

Indian J. Fish., **51**(1) : 11-15, Jan.-Mar., 2004

Ovarian maturation in the banana shrimp, *Fenneropenaeus merguensis* (De Man) by Serotonin (5-hydroxytryptamine) injection

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ABSTRACT

The effect of serotonin (5-hydroxytryptamine, 5-HT) on the histological structure of ovaries of the banana prawn *Fenneropenaeus merguensis* (Crustacea: Decapoda) was studied. The Gonadosomatic index (GSI) increased significantly in the treated females, with a majority of oocytes reaching early vitellogenic stage, indicating that ovarian maturation could be induced and accelerated by 5-HT. The changes were also associated with a significant increase ($P < 0.01$) in the female specific protein (FSP) level in the treated females compared to the controls. 5-HT exerts its stimulatory effect on the ovaries of crustaceans indirectly by triggering release of gonad stimulating hormone (GSH) from the brain and thoracic ganglion. Thus, a programme incorporating 5-HT, high salinity and good water quality seems to be a practical alternative to eyestalk ablation to induce maturation in *F. merguensis* in captivity.

Introduction

Domestication of candidate species of shrimps for culture is dependent on captive broodstock development and breeding under controlled conditions. Unilateral eyestalk ablation technique is currently used world-wide for induced maturation of broodstock of penaeid shrimps. Eyestalk ablation suspends the controls on a large number of body functions so that females divert all their energies into egg production, leading to decline in egg quality. The development of an alternative technique that would allow the production of high quality eggs over time would be of immense benefit to the penaeid shrimp culture industry.

Neurotransmitters regulate gonadal maturation in crustaceans (Fingerman 1997). Neurotransmitters induce the

release of neurohormones that regulate the reproductive activities in both vertebrates (Crim *et al.*, 1984) and invertebrates (Fingerman, 1987). Serotonin, 5-hydroxytryptamine (5-HT) found in the central nervous system of crustaceans, is a biogenic amine functioning as a neurotransmitter/neuromodulator / neurohormone in a wide variety of species including crustaceans (Elofsson *et al.*, 1982; Elofsson 1983; Kulkarni and Fingerman, 1992). In crustacea some of these amines also serve the function of neuroregulators to control the release of neurohormones by stimulating the release of several eyestalk neuropeptides including moult-inhibiting hormone. Other functions attributed to 5-HT include migration of the proximal retinal pigment, pericardial organ

neurohormone, stomatogastric ganglion neuromodulator or neurohormone, behavioural responses, osmoregulation and mechano-reception (Fingerman, 1997).

5-HT stimulated ovarian development in fiddler crab, *Uca pugilator* (Richardson *et al.*, 1991; Kulkarni and Fingerman, 1992), *Procambarus clarkii* (Kulkarni *et al.*, 1992; Sarojini *et al.*, 1995), *Penaeus vannamei* (Vaca and Alfaro, 2000), *P. penicillatus* (Oliviera and Correa, 1999) and *Paratelphusa hydrodromous* (Ragunathan and Arivazhagan, 1999). Kulkarni *et al.* (1992) reported that *Procambarus clarkii* when given 5-HT *in vivo* showed significant increase in ovarian index and oocyte size over the concurrent controls. 5-HT stimulates ovarian development in crustaceans indirectly by triggering release of gonad stimulating hormone (GSH) from the brain and thoracic ganglion. The present study was conducted to investigate the effect of Serotonin, (5-HT) on ovarian development of banana prawn, *Fenneropenaeus merguensis* (de Man)

Materials and methods

Healthy adult *F. merguensis* females (above 120mm in length and 15-20g in weight) were collected from sea. They were disinfected with 50mg/l of formalin for 1-2 minutes in the laboratory and transferred to aerated plastic pools containing seawater (salinity 25 ± 2 ppt; temperature: $29 \pm 2^\circ\text{C}$). The shrimps were fed with fresh clams and squids *ad libitum* once daily at the rate of 10% of their body weight. After an initial acclimatisation period for 48 hours, five sexually immature females in the intermoult stage were randomly distributed into each of the experimental units (fish breeding hapa of 2x1x1 m;

mesh size - 225 mesh /sq. inch; two numbers) set up in Karwar bay. The animals were fed with clams and squids *ad libitum* once daily. The experimental females were given three injections of Serotonin, 5-HT creatinine sulfate @ $15\mu\text{g} / \text{g}$ body weight intramuscularly through the first abdominal somite using a hypodermic syringe on the first, fifth and tenth day. Control shrimps reared under similar experimental conditions were injected with 100 μl of normal ethanol. Males were introduced into the rearing units in the ratio of one male to two females. The nets were thoroughly cleaned of any encrustation on its surface when the shrimps were removed for hormone injections. The experiment lasted for fifteen days.

At the termination of the experiment, females were removed, weighed and the gonadosomatic index was determined using the formula

$$\text{Gonadosomatic index} = \frac{\text{Wet weight of the ovary} \times 100}{\text{Wet weight of the shrimp}}$$

Histology : Samples from the middle lobe of the ovary were fixed in Bouin's solution and prepared for histological observation. After 24h of fixation, the ovaries were dehydrated in an alcohol series and embedded in paraffin (mp 56-58 $^\circ\text{C}$). Seven-micrometer sections were prepared and stained with haematoxylin and eosin stain. One hundred oocytes from each group were measured to determine their diameters by using ocular micrometer with a light microscope.

Electrophoresis : Haemolymph was collected from the shrimps through the pericardial cavity using needles rinsed with 0.2M Ethylenediamine tetra acetic acid disodium salt (EDTA). Approximately 0.5ml of serum, diluted

to 5ml, was subjected to ammonium sulphate precipitation (Wallace *et al.*, 1967). Vitellogenin precipitated at 60% saturation of ammonium sulphate under ice-cold condition was centrifuged at 10,000 g for 15 minutes. The pellet thus obtained was dissolved in 200µl of distilled water and analysed electrophoretically in a 6.5% Native Polyacrylamide Gel (PAGE) using 1.8M Tris-HCl buffer (pH 8.9) (Davis, 1964). Electrophoresis was conducted at constant current of 65mA at 10°C. The gels were stained for proteins with 0.1% Coomassie brilliant blue R250 according to conventional methods and destained in a solution of 50% methanol containing 10% acetic acid. The data was statistically analysed with one-way analysis of variance.

Results

In the present investigation, ovarian index of females treated with serotonin exhibited significant increase compared to the controls. The average GSI of 5-HT treated females was 0.7796, which was higher than the control (0.336). Histologically the treated females were

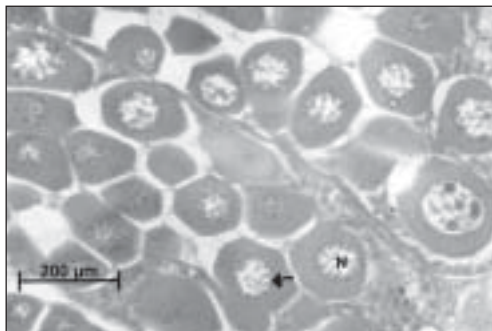


Fig. 1a Cross-section of the ovaries of female *F.merguensis* injected with 5HT (x 100) showing oocytes with granular cytoplasm and a large round nucleus with nucleolar material arranged as a ring around the peiphery of nucleus (←). (N. Nucleus)

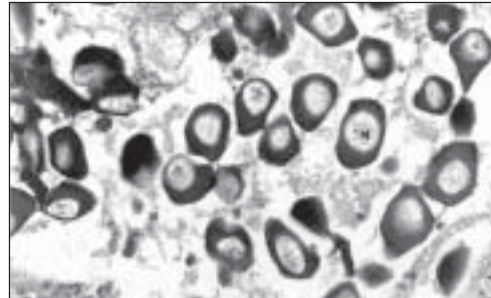


Fig. 1b Cross-section of the control ovaries of female *F. merguensis* (X 100) showing oocytes with a centrally located prominent nucleus with numerous nucleoli. (N-Nucleus)

in the advanced second stage of maturation with nearly all the oocytes in the early phase of vitellogenesis loaded with dense yolk globules and with increased cell size (140-188µ), when compared with ovaries of control females (12-45µ). The nuclei of these oocytes were compact with peripherally located nucleoli. Follicular cells were observed surrounding the oocytes (Fig. 1a). In the control females, the oocytes showed clear nucleus occupying a major portion of the oocyte (Fig. 1b).

Electrophoretic analysis of the haemolymph samples from females treated with 5-HT revealed that the expression of female specific protein (FSP) in these females

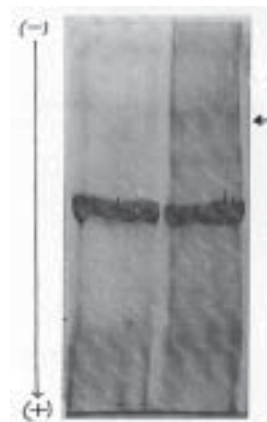


Fig. 2 Native PAGE showing vitellogenin expression in the haemolymph of females treated with serotonin.

Lane 1. Control
 Lane 2. Haemolymph from females treated with serotonin
 → Vitellogenin

TABLE 1. Ova diameter and Gonadosomatic indices of control and 5-HT treated *F. merguensis* (Anova, $P < 0.01$)

Treatment	Ova diameter (μ)	GSI
Control	12 - 45	0.336 \pm 0.171
Serotonin	140 - 188	0.7796 \pm 0.10

was comparable to that of early maturing females (Fig.2).

Statistical analysis by means of one-way analysis of variance (ANOVA) of the GSI of females treated with 5-HT showed that the ovarian maturation in the treated females was significantly higher ($P < 0.01$) than that of controls confirming the stimulatory role of 5-HT on ovarian maturation (Table 1).

Discussion

Administration of the neurotransmitter, 5-hydroxytryptamine to female *F. merguensis* showed pronounced ovarian development, with corresponding increase in the average GSI than the controls, indicating the possible stimulatory role of 5-HT on ovarian maturation. The histological changes observed in the ovary of the treated groups were similar to those observed in females undergoing natural maturation, suggesting normal development or vitellogenesis under the stimulation of 5-HT.

Several reports exist on the effects of 5-HT on gonadal development of crustaceans. A significant increase in ovarian index and oocyte size of crayfish *P. clarkii* when given 5-HT, was reported by Kulkarni *et al.* (1992). Richardson *et al.* (1991) have demonstrated increased dose dependent ovarian development in the fiddler crab *Uca pugilator*, while Kulkarni *et al.* (1992) and Kulkarni and Fingerman (1992) have given supporting evidence showing the stimulatory role of 5-HT in the reproduction of female

crustaceans. Rangunathan and Arivazhagan (1999) showed the effect of eyestalk ablation together with 5-hydroxytryptamine in the ovarian maturation of freshwater crab *Paratelphusa hydrodromous*. According to Vaca and Alfaro (2000), *P. vannamei* injected with serotonin showed higher rate of maturation and spawning. Similarly, in the present study, the significant increase in the GSI observed after 15 days of administration of 5-HT may also be concluded to be due to its stimulatory effect on central nervous system, triggering vitellogenin synthesis and its release into the serum, resulting in induction of ovarian maturation in *F. merguensis*. It is suggested that 5-HT, which is present in the central nervous system of crustaceans exerts its effect indirectly, by stimulating release of the ovary-stimulating hormone (GSH) which in turn acts directly on the gonad.

In *F. merguensis*, full maturation and spawning was not achieved by injection of 5HT, even though indications of initiation of ovarian maturation were observed. This may probably be due to the low dose (15 μ g / g body weight) injected. Vaca and Alfaro (2000) achieved maturation and spawning in *P. vannamei* when injected with serotonin @ 15mg/g body weight.

Acknowledgements

The authors are thankful to Director, CMFRI, Cochin, for providing facilities. The first author wishes to express thanks to the Indian Council of Agricultural Research, New Delhi for the

award of fellowship.

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