CHAPTER III
LITERATURE REVIEW

3.1 Biology of the giant freshwater prawn, *Macrobrachium rosenbergii*

3.1.1 Biological classification and distribution

The giant freshwater prawn, *Macrobrachium rosenbergii*, is classified in phylum Arthropoda, subphylum Crustacea, class Malacostraca, order Decapoda, family Palaemonidae, genus *Macrobrachium*, and species *rosenbergii* by De Man (1879). The giant freshwater prawn is an important economic species in Asian countries, including India, Myanmar, Vietnam, Indonesia, Philippines, and Thailand (Tayamen, 2001). They are found in natural freshwater sources throughout the Pacific regions in the tropic zone (Figure 3.1). This prawn is a freshwater species but sometimes they live in brackish water, especially during reproduction and metamorphosis.

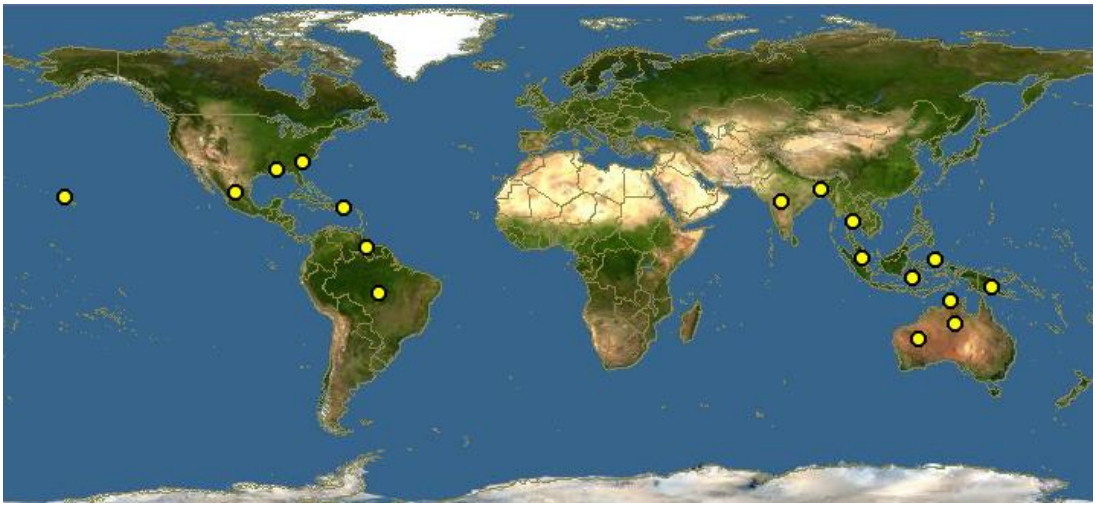

Figure 3.1 The distribution of *M. rosenbergii*. ○ shows the area where the giant freshwater prawn are found in nature habitats the tropical area. (http://www.discoverlife.org/20/m?kind=Macrobrachium+rosenbergii&b=EOL/pages/344690)

3.1.2 Morphology

The adult prawn has blue-green color, sometimes brown color may appear because the color is depending on the type of food being consumed (D'Abramo and Brunson, 1996). The male giant freshwater prawn is favored as it grows faster, reaching a larger size and having larger amount of flesh than female (Phoungpetchara et al., 2011).

Both sex can be distinguished easily, the male has large second periopods with terminal claws and larger head than female (Figure 3.2), as well as exhibiting appendix musculina and the opening of male gonopores at the base of the 5th periopods. In female, the gonopores open at the base of 3rd periopods, and the space between pairs of periopods is larger than male (Figure 3.3).

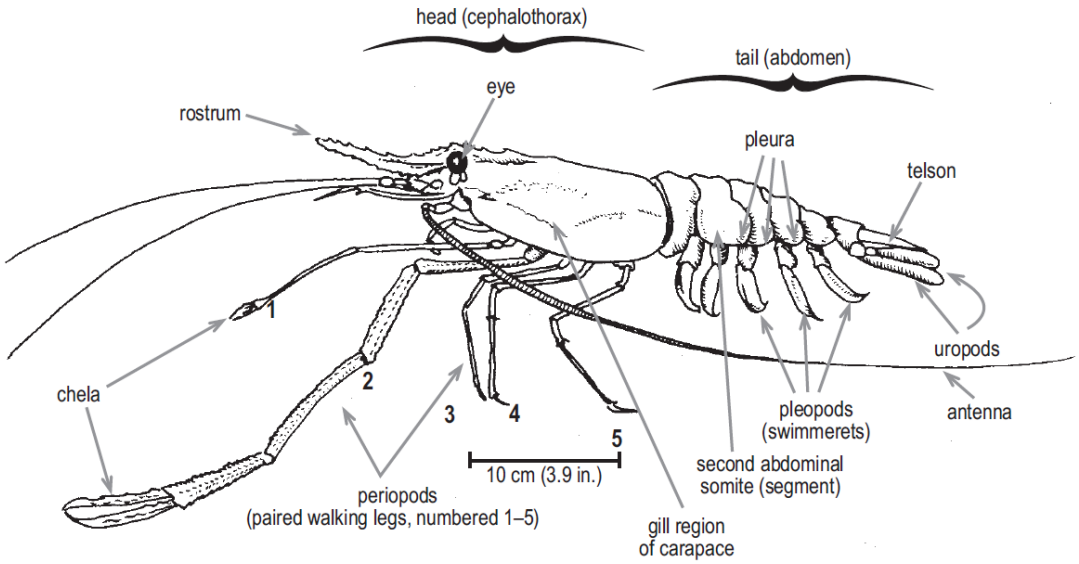

Figure 3.2 The External morphology of *M. rosenbergii*. The androgenic gland (AG) lies along the ejaculatory bulb at the base of the fifth periopods. (Nandlal and Pickering, 2005)

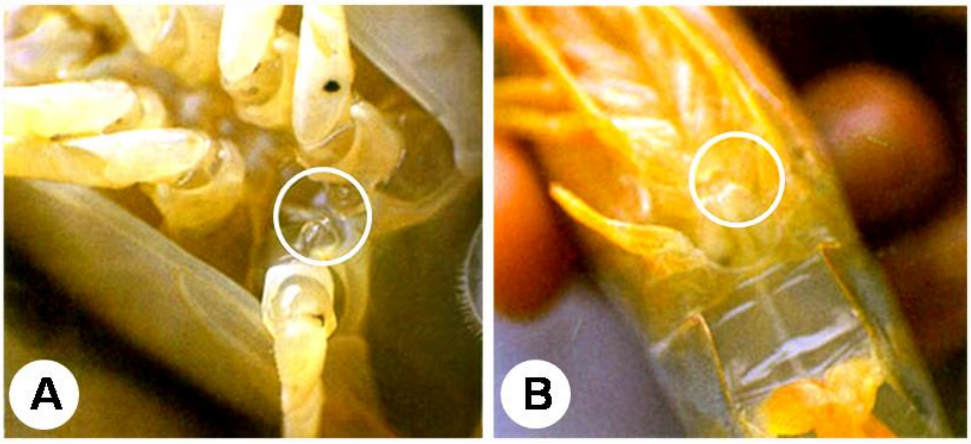

Figure 3.3 The difference of male and female gonopore in *M. rosenbergii*. (A) Male gonopore at the base of fifth periopod. (B) Female gonopore at the base of the third periopod. (Modified from http://203.172.205.22/web/thai Enclycropedia /book 13/b13p254.htm)

3.1.3 Male morphotype

The males are characterized into three morphotype, including blue-claw male (BC) or fully mature male, orange-claw male (OC), and small male (SM) (Okumura and Hara, 2004). BC is the reproductive stage, biggest in size with extra ordinarily large spiny blue claws. SM the smallest sneaking copulation stage (Okumura and Hara, 2004). OC is at medium size, reproductively less active than BC males, but are growing more rapidly than young SM and mature BC males (Okumura and Hara, 2004).

3.1.4 Breeding

Mature male and female around the age of 6 months have highest efficiency in breeding. Female prawns turn into soft-shelled molting female before mating; while male has hard-shell at mating. The male inserts the jelly-like sperm mass into the gap between female periopods and then female release eggs, after which fertilization occur. The eggs remain bound to the abdomen until hatching. There are four stages of fertilized eggs; bright-yellow, orange, brown and then grey which appear before hatching (D'Abramo and Brunson, 1996). After hatching, larval prawns are released and metamorphosis then occurs sequentially.

3.1.5 Life cycle and larval and post-larval stage

The larvae (L) have 11 stages of metamorphosis and 11 molting cycles (Figure 3.4). They are swimming upside down with tail first (D'Abramo and Brunson, 1996); sometimes they use their tails hanging to the surface planktons in brackish water, with salinities of 12 to 18 ppt. They feed on small planktons and each other (cannibalism). After last molt (25-40 days), the larvae may flip over from supine to prone position, as in adult. This flipping prawn is called post-larvae (PL) stage which lasts about 40 days.

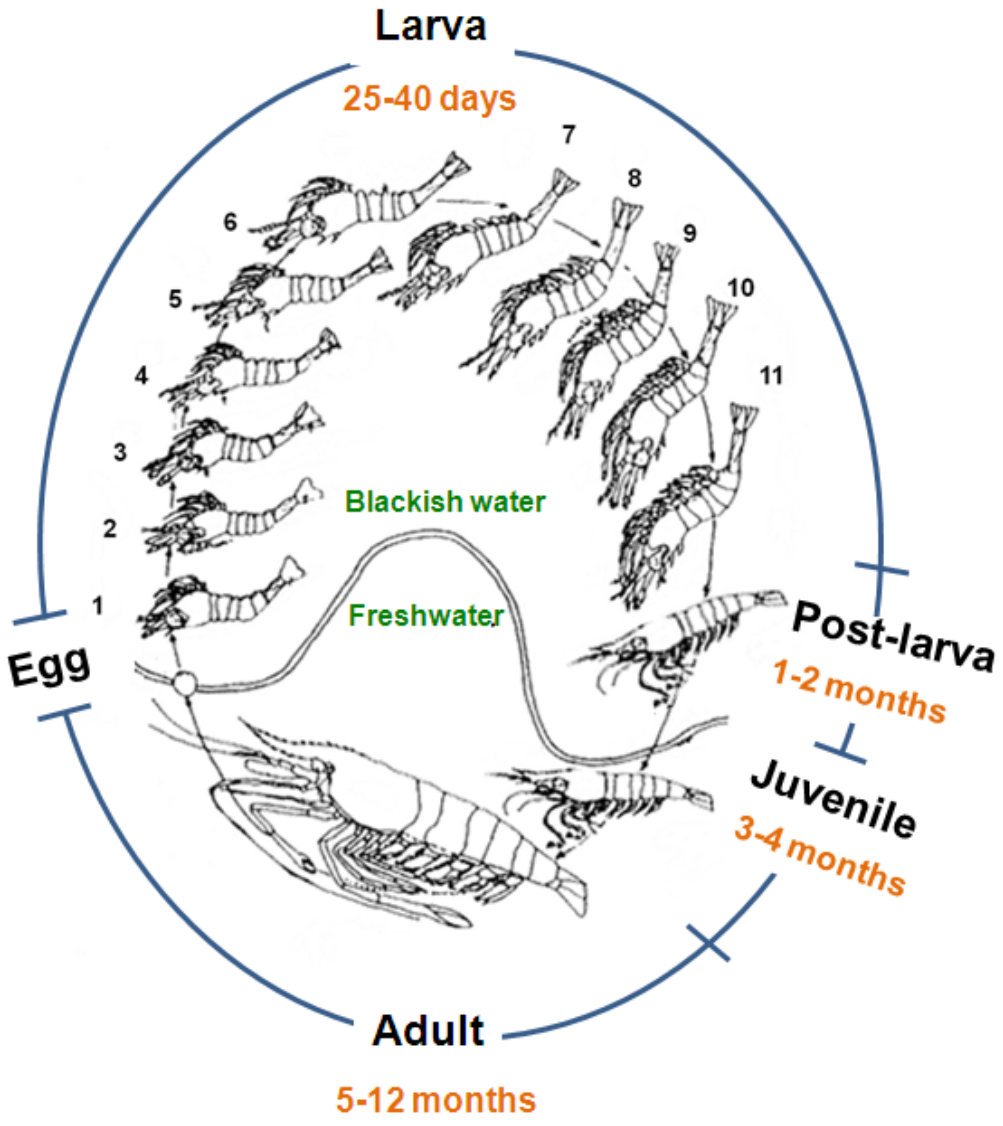

Figure 3.4 Life cycle of *M. rosenbergii*. (Modified from http://coursewares.mju.ac.th :81/elearning47/section2/fa301/img_ lession/l2/2-7.gif)

The PL prawns are translucent and look like adult prawn (Figure 3.5), but have less developed internal organs and external hard-shell. There are around 0.7 – 1.0 cm. in length. The survival rate is high in this stage because they changes to benthic animal that can feed on more variety of food than larvae, and the prawn can survive when transferred from brackish to fresh water. After growth and development for 40-45 days, the PLs become juveniles that begin to express secondary sex characteristics and become fully grown adult in 6 months after hatching.

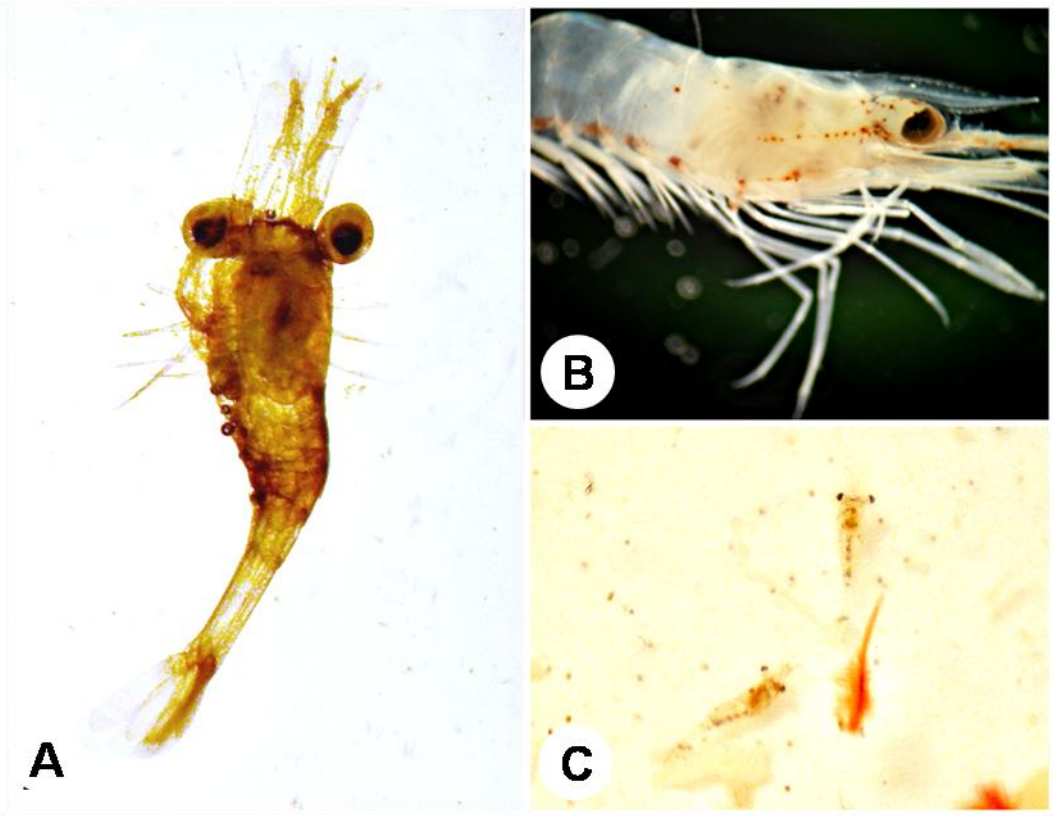

Figure 3.5 Larval and post-larval stages of *M. rosenbergii*. (A) Larva (L) in bright field. (B) Post-larva (PL) and (C) PL in swimming position captured in dark field microscope.

3.2 The male reproductive system

In male *M. rosenbergii*, the testes are located in the head under the carapace and divided into two parts; the anterior testis (Ant. Tes) and posterior testis (Post. Tes). The spermatic ducts (SD) have three parts, including proximal spermatic duct (PSD), middle spermatic duct (MSD), distal spermatic duct (DSD) and ejaculatory bulb (EB) that is linked to the androgenic gland (AG) (Figure 3.6). The end of EB is attached to the base of the fifth walking leg for sperm releasing through the gonopore (Phoungpetchara et al., 2011).

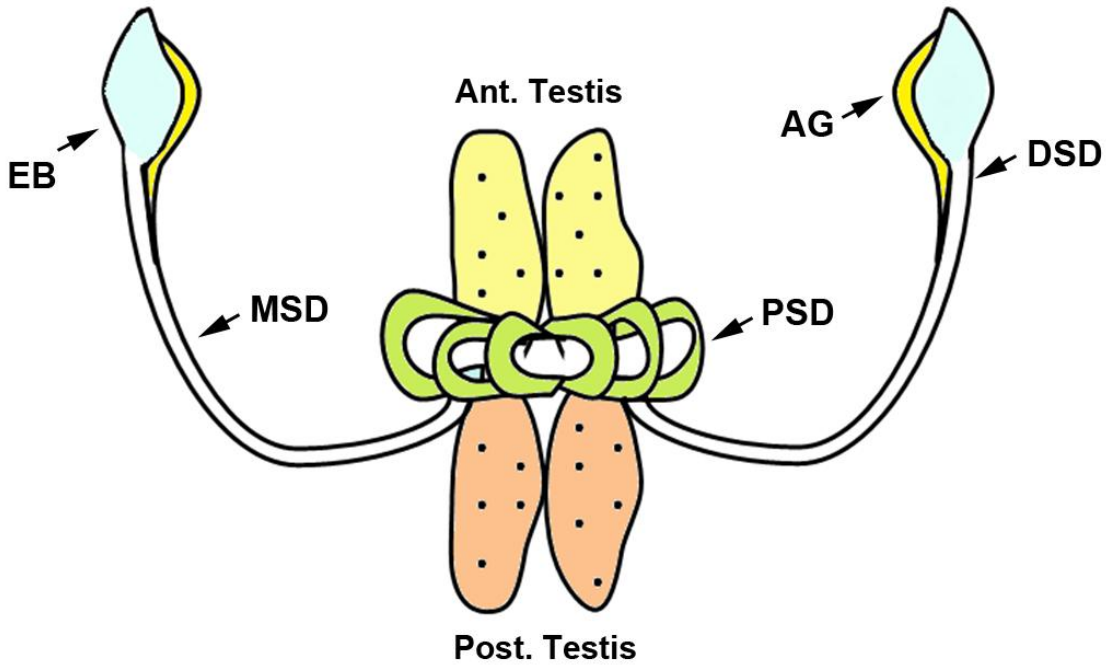

Figure 3.6 The male reproductive organs and location of the AG in an adult male prawn. Anterior testis = Ant. Tes; posterior testis = Post. Tes; proximal spermatic duct = PSD; middle spermatic duct = MSD; distal spermatic duct = DSD; ejaculatory bulb = EB; androgenic gland = AG

3.2.1 Structure of the testes

A pair of the testes in this prawn is composed of two parts: anterior and posterior (Okumura and Hara, 2004; Poljaroen et al., 2010) (Figure 3.6). It is located dorsally in the thorax between the hepatopancreas and the carapace. Each part of the testes is wrapped with thin connective tissues (CT) capsule (Figure 3.7 A) and contains numerous white seminiferous tubules (STs) and surrounded by intertubular area (IT). The epithelium of STs is sperm production site that consists of several stages of the developing germ cells (black arrowhead) (Poljaroen et al., 2010) (Figure 3.7 B).

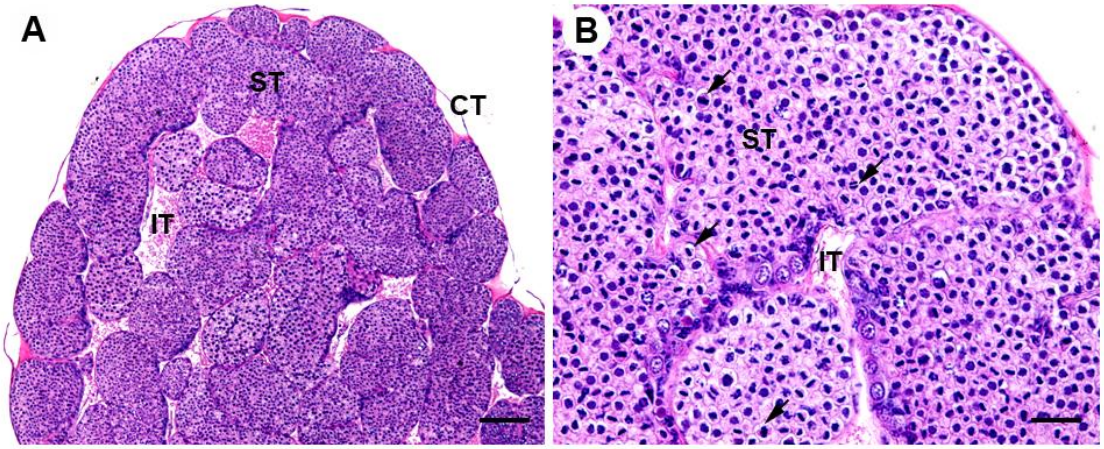

Figure 3.7 Structure of testis in adult male prawn which is composed of seminiferous tubules (STs). (A) Low magnification image shows location of connective tissues (CT) and intertubular area (IT). (B) High magnification image shows the developing germ cells within STs (black arrowed).

3.2.2 Seminiferous tubules (STs) and spermatogenesis

In all animal species, the epithelium of seminiferous tubule consists of (1) developing germ cells i.e. spermatogonia (Sg), spermatocytes (Sc), spermatids (St); (2) mature cell or spermatozoa (Sz); and (3) nurse cells (Nc) located close to the basement membrane.

Normally, spermatogenesis in mammals occurs in the seminiferous tubules (STs) following puberty, which starts from mitotic divisions of type B spermatogonia into primary spermatocytes (Sadler, 2009) (Figure 3.8). The primary spermatocytes (spermatocytes I) then go through meiosis I to produce secondary spermatocytes (spermatocytes II), during meiosis II the latter produce haploid spermatids which differentiate into spermatozoa that contain little cytoplasm (Sadler, 2009) (Figure 3.8). Furthermore, germ cells in STs are supported by Sertoli cells or nurse cells which maintain suitable microenvironment (niche) for developing germ cells (Sprando and Russell, 1987; Sadler, 2009). Therefore, each mammalian ST contains a mixture of developing germ cells and spermatozoa designated as cellular association, which can be classified into 14 stages in rodents (Ross and Pawlina, 2011), 12 stages in human (Ross and Pawlina, 2011).

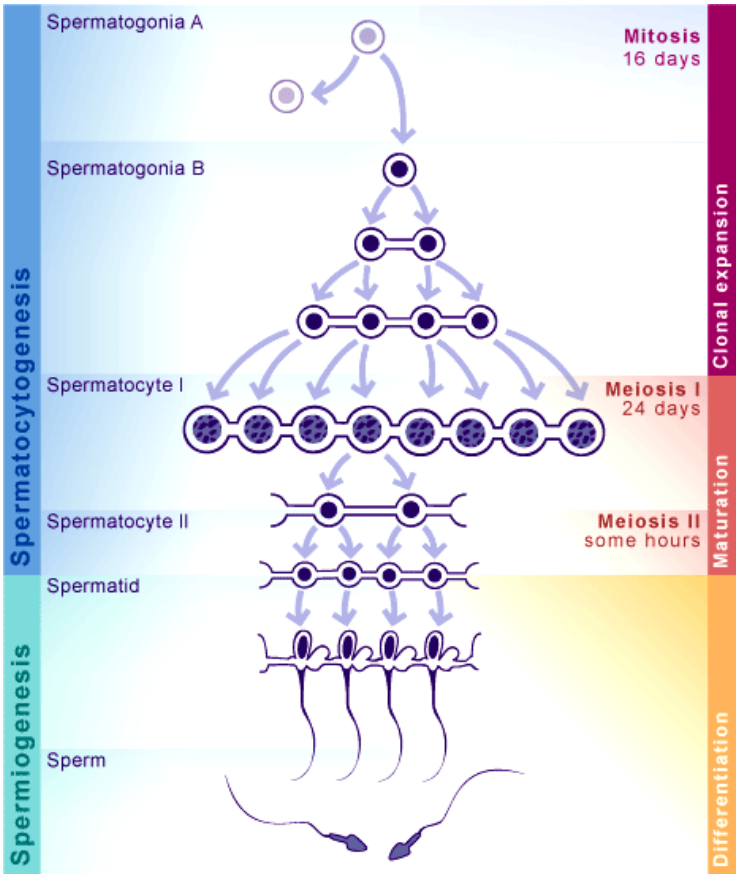

Figure 3.8 Mammalian spermatogenesis.
(http://www.embryology.ch/anglais/cgameto gen/spermato03.html)

In contrast, the STs of *M. rosenbergii* have been characterized into 9 maturation stages (i.e., stages I to IX), according to cellular association (Poljaroen et al., 2011) (Table 3.1). STs at stages I-V contain mostly spermatocytes (Table 3.1; Figure 3.9 A-E). STs at stages VI-VIII contain mostly spermatids (Table 3.1; Figures 3.9 F-H). STs at stage IX contain mostly mature spermatozoa with umbrella-like shape (Table 3.1; Figure 3.9 I). In all stages of STs, nurse cells are located close to the basement membrane of the tubule under the spermatogonia.

Table 3.1 Type of germ cells in the 9 stages of seminiferous tubules in *M. rosenbergii*

Stage of STs	Type of cell
I	leptotene spermatocytes
II	zygotene and pachytene spermatocytes
III	diplotene spermatocytes
IV	diplotene and metaphase spermatocytes
V	metaphase spermatocytes
VI	early and mid-stage developing spermatids
VII	late-stage spermatids
VIII	mature sperm (condensed chromatin)
IX	mature sperm (decondensed chromatin)

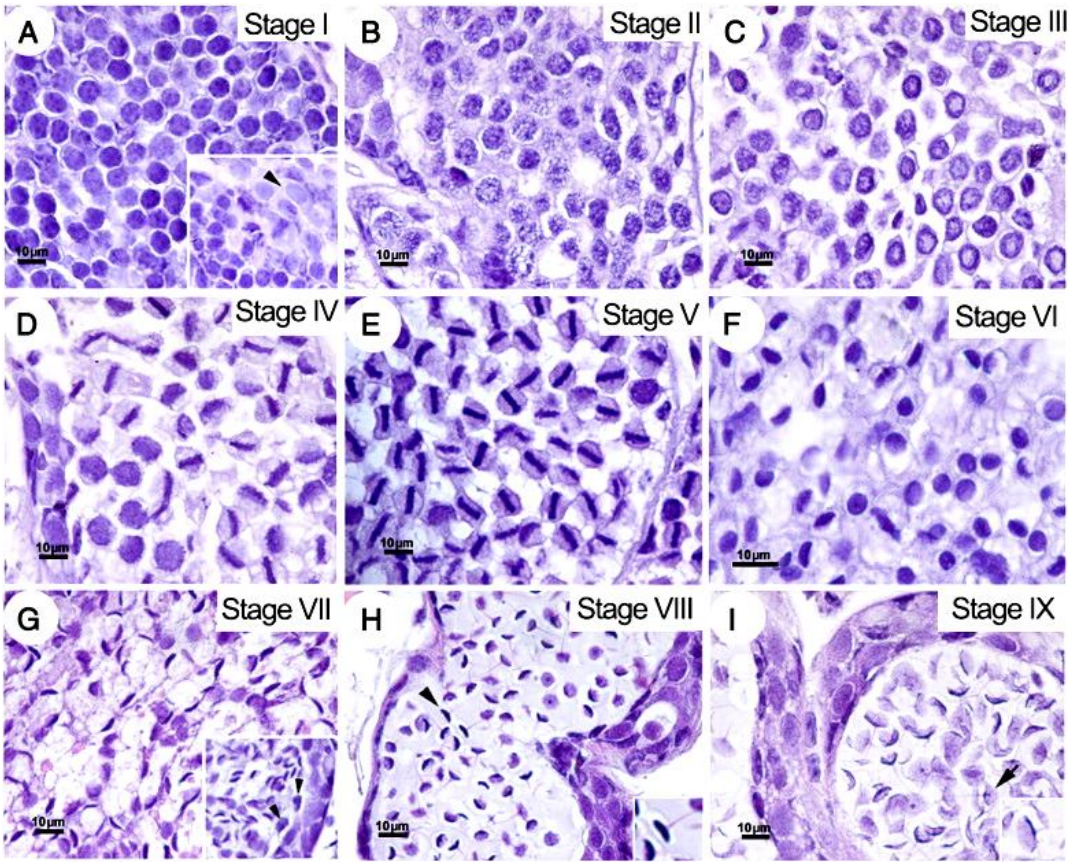

Figure 3.9 H & E stained micrographs showing 9 stages of seminiferous tubules in *M. rosenbergii*. (A-E) Stages I to V contain mostly primary and secondary spermatocytes. (F-H) Stages VI to VIII contain mostly spermatids (early, middle, and late spermatids). (I) Stage IX contain mostly spermatozoa with decondensed chromatin. In all stages, the nurse cells and spermatogonia are always located on the basement membrane (Poljaroen et al., 2011).

3.2.3 Structure of the spermatic duct (SD)

The spermatic duct is a sperm transportation tube that may contract to release the jelly-like sperm mass from the testis to the ejaculatory bulb. It is a connecting tube between testis and ejaculatory bulb (Figure 3.10 A). Each of spermatic duct is divided into 3 parts; proximal spermatic ducts (PSD) (coiled duct), middle spermatic ducts (MSD) (straight duct), and distal spermatic ducts (DSD) that continuous in to the ejaculatory bulb (Figure 3.10 A).

The PSD is the coiled duct lying beside the testis and, contains 2 striated muscular layers, including the inner longitudinal and the outer circular (Phoungpetchara et al., 2012). The epithelium type is a simple tall columnar with very high simple columnar epithelium (He) on one side (Phoungpetchara et al., 2012) (Figure 3.10 B-D). The MSD is the straight and long duct. It also contains inner longitudinal and outer circular muscle. The epithelium is also a simple columnar epithelium (Phoungpetchara et al., 2012) (Figure 3.10 E-G).

The DSD is the thickest part of SD. It contains thick inner longitudinal and outer circular layer muscle. The epithelium type is a simple columnar (Phoungpetchara et al., 2012) (Figure 3.10 H-J).

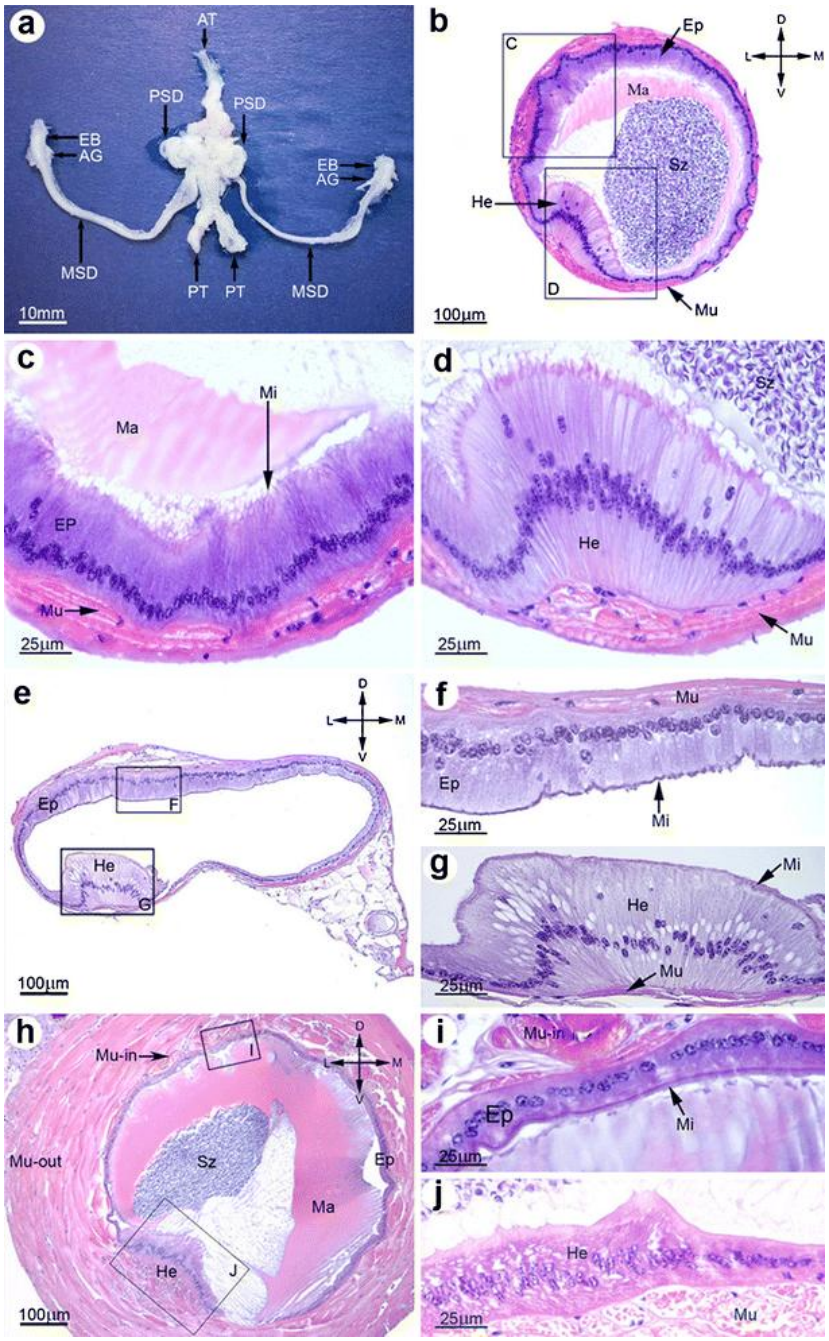

Figure 3.10 The overall structure (a) of the male reproductive organs, showing especially the SD. (b-d) The cross section of the PSD showing the high epithelium (He), inner longitudinal (Mi) and the outer circular (Mo). (e-g) The cross section of MSD and DSD (h-j) also show the same structure but different in the thickness of muscle and epithelium height. Ep = epithelium; Sz = spermatozoa; Ma = matrix. (Phoungpetchara et al., 2012)

3.2.4 The androgenic gland (AG)

3.2.4.1 Discovery of AG

Androgenic gland is an accessory endocrine gland that controls male sex differentiation in most crustaceans. The AG was first discovered by Cronin near the sperm duct of male blue swimming crab (*Callinectes sapidus*) (Cronin, 1947), but at that time the function of this gland was not known. In 1953-1955, this gland was discovered in amphipod, *Orchestia gammarella*, and named the androgenic gland (AG) by Charniaux-Cotton (Charniaux-Cotton, 1953, 1954, 1955). Moreover, she described that AG controls primary and secondary male sex characteristics through the experiments with AG removal and implantation in both sex of amphipod (Charniaux-Cotton, 1962). A terrestrial isopod, *Armadillidium vulgare*, was a successfully model in sex reversal, as the seminal vesicles and vas deferens occur in the AG-implanted females (Suzuki and Yamasaki, 1991). Similar experiments on AG ablation and implantation have been done in other crustaceans, including Australian red-claw crayfish *Cherax quadricarinatus* (Karplus et al., 2003; Manor et al., 2004), giant freshwater prawn *M. rosenbergii* (Nagamine et al., 1980; Ventura et al., 2009 and 2011; Phoungpetchara et al., 2011) and blue swimming crab *Portunus pelagicus* (Sroyraya et al., 2010).

3.2.4.2 Structure of AG

In adult male *M. rosenbergii,* AG is a triangular tissue mass surrounded by a thin connective tissue capsule attached to the EB, and it is composed of several lobules and three types of cells (Figures 3.11 and 3.12; Table 3.2). Type I cells are small, polygonal shaped-cells whose cytoplasm is deeply stained with H&E. They have abundant multilayered flattened rough endoplasmic reticulum (rER), and nuclei containing mostly heterochromatin. Type II cells are larger than type I, and have a polygonal shape. Their cytoplasm is lightly stained with H&E and the nuclei contain mostly euchromatin, the cytoplasm contains rER with dilated cisternae and few smooth endoplasmic reticulum (sER). Type III cells are similar in size to type I

cells, but the cytoplasm is much clearer because they are not stained with H&E, and their cytoplasm contains abundant sER (Phoungpetchara et al., 2011).

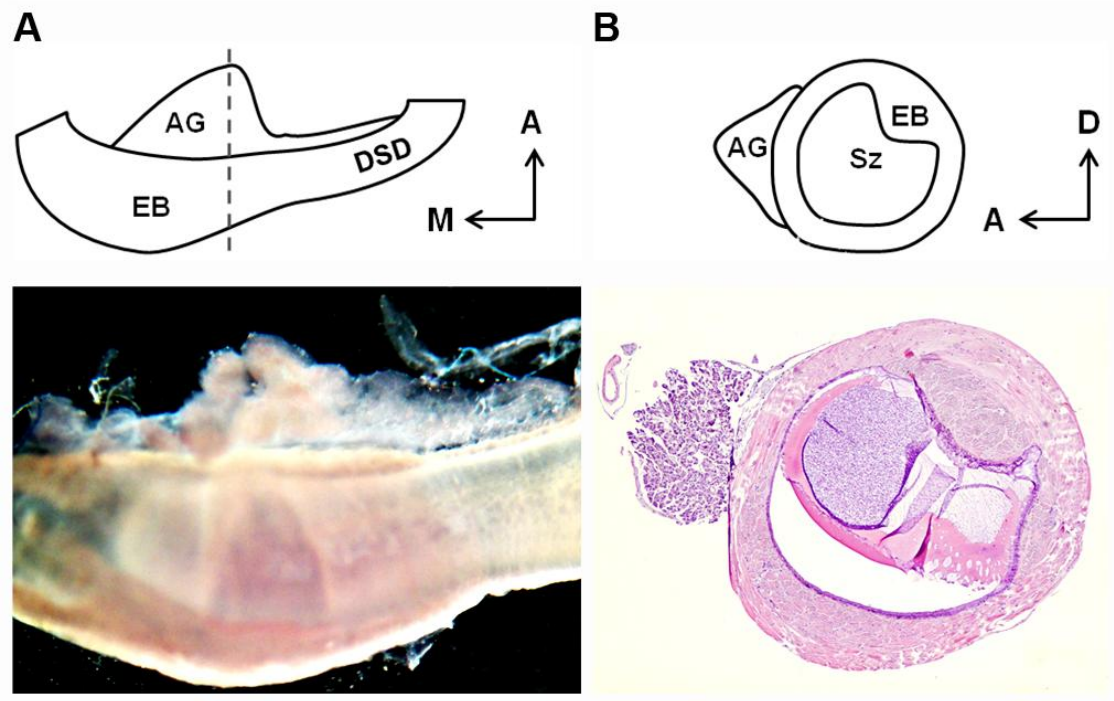

Figure 3.11 The structure of the spermatic ducts (DSD, EB) and AG showing (A) external morphology; and (B) H&E stained section at the dash line in (A). EB = ejaculatory bulb; DSD = distal spermatic duct; Sz = spermatozoa; A = anterior; M = medial; D = dorsal

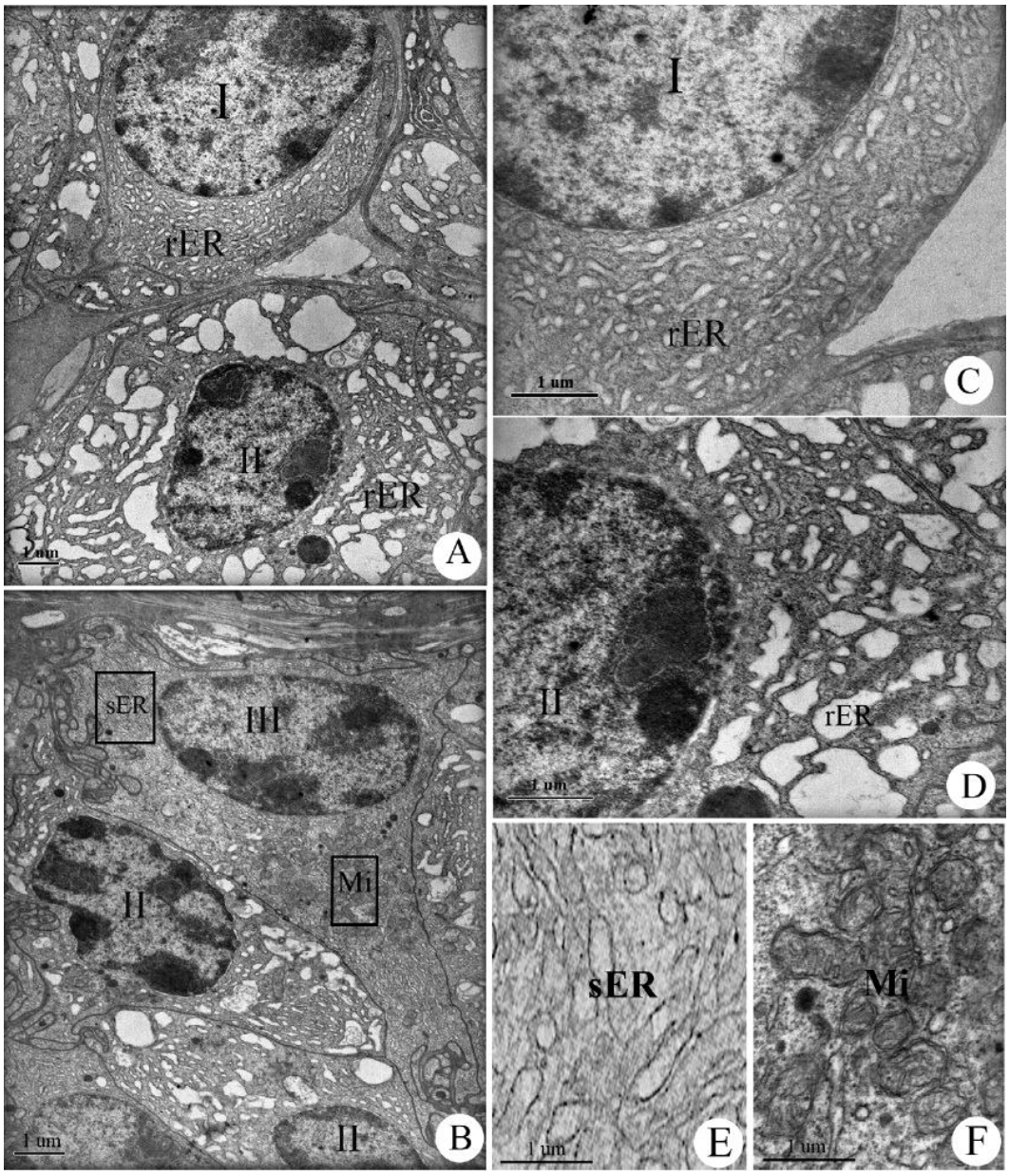

Figure 3.12 TEM electron micrographs of the AG cells in *M. rosenbergii*.

(A, C) Type I cells contain flattened rough endoplasmic reticulum (rER).

(A, D) Type II cells contain rER and a clock face nucleus.

(B, E) Type III cells contain fewer rER, sER and mitochondria (Mi).

(Modified from Phoungpetchara et al., 2011)

Table 3.2 The three types of AG cells in adult male *M. rosenbergii* which are different in sizes, characteristics, and possible function. (Phoungpetchara et al., 2011)

	Type of AG cell		
	I	**II**	**III**
Size (diameter**)**	13.42 ± 1.7 µm	18.58 ± 2.39 µm	13.97 ± 1.37 µm
Shape	polygonal shaped-cells	polygonal shaped-cells	polygonal shaped-cells
Feature	- cytoplasm deeply stains with H&E - nuclei containing mostly heteroch romatin - abundant rER	- cytoplasm lightly stains with H&E - nuclei containing mostly euch romatin - prominent nuclei - exhibit rER with dilated cistenae	- clear cytoplasm - exhibit abundant sER - mitochondria with tubular cristae
Population	50.6 ± 1.14%	48..2 ± 0.84%	1.2 ± 0.45%
Finction	protein synthesis	protein synthesis	steroid synthesis

3.2.4.3 AG structure in each morphotype

The AG is highly developed and appears largest in a blue-claw male, but less developed in orange-claw males and small males (Okumura and Hara, 2004). However, there are no different in cell types between the AG in the three morphotypes (Figure 3.13). The morphological characteristics of the three cell types and their actual appearances are illustrated in Figures 3.12 from the report by Phuangpetchara et al., (2011).

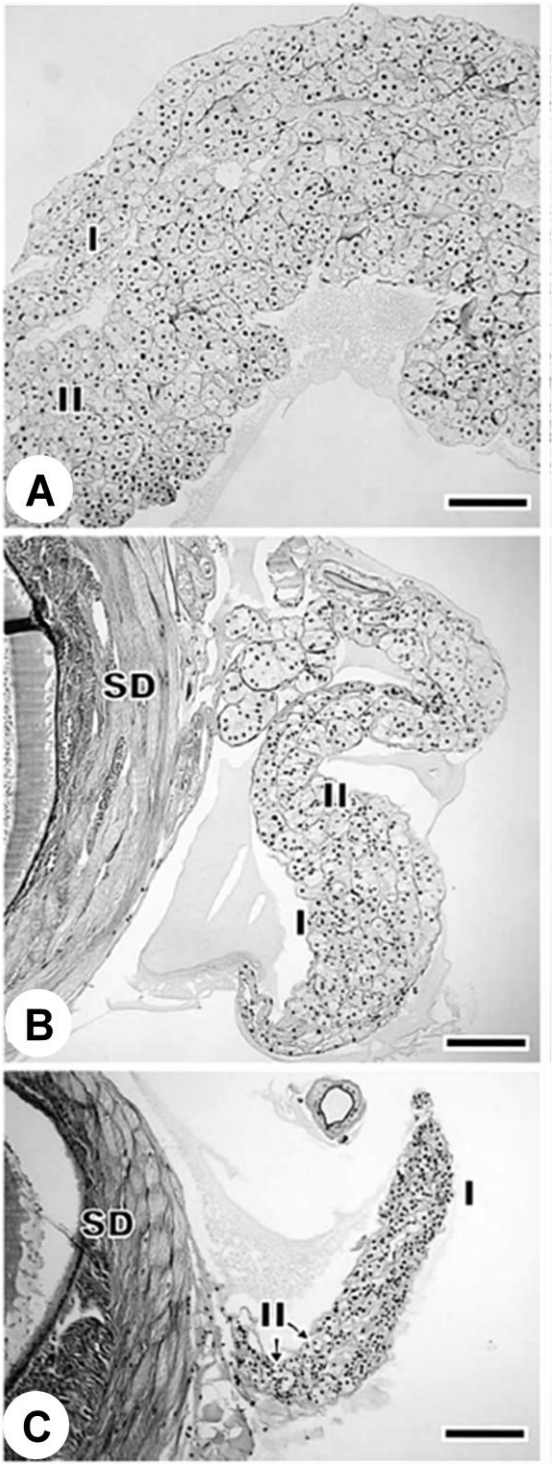

Figure 3.13 The AG in three male morphotype of *M. rosenbergii*. (A) Blue-claw male AG is well developing, (B) orange-claw male and (C) small male is in developing process. (Modified from Okumura and Hara, 2004)

3.2.5 The AG hormone (AGH) and insulin-like androgenic gland factors (IAGs)

The AGH is a crustacean peptide hormone with 17 kDa in molecular weight. It was firstly identified in *A. vulgare* (Hasegawa et al., 1987) and confirmed by Martin et al., 1990 (Martin et al., 1990). The cloned cDNA of AG was successful obtained in *A. vulgare* several years after (Okuno et al., 1999).

AGH is synthesized as a preprohormone that consists of a signal peptide that is cleaved off into B, C and A chains. Three disulfide bonds are found as inter-chain and intra-chain linkages. This structure resembles insulin-like peptides. So, this peptide is called "AG-specific insulin-like peptide" (Figures 3.14 and 3.15).

Using subtractive suppression hybridization (SSH) of cDNA from AG and peripheral glands (a mix of mandibular and Y-organs). Insulin-like peptide was identified in *C. quadricarinatus* (*Cq*) and named *Cq*IAG (*C. quadricarinatus* insulin-like androgenic gland factor) (Manor *et al*., 2007) (Figures 3.14 and 3.15). They also found that *CqIAG* was expressed at the early stage of male juvenile prawn (about 8 days after released from the mother). Two years later, *M. rosenbergii* AG hormone (*Mr*IAG) was found by using SSH of cDNA libraries (Ventura et al., 2009) with full-length sequence. Moreover, the authors test the function of *MrIAG* in male by silencing this gene. In recent year, IAG from other decapod crustaceans were discovered including in black tiger shrimp *P. monodon* (Mareddy et al., 2011), blue swimmer crab *P. pelagicus* (Sroyraya et al., 2010), and blue crab *C. sapidus* and their phylogenetic relationship could be developed (Chung et al., 2011) (Figure 3.16).

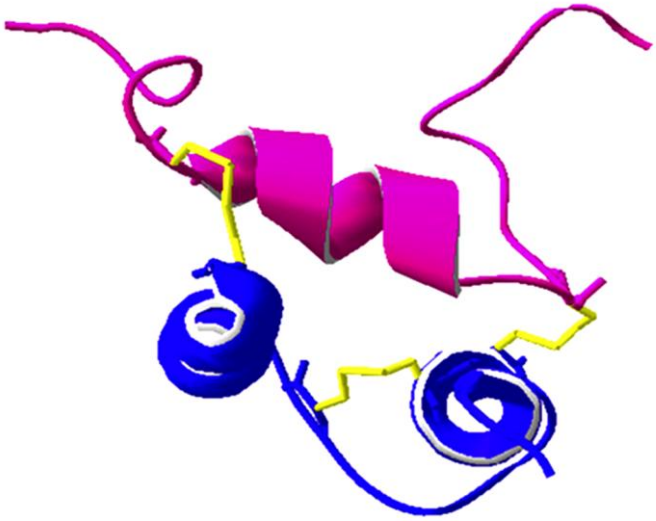

Figure 3.14 Three-dimensional model of *CqIAG* was constructed by EsyPred3D and edited by Swiss-PdbViewer. The 3D model of mature CqIAG is based on insulin-like peptide from the silk moth *Bombyx mori*. (Modified from Ventura et al., 2011)

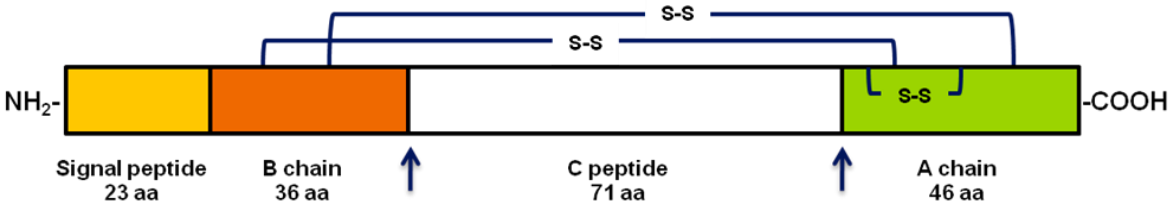

Figure 3.15 Linear model of *CqIAG* representing the preprohormone that consists of signal peptides, B chain, C peptide and A chain. (Modified from Ventura et al., 2011)

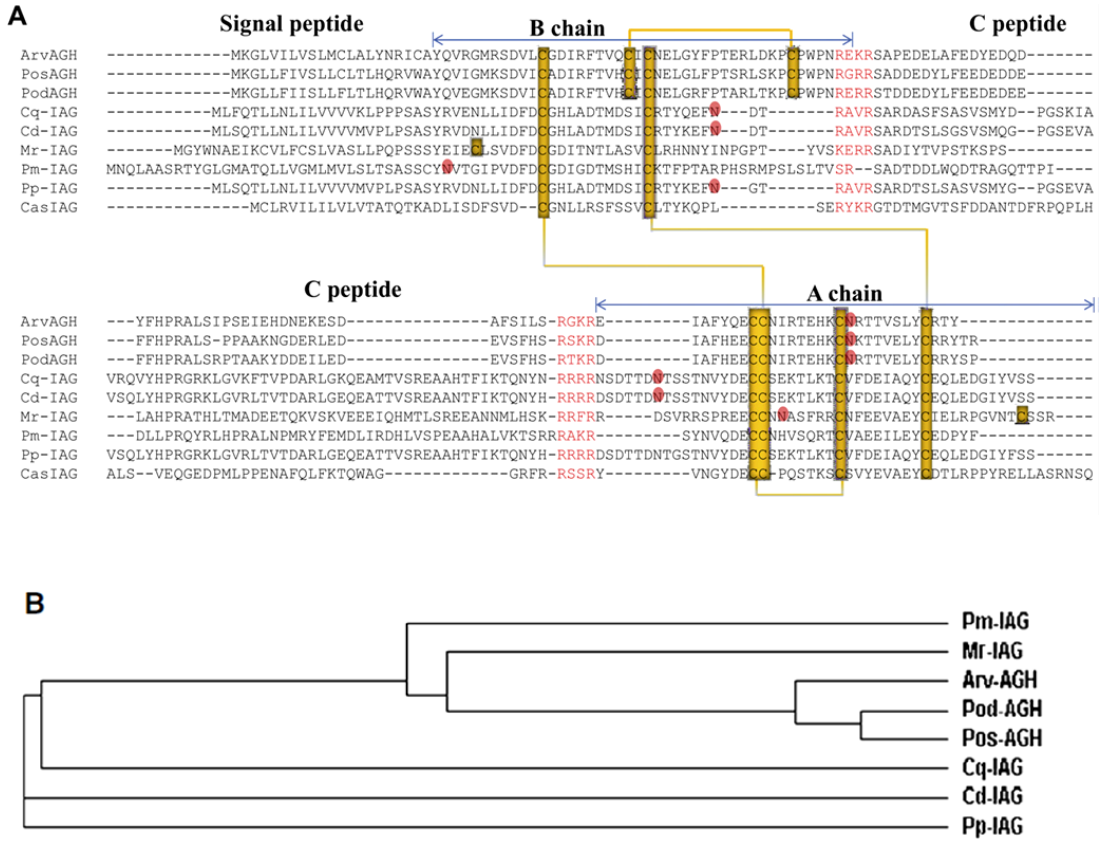

Figure 3.16 (A) Multiple sequence alignment of IAGs using ClustalW analysis (Modified from Ventura et al., 2011). (B) Cladogram of IAGs from 5 decapod and 3 isopod species using ClustalW analysis. (Modified from Mareddy et al., 2011)

3.2.6 The functions of insulin-like androgenic gland factors (IAGs)

In *M. rosenbergii*, *MrIAG* could be silenced by injections of *MrIAG* dsRNA to juvenile males. After the injection the male characteristics, including appendix masculina and spermatogenesis were decreased (Ventura et al., 2009). Full sex reversal was described in *C. quadricarinatus* by applying *CqIAG* silencing to create intersex prawn. Thus the authors suggested that this gene is a key for controlling male sex. As well, after injecting with *CqIAG* RNAi spermatogenesis and testicular function were inhibited but vitellogenesis occured (Figure 3.17 A). Moreover, the prawn changed the setae on the pleopods to ovigerous seta that is a part of maternal care-related adaptation behavior (Rosen et al., 2010) (Figure 3.17 B).

The manipulation of the sex reversal of males has been tried in various other crustaceans with variable results as summarized in Figure 3.18.

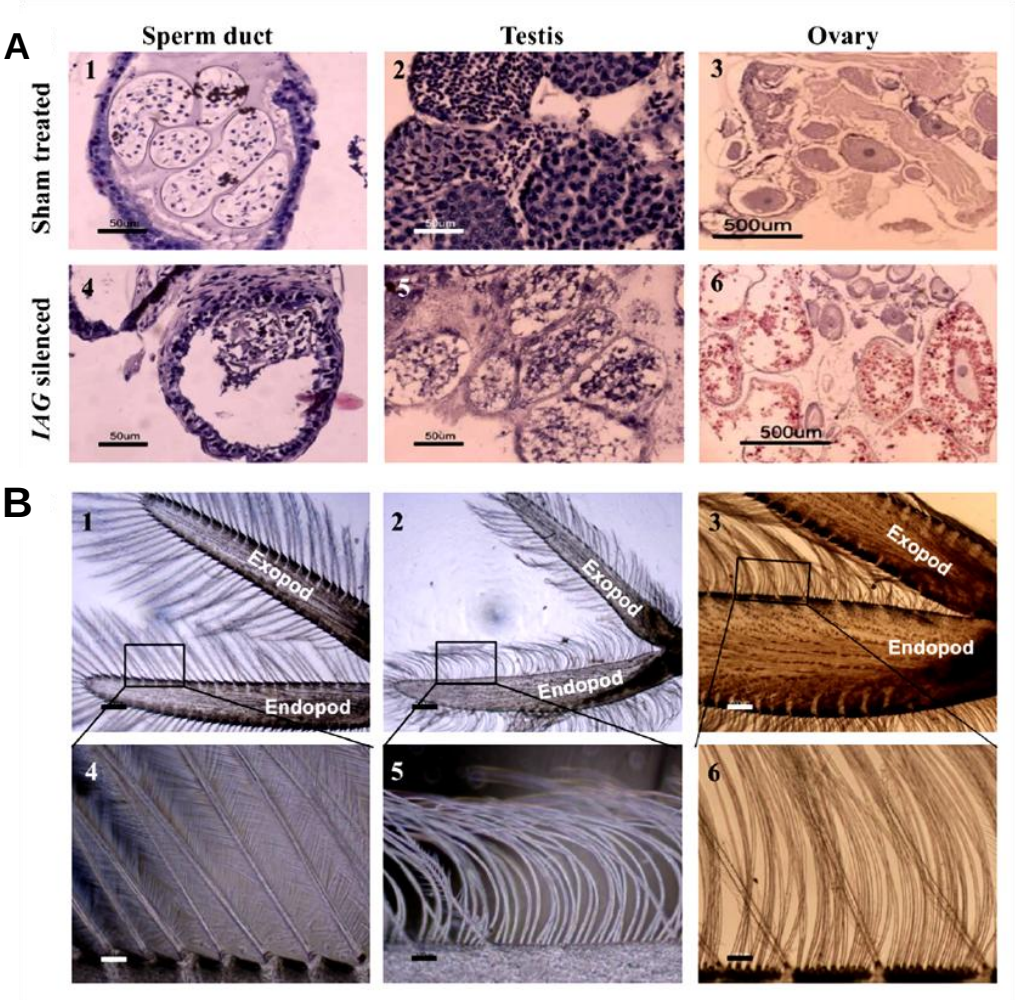

Figure 3.17 The morphological change of testis and spermatic duct during sex reversal in intersex prawn in *C. quadricarinatus* using *CqIAG* silencing. (A) Testis degeneration and oogenesis occurs and spermatic turns into oviduct. (B) Setae at the pleopods change to ovigerous type setae. (Modified from Rosen et al., 2010)

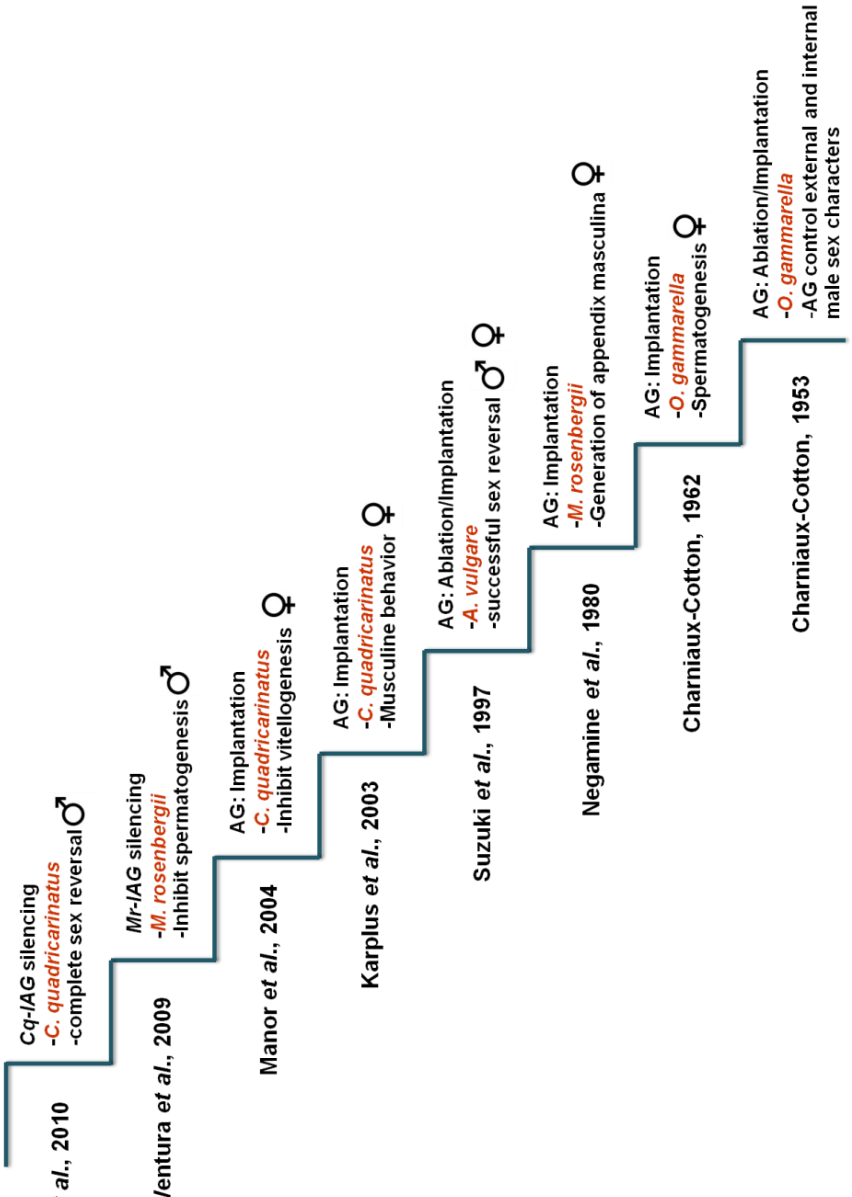

Figure 3.18 Summary of androgenic gland manipulation experiments in crustaceans up to the present.

3.3 The neuro-endocrine controls of crustacean male reproduction and spermatogenesis

We believe that the main axis of neuroendocrine control of male reproduction exercised by the brain is most likely conserved in higher vertebrates and invertebrates. Since most studies have been carried out in vertebrates, especially mammals, we would like to review the central neuro-endocrine control of reproduction in mammals as a working model.

3.3.1 Hypothalamic-pituitary-gonadal axis (HPG axis) in mammals

The hypothalamic-pituitary-gonadal axis (or HPG axis) is the interactive relationship among the hypothalamus, pituitary gland, and gonads. This axis controls the development and reproduction in vertebrates, which is active in both sexes during three parts of life, i.e.,the fetal, neonatal, and puberty period.

The HPG axis is composed of hypothalamus, anterior pituitary gland, and gonads (Figure 3.19). The pulsatile secretion of gonadotropin-releasing hormone (GnRH) from GnRH cells is controlled by the Kisspeptin via kisspeptin-GPR54 (kisspeptin or KISS1 receptor) signaling pathway. Mutations of GPR54 result in downregulation of pulsatile GnRH secretion and infertility (Matzuk and Lamb, 2008). Kisspeptin is represented by *Kiss1*, which is secreted from the neurons in the anteroventral periventricular (AVPV) and arcuate (ARC) nucleus of the hypothalamus. Kisspeptin secretion is under the control of leptin because leptin receptor is expressed in kisspeptin neurons of Arcuate Nucleus. Leptin is synthesized and secreted by adipocytes, which are the fat storage cells. The function of leptin is dependent on the nutritional status, especially the levels of lipids in fat cells and blood circulation. The leptin deficient ob/ob mice are infertile or having a delayed puberty (The Giovanni's house, 2011).

The GnRH interacts with the GnRH receptor (GnRHR) on the membranes of gonadotrophic hormone-producing cells and stimulates the syntheses and secretions of FSH and LH. In male, FSH stimulates Sertoli cells to produce androgen-binding protein (ABP) that binds and raises the local (testicular concentration) of testosterone, and LH activates the Leydig cells to produce testosterone that stimulates the processes

of spermatogenesis. In turn, testosterone also controls the GnRH level by negative feedback (Matzuk and Lamb, 2008).

In female the HPG axis acts similarly. However, FSH acts on the oocytes and follicular cells to promote the formation of oocyte follicles and the synthesis of female sex steroid (estrogen), while LH acts on thecal cells and granulosa cells to synthesize progesterone. These two female sex steroids are involved in the controls of endometrium which is the implanting and nourishment site for embryo and fetus. As well, they also control female external genital differentiation and growth.

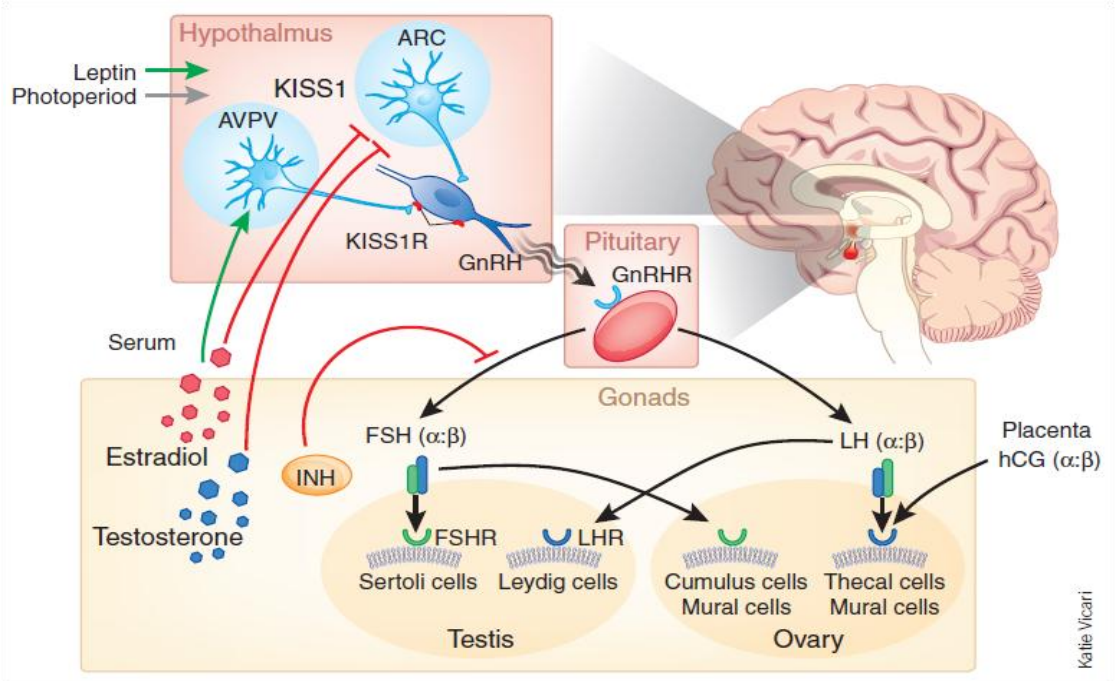

Figure 3.19 The hypothalamic-pituitary-gonadal axis in mammals. The pulsatile secretion of GnRH is controlled by the Kisspeptin (KISS1), secreted from the neurons in the anteroventral periventricular (AVPV) and arcuate (ARC) nucleus of the hypothalamus. The GnRH act on the GnRH receptors in cells of the pituitary gland that secretes FSH and LH to control the synthesis and secretion of sex steroids in the testis and ovary. (Modified from: Matzuk and Lamb, 2008)

3.3.2 The higher control of reproduction in Crustaceans

Major neurotransmitters that may be involved in the higher control of the male gonadal development and gamete production are serotonin (5-HT) and dopamine (DA) (Sarojini et al., 1995; Fingerman, 1997; Tinikul et al., 2008, 2009). In addition, neurohormones that could exercise control over testicular development may include GSH which is still unidentified, and possibly is the GnRHs. It is not yet known whether these two hormones exercise similar control over the AG, testicular development as well as other male characteristics. A hypothesis for this network of control was proposed by Sobhon, 2014 (Unpublished data) (Figure 3.20).

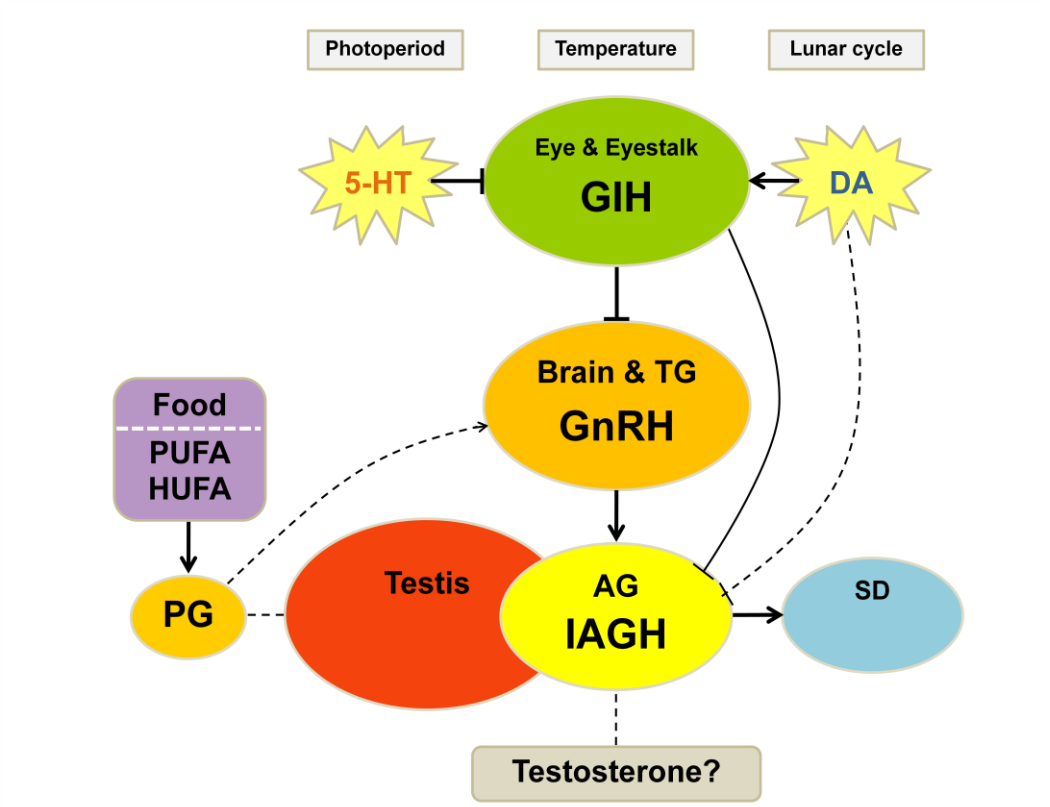

Figure 3.20 A diagram showing possible roles of Eye-Brain/Thoracic ganglia-Androgenic gland-Testis axis in male decapods crustacean. The secretion of GIH from the eyestalk is controlled by antagonistic neurotransmitter 5-HT (inhibitory effect) and DA (stimulatory effect), secreted from the CNS and eyestalk. The GIH has an inhihitory effect on GnRH and IAG secretion both directly and indirectly. Secretion of GnRH from the brain and TG stimulates AG function to control male sex characteristic, enlargement of the SD, testis maturation, spermatogenesis, and secretion of testosterone. Food, especially unsaturated fatty acids (PUFA, HUFA) may directly control the development of testis and the AG or mediated by prostaglandin especially PGE_2 and PGF_2. PGs may also provide a positive feedback on GnRH synthesis and secretion as reported in vertebrates. This diagram also shows the extrinsic factors that controls reproduction, including photoperiod, lunar cycle, and temperature. (Modified from Sobhon, 2014)

3.3.2.1 Serotonin (5-HT)

5-HT is found in the central nervous system (CNS) of crustaceans, including *M. rosenbergii, Homarus americanus, P. clarkii,* and *L. vannamei* (Fingerman et al., 1994; Tinikul et al., 2008). Injection of 5-HT increases ovarian maturation, oocyte diameter and ovarian-somatic index (Kulkarni et al., 1992; Sarojini et al., 1995) in *P. clarkii* (Sarojini et al., 1995), *L. vannamei* (Vaca and Alfaro, 2000), *L. stylirostris* and *L. vannamei* (Alfaro et al., 2004), *P. monodon* (Wongprasert et al., 2006), and *M. rosenbergii* (Meeratana et al., 2006; Tinikul et al., 2009). Similarly in male, 5-HT increases the testis-somatic index (TSI), diameter of the tubules (DTT), and cell proliferation in a small male *M. rosenbergii* (Poljaroen et al., 2011). At present, it is not known how 5-HT controls the gonad inhibitory hormone (GIH), molt inhibiting hormone (MIH), and mandibular organ inhibiting hormone (MOIH) as well as IAG.

3.3.2.2 Dopamine (DA)

DA is also found in various parts of the CNS of *Pacifastacus leniusculus* (Laxmyr, 1984), *L. vannamei* (Tinikul et al., 2011) *P. clarkii* (Mercier et al., 1991; Alvarez et al., 2005). DA delays ovarian maturation (Kulkarni et al., 1992; Sarojini et al., 1995) in *Uca pugilator* (Sarojini et al., 1995), and *M. rosenbergii* (Chen et al., 2003; Tinikul et al., 2009). In addition, DA delays testicular maturation in *U. pugilator* (Sarojini et al., 1995) and small male *M. rosenbergii* (Poljaroen et al., 2011). Like in case of 5-HT, the inhibition mechanism of DA is still unknown.

3.3.2.3 Eyestalk hormones (GIH, MIH, MOIH, CHH)

The eyestalk in crustacean is a very important region of the central nervous system (CNS) because it can produce the hormones that moderate several physiological parameters of the prawn including reproduction. The eyestalk ablation could stimulate ovarian and testicular maturation, spermatogenesis, and also hypertrophy of the AGs (Nagaraju, 2011; Phoungpetchara et al., 2011; Treerattrakool et al., 2014). There are 4 major neurohormones belonging to the CHH-peptide family synthesized by X-organ-sinus gland complex (XO-SG) of the eyestalk, including

gonad-inhibiting hormone (GIH), molt-inhibiting hormone (MIH), mandibular organ-inhibiting hormone (MOIH), and crustacean hyperglycemic hormone (CHH).

GIH is produced in the medulla terminalis X-organ (XO) and stored in the sinus gland (SG) (Treerattrakool et al., 2014). In female, GIH could inhibit vitellogenin synthesis that is the key factor for ovarian maturation (Wilder et al., 2010). It was found that eyestalk ablation in male *L. vannamei* increased testis size because of GIH and MIH were absent (Nagaraju, 2011).

MIH is synthesized in the medulla terminalis XO as well and then transported to the SG (Huang et al., 2014). Eyestalk ablation could increase ecdysteroid in the hemolymph and molting behavior in *U. pugilator* (Hopkins, 1982). Moreover, injection of the eyestalk extract could decrease ecdysteroid and also delay molting in *Homarus americanus* (Bruce and Chang, 1984; Chang et al., 1987). Therefore, it was suggested that MIH controls molting and ecdysteroid production. In female *Scylla paramamosain*, the MIH mRNA increased from stage 1 to 3 of ovarian cycle (Huang et al., 2014), therefore, MIH might also play some part in controlling the ovarian maturation.

MOIH controls the mandibular organ (MO) functions and the secretion of methyl farnesoate (MF) (Nagaraju et al., 2005). MF is a hormone that is involveed in reproduction, molting, and morphogenesis (Nagaraju et al., 2005). It has been reported that purified MOIH inhibits MF synthesis by inhibiting cytosolic S-adenosyl-L-methionine, farnesolic acid methyltransferase enzyme in MO (Wainwright et al., 1999, Nagaraju et al., 2005).While MF could assist in stimulating ovarian and testicular maturation.

CHH controls hemolymph glucose level (Aquiloni et al., 2012). It is also involved in lipid metabolism (Santos et al., 1997), stress responses in *Palaemon elegans* (Chang et al., 1999; Lorenzon et al., 2000; Lorenzon et al., 2004), and also indirectly reproduction (De Kleijn et al., 1998; De Kleijn and van Herp, 1998).

3.3.2.4. Gonad-stimulating hormone (GSH)

GSH is a putative hormone thought to be produced in the brain and thoracic ganglia; however, its real identity is still undetermined. It was reported that the extract from brain and thoracic ganglia could induce ovarian growth both *in vivo* and *in vitro* (Fingerman, 1997). Implantation of brain tissue also increased the development of oocytes and induced ovarian growth in *Paratelphusa hydrodromus, Parapenaeopsis hardwickii, M. kistnesis, Paratya compressa* and *L. vannamei* (Gomez, 1965; Kulkarni et al., 1981; Sarojini et al., 1983; Takayanagi et al., 1986; Yano and Wyban, 1993). In male, injection of the TG extract could induce hypertrophy of the AGs, increase gonad index and vas deferens, and stimulate spermatogenesis in *Potamon koolooense* (Joshi and Khanna, 1984). Since our immuhistochemical studies revealed very strong immunoreactivities of many GnRH isotypes in the brain and thoracic ganglia, and these GnRHs could also stimulate ovarian maturation (Ngernsoungnern et al., 2008 and 2009), we believed that GSH may actually be GSH.

3.3.2.5 GnRHs in invertebrates

GnRHs are principal decapeptides that regulate the reproduction in vertebrates and invertebrates, with endocrine and neuromodulatory roles (Sherwood and Adams, 2005; Tsai, 2006; Roch et al., 2011). In vertebrates it is synthesized by GnRH neurons in the hypothalamus (Radovick et al., 1991; Whitlock, 2005). In particular, vertebrate GnRHs contains N-terminal pyro-glutamate and amidated C-terminal end (Roch et al., 2011) (Figures 3.21 and 3.24). There are 10 amino acid (decapeptide) with highly conserve 4 amino acids at positions 1-4, 9, and 10, arranged in a horse-shoe shape structure which is bended at the glycine position 6 (Gly6) (Figures 3.21 and 3.24). In invertebrates the GnRHs are 12 amino acid peptides (dodecapeptide) (Roch et al., 2011; Millar and Newton, 2013) (Figure 3.24).

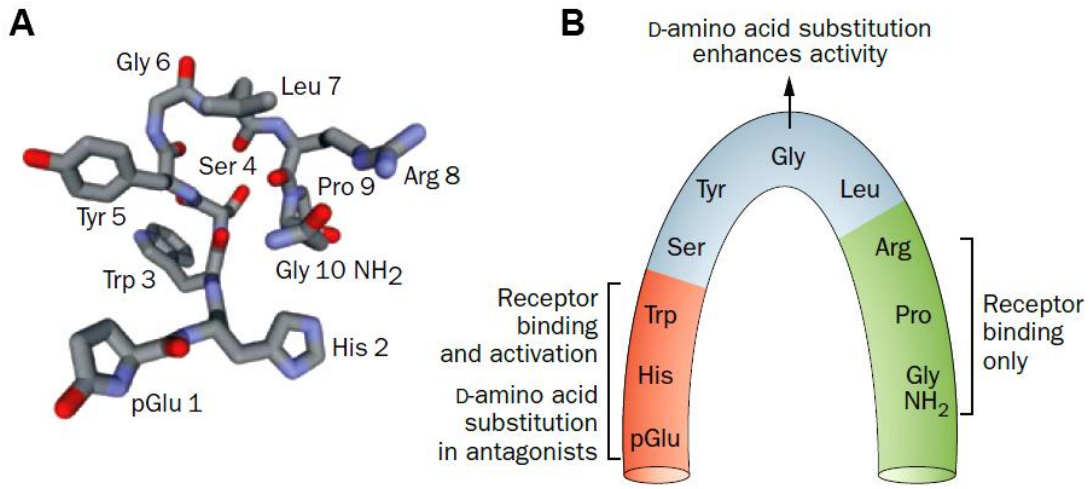

Figure 3.21 The structure of vertebrate GnRH. (A) The NMR structure showing amino acid folding near glycine in position 6 (Gly6). (B) Diagram showing conformation of GnRH and receptor binding and activation at N-terminal (red) and C-terminal (green). (Modified from Millar and Newton, 2013)

Normally, the GnRH gene encodes GnRH and the GnRH-associated peptide or GAP (Fernald and White, 1999). There are 4 exons in this preprohormone gene: the 58-UTR encoded by exon 1; signal peptide, decapeptide, proteolytic cleavage site, and N-terminal of GAP encoded by exon 2; the central part of GAP encoded by exon 3; the C terminal of GAP with 38-UTR encoded by exon 4 (Fernald and White, 1999) (Figure 3.22). The GnRH preprohormones differ only in the GAP coding sequences (Fernald and White, 1999).

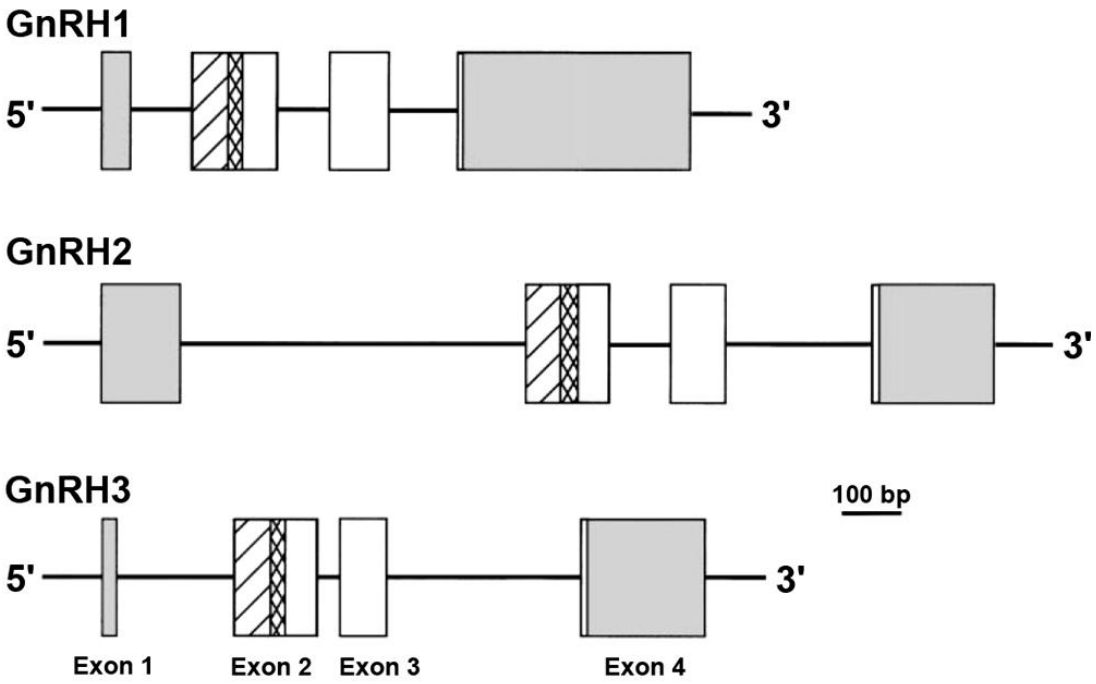

Figure 3.22 A diagram showing the organization of three different forms of GnRH1, GnRH2, and GnRH3. The grey exon encodes preprohormone. Single hatched bars exon encodes signal sequence coding region. Cross-hatched bars exon encodes GnRH-coding region. Open bars encodes GAP. (Fernald and White, 1999)

The GnRH receptor (GnRHR) is a 60 kDa 7-transmembrane receptor grouped into G-protein coupled receptor (GPCR) family (Millar, 2005) (Figure 3.23). It is found in the pituitary gonadotroph cells and others target organs (Millar, 2005). GnRHR combines with G-proteins and then activates phosphatidylinositol (PI) resulting in the releasing of FSH and LH (Millar, 2005). The GnRHRs are also conserved in various species (Millar, 2005).

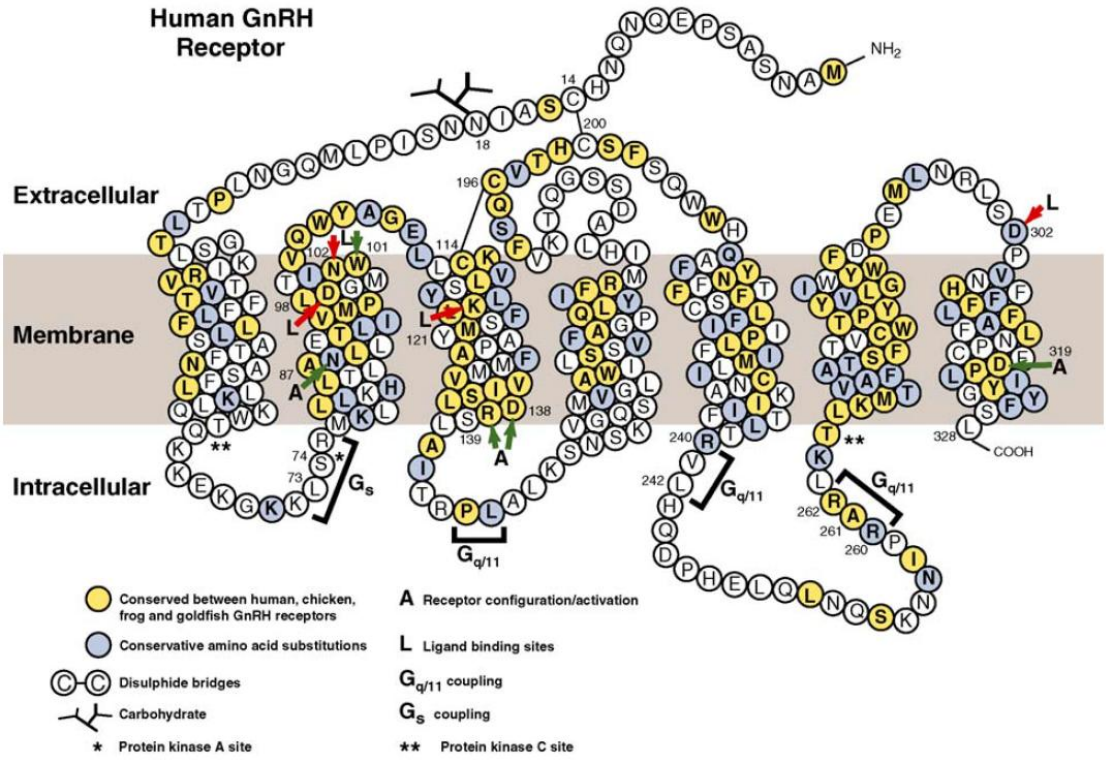

Figure 3.23 A diagram showing GnRH receptor amino acids sequences. The yellow amino acids represent conserved parts between vertebrate GnRHRs. The ligand binding sites (L) and G-protein coupling site are shown. (Millar, 2005)

GnRH has many isoforms and up to date 5 distinct groups have been reported, including GnRH1, GnRH2, GnRH3, GnRH4, and GnRH5 (Roch et al., 2011) (Figures 3.24 and 3.25). The GnRH-I is detected in hypothalamus and plays a major role in reproduction through its control of HPG axis (Roch et al., 2011) (Figures 3.24 and 3.25). GnRH II is conserved in fish and humans and distributed in the central (midbrain) and peripheral nervous systems, nonneural tissues, and reproductive tissues (Millar, 2003) (Figures 3.24 and 3.25). This GnRHs type may act as neuromodulator in the central nervous system especially with the reproduction-associated behavior (Millar, 2003). GnRH-III is found in olfactory bulb and telencephalon of bony fish (Roch et al., 2011) (Figures 3.24 and 3.25). GnRH-IV is in the hypothalamus, diencephalon and ventricular region in lamprey, a primitive cyclostome (Figures 3.24

and 3.25). GnRH-V is invertebrate or protostomian GnRHs that are present in mollusks and octopus (Tsai, 2006) (Figures 3.24 and 3.25). This GnRH contains 11-12 amino acids which differ from vertebrate GnRH by the insertion of 2 amino acids after amino acid position 1 and some variation in amino acid at position 9 (Roch et al., 2011) (Figure 3.24). Normally, vertebrate GnRHs end with amidated glycine residue at the C-terminal (Roch et al., 2011). However, in invertebrates, including limpet, marine worm and leech, the terminal glycine at C terminal is absent (Roch et al., 2011). The other group of GnRHs which may be regarded as group VI are all protochordate tunicates which contains 9 different isoforms (Roch et al., 2011) (Figures 3.24 and 3.25). It was found that tunicate GnRHs could also induce gamete release (Roch et al., 2011).

Eventhough, the actual GnRHs in decapods crustaceans have not yet been identified, the GnRH-like immunoreactivities have been detected in the CNS and ovaries of several species of prawns, including *M. rosenbergii, P. monodon* and injections of some exogenous GnRHs could stimulate ovarian maturation in *P. monodon* (Nernsoungnern et al., 2008, 2009), *M. rosenbergii* (Nernsoungnern et al., 2009) and *L. vannamei* (Tinikul et al., 2014). As well, GnRHs induce ovarian development and spawning in pond snails (Young et al., 1997), tunicates (Terakado, 2001) and corals (Twan et al., 2006) In male *M. rosenbergii*, testicular maturation is also increased after l-GnRH-III and oct-GnRH injections into small males (Poljaroen et al., 2011).

Recently, GnRH peptide was detected in the ovaries of *P. clarkii* by chemical sequence analysis (Guan et al., 2014). The structure of pcGnRH is pQSYHFSLGWKP-NH_2. After bioassay in female, this peptide could stimulate ovarian maturation (Guan et al., 2014).

Name	Original name	1	-	-	2	3	4	5	6	7	8	9	10
GnRH1	Mammals	pQ	-	-	H	W	S	Y	G	L	R	P	G amide
1	Guinea pig	pQ	-	-	Y	W	S	Y	G	V	R	P	G amide
1	Chicken-I	pQ	-	-	H	W	S	Y	G	L	Q	P	G amide
1	Frog	pQ	-	-	H	W	S	Y	G	L	W	P	G amide
1	Sea bream	pQ	-	-	H	W	S	Y	G	L	S	P	G amide
1	Pejerrey/Medaka	pQ	-	-	H	W	S	F	G	L	S	P	G amide
1	Herring	pQ	-	-	H	W	S	H	G	L	S	P	G amide
1	Catfish	pQ	-	-	H	W	S	H	G	L	N	P	G amide
1	Whitefish	pQ	-	-	H	W	S	Y	G	M	N	P	G amide
GnRH2	Chicken-II	pQ	-	-	H	W	S	H	G	W	Y	P	G amide
2	Dogfish	pQ	-	-	H	W	S	H	G	W	L	P	G amide
2	Lamprey-II	pQ	-	-	H	W	S	H	G	W	F	P	G amide
GnRH3	Salmon	pQ	-	-	H	W	S	Y	G	W	L	P	G amide
GnRH4	Lamprey-III	pQ	-	-	H	W	S	H	D	W	K	P	G amide
4	Lamprey-I	pQ	-	-	H	Y	S	L	E	W	K	P	G amide
GnRH 6	Tunicate-1	pQ	-	-	H	W	S	D	Y	F	K	P	G amide
	Tunicate-2	pQ	-	-	H	W	S	L	C	H	A	P	G amide
	Tunicate-3	pQ	-	-	H	W	S	Y	E	F	M	P	G amide
	Tunicate-4	pQ	-	-	H	W	S	N	Q	L	T	P	G amide
	Tunicate-5	pQ	-	-	H	W	S	Y	E	Y	M	P	G amide
	Tunicate-6	pQ	-	-	H	W	S	K	G	Y	S	P	G amide
	Tunicate-7	pQ	-	-	H	W	S	Y	A	L	S	P	G amide
	Tunicate-8	pQ	-	-	H	W	S	L	A	L	S	P	G amide
	Tunicate-9	pQ	-	-	H	W	S	N	K	L	A	P	G amide
GnRH 5	Sea Urchin*	pQ	V	H	H	R	F	S	G	W	R	P	G amide
	Octopus	pQ	N	Y	H	F	S	N	G	W	H	P	G amide
	Aplysia	pQ	N	Y	H	F	S	N	G	W	Y	A	- amide
	Limpet	pQ	H	Y	H	F	S	N	G	W	K	S	- amide
	Marine worm	pQ	A	Y	H	F	S	H	G	W	F	P	- amide
	Leech*	pQ	S	I	H	F	S	R	S	W	Q	P	- amide

Figure 3.24 Amino acid sequences of GnRHs in vertebrates and invertebrates. Blue shadings show identical residues compared with mammalian GnRH1. (Modified from Roch et al., 2011)

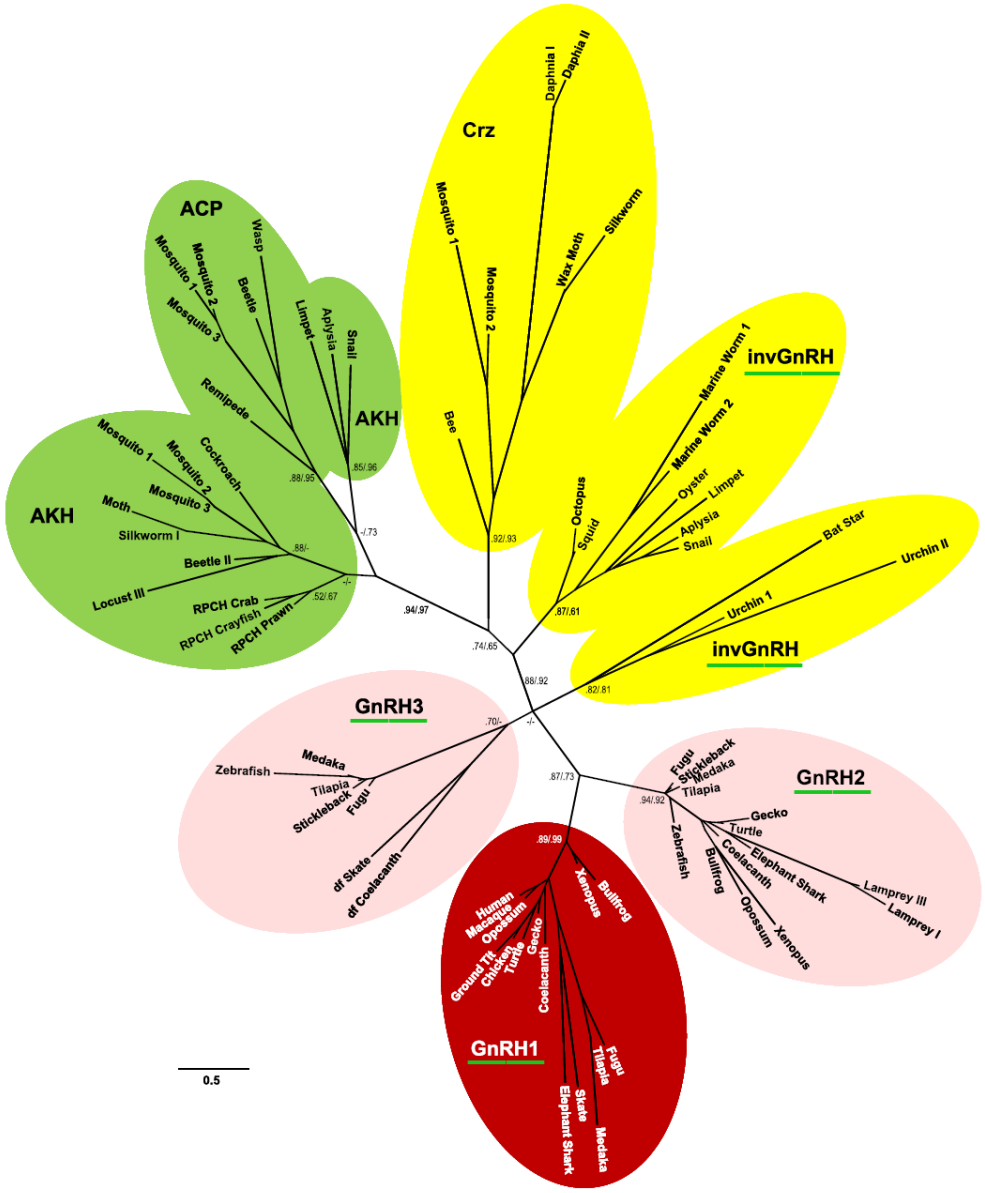

Figure 3.25 Phylogeny of GnRH superfamily based on sequences of propeptides. Scale bar = amino acid substitutions per site. (Modified from Roch et al., 2014)

3.3.2.6 Other hormones in the GnRH superfamily (Red pigment concentrating hormone, RPCH; adipokinetic hormone, AKH; and corazonin, Crz)

RPCH is a peptide hormone that controls the distribution of pigment for the animals' adaptation (Rao, 2001). It is produced in XO-SG of the eyestalk (Mangerich et al., 1986). RPCH peptide sequences are known to be highly conserved in decapods crustacean with the pQLNFSPGWamide sequence (Rodríguez-Sosa et al., 1994; Kornthong et al., 2013). The mRNA transcript of RPCH was detected in the eyestalk, brain, thoracic ganglia, and abdominal ganglia (Kornthong et al., 2013). Moreover, RPCH could stimulate the MF synthesis in MO which regulates the reproduction in *P. clarkii* (Laufer et al., 1998). Injection of RPCH also increased oocyte diameter and ovarian index in *P. Clarkii* (Sarojini et al., 1995).

AKH is a decapeptide involved in the pigment concentration in some crustaceans, and the mobilization of energy in insects (Kornthong et al., 2013). The AKH sequences are grouped into the same family with RPCH because they share some similar amino acids, and thus called AKH/RPCH sub family (Kornthong et al., 2013) (Figure 3.25).

Corazonin is a 11 amino-acid peptide with the sequence Glu-Thr-Phe-Gln-Tyr-Ser-Arg-Gly-Trp-Thr-Asn-amide (Nässel, 2002). It has been identified in most arthropods. In 1989, Crz was identified as a cardio-accelerating agent in cockroaches. Thus, the Crz name comes from ‘‘corazon”, a Spanish word for ‘‘heart” (Veenstra et al., 1989). Moreover, the Crz-receptor sequence resembles that of GnRH-AKH receptors. Thus Crz may bind to these receptors and affect the reproduction and metabolism (Boerjan et al., 2010).

Corazonin (Crz) has been reported to be present in the CNS and other tissues of arthropods, especially insects. Some studies have shown that Crz is involved in various physiological functions, including being a stress hormone (Veenstra et al., 1993; Boerjan et al., 2010), stimulating heart beating in cockroaches (Veenstra et al., 1989), stimulating pigment production in locusts (Hua et al., 2000) and reduce silk making in caterpillar (Tanaka et al., 2002).

In decapods crustaceans, Crz was first reported in the CNS (the eyestalk ganglia (including the sinus gland), the supraoesophageal ganglion (brain) and the ventral nerve cord of *L. vannamei* by mass spectrometry (Ma et al., 2010) and in the CNS of *Cancer borealis* (Huybrechts et al., 2003). It is not yet known what function Crz plays in decapods crustaceans, eventhough preliminary study indicated that it could suppress spermatogenesis in *M. rosenbergii* (Poljaroen et al., 2011). It will be interesting to see whether Crz also affects the AG activity as AG hormones (AGH, IAG) are known to control spermatogenesis in isopod, amphipod and decapods crustaceans.

3.4 The roles of lipids in reproduction

Lipids are molecules that play important roles in homeostasis and physiology in all organisms, including energy storage, maintenance of cell structure and in signaling pathway (Subramaniam et al., 2011).

3.4.1 Types of lipids

Lipids are classified into 4 major class, including glycerophospholipids, sphingolipids, glycerolipids, and fatty acid.

3.4.1.1 Glycerolipids

Glycerolipids consist of mono, di, and tri glycerol. The well known glycerolipids is called triacylglycerols (Figure 3.26). The function of glycerolipids is concerned with energy storage in the adipose tissue (Subramaniam et al., 2011).

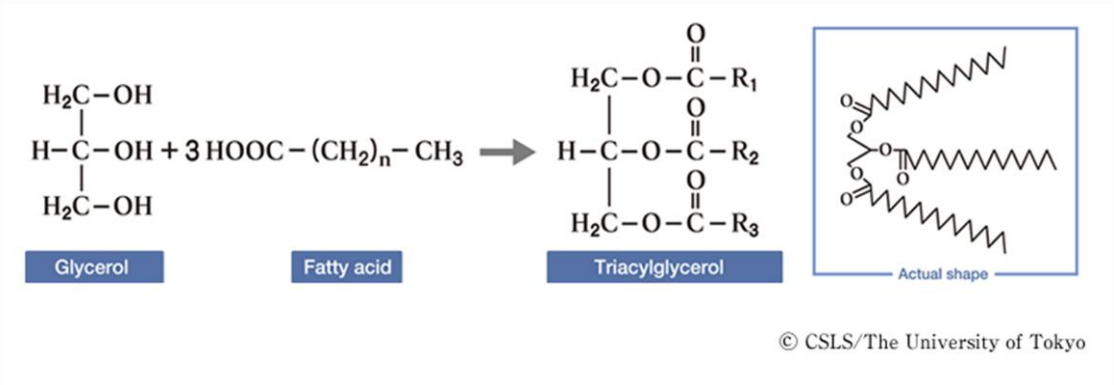

Figure 3.26 The component of triacylglycerols.
(http://csls-text3.c.u-tokyo.ac.jp/large_fig/fig06_04a.html)

3.4.1.2 Glycerophospholipids

Glycerophospholipids usually called phospholipids (PL) which is the key components in bilayered membrane (Fahy, 2005; Subramaniam et al., 2011). There are classified by the head groups on the glycerol backbone that connect to the phosphate groups (Figure 3.27); and they include phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS) (Fahy, 2005; Subramaniam et al., 2011).

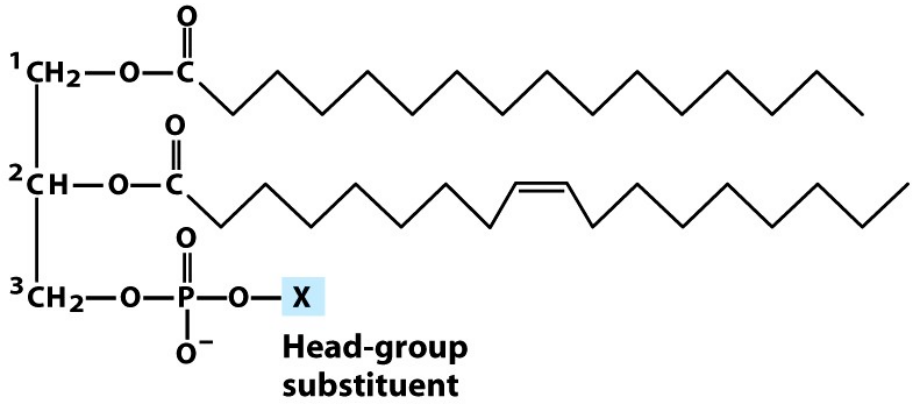

Figure 3.27 The common structure of phospholipids which contains various head groups attached to the phosphate group. (https://classconnection.s3.Amazonaws.com/548/flashcards/170548/png/glycerophospholipid_(gen._structure)1323189443594.png)

3.4.1.3 Sphingolipids

A sphingolipid is composed of a long carbon chain called sphingosine (Figure 3.28). It is combined with fatty acids by amide linkages to form a common structure called ceramides, which bound to only one hydrogen. In mammals, the major sphingolipids are sphingomyelins or ceramide phosphocholines (Hori and Sugita, 1993).

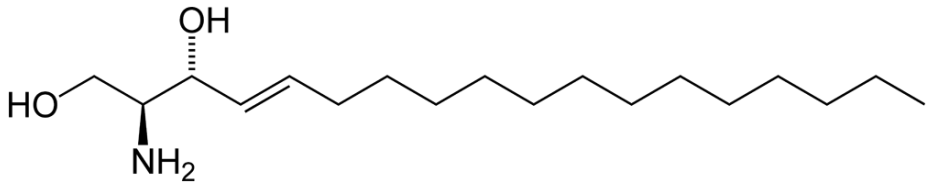

Figure 3.28 The structure of long carbon chain of sphingosine. (http://upload.wikimedia.org/wikipedia/commons/f/fd/Sphingosine-2D-skeletal.png)

3.4.1.4 Fatty acids (FA)

FA is composed of a carboxylic acid with long aliphatic tail. It is divided into 2 major groups characterized by the presence and absence of double-bond inside the chain, including saturated fatty acid and unsaturated fatty acid (IUPAC, 2014). Saturated fatty acids are the FAs that have no double bonds. They are the most abundant FAs in plants and animals. Unsaturated fatty acids contain varying numbers of double bonds and are divided into 2 groups, including monounsaturated FA (contain 1 double bond) and polyunsaturated FA (contain >1 double bond). The omega-6 has the first double bond in the *n*-6 position whereas omega-3 has the first double bond in the *n*-3 position.

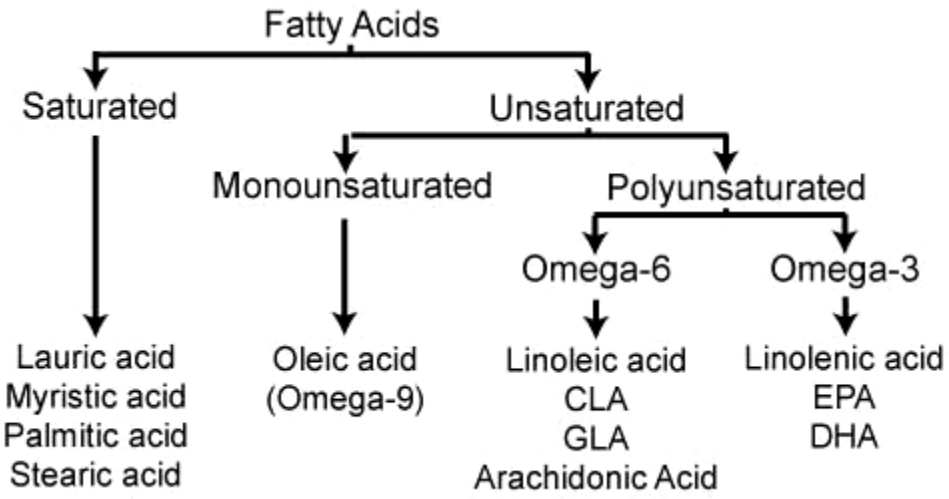

Figure 3.29 Types of fatty acids. (http://modernherbalmedicine.com/images/Types-of-Fatty-Acids.jpg)

3.4.2 Lipids are involved in male reproduction

Lipids are known to be the major components of the plasma membrane which is important to sperm fertilizability (Wassall and Stillwell, 2009; Mandal et al., 2014). The PUFA groups are known to control the membrane flexibility and fluidity (Israelachvili et al., 1980; Fleming and Yanagimachi, 1981; Meizel and Turner, 1983). The most important lipids of the sperm cell membranes is PL, including PC, PE, sphyngomyelin, and ceramide (Mann and Lutwak-Mann, 1981) which have varying amounts in each species (Swain and Miller, 2000; Miller et al., 2005). It has been reported that the sperm plasma membrane changes during testis maturation in ram,bull, boar, and goat in which PLs were decreased during maturation (Dawson and Scott, 1964; Grogan et al., 1966; Quinn and White, 1967; Poulos et al., 1973; Poulos et al, 1975; Terner et al., 1975; Evans and B. P. Setchell , 1979; Parks and Hammerstedt, 1985; Nikolopoulou et al., 1985; Rana et al., 1991; Aveldaño et al., 1992). Moreover, the cholesterol also decreases in sperm membrane of rat, ram, and hamster during testis maturation (Scott et al., 1967; Bleau and VandenHeuvel, 1974; Legault et al., 1979).

3.4.3 Phosphatidylcoline (PC) and its function in reproduction

The PLs, especially PCs, are major integral components of plasma membranes, and they are also involved in sperm membrane permeability and fluidity (Davis et al., 1966; Lenzi et al., 1996; Lin et al., 2004; Zaniboni et al., 2006), acrosomal reactions (Cross, 1994), and sperm motility (Infante and Huszagh, 1985). PCs are composed of choline head groups, glycerol, and two fatty acid side chains that can be saturated and/or unsaturated (Figure 3.30). PC treatments prevents lipid peroxidation or degradation of enzymes in stored semen of the turkey (Long and Conn, 2012), and improved acrosomal responses in human sperm (Cross, 1994). It has been reported that PC is also involved in energy metabolism in the sperm of sea urchin (Mita et al., 1990) and *in vitro* acrosomal response in human spermatozoa (Cross, 1994). In female, lipids are also involved in ovarian maturation and oocyte differentiation (Glencross and Smoth, 2001). It has been reported that PC and TAG are a major component of ovary that contribute to successful embryogenesis, and offsprings quality in shrimp (Wouters at al., 2001).

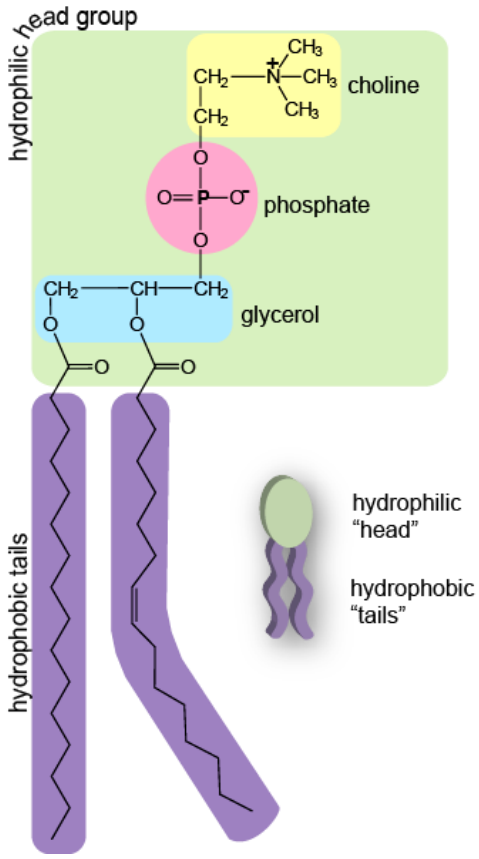

Figure 3.30 The structure of phosphatidylcholine. (http://media1.shmoop.com/images /biology/biobook_biomol_22.png)

3.4.4 Polyunsaturated fatty acids (PUFA) and highly unsaturated fatty acids (HUFA) and their roles in reproduction

It has been reported that PUFA and HUFA play important roles in reproduction (Harlioğlu et al., 2013; Lin et al., 2004; Ahluwalia and Holman, 1966; Ewing et al., 1966; Evans and Setchell, 1979a; Evans and Setchell, 1979b; Roqueta-Rivera et al., 2010). The three best known HUFA involved with reproduction, especially gonadal development,are ARA, EPA, and DHA. DHA is associated with Sertoli cells activity and functions in mammal (Saether et al., 2007). Normally, 20 and 22 carbon n-3 and n-6 PUFAs are accumulated in developing male germ cells and spermatozoa of mammals (Saether et al., 2007). ARA, an omega 6 FA, is a precursor of series II prostaglandins (PGs) (Figure 3.31), whereas EPA is a precursor of series III PGs (Stacey and Goetz, 1982) (Figure 3.31). Both PGs are involved in steroid production (Wade et al., 1994). Recently, our group reported that injection of PGEs in female *M. rosenbergii* could stimulate ovarian maturation (Sumpownon et al., 2015).

The role of ARA, EPA, and DHA in reproduction has been studied in the goldfish (Wade et al., 1994), and it was found that they control steroidogenesis in the testis, and that EPA deficiency delays spermiation and decrease fertilization rate. For penaeid shrimps, including *P. monodon* and *L. vannamei*, it was found that the diet containing polychaetes, mollusk, squids, fish, vegetable oils which are rich in HUFA and PUFA, especially ARA, EPA, and DHA, could improve the quality of spermatophores and sperm (Perez-Velazquez and González-Félix, 2003; Meunpol et al., 2005; Chimsung, 2014; Shailender et al., 2012). Similarly, diet containing these FAs could also enhance male reproductive performance in *M. malcolmsonii* (Samuel et al., 1999). Moreover, diet containing n-3 HUFA could improve survival and growth rate in larvae and postlarvae of *P. monodon* (Millamena et al., 1988; Rees et al., 1994), *P. japonicus* (Kontara et al., 1997), *L. vannamei* (Lim et al., 1997; Wouters et al., 1997), *Fenneropenaeus chinensis* (Xu et al., 1993), *P. trituberculatus* (Takeuchi et al., 1999), and *Scylla serrata* (Davis, 2004; Suprayudi et al., 2004). Another study reported that the EPA-containing diet enhanced sperm production in the freshwater crayfish, *Astacus leptodactylus* (Harlioğlu et al., 2013), and HUFA was found to increase the recovery of spermatogenesis in n-3 desaturase-null mice that cannot synthesize HUFA (Roqueta-Rivera et al., 2010).

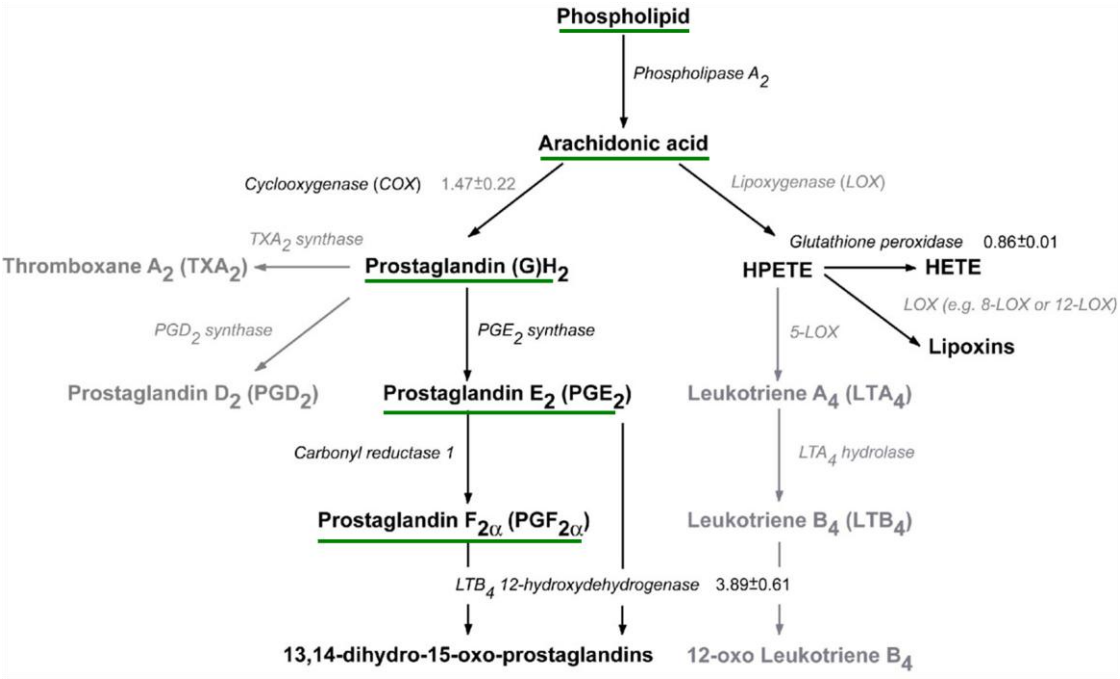

Figure 3.31 Eicosanoid biosynthetic pathways showing the essential molecules (green underlined) and putative genes (italic). An ARA side chain of the phospholipids is converted to prostaglandin H_2 (PGH_2) by cyclooxygenase enzyme (COX). Then PGH_2 is converted to PGE_2 and $PGF_{2\alpha}$ by PGE_2 and $PGF_{2\alpha}$ synthases and carbonyl reductase 1 enzyme. (Modified from Heckmann et al., 2008)

3.4.5 Lipid profiles in the gonads of crustaceans and the roles of lipids in crustacean reproduction

In crustacean females, there are a number of reports on lipid profiles in the ovaries, and these have been used as the basis for formulating a balanced diet. For example, ovarian lipids made of TAGs and PLs, especially PC, PE, PS, PI, have been reported in *Serolis pagenstecheri* (Clarke, 1984), *S. cornuta* (Clarke, 1984), *P. monodon* (Millamena and Pascual, 1990), *P. semisulcatus* (Ravid et al., 1999), *M. rosenbergii* (Cavalli et al., 2001), *L.* v*annamei* (Wouters et al., 2001), *Fenneropenaeus indicus* (Boucard et al., 2002), *C. quadricarinatus* (Rodríguez-González et al., 2006), *P. sanguinolentus* (Ravichandran et al., 2009), *Albunea symmysta* (Srinivasan et al., 2012), and *P. merguiensis* (Chansela et al., 2012). Besides, major fatty acids in this species are composed of 14:0, 15:0, 16:0, 17:0, 18:0, 16:1, 18:1, 18:2, 20:1, 20:2, 20:4, 20:5, and 22:6. Lipid changes are associated with ovarian maturation, hatching, embryonic development, and larval development (Wouters et al., 2001; Chansela et al., 2012). Appropriate amount of lipids and FA components are critical for the formulation of balanced lipid diets for female broodstock.

On the other hand, studies in males have focused on testicular lipids, including TAGs and PLs, in *S. pagenstecheri* (Clarke, 1984), *S. cornuta* (Clarke, 1984), *Pleoticus muelleri* (Jeckel et al., 1989), *P. monodon* (Millamena and Pascual, 1990), and *M. nipponense* (Wang et al., 2010). These reports indicated that the amount of lipids in the testes were lower than in the ovaries, and usually contain eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). However, arachidonic acid (ARA) was found to be higher than EPA and DHA in the spermatophores of *P. monodon* (Meunpol et al., 2005). By and large, the profiling of lipids in the testes of decapods is still the understudied area. A knowledge of lipid composition in the testes of developing males of *M. rosenbergii* is now needed so that the information could be used for the formulation of balanced diets to improve the male fecundity.