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RESEARCH ARTICLE

IMPACT OF SEROTONIN ON MOULTING, OVARIAN MATURATION AND SPAWNING IN A MOLE CRAB, *EMERITA EMERITUS*.

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Abstract

The commercial brood production through eyestalk ablation is often associated with high mortality in the brood stock and inferior quality of offspring and it is necessary to find an alternative for worldwide expansion of fishculture. The present study was designed to find the possible role of serotonin (5-hydroxytryptamine, 5-HT) in moulting, reproduction and spawning in the mole crab *Emerita emeritus*. Serotonin in brain, hemolymph and ovary of the mole crab was correlated with moulting and reproduction to elucidate its modulatory effect. Immuno cytochemical observation indicated positivity to serotonin in neurosecretary cells and ovarian cells at various phases of development. Quantitative analysis of serotonin, using HPLC clearly exhibited a gradual increase in brain and thoracic ganglia during intermoult. Similarly ovary and hemolymph showed highest concentration, whereas the concentration of serotonin gradually declined from premoult stages indicating that the amine act as a neurotransmitter through the mediation of eyestalk neuropeptide hormone. To augument the impact of serotonin, exogenous 5 HT, at various concentration ($1x10^{-6}$, $1x10^{-7}$ and$1x10^{-8}$ mol/crab) were injected into the crab at immature stage of ovarian development. The effect was compared against a control group, which received the injection of the sterile vehicle solution. Serotonin induced ovarian maturation and increased the spawning rate at a dose of $1x10^{-6}$ mol/crab. Thus exogenous form of 5-HT influenced ovarian maturation and enhanced the spawn on 12th day while the control crabs spawned only on the 22nd day of the experiment. From the results we conclude that, serotonin regulated ovarian maturation and advanced spawning by overcoming the inhibition of gonad inhibitory hormone from the optic lobe of the mole crab or in the brain and thoracic ganglia to stimulate gonad stimulating hormone synthesis or indirect effect on the ovary.

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Introduction:-

Crustaceans are one of the nutritional aquatic food sources worldwide, but the major bottleneck in crustacean aquaculture industry is the availability of seed. The commercial brood production through hatchery operation has successfully been carried out in shrimps, lobsters, and to certain extent in crabs through eyestalk ablation (Huberman, 2000). However, the technique could lead to a loss in egg quality and eventual death of the spawner (Benzie, 1998). To overcome the above problems several non- surgical procedures have been attempted to induce the ovarian maturation, such as injections of GIH antibody, hormones, neurotransmitters and double- stranded RNA to knockoff GIH mRNA; results from some of these experiments are promising (Poltana *et al*., 2005; Treerattrakool *et al*., 2005). In crustaceans reproduction is regulated by two antagonistic hormones, the gonad inhibiting hormone (GIH) from X-organ sinus gland complex in the eyestalk (Panouse, 1943) and gonad stimulating hormone (GSH) in the brain and thoracic ganglion (Otsu, 1963). The coordination between these two hormones is crucial in the regulation of ovarian maturation. The synthesis and release of GIH and GSH from XO/SG complex, brain and thoracic ganglia, respectively are believed to be modulated by biogenic amines (Richardson *et al*., 1991; Fingerman, 1997). Biogenic amines are conserved molecules and involved in the regulation of a wide array of physiological activities in decapods (Fingerman, 1997). They function as neuroregulators or neurohormones and exert their effect on target tissues (Fingerman et al., 1994; Luschen *et al*., 1993; Sneddon *et al.*, 2000).

Several previous studies have reported astimulatory effect of 5-HT on gonadal maturation in some decapods, including *Procambarusclarkii* (Kulkarni *et al*., 1992), *Penaeusmonodon* (Wongprasert *et al.*, 2006), and *M.rosenbergii* (Meeratana *et al*., 2006). Serotonin had been found to be distributed widely in the crayfishcentral nervous system (Fong et al., 1994), eyestalk (Martinez, 1991), brain (Barley, 1985), subesophageal, thoracic and abdominal ganglia (Matsutani, 1990). Serotonin and the nervous tissue extract have been found to stimulate ovarian maturation and spawning in freshwater shrimp, *Paratyacompressa* (Mattson and Spaziani, 1985), in red swamp crayfish, *P. clarkii* (Bauchau and Mengeot, 1966), in *Litopenaeus stylirostris* and *L. vannamei* (Alfaro *et al*., 2004).

Serotonin have been detected by HPLC in various regions of the CNS of *Pacifastacusleniusculus* (Elofsson *et al*., 1982; Laxmyr, 1984), the eyestalks of *P. clarkii* (Alvarez *et al*., 2005), and the intestinal nerve of *P. clarkii* (Mercier *et al*., 1991). Major tissues that exhibited a high level of 5-HT are the optic ganglion, cerebral ganglion, circumoesophageal connectives, stomato gastric ganglion, and thoracic ganglia (Aramant and Elofsson, 1976; Beltz*et al*., 1984). However, quantification of the levels of this neurotransmitter in correlation with different phases of the decapod crustacean ovarian and moulting cycle has not yet been performed. Serotonin like immunoreactivity has been reported in the eyestalk (Rodriguez-Sosa *et al*., 1997) and in the brain of *P.clarkii* (Sullivan and Beltz, 2001), in the brain of the *P. leniusculus* (Eloffson, 1983) and lobster (Sullivan and Beltz, 2001), in the ventral nerve cord of crayfish *Cherax destructor* (Harrison *et al*., 1995), and thoracic ganglia of crayfish *P. clarkia* and fresh water prawn *Macrobrachium rosenbergii.* .

Serotonin has stimulated ovarian maturation and hemolymph vitellogenin in the Indian white shrimp, *F. indicus* (Santhoshi *et al.*, 2008). Serotonin injection induces maturation and spawning in *P. vannamei* but at rates lower than unilateral eyestalk abalation (Vaca and Alfaro, 2000). Furthermore, serotonin has been stimulated ovarian maturation and spawning in *P. monodon* (Wongprasert*et al*., 2006) and in *M. rosenbergii* (Meeratana *et al*., 2006). The present study was undertaken to examine the role of serotonin on ovarian maturation and spawning in the mole crab *E. emeritus* and to localize and determine the levels of serotonin in the brain, thoracic ganglia, hemolymph and ovary of the crab. The experimental model, *Emerita emeritus* selected for the present study is a burrowing mole crab in which the paired eyestalks are secondarily reduced. In the absence of any neurosecretory centres in the eyestalk of *E. emeritus* the X-organ/ sinus gland complex is embedded in the brain (Hanstrom, 1939). Therefore in this study an attempt has been made to stimulate ovarian maturation and spawning with exogenous serotonin as an alternative for eyestalk abalation.

Material and methods:-

Collection and maintenance of experimental animals:-

Mole crab *Emerita emeritus*, in size range of 10-22 mm carapace length (CL) were collected from the intertidal region of Elliot's beach at Besant Nagar, Chennai, India. The female crabs were identified by the occurrence of three

pairs of pleopods. The animals were maintained in plastic tanks with sufficient aeration with clean sand spread in slanting position. The sea water was changed daily and sand was changed once in a week.

Classification of ovarian and embryo developmental stages:-

Ovarian developmental stages were determined by observing colour changes in the ovary (Kerr, 1969: Wolin *et al.*, 1973) and by direct microscopical examination of the oocytes. Embryonic development has been divided into nine stages based on colour change and other concomitant morphological features of the embryo, as detailed in (Subramoniam, 1991).

Analysis of ovarian stages:-

Histology:-

Fragments of ovary of the female crabs were fixed in 4% formaldehyde in buffered saline (pH 7.5), dehydrated through graded alcohol series, embedded in paraffin, sectioned (4-5 µm), deparaffinized and rehydrated (Pantin,1934). Sections were stained with haematoxylin and eosin, mounted on DPX, observed and photographed on a Carl Zeiss ACE Axioplan microscope (Carl Zeiss, Germany).

Measurement of gonadosomatic index (GSI):-

Crabs were blotted dry with a filter paper and total weights recorded to the nearest milligram using an electronic balance. The gonads were carefully dissected and blotted with a filter paper to remove moisture. Total weight of the reproductive tissue was recorded to the nearest milligram. The gonadosomatic index was calculated using the following formula

$$\text{GSI} = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100$$

Measurement of hepatosomatic index (HSI):-

Crabs were blotted dry with a filter paper and total weights recorded tothe nearest milligram using an electronic balance. The hepatopancreas was removed from each crab, blotted dry with a filter paper and weight was recorded to the nearest milligram. The hepatosomatic index of each crab was calculated by using the following formula,

$$\text{HSI} = \frac{\text{Hepatopancreas weight}}{\text{Body weight}} \times 100$$

Measurement of oocyte diameter:-

Oocyte diameter was determined by light microscope. For each crab, the diameter of as many as 30 oocytes were measured and mean oocyte diameter was calculated. Because germcells as well as developing oocytes of different stages were present, the stage of development was characterized based on the number of oocytes confined to their development. The ovaries at various stages of development were fixed and sectioned as per the method described earlier. The stained, mounted sections were viewed under light microscope. For each group of sample, oocytes were measured using an ocular micrometer calibrated with a stage micrometer.

Analysis of moult stages:-

The moult stages of *E. emeritus* were determined based on microscopic observations of morphogenesis in pleopodal setae such as epidermal retraction and new setal development and by morphological criteria including carapace hardness, rigidity, exoskeletal suture and by behavioral variables (Gunamalai and Subramoniam, 2002).

Quantification of 5-HT by HPLC:

To detect serotonin level in tissues such as brain, thoracic ganglia, ovary and hemolymph were collected from ten animals at each ovarian and moulting stages. Each organ was carefully dissected, and its wet mass was determined. Serotonin and all other chemicals used in the experiments were obtained from Sigma Aldrich (USA). Samples were placed in 50µl 0.1M perchloric acid. The mixture was then sonicated and centrifuged at 14,000g at 4ºC. The supernatants were then collected and filtered through a 0.22µm Spin-x centrifugal filter tube before injecting into the HPLC column (Kim *et al.*, 1987). The average concentrations of 5-HT were estimated in three replicates. Standard solutions were freshly prepared by dissolving 5-HT in ice-cold 0.1 M perchloric acid on the day of analysis and were stored in ice between injections onto the HPLC system.

Supernatants from the extracts were injected into a phenomenex column (Reverse phase C18, particle size 3 µ, 250 x 4.6 mm): A glassy carbon electrode served as a working electrode and was used with an Ag/AgCl reference electrode. The detector potential was set at +0.45 V versus Ag/AgCl electrode. The sensitivity of the detector was maintained at 100 nA with full scale deflection. The mobile phase consisted of 32 mM citric acid; 3.54 g Na2PO4; 0.037 g EDTA and 0.236 g octyl sodium sulphate. The pH was adjusted to 4.2. The mixture was sonicated, centrifuged at 14.000g at 40 C. The supernatant were collected and then filtered through a 0.22 µm Spin-x centrifugal filter tube before injection. Samples were injected into a 20 µl injection loop. 5-HT levels were quantified using the external standard method. Peaks corresponding to 5-HT were detected in the extracts at the same elution times as their corresponding standards. The identities of the peaks in each sample were further verified by spiking the tissue extracts with known amounts of 5-HT standards. All samples were freshly prepared and analyzed within the same day.

Immunocytochemical localization of 5-HT:-

The brain, thoracic ganglia and ovaries of different stages of reproduction were dissected and fixed in Bouin's fluid for 24 hrs and processed for paraffin embedding on gelatin coated slides. The sections were hydrated and processed for PAP staining as described by Sternberger (1986). Rat anti- 5-HT (Biogenex, SC-58031, Bangalore) was used as the primary antibody (1:5000 dilution, incubation at 4ºC for 74 hrs). Goat anti-rat serum (IgG (R-3128), Genei) was used as secondary antibody (1:2000 dilution, incubation at room temperature for 1 hr). After the immune-reaction, sections were incubated in a medium consisting of diamino benzidine (Sigma, United States; 5 mg in 10 ml Tris-HCl and 0.01% fresh H2O2 for 5 min. at room temperature). The specificity of the immunoreactions was tested with non-immune rabbit serum in the place of antiserum and with the centrifuged supernatant of 5-HT saturated antiserum. In both examples, there was no immunoreaction. Non-specific staining was checked by conducting the histochemical reaction in hydrated sections or in sections pretreated with 0.5-1.0% H2O2 for 10 min. The endogenous peroxidase activity was negligible and did not interfere with immuno staining in the brain and thoracic ganglia and ovary of this species.

Experiments on exogenous serotonin to optimize ovarian maturation and spawning:-

To determine the effect of serotonin on ovarian maturation and their dose dependant action, as many as 198 mole crabs were divided into five groups. Group I consisted of 6 crabs served as initial control and sacrificed on the day '0' of the experiment. Remaining 192 crabs were divided into four groups of 48 crabs each. Group II served as concurrent control and Group III received serotonin at a concentration of $1x10^{-6}$ mol/crab, Group IV received $1x10^{-7}$ mol/crab, Group V received $1x10^{-8}$ mol/crab. For convenient analysis 48 animals in each group were further divided into four groups each consisting of 12 animals were observed on 1^{st}, 6^{th}, 12^{th} and 18^{th} day. The first group served as control which did not receive any treatment. The second group served as control and received 20µl of physiological saline. Crabs of the third, fourth and fifth groups were injected with serotonin creatinine sulphate with a hypodermal syringe (1 ml 26 G1/2) and Precision Glide Needle through the arthrodial membrane of the last appendage with concentrations of $1x10^{-6}$, $1x10^{-7}$ and $1x10^{-8}$ mol/crab, respectively in a final volume of 20µl/crab. The test doses were selected based on earlier dose response studies (Pushpalatha and Reddy, 2007; Sainath and Reddy, 2010). The effect of serotonin on the ovarian maturation of the crab was detected through ovarian index, oocyte diameter and histology of the ovary as per the routine standard method. Reproductive performance of the crabs subjected to various concentrations of serotonin was also monitored upto their spawning.

Statistical analysis:-

All statistics were performed using SPSS 10 for Windows package (SPSS Inc., Chicago, IL, USA), and data are given as arithmetic Mean±Standard deviations. All the data obtained on the levels of serotonin during different ovarian maturation and moulting were subjected to one - way analysis of variance (ANOVA) followed by Multiple Newman- Kuel's test (MNK). A probability value of 0.05 or less of the statistical tests between the control and experimental values was considered significant.

Results:-

Fluctuation of serotonin during moulting stages:-

The concentration of serotonin in brain increased gradually through intermoult (C1) 0.09±0.02ng/mg, (C2), 0.16±0.02ng/mg and finally reached 0.47±0.01ng/mg at the (C3) stage (Figure 2). However, the concentration of serotonin in brain gradually declined from premoult (D_0) 0.22±0.04ng/mg to 0.12±0.01ng/mg at the (D3-4) stage. The concentration of serotonin in thoracic ganglia also decreased from premoult (D_0) 0.35±0.03ng/mg, and was minimum in premoult (D3-4) stage 0.26±0.01ng/mg. In the ovary, serotonin concentration was highest

(0.96±0.08ng/mg) in intermoult (C3) stage and then decreased from premoult stages (D_0) to (D3-4) (0.41±0.04, 0.38±0.01, 0.34±0.01, 0.29±0.03 ng/mg, respectively). At premoult (D3-4) stage, the concentration of serotonin in the ovary was 3.3 fold lower ($P<0.05$) than in the intermoult (C3) stage. In hemolymph, serotonin concentration increased from intermoult (C1) stage (0.15±0.02ng/mg), became highest in intermoult (C3) stage (0.74±0.06ng/mg), and then decreased in premoult stages (D_0), (D1), (D2) and (D3-4) (0.37±0.04, 0.34±0.02, 0.30±0.01 and 0.27±0.02 ng/mg respectively). Serotonin content in the postmoult stage increased than that of the premoult stages ($P<0.05$).

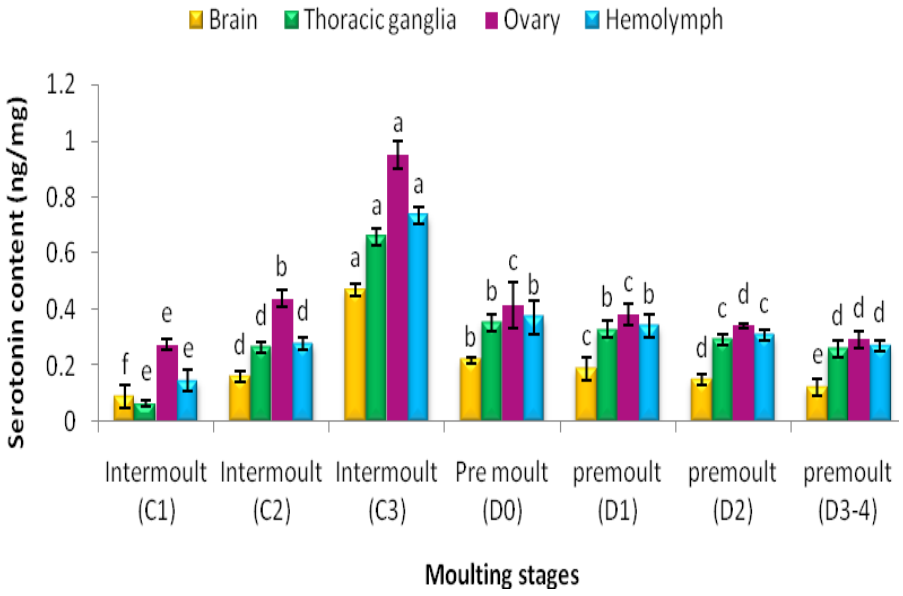

Figure 2:- Serotonin concentrations in various tissues during moulting stages.

Serotonin titers in different tissues in relation to ovarian development:-

The concentration of serotonin in the brain, thoracic ganglia, ovary and hemolymph exhibited a gradual increase from immature to vitellogenic stages (Figure 1). At immature stage the concentration of serotonin in the brain was 0.083±0.01ng/mg, and it gradually increased through the early pre-vitellogenic stage (0.144±0.01 ng/mg), pre-vitellogenic stage (0.379±0.02ng/mg) and finally reached the highest level (0.558±0.04ng/mg) in vitellogenic stage, A similar pattern was observed in the ovary and hemolymph, where the serotonin concentration in ovary was 0.251±0.01ng/mg at immature stage, 0.425±0.04ng/mg at early pre-vitellogenic, 0.799±0.01ng/mg at pre-vitellogenic stage and finally 1.253±0.03ng/mg at vitellogenic stage. In the hemolymph the serotonin concentration increased from 0.155±0.03ng/mg at immature stage to 0.912±0.04ng/mg at vitellogenic stage. The serotonin concentration of thoracic ganglia at immature stage was lesser (0.075±0.02 ng/mg) than of brain tissue. However the serotonin concentration increased in other stages of ovarian development when compared to that of brain. When compared within a tissue the concentration of serotonin was about 6 times higher in the brain at vitellogenic stage ($P<0.05$) than in the brain at immature stage, respectively. The concentration of serotonin was about 3, 6, 11times higher in the thoracic ganglia at early pre-vitellogenic, pre-vitellogenic and vitellogenic stage ($P<0.05$) than in the thoracic ganglia at immature stage, respectively. Furthermore, the absolute quantity of serotonin in tested tissue was higher in the vitellogenic stage when compared to the immature stage.

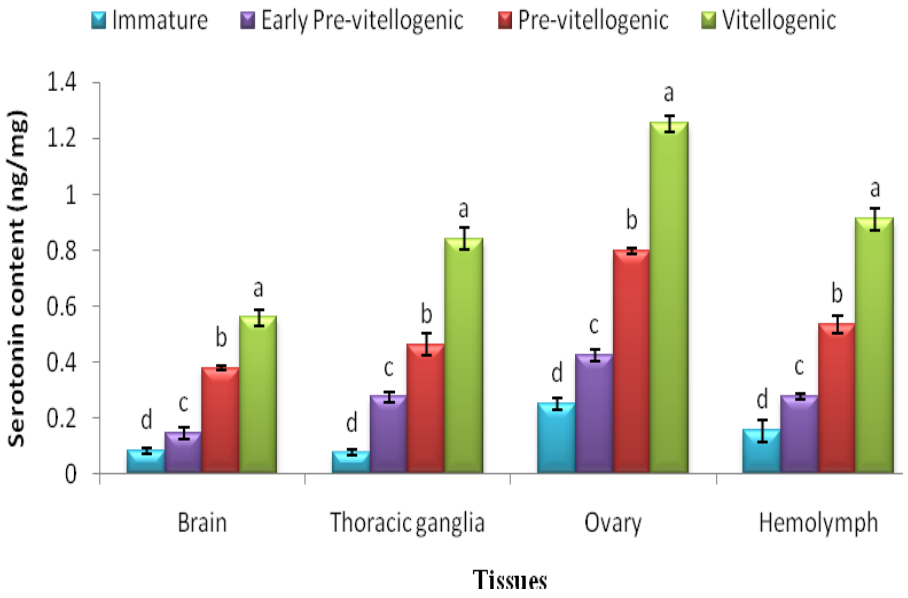

Figure 1:- Serotonin concentration in various tissues at ovarian development stages.

Serotonin activity in brain, thoracic ganglia and ovarian tissues:-

The brain of immature and early pre-vitellogenic stages of ovarian development showed fewer number of immunoreactive cells and some of the NSC's were non-immunoreactive in areas of the brain(Figure 3). On the other hand, the brain of pre-vitellogenic and vitellogenic stages of ovarian development showed strong and moderately differentiated neurosecretory cells correlating with the advancement of ovarian maturation. The number of immunoreactive cells and the extent of their immunostaining in thoracic ganglionic mass exceeded that of brain.More number of well differentiated neurosecretory cells could be seen in the thoracic ganglion of pre-vitellogenic and vitellogenic stages of ovarian development. In immature ovary, serotonin immunoreactivity was pronounced in the ooplasm of the follicular cells and in the primary oocytes (Figure 4). At that stage the oocytes showed less positive reaction. The immunoreactivity was pronounced in the ooplasm of the primary oocyte and was intense in early pre-vitellogenic stage of ovary. The pre-vitellogenic and vitellogenic stage oocytes also showed intense positive staining for serotonin.

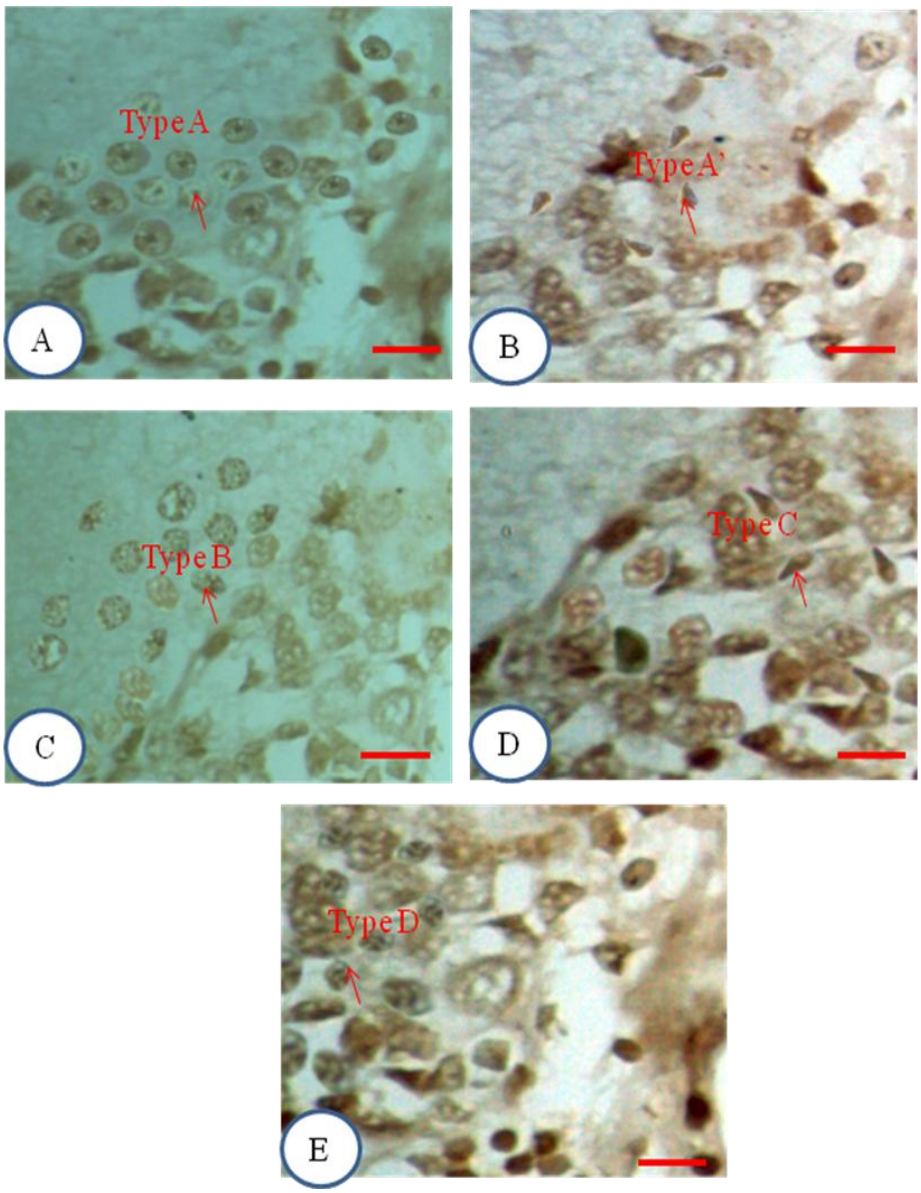

Figure 3:- Photomicrographs of cross section through the brain showing serotonin immune reactivity in *E.emeritus.*
(A) Brain section showing type A NSC with moderate serotonin immune reactivity at early pre-vitellogenic stage of ovarian development.
(B) Type A' NSC's showing weak serotonin immune reactivity at vitellogenic stage of ovarian development.
C) Type D NSC's showing strong serotonin immune reactivity at vitellogenic stage of ovarian development.

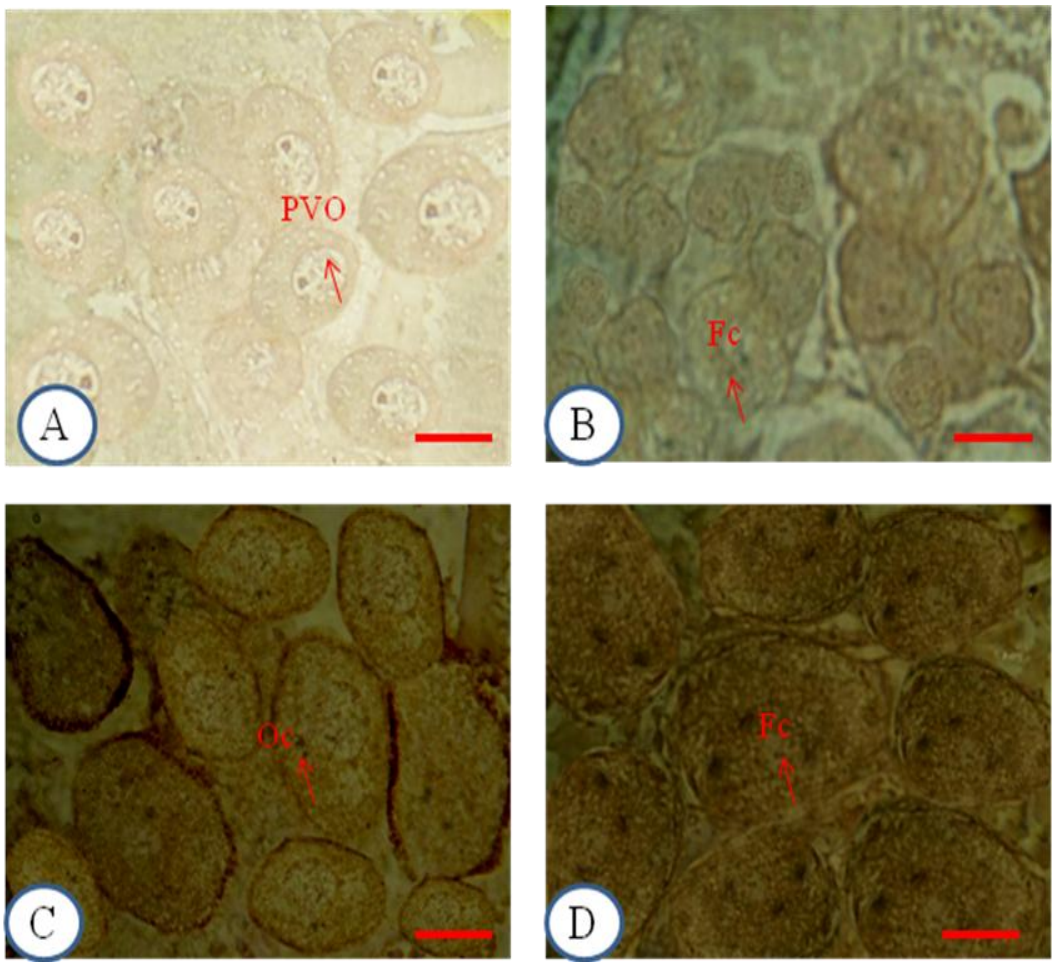

Figure 4:- Photomicrographs showing serotonin immune reactivity in the ovary of *E.emeritus.*
(A) Immature ovarian section showing weakly stained pre vitellogenic oocytes (PVO).
(B) Early pre-vitellogenic ovarian section showing moderately stained follicle cells (Fc) surrounding the vitellogenic oocytes.
(C) Pre- vitellogenic ovarian section showing intensely stained oocytes (Oc).
(D) Vitellogenic ovarian section showing flattened follicle cells (Fc) with intense immunoreactivity.

Exogenous serotonin on ovarian growth and spawning:-

The ovarian index and oocyte diameter in initial control crabs and control crabs were 0.92±0.06 (WW %), 64.13±0.99 µm and 0.92±0.05 (WW %), 64.14±1.71µm, respectively (Figure 5). The ovarian index and oocyte diameter significantly increased ($P<0.05$) from 0-18th day of the experiment in the control groups. The GSI values showed no much variation in the first day of the experiment. In $1x10^{-6}$ mol/crab serotonin injected group, the GSI value increased to 3.16±0.14% after 6 days exposure. Whereas in the lower concentration (of $1x10^{-8}$ mol/crab) increased the GSI to 3.58±0.17% only after 18 days of exposure. Similarly at lower concentration of serotonin the oocyte diameter did not show much variation compared to control. At higher concentration of serotonin, the oocyte diameter drastically increased to 297.7±1.48µm on 6th day of the experiment indicating that the oocytes are at vitellogenic stage of development. The occurrence of minimum size of oocyte on 12th day of the experiment at higher concentration revealed that the crab had undergone spawning, having proliferating immature oocytes, whereas the control crabs spawned only on the 22nd day of the experiment (Figure 6). In the moderate concentration of $1x10^{-7}$mol/crab the immature oocytes could be seen only on 18th day of the experiment. On the same time at low concentration of serotonin $1x10^{-8}$ mol/crab vitellogenic oocytes could be seen only on the 18th day of experiment. No mortality of the crabs was observed in either experimental groups or in the control groups throughout the study.

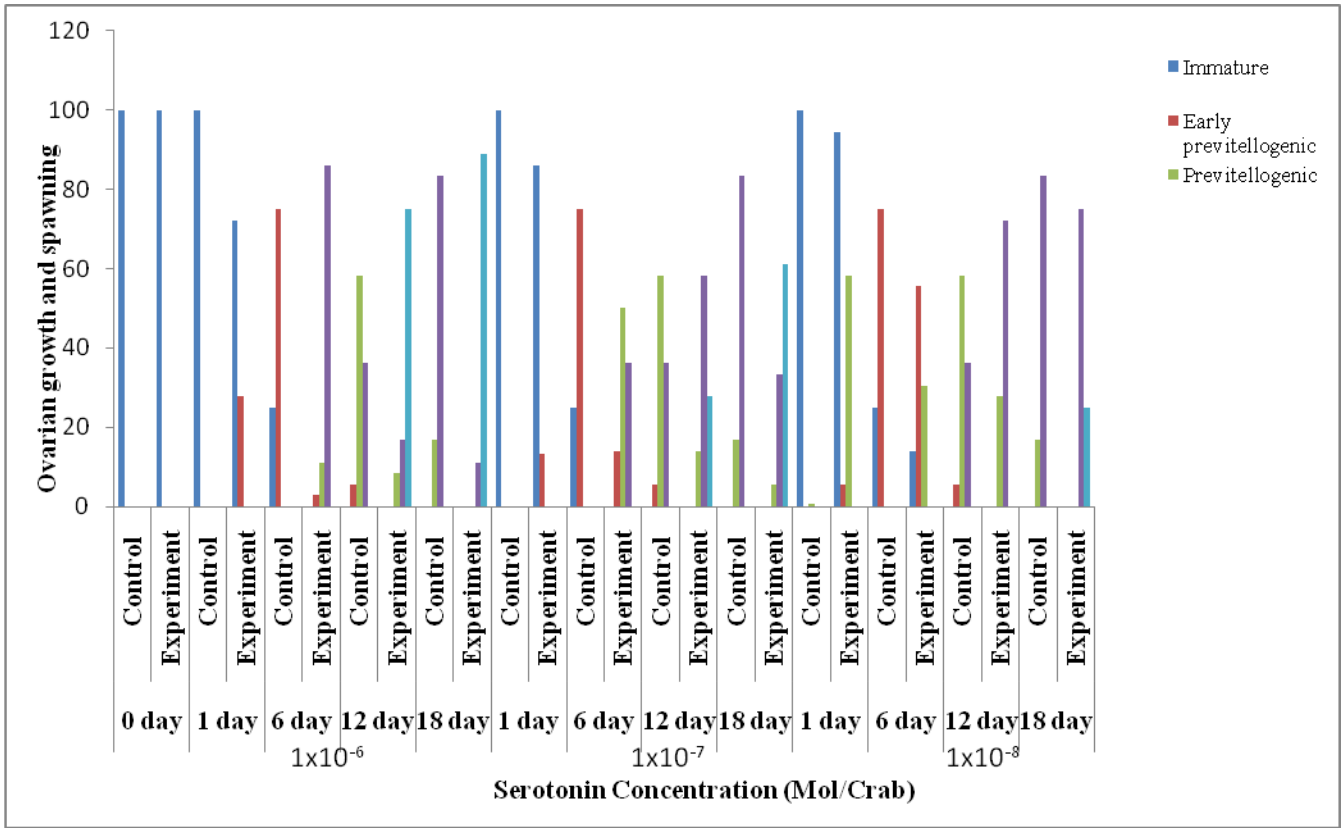

Figure 5:- Ovarian development and spawning at different serotonin concentrations.

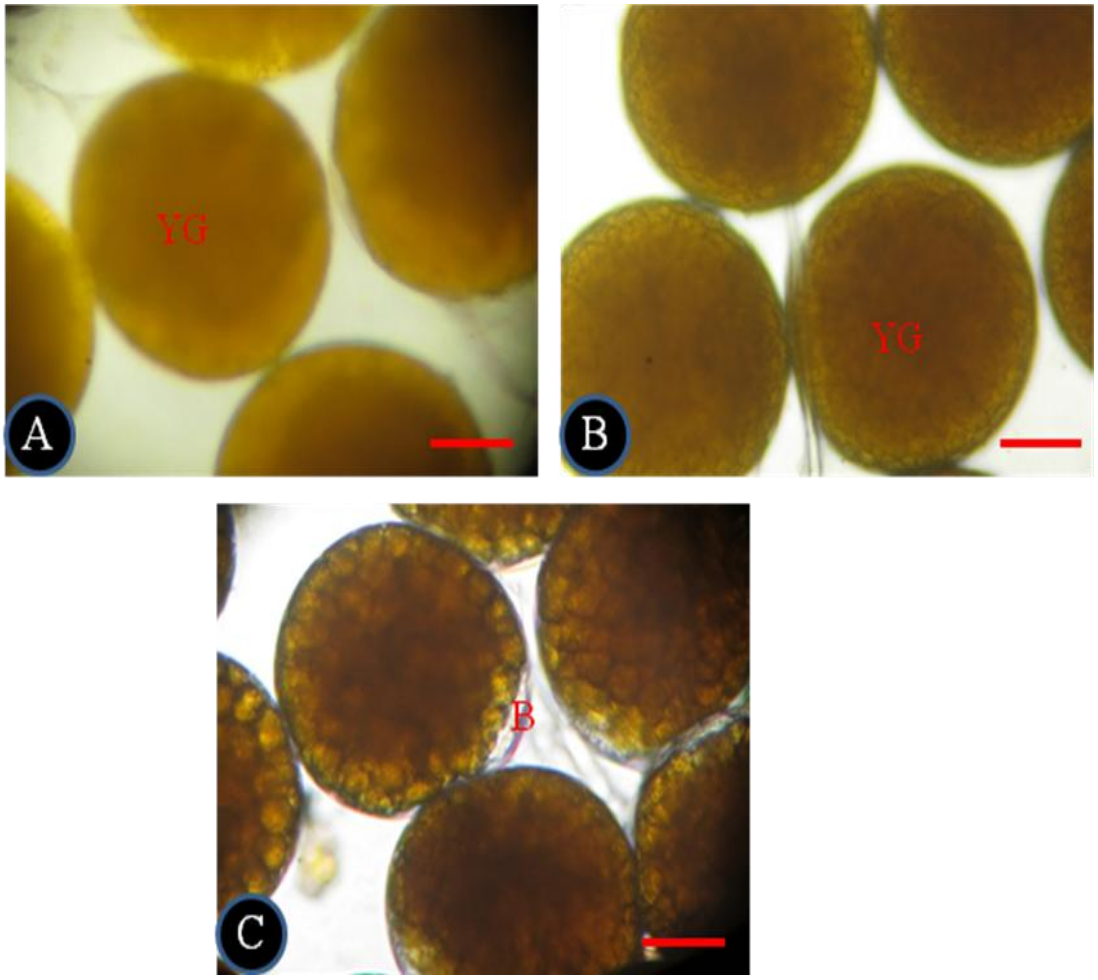

Figure 6:- Photomicrgraphs showing embryonic stages of eggs in the crabs injected with serotonin ($1x10^{-6}$ mol/crab).
(A) Eggs of the control crab spawned on 22nd day of the experiment showing fresh egg with dense yolk granules (YG).
(B) Spawned crab, treated with serotonin on 12th of the experiment showing fresh egg with dense yolk granules (YG).
(C) Eggs of 12th day spawned crabs on 18th day of the experiment showing the advance stage of development.

Histological observations were prominent in the ovary of the crab treated with $1x10^{-6}$ mol/crab of serotonin. On the 6th day of the experiment the ovary showed normal development with vitellogenic oocytes containing distinct ooplasm filled with yolk granules. On 12th day of the experiment, the ovary showed immature oocytes, reduction in the size of the oocyte and absence of yolk material indicating that the crab has spawned having immature ovary. Whereas on 18th day observation of 12th day spawned ovaries indicated that they were in early pre- vitellogenic stage of development (Figure 7). Gradual increase was observed in the ovarian index, gonadosomatic index as well as oocytes diameter in through the maturation stages. In $1x10^{-6}$ mol/crab serotonin injected group, the oocyte diameter drastically increased to 297.7±1.48 µm on 6th day of the experiment indicating that the oocytes are at vitellogenic stage of development. The occurrence of minimum size of oocyte on 12th day of the experiment at higher concentration revealed that the crab had undergone spawning, having proliferating immature oocytes, whereas the control crabs spawned only on the 22nd day of the experiment.

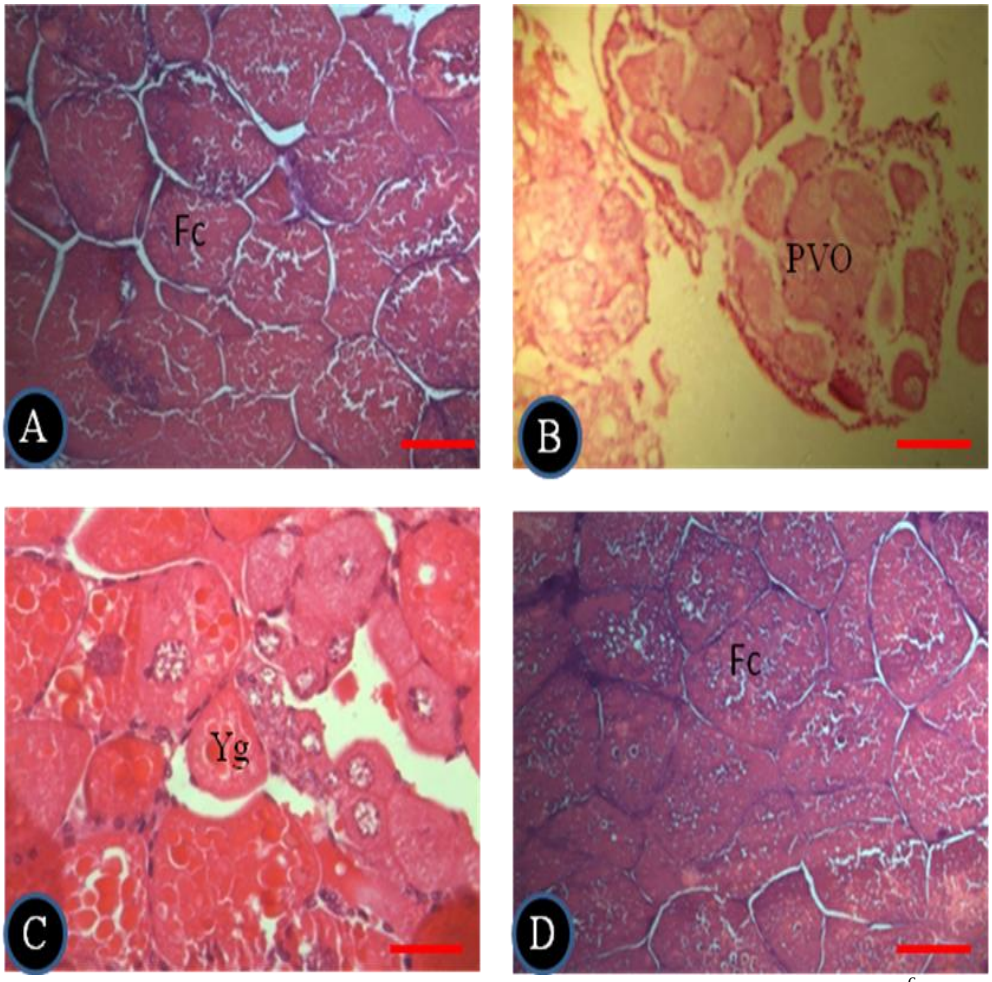

Figure 7:- Photomicrographs of section through the ovary at crabs injected with serotonin ($1x10^{-6}$ mol/crab).
(A) Ovary of *E.emeritus* on 6th day of the experiment showing vitellogenic oocytes with fully occupied large yolk granules along with flattened and attached follicle cells (Fc).
(B) Ovary of *E. emeritus* on 12th day of the experiment showing pre-vitellogenic oocytes (PVO) with germarium and follicle cells.
(C) Ovary of *E. emeritus* on 12th day spawned crabs on 18th day of the experiment showing yolk granules (YG) deposited at the pheriphery with prominent nucleus.
(D) Ovary of *E. emeritus* in control crab on 18th day of the experiment showing vitellogenic oocytes with flattened and attached follicle cells (Fc.)

Discussion:-

The present study documents how endogenous levels of serotonin in the ovary and nervous system of *E.emeritus* changes influence ovarian and moulting cycle. Variations in the concentration of 5-HT in the ovaries appear to be correlated with changes in brain and thoracic ganglia throughout the four stages of the cycle. Specifically, 5-HT levels rose steadily in all ovary, brain and thoracic ganglia from ovarian stage I to ovarian stage IV. In contrast, during moulting cycle the levels of serotonin declined during the premoult cycle. Thus changes in serotonin concentration during moulting were essentially opposite to those during ovarian maturation. This suggests a possible interrelationship between X-organ and Y-organ in crustacean moulting (Skinner 1985). The rate of synthesis and secretion of ecdysone by the Y-organ is negatively regulated by the X-organ sinus gland complex. It was also reported that serotonin levels in the brain and thoracic ganglion reaches maximum at ovarian stage IV in *P. monodon* (Wongprasert *et al.*, 2006) and *M. rosenbergii* (Tinikul *et al.*, 2008).The thoracic ganglia containedhigh levels of 5-HT and DA implies that this part of CNS may play a leading role in controlling the prawn's ovarian maturation, andthat the brain may play a secondary role.

The distribution of serotonin has been extensively mapped using immuno histochemistry on the CNS of various crustaceans, including the crayfish, *Cherax destructor* , *P. leniusculus, P. clarkii*, thelobster, *Homarus americanus* , and *squa tlobster*, *Munida quadrispina* (Antonsen and Paul, 2001). Although immuno histochemistry is a useful method for visualizing and mapping neuronal populations containing biogenic amines, it is not an effective method for estimating and comparing the quantities of these neurotransmitters in various regions of the CNS. HPLC is a better method for this purpose, as pointed out by Hardie and Hirsh (2006). In the crayfish, *P. clarkii*, levels of biogenicamines were quantified in various regions of the CNS, eyestalk, and intestinal nerve using HPLC (Mercier *et al*., 1991; Kulkarni *et al*., 1992). In this species, the total concentration of 5-HT in the brain (0.581±0.36 lg/g) was higher than in the eyestalks (0.299±0.15 lg/g). In the spiny lobster, *P.homarus*, the level of 5-HT was higher in thoracic ganglia than in the brain at ovarian stage IV, and as a result it was suggested that 5-HT could be involved in controlling the ovarian development (Kirubagaran *et al*., 2005). In the lobsters, *Palinurus interruptus, H. americanus,* and the crab, *Cancer irroratus*, major tissues that exhibited high levels of 5-HT were the optic ganglion, cerebral ganglion, circumoesophageal connectives, stomatogastric ganglion and thoracic ganglia (Beltz *et al*., 1984).The findings of 5HT localization in the oocytes and the increasing levels of 5HT as the ovary was maturing, as well as the finding of 5HT1 receptors in the ovary (Ongvarrasopone *et al*., 2006) seems to suggest a direct role of 5HTon *E.emeritus* ovary. In this study, however, two findings concerning 5HT are: (1) exogenous 5HT induced ovarian maturation and spawning; (2) endogenous 5HT was present in the ovary, brain, thoracic ganglia and hemolymph; and how these two findings are connected cannot be answered by the present data. Without exogenous source, 5HT is already in the oocytic cytoplasm. Its presence could mean 5HT is synthesized in the oocyte or 5HT from hemolymph. Since vitellogenin accumulation is an important process of the ovary to become mature, increasing level of 5HT in the oocyte might be involved in this process. HPLC analysis of serotonin showed a gradual increment in the brain and thoracic ganglia implying its major role in differentiation of oocytes from the immature to the vitellogenic stage. The presence of several immuno positive 5-HT Cell bodies seen in the brain and thoracic ganglia support this view. Further studies on the localization of 5-HT receptors in the ovary would be helpful to understand the maturational processes more efficiently.

Results clearly indicated that the injection of 5HT at $1x10^{-6}$ mol/crab induced ovarian maturation and spawning at a faster rate in the mole crab *E.emeritus.* The treatment significantly increased ovarian index and oocyte diameter in the crab. These results are contrary with previous data that showed serotonin induced ovarian maturation is mediated by triggering release of GSH from neuroendocrine centres which would bring about ovarian maturation (Richardson *et al*., 1991; Kulkarni *et al*., 1991; Sarojini*et al*., 1995; Vaca and Alfaro, 2000; Kumlu, 2005; Meeratana*et al*., 2006). Treatment of the crayfish, *P. clarkii*, with 5-HT causes significant increases in ovarian index and oocyte diameter (Kulkarni*et al*., 1992). Kulkarni *et al*., (1991) reported that brain and thoracic ganglia of *P. clarkia* stimulated incorporation of leucine into ovarian protein. In the freshwater prawn, *M. rosenbergii*, treatment with 5-HT also showed a significant increase in the gonadosomatic (GSI) index (Meeratana *et al*., 2006). In *L. stylirostris,*the combined injection of 5-HT and spiperone elicited higher rates of ovarian maturation compared to application of 5-HT alone (Alfaro *et al*., 2004). Furthermore, the ovaries of prawns injected with the culture medium of 5-HT-primed thoracic ganglia exhibited an increase in the number of oocytes developing to vitellogenic and mature stages. These findings suggest that 5-HT and/or other hormonal factors from the thoracic ganglia could be major factors that stimulate the ovarian maturation (Meeratana *et al.*, 2006).The mechanism(s) of 5HT in the induction of ovarian maturation and spawning in *E. emeritus* as well as in other crustaceans. Most investigators suggested that 5HT may act inside the optic lobe to inhibit GIH synthesis/secretion, or in the brain and thoracic ganglion to stimulate GSH synthesis/secretion, thus indirectly influence ovarian maturation (Sarojini *et al.*, 1995; Fingerman, 1997). In serotonin injected crabs, the histological architecture of the ovary indicated accumulation of yolk granules which is a characteristic feature of vitellogenesis.

Conclusion:-

The results clearly indicate that serotonin is involved in the induction of ovarian maturation and spawning in the crab *E. emeritus* as these mole crabs lack prominent eyestalk as in other crustaceans. The rudimentary eyestalk present in this crab probably apt for synthesis of biogenic amines as alternative for eyestalk abalation for ovarian maturation and spawning.

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