Endocrine regulation of egg production in economically important crustaceans

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Significant advances have been made in crustacean reproductive endocrinology, especially in view of its application potential in aquaculture. Major contribution is in the isolation, characterization as well as functional role of eye-stalk neuropeptides, which control diversified physiological functions, including egg production. Their specific control over proximate endocrine glands such as mandibular organ and Y-organ has relevance to the formulation of control methods to enhance egg production in the commercially important crustaceans. Neuropeptide diversity as well as multiple peptide actions in the crustaceans call for intensified work in the field to gain greater understanding towards their possible use in reproductive control. Plausibility of biogenic amines and endogenous vertebrate-type steroids in the control of reproduction indicates the complexity of crustacean endocrinology as well as their use in the formulation of control measures. A need for understanding the vitellogenic processes together with the tissue response to various endocrine factors in controlling reproduction is also stressed.

AQUACULTURE of marine crustaceans, especially the various shrimp species, has been traditionally practised in many south-east Asian countries by a system of trapping and holding the juveniles. The tidal waters carrying the fries of shrimps and fishes during the high tide are trapped and allowed to grow in ponds using the naturally available feed. In India, similar traditional practices are found in the brackish waters of Kerala, parts of Karnataka, Goa and Orissa, where paddy and shrimps are grown in low-lying areas inundated during the high tides.

As the commercial importance of shrimp culture has been realized, semi-intensive culture gained prominence all along the east and west coast, using the seeds collected from the wild. Collection of seeds from the wild was not without difficulties. It entailed the destruction of a large number of non-essential fries of other organisms. Again, the seed collection in nature is highly seasonal, and has been affected adversely in recent years due to pollution in the backwaters. Hence, the better alternative is to go for the hatchery production of shrimp seeds under controlled conditions. In this method, the females, captured from the wild, undergo ovarian maturation and spawning in captivity. Although quality seeds are obtained here, the females require a longer period for rematuration of ovaries after successive spawnings.

The commercial seed production through hatchery operation has successfully been carried out in the shrimp species, lobsters, and to certain extent in crabs. In evolving a suitable method for optimal production of seeds in the hatchery, an understanding of female reproductive physiology with special reference to the egg production in the ovary and their spawning mechanism is imperative. Furthermore, unlike in the insects, most crustaceans continue to grow and moult even after sexual maturity, thereby imposing competition for storage nutritive materials for the egg production and new cuticle formation. Therefore, crustaceans have evolved a regulatory mechanism for moulting and reproduction that delicately interlinks the two processes relegating most of the reproductive activities to the intermoult period. Female reproduction, particularly the egg formation, within the ovary is controlled both by extrinsic as well as intrinsic factors. This review attempts to critically appraise the exhaustive information available on the control of egg maturation and their utility in evolving manipulative techniques to increase seed production.

Process of egg formation

In the reproducing crustaceans, the paired ovaries are located either dorsal or dorsolateral to the gut. Its limbs may be fused anteriorly as in the peracarids or other lower crustaceans or in the midline as in the macrurans and stomatopods[1]. In the shrimps, the ovaries are paired, partly fused, bilaterally symmetrical, extending from the pericardial region of the stomach to the telson[2]. The oviducts are lateral extensions in the ovary to connect the latter with the gonopores. The proximal part of the oviduct in Brachyura differentiates into a spermatheca, lined with glandular epithelium.

Oogenesis, as occurring in the ovary, consists of proliferative and differentiative phases. In the proliferative phase, the oogonial cells multiply mitotically inside the germinal zone. The primary oocyte, derived from the secondary oogonial cells, undergo differentiation and maturation in the growth zone. In general, the germarium may be found as peripheral epithelium or central germinal

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cord or germ nests distributed throughout the ovary[3]. In the shrimp *Penaeus setiferus*, a germinal epithelium is found in the medio-ventral region of the ovarian wall.

For a proper understanding of the hormonal control of egg production in crustaceans, a detailed knowledge on the egg formation with reference to the mode of yolk formation is necessary. Decapod crustaceans develop a large number of heavily-yolked eggs in the ovary. Hence, vitellogenesis, the process of yolk synthesis and deposition, is a crucial event in the female gametogenesis. Vitellin or lipovitellin is the major yolk protein that accumulates in the ovary during this process[3]. The growing oocytes initially synthesize the yolk materials using their own metabolic machinery. This stage is followed by an intensive accumulation of yolk from the hemolymph by endocytosis. These two processes have been designated as primary and secondary vitellogenesis. Nevertheless, crustaceans show diverse patterns in respect of yolk formation within the ovary. Recent evidences show that in majority of the shrimps, yolk synthesis occurs almost exclusively within the oocytes[4]. However, in lobsters, crabs and other crustaceans, there is evidence for extraovarian yolk synthesis followed by sequestration into the growing oocytes. Variations in the mode of yolk formation in these two groups of decapod crustaceans may be related to the lecithality of the eggs and the mode of larval development. In the shrimps, the yolk content of the egg is meagre, and hence the oocytes may be in a position to synthesize most of them, with very little contribution from extra ovarian sites. Conversely, in the crabs and lobsters, the oocytes are heavily yolk-laden and have to depend on extraneous sources for synthesis of yolk protein. Accordingly, the embryonic development is abbreviated in the shrimps, and protracted in other decapods.

The relative role of the ovaries as well as other somatic organs in contributing to the final yolk products is not well defined in several crustaceans. However, the identification of the tissues that participate in the synthesis of the yolk precursor molecules is a critical prerequisite for studying vitellogenesis at the endocrine and cellular levels. The synthesis of the yolk precursor proteins was authentically indicated first in the subepidermal adipose tissue (SAT) of isopods[5] and amphipods[6]. Convincing evidence is also available for the synthesis of yolk precursors by the hepatopancreas (HP) of several decapod crustaceans[7–9]. In a recent study, employing radiolabelled amino acids, Rani and Subramoniam[10] demonstrated high synthetic activity in SAT in the initial phase of vitellogenesis followed by an intense synthesis and storage of vitellogenin in the hepatopancreas of the mud crab, *Scylla serrata*. Using cDNA cloning techniques, a single gene coding for both vitellin in the ovary as well as vitellogenin in the hepatopancreas has been discovered in the shrimp *Penaeus semisulcatus*[11]. Understandably, the differential expression of this gene in the ovary and hepatopancreas in the shrimp and the other decapods is responsible for the differences in their mode of yolk formation.

Endocrine regulation of egg production

Crustaceans possess a full range of nervous structures for perception of environmental cues and signals and use a complex neuroendocrine system for transduction of the messages to the endocrine glands, which produce the factors regulating the activity of organs concerning reproduction. The various neuroendocrine and nonneural hormonal centers are depicted in Figure 1. The chief hormonal factors that originate from various endocrine glands can be grouped under three chemical classes such as steroids (ecdysteroids – Y organ), peptides (various eyestalk neurohormones), and terpenoids (methyl farnesoate – mandibular organ). Reproduction in Crustacea has been hypothesized to be controlled by dual endocrine factors, one inhibitory and other stimulatory, both originating from the neuroendocrine centers such as the eyestalk ganglia and the brain/thoracic ganglia[3,12]. In the majority of malacostracan crustaceans, eyestalk is the pivotal organ for housing various neuropeptides (Figure 2)[13]. The optic peduncle within the stalked eye of the crustaceans consists of three ganglionic formations such as medulla externa, medulla interna and medulla terminalis. Among the three, the medulla terminalis contains a cluster of cell bodies called, medulla terminalis X-organ or simply X-organ[14]. A neurohemal organ associated with the X-organ is the sinus gland located at the peripheral junction between the medulla externa and medulla interna. This gland is formed by the cluster of neurosecretory axonal terminals extending chiefly from the perikaria of the X-organ. The X-organ

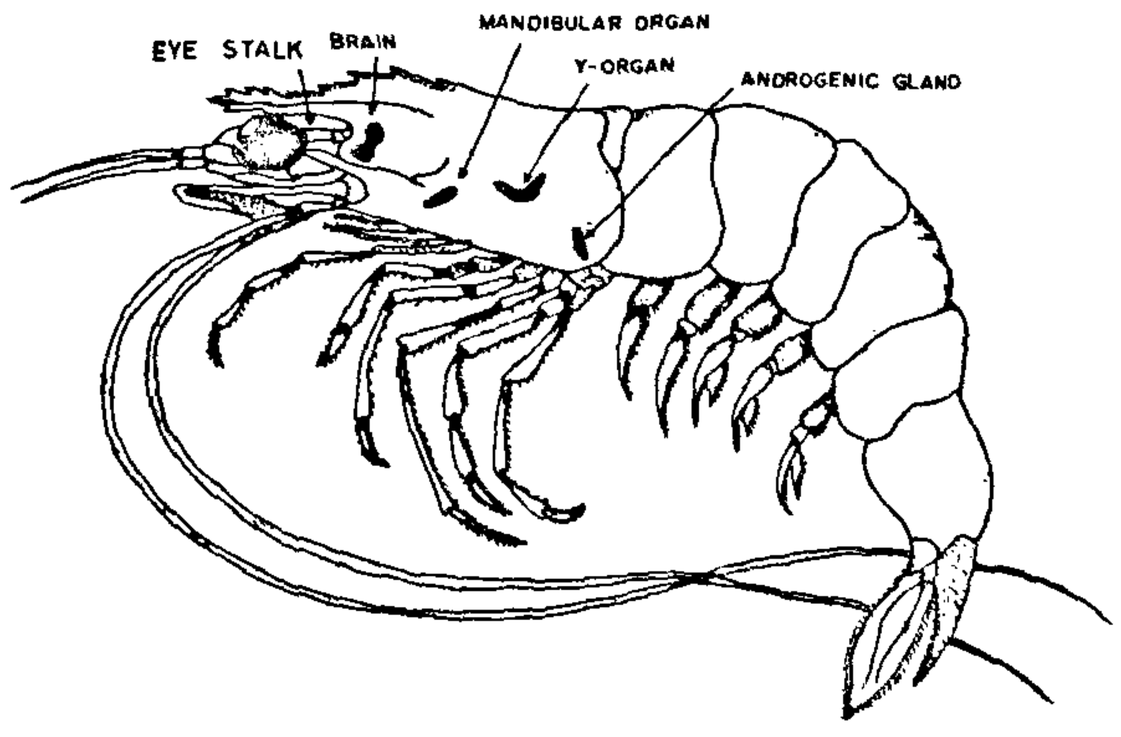

Figure 1. Diagrammatic location of major endocrine glands in a penaeid shrimp.

sinus gland system is structurally and functionally analogous to the vertebrate hypothalamic-neurohypophysial system as well as to the corpus cardiacum of the insects[15,16].

A remarkable discovery of the endocrine regulation of female reproduction in crustaceans was made over 50 years ago by Panouse[17], when he demonstrated rapid ovarian maturation by ablating the eyestalk of the shrimp *Palaemon serratus*. The occurrence of the gonad inhibitory principle in the eyestalk and its deprivation causing accelerated ovarian growth resulted in the adoption of a simple technique of induced ovarian maturation by removing the eyestalk. The eyestalk ablation technique for inducing gonadal maturation is now practised in the shrimp farms worldwide. Over 20 species of commercially important shrimp species have responded positively to eyestalk ablation under captive conditions (Table 1). In practice, unilateral eyestalk ablation has been found to be adequate to stimulate ovarian maturation and spawning. This may indicate that the lowering titre of the gonad inhibitory hormone (GIH) below a certain threshold level by single eyestalk ablation is sufficient enough to stop its inhibitory effect on the stimulatory neuropeptides or other putative gonadotropic hormonal sources. This threshold level of GIH to inhibit or restrain the gonadal development has not been determined for any shrimp species.

The eyestalk ablation technique involves: (i) simple pinching of the eyestalk at the base, (ii) enucleation, (iii) cauterizing the eyestalk with an electrocautery device or a red hot wire or forceps, and (iv) ligation by tying of the eyestalk tightly with a hair or surgical thread. The increased use of eyestalk ablation technique in the captive breeding of shrimps and lobsters have brought forth both positive and negative effects on the quality of spawning and the seeds produced (see Bray and Lawrence)[18]. In most penaeid shrimps, there is a natural inhibition of ovarian maturation and spawning under captive conditions; the eyestalk ablation removes it. Even in other species which develop ovary and spawn in captivity, the use of eyestalk ablation reduces the interbreeding time significantly (Figure 3), thus augmenting total egg production in a given time. In addition, eyestalk ablation is helpful in extending the period of annual breeding cycle in the shrimps.

The main criticism levelled against the eyestalk ablation-induced reproduction is the poorer quality of offspring produced. This may be due to incomplete diets which could not provide nutrients quick enough for the consequent accelerated ovarian maturation. In other cases, non-fertile eggs were reported after eyestalk ablation. However, Bray and Lawrence[18] argue that in these shrimps mating would not have occurred to produce the fertilized eggs and nauplii. Besides inadequate diet for the females, poor quality of the sea water, toxicity, environmental fluctuations or stress may contribute to the poor quality seed. At the histological or biochemical level, Lawrence *et al.*[19] could not find any distinction between ovarian maturation with or without eyestalk ablation in penaeid shrimps. It may be concluded from the above discussion that provided quality diet and suitable environmental conditions, eyestalk ablation per se does induce and accelerate ovarian growth, leading to normal spawning and healthy offsprings in the pond-reared as well as wild-caught females. Interestingly, eyestalk ablation during the nonbreeding seasons in a freshwater crab, *Paratelphusa hydrodromous* caused biochemical impoverization in the induced ovary[20].

Gonad inhibitory hormone

Although the effect of eyestalk ablation on the egg maturation and spawning through its inhibitory effect was known for a long time, the nature of this inhibitory

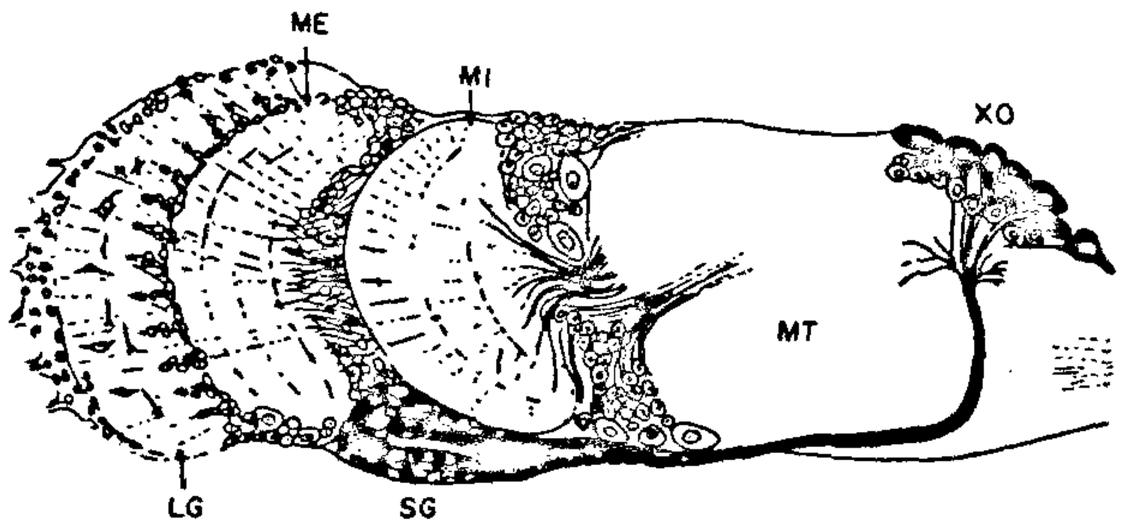

Figure 2. The XO–SG neurosecretory pathway in the eyestalk of *Carcinus meanas*. Neurosecretory structures (drawn in black) include a group, a perikarya in medulla terminalis ganglionic X-organ (XO), their axons with proximal dendritic branches, the XO–SG tract of axons and terminii at the sinus gland (SG). LG, lamina ganglionaris; ME; MI; MT; medulla externa, interna, and terminalis, respectively (Redrawn from Keller and Sedlmeir[13]).

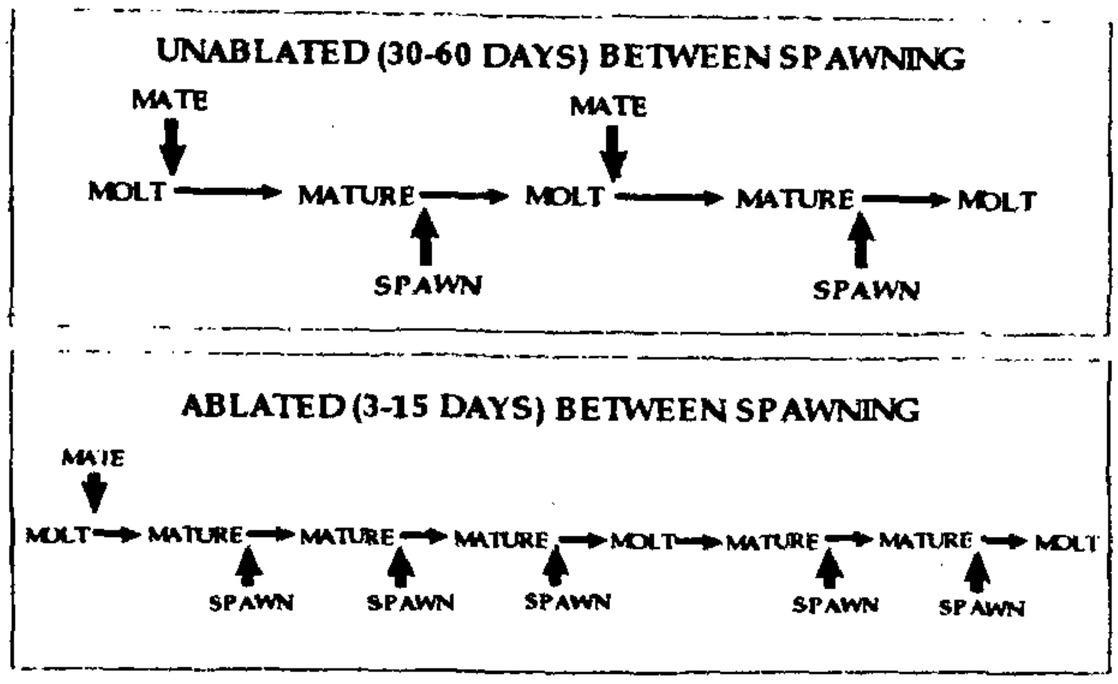

Figure 3. The effect of eyestalk ablation in reducing the interbreeding period in *Penaeus monodon* (data based on Primavera[123]).

Table 1. Effect of eyestalk ablation on ovarian maturation and spawning in decapod crustaceans

Species	Type of ablation	Effect of ablation	Ref.
Penaeus monodon	Ablation	Absence of spermatophore in the seminal receptacle; Ovarian maturation, spawning and ecdysis	Lin *et al.*[72]
P. monodon	Unilateral ablation	Induction of ovarian maturation	AQUACOP[73]
P. monodon	Bilateral Ablation	Higher number of eggs and nauplii	Primavera and Cuballero[74]
P. monodon	Ablation	Maturation and spawning induced	Halder[75]
P. monodon	Unilateral	Spawning with viable nauplii	Primavera[76]
P. monodon	Unilateral	Spawning with viable eggs	Beard and Wickins[77]
P. monodon	Unilateral	Rematuration accelerated	Primavera and Borlongan[78]
P. monodon *P. monodon*	Unilateral Bilateral	– Induced gonadal development – High mortality	Santiago (Jr)[79]
P. monodon	Unilateral	Ovarian maturation induced; bigger oocytes	Tan-Fermin[80]
P. monodon	Unilateral	Spawning index and egg fertilisation rates not affected	Kelemee and Smith[81]
P. monodon	Unilateral	Ovarian maturation induced	Pudadeva and Primavera[82]
P. monodon	Ablated	Ovarian maturation induced	Ruangpanit *et al.*[83]
P. monodon	Unilateral	Maximum growth with fresh feed	Murugesan *et al.*[84]
P. japonicus	Bilateral tying/ cutting	Ovarian maturation Increased ovarian lipid Decrease in hepatopancreas lipids	Teshima *et al.*[85]
P. japonicus	Bilateral	Lipid transfer from hepatopancreas to ovaries	Teshima *et al.*[86]
P. notalis and P. schmitti	Unilateral	Maturation induced; low hatching rate	Trujillo and Primavera[87]
P. schmitti	Unilateral	Maturation induced	Nascimento *et al.*[88]
P. stylirostris	Unilateral	Ovarian maturation but the spawn not fertilizable	Chamberlain and Gervais[89]
P. stylirostris	Unilateral		Chamberlain and Lawrence[90]
P. setiferus	Unilateral	Fertile eggs produced	Lawrence *et al.*[91]
P. setiferus	Unilateral	Egg production increased	Brown *et al.*[92]
P. indicus	Unilateral (pinched/tied)	Ovarian maturation induced	Makinouchi and Primavera[93]
P. indicus	Ablated	Induced ovarian maturation	Emmerson[94]
P. orientalis	Unilateral	Induced ovarian maturation	Arnstein and Beard[95]
P. semisulcatus	Unilateral	Increased energy demand; Growth decreased; Egg quality not affected; Spawning induced	Browdy and Samocha[96,97]
P. notialis	Unilateral	Ovarian development quickened	Ramos-Trujillo and Gonzale Flores[98]
Macrobrachium nobili	Unilateral	Growth and maturation induced by ablation alone	Kumari and Pandian[99]
P. plebejus	Unilateral	Induced ovarian development	Kelemee and Smith[100]
P. monodon and Metapenaeus dobsoni	Not given	Enhanced ovarian maturation and spawning	Muthu and Lakshmi Narayan[1
Parapenaeopsis stylifera	Unilateral	Induced ovarian maturation and spawning	Emmerson[102]
M. rosenbergii	Snipping both eye stalks	Serum vitellogenin appeared, increased and then decreased	Wilder *et al.*[103]
M. lanchestri	Bilateral	High maturity and increase in growth rate	Ponnuchamy *et al.*[104]
M. malcolmsonii	Unilateral	Increased fecundity	Murugadass *et al.*[105]
M. nipponese	Ablated	Ovarian maturation induced	Bingru *et al.*[106]
Palaemon serratus	Uni-/bi-lateral	Induced ovarian development	Panouse[17]
Panulirus argus	Ablated	Increase in the gonadal size	Quackenbush and Hernkind[107]
Paratelphusa hydrodromous	Ablated	– Low level of organic substances – More oocytes – Lipid: protein ratio in ovary high	Anilkumar and Adiyodi[20]
Astacus astacus	Bilateral	Molting rate increased	Huner and Lindquist[108]

hormone remained unknown. With the introduction of modern methods in the microanalytical chemistry and molecular biology into crustacean research, the separation as well as the structural elucidation of several peptides including the gonad inhibitory hormone (GIH) were made possible[21]. The first isolation and partial characterization of GIH were made in the crab *Cancer magister* using gel chromatographic techniques[22]. It was found to be a 2 kDa peptide with inhibitory effect on the ovarian growth of the shrimp *Crangon crangon*. Quackenbush and Keely[23] also purified the GIH from the crude extract of the shrimp *Penaeus setiferus* using sephadex G-25 chromatography and determined the molecular weight as 3,300 Da. This peptide inhibited the ^{14}C-leucine incorporation into the vitellogenin of the cultured ovary of *Uca pugilator*, while the incorporation of the radioactivity in the other proteins remained unaffected. The GIH of *P. setiferus* also showed the same inhibitory effect on the yolk protein synthesis in the ovary and hepatopancreas of another penaeid shrimp *P. vannamei*[24]. By virtue of its specific inhibitory role in the yolk synthesis, the GIH is rechristened as vitellogenesis inhibitory hormone (VIH)[25]. Using RP-HPLC, Soyez *et al.*[26] isolated the VIH from the sinus gland of *Homarus americanus* and determined the amino acid sequence employing gas phase microsequencing and mass spectrometry. The VIH of this lobster occurs in two isoforms, the Hoa-VIH-I and Hoa-VIH-II. Both forms have the same primary structure with 77 amino acid residues with six cysteins, a free *N*-terminus, a pI of 6.8 and molecular weight of 9315 Da. Interestingly, VIH I only showed effect on vitellogenesis. Following this, Aguilar *et al.*[27] reported the identification and characterization of VIH from the Mexican crayfish *Procambarus bouvierii*. This neuropeptide has a molecular weight of 8388 Da but a blocked *N*-terminus. It consists of 72–74 amino acid residues with specific absence of tyrosine, histidine and methionine.

Immunocytochemical localization of VIH in X-organ – sinus gland

Immunocytochemical studies have revealed the co-localization of VIH with another peptide, crustacean hyperglycemic hormone (CHH) in the same X-organ neurosecretory perikarya[28]. That VIH and CHH are two different peptides have however been shown by the fact that the preabsorption of the antiVIH serum with purified CHH did not abolish its immunoreactivity in the axon terminals of the sinus glands. The ability to develop nonradioactively labelled mRNA probes for VIH and CHH has enabled investigation of the mRNAs encoding these neuropeptides[29]. *In situ* hybridization, using these RNA probes, has also revealed co-localization of VIH and CHH in the same neurosecretory cells of X-organ. These results suggest that CHH and VIH may be synthesized in one cell group of the X-organ and that the variations in staining reflects the differences in the synthetic activity of the individual cells. Lobster VIH mRNA is only found in the eyestalks ganglia, indicating that VIH is not produced in other parts of the nervous system[30]. The level of VIH mRNA expression in the X-organ correlates well with the VIH titre in the hemolymph of the lobster *H. americanus*[31]. The level of VIH mRNA expression in the eyestalk is significantly lower immediately after spawning (just before vitellogenesis resumes) than at other stages in the ovarian cycle. Hemolymph levels of VIH are also significantly lower during secondary vitellogenesis than they are at other stages in the reproductive cycle. This level increases significantly just before spawning when vitellogenin levels are declining. The VIH titre in the hemolymph however remains high after spawning and during primary vitellogenesis.

VIH's relationship with other eyestalk neuropeptides

Recent microanalytical investigations on the primary structure of VIH have revealed its close relationship with two other neuropeptides, namely, crustacean hyperglycemic hormone (CHH) and moult inhibiting hormone (MIH). Significantly, these three hormones constitute a novel family of large peptides (CHH family peptides) from the X-organ–sinus-gland system with characteristics unique to crustaceans only.

They comprise 72–78 amino acid residues with six conserved Cys residues. These peptides also occur in several isoforms. Based on the sequence homology of amino acids, two types of CHH-family peptides may be discerned. Type I includes all CHHs and Hoa-MIH, and type II comprises Cam-MIH and Hoa-VIH (Figure 4). The characteristics of the type I molecules are (1) the total number of the amino acid residues are 72 or 73, (2) the amino terminus is blocked by a pyroglutamate residue with one exception (not shown in the figure), and (3) the carboxyl terminus is also blocked by an amide without exception. Conversely, the type II molecules consist of 77 or 78 amino acid residues and their amino- and carboxyl-termini are both free. Again the type II molecules have an insertion of the -gly-residue at position 12 and hence the conservation of relative distribution of six -cys-residues is slightly incomplete[32].

The structural relationship among CHH/MIH/VIH is further highlighted by the structural elucidation of their preprohormones, as deduced from the cloning and sequencing of their cDNAs[30]. Alignment of the prepro VIH, prepro MIH and prepro CHH (Figure 2 of de Kleijn *et al.*[30]) from the lobsters, crabs and crayfishes reveals the presence of two groups among the CHH-family peptides. The preprohormones for the CHHs contain an additional peptide preceding the hormone,

namely, the CHH precursor related peptide (CPRP). However, CPRP is lacking in the VIH/MIH precursors, suggesting an early separation between the two, possibly by a deletion in ancestral CHH/MIH/VIH gene[30]. In addition to the close structural homology between MIH and VIH, the well-known functional homology is that both the hormones have similar inhibitory type of effects on the two energy-demanding physiological processes, namely, moulting and vitellogenesis.

Cross-functions in CHH-family peptides

Structural similarities found among the CHH/MIH/VIH peptide groups are also reflected in their overlapping physiological functions. For instance, the lobster MIH has significant CHH activity[33]. Similarly, Tensen *et al.*[34] had earlier demonstrated a gonad stimulatory role in one or both of the isoforms of lobster CHH-B on the shrimp oocyte growth. Further expression studies on the mRNA of CHH-B hormones have indicated their occurrence throughout the nervous system, in addition to the eyestalk ganglia[31]. Levels of lobster CHH-B mRNA are low immediately after spawning, but increase significantly after the onset of vitellogenesis. The CHH-B isoforms have less hyperglycemic effect, thereby deviating from their primary role of glucose metabolism[31]. Conversely, CHH-A isoforms are the most potent crustacean hyperglycemic hormone with little activity on the ovarian maturation. Certain sinus gland peptides, not belonging to the CHH-family, also show sequence homology as well as functional identity with MIH and CHH[32]. Peptide G1, also originating from the sinus gland, appears to have identical sequence with MIH and one of the CHH isoforms isolated from *H. americanus*[35].

Penaeid eye stalk neuropeptides

A feature of interest in the neuropeptide research is its confinement to the lobsters, crayfishes and to a certain extent, to the shore crabs. By far, the CHH predominates over all other eyestalk neuropeptides by constituting as much as 60%. Also, the multifunctional roles of CHH of the lobsters have been clearly revealed in the recent bioassay studies. In contrast, molecular studies on the neuropeptide of the shrimp species are limited, although the effect of eyestalk ablation in ovarian maturation is well evidenced. Nevertheless, a few reports on the CHH and MIH peptides from the shrimp species have appeared (see Subramoniam *et al.*[36] for reference). All these studies indicate the presence of multiple CHH forms in the shrimp, as reported in lobsters and crayfishes. A very recent study on the CHH in the kuruma shrimp, *Penaeus japonicus*, has revealed the presence of as many as seven isoforms[37]. Notably, all these isoforms inhibit protein and mRNA synthesis in *in vitro*-incubated ovarian fragments of vitellogenic *P. semisulcatus*. The ovarian proteins inhibited by the CHH peptides include the vitellins also. However, the non-CHH-family peptides did not inhibit protein synthesis. Previous studies have shown that these peptides isolated from the sinus gland of *P. japonicus* have both CHH and MIH activities[38,39]. Hence, their inhibitory effect on ovarian protein synthesis demonstrates additional biological effect. Even here, not all the isoforms show similarity in their inhibitory effect. Based on their differential inhibitory functions, two groups of CHH peptides could be thus discerned in *P. japonicus*. In the first group, all the CHH-family peptides excepting fraction IV have been included. They all show high inhibitory effect on ovarian protein synthesis. Group II includes fraction IV which exhibits a very weak inhibition on protein synthesis in the ovary. Confirmatory evidences from the other shrimp species CHH will be highly rewarding to generalize their gonad inhibitory property in the shrimps. Obviously, penaeid CHH peptides differ from those of the other decapods, in their amino acid composition[36], unblocked C-terminus[36,37] as well as their functional role in the inhibition of protein synthesis, as discussed above.

It is again of interest to note in this context the presence of CHH receptors in several tissues including oocyte membranes in the crabs, *Carcinus meanas* and *C. pagurus*[40]. Understandably, different target tissues involved in the carbohydrate metabolism (hepatopancreas), moult inhibition (Y-organ) and vitellogenesis (ovary) possess CHH receptors, but their ultimate use by these peptides in their respective physiological control mechanisms remains elusive. As for their reproductive control, though it warrants confirmation from other

Amino Acid Sequences of CHH-Family Peptides

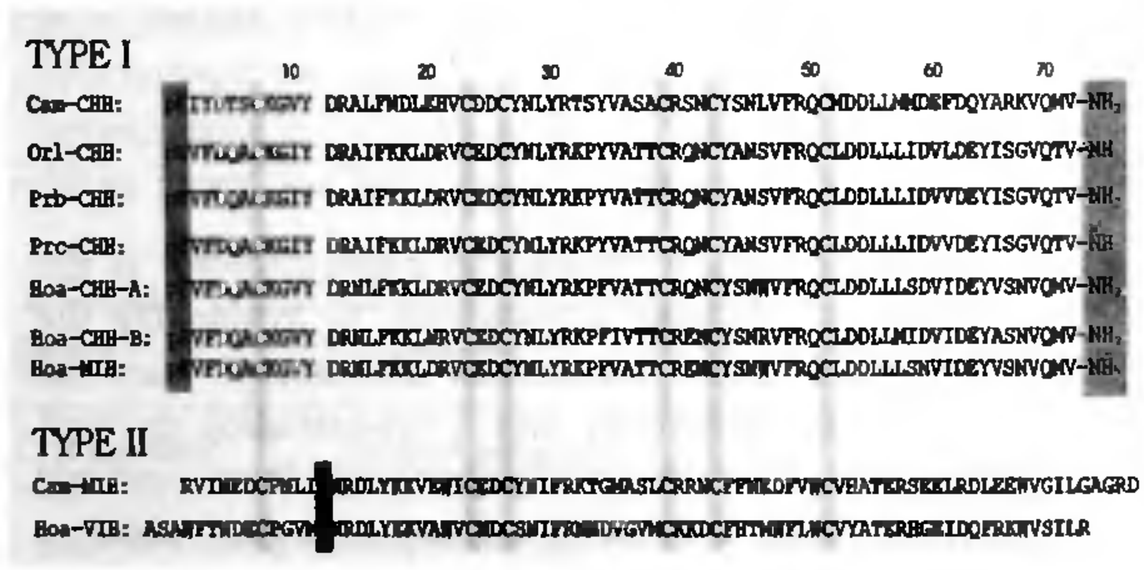

Figure 4. Comparison of amino acid sequences of CHH-family neuropetides. Data based on various sources and Keller[21]. Cam-CHH *Carcinus maenas*, crustacean hyperglycemic hormone; Orl-CHH *Orconectus limosus*, crustacean hyperglycemic hormone; Prb-CHH *Procambarus*, crustacean hyperglycemic hormone; Prc-CHH *Procambarus clarkii*, crustacean hyperglycemic hormone; Hoa-CHH-A *Homarus americanus*, crustacean hyperglycemic hormone; Hoa-CHH-B *Homarus americanus*, crustacean hyperglycemic hormone; Hoa-MIH *Homarus americanus*, molt inhibiting hormone; Cam-MIH *Carcinus maenas*, molt inhibiting hormone; Hoa-VIH *Homarus americanus*, vitellogenesis inhibiting hormone.

penaeid species, these results are significant in the sense that majority of the egg yolk in the shrimp ovary is synthesized within the oocytes. The ubiquitous response of all the shrimp species for eyestalk ablation towards ovarian rematuration also calls for the identification of vitellogenesis inhibitory hormone in them. It remains to be known whether the so-called VIH is one or more of these CHH isoforms or is a peptide, distinct from the CHH isoforms. Recent work from our laboratory has shed some light on the inhibitory effect of HPLC separated eyestalk peptides on vitellogenesis in *P. monodon*[41]. At least two fractions of RP-HPLC separated X-organ sinus gland extracts inhibited vitellogenin synthesis in the eyestalk-ablated *P. monodon*. Amino acid sequencing of the shrimp VIH would reveal their identity with the known CHH-family peptides.

Gonad stimulatory hormone. A gonad stimulatory neuropeptide present in the central nervous system was discovered in the crustaceans much later than its antagonistic inhibitory neuropeptide in the eyestalk. Otsu[42] and Gomez and Nayar[43] showed that thoracic and supraoesophageal ganglion implants from the mature female induced ovarian growth in immature freshwater prawn and crab, respectively. Injection of the brain extracts from the lobster *Homarus americanus* resulted in the induced ovarian maturation in the shrimp *P. vannamei*[44]. According to them, the brain produces some peptides that function as gonad stimulatory hormone (GSH) releasing factor, so as to act on the thoracic ganglia to release the GSH. Interestingly, a gonad-stimulatory neuropeptide has also been reported in the sinus gland extract of the Mexican crayfish, *Procambarus bouverri*[45]. However, the gonad stimulatory neuropeptide has neither been isolated nor characterized in any crustacean species.

Mandibular organ and gonadal control

It is evident from the preceding section that the egg production in decapod crustaceans is under the control of various peptidergic hormonal factors. Notwithstanding the inhibitory and stimulatory role of the neuropeptides on ovarian maturation, the involvement of nonneural endocrine glands in crustacean reproduction has also come to light in recent years. Mandibular organ (MO) synthesizing a sesquiterpenoid, methyl farnesoate (MF), is a standing example. The first evidence for MO's role in vitellogenesis was provided by Hinsch[46] from her observation that the active MO implants stimulated ovarian growth in the immature female spider crab, *Libinia emarginata*. Subsequently, Laufer *et al.*[47] demonstrated the increasing titre of MF in the hemolymph of this crab during vitellogenesis, thus introducing a new gonad-stimulating hormone. MF is the unepoxidated form of the insect juvenile hormone (JH III), and hence appears to have a gonadotropic role, like JH in insects[48–51]. MO, as the source of MF in other crustaceans such as lobsters, crayfish, and shrimps, has also been reported. In the shrimp species, *P. duorarum* and *P. vannamei*, the MO is small in the nonreproductive females, but becomes larger and active in reproductive specimens[52,53]. Recently, Laufer *et al.*[54] reported stimulation of ovarian maturation in the prereproductive females of crayfish *Procambarus clarkii* by treatment with extraneous MF. A contrasting result has however been reported for the noninvolvement of MO in the reproduction of the American lobster, *Homarus americanus*. In this lobster even after the removal of MO normal spawning rhythm is maintained. MF level in the hemolymph also does not positively correlate with vitellogenic period. It is very high in the preovulatory females during winter; whereas, the level drops to undetectable level during spring when there is active vitellogenesis[55].

Despite its conflicting role in the control of vitellogenesis among different decapods, MO's activity seems to be under the control of the eyestalk neuropeptides, as evidenced by several eyestalk ablation studies. Tsukimura and Borst[56] showed a distinctive elevation of MF level in hemolymph and MO after eyestalk ablation in *H. americanus*. The involvement of cGMP as a second messenger in the inhibitory activity of the sinus gland factor on the MF synthesis was demonstrated by Tsukimura *et al.*[57]. Although the sinus gland factor regulating the MF synthesis has not yet been identified, several known neuropeptides have been implicated in the control of its activity. The red pigment concentrating hormone (RPCH) is able to stimulate MF synthesis, while the pigment dispersing hormone (PDH) inhibits MF synthesis in the crayfish *Procambarus clarkii*[58]. More recently, Liu and Laufer[59], and Liu *et al.*[60] have demonstrated that a sinus gland neuropeptide, with CHH activity, also inhibits mandibular activity in the crab *Libinia emarginata*.

Vertebrate-like steroids

An interesting aspect of crustacean endocrinology is the ability of several decapod crustaceans to synthesize the vertebrate-type steroid hormones. Hormones such as pregnenolone, progesterone, and 17-β-estradiol as well as testosterone have been documented from various nonreproductive as well as reproductive organs such as the mandibular organ, kidney, hepatopancreas, hemolymph, ovary and testis[35]. Furthermore, crustaceans have been found to be responding to the injection of vertebrate gonadotrophins such as human chorionic gonadotrophin, follicle-stimulating hormone and lutenizing hormone by stimulating vitellogenesis[61]. Although the mode of vertebrate hormone action on the crustacean egg production is not properly understood, there seems to be tremendous application potential in using these

hormones to stimulate gonadal maturation in the aquaculture species. It may be seen from Table 2 that estradiol and progesterone levels in the hemolymph fluctuate closely to the ovarian maturity stages. In addition, their tissue levels, especially in the ovary and hepatopancreas, also correlate positively with the ovarian maturity stages[62]. It is not possible to know at this stage whether their fluctuation during reproductive cycle is really indicative of their controlling role in vitellogenesis or not. Recently, Yano and Itakura[63] reported the synthesis of vitellogenin by ovarian pieces under *in vitro* conditions when incubated with 17-β-estradiol. In the oviparous vertebrates like chicken, transcriptional activation of the vitellogenin gene in the hepatic cells is estrogen-dependent[64]. In a recent study on the marine shrimp, *Penaeus semisulcatus*, it has been shown that both the vitellogenin (Vg) synthesis in the hepatopancreas and vitellin (Vt) synthesis in the oocytes are coded by one gene[11]. Yet Vg/Vt gene expression by the steroids, estrogen or progesterone in the crustaceans remains to be demonstrated.

Integrative role of biogenic amines in crustacean egg formation

In Crustacea, biogenic amines function mainly as neurotransmitters and neuromodulators. Some of these amines also subserve the functions of neuroregulators to control the release of crustacean neurohormones (Table 3). For example, 5-hydroxytryptamine (5-HT) stimulates release of several eyestalk neuropeptides. The role of 5-HT on the ovarian development was indicated by its stimulatory effect on the gonad stimulatory hormone in *Uca pugilator*[65] as well as crayfish, *Procambarus clarkii*[66]. However, dopamine inhibited the 5-HT-stimulated ovarian maturation under *in vivo* conditions[66]. Again, an opioid, met-enkephalin has been shown to inhibit ovarian development by specific stimulation of VIH from the X-organ and/or by a concomitant inhibition of the GSH release from the brain/thoracic ganglia[66].

Influence of moulting hormones in reproduction

Crustaceans, with a few exceptions, continue to moult after attainment of sexual maturity. As egg production and moulting are important energy demanding processes, crustaceans have evolved an endocrine mechanism that would allow only one of these processes to occur at a time. Relegation of reproductive processes to intermoult period is important for utilization of reserve nutritive materials without any competition from moulting events such as new cuticle formation. In certain crab species, such as *Paratelphusa hydrodromus*, with annual breeding cycle, moulting never occurs during the breeding season[67], whereas in other decapods, such as the penaeid shrimps and the sand crab *Emerita asiatica*, moulting almost alternates with female reproductive cycle[68]. Furthermore, the incompatibility between moulting and reproduction is high in decapods like lobsters and crabs, where the egg development in the pleopod prevents moulting, but in broadcast spawners like penaeid shrimps, the moulting can possibly occur soon after spawning without a time gap.

In Crustacea, moulting and reproduction have been generally believed to be under the control of a bihormonal system[12]. This system includes a moulting hormone, crustecdysteroid synthesized in the Y-organ and an inhibitory neuropeptide (MIH) originating in the X-organ sinus gland complex[61]. This moult controlling system works in conjunction with a similar bihormonal mechanism controlling female reproduction. During the period of moulting, the animal is under the influence of the moulting hormone, without any intervention from moult inhibitory neuropeptide. Conversely, during the female reproduction the titre of MIH is high, keeping the moult activities under rest, but at the same time, allowing reproduction to proceed. Concurrently, the gonad inhibitory hormone is at its lowest ebb, and the gonad stimulatory neuropeptides are most active.

With the discovery of many more controlling factors in crustaceans, the above hypothetical regulatory mechanism on moulting and reproduction are placed under critical

Table 2. Naturally occurring vertebrate-type steroid hormones in the crustaceans

Steroid	Species	Detected sites	Ref.
17 β-Estradiol	*Penaeus monodon*	Ovary, hepatopancreas and hemolymph, where the concentration varies with the maturing stages of ovary	Faires *et al.*[109] Quinitio *et al.*[62]
	Parapenaeus fissures	Ovary	Jeng *et al.*[110]
	Macrobrachium rosenbergii	Ovary and hemolymph	Ghosh and Ray[111]
	Homarus americanus	Ovary and eggs	Lisk[112]; Couch *et al.*[113]
	Pandalus kessleri	Hemolymph, wherein the concentration varies according to ovarian maturity stages	Quinitio *et al.*[114]
	Nephrops norvegicus	Ovary, eggs and hemolymph	Faires *et al.*[115]
Progesterone	*Penaeus monodon*	Ovary, hepatopancreas and hemolymph	Quinitio *et al.*[62]
	Homarus americanus	Ovary	Couch *et al.*[113]
	Pandalus kessleri	Hemolymph with varying concentrations with ovarian maturation	Quinitio *et al.*[114]
	Homarus americanus	Testis and serum	Burns *et al.*[116]

review. Despite its purported role in egg production, the methyl farnesoate originating from the mandibular organ is shown to have its influence on moulting too[69]. Hormonal extract of MO from the crayfish, *Procambarus clarkii*, accelerated moulting in the shrimp, *Caridina denticulata*[70]. By co-culturing the Y-organs of the *Cancer magister* with the mandibular organ[33] or with (2E, 6E)-MF[71], an increased release of ecdysone into the culture medium was noticed. Obviously, the moulting gland is under the influence of a stimulatory factor from a glandular product (MF) and an inhibitory neuropeptide (MIH) from the eyestalk. Interestingly, the MO is itself under the inhibitory control of an eyestalk neuropeptide. The presence of a dual controlling mechanism on the Y-organ activity is highly advantageous for a temporal spacing of moulting from reproduction (egg production). Again, the seemingly complicated control mechanism operating on MO activity demonstrates how delicately moulting and reproductive events are related.

Conclusion and future directions

The concept of crustacean reproductive endocrinology has significantly changed in the recent years, thanks to the discovery of new endocrine organs as well as a host of new molecules with putative functions in the control of reproduction. For instance, the mandibular organ, synthesizing primarily the insectan JH-like substance, the MF, has turned out to be the major gonadotropic source for the control of ovarian maturation in many decapods. However, the old bihormonal concept of ovarian maturation by inhibitory and stimulatory neuropeptides originating from the central nervous system has not changed. Instead, a number of neuropeptides from the chief endocrine centres such as X-organ-sinus-gland complex, brain and thoracic ganglion have been isolated and their functional role in reproduction deciphered. It will be interesting to know if they also control the activity of other known gonadotrophic centres such as mandibular organ. However, available evidence indicates that the mandibular organ is controlled both positively as well as negatively by other eye stalk neuropeptides such as pigment dispersing hormone, and red pigment concentrating hormone.

The discovery of several isoforms of CHH-family neuropeptide has also revealed the complexity of crustacean reproductive endocrine control. This is especially true with penaeid shrimps. In *P. japonicus*, several of the CHH isoforms (CHH type I) showed significant inhibitory control over vitellogenesis. In the event of newer neuropeptides being discovered, with undescribed functional roles in reproduction, further work with more commercially important species is necessary. With the possibility of these neuroepeptides being synthesized in the laboratory, novel manipulative methods to enhance egg production could be in the offing.

Another feature of interest in the crustacean endocrinology is the role played by biogenic amines in the indirect control of reproductive activities. The biogenic amines such as 5-HT and dopamine either stimulate or inhibit the release of the neuropeptides concerned with the reproductive control. Again, the increasing evidence that the crustacean ovary responds to endogenous vertebrate-type steroids indicates the possibility of introducing new strategies in induced ovarian maturation in aquaculture

Table 3. Reproductive role of biogenic amines in crustaceans

Amines	Species	Functions	Ref.
Serotonin	*Uca pugilator*	Stimulation of ovarian development	Richardson *et al.*[65] Kulkarni and Fingerman[117]
		Agonists-induced ovarian maturation	Kulkarni and Fingerman[117]
		Stimulation of testicular development by 5-HT and its agonists	Sarojini *et al.*[118]
	Procambarus clarkii	Stimulation of ovarian maturation *in vivo* but *in vitro*	Kulkarni *et al.*[119]
		Stimulation of ovarian development during *in vitro* incubation with brain and thoracic ganglia but not with muscle	Sarojini *et al.*[66]
	Homarus americanus	Low enhancement of egg extruding process	Howard and Talbot[120]
	Libinia emarginata	Inhibits synthesis of MF from MO	Homola *et al.*[121]
Dopamine	*Uca pugilator*	No effect on ovarian development	Richardson *et al.*[65]
	Procambarus clarkii	Inhibits the ovary stimulating action of 5-HT	Sarojini *et al.*[122]
	Libinia emarginata	No effect on MF synthesis	Homola *et al.*[121]
	Homarus americanus	No effect on egg extruding process	Howard and Talbot[120]
Octopamine	*Procambarus clarkii*	No change in ovarian index or oocyte size	Kulkarni *et al.*[119]
	Homarus americanus	Strongly enhances the egg extruding process	Howard and Talbot[120]
	Libinia emarginata	Inhibition of MF synthesis	Homola *et al.*[121]
	Uca pugilator	No effect on ovarian development	Richardson *et al.*[65]
Norepinephrine	*Procambarus clarkii*	No change in ovarian index or oocyte size	Kulkarni *et al.*[119]

operations. Yet again, the mandibular organ is a common centre for stimulation of reproduction as well as Y-organ synthesis. Understandably, the co-ordination between reproduction and moulting is achieved by a delicate network of different hormonal factors including the neuropeptides and biogenic amines helping in the transduction of environmental cues to internal endocrine organs which ultimately control egg production and moulting.

To sum up, the crustacean egg production is controlled by a cascade of hormonal activities elaborated by both the central nervous system and the endocrine glands. Prominent among these are the neuropeptides as well as the biogenic amines which integrate the functional role of the proximate endocrine glands. Hence, it is recommended that any manipulative method to enhance egg production should involve the use of the neuropeptides/biogenic amines in order that other normal physiological activities of the crustaceans may not be disrupted.

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