Chapter

Endocrinology of Reproduction in Crustaceans

Ramachandra Reddy Pamuru

Abstract

Crustaceans have become the most popular proteinacious foods to meet the food demand of ever growing human population in the World. But, the culturing of crustacean species has many problems, including limited availability of quality seed. Out of all conventional methods practiced to increase seed of good quality and quantity, manipulation of the endocrine system of brood stock is found to be one of the best methods. Regulation of crustacean reproduction is under the control of many hormones and factors. The eyestalk hormones, namely gonad/vitellogenin-inhibiting hormone (VIH) and mandibular organ inhibiting hormone (MOIH) show negative effects on maturation, whereas the other eyestalk hormones show mixed effects on maturation. The non-eyestalk hormones namely gonad stimulating hormone (GSH), methyl farnesoate (MF) and ecdysteroids are ovarian maturation inducers in crustaceans. The pros and cons of endocrine manipulation in crustaceans are discussed in this chapter.

Keywords: crustaceans, endocrine hormones, regulation of reproduction

1. Introduction

Crustaceans, a major group of animals which serve as food for humans and animals come under phylum Arthropoda. There are about 45,000 crustacean species distributed throughout the World. The crab, marine shrimps, crayfishes, lobsters and freshwater prawns are edible and they belong to crustacea. This group of animals is free-living and the habitat of most of them is freshwater or marine, where few of them are semi terrestrial. Edible crustaceans have lots of importance because of its role in acting as rich protein food, sustainability in culturing and trading. They possess significant economic value in Nations of developing which undoubtedly provide food security in both the production and transportation to within and to other Nations. The acceptance of crustacean food in World has also increased due to its softness, flavor, easy digestion and numerous health benefits due to the presence of protein, minerals and vitamins which are known to prevent a range of diseases. The seafood, especially crustacean proteinacious food is famous in many countries and has its own demand. Most of crustacean food is produced in China and other Asian countries. The fresh, the frozen and the snacks are different forms of crustacean food available throughout the World, supplied from the food industry. Besides food industry, other major industries that use crustaceans are pharmaceutical and cosmetic industries. The crustacean pigments and natural compounds of shell are holding high value in the cosmetic industry. Globally, the edible crustacean production is about 10 million tons per year through fisheries

and aquaculture farming. Due to its significance, the crustacean World market has reached to US $ 147 in the year 2017 and is anticipated to grow at a reasonable rate in the years to follow. Though the crustacean production is increasing steadily in the global market, it is not at a great rate to meet the demands of the ever growing human population on the globe. Due to several reasons, crustacean aquaculture industry is facing numerous troubles in obtaining expected productivity. One of such reasons is limited availability of good quality seed, a potential startup for high yield of protein.

Crustaceans are mostly unisexual and can be easily distinguished into the male and female by morphology. Male and female reproductive organs are developed to produce and reproduce young ones in the brood sack of the female. The usage of quality seed is a key to get increased productivity of the quality protein in aquaculture. Naturally captured crustacean seed is found the most promising one to get more protein through culture. But, the availability of seed in the nature is limited and tedious to obtain. To overcome this, a classical eyestalk ablation method is introduced to induce spawning in the brood stock [1]. Removal of eyestalk eliminates the synthesis and release of eyestalk gonad inhibitory principle from neurohemal X-organ sinus gland complex, which promotes maturation [2]. Because of cautery of eyestalk, this method causes the highest mortality in the brood stock and loss of hemolymph very often leads to production of inferior quality seed. In search of an alternative, the researchers are working in multiple directions to get quality seed. Among all, the endocrine manipulation of crustacean hormones is one of the best methods for quality seed production.

2. Maturation and hormones

The proper maturation of gonads in crustacea provides the quality seed production. The maturation in crustaceans happens mainly due to activation of biosynthesis of female reproductive egg protein called vitellogenin. Vitellogenin is a lipoprotein used for the nourishment (energy requirement) of developing embryo. The site of vitellogenesis in crustaceans is various tissues, including the ovary. The major sites for vitellogenesis are ovary, hepatopancreas and epidermis. Most of the crabs are holding hepatopancreas as vitellogenin synthesizing site whereas in other crustaceans, it is epidermis or hepatopancreas along with the ovary. In natural maturation vitellogenin is produced as pre-vitellogenin and it undergoes cleavage to produce the mature vitellogenin/vitellin molecules of bearing different molecular sizes. The size and number of vitellins varies from one species to other. These vitellins via hemolymph are transported to ovary and they deposit in the developing oocytes. The accumulation of vitellin in the developing ovary is continued until the ovary reaches to fully matured stage [3]. The female reproductive protein vitellin/vitellogenin is a good indicator of reproductive activity in crustaceans and is frequently used to determine the regulatory functions of endocrine hormones [4].

There are many factors that regulate the maturation of ovary in crustacean brood stock. The eyestalk neural tissue in crustacea secrets a number of neuroendocrine hormones and is analogous to vertebrate pituitary-hypothalamus. The vitellogenesis/gonad-inhibiting hormone (VIH/GIH), mandibular organ-inhibiting hormone (MOIH), crustacean hyperglycemic hormone (CHH), molt-inhibiting hormone (MIH), biogenic amines and opioids are the major secretory products of eyestalk. These eyestalk products play a crucial role in regulating the maturation in crustaceans. The products of Y-organs and mandibular organs called ecdysteroids and methyl farnesoate (MF) respectively and gonad stimulating hormone (GSH) are non-eyestalk hormones involved in the regulation of ovarian maturation [5, 6].

Besides these, the ovarian maturation is also regulated by the external factors such as temperature, salinity, pH, food availability in the pond and heavy metals present in the water through endocrine regulation. However, the regulation of reproduction is a complex process in crustacea and its understanding requires the detailed description of action of internal and external factors.

3. Factors involved in the regulation of reproduction

Endocrine hormones such as eyestalk neuropeptides, GSH, opioids, biogenic amines, ecdysteroids and MF are endogenous factors that regulate reproduction in crustaceans [6]. The internal factors are of two types based on their action in the regulation of reproduction. Hormones which promote reproduction come under "positive regulators", whereas those that suppress the reproduction are called "negative regulators". The positive regulators are MF, ecdysteroids and GSH, and the negative regulators are VIH/GIH and MOIH. Some factors like CHH, MIH, biogenic amines and opioids are found to be having both the actions. The Y-organs, mandibular organs, brain, thoracic ganglia and ovary are responsible for the release of a variety of reproductive regulatory hormones and factors beside the major endocrine centers of the eyestalks. The role of these hormones and factors in the regulation of crustacean maturation, especially in the ovarian growth is discussed here.

3.1 Factors of X-organ sinus gland complex

The eyestalk neural tissue is divided into medulla extern interna and terminalis. Neural clusters located in these three parts of neural tissue are responsible for the secretion of all eyestalk peptide hormones and other factors. These secretions are initially stored in a neurohemal organ sinus gland located in the eyestalk and it finally secretes into the stream of hemolymph to show its action at the target tissue. The eyestalk neural tissue, cluster of neuronal cells and sinus gland are collectively called "neurohemal X-organ sinus gland complex". The hormones of eyestalk such as CHH, VIH, MIH, MOIH and other peptides are collectively called as CHH-family peptides. These peptide hormones are classified as Type I and II based on the presence or absence of Glycine residue at 12th position of their mature peptides [7]. The secretions of eyestalk are as follows and are discussed in Section 3.1.1.

3.1.1 Vitellogenesis/gonad-inhibiting hormone

The prime role of eyestalk GIH/VIH in crustacea is inhibition of ovarian/gonad maturation. Panouse [1] first identified the presence of GIH in *Palaemon* (*Leander*) *serratus (P. serratus*). He founded the speedy rise in size of ovary and precocious egg deposition in the ovaries by the removal of eyestalk gonad inhibitory factor (GIH) from immature *P. serratus*. Later the same was reported in other crustacean species (See review [3, 8]). Since the lack of exact bioassay, identification and characterization of VIH neuropeptides the literature available is limited. However, VIH was identified and characterized in a variety of crustaceans. The ovarian fragments of *Procambarus bouvieri* were incubated *in vitro* with isolated VIH from the same species and inhibition of vitellogenin m-RNA levels were found in ovarian fragments [9]. Soyez et al. [10] first reported the complete structure of VIH in American lobster, *Homarus americanus* (*H. americanus*), and later Gréve et al. [11] in the terrestrial isopod *Armadillidium vulgare*.

In the recent past, recombinant proteins were identified as potential molecules for altering the biological functions. The recombinant GIH/VIH protein was synthesized for a variety of crustaceans. The r-GIH was synthesized for lobster *Nephrops norvegicus*

and using this identified the GIH synthesizing cells in eyestalk neural tissue [12]. The VIH activity of secondary structure of *H. americanus* recombinant VIH (rHoa-VIH) which was assured by bacterial expression system was incubated with *Marsupenaeus japonicus* (*M. japonicus*) ovarian fragments *in vitro* [13]. Ohira et al. [13] was tested the reduced vitellogenin m-RNA levels in the ovary of *M. japonicus* incubated with c-terminus amidated rHoa-VIH. Advancement of technology reveals the gene function and is not restricted to characterize the crustacean genes. The gene –silencing using RNAi methodology is in use to silence the inhibitory CHH-family peptides, to activate reproduction and growth in crustaceans. VIH gene silencing was demonstrated in *Penaeus monodon* (*P. monodon*). The *in vivo* functional gene silencing of *P. monodon* VIH (Pem-VIH) with increased vitellogenin m-RNA levels was reported by administration of Pem-VIH ds m-RNA [14].

Though the mechanism of VIH/GIH induced inhibition of reproduction is not clear, from the available literature, it is identified that VIH shows its action in two intracellular signaling pathways in the ovary such as a) cyclic nucleotide signaling and b) calcium ion and protein kinase C signaling [15]. In one way, VIH induces its inhibitory action by activating the cyclic nucleotides adenylyl cyclase or guanylyl cyclase or both (**Figure 1**). Binding of VIH to G protein receptor activates the G protein which activates adenylyl cyclase that produce cAMP from ATP. On the other hand VIH may also stimulate the guanylyl cyclase through its receptors (GCR)

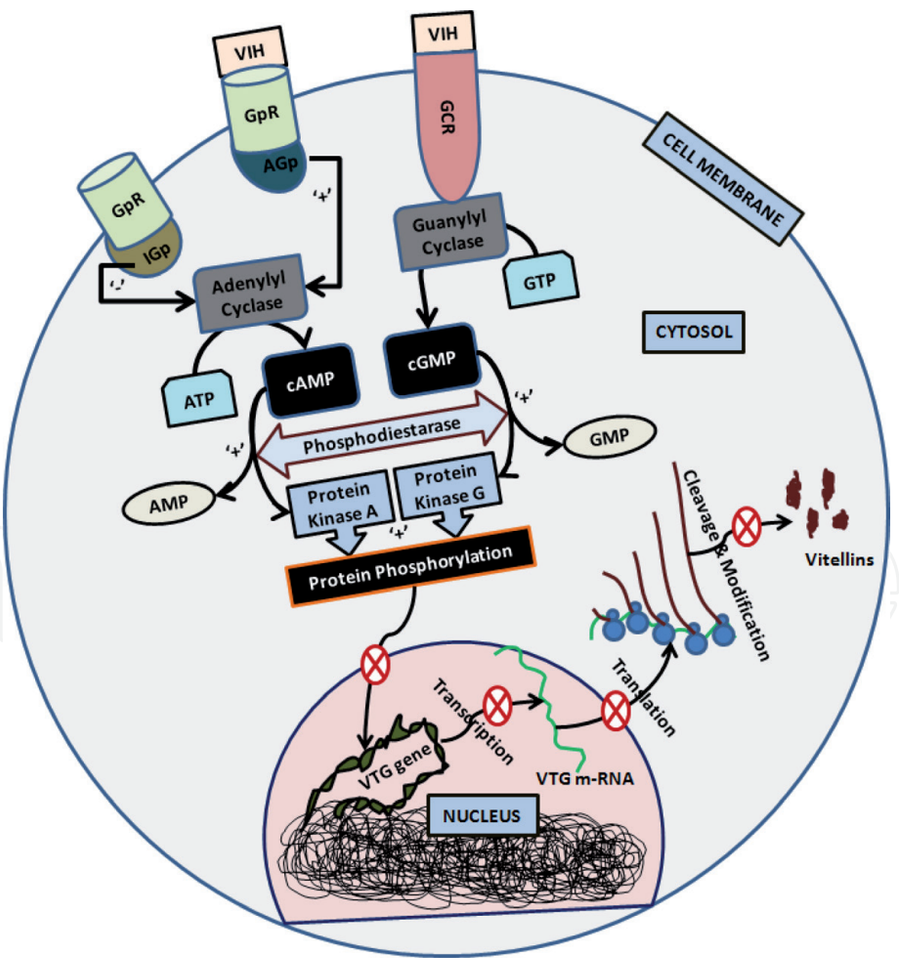

Figure 1.
Schematic diagram showing the mechanistic inhibitory action of VIH on vitellogenin synthesis through secondary messenger cAMP and cGMP signaling pathway. VIH: Vitellogenesis-inhibiting hormone; GpR: G protein receptor; GCR: Guanylyl cyclase activator; SGp: Stimulated (Active) G protein; IGp: Inactive G protein; ATP: Adenosine triphosphate; GTP: Guanosine triphosphate; cAMP: Cyclic adenosine monophosphate; cGMP: Cyclic guanosine monophosphate; AMP: Adenosine monophosphate; GMP: Guanosine monophosphate; VTG: Vitellogenin; '+' and '-' denotes activation and inhibition respectively; ⊗: supression of the reaction.

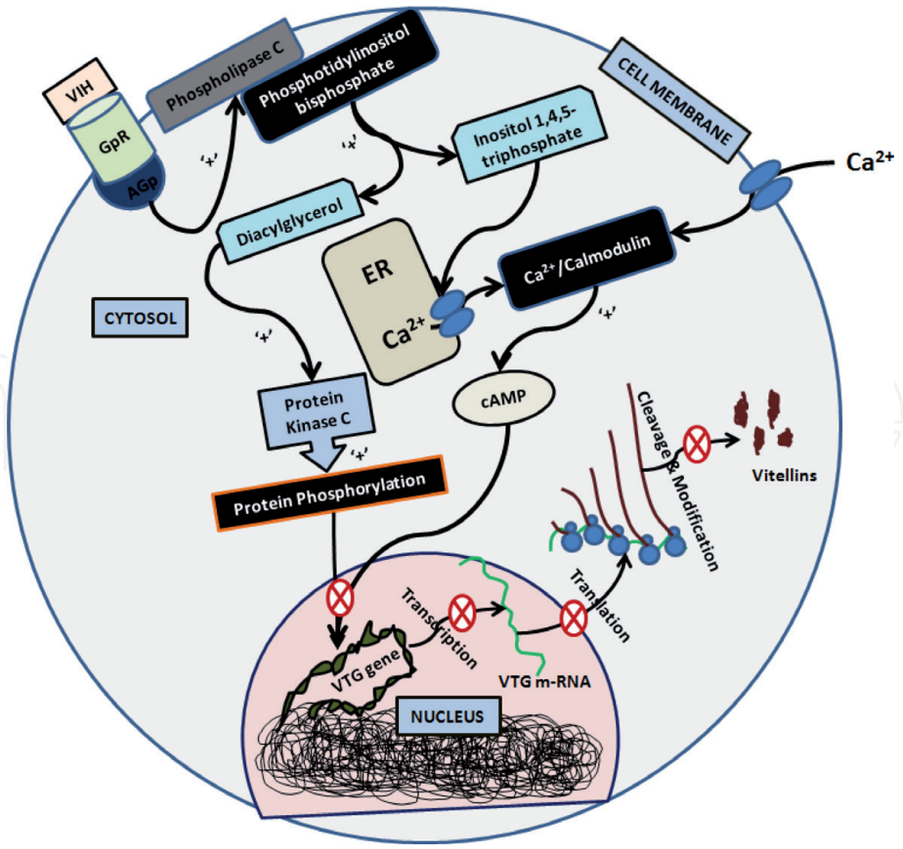

Figure 2.
Schematic diagram showing the mechanistic inhibitory action of VIH on vitellogenin synthesis through Ca^{2+} and protein kinase C signaling pathway. VIH: Vitellogenesis-inhibiting hormone; GpR: G protein receptor; AGp: Active G protein; cAMP: Cyclic adenosine monophosphate; ER: Endoplasmic reticulum; Ca^{2+}: Calcium ion; VTG: Vitellogenin; '+' and '-' denotes activation and inhibition respectively; ⊗: the negetive regulation.

which leads to conversion of GTP into cGMP. Both the cyclic nucleotides by the action of phosphodiesterase activate protein kinase A and G respectively for cAMP and cGMP. Increased protein phosphorylation induced by protein kinase A or G or both suppresses the gene expression of vitellogenin in the nucleus, thereby reduces the vitellogenin protein levels in the ovarian follicles [16].

Figure 2 explains the second mechanistic action of VIH inhibitory pathway through activation of protein kinase C by diacylglycerol, inosital and calcium ions (calmodulin). The high titer of circulatory VIH, binds to G protein receptors (GpR) in the developing ovarian follicles. Upon binding of GpR with VIH, activates the G protein, which initiates the conversion of phosphotidyl bisphosphate into diacylglycerol and inositol 1, 4, 5 – triphosphate upon activation of phoshpolipase C. The diacylglycerol activates the protein phosphorylation through protein kinase C. On the other hand inositol 1, 4, 5 – triphosphate induces the increase of intracellular free calcium (Ca^{2+}) from the endoplasmic reticulum in addition to the calcium available through calcium transported from the extracellular space to cytosol. Calmodulin bound or free Ca^{2+} activates the synthesis of cAMP, which directly inhibits the gene expression of vitellogenin gene in the nucleus along with high levels of protein phosphorylation [16].

3.1.2 Mandibular organ-inhibiting hormone

The MOIH, an eyestalk neuropeptide known negative regulator of reproduction is identified in many crustaceans [17]. More specifically, mandibular organs (MOs) involved in maturation of crustaceans through its secretory product methyl

farnesoate are under negative control of MOIH. The elevated MF levels of 54.1 and 106.9 ng/gland in MO are reported respectively, after unilateral and bilateral eyestalk ablation respectively in *H. americanus* [18]. In the same study the suppressed MF levels are recorded in MOs after injection of sinus gland extracts to bilateral eyestalk ablated *H. americanus*. Chaves [19] demonstrated the negative effect of MOIH on MF synthesis from MO in crayfish *Procambarus clarkii (P. clarkii*) by measuring the activity of farnesoic acid O-methyl transferase an enzyme involved in MF synthesis and also found 20–100 fold rise in hemolymph MF levels during 8th–12th day after eyestalk ablation. An increase in MO size is also recorded in *H. americanus* during ovarian maturation [20]. Premature vitellogenesis is observed in juvenile *Libinia emarginata* after MO implantation [21]. During the ovarian maturation process an increase in the size of MO along with an increase in ovarian index and oocyte diameter is reported in the natural reproductive cycle of crab *Oziothelphusa senex senex* (*O. senex senex*) [22]. Direct studies showing the MOIH action against the release of MF from MO are demonstrated in many studies. The above mentioned studies are evidenced in the stimulatory role of MO and MF in crustacean reproduction. However, the role of MOIH in the regulation of reproduction by inhibiting the synthesis and secretion of MF from MO is clear from the limited literature, but the mechanistic action of MOIH is not clear and further studies are needed to establish it.

3.1.3 Crustacean hyperglycemic hormone

The major CHH-family peptide synthesized and released from the eyestalk neural tissue is a crustacean hyperglycemic hormone (CHH). The onset of vitellogenesis is regulated by CHH besides its principal activity in carbohydrate metabolism [23]. Studies on CHH regulated reproduction are limited. At first, the role of CHH-A and CHH-B on reproduction is demonstrated in *H. americanus* [9, 23]. De Kleijn et al. [24] has found that the onset of vitellogenin synthesis is triggered by Hoa-CHH-A and oocyte development in late stages of maturation is by Hoa-CHH-B. Similarly, in *Metapenaeus ensis* (*M. ensis*) isolated two CHH-family peptides involved in ovarian development, especially in early stage (CHH-A) and, middle and later stage (CHH-B) [25]. In contrast, *in vitro* inhibition of vitellogenin and its expression in *Penaeus semisulcatus* ovarian fragments by *Penaeus japonicus* CHH are reported [26].

The isoforms of CHH may be received by different types of receptors responsible for a variety of physiological functions including reproduction. It is evidenced through occurrence of CHH receptors on the membrane of Y-organs the principle glands synthesize ecdysteroids in *Carcinus maenas* (*C. maenas*) and *Gecarcinus lateralis* [27, 28]. The recombinant CHH reduced 51% of ecdysteroid levels from Y-organs of crab *C. maenas* [27]. Since CHH isoforms are showing similarity with other CHH-family peptides and its pleiotropic nature, they might display functions of important depends on the tissue they reach. Due to the CHH importance in crustacean reproduction and endocrine manipulation, further mechanistic actions are necessary to elucidate.

3.1.4 Molt-inhibiting hormone

Molt inhibition is the fundamental role of eyestalk MIH. Through inhibiting the synthesis and secretion of molting hormone (ecdysteroids) from Y-organs (molting glands) MIH regulates molting. Due to its pleiotropic nature, MIH is reported for its involvement in the onset of vitellogenesis in crustaceans. The MIH mediated reproduction is not very clear and reports are showing both positive and negative effects on maturation. The concealed onset of vitellogenesis by MIH is identified in shrimp

M. ensis [29]. In the same species also reported recombinant Me-MIH-B induced vitellogenin expression in ovary and hepatopancreas in a dose dependent manner *in vitro*. Recent studies identified and characterized the hepatopancreatic MIH binding proteins in maturing female crab *Callinectes sapidus* (*C. sapidus*) [30]. Zmora et al. [30] Identified high cAMP (a secondary messenger) levels in hepatopancreatic tissue incubated with *C. sapidus* MIH and predicted the mechanistic action of MIH in the onset of reproduction. Zmora et al. [30] reported the dynamics of MIH during different stages of ovarian maturation in *C. sapidus*. They reported the antagonistic action of MIH on growth (inhibition) and reproduction (induction) by comparing the MIH titers at premature (low) and mature stages (four fold higher) of ovary. Moreover, the gonad stimulatory effect of MIH-B isolated from *M. ensis* is tested and its pattern of expression in eyestalk neural tissue is correlated with ovarian maturation cycle [31]. Tiu and Chan [29] found elevated hemolymph and ovarian vitellogenin protein levels along with hepatopancreatic and ovarian vitellogenin m-RNA levels upon administration with Me-MIH-B *in vivo*. The ds-RNA of Me-MIH-B injection concealed the vitellogenin m-RNA levels in hepatopancreas and ovary along with lowered vitellogenin protein levels in hemolymph and ovary in *M. ensis* [29]. However, the growth and reproduction are antagonistic in crabs and sequential in prawns and shrimps. Furthermore, studies are immensely needed on MIH for manipulation of crustacean reproduction in order to uphold sustainable aquaculture.

3.1.5 Opioid peptides

Small peptides containing five amino acid residues released from eyestalks are called opioids. Their presence is well defined in vertebrates [32] and is also reported in a few invertebrates including crustaceans using modern techniques like radioimmunoassay (RIA), high performance liquid chromatography (HPLC) and immunohistochemistry (IHC). In crustaceans, opioids are identified in the eyestalk neural tissue and are released from sinus glands into the hemolymph. The endogenous opioids found in crustaceans are named as leucine-enkephalin and methionine-enkephalin. Limited reports are available for opioids induced reproduction in crustaceans [33]. The *in vivo* experiment was conducted to investigate the role of endogenous opioids on reproduction in crab *Uca pugilator*. Whereas, observed dose dependent inhibition of ovarian development by injecting methionine-enkephalin. In *P. clarkii*, it is proved that incubation of thoracic ganglion alone with ovarian fragments *in vitro* does not cause any change in the ovary, whereas incubation along with methionine-enkephalin reduced the ovarian maturation in a dose dependent manner [34]. Out of two enkephalins identified, only leucin-enkehpalin is found to be involved in the regulation of maturation in prawn *Penaeus indicus* (*P. indicus*) [35]. The induction of maturation by leucine-enkephalin is tested in *P. indicus* and *O. senex senex* [34, 35]. However, the role of opioids in crustaceans is controversial and is required to conduct more studies to determine its specific mechanistic action and use in endocrine manipulation of reproduction.

3.1.6 Biogenic amines

In decapods, biogenic amines are well reported as regulators of reproduction. Initially these amines act as neuroregulators and later as neurohormones. They play an important role in synthesis and releasing of endocrine hormones involved in the regulation of reproduction [36]. The hormones like CHH-family peptides (GIH, CHH, MIH, RPDH and BPDH) and GSH are influenced by biogenic amines [37]. The identified biogenic amines in crustaceans are serotonin (5-hydroxy tryptamine; 5-HT) and dopamine, and they influence the ovarian maturation (**Table 1**).

S.no.	Crustacean species	Biogenic amine and its function	References
1	*Oziothelphusa senex senex*	Serotonin—stimulates ovarian index and oocyte size	Sainath et al. [38]
2	*Ucca pugilator*	5-HT—gonadal development and maturation; stimulates release of GSH Dopamine—stimulates the testicular maturation	Sarojini et al. [39]; Richardson et al. [37]
3	*Fenneropenaeus merguiensis*	5-HT—female gonadal maturation	Makkapan et al. [40]
4	*Litopenaeus vannamei*	5-HT—female gonadal maturation	Vaca and Alfaro [41]
5	*Procambarus clarkii*	Dopamine—inhibits ovarian maturation 5-HT—stimulates ovarian index and oocyte size; stimulates release of GSH	Sarojini et al. [42]; Luschen et al. [43]
6	*Paratelphusa hydrodromus*	5-HT—stimulates ovarian index in eyestalk ablated animals	Sarojini et al. [39]
7	*Penaeus semisulcatus*	5-HT—stimulates ovarian maturation and spawning	Kumlu [44]
8	*Cherax quadricarinatus*	5-HT—stimulates ovarian maturation in presence of thoracic ganglia	Cahansky et al. [45]
9.	*Scylla serrata*	5-HT—Stimulates reproduction by inducing ecdysteroid and MF secretions.	Girish et al. [46]

Table 1.
The biogenic amines identified and its effect found in different crustacean species. 5-HT: 5-Hydroxytryptamine.

Serotonin induces the release of GSH from thoracic ganglion, whereas dopamine promotes release of eyestalk GIH and reduces thoracic ganglion GSH [47]. The GSH triggered ovarian development by 5-HT in both *in vitro* and *in vivo* is reported in *Penaeus vannamei* [41]. Recently Sainath et al. [38] well demonstrated the reproductive function of serotonin in freshwater rice field crab *O. senex senex*. Since its importance in maturation, biogenic amines are potential candidate molecules for endocrine manipulation of reproduction in crustaceans.

3.2 Gonad stimulating hormone

The gonad stimulating hormone (GSH) is found to be synthesized and released from the crustacean brain and thoracic ganglia. Many *in vivo* and *in vitro* studies have explained the ovarian stimulating effects of brain and thoracic ganglia [48] without isolation and characterization of GSH principle. At first, induced ovarian growth is reported by implanting the brain in *Paratelphusa hydrodromous* [49], later in *Macrobrachium kistnesis, Parapenaeopsis hardwickii* [50, 51]. The stimulatory role of implanted thoracic ganglia is reported for the first time in *Potamon dehaani* [52] and later in *Cherax quadricarinatus* [53]. The combined effect of brain and thoracic ganglia on ovarian development is demonstrated in *P. clarkii* [42]. The role of copper and cadmium on ovarian development through GSH is tested in *Chasmagnathus granulata,* where they incubated ovarian explants and thoracic ganglia along with heavy metals and found inhibition of ovarian maturation [54]. Though the GSH has lots of importance in regulating crustacean reproduction, it could not be used in endocrine manipulation until its structural characterization done in crustaceans.

3.3 Ecdysteroids

In crustaceans, ecdysteroids are polyhydroxylated ketosteoroids synthesized and released from the molting gland called Y-organ with a primary role in regulation of growth. Y-organs are homologous to prothoracic glands of insects and are non-neural paired endocrine glands. Besides molting, ecdysteroids regulate crustacean reproduction and embroyogenesis [55]. The mechanistic action of crustecdysone is explained after the discovery of its receptors called ecdysone receptors (EcR) [56]. EcR binds with retinoid X receptor (RXR) to form heterodimer and stimulates DNA regulatory elements of ecdysteroid signaling pathway [56, 57]. Various physiological functions of ecdysteroids are reported with the EcR and its domain activation, which decides to exert the physiological function by activation or suppression of gene function [58, 59].

At first Charniaux-Cotton and Touir [60] has identified the role of ecdysteroids in the maturation of oocyte of *Lysmata seticaudata* by Y-organectomy, later in *Orchestia gammarella* [32]. The levels of ecdysteroids at the initial stages of spermatogonial mitoses and ovarian maturation are found along with expression of vitellogenin gene in crustaceans [61]. The ecdysteroids and its role is well established in crustaceans. Furthermore, the endocrine manipulation can be executed by supplementing the ecdysteroids through feed to the brood stock to induce reproduction. As a limitation, this method takes longer time to induce ovarian maturation and the animal may enter molt instead of the reproduction.

3.4 Methyl farnesoate

The terpenoid hormone identified through reverse endocrinology is called the methyl farneosate (MF) an analog of insect juvenile hormone III confined only to decapoda and is a secretory product of mandibular organ [62]. MF synthesis and secretion from MO is repressed by eyestalk neuropeptide MOIH [22]. The hemolymph titers of MF are founded high during ovarian maturation and it has a direct relation to promoting maturation in crustaceans [63]. The mechanism of MF induced maturation is found through ecdysteroid synthesis in Y-organs. The mandibular organ disruption due to exposure of environmental pollutants decreases the hemolymph levels of 20-hydroxyecdysone, which is a stimulator product of MF [64]. MF acts as a ligand for retinoid-X-receptor (RXR) and synergize with ecdysteroids to stimulate RXR-EcR heterodimer complex for expression of combined regulatory genes of these two hormones [56, 57]. In *C. maenas* administration of MF elevated the levels of RXR m-RNA in both *in vitro* and *in vivo* [65]. MF is the best suited hormone for endocrine manipulation of reproduction in crustaceans.

4. Conclusion

Seed production is purely done in hatcheries and is supplied to the farmers. Though the reproductive manipulation is not directly influencing the high amount of quality protein production, it is an initial key factor to produce quality feed. The quality seed usage in culture pond is one of the key for high amount of quality protein production. Healthy seed is always superior to maintain and is not easily affected by bacterial, viral and fungal pathogens. More promisingly, superior seed get adjusted easily to new environment and is not affected by fluctuations in the pond management. Moreover, decreased mortality rate in seed, increases the population which reflects the amount of protein/yield at the harvesting time. It is most important that, crustacean hatchery industry should improve the seed quality by following the new methodologies developed

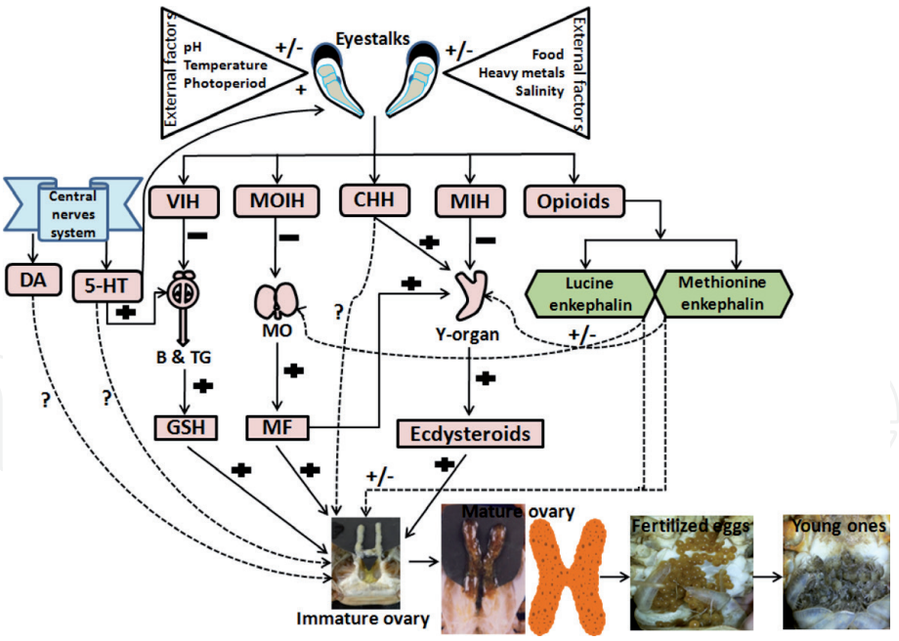

Figure 3.
Influence of internal and external factors in the regulation of crustacean reproduction. DA: Dopamine; 5-HT: 5-Hydroxytryptamine; VIH: Vitellogenesis-inhibiting hormone; MOIH: Mandibular organ-inhibiting hormone; CHH: Crustacean hyperglycemic hormone; MIH: Molt-inhibiting hormone; B & TG: Brain and thoracic ganglia; GSH: Gonad stimulating hormone; MO: Mandibular organ; MF: Methyl farnesoate; '+', '-' and '+/-' denotes activation, inhibition and activation or inhibition, respectively.

from time to time. The systematic pond management and adequate food supply increases protein yield. The high yield influences (increases) the socioeconomic status of farmers ultimately improve the economy of country besides supplementing quality protein forever increasing population. The waste generated (other than protein) in aqua industry is used as feed for poultry, pig, fish and other farm animals, and also in cosmetic industry.

The factors such as pH, temperature, photoperiod, availability of food, salinity of water and heavy metal contamination influences the synthesis and release of CHH-family and other eyestalk secretions in cultured crustaceans.

Regulation of reproduction in crustaceans is a complex process, where a number of internal and external factors are involved. The endocrine hormones and other internal factors act accordingly along with external factors and regulate maturation. The role of well-known internal and external factors is depicted in **Figure 3**. Established endocrine authoritarian mechanisms of crustacean maturation were interpreted in the present chapter. The best method to manipulate endocrine hormones in crustaceans is through supplementation of reproduction positive regulators by means of the feed and is a best alternative for eyestalk ablation technique to improve good quality and quantity of seed from brood stock. Though the administration of positive regulators yielding quality seed, it may injure the animal and increases stress and sometimes it may lead to mortality. The promising method emerging in recent years is gene knockout studies, where the functional genes (negative regulatory genes of reproduction) made inactive. However, knockout studies are not initiated yet in crustacean aquaculture. The identified and reported method for temporary knockdown of gene function in crustaceans is RNAi silencing technology. Silencing of VIH and other genes inhibit reproduction may help to induce maturation thereby quality seed [23]. Controlled external factors with manipulation of internal factors (neither positive nor negative) provide fruitful results without any stress in the brood stock.

Acknowledgements

Author is great full to Prof. P. Sreenivasula Reddy encouragement guidance in successful completion of this Chapter.

Conflict of interest

Authors declare no conflict of interest.

Author details

Ramachandra Reddy Pamuru
Department of Biochemistry, Yogi Vemana University, Kadapa, Andhra Pradesh, India

*Address all correspondence to: reddyprbiotech@gmail.com

References

[1] Panouse JB. Influence de l'ablation du pédoncle oculaire sur la croissance de l'ovaire chez la crevette Leander serratus. Comptes rendus de l'Académie des Sciences. 1943;**217**:553-555

[2] Carlisle DB. Studies on Lysmata seticaudata Risso (Crustacea: Decapoda) V. The ovarian inhibiting hormone and the hormonal inhibition of sex-reversal. Pubblicazioni della Stazione Zoologica di Napoli. 1953;**24**:355-372

[3] Subramoniam T. Mechanisms and control of vitellogenesis in crustaceans. Fisheries Science. 2011;**77**:1-21

[4] Tsukimura B. Crustacean vitellogenesis: Its role in oocyte development. American Zoologist. 2001;**41**:465-476

[5] Reddy PS, Ramamurthi R. Recent trends in crustacean endocrine research. Proceedings of the Indian National Science Academy. 1999;**65**:15-32

[6] Swetha CH, Sainath SB, Reddy PR, Reddy PS. Reproductive endocrinology of female crustaceans: Perspective and prospective. Journal of Marine Science: Research and Development. 2011;**S3**:001

[7] Yang WJ, Aida K, Nagasawa H. Amino acid sequences and activities of multiple hyperglycemic hormones from the kuruma prawn, *Penaeus japonicus*. Peptides. 1997;**18**:479-485

[8] Wilder MN, Okumura T, Tsutsui N. Reproductive mechanisms in crustacea focusing on selected prawn species: Vitellogenin structure, processing and synthetic control. Aqua BioScience Monographs. 2010;**3**:73-110

[9] De Kleijn DP, De Leeuw EP, Van Den Burg BC, Martens GJ, Van Herp F. Cloning and expression of two mRNAs encoding structurally different crustacean hyperglycemic hormone precursors in the lobster *Homarus americanus*. Biochimica et Biophysica Acta. 1995;**1260**:62-66

[10] Soyez D, le Caer JP, Noel PY, Rossier J. Primary structure of two isoforms of the vitellogenesis inhibiting hormone from the lobster *Homarus americanus*. Neuropeptides. 1991;**20**:25-32

[11] Grève P, Sorokine O, Berges T, Lacombe C, Van Dorsselaer A, et al. Isolation and amino acid sequence of a peptide with vitellogenesis inhibiting activity from the terrestrial isopod *Armadillidium vulgare* (Crustacea). General and Comparative Endocrinology. 1999;**115**:406-414

[12] Edomi P, Azzoni E, Mettulio R, Pandolfelli N, Ferrero EA, et al. Gonad inhibiting hormone (GIH) of the Norway lobster (*Nephrops norvegicus*): cDNA cloning, expression, recombinant protein production, and immunolocalization. Gene. 2002;**284**:93-102

[13] Ohira T, Okumura T, Suzuki M, Yajima Y, Tsutsui N, et al. Production and characterization of recombinant vitellogenesis-inhibiting hormone from the American lobster *Homarus americanus*. Peptides. 2006;**27**:1251-1258

[14] Treerattrakool S, Panyim S, Chan SM, Withyachumnarnkul B, Udomkit A. Molecular characterization of gonad-inhibiting hormone of *Penaeus monodon* and elucidation of its inhibitory role in vitellogenin expression by RNA interference. FEBS Journal. 2008;**275**:970-980

[15] Okumura T. Effects of cyclic nucleotides, calcium ionophore, and phorbol ester on vitellogenin mRNA levels in incubated ovarian fragments of the kuruma prawn *Marsupenaeus japonicus*. General and Comparative Endocrinology. 2006;**148**:245-251

[16] Okumura T. Intracellular signaling pathways for vitellogenin synthesis in the ovary of the kuruma prawn, *Penaeus* (*marsupenaeus*) *japonicus*. Bulletin of Fisheries Research Agency. 2008;**26**:135-141. (in Japanese)

[17] Wainwright G, Webster SG, Wilkinson MC, Chung JS, Rees HH. Structure and significance of mandibular organ-inhibiting hormone in the crab, Cancer pagurus. Involvement in multihormonal regulation of growth and reproduction. The Journal of Biological Chemistry. 1996;**271**:12749-12754

[18] Tsukimura B, Borst DW. Regulation of methyl farnesoate in the hemolymph and mandibular organ of the lobster, *Homarus americanus*. General and Comparative Endocrinology. 1992;**2**:296-303

[19] Chaves AR. Effects of sinus gland extract on mandibular organ size and methyl farnesoate synthesis in the crayfish. Comparative Biochemistry and Physiology. Part A: Molecular and Integrative Physiology. 2001;**128**:327-333

[20] Waddy SL, Aiken DE, De Kleijn DPV. Control of reproduction. In: Factor JR, editor. Biology of the lobster, *Homarus americanus*. San Diego, USA: Academic Press; 1995. pp. 217-266

[21] Borst DW, Tsukimura B, Laufer H, Couch E. Regional differences in methyl farnesoate production by the lobster mandibular organ. The Biological Bulletin. 1994;**186**:916

[22] Nagaraju GPC, Reddy PR, Reddy PS. Mandibular organ: It's relation to body weight, sex, molt and reproduction in the crab, Oziotelphusa senex senex Fabricius (1791). Aquaculture. 2004;**232**:603-612

[23] Lugo JM, Morera Y, Rodríguez T, Huberman A, Ramos L, et al. Molecular cloning and characterization of the crustacean hyperglycemic hormone cDNA from *Litopenaeus schmitti*; functional analysis by double stranded RNA interference technique. FEBS Journal. 2006;**273**:5669-5677

[24] De Kleijn DP, Janssen KP, Waddy SL, Hegeman R, Lie WY, et al. Expression of the crustacean hyperglycemic hormones and the gonad inhibiting hormone during the reproductive cycle of the female American lobster *Homarus americanus*. Journal of Endocrinology. 1998;**156**:291-298

[25] Gu PL, Yu KL, Chan SM. Molecular characterization of an additional shrimp hyperglycemic hormone: cDNA cloning, gene organization, expression and biological assay of recombinant proteins. FEBS Letters. 2000;**472**:122-128

[26] Van Herp F. Molecular, cytological and physiological aspects of the crustacean hyperglycemic hormone family. In: Cost EM, Webster SG, editors. Recent Advances in Arthropod Endocrinology. Vol. 65. Cambridge, UK: University Press; 1998. pp. 53-70

[27] Chung JS, Webster SG. Moult cycle-related changes in biological activity of moult-inhibiting hormone (MIH) and crustacean hyperglycaemic hormone (CHH) in the crab, *Carcinus maenas*. From target to transcript. European Journal of Biochemistry. 2003;**270**:3280-3288

[28] Zarubin TP, Chang ES, Mykles DL. Expression of recombinant eyestalk crustacean hyperglycemic hormone from the tropical land crab, *Gecarcinus lateralis*, that inhibits Y-organ ecdysteroidogenesis in vitro. Molecular Biology Reports. 2009;**36**:1231-1237

[29] Tiu SH, Chan SM. The use of recombinant protein and RNA interference approaches to study the reproductive functions of a

gonad-stimulating hormone from the shrimp *Metapenaeus ensis*. FEBS Journal. 2007;**274**:4385-4395

[30] Zmora N, Trant J, Zohar Y, Chung JS. Molt-inhibiting hormone stimulates vitellogenesis at advanced ovarian developmental stages in the female blue crab, Callinectes sapidus 1: An ovarian stage dependent involvement. Saline Systems. 2009;**5**:7

[31] Gu PL, Tobe SS, Chow BKC, Chu KH, He JG, Chan S-M. Characterization of an additional molt inhibiting hormonelike neuropeptide from the shrimp *Metapenaeus ensis*. Peptides. 2002;**23**:1875-1883

[32] Meusy JJ, Blanche MF, Junera H. Molting and vitellogenesis in the crustacean amphipod Orchestia gammarella Pallas. II. Synthesis of vitellogenin (female protein fraction of hemolymph) after the destruction of Y organs. General and Comparative Endocrinology. 1977;**33**:35-40

[33] Kishori B, Reddy PS. Antagonistic effects of opioid peptides in the regulation of ovarian growth of the Indian rice field crab, Oziotelphusa senex senex Fabricius. Invertebrate Reproduction and Development. 2000;**37**:107-111

[34] Kishori B, Reddy PS. Influence of leucine-enkephalin on moulting and vitellogenesis in the freshwater crab, Oziotelphusa senex senex (Fabricius, 1791) (Decapoda, Brachyura). Crustaceana. 2004;**76**:1281-1290

[35] Reddy PS. Involvement of opioid peptides in the regulation of reproduction in the prawn *Penaeus indicus*. Naturwissenschaften. 2000;**87**:535-538

[36] Keller R, Beyer J. Zur hyperglyka¨mischen Wirkung von Serotonin und Augenstiel extrakt beimFlußkrebs *Orconectes limosus*. Zeitschrift für vergleichende Physiologie. 1968;**59**:78-85

[37] Richardson HG, Deecaraman M, Fingerman M. The effect of biogenic amines on ovarian development in fiddler crab, Uca pugilator. Comparative Biochemistry and Physiology. 1991;**99**:53-56

[38] Sainath SB, Reddy PS. Effect of selected biogenic amines on reproduction in the freshwater edible crab Oziotelphusa senex senex. Aquaculture. 2011;**313**:144-148

[39] Sarojini R, Nagabhushanam R, Fingerman M. Evidence for opioid involvement in the regulation ofovarian maturation of the fiddler crab, Uca pugilator. Comparative Biochemistry and Physiology. 1995a;**111A**:279-282

[40] Makkapan W, Maikaeo L, Miyazaki T, Chotigeat W. Molecular mechanism of serotonin via methyl farnesoate in ovarian development of white shrimp: Fenneropenaeus merguiensis de Man. Aquaculture. 2011;**321**:101-107

[41] Vaca A, Alfaro AJ. Ovarian maturation and spawning in the white shrimp, Penaeus vannamei, by serotonin injection. Aquaculture. 2000;**182**:373-385

[42] Sarojini R, Nagabhushanam R, Fingerman M. Mode of action of the neurotransmitter 5-hydroxytryptamine in stimulating ovarian maturation in the red swamp crayfish, *Procambarus clarkii*: An in vivo and in vitro study. Journal of Experimental Zoology. 1995b;**271**:395-400

[43] Luschen W, Wilig A, Jaros PP. The role of biogenic amines in the control of blood glucose level in the decapod crustacean *Carcinus maenas L*. Comparative Biochemistry and Physiology. 1993;**105**:291-296

[44] Kumlu M. Gonadal maturation and spawning in Penaeus semisulcatus de Hann, 1844 by hormone injection. Turkish Journal of Zoology. 2005;**29**:193-199

[45] Cahansky AV, Medesani DA, Chaulet A, Rodríguez EM. In vitro effects of both dopaminergic and enkephalinergic antagonists on the ovarian growth of *Cherax quadricarinatus* (Decapoda, Parastacidae), at different periods of the reproductive cycle. Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology. 2011;**158**:126-131

[46] Girish BP, Swetha CH, Reddy PS. Serotonin induces ecdysteroidogenesis and methyl farnesoate synthesis in the mud crab, *Scylla serrata*. Biochemical and Biophysical Research Communication. 2017;**490**:1340-1345

[47] Fingerman M. Roles of neurotransmitters in regulating reproductive hormone release and gonadal maturation in decapod crustaceans. Invertebrate Reproduction and Development. 1997;**31**:47-54

[48] Chang ES. Hormonal control of molting in decapod crustacea. American Zoologist. 1985;**25**:179-185

[49] Gomez R. Acceleration of development of gonads by implantations of brain in the crab *Paratelphusa hydrodromous*. Naturwiss. 1965;**52**:216

[50] Sarojini R, Mirajkar MS, Nagabhushanam R. Bihormonal control of oogenesis in the freshwater prawn, *Macrobrachium kistnensis*. Acta Physiologica Hungarica. 1983;**61**:5-10

[51] Kulkarni G, Nagabhushanam R, Joshi P. Effect of progesterone on ovarian maturation in a marine penaeid prawn *Parapenaeopsis hardwickii* (Miers, 1878). Indian Journal of Experimental Biology. 1979;**17**:986-987

[52] Otsu T. Bihormonal control of sexual cycle in the freshwater crab, *Potoman dehaani*. Embryologia. 1963;**8**:1-20

[53] Von Hentig R. Influence of salinity and temperature on development, growth, reproduction and energy budget of Artemia Salina. Marine Biology. 1971;**9**:145-182

[54] Medesani DA, López Greco LS, Rodríguez EM. Interference of cadmium and copper with the endocrine control of ovarian growth, in the estuarine crab *Chasmagnathus granulata*. Aquatic Toxicology. 2004;**69**:165-174

[55] Subramoniam T. Crustacean ecdysteroids in reproduction and embryogenesis. Comparative Biochemistry and Physiology. 2000;**125**:135-156

[56] Tarrant AM, Behrendt L, Stegeman JJ, Verslycke T. Ecdysteroid receptor from the American lobster *Homarus americanus*: EcR/RXR isoform cloning and ligand-binding properties. General and Comparative Endocrinology. 2011;**173**:346-355

[57] Hill RJ, Billas IM, Bonneton F, Graham LD, Lawrence MC. Ecdysone receptors: From the Ashburner model to structural biology. Annual Review of Entomology. 2013;**58**:251-271

[58] Tan A, Palli SR. Ecdysone receptor isoforms play distinct roles in controlling molting and metamorphosis in the red floor beatle, *Tribolium castaneum*. Molecular and Cellular Endocrinology. 2008;**291**:42-49

[59] Schwedes C, Tulsiani S, Carney GE. Ecdysone receptor expression and activity in adult *Drosophila melanogaster*. Journal of Insect Physiology. 2011;**57**:899-907

[60] Charniaux-Cotton H, Toui A. Contrale de la prgvitellogenese et

de la vitellogenese chez la crevette hermaphrodite Lysmata seticaudata Risso. Comptes rendus de l'Académie des Sciences. 1973;**276**:2717-2720

[61] Tiu SH, Chan SM, Tobe SS. The effects of farnesoic acid and 20-hydroxyecdysone on vitellogenin gene expression in the lobster, *Homarus americanus*, and possible roles in the reproductive process. General and Comparative Endocrinology. 2010;**166**:337-345

[62] Laufer H, Biggers WJ, Ahl JS. Stimulation of ovarian maturation in the crayfish *Procambarus clarkii* by methyl farnesoate. General and Comparative Endocrinology. 1998;**111**:113-118

[63] Reddy PR, Nagaraju GPC, Reddy PS. Involvement of methyl farnesoate in the regulation of molting and reproduction in the freshwater crab Oziotelphusa senex senex. Journal of Crustacean Biology. 2004;**24**:511-515

[64] Hyne RV. Review of the reproductive biology of amphipods and their endocrine regulation: Identification of mechanistic pathways for reproductive toxicants. Environmental Toxicology and Chemistry. 2011;**30**:2647-2657

[65] Nagaraju GPC. Reproductive regulators in decapod crustaceans: An overview. Journal of Experimental Biology. 2011;**214**:3-16