CHAPTER VII
CONCLUSIONS

7.1 Effects of serotonin, dopamine, gonadotropin-releasing hormones, and corazonin, on the androgenic gland of the giant freshwater prawn

7.1.1 Morphological and histological changes of the AG

The AG of the prawns treated with 2.5×10^{-6} mol/prawn 5-HT, 500 ng/g BW l-GnRH-III were larger than the AG of the prawns treated with 2.5×10^{-6} mol/prawn DA, 500 ng/g BW Crz, and VC group.

7.1.2 The AG-somatic index (ASI)

The ASI values of prawns treated with 2.5×10^{-6} mol/prawn 5-HT and 50, 500 ng/g BW l-GnRH-III were significantly higher than the VC (P<0.05). In contrast ASI values of prawns treated with 2.5×10^{-6}, 2.5×10^{-7} mol/prawn DA and 50, 500 ng/g BW Crz were significantly lower than the VC (P<0.05).

7.1.3 Cell proliferation assay using 5-bromo-2'-deoxyuridine (BrdU)

The groups treated with 5-HT and l-GnRH-III showed significantly increased number of dividing cells (P<0.05). In contrast, the number of labeled nuclei decreased in the group treated with DA and Crz when compared with the VC group (P<0.05).

7.1.4 Specificity of rabbit antiserum against *Mr*IAG (anti-*Mr*IAG)

The specificity of anti-*Mr*IAG was tested by dot blot analysis. The positive results were detected in *Mr*IAG peptide and the AG extract. For recombinant IAG production, the pro-*Mr*IAG protein could be expressed and showed MW at ~20 kDa with the mature peptide at 17.6 kDa which corresponding to the native IAG probed by antibody against IAG.

7.1.5 Detection of *Mr*IAG using immunofluorescence

The immunoreactivity of *Mr*IAG in the AG of the group treated with 5-HT and l-GnRH-III were shown to be very intense in cells throughout the AG but the *Mr*IAG-*ir* was very weak in the groups treated with DA and Crz.

7.1.6 Estimation of the relative amounts of *Mr*IAG by ELISA

*Mr*IAG concentration in the group treated with 5HT and l-GnRH-III were significantly higher than in the VC group ($P<0.05$). However, the concentration of *Mr*IAG of the groups treated with DA and Crz were significantly lower than that of the VC group.

7.2 Temporal expressions of *MrIAG* in larvae and postlarvae, and the use this gene as a marker for selecting male offsprings

7.2.1 Performance of postlarvae culture

The survival trend of PL1 during experiments was highest in PL1D7 at 95.6 %, followed by PL1D6 at 93.8 and PL1D5 93.0 %, whereas PL1D2 showed only 73.8 % survival.

7.2.2 Morphology of postlarvae

The PL prawns in crops PL1D1-PL1D13 showed similar external features but different only in the body length.

7.2.3 Temporal expression of *MrIAG*

MrIAG expression of *M. rosenbergii* could be detected in PL1D1 prawns or first day of flipping with the length of 394 bp.

7.2.4 *MrIAG* expression in a single prawn of each crop

The results showed that PL1D8 or first crop of flipping prawn had 80% male with high and 20% male with low IAG expression. The second and third crops of flipping prawns (PL2D8 and PL3D8) had 33.33% male with high and 26.67% male with low expression of *IAG*. The forth crop of flipping prawns (PL4D8) had only 20% male with high and 40% male with low *IAG* expression.

7.2.5 The percent of male prawn in each flipping day as determined by observing the presence of male gonopore complex

After culturing for 4 months the percent of male in PL1 was 81.5% and PL2 was 76.1% which were significantly different from PL3 at 64.6%. So, we recommend that to obtain mostly male the prawns should be collected and separated at PL stages at flipping day 1 to 2 where male could be more than 75%.

7.3 Changes of phosphatidylcholine and fatty acids in germ cells during testicular maturation in three developmental male morphotypes of *M. rosenbergii* revealed by imaging mass spectrometry

7.3.1 Histology of the seminiferous tubules

The stages of the STs were separated into three groups representing early, middle, and late stages of spermatogenesis. Group A contained Sg and nurse cells, and mostly Sc. Group B contained some Sg and Sc but mostly St and immature Sz with condensed chromatin. Group C contained mostly mature Sz with de-condensed chromatin. In all stages, the STs were surrounded by intertubular area.

7.3.2 Quantification of lipids by thin layer chromatography

PC band showed the highest intensity in the STs of group B when compared with group A and C ($P<0.05$). Moreover, the highest amounts of PCs could be observed in the OC males and the lowest amount in BC males.

7.3.3 Identification of lipids by tandem mass spectrometry (MS/MS)

All signals from ion images were identified and comprised of *m/z* 756.5, 760.5, 782.5, 798.5, 780.5, 796.5, 784.5, 800.5, 804.5, 806.5, 808.5, 824.5, and 810.5. The signals that represented omega-3 FAs were 826.5 (EPA-containing), 846.5 (EPA-containing), 828.5 (DHA-containing), 844.5 (DHA-containing), 870.5 (DHA-containing), and 872.5 (DHA-containing), and the signals that represented omega-6 were 820.5 (ARA-containing), 830.5 (ARA-containing), and 832.5 (ARA-containing).

7.3.4 Distributions of lipids by imaging mass spectrometry (IMS)

Ion images indicating the four distributions pattern of PCs: (1) the PCs presented in all group*s* of STs showed high signal intensities in developing germ cells area containing Sg, Sc, and St and the IT. (2) The PCs presented in the STs of groups A and B showed the highest signal intensities in developing germ cell areas and the IT. (3) The PCs presented in the STs of groups B and C also showed high signal

intensities in the IT. (4) Lastly, the PCs presented only in the STs of group A showed high signal intensities in developing germ cell areas.

7.3.5 Quantification of fatty acids by gas chromatography-mass spectrometry (GC-MS)

During the development from SM to mature BC, the OC testes contained the highest amounts of FAs. The major FAs that were detected in the testes of SM, OC, BC were 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, with the SM and OC having higher mounts of these FAs than BC. However, FA ratios showed that the testes of SM contained higher accumulations of 17:0, 20:1, 20:2, 20:5 (EPA) and 22:6 (DHA) when compared with OC while there were no significant differences between SM and BC. Similarly, STs of group B showed higher amount of 15:0, 16:0, 17:0, 18:0, 18:1, 18:2, 20:2, and 20:5 when compared with group C.