed at 24 hrs and 48 hrs of experimentation (Fig.4)

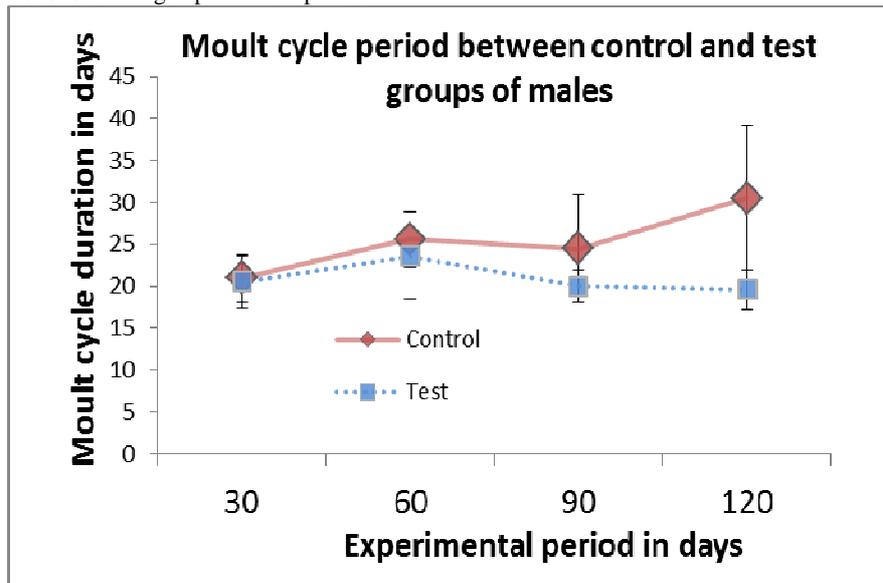
### Discussion

The present study demonstrates that testosterone hormone enhances the reproductive performance in male tiger shrimp *Penaeus monodon* by stimulating Androgenic gland especially with more intensity during first 60 days of the experiment as well as stimulating the Y-organ to fasten the moult cycle by reducing duration of moult cycle during second part (61-120 days) of the experiment.

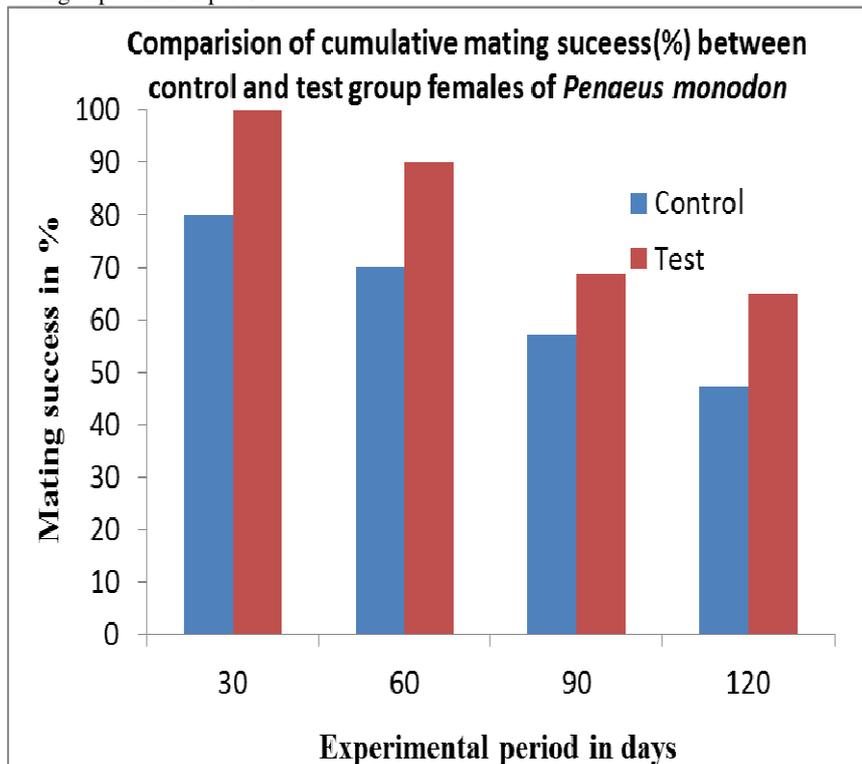
#### 4.1 Androgenic gland

The purpose of the Androgenic gland (AG) in regulating expansion and maturation of the crustacean male reproductive system and secondary sexual characteristics was first explained by Charniaux-Cotton (1954). Androgenic gland in crustaceans produces Androgenic hormone that is responsible for development of male characteristics and promotes spermatogenesis. Within male crustaceans, gonad inhibiting hormone (GIH) from eye stalk and gonad stimulating hormone (GSH) from thoracic ganglia and brain and the Androgenic gland hormone (AGH) from Androgenic gland regulate the spermatogenesis (Nagaraju, 2011). The eyestalk peptide GIH seems to affect the testes indirectly, by inhibiting the AGs. Further GSH is essential to trigger AG spermatogenesis (Juchault and Legrand, 1965). Eye Stock Ablation, thereby eliminating the preliminary place of GIH, results in hypertrophy of the AGs and a rapid increase in spermatogenesis (Payen et al., 1971). In the present study, from the histological observation of hypertrophy of epithelial cells of Androgenic gland (Fig. 3), it is evident that testosterone hormone stimulates the Androgenic gland to release AGH that promotes spermatogenesis and subsequent mating behavior to increase mating success. Similar observations were made by Kulkarni et al. (1984) in the Androgenic gland of male *Parapenaeopsis hardwickii* when the eye stalks were ablated and when the abstracts of brain and thoracic ganglia were injected. Naghabhushanam and Kulakarni (1981) reported that hypertrophy and hyperplasia in Androgenic gland and increased diameter of testicular follicles of *Parapenaeopsis hardwickii* when animals were

**Figure 1:** Comparison of mean moult cycle duration (Mean±SD) in days between control and test groups of male *Penaeus monodon* during experimental period

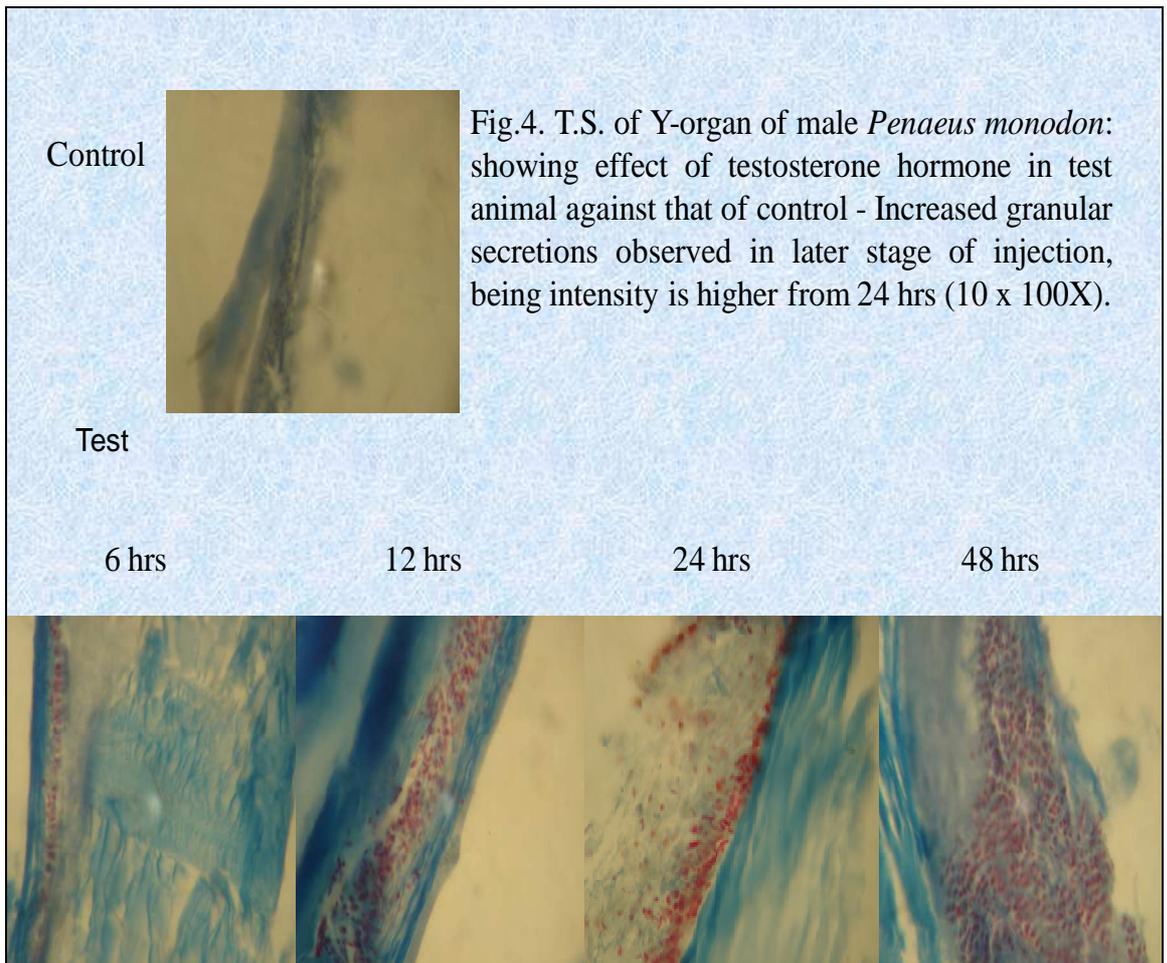
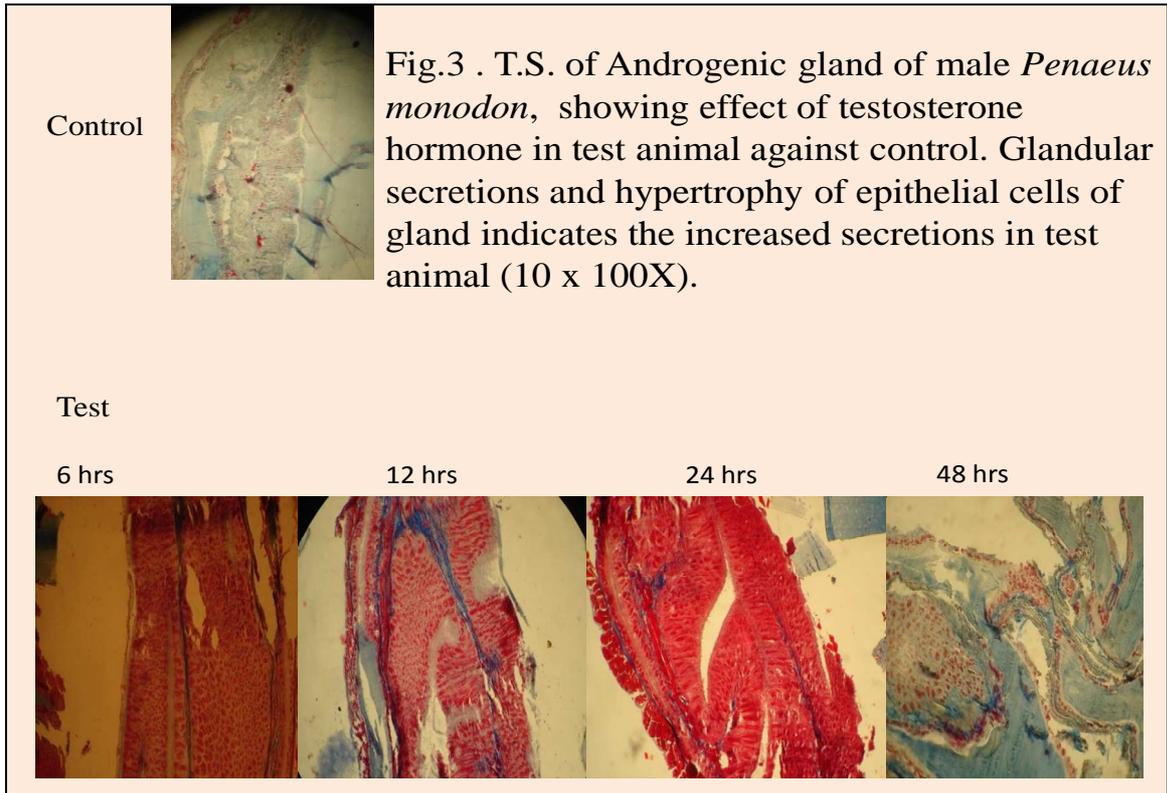


**Figure 2:** Comparison of cumulative mating success (%) between control and test groups of female *Penaeus monodon* during experimental period



**Table 1:** Water temperature (°C), salinity (ppt), pH and ammonia (mg/L) levels in the experimental tanks during the 120 days experimental period

Duration (days)	Temperature(°C)	Salinity(ppt)	pH	Ammonia(mg/L)
1-30	28.25±1.76	25.5±3.53	8.0	0.00105±0.00134
31-60	26.0±0.70	26.0±1.41	8.0	0.00105±0.00134
61-90	25.25±2.47	27.5±3.53	8.1	0.000685±0.00087
91-120	27.75±1.76	29.5±0.70	8.1	0.00065±0.00063



**Table 2:** Comparison of parameters between Control and Test groups of *Penaeus monodon*

S.No	Parameter	Control	Test	Probability
<b>Males</b>				
1	Mean total length (mm)	198.2±4.65	200.2±10.03	ns
2	Mean weight (g)	74±5.47	75±7.07	ns
3	Survival at the termination of experiment (%)	20	40	ns
4	Number of days survived/animal	95.8±23.36	101.8±26.47	ns
5	Number of moults undergone/ animal	3.6±1.51	5±1.22	ns
6	Average duration of moult cycle in days	26.2±4.25	20.85±2.37	$P < 0.01$
<b>Females</b>				
1	Mean total length (mm)	228.8±11.23	223.6±20.79	ns
2	Mean weight (g)	116.6±17.54	112.6±29.09	ns
3	Survival at the termination of experiment (%)	20	20	ns
4	Number of days survived/animal	105±29.09	97.8±39.12	ns
5	Number of moults undergone/ animal	3.8±1.09	4.0±1.73	ns
6	Average duration of moult cycle in days	26.74±4.75	24.7±2.11	ns
7	Number of matings/ animal	1.8±0.83	2.6±0.89	ns
8	Mating success/animal (%)	47.4±19.49	65±17.46	$P < 0.05$
<b>Pooled</b>				
1	Feed consumption (% of biomass)	6.37	6.65	ns

ns= not significant at  $P > 0.05$  level

injected with testosterone acetate and testosterone propionate at 10 µg /g. body weight. Yashiro et al. (1998) reported that duration of time for formation of sperm sacs was reduced by injecting methyl testosterone at 100 ng/g. body weight in pond reared male *P. monodon*. Altered sex ratio, increasing male population was achieved when larvae of *Macrobrachium rosenbergii* were fed with 17α-methyl testosterone hormone enriched *Artemia* nauplii for 50 days (Baghel et al., 2004). The above studies reported that testosterone hormone induces sperm development in *P. monodon*; induces hypertrophy and hyperplasia in Androgenic gland of *Parapenaeopsis hardwickii*; and altering sex ratio increasing male population in *M. rosenbergii*. The present study resulted in further advancement in the effect of hormone by enhancing mating success in *P. monodon*.

#### 4.2 Importance of mating success in selective breeding

In domestication programme of tiger shrimp when mating is failed between targeted groups/families artificial insemination is resorted to overcome the situation (Maheswarudu et al., 2000). But, artificial insemination is possible when male of desired group is able to produce sperm/spermatophore. The present study indicates that testosterone hormone can be employed to enhance sperm development and subsequent mating success in long term goal of development of disease resistant broodstock of *P. monodon* (Maheswarudu, 2014).

#### 4.3 Y-organ

The endocrine function of the Y-organ in control of moulting in crustaceans was suggested by Gabe (1953, 1956). In vitro culture of the Y-organ

provided direct evidence that it produces ecdysteroids in *Procambarus clarkii* (Sonobe et al., 1991) and *Penaeus vannamei* (Blais et al., 1994). The rate of ecdysteroid synthesis in Y-organ is generally controlled by moult inhibiting hormone (MIH) from the X-organ sinus gland complex (Diwan, 2005); the moulting process is regulated through ecdysteroid hormones from the Y-organs during proecdysis and moult-inhibiting hormone (MIH) produced by a group of neurosecretory cells (NSC) in the X-organ sinus gland complex of the eyestalks of shrimp during intermoult period (Diwan and Nagabhushanam, 1975; Aiken, 1980; Skinner, 1985; Chang, 1989). In the present study moult cycle duration significantly reduced in test group males especially during second part of the experiment suggesting that testosterone hormone stimulates Y-organ to release ecdysteroid hormones that fastens moulting process especially during second part of the experiment. Histological observations, Y-organ showing increased granular secretions (Fig. 4), confirm the hormone activity on Y-organ to stimulate for releasing ecdysteroids.

#### 4.4 Effect of hormone on mating success and moulting

Mating success was more pronounced in test group females during first 60 days of experiment than that of during subsequent period (61-120 days) (Fig.2). Duration of moult cycle in test group males significantly reduced during 61-120 days than that of 1-60 days (Fig.1). These results are suggesting that testosterone hormone is more effective on Androgenic gland during 1-60 days and on Y-organ during 61-120 days. This type of differential intensity in the activity of hormone is also evident from histological study that hypertrophied

condition of Androgenic gland cells during 6-24 hrs and increased granular secretions in Y-organ during 24-48 hrs.

### Research Highlights

1. Testosterone hormone induces sperm development and subsequent mating success while injecting males with hormone at 5 ug/g. body weight, more pronouncing with 90% mating success during first 60 days of the experiment than that of subsequent period of 60 days.
2. During the second half of the experiment hormone induced moulting in test group males, resulting reduced moult cycle period.
3. Histological study reveals that hormone induces Androgenic gland to stimulate spermatogenesis during 6 hrs, 12 hrs and 24 hrs and its effect reduced at 48 hrs.
4. Histological study also reveals that hormone induces Y-organ to stimulate moulting especially at 24 hrs and 48 hrs.
5. Mating success was more pronounced in test group females during first 60 days of experiment than that of during subsequent period (61-120 days) (Fig.2). Duration of moult cycle in test group males significantly reduced during 61-120 days than that of 1-60 days (Fig.1). These results are suggesting that testosterone hormone is more effective on Androgenic gland during 1-60 days and on Y-organ during 61-120 days. This type of differential intensity in the activity of hormone is also evident from histological study that hypertrophied condition of Androgenic gland cells during 6-24 hrs and increased granular secretions in Y-organ during 24-48 hrs.

### Conclusion

Thus the present study is suggesting that the hormone is stimulating (Androgenic gland) sexual activity initially for 60 days and then enhancing the growth by stimulating (Y-organ) the moulting pattern especially during second part of experiment. Hence it is advisable to use testosterone hormone to enhance reproductive performance in male tiger shrimp to achieve mating success between desired families/ groups in domestication programme.

### Limitations

As the corresponding author experienced mortality of male broodstock when hormone was injected at 10 µg/g. body weight for F<sub>3</sub> generation males for inducing spermatogenesis in domestication

programme of black tiger shrimp *Penaeus monodon* the present study was conducted to evaluate the effect of testosterone hormone on reproductive performance of male broodstock by injecting hormone at 5 µg/g. body weight. However, efforts can be made to evaluate the effect of hormone at different rates.

### Recommendations for Further Study

1. Histological study reveals that hormone stimulates Androgenic gland to release Androgenic hormone; and also stimulates Y-organ to release moulting hormone (Ecdysteroid). However, the activity and secretory pathways of the hormonal effect on reproduction and moulting needs to be investigated by isolation of the hormone in tissues of targeted organs at different levels of activity by hormonal assay.
2. Histological study reveals that the hormone is stimulating the Androgenic gland to release Androgenic hormone that induces sperm development and subsequent mating success. To confirm whether testosterone hormone directly acting on Androgenic gland or indirectly acting by acting on thoracic ganglia and brain to release gonad stimulating hormone that induces Androgenic gland to release Androgenic hormone further studies are needed.
3. Though the present study was aimed at enhancement of mating success by usage of testosterone hormone the results besides fulfilling the aimed objective, suggests that when hormone is used for prolonged period of 120 days it promotes moulting reducing moulting period duration during second part of the experiment, scoping the possibility to promote growth by employing hormone. Further studies are needed to exploit the beneficial effects of this new venture.
4. Histological study reveals that the hormone is stimulating the Y-organ to release ecdysteroids that induces moulting. To confirm whether testosterone hormone directly acting on Y-organ or indirectly acting by acting on a group of neurosecretory cells (NSC) in the X-organ sinus gland complex of the eyestalks to reduce the release of moult inhibiting hormone (MIH) that inhibits the moulting process, giving scope to Y-organ to promote moulting process, further studies are needed.
5. These results of the present study are suggesting that testosterone hormone is more effective on Androgenic gland during 1-60 days and on Y-organ during 61-120 days. This type of differential activity of hormone is also evident from histological study that hypertrophied condition of Androgenic gland cells during 6-24 hrs and

increased granular secretions in Y-organ during 24-48 hrs. Detailed histological study and hormonal assay are needed to explain the logics behind the intensity of hormonal effect on two targeted organs at different durations.

### Funding and Policy Aspects

Though efforts were made by different countries to develop captive broodstock of tiger shrimp but it has not reached commercial level and still shrimp culture industry is awaiting for captive broodstock of tiger shrimp. Advantages and disadvantages of disease resistant broodstock versus sprcific pathogen free brood stock were discussed by Maheswarudu (2007; 2014). More efforts should be made towards development of disease resistant broodstock as it safeguards the industry even at the negligence of biosecurity by shrimp farmers in grow-out sector. Priority should be given to wards development of disease resistant broodstock by allotting adequate funds for concerned research programmes. As domestication programme involves continuous monitoring for prolonged period dedicated research team should be identified before funding the research programmes and due incentives should be granted to the team to encourage team spirit and integrity. The present study, usage of testosterone hormone to enhance mating success; and the study by Maheswarudu and Vineetha (2013), identification of arachidonic acid as shrimp (female) maturation stimulator from littoral oligochaete *Pontodrilus bermudensis*, will aide to lead the shrimp domestication programme successfully to successive generations for developing captive broodstock with desired traits.

### Acknowledgement

The authors are grateful to Prof. Mohan Joseph Modayil, former Director of CMFRI and Dr. G. Syda Rao, former Scientist-in-Charge, Visakhapatnam Regional Centre of CMFRI for providing facilities; and Dr. A. Gopalakrishnan, present Director of CMFRI, Dr. E.V. Radhakrishnan, former Head of Crustacean Fisheries Division for encouragement.

The Corresponding author (GM) is grateful to Department of Biotechnology, Government of India for providing DBT overseas associateship during 1995 to work under Prof. Milton Fingerma, Tulane University, New Orleans, USA as the result of the present study is outcome of the experience gained during the associateship period.

### Author's Contribution and Competing Interests

The corresponding author (GM) and contributing author (U.R) designed the experiment and carried

out the work. The contributing author (MS) carried out water parameters analysis. The corresponding author and contributing author (U.R) designed the histological experiment and carried out the work and the contributing author (MSC) done the histology study. The contributing author (CS) has taken the care of experimental animal maintenance. The corresponding author (GM) and contributing author (U.R) prepared the manuscript and the contributing author (MSC) reviewed the manuscript.

The authors are not competing for financial interests.

### References

- Aiken D.E., Molting and growth, in Cobb J.S. and Phillips B.F.(Editors) 1980;The biology and management of lobsters, Vol.1, Academic Press, New York, 91.
- Baghel D.S.,Lakara W.S., Rao G.P.S., 2004. Altered sex ratio in giant fresh water prawn, *Macrobrachium rosenbergii* (de Man) using hormone bioencapsulated live *Artemia* feed. *Aquaculture Research*, 35, 943-947.
- Blais C, Sefiani M., Toullec J.Y., Soye D.,1994. In vitro production of ecdysteroids by Y-organs of *Penaeus vannamei* (Crustacea, Decapoda), correlation with haemolymph titers. *Invertebrate Reproduction and Development*, 26 (1), 3-11.
- Burns B.G., Sangalang, G.B., Freeman H.C., McMenemy, 1984. Isolation and identification of testosterone from the serum and testes of the American lobster (*Homarus americanus*). *General and Comparative Endocrinology*, 52,429-432.
- Chang E. S., 1989. Endocrine regulation of molting in Crustacea. *Reviews in Aquatic Science*, 1,131-157.
- Charniaux-Cotton H., 1954. Découverte chez un Crustacé Amphipode (Orchestiagammarella) d'une glande endocrine responsable de la différenciation des caractères sexuels primaires et secondaires mâles. *CR Acad. Sci. Paris*, 239, 780-782.
- Diwan A.D., 2005. Current progress in shrimp endocrinology—A review. *Indian Journal of Experimental Biology*, 43, 2009-223.
- Diwan A .D., Nagabhusanam R., 1975. The neurosecretory cells of the central nervous system of the freshwater crab *Barytelphusa cunicularis* (Westwood 1836). *Rivista di Biologia*, 28, 79-115.
- Fairs N. J., Evershed R. P., Quinlan P. T., Goad, L. J., 1989. Detection of unconjugated and conjugated steroids in the ovary, eggs, and haemolymph of the decapod crustacean *Nephrops norvegicus*. *General and Comparative Endocrinology*, 4, 199–208.
- Gabe M., 1953. Sur l'existence chez quelques Crustacés Malacostracés d'un organe comparable a la glande de mue des Insectes. *C.R. Acad. Sci. Paris*, 237, 1111-1113.

- Gabe M., 1956. Histologie comparee de la glande de mue (organe Y) des Crustaces Malacostraces. Annales Des Sciences Naturelles-Zoologie Et Biologie Animale, 18, 145-152.
- Juchault P., Legrand J., 1965. Contribution a letude experimentable de l intervention de neurohormones dans le changement de sexe d Anlocra physodes (Crustace, Isopode, Cymothoidae). Comptes Rendus Hebdomadaires des Seances del Academie des Sciences, 260,1783-1786.
- Kulkarni G., Nagabhushanam R., Joshi P.K., 1979. Effect of progesterone on ovarian maturation in a marine penaeid prawn *Parapenaeopsis hardwickii* (Miers, 1878). Indian Journal of Experimental biology, 17, 986-987.
- Kulkarni, G., Nagabhushanam R., Joshi P.K., 1984. Neuroendocrine control of reproduction in the male penaeid prawn, *Parapenaeus hardwickii* (Meirs) (crustacea, Decapoda, penaeidae). Hydrobiologia, 108, 281-289.
- Maheswarudu G., Evidence of the existence of resistance/tolerance to WSSV in *Penaeus monodon*., In Bright Singh, I S, Joseph, Valsamma, Philip, Rosamma, Mohandas, A (Editors) 2007; Aquaculture and Marine Biotechnology, National Centre for Aquatic Animal Health, Cochin University of Science and Technology (CUSAT), Cochin, 30-35.
- Maheswarudu G., 2014. Selective breeding and development of disease resistant broodstock of *Penaeus monodon*. The Journal of Genetics. Photon.
- Maheswarudu G., Vineetha A., 2013. Littoral oligochaete *Pontodrilus bermudensis* Beddard: A potential source for Archidonic acid that stimulates maturation in penaeid shrimp. The Journal of Veterinary Science. Photon 14,290-300.
- Maheswarudu G., Radhakrishnan E. V., Pillai N. N., Mohan S., Arputharaj M. R., Ramakrishnan A., Vairamani A., 1996. Repetitive spawning of *Penaeus indicus* without eyestalk ablation, hatching rate and growth upto juveniles. Fishing Chimes,16 (3), 21-23.
- Maheswarudu G., Pillai N. N., Mohan S., Arputharaj M. R., Ramakrishnan A., Vairamani A., 2000. A technique for artificial insemination of *P. monodon* and its use in hybridisation studies of penaeids. In: Abstracts-Symposium on Ecofriendly Mariculture Packages- An Update, Marine Biological Association of India, 30-31.
- Miriam Paul Sreeram, Maheswarudu G., Varma J. B., 2004. Trade of live brooders of the black tiger shrimp, *Penaeus (Penaeus) monodon* Fabricius 1798 at Visakhapatnam, Andhra Pradesh. Journal of Indian Fisheries Association, 31, 37-46.
- Mohan C.V., Sudha P.M., Shankar K.M., Hegde A., 1997. Vertical transmission of white spot syndrome baculovirus in shrimps-a possibility? Current science, 74, 109-110.
- Nagabhushanam R., Kulkarni G. K., 1981. Effect of exogenous testosterone on the Androgenic gland and testis of a marine penaeid prawn, *Parapenaeopsis hardwickii* (Miers) (Crustacea, Decapoda, Penaeidae). Aquaculture, 23, 19-27.
- Nagaraju G.P.C., 2011. Reproductive regulators in decapod crustaceans: an overview. The Journal of Experimental Biology, 214, 3-16.
- Ollevier F., de Clerck D., Diederik H., de Loof A., 1986. Identification of non ecdysteroids in the haemolymph of both male and female *Astacus leptodactylus* (crustacea) by gas chromatography- mass spectrometry. General and Comparative endocrinology, 61, 214-228.
- Payen G., Costlow Jr J. D., Charniaux-Cotton H., 1971. Comparative study of the ultrastructure of Androgenic glands from normal and eyestalkless crabs during larval life or after puberty in the species *Rhithropanopeus hemsii* (Gould) and *Callinectes sapidus* Rathbun. General and Comparative endocrinology, 17, 526-542.
- Pearse, A.G., 1968. Histochemistry- Theoretical and Applied, 3rd edition, J.A. Churchill Ltd., London, 1.
- Quackenbush L.S., Keely L.L., 1986. Vitellogenesis in the shrimp, *Penaeus vannamei*. American Zoologist, 26, 56.
- Radhakrishnan E.V., Maheswarudu G., Arputharaj M. R., Ramakrishnan A., 2000. Repetitive maturation and spawning of the green tiger prawn *Penaeus semisulcatus* by environmental regulation in closed seawater recirculation systems. Journal of the Marine Biological Association of India, 42 (1 & 2), 91-100.
- Sakthivel M., Ramamurthy B., 2003. What ails shrimp farming and its future development? Fishing Chimes, 22, 33-36.
- Sarojini S., 1963. Comparison of effects of Androgenic hormone and testosterone propionate on the female ocy pod crab. Current Science, 9, 411-412.
- Skinner D. M., Molting and regeneration, in Bliss D.E., Mantel L.H. (Editors)1985; The Biology of crustacea, Academic Press, New York, Vol. 9, 43.
- Sonobe H., Kamba M., Ohta K., Ikeda M., Naya Y., 1991. In vitro secretion of ecdysteroids by Y-organs of the crayfish, *Procambarus clarkii*, Experientia, 47, 948-952.
- Tsai, M.F, Lo C.F., Liu H.C., Liu K.F., Kou, G.H., 1997. Detection of white spot syndrome virus (WSSV) in off springs of a WSSV carrier brooder by *in situ* hybridization. Proceedings of the International Symposium on Diseases in Marine Aquaculture, Oct 1997, Japan.
- Tsukimura, B., Kamemoto F.I., 1988. Organ culture assay of the effects of putative reproductive hormones on immature *Penaeus vannamei* ovaries. Journal of World Aquaculture Society, 19, 188.

Tim Verslycke, Katia De Wasch, Hubert F. De Brabander, Colin R. Janssen, 2002. Testosterone Metabolism in the Estuarine Mysid *Neomysis integer* (Crustacea; Mysidacea): Identification of Testosterone Metabolites and Endogenous Vertebrate-Type Steroids. *General and Comparative Endocrinology*, 126, 190–199.

Vijayan K. K., Mohamed K. S., Diwan A. D., 1993. On the structure and moult controlling function of the Y-organ in the prawn *Penaeus indicus* H. Milne Edwards. *Journal of the World Aquaculture Society*, 24, 516-521.

Yano I., 1985. Induced ovarian maturation and spawning of Greasy back shrimp *Metapenaeus ensis* by progesterone. *Aquaculture*, 47, 223-229.

Yano I., 1987. Effect of 17  $\alpha$  hydroxy progesterone on vitellogenin secretion I kuruma prawn *Penaeus japonicus*. *Aquaculture*, 61, 49–57.

Yashiro R., Na-anant P., Dumchum, V., Effect of Methyl testosterone and 17 $\alpha$ -hydroxy-progesterone on spermatogenesis in the black tiger shrimp, *Penaeus monodon*, in Flegel T.W. (Editors) 1998; *Advances in shrimp biotechnology*, National center for Genetic Engineering and Biotechnology, Bangkok, 33-36.

For publications/ Enquiries/ Submissions:  
**Email: [photonjournal@yahoo.com](mailto:photonjournal@yahoo.com)**