CHAPTER I
INTRODUCTION

The giant freshwater prawn, *Macrobrachium rosenbergii*, is one of the important aquatic economic animals in Asia-Pacific countries including Thailand. Knowledge and understanding of its reproductive process will help to increase the aquaculture of this species, especially the monoculture of male prawns which yield larger proportional flesh and thus more valuable than female. During maturation, the prawns gonads are regulated by various agents, including neurotransmitters, neurohormones, pheromones, and lipids from feed which play synergistic roles in controlling the gonadal maturation and germ cell production through the eye-brain/thoracic ganglia-gonad axis, much like the hypothalamic-pituitary-gonadal axis (HPG axis) in mammals.

In this study, we focus on (1) the control of the above-mentioned axis on the-androgenic gland (AG) in male *M. rosenbergii* which is mediated by neurotransmitters (serotonin, 5-HT and dopamine, DA), neurohormones (gonadotropin-releasing hormones), and lipids (polyunsaturated fatty acids, PUFA; and highly unsaturated fatty acids, HUFA). The androgenic gland (AG) is the chief endocrine gland that controls male sex differentiation, the testicular development and sperm production. (2) The temporal expression and application of insulin-like androgenic gland hormone (*Mr*IAG) secreted from the AG as a marker for male sex for selecting male offsprings for monoculture. (3) The profile of lipids especially the saturated and unsaturated fatty acids that could play roles in growth and maturation of the testis.

Firstly, at the higher control level the X-organ-sinus gland located at the eyestalk secretes the antagonist neurotransmitters called 5-HT to inhibit and DA to promote gonad inhibiting hormone (GIH) which inhibit gonadal development. From previous study, DA delays ovarian maturation in *Uca pugilator* (Sarojini et al., 1995), and *M. rosenbergii* (Chen et al., 2003; Tinikul et al., 2009) and delays testicular

maturation in *U. pugilator* (Sarojini et al., 1995) and small male *M. rosenbergii* (Poljaroen et al., 2011). On the other hand, 5-HT promotes ovarian maturation in *Procambarus clarkii* (Sarojini et al., 1995), *Litopenaeus vannamei* (Vaca and Alfaro, 2000), *L. stylirostris* and *L. vannamei* (Alfaro et al., 2004), *Penaeus monodon* (Wongprasert et al., 2006), and *M. rosenbergii* (Meeratana et al., 2006; Tinikul et al., 2009), and testis maturation in small male *M. rosenbergii* (Poljaroen et al., 2011).

Secondly, the brain and thoracic ganglia may secrete a putative neurohormone called gonad stimulating hormone (GSH) which could be the GnRH-like hormone synthesized by neurons in the brain and thoracic ganglia which, like in vertebrates, also stimulate the prawn reproductive system (Sherwood and Adams., 2005; Tsai, 2006). Eventhough the actual GnRHs in decapods crustaceans have not yet been identified. There are some evidence to support the existence of GnRH like peptide in crustaceans by immunohistochemistry (Nernsoungnern et al., 2008a, 2008b; Saetan et al., 2013) and the injection of few GnRH isotypes could promote gonad maturation in *M. rosenbergii* (Nernsoungnern et al., 2009; Poljaroen et al., 2011). Moreover, there are other hormones in the GnRH family, including adipokinetic hormone (AKH), red pigment-concentrating hormone (RPCH), and corazonin (Crz) that may play roles in controlling gonadal maturation. There is a report showing that the Crz may have a negative effect on testicular development which is an opposite effect to GnRHs (Poljaroen et al., 2011).

Thirdly, lipids also play a role in reproduction through fatty acid molecules especially PUFA and HUFAs which are the precursors of prostaglandins (PGs) that may provide positive feedback to the higher controls at the 5-HT, DA and GSH/GnRH levels. In a balanced condition, lipids especially PUFAs and HUFAs could stimulate ovarian maturation and increase oocyte maturation which affects embryogenesis (Chansela et al., 2012). HUFAs are 20-carbon unsaturated fatty acids which usually are side chains of phospholipids. It is reported that three HUFAs, including 20:3 n-6 (dihomo-gamma-linolenic acid, DGLA), 20:4 n-6 (arachidonic acid, ARA), and 20:5 n-3 (eicosapentaenoic acid, EPA) are key factors that promote ovarian and testicular development and are the precursors for PGs synthesis (Smith et al., 2000). Recently, our group reported that injection of PGEs in female *M. rosenbergii* could stimulate ovarian maturation (Sumpownon et al., 2015). However, there is no study on the lipid

profiles and their roles (including that of PGs) in controlling the reproduction of male prawns.

Finally, the last endocrine organ that secreted *Mr*IAG to control testis maturation, spermatogenesis, and also male sex characteristics is the AG. This gland can be regulated by neurotransmitter, neurohormones, and lipids in the direct and/or indirect manners but these roles are still unstudied. Manipulations of AG could lead to economic benefit in obtain high quality male broodstock or all male offspring for monoculture because male grow faster and produce more muscle mass than female (Phoungpetchara et al., 2011). Using an AGs as a male marker could help in screening and selecting male larvae for momoculture.

Hence, this dissertation aims to: (1) study the possible role of the eyestalk-brain/thoracic ganglion-AG axis especially that mediated by neurotransmitters 5-HT, and DA and neurohormones (GnRH, Crz) in controlling male reproduction through their actions on the AG; (2) to assess the use of *MrIAG* as a male marker and find a simple and practical way to obtain all male offspring; and (3) to study the profile and localize lipids and fatty acids, especially HUFAs and PUFAs, in the testes of three male morphotypes that represent the stages of male maturation by mass spectrometry, and associated techniques. The data obtained could be use as the basis for formulating a balanced feed for male broodstock. All knowledge would be useful for increasing productivity in prawn culture through the manipulation and promotion of male sexuality.