CHAPTER II

Literature review

Biology of *Penaeus monodon* Fabricius, 1798

Giant tiger prawn, *Penaeus monodon*, is one of the largest penaeid prawns in the world. The prawn *P. monodon* is widely distributed throughout the greater part of the Indo-Pacific region, ranging northward to Japan and Taiwan, eastward to Tahiti, southward to Australia, and westward to Africa. In general, *P. monodon* is distributed from 30 ^{o}E to 155 ^{o}E in longitude and from 35 ^{o}N to 35 ^{o}S in latitude with the main fishing grounds located in tropical countries (Solis, 1988).

Morphology

Live adult female and male giant tiger prawn have the following characteristic coloration (Fig. 2.1) : carapace and abdomen are transversely banded with red and white, the antennae are grayish brown, and the pereopods and pleopods are brown with crimson fringing setae. In shallow brackish waters or when cultured in ponds, the color changes to dark and, often, to blackish brown (Solis, 1988).

The shell is smooth, polished and glabrous. The rostrum extends beyond the tip of the antennular peduncle, is sigmoidal in shape, and possesses 6-8 dorsal and 2-4 ventral teeth, mostly 7 and 3, respectively. The Carapace is carinated with the adrostral carina almost reaching the posterior margin of the carapace. The gastro-orbital carina occupies the posterior one-third to one-half distance between the post-orbital margin of the carapace and the hepatic spine. The hepatic carina is prominent and almost horizontal. The antennular flagellum is subequal to or slightly longer than the peduncle. Exopods are present on the first four pereopods but absent in the fifth. The abdomen is carinated dorsally from the anterior one-third of the fourth, to the posterior end of the sixth, somites. The telson has a median groove but without dorso-lateral spines (Fig. 2.2) (Farfante and Kensley, 1997; Solis, 1988).

Figure 2.1 The giant tiger prawn, *Penaeus monodon*, female (top) and male (bottom).

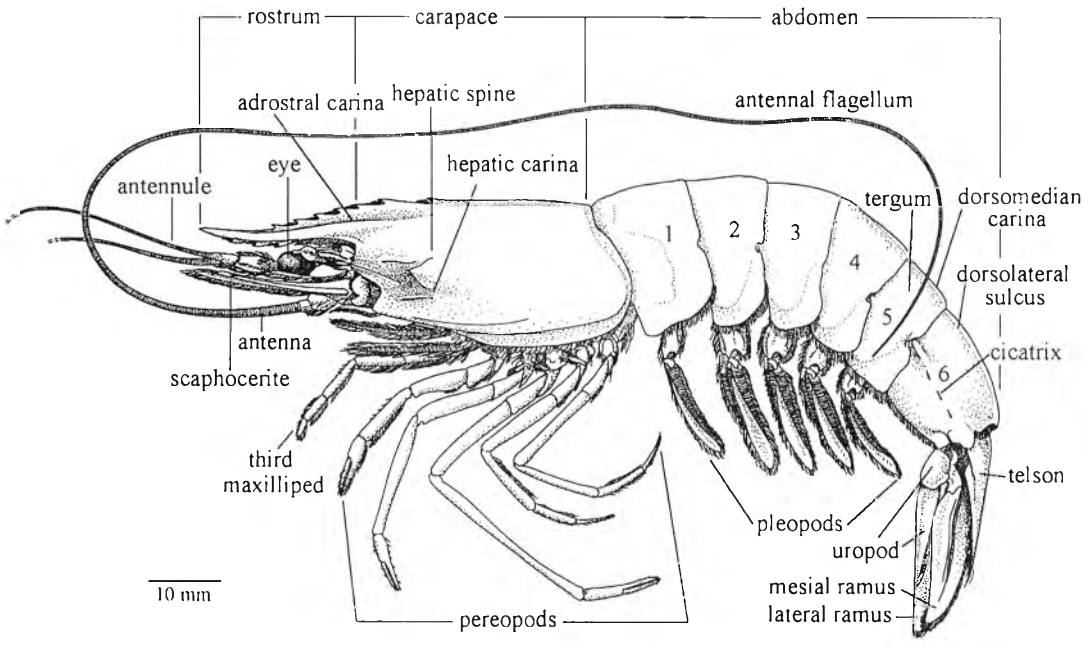

Figure 2.2 Lateral view of giant tiger prawn, *Penaeus monodon* (Farfante and Kensley, 1997).

External morphology of reproductive system

The *P. monodon* is heterosexual. The female attains a relatively larger size than the male. The sexually mature prawn can be distinguished by the presence of the external genital organs: joined petasma, a pair of appendix masculina on the exopods of the second pleopods, and a genital opening on the coxa of the fifth of pereopod for the male. In females, the thelycum is situated between the fourth and fifth pair of pleopods, consists of an anterior and a pair of lateral plates. (Fig. 2.3) It receives the spermatophores during mating. In *P. monodon*, the thelycum is classified as closed type. The genital opening is on the coxa of the third pereopod (Farfante and Kensley, 1997; Solis, 1988).

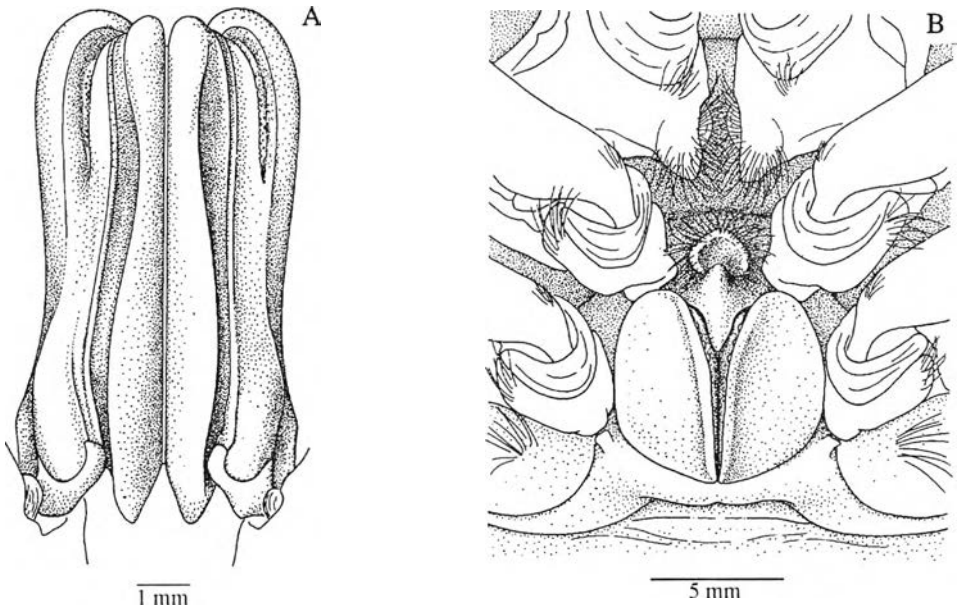

Figure 2.3 External genitalia of male, petasma (A) and female, thelycum (B) of *Penaeus monodon* (Farfante and Kensley, 1997).

Ovarian development

In penaeid shrimps, the ovary extends from the cephalothorax along the length of the abdomen to the telson. The paired ovaries are often connected by bridges of ovarian tissue. The ovary is partially fused in the cephalothoracic region and consists of a pair of anterolateral horns and several lateral lobes. A pair of lobes extends the length of the abdomen. A connective tissue capsule, or wall, surrounds the decapod ovary. The germinal epithelium from which the oogonia and follicle cells are derived lies inside this wall. The ovarian wall consists of one or more layers of connective tissue. The processes of connective tissue separate the ovary into subunits, termed nodules or cysts, consisting of maturing germinal and follicle cells. The ovarian maturation of *P. monodon* has been categorized into five stages, the classification of which is based on ovum size, gonad expansion and coloration (Primavera, 1983 and Tan-Fermin and Pudadera, 1989).

Stage I (undeveloped stage). Ovaries are thin, transparent and not visible through the dorsal exoskeleton.

Stage II (developing stage). The ovaries are flaccid and white to olive green in color and discernible as a linear band through the exoskeleton.

Stage III (maturing stage and nearly ripe stage). Ovaries have glaucous color with the anterior portion thick and expanded. They are very visible through the exoskeleton, particularly at the first abdominal segment.

Stage IV (ripe stage). The ovary is diamond-shaped, expanding through the exoskeleton of the first abdominal segment. The isolated ovary appears dark olive green, filling up all the available space in the body cavity.

Stage V (spent stage). Ovaries are observed to be discontinuous, i.e. white in color in either the anterior or posterior portions with olive green color in the opposite ends. And in this stage also contains some yolky oocytes, thicker follicle layer, or irregularly shaped perinucleolar oocytes.

Oogenesis

Krol et al. (1992) described that stages of ovarian development in decapods can be divided into previtellogenesis, vitellogenesis, oocyte maturation. Oogenesis can be classified into 1) a proliferative phase in which oogonia are produced and 2) differentiative phases in which the oogonia develop from the previtellogenesis stage through maturation.

In the proliferative phase, clustered secondary oogonia are produced by mitosis in the germinal zone throughout the reproductive life of the female. The secondary oogonia remain in the germinal zone until spawning or sexual responses trigger gametogenesis. In the differentiate phase, primary oocytes derived from the secondary oogonial cells are displaced toward the growth zone, where they immediately enter prophase of meiosis. At the pachytene stage of meiosis I prophase, cell division halts and metabolic activity of the primary oocyte overrides meiotic division. At this point nuclear and cytoplasmic changes occur, the oocyte increases in size, and the oocytes arrange themselves in their final position within the ovary. When the oocytes mature and ovulation occurs, meiotic division progresses to the metaphase of primary maturation division. Early oocyte growth involves development of the germ cell prior to vitellogenesis. When oogonia leave the germinal zone, they progress from immature oogonia with large round nuclei containing granular chromatin to previtellogenic oocytes with central nuclei contain large nucleoli (Krol et al., 1992).

Previtellogenesis in decapods is characterized by an increase in activity of various cytoplasmic organelles. Accumulation of ribosomes and development of rough endoplasmic reticulum give rise to vesicular elements. The vesicles are filled with granular glycoprotein. In previtellogenic oocytes, golgi complexes, smooth endoplasmic reticulum, and annulate lamellae appear first near the nucleus and then disperse throughout the cytoplasm. In the late previtellogenic oocytes, a large cytoplasmic inclusion body called a perinuclear yolk-nucleus complex is prominent

and the yolk-nucleus complex disappears, at least as a separate entity, when vitellogenesis begins (Krol et al., 1992).

Vitellogenesis is an important physiological process in crustacean reproduction. It is the process through which food is progressively stored in the growing oocytes of oviparous animals, making up the yolk of the mature egg. The food reserves are produced outside the oocytes and transported through the body fluids by soluble protein. Vitellogenesis involves the assembly of organic and inorganic components of yolk in the developing stage. Yolk consists of water, carbohydrate, proteins, and lipids which are necessary nutrients for the developing embryo. Yolk provides structural materials for tissue formation and fuel. The main protein involved in the process is called vitellogenin and also called a female-specific protein in the haemolymph of crustacean. Vitellogenin is its precursor molecule. The uptake of vitellogenin by the oocytes is mediated by specific receptors, where it is incorporated into yolk proteins (vitellin). Vitellin is the major yolk protein in eggs of crustacean with a high molecular mass ranging from 290 to 560 kD. The main protein of yolk composes of lipids, carbohydrates, phosphate and often associates with carotenoid pigment, which possibly serves as a shield to protect the embryo against solar radiation called lipo-glyco-phospho-carotenoprotein. The molecule has 2 to 11 polypeptide subunits with a molecular mass ranging from 40 to 200 kD in various crustacean species (Lui and O' Connor, 1976, 1977 Eastman-Reks and Fingerman, 1985). Vitellin has also been found to be necessary as a source of nutrients for the developing embryo. At a period of 36 to 48 hours after hatching, penaeid shrimp nauplii subsist entirely on the yolk retained in the egg. The quality and quantity of egg yolk is therefore crucial for the maintenance of early life in shrimp larvae (Krol et al., 1992).

As early vitellogenesis begins, several cytoplasmic changes occur in the oocyte. Dense granular materials accumulate in vesicle of the rough endoplasmic

reticulum. Vitellogenic oocytes of some decapod contain highly developed rough endoplasmic reticulum. Cisternae of rough endoplasmic reticulum in the oocytic cytoplasm contain an electron-dense material and granules. In several decapods, nucleolar material moves from the nucleus to the cytoplasm at the end of previtellogenesis, probably as a prelude to yolk synthesis. Transfer of nucleolar material occurs either when the membrane-bound vesicle fuse with the inner membrane of the nuclear envelope and the outer envelop blebs out or when the material passes through nuclear pores. This movement of nucleolar material may represent a transfer of RNA or mRNA from the cell nucleus to the cytoplasm (Krol et al., 1992).

As vitellogenesis proceed, oocytes mature synchronously as yolk accumulates. The color of the ovary results from the accumulation of vitellogenin-containing carotenoid pigment. In decapods, an electron-dense material is deposited in the perivitelline space along the oolemma and among the microvilli. In later stage, this material produces the chorion, or outer envelop, of the oocyte. The chorion becomes a continuous layer separating the oocyte from the follicle cells. These oocytic investments form toward the end of vitellogenesis in decapods (Krol et al., 1992).

By the end of vitellogenesis, the microvilli disappear, the oocyte plasma membrane becomes smooth, and cortical granules appear in the oocyte cytoplasm. Endogenous yolk is a component of the cortical granules, cortical rod appear first as spherical bodies near the periphery of the oocyte cytoplasm and elongate toward the nucleus as maturation proceeds. The cortical rod contain a polypeptide found in vesicles formed from dilated endoplasmic reticulum. This type of vesicle is the most abundant of the three types found in the cytoplasm and the other two types of vesicles are components of yolk. The cortical rod of shrimp as lying in extraoocytic crypts forms in the cytoplasmic membrane of the oocyte. The crypts are apparently formed by the fusion of materials in pinocytotic vesicles secreted by the oocyte. Overlying

the crypt contents and lying adjacent to the follicle cells is a vitelline envelope which appears to be formed by the follicle cells (Krol et al., 1992).

Several events mark the culmination of oocyte maturation in decapods. In the mature oocyte of shrimp, the nuclear membrane disappears and germinal vesicle breakdown occurs as a prelude to further meiotic division in the cell. This event occurs several hours before spawning. The ovulation occurs when the follicle cells disappear from around the oocytes (Krol et al., 1992).

At spawning, the cortical rods extrude when they contact sea water, then they dissipate and form a jelly-like substance that surround the oocyte. Mature oocytes that are not released from the ovary are resorbed. In crab, spawning occurs when the muscular layer of the ovarian wall extrudes mature oocytes. Decapod cephalothoracic and abdominal muscles around the ovary may force mature oocytes to the oviduct. Most malacostracans apparently do not retain mature oocytes in the oviduct. Both follicle cells and haemocytes play role in resorption of oocytes. Yolk undergoes resorption in the reverse order to its formation, i.e., first carbohydrate, the lipid, and finally protein is resorbed. In the penaeid shrimps, resorption of unspawned ripe oocytes and ovarian repair occur during rematuration (Krol et al., 1992). In certain species of decapods, the vitellogenin is believed to be synthesized by the ovary such as in *Procambarus sp.* (Lui and O'Connor, 1976), *Penaeus japonicus* (Yano and Chinzei, 1987), *Penaeus vannamei* (Quackenbush, 1989a), and *Penaeus semisalcatus* (Browdy et al., 1990). By using immunocytochemical techniques in the crabs, *Carcinus maenas* and *Libinia emerginata,* Paulus and Laufer (1987) found that the vitellogenin was localized in hepatopancreas specialized cell and designated vitellogenocyte.

Characterization of vitellin and vitellogenin in decapod crustacean

Several researchers have been studying vitellin and vitellogenin of ovary and haemolymph in decapod crustaceans as summarized in Table 2.1.

In 1985, Eastmans-Rek and Fingerman incubated ovaries of the crab *Uca pugilator* in a 4,5-^{3}H-leucine medium. Using SDS-PAGE, they demonstrated that the ovaries were capable of synthesizing vitellin. Labelled leucine was incorported equally into two subunits of vitellin which had molecular mass of 100.6 and 123.5 kD.

Derelle et al. (1986) demonstrated that vitellin of the prawn *Macrobrachium rosenbergii* had a molecular mass of 330 kD and consisted of two subunits with molecular mass of 84 and 92 kD. By using sandwich ELISA, they found that the circulating vitellogenin level was present at a very low amount approximately 1 mg/ml in the early reproductive stage. The vitellogenin level increased during the period of intense uptake of vitellogenin by the oocytes and remained high during nearly molting stage at 10 to 15 mg/ml. Thereafter the vitellogenin level rapidly decreased and reached very low concentration again at the end of the molting/reproductive cycles, before and just after ovulation.

Yano and Chinzei (1987) found that ovaries were capable of synthesizing vitellin by incubating the ovary and hepatopancreas tissues of the shrimp, *Penaeus japonicus* in a ^{14}C-amino acid medium. The synthesized vitellin was further studied by immunoelectrophoresis and SDS-PAGE.

Tom et al. (1987a) isolated vitellin from ovaries of the shrimp, *Parapenaeus longirostris* using gel filtration on Sepharose 6B and DEAE-Sepharose ion exchange chromatography. The purified vitellin, consisted of two subunits with molecular mass of 45 and 66 kD, was a glycoprotein. Using immunodiffusion, immunoelectrophoresis and immunofluorescence methods, Tom et al. (1987b) demonstrated that the vitellin was present in the developing oocyte, subepidermal adipose tissue and in

haemolymph of *P. longirostris*, but not found in the early developing oocyte indicating that vitellin was synthesized in an extraovarian sites.

Lee and Puppione (1988) isolated and characterized lipoproteins in the haemolymph of the blue crab *Callinectes sapidus* by using ultracentrifuge and electrophoresis. They found two distinct lipoproteins (lipoprotein I and II). Lipoprotein I, a single peptide with molecular mass of 112 kD, was always present at low concentration in the haemolymph of both male and female. Lipoprotein II was female specific and found in high concentration in crabs with large and well-developed ovaries. Lipoprotein II composed of three peptides with the molecular mass of 78, 107 and 190 kD and had similar to vitellogenin of the crab ovaries. Lipoprotein II probably plays an important role in carrying lipid and specific peptides to ovarian tissues for vitellogenesis.

Quackenbush (1989a) isolated yolk protein from ovaries of the shrimp, *Penaeus vannamei* using $(NH_4)_2SO_4$ precipitation and Sephadex G-200 column chromatography. The isolated yolk protein was used as an antigen for antibody production in rabbits. Western blot analysis revealed that the antibody reacted with the four subunits of 76, 95, 97 and 103 kD in the ovary extracts but in the hepatopancreas, only two subunits, 97 and 102 kD were found. The eyestalk extract from *Penaeus setiferus* could inhibit *in vitro* protein synthesis by using the machinaries from the hepatopancreas and the ovary. The inhibitory factor from the eyestalk extract with a molecular mass of 3,300 dalton was isolated by boiling with distilled water and purified by Sephadex G-25 column indicating that it was heat stable.

Quinitio et al. (1989) purified vitellin from vitellogenic ovaries of the hermaphrodite shrimp, *Pandalus kessleri* using hydroxylapatite, DEAE (diethyl-aminoethyl) cellulose and sepharose 6B column chromatography. The purified vitellin had a molecular mass of approximately 560 kD and composed of two

subunits, 81 and 110 kD. Using immunodiffusion and immunoelectrophoresis, they demonstrated the presence of a female specific protein, vitellogenin, in the haemolymph. The haemolymph vitellogenin concentration increased as vitellogenesis in the ovary oocytes advances and fell down after the egg laying.

In 1990, vitellin from the mature ovaries of *P. monodon* was purified by hydroxylapatite and sepharose 6B column. The isolated vitellin was a glyco-lipo-protein with a molecular mass of 540 kD and composed of four major subunits, 74, 83, 104 and 168 kD and 1 minor subunit, 90 kD. The female specific protein (FSP, vitellogenin) in haemolymph and its related vitellin were identified and characterized by electrophoretical and immunological procedures (Quinitio et al., 1990). Using double immunodiffusion method, Quinitio et al. demonstrated that the antiserum specific to vitellin of *P. monodon* cross-reacted with ovary extracts of *Penaeus indicaus*, *Penaeus merquiensis* and *Penaeus semisulcatus*, but not with *Pandalus kessleri*. This indicated that there are no antigenic differences within these penaeid species.

Browdy et al. (1990) incubated ovaries of the penaeid shrimp *P. semisulcatus* form various stages of oocyte development with ^{14}C or ^{3}H-leucine. The vitellin specific synthesis was analyzed by radioimmunoprecipitation. The molecular mass of vitellin as determined by gel filtration on sepharose 6B was 283 kD. The SDS-PAGE analysis of this isolated vitellin showed four subunits with molecular mass of 50, 63, 80 and 90 kD.

Tom et al. (1992) studied vitellin from ovaries of the two different penaeid species, *Penaeus semisulcatus* and *Penaeus vannamei* using gel filtration, electrophoresis, Western blot and amino acid analyses. The molecular mass of native vitellin molecules of *P. semisulcatus* and *P. vannamei* were found to be almost identical at 283 and 289 kD, respectively. The two subunits of denatured vitellin were 86 and 95 kD for *P. semisulcatus* and 61 and 69 kD for *P. vannamei*.

Chen and Chen (1993) isolated vitellin from the egg of the giant tiger prawn *P. monodon* using PAGE, SDS-PAGE, gel elution and Western blot. The isolated vitellin consisted of four subunits with molecular mass of 74, 83, 104 and 168 kD. Chang et al. (1993a) purified vitellin from the mature ovaries of *P. monodon* by gel filtration, hydroxylapatite, DEAE-Sephacel and PAGE. The isolated vitellin, consisted of eight subunits at 35, 45, 49, 58, 64, 68, 82 and 91 kD, was a glyco-lipo-carotenoprotein with a molecular mass of 492 kD.

Chang et al. (1993b) purified vitellin from the mature ovaries of *Macrobrachium rosenbergii* using gel filtration, DEAE-Sephacel, HPLC and PAGE. The purified vitellin of *M. rosenbergii* was a lipoglycoprotein and was classified into three groups with the molecular mass of 240, 450 and 780 kD. Each vitellin consistently had two polypeptide subunits with molecular mass of 90 and 104 kD.

In 1994, Chang et al. purified and characterized the female specific protein or vitellogenin from the mature female haemolymph of *P. monodon*. The haemolymph was stained with Sudan Black B (SBB) and fractionated by an ultracentrifuge. The fourth fraction of the female haemolymph was further purified by PAGE and electro-elution and the vitellogenin was determined by Western blot. The purified vitellogenin were a lipo-glyco-phosphoprotein and composed of two polypeptide subunits with molecular mass of 82 and 170 kD. Using immunoprecipitation, immunofluorescence, SDS-PAGE and Western blot, Chen and Chen (1994) reported that the vitellogenin from female haemolymph of *P. monodon* consisted of four peptide subunits with molecular mass of 74, 83, 104 and 168 kD.

Khayat et al. (1994) isolated vitellin from the ovaries of vitellogenic female prawns *Penaeus semisulcatus* by ion-exchange chromatography, and PAGE. Western blot analysis revealed that vitellin composed of three major subunits of apparent molecular mass of 80, 96 and 158 kD.

Lee and Watson (1994) detected vitellin and vitellogenin from the blue crab *Callinectes sapidus*. Vitellin was purified by gel filtration chromatography and the anti-vitellin immune serum was raised in rabbits. The purified vitellin consisted of four subunits of 86, 109, 168 and 188 kD. An ELISA method for quantitating vitellogenin level in haemolymph showed that the vitellin standard curve was linear over the range of 62.5 to 1,500 ng and the sensitivity of this assay was 148 ng/ml.

In the freshwater prawn *Macrobrachium rosenbergii*, Chang and Shih (1995) divided the ovarian development into five stages. During reproductive cycle, the haemolymph vitellogenin levels increased in the early stages of ovarian development (stage I or II) and were maintained at high levels through stage V. The vitellogenin levels decreased during oviposition and remained at low levels until the embryos hatched.

Chang and Jeng (1995) isolated and characterized the female specific protein or vitellogenin from the mature female haemolymph of *Penaeus chinensis*. The haemolymph was stained with Sudan Black B and fractionated by an ultracentrifugation. The vitellogenin fraction was further purified by PAGE, electroelution and determined by Western blot and immunoprecipitation. The purified vitellogenin was considered as a lipo-glyco-phospho-protein and consisted of two polypeptide subunits with molecular mass of 85 and 191 kD.

In 1996, Chang et al. purified and characterized vitellin from the mature ovaries of *P. chinensis* by gel filtration, hydroxylapatite, PAGE and electro-elution. The purified vitellin had two forms with apparent molecular mass of 380 (Vn1) and 500 (Vn2) kD. The Vn1 composed of five subunits with molecular mass of 40, 58, 78, 85 and 105 kD; whereas, the Vn2 composed of three subunits with 78, 85 and 155 kD. Vn1 and Vn2 were determined as a lipo-glyco-caroteno-phospho-protein and a glyco-phospho-protein, respectively.

Lubzens et al. (1997) produced rabbit antiserum against vitellin that was isolated from vitellogenic ovaries of the shrimp *Penaeus semisulcatus* by gel filtration and ion exchange chromatography. The haemolymph vitellogenin was stained with Sudan Black B and isolated by an ultracentrifugation. The isolated vitellogenin positively reacted with the antiserum against vitellin. Both vitellin and vitellogenin consisted mainly of three polypeptide subunits with molecular mass of 80, 120 and 200 kD as revealed by SDS-PAGE and Western blot.

Qiu et al. (1997) isolated vitellin from the ovaries of *Metapenaeus ensis* using Sephadex G-200 gel filtration and DEAE-Sepharose anion exchange chromatography. The purified vitellin had a molecular mass of 350 kD and composed of two major subunits with molecular mass of 76 and 102 kD.

Pateraki and Stratakis (1997) characterized vitellogenin and vitellin from the land crab *Potamon potamios*. Vitellin from the ovaries and vitellogenin from haemolymph were isolated by KBr differential density gradient ultracentrifugation and further characterized by PAGE, SDS-PAGE and Western blot. The purified vitellin had a molecular mass of 510 kD and consisted of three subunits with molecular mass of 85, 105 and 115 kD. The vitellogenin had a molecular mass of 551 kD with four subunits of 85, 105, 115 and 181 kD.

The vitellogenin levels and protein in haemolymph, ovary and hepatopancreas of freshwater prawn *Macrobrachuim rosenbergii*, in different stages of ovarian development were measured by ELISA and Bradford methods, respectively. Haemolymph vitellogenin levels increased in the early stages of ovarian development and remained at high levels until stage V. The concentrations of proteins in haemolymph and hepatopancreas remained constant during various stages of ovarian development. The concentrations of vitellogenin and protein in haemolymph and hepatopancreas did not closely correlate with ovarian development but the concentrations of ovarian vitellin and protein were closely associated with ovarian

stage (Lee and Chang, 1997). And Lee et al. (1997) purified and characterized vitellogenin from the haemolymph of the same prawn. Haemolymph vitellogenin was purified with DEAE, hydroxylapatite and DEAE chromatographic column. Using ELISA, Western blotting, immunoprecipitation and SDS-PAGE, they demonstrated that the purified vitellogenin had a molecular mass of 700 kD with the three subunits of 170, 100 and 89 kD; whereas, the vitellin had the two subunits of 100 and 89 kD.

Identification of GIH and GIH assay

The fact that the crustacean reproduction is under hormonal control has been known for more than 50 years. Panouse (1943, 1944) (cited from Fingerman. 1997) found that removal of both eyestalks resulted in precocious ovarian development in shrimp *Leander serratus*. By implantation of sinus gland, it was demonstrated that this effect was due to the fact that the sinus gland in the eyestalk contained a gonad inhibiting hormone (GIH). The attempts to identify GIH in various crustacean species has been continuously studied with very few successful reports.

Bomirski et al. (1981) isolated gonad inhibiting hormone from the eyestalk of crab *Cancer magister* by boiling and using Sephadex G 25 column chromatography. The eyestalk extract was assayed by injecting into the eye-ablated female shrimp *Crangon crangon.* The ovarian index (OI) was determinated by comparing with OI of the shrimp that was not injected with the extact. The GIH was elucidated as a peptide with a molecular mass of 2,000 daltons.

In 1983, Quackenbush and Herrnkind isolated vitellogenesis inhibiting hormone (VIH) from the eyestalk of the *Panulirus argus* by Sephadex gel chromatography and polyacrylamide gel electrophoresis. The VIH was a peptide and had a molecular mass of 5,000 daltons. The hormone was assayed in the eye-ablated crab *Uca pugilator*. Later Quackenbush (1989b) tried to isolate VIH from the eyestalk of shrimp *Penaeus vannamei* by using Sephadex G 25 gel chromatography.

The hormone inhibited *in vitro* protein synthesis in ovaries and hepatopancreas of shrimp.

Soyez et al. (1987) isolated VIH from the sinus glands of lobster *Homarus americanus* by reversed phase high performance liquid chromatography (RP-HPLC) and sodium dodecyl sulfate urea polyacrylamide gel electrophoresis (SDS-urea-PAGE). The hormone characterization was based on a bioassay developed in the bilateral eyestalk ablated female shrimp *Palaemonetes varians* by detecting the inhibition of oocyte development. The VIH was a peptide with a molecular mass of 7,000 to 8,000 daltons. In 1991, Soyez et al. purified VIH from *H. americanus* for studying the structure, component and amino acid sequences of this hormone by using gas phase microsequencing and fast atom bombardment mass spectrometry. The hormone composed of 77 amino acid residues with molecular mass of 9,135 and had a free N-terminus. The amino acid sequences of this hormone showed homology to crustacean hyperglycemic hormone (CHH) and molt inhibiting hormone (MIH).

In 1992, the VIH was purified from sinus glands of the Mexican crayfish *Procambarus bouvieri* by a single step RP-HPLC on a μBondapak-Phenyl column. The biological activity of the hormone was *in vitro* tested with bioassay culture of *P. vannamei* ovaries. The VIH was a peptide with a molecular mass of 8,388 $\pm$ 2 and consisted of 72 to 74 amino acid residues (Aguilar et al., 1992).

PCR strategy was used to characterize the GIH gene based on the GIH amino acid sequences of *H. americanus*. Alignment of this hormone with MIH from *Carcinus maenas* showed 53% amino acid sequence identity. When the different CHH from various species were compared, much lower degree of identity (19%) was demonstrated. Unlike CHH, both MIH and GIH gene lacked CHH-precursor-related peptide (CPRP) indicating that CHH, MIH and GIH preprohormones belonged to two distinct subgroups of the CHH/MIH/GIH family (De Kleijn et al., 1994).

Presently the studies of mechanisms underlined hormonal control of ovarian maturation have been progressed slowly. The eye ablation has been practically used for induction of ovarian maturation in various crustaceans including *P. monodon*. Our studies were preliminary investigations in order to identify the hormone involved in ovarian maturation of *P. monodon* and the hormonal control mechanisms. Hopefully these studies will provide basic knowledges leading to further studies on sustainable aquaculture of this species and other species in the future.

Table 2.1 Molecular mass of vitellin and vitellogenin subunits reported in decapod crustacean species.

Species	Study	Methods	Molecular mass (kD)		References
			Holo-Protein	Subunits	
Uca pugilator	Vitellin	4,5-^{3}H leucine SDS-PAGE	ND	100.6 123.5	Eastman-Rek and Fingerman, 1985.
Macrobrachium rosenbergii	Vitellin Vitellogenin	Gel filtration SDS-PAGE Sandwich ELISA	330	84 92	Derelle et al., 1986.
Penaeus japonicus	Vitellin	^{14}C-amino acid Immunoelectrophoresis SDS-PAGE Immunohistochemistry	ND	ND	Yano and Chinzei, 1987.
Parapenaeus longirostris	Vitellin	Sepharose 6B DEAE-sepharose PAGE SDS-PAGE	ND	45 66	Tom et al., 1987a, b.
Callinectes sapidus	Vitellin Vitellogenin	Ulatracentrifugation PAGE SDS-PAGE	ND	78 107 78 107 190	Lee and Puppione, 1988.
Penaeus vannamei	Vitellin	$(NH_4)_2SO_4$ Sephadex G-200 SDS-PAGE Western blot	ND	76 95 97 103	Quackenbush, 1989a.
Pandalus kessleri	Vitellin	Hydroxylapatite DEAE cellulose Sepharose 6B	560	81 110	Quinitio et al., 1989.
Penaeus monodon	Vitellin	Hydroxylapatite Sepharose 6B	540	74 83 90 104 168	Quinitio et al., 1990.
Penaeus semisulcatus	Vitellin	Sepharose 6B DEAE Sepharose SDS-PAGE	283	50 63 80 90	Browdy et al., 1990.

Table 2.1 (continue)

Species	Study	Methods	Molecular mass (kD)		References
			Holo-Protein	Subunits	
P. semisulcatus	Vitellin	Gel filtration PAGE SDS-PAGE Western blot Amino acid analysis	283	86 95	Tom et al., 1992.
P. vannamei			289	61 69	
P. monodon	Vitellin	PAGE SDS-PAGE Gel elution Western blot	ND	74 83 104 168	Chen and Chen, 1993.
P. monodon	Vitellin	Gel filtration Hydroxylapatite DEAE-Sephacel PAGE	492	35 45 49 58 64 68 82 91	Chang et al., 1993a.
M. rosenbergii	Vitellin	Gel filtration DEAE-Sephacel HPLC PAGE	240 450 780	90 104	Chang et al., 1993b.
P. monodon	Vitellogenin	Ultracentrifugation PAGE SDS-PAGE Western blot	ND	82 170	Chang et al., 1994.
P. monodon	Vitellogenin	Immunoprecipitation Immunofluorescence SDS-PAGE Western blot	ND	74 83 104 168	Chen and Chen, 1994.
P. semisulcatus	Vitellin	Ion-exchange chroma-tography PAGE SDS-PAGE Western Blot	ND	80 96 158	Khayat et al., 1994.
C. sapidus	Vitellin Vitellogenin	PAGE SDS-PAGE Western blot ELISA	ND	86 109 168 188	Lee and Watson, 1994.
Penaeus chinensis	Vitellogenin	Ultracentrifugation PAGE Immunoprecipitate Gel elution SDS-PAGE Western blot	ND	85 191	Chang and Jeng, 1995.

Table 2.1 (continue)

Species	Study	Methods	Molecular mass (kD)		References
			Holo-Protein	Subunits	
P. chinensis	Vitellin	Gel filtration Hydroxylapatite PAGE Gel elution SDS-PAGE	380 500	40 58 78 85 105 78 85 155	Chang et al., 1996.
P. semisulcatus	Vitellin Vitellogenin	Ultracentrifugation SDS-PAGE Western blot	ND	80 120 200 80 120 200	Lubzens et al., 1997.
Metapenaeus ensis	Vitellin	Gel filtration Anion exchange chromatography PAGE SDS-PAGE Western blot Amino acid analysis	350	76 102	Qiu et al., 1997.
Potamon potamios	Vitellin Vitellogenin	Ultracentrifugation Sepharose CL-6B PAGE SDS-PAGE	510 551	85 105 115 85 105 115 181	Pateraki and Stratakis, 1997.
M. rosenbergii	Vitellogenin	ELISA	ND	ND	Lee and Chang, 1997.
M. rosenbergii	Vitellogenin Vitellin	DEAE Hydroxylapatite chromatography PAGE ELISA Immunoprecipitation SDS-PAGE Western blot	700 ND	89 100 170 89 100	Lee et al., 1997.

ND = Not determined

Table 2.2 Vitellogenesis or gonad inhibiting hormone (VIH or GIH) reported in decapod crustacean species.

Species	Study	Methods	Molecular mass (Dalton)	References
Cancer magister	GIH	Boiling Sephadex G 25	2,000	Bomirski et al., 1981
Panulirus argus	VIH	Sephadex PAGE	5,000	Quackenbush and Herrnkind, 1983
Homarus americanus	VIH	RP-HPLC SDS-urea-PAGE	7,000-8,000	Soyez et al., 1987
Penaeus vannamei	VIH	Sephadex G 25	ND	Quackenbush, 1989b.
H. americanus	VIH	Gas phase microsequencing Fast atom bombardment mass spectrometry	9,135	Soyez et al., 1991.
Procambarus bouvieri	VIH	RP-HPLC	8,388	Aguilar et al., 1992
H. americanus	VIH	PCR	ND	De Kleijn et al., 1994

ND = Not determined