# CHAPTER I

# Introduction

Marine prawn culture, particularly the giant tiger prawn, *Penaeus monodon*, has become an important revenue for Thailand. The value of exported prawn products has been increased up to the levels of 4 to 5 thousand million bahts per year. However, the continued operation at optimal levels has encountered various problems due to the lack of basic knowledge in biology of these marine prawn species. One serious problem is an insufficient supply of the broodstock for fry production. The current practice is now using mature female prawns caught from the deep sea by the trawlers. The prawns are kept in captivity are induced for ovarian maturation by eyestalk ablation (Anilkumar and Adiyodi, 1980; Emmerson, 1983). Eyestalk ablation destroys the X-organ and sinus gland, the sites that produces and stores, respectively, the gonad inhibiting hormone (GIH) or vitellogenesis inhibiting hormone (VIH) which controls ovarian maturation (Aguilar et al., 1992; Hasegawa et al., 1993; Soyez et al., 1987). Eyestalk contains not only GIH but also other hormones such as crustacean hyperglycemic hormone-CHH (Huberman and Aguilar. 1988), molting inhibiting hormone-MIH (Chang et al., 1987), red pigment concentrating hormone-RPCH (Fernlund and Josefsson. 1972) and pigment dispersing hormone-PDH (Rao and Riehm. 1988) involved in important physiological regulatory processes as well as hormones involved in ion regulation. Therefore, eyestalk ablation can cause severe unwanted side effects and may cause a direct effect upon egg quality, mating efficiency, survival rates of larvae, and often inducing prematured death. (Primavera, 1985).

Various attempts for induction of ovarian development without destroying other hormonal systems have been investigated. They include manipulation of light intensity, photoperiod, light quality (wavelength), salinity, pH, tank management and

nutrition (Hillier, 1984; Wurt and Stickney, 1984; Primavera, 1985; Crocos and Kerr, 1986). However, for most marine shrimps, satisfied results have not been obtained without eye-ablation.

Another hypothesis about regulation of the ovarian maturation is based on the principle that ovarian development is achieved through the balance of the antagonistic actions of the two hormones, GIH and gonad stimulating hormone (GSH) or vitellogenesis stimulating hormone (VSH) (Hasegawa et al., 1993; Fingerman, 1997). It has been explored by treating the shrimps with extracts from various neuroendocrine tissues such as brain and thoracic ganglion (Eastman-Reks and Fingerman, 1984; Takayanagi et al., 1986) or with various known vertebrate hormones such as 17 α-hydroxy progesterone (Yano, 1985 and 1987), human chorionic gonadotropin (Bomirski and Klek-Kawinska, 1976; Nagabhushanam and Sarojini, 1987), hypophyseal gonadotropin (Reddy et al., 1987), 5-hydroxytryptamine (5HT) (Kulkarni et al., 1992), and 5HT in combination with brain and thoracic ganglion extract (Sarojini et al., 1995). However, the results were not encouraging and the practical use of these hormones has not been established for most of the marine shrimps.

As mentioned above, the role of GIH on ovarian development in various crustaceans has been established. In order to understand the control mechanisms of GIH on ovarian development, the GIH has to be isolated and identified. Therefore, GIH assay is required in order to be able to detect the GIH during purification steps. The classical GIH assay in shrimp and crab has been performed by bilateral eye-ablation and reinjection of the eyestalk extract back into the eye-ablated animals. The inhibition of ovarian growth is examined and compared to the control groups (Bomirski et al., 1981; Quackenbush and Herrnkind, 1983; Soyez et al., 1987; Sithigorngul et al., 1989). Even though this assay exhibits the affects of GIH on ovarian growth directly, there are several disadvantages that have to be considered

other than the laborious processes and time consuming of the assay. These factors are high variations of nutritional condition and maturation stage in each animal which sometimes can conceal the effect of eyestalk products.

An alternative method is to determine the inhibitory effects on vitellogenin synthesis (vitellogenin is a precursor of vitellin, a major yolk protein stored in the oocyte) by incubating the ovarian tissues with radioactive labeled amino acid and determining the inhibition efficiency of eyestalk products upon incorporation of radioactive labeled amino acid into ovarian protein (Browdy et al., 1990; Eastmans-Rek and Fingerman, 1985; Lui and O'Conner, 1976, 1977; Yano and Chinzei, 1987). In this assay, the antibody specific to vitellin is required to identify the labeled vitellin. However, many factors may effect nospecifically on the incorporation of radioactive labeled amino acid into yolk protein. Therefore, this assay must be performed with caution by using appropriate controls.

During reproductive cycle, vitellin is accumulated in the developing oocyte for providing a primary nutrition for embryogenesis and early larval development. Vitellogenin, a female-specific protein (FSP) is immunologically identical to ovarian vitellin demonstrated in several decapod species such as *Callinectes sapidus* (Kerr, 1969), *Uca pugilator* (Wolin et al., 1973), *Pandalus kessleri* (Quinito et al., 1989), *P. monodon* (Quinitio et al., 1990) and *Macrobrachium rosenbergii* (Chang et al., 1993b). It is synthesized and delivered to ovary via haemolymph circulation. The site of vitellin synthesis in decapod crustaceans has been demonstrated in several organs including ovary (Lui and O'Connor, 1976; Yano and Chinzei, 1987; Quackenbush, 1989; Browdy et al., 1990) and hepatopancreas (Paulus and laufer, 1987). In *Parapenaeus longirostris*, the vitellin synthesis was identified in adipose tissue (Tom et al., 1987a). Vitellogenin is transported into developing oocytes and incorporated into yolk protein. (Charniaux-Cotton, 1985; Tom et al., 1987b). The changed levels of vitellogenin in haemolymph were observed during the reproductive cycle. (Derelle et

al., 1986; Shafir et al., 1992; Chang and Shih, 1995). It has been hypothesized that vitellogenin levels were controlled by GIH or VIH (Soyez et al., 1987; Aguilar et al., 1992; Hasegawa et al., 1993) and GSH or VSH (Hasegawa et al., 1993; Fingerman, 1997). Therefore, the determination of haemolymph vitellogenin levels could be used as an indicator for the action of hormones instead of determination of ovarian growth and incorporation of amino acids during protein synthesis in GIH assay.

The objectives of the present study were to produce monoclonal antibodies (MAbs) specific to vitellin and vitellogenin, to use these antibodies for determining the haemolymph vitellogenin levels, to determine relationship between haemolymph vitellogenin levels and ovarian development and to develop the GIH assay in *P. monodon*, an economically important shrimp widely cultivated in Thailand. A monoclonal antibody approach was selected because it has several advantages over the conventional polyclonal antiserum. Monoclonal antibody production does not require highly purified antigens. A complex mixture of antigens can be used and hybridoma clones that produce monoclonal antibodies against desired antigens can be selected during screening processes (Sithigorngul et al., 1989). Once the established cell lines are obtained, homogeneous monospecific antibodies can be produced in unlimited amounts. Due to its mono-specificity, the monoclonal antibodies can be used to clarify the molecular nature of vitellin in *P. monodon*.

In chapter II, a literature review, will describe the biology, reproduction of *P. monodon*, methodologies for characterization of vitellin and vitellogenin and assay for GIH. Chapter III will be a preliminary experiment on the production of monoclonal antibodies using native vitellin as immunogen and using these MAbs for quantitative analysis of vitellogenin levels in the haemolymph. Chapter IV will be a refinement procedure in order to obtain a complete set of MAbs against each vitellin subunits and using them as a tool to clarify the confusion about vitellin subunits proposed by various investigators (Quinitio et al., 1990; Chen and Chen, 1993; 1994; Chang et al.,

1993a; 1994). Chapter V will be the application of MAbs to study the correlation of haemolymph vitellogenin levels and ovarian development which will lead to the development of GIH assay in the subsequent experiments. Chapter VI will summarize the overall results and a brief discussion of the potential application of MAbs for further studies.