Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Research Article

Effect of *β*- estradiol hormone and eye stalk ablation on ovarian maturation in fresh water crab, *Barytelphusa cunicularis*

Kale RS

Department of Zoology, Arts, Science and Commerce College Manmad,
Tal. Nandgaon, Nashik-423 104 (M.S. India)
drrahulskale@yahoo.in

Article Info

Received: 20-02-2017,
Revised: 02-03-2017,
Accepted: 09-03-2017

Keywords:
Barytelphusa cunicularis, *β*-estradiol hormone, eyestalk ablation, ovarian maturation.

Abstract

To investigate the role of *β*- estradiol hormone and eye stalk ablation on the ovarian maturation in the freshwater crab, *Barytelphusa cunicularis* by GSI (Gonad Somatic Index) as a biomarker. Females of *B. cunicularis* were treated with *β*- estradiol hormone and eye stalk ablation for 21 days and compared with normal 1% ethanol injected crab's ovaries.

INTRODUCTION

Crustacean female reproductive physiology is governed by a variety of hormonal and neuronal factors (Subramoniam, 2000). These include the neuropeptide hormones, such as the gonad stimulating hormone (GSH) and the Vitellogenin inhibiting hormone (VIH), which have as agonist-antagonist effect, respectively on vitellogenesis. The next class of factors, which are the terpenoids, such as methyl farnesoate a stimulator of vitellogenesis; ketosteroids, such as ecdysteroids; and sex steroids such as estradiol and progesterone (Zapata *et al.*, 2003; Nagaraju *et al*., 2006). These steroid hormones are biologically active in crustaceans which control vitellogenesis and are apparently necessary for both reproduction and moulting processes; the important high energy demanding physiological process (Adiyodi, 1969; Stevenson *et al*., 1979; Quackenbush, 2001). Vertebrate-type steroids have been observed in the hepatopancreas, ovary, hemolymph of crustaceans and their correlation with the oocyte maturation cycle (Lafont and Mathieu, 2007). Sarojini *et al.* (1990) studied the effect of steroids on the ovaries of the marine crab, *Scylla serrata* that revealed acceleration in ovarian maturation.17 *β* - estradiol and 17*α* - hydroxyprogesterone produced a significant increase in the gonad somatic index of *Procambarus clarkii*, followed by subsequent increase in oocyte diameter (Rodriguez *et al*., 2002b).Reddy *et al.* (2006) demonstrated that 17 *α* - hydroxyprogesterone hormone induced ovarian growth and ovarian VTG synthesis in the freshwater crab, *Oziotelphusa senex senex*. Kale *et al*. (2008) observed increase in ovarian rematuration by 17 *α* - hydroxyprogesterone hormone in freshwater crab**,** *Barytelphusa cunicularis.* Muhd-Farouk *et al.* (2014) studied effect of vertebrate steroid hormones on the ovarian maturation stages of orange mud crab, *Scylla olivacea* and found enhancement in the ovarian maturation.The role of vertebrate-type steroid hormones in ovarian maturation of crustaceans has also been studied by several authors

(Kanazawa and Teshima, 1971; Jeng *et al*., 1978; Nagabhushanam *et al*., 1980; Shih, 1997; Yano, 2000; Warrier *et al.,* 2001; Zapata, 2003; Okumura, 2004; Kirubagaran *et al*., 2005; Muhd-Farouk *et al.,* 2014). In contrast of this, lack of response to vertebrate-like steroid hormones in decapod crustacean's ovarian maturation were reported by Teresa *et al*., 2003; Okumura and Sakiyama, 2004; Kirubagaran *et al*., 2005; Gunamalai *et al*., 2006.

The neuroendocrine X-organ and sinus gland complex in the eyestalk is also a critical factor which control crustacean reproduction and molting by a series of inhibitory neurosecretory and hormonal factors such as vitellogenesis inhibiting hormone (VIH), which effectively targets the ovaries and hepatopancreas (Laufer *et al*., 1998; Shechter *et al.,* 2005).The presence of VIH in the eyestalk has been well recognized; which is expected to remove the source of VIH by eyestalk ablation for the acceleration of ovarian maturation, but less is known about VSH, which is thought to originate in the brain and thoracic ganglia (Charniaux-cotton and Payen, 1988). Khazraeenia & Khazraiinia (2009) studied effects of bilateral eyestalk ablation on gonadal maturity, moulting and biochemical changes in the hemolymph of female crab, *Potamon persicum* and noticed upbeat results. Varalakshmi & Reddy (2010) noticed acceleration in the growth and ovarian maturation due to eyestalk ablations in freshwater prawn, *Macrobrachium lanchesteri* (de Man). Pervaiz *et al.* (2011) observed positive results on gonadal development of *Macrobrachium dayanum* due to unilateral eyestalk ablation. Wu *et al*. (2013) studied the effect of eyestalk ablation in crab, *Eriocheir sinensis* on physiological and biochemical metabolism and found to induce gonadal maturation. Samyappan *et al.* (2015) also found marked decrease in lipid content in the hepatopancreas and a significant increase in ovarian tissue due to unilateral eyestalk ablation in freshwater female crab, *Oziotelphusa senex senex* showing ovarian growth. Similarly, Sarojini *et al.* (2016) studied impact of unilateral eyestalk ablation on protein content in freshwater crab, *Spiralothelphusa hydrodroma* and found significant increase in ovarian maturation.

In this ray of light we investigated the comparative effect of *β*-estradiol hormone and eyestalk ablation on ovarian maturation in freshwater crab, *Barytelphusa cunicularis* using GSI (Gonad Somatic Index).

MATERIALS AND METHODS

Barytelphusa cunicularis used in present investigation were collected from Godavari River near Kaigaon Toka, Newasa. Female crabs were selected and kept in the laboratory for acclimatization for 10 days in plastic troughs. Healthy well-adapted crabs of approximately same weights and size ranging between 35-40g body weights probably of intermoult stage were used in the experiments. Water in the troughs was changed daily and crabs fed by small pieces of earthworm and bivalve flesh on alternate days. Other parameters like temperature, pH, salinity, photoperiod etc. were maintained constant as far as possible. Records were maintained of gonad somatic index and gonad colour for results. The gonad somatic index was calculated according to the formula given by, Farmanfarmaian *et al*.(1958) viz., G.I. = (Wet weight of gonad) / (Wet weight of animal) X 100. The mean values of the indices for 10 female crabs were considered.

Preparation of *β*-estradiol hormone injection:10mg of *β*-estradiol hormone (Sigma Chem., USA) was dissolved in 1ml of 1% ethanol and resulting solution diluted to 10ml by adding glass distill water. The final concentration of the hormone preparation was 1µl = 1µg. From this preparation hormone was injected to crab receiving a dose of 20µl hormone/crab.

Experimental design: Total 40 female crabs were selected and divided into four groups each containing 10 crabs. The first group was served as base control (normal) and crabs were sacrificed on o-day, second group as experimental injected by 1% ethanol, third group was injected by *β*-estradiol hormone with the help of hypodermic syringe having a 27-guaze-needle, hormone injections were administered through 3rd walking legs in the arthrodial membrane into the abdominal musculature female crabs receiving a dose of 20 µl /crab. Fourth group consisted of surgically eyestalk ablated crabs. The whole experiment was conducted for a period of 21 days and the crabs from second, third and fourth were sacrificed on 21st day of the experiment and their ovarian indices were recorded. Weight of crab, wet weight of gonad and colour of gonads were recorded accurately for more relevant results.

RESULTS AND DISCUSSION

Base control ovary: Base control crab ovaries were sacrificed on '0' day of the experiment and ovaries were dissected out. The ovarian index was recorded to be 0.2065 ± 0.0025 (Table -1& Fig. 1). Morphologically the ovaries were small in size and pale yellow in colour.

Experimental control ovary: Experimental control crabs injected by 1% ethanol were sacrificed on 21st day of the experiment. The ovarian index was recorded to be 0.2835 ± 0.0020 (Table -1 & Fig. 1). The ovaries were yellow in colour.

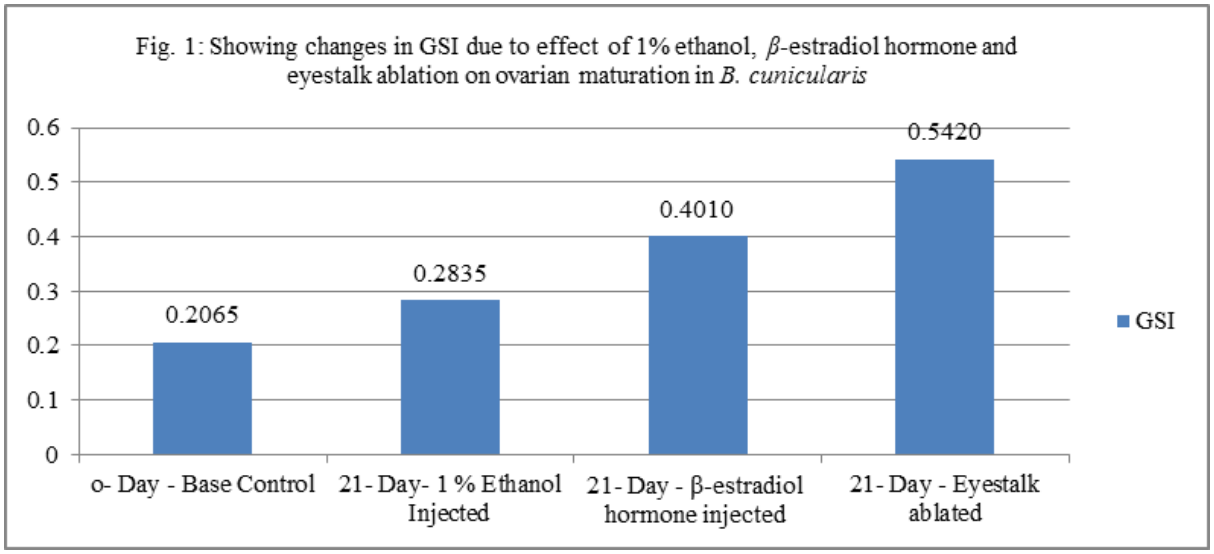
***β*- estradiol hormone injected ovary:** The crabs injected with *β*- estradiol hormone were sacrificed on 21st day of the experiment and the ovaries over dissected out. The ovarian index was recorded to be 0.4010 ± 0.0010 (Table -1 & Fig. 1) and the ovaries were noticed to be dark yellow in colour. The ovarian index was increased over the base control and experimental control.

Eyestalk ablated ovary: Eyestalk ablated crabs were sacrificed on 21st day of the experiment and ovaries were dissected out for the observations. The ovarian index was recorded 0.5420 ± 0.0020 (Table -1 & Fig. 1). The colour of the ovaries observed slightly orange. The ovarian index was found to increase over the ovarian index of base control, experimental control and hormone-injected ovaries.

Table 1: Showing effect of *β*-estradiol hormone and eyestalk ablation on ovarian maturation in *B. cunicularis*.

Animal Category	No. of Crab	Exp. Day	Ovarian index ± S.D.	Colour of the ovary
Group-I Base Control	**10**	**0-Day**	0.2065±0.0025	Pale yellow
Group-II (1% ethanol injected)	10	**21-Day**	0.2835±0.0020	Yellow
Group-III *β*-estradiol hormone injected crabs	10	21-Day	0.4010±0.0010	Dark yellow
Group-IV Eyestalk ablated crabs	10	21-Day	0.5420 ±0.0020	Slightly orange

± S.D. - Mean Standard Deviation



The physiology of reproduction in a species is influenced by the endogenous steroid hormones in many crustaceans which control gametogenesis and the exogenous factors which also have an important role in regulating the gonadal maturation and reproduction in number of invertebrates. Ovary synthesizes estradiol and releases it into hemolymph from where it reaches the hepatopancreas to stimulate vitellogenin synthesis and progesterone for post vitellogenic maturation of the oocytes as in vertebrates.

Synthesis and conversion of cholesterol to steroidal hormones in various tissues of crustaceans has been observed by (Kanazawa and Teshima, 1971; Shih and Liao, 1998; Wilder *et al*., 2002; Kirubagaran *et al*., 2005; Gunamalai *et al*., 2006).

In the present study *β*- estradiol hormone was used for the detection of stimulation of ovarian maturation in freshwater crab, *Barytelphusa cunicularis.* The ovarian development by this hormone was compared with eyestalk ablation simultaneously. Experiment carried for early reproductive phase, ovarian indices (OI) were found to be 0.2065 ± 0.0025, 0.2835 ± 0.0020, 0.4010 ± 0.0010 and 0.5420 ± 0.0020 for base control, experimental control, *β*- estradiol hormone injected and eyestalk ablated crabs respectively (Table-1& Fig. 1). In crustaceans there are several tools to determine ovarian maturation like gonad somatic index, oocyte diameter, change in colour of ovary, concentration of vitellogenin in hemolymph and accumulation of yolk globules in oocytes (Charniaux- Cotton and Payen, 1988; Tsukimura, 2001). In this study gonad somatic index and change in colour of ovary has been considered as a tool to know the ovarian maturation. Observations revealed that ovarian indices (OI) were notably higher in *β*- estradiol hormone injected and eyestalk ablated crabs as compared to the base control and experimental control crabs. Ovarian colour was also found to be pale yellow, yellow, dark yellow and slightly orange in base control, experimental control, *β*- estradiol hormone injected and eyestalk ablated crabs respectively which clearly demonstrates that the *β*- estradiol and eyestalk ablation have significantly enhanced the ovarian index (OI) of freshwater crab, *Barytelphusa cunicularis.* Present results are in good agreement with the results of Sujathamma and Dayakar (2015) in which they studied the effect of estradiol and 17α-hydroxyprogesterone on ovarian development of freshwater paddy field crab, *Oziotelphusa senex senex (Fabricius).* Both estradiol and 17α-hydroxyprogesterone hormones are noticed to be stimulating the gonadal growth and reproduction.The gonad somatic index (GSI) and oocyte diameter (OD)were found to be increased significantly due to the injection of estradiol hormone which was more effective than 17α-hydroxyprogesterone when compared with controls. Muhd- Farouk (2014) also observed positive effects of 17α- hydroxyprogesterone and 17α-hydroxypregnenolone on ovarian morphology of orange mud crab, *Scylla olivacea.*

Similarly, Nagabhushanam *et al.* (1987) has reported rapid ovarian development by the injection steroid hormone in *Parapenaeopsis stylifera*. (Rodriguez *et al.,* 2002b) noticed significant increase in the gonad somatic index and oocyte diameter in *Procambarus clarkii* by the administration of 17 *β*-estradiol and 17α-hydroxyprogesterone. Zapata *et al*. (2003) observed ovarian growth in the crab, *Chasmagnathus granulata* by the induction of 17α-hydroxyprogesterone and Juvenile hormone III. Reddy *et al.* (2006) demonstrated that 17α-hydroxyprogesterone hormone induced ovarian growth and ovarian VTG synthesis in the freshwater crab, *Oziotelphusa senex senex*. Kale *et al*. (2008) observed increase in ovarian rematuration by 17 *α* -hydroxyprogesterone hormone in freshwater crab**,** *Barytelphusa cunicularis.* Similarly, Coccia *et al.*(2010) reported affirmative effect of estradiol & progesterone on the reproduction of freshwater crayfish, *Cherax albidus*. A positive correlation between vitellogenin (VTG) circulating levels and hemolymph levels of progesterone and 17β-estradiol have been reported in crabs (Shih, 1997; Warrier *et al.,* 2001; Zapata *et al.,* 2003).

Eyestalk ablation is expected to remove the source of vitellogenesis inhibiting hormone for the acceleration of ovarian maturation and regulation of molting. It also influence lipid metabolism, protein metabolism, carbohydrate metabolism, hydromineral regulation, gonad inhibition and limb growth (Charniaux-cotton and Payen, 1988; De Kleijn and Van Herp, 1995; Laufer *et al*., 1998; Wilder *et al*., 2002; Longyant *et al*., 2003; Uawisetwathana *et al.,* 2011). The ovarian index recorded in this investigation of eyestalk ablated crabs was 0.5420 ± 0.0020 which indicated active gonad development as compared to all other groups. Similarly, Wu *et al.* (2013) found significant physiological and biochemical metabolism in ovarian maturation by eyestalk ablation in *Eriocheir sinensis.* Pervaiz *et al.* (2014) noticed incresead gonadal development of *Macrobrachium dayanum* due to bilateral eyestalk ablation. Samyappan *et al.* (2015) also found marked decrease in lipid content in the hepatopancreas and a significant increase in ovarian tissue due to unilateral eyestalk ablation in freshwater female crab, *Oziotelphusa senex senex* suggesting active ovarian maturation. Available literature and results of present investigations are in good accord. Although this technique is found to be stimulating ovarian maturation this method is not repeatable and some time may cause high mortality,

deterioration in spawn quality, spawner, larval quality and quantity over time (Browdy, 1992; Aktas and Kumlu, 1999).

In conclusion the result of the present examination clearly demonstrates that *β*- estradiol hormone and eyestalk ablation found to be stimulating the ovarian maturation in freshwater female crab, *B. cunicularis*. New technological advances in such type of hormonal manipulation, further progress in the understanding of crustacean endocrinology is essential for the exploration of aquaculture.

AKNOWLEDGEMENT

The authors are grateful to BCUD, Savitribai Phule Pune University, Pune for funding this research work. Authors are also thankful to Principal, Vice-Principal and all those who have helped directly indirectly for completion of this research work.

REFERENCES

Adiyodi RG, 1969. Protein metabolism in relation to reproduction and moulting in the crab, *Paratelphusa hydrodromous* (Herbst) III: RNA activity and protein yolk biosynthesis during normal vitellogenesis and under conditions of acute inamination. *Indian J. Exp. Biol*., **7**: 13-16.

Aktas M and Kumlu M, 1999. Gonadal maturation and spawning of *Penaeus semisulcatus* (Penaeidae: Decapoda). *Tr. J. of Zoology*, **23**: 61-66.

Browdy CK, 1992. A review of the reproductive biology of penaeid species: Perspectives on controlled shrimp maturation systems for high quality nauplii production. In: J. Wyban. (Ed.), proceeding of the special session on shrimp farming. *World Aquaculture society*, Baton Rouge, LA, USA, pp. 22-51.

Charniaux-Cotton H and Payen G, 1988. Crustacean reproduction. In: Laufer, H, Downer, RGH (eds). *Endocrinology of Selected Invertebrate Types*. *Alan R. Liss, New York*, 279–303.

Coccia E, De Lisa E, Di Cristo C, Di Cosmo A and Paolucci M, 2010. Effects of estradiol and progesterone on the reproduction of the freshwater Crayfish, *Cherax albidus*.*Biol. Bull*. **218**: 36–47.

De Kleijn DVP and Van Herp F, 1995. Molecular biology of neurohormone precursors in the eyestalk of crustacea. *Comp. Biochem. Physiol*., **112B**: 573-579.

Farmanfarmaian A, Giese AC, Boolootian RA and Benett J, 1958. Annual reproductive cycle in four species of West coast starfishes. *J. Exp. Zool*., **138**: 355-367.

Gunamalai V, Kirubagaran R and Subramoniam T, 2006. Vertebrate steroids and the control of female reproduction in two decapod crustaceans, *Emerita asiatica* and *Macrobrachium rosenbergii*. *Current science*, **90** (1):10

Jeng SS, Wan WC and Chang CF, 1978. Existence of an estrogen-like compound in the ovary of the shrimp, *Parapenaeus fissures*.*Gen. Comp. Endocrinol*., **36**: 211-214.

Kale RS, Shejule KB, Kharat PS and Sonawane AK, 2008. Induce ovarian maturation by 17 *α* - hydroxyprogesterone hormone in freshwater crab**,** *Barytelphusa cunicularis. J. Aqua. Biol*., **23** (2) : 109 - 112.

Kanazawa A and Teshima SI, 1971. In vivo conversion of cholesterol to steroid hormones in the spiny lobster, *Panulirus japonica*. *Bull. Jpn. Soc. Sci. Fish*., **37**: 891-898.

Khazraeenia S and Khazraiinia P, 2009. Effects of bilateral eyestalk ablation on gonadal maturity, moulting and biochemical changes in the hemolymph of female crabs (Decapoda, Brachyura, Potamidae). *Int. J. Vet. Res.* **3**, 2*:*143-150.

Kirubagaran R, Peter DM, Dharani G, Vinithkumar NV, Sreeraj G and Muthukamatchi R, 2005. Changes in vertebrate-type steroids and 5-hydroxytryptamine during ovarian recrudescence in the Indian spiny lobster, *Panulirus homarus*. *New Zealand Journal of Marine and Freshwater Research*,**39**: 527–537.

Lafont R and Mathieu M, 2007. Steroids in aquatic invertebrates. *Ecotoxicology*,**16**:109–130.

Laufer H, Bigger, WJ and Ahl JSB, 1998. Stimulation of ovarian maturation in the crayfish, *Procambarus clarkii* by methyl farnesoate. *Gen. Comp. Endocrinol*., **111**: 113-118.

Longyant S, Sithigorngul P, Sithigorngul W, Chaivisuthangkura P, Thammapalerd N and Menasveta P, 2003. The effect of eyestalk extract on vitellogenin levels in the haemolymph of the giant tiger prawn, *Penaeus monodon. Science Asia*, **29**: 371-381.

Muhd-Farouk H, Abol Munafi AB, Jasmini S and Ikhwanuddin M, 2014. Effect of steroid hormones 17 *α* - hydroxyprogesterone and 17 *α* - hydroxypregnenolone on ovary external morphology of orange mud crab, *Scylla olivacea. Asian Journal of Cell Biology:* **9**(1)23-28.

Nagabhushanam R, Joshi PK and Sarojini R, 1987. Effect of exogenous steroids on reproduction in *Parapenaeopsis stylifera*. *Advances in aquatic biology and fisheries*, pp.201-206.

Nagaraju GPC, Reddy PR and Reddy PS, 2006. In vitro methyl farnesoate secretion by mandibular organs isolated from different molt and reproductive stages of the crab, *Oziotelphusa senex senex*. *Fisheries Science*, **72**: 410-414.

Okumura T and Sakiyama K, 2004. Hemolymph levels of vertebrate-type steroid hormones in female kuruma prawn, *Marsupenaeus japonicus* (Crustacea: Decapoda: Penaeidae) during natural reproductive cycle and induced ovarian development by eyestalk ablation. *Fish. Sci.*,**70** (3): 372-380.

Okumura T, 2004. Perspectives on hormonal manipulation of shrimp reproduction. *JARQ*, 38(1): 49-54.

Pervaiz PA, Jhon SM, Sikdar-Bar M, Khan HA and Wani AA, 2011. Studies on the effect of unilateral eyestalk ablation in maturation of gonads of a freshwater prawn, *Macrobrachium dayanum*.*World Journal of Zoology*, **6**: 159-163

Quackenbush LS, 2001. Yolk synthesis in the marine shrimp, *Penaeus vannamei*.*Amer. Zool*., **41**: 458-464.

Reddy PR, Kiranmayi PK, Kumari T and Reddy PS, 2006. 17*β* -Hydroxyprogesterone induced ovarian growth and vitellogenesis in the freshwater rice field crab, *Oziotelphusa senex senex*. *Aquaculture*, **254**:768–775

Rodriguez EM, Medesani DA, Lopez Greco LS and Fingerman M, 2002b. Effects of some steroids and other compounds on ovarian growth of the red swamp crayfish, *Procambarus clarkii*, during early vitellogenesis. *J. Exp. Zool.* **292:** 82–87.

Samyappan K, Saravanan R, Vijayakanth T and Prabakaran D, 2015. Impact of unilateral eyestalk ablation on lipid profiles in freshwater female crab, *Oziotelphusa senex senex*. *Asian Journal of Science and Technology*, **6**: 1474-1478.

Sarojini R, Sambasivarao S and Jayalakshmi K, 1990. Effect of steroids (Estradiol and estrone) on the ovaries of the marine crab, *Scylla serrata*.*Comp. Physiol. Ecol.*,**15**(1): 21-26.

Sarojini N, Bharathi A and Padmaja M, 2016. Impact of unilateral eyestalk ablation on protein content in freshwater crab, *Spiralothelphusa hydrodroma*. G.J.B.B., **5** (1) 2016: 31-34.

Shechter A, Aflalo ED, Davis C and Sagi A, 2005. Expression of the reproductive female-specific vitellogenin gene in endocrinologically induced male and intersex *Cherax quadricarinatus* crayfish. *Biol. Reprod.***73:** 72–79.

Shih JT and Liao CF, 1998. Conversion of cholesterol to sex steroid life substances by tissues of *Mictyris brevidactylus* in vitro. *Zool. Studies*, **37**(2): 102-110.

Shih JT, 1997. Sex steroid-like substances in the ovaries, hepatopancreas and body fluid of female, *Mictyris brevidactylus*.*Zool. Stud*., **36**: 136-145.

Stevenson JR, Armstrong PW, Cheng ES and O'Conner JD, 1979. Ecdysone titers during molt cycle of the crayfish, *Orconectes samborni*. *Gen. Comp. Endocrinol*., **39**: 20-25.

Subramoniam T, 2000. Crustacean ecdysteroids in reproduction and embryogenesis.*Comp. Biochem. Physiol.*, **125**: 135–156.

SujathammaA and Dayakar Y, 2015. Effect of estradiol and 17α-hydroxyprogesterone on ovarian development of freshwater paddy field crab, *Oziotelphusa senex senex (Fabricius)European Journal of Experimental Biology*, **5**(9):27-30.

Teresa S, Monteiro PRR, Reis- Henriques MA and Coimbra J, 2003. In viro metabolism of steroid hormones by ovary and hepatopancreas of the crustacean penaeid shrimp, *Marsupenaeus japonicus*. *Sci. Mar*., **67**(3): 299-306.

Tsukimura B, 2001. Crustacean vitellogenesis: Its role in oocyte development. *Integrative and comparative biology*, **41**(3): 465-476.

Varalakshmi KN and Reddy R, 2010. Effects of eyestalk ablation on growth and ovarian maturation of the freshwater prawns, *Macrobrachium lanchesteri*. *Turk.J. Fish. & Aquatic Sci.*, **10**: 403-410.

Warrier SR, Tirumalai R and Subramoniam T, 2001. Occurrence of vertebrate steroids, estradiol 17β and progesterone in the reproducing females of the mud crab, *Scylla serrata*.*Comparative Biochemistry and Physiology Part A*, **130**: 283-294.

Wilder MN, Subramoniam T and Aida K, 2002. Yolk proteins of crustacea in reproductive biology of invertebrates, Vol. XII A, progress in vitellogenesis, eds. Raikhel, A.S. and Sappington, T.W., *Science Publishers, Enfield, NH, USA*, 131-174.

Wu J, Kang X, Mu S and Tian Z, 2013. Effect of eyestalk ablation in *Eriocheir sinensis* on physiological and biochemical metabolism. *Agricultural Sciences*, **4**: 25-29

Zapata V, Lopez Greco LS, Medesani D, Rodriguez EM, 2003. Ovarian growth in the crab, *Chasmagnathus granulata* induced by hormones and neuroregulators throughout the year. In vivo and in vitro studies. *Aquaculture*, **224** (1-4): 339.

How to Cite this Article:

Kale RS, 2017. Effect of *β*- estradiol hormone and eye stalk ablation on ovarian maturation in fresh water crab, *Barytelphusa cunicularis***,** *Bioscience Discovery*, **8**(2):140-145.