Effect of Salinity on Previtellogenic Development of Female Pacific White Shrimp (*Litopenaeus vannamei* Boone, 1931)

Ekachai Duangjai[1*], Wanvipa Suthikrai[2], Narongsak Puanglarp[3], Prapansak Srisapoome[1], Panida Unagul[4] and Oraporn Meunpol[1]

ABSTRACT

Successful induced maturation of female shrimp consists of three stages of gonadal development, namely, previtellogensis, vitellogensis, and final maturation. Previous research in shrimp reproduction investigated mostly the final maturation stage and spawning process, and few studies were conducted on previtellogenic ovarian development. Several factors influence previtellogenesis of female Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931). In this study, the effect of salinity, one of the most powerful factors, was investigated. Female *L. vannamei*, (45 g body weight, 120 days old) with previtellogenic ovaries were reared in cages (5x5 m^2) installed in two earthen ponds. Salinity levels were 15 and 30 ppt. Haemolymph, hepatopancreas and ovaries of the experimental shrimp were collected monthly for hormone analysis. P_4, E_2 and $PGF_{2\alpha}$ levels in haemolymph of the shrimp samples were measured by either RP-HPLC or RIA. Biological marker (Primer) specific for 357 bp of Vg and 314 bp of FAMeT gene modified from NCBI were developed from the hepatopancreas of female *L. vannamei*. Vg and FAMeT gene expression levels using 1stcDNA synthesis from hepatopancreas and ovary tissues were determined by RT-PCR. Results showed that the levels of Vg and FAMeT gene expression in the ovaries and hepatopancreas of shrimp reared in 30 ppt were significantly higher than those of shrimp reared in 15 ppt ($P<0.05$). The mean expression level of Vg and FAMeT in shrimp hepatopancreas was higher than in the ovaries. Similarly, changes in P_4, E_2 and $PGF_{2\alpha}$ concentrations in haemolymph and GSI of shrimp reared in 30 ppt were significantly higher than those of shrimp reared in 15 ppt ($P<0.05$). In conclusion, the experiment showed that salinity played an important role in shrimp ovarian development at different levels of changes, namely morphological (GSI), hormonal and gene expression levels.

Keywords: Salinity, previtellogenic stage, reproductive hormones and genes, ovarian development

---

[1] Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok 10900 Thailand+
[2] Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330 Thailand
[3] Center of Excellence for Marine Biotechnology, Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330 Thailand
[4] National Center for Genetic Engineering and Biotechnology (BIOTEC), Genome Institute, Thailand
* Corresponding author, email: Mr1fisheries@hotmail.com

INTRODUCTION

The Pacific white shrimp (*Litopenaeus vannamei* Boone, 1931) is an internationally significant aquaculture species and one of the most common marine shrimp species cultured in both coastal and low salinity areas in Thailand (Limsuwan and Chanratchakool, 2004). Unilateral eyestalk ablation is a traditional technique which is still being used to induce gonad maturation and spawning of captive female shrimp, although it has been reported that it causes negative effects on female shrimp health (Treerattrakool *et al*., 2011). Today, most studies focus on shrimp culture techniques, female broodstock enhancement, and shrimp genetic improvement (Coman *et al*., 2007; Hoa *et al*., 2009; Meunpol *et al*., 2010). However, few studies have been conducted on reproductive physiology changes especially on gonad maturation affected by changes in environmental factors (Okumura *et al*., 2004). It is necessary to understand the changes in reproductive physiology which is controlled by neuroendocrine gland of crustaceans (Fingerman, 1995; Palacios *et al*., 1999). Gonad maturation in crustaceans appears to be principally regulated by two antagonistic neuropeptides: Gonad-inhibiting hormone, GIH (also called vitellogenesis-inhibiting hormone, VIH, in females) and gonad stimulating hormone (GSH) (Sainz-Hernandez *et al*., 2008; Nguyen *et al*., 2010). It has been reported that non-peptidergic hormones prompted predictions that control crustacean reproduction activity (Chen *et al*., 2004; Morera *et al*., 2013). Recently, the seasonal reproductive activity of female crustaceans is characterized by examining hemolymph vertebrate-type steroid concentration (Okumura *et al*., 2004; Meunpol *et al*., 2007, 2010). The importance of vertebrate-type steroid has been demonstrated as gonad-stimulating hormone which play an important role in reproductive physiology control including ovarian development, ovulation, and spawning (Okumura *et al*., 2004; Meunpol *et al*., 2007, 2010). However, during synthesis, it is affected by several external factors especially water salinity due to seasonal and environmental variations (Shinji *et al*., 2012). Understanding the importance of the changes in vertebrate-type steroid hormone at previtellogenic stage of ovaries in marine shrimp will allow us to establish their productive tools for controlling populations of captive-reared shrimp via effective breeding without traditional eyestalk ablation. Therefore, this study aimed to monitor some of the reproductive hormones and genes of female Pacific white shrimp (*L. vannamei*) at previtellogenic stage of ovarian development in the hope that this will lead to natural final maturation and also spawning without destroying the eyes and lives of the shrimp broodstock.

MATERIALS AND METHODS

Experimental design

Female *L. vannamei* (45 g.bw, 120 days approx.) at previtellogenic stage were reared in two different salinities, i.e. 15 and 30 ppt, in earthen ponds at the Chachoengsao Coastal Aquaculture Development Center, Department of Fisheries, Chachoengsao Province, Thailand, from June to September, 2011. The stocking density was 2 shrimp/m^2. They were fed twice a day (08.00 am and

17.00 pm) with 50% commercial diet (3% bodyweight per day) and 50% natural feeds (5% bodyweight per day). Natural feed including octopus (*Octopus* sp.) and oyster (*Crassostrea* sp.) were used. Water was changed monthly at the rate of 40% of total volume. Uninterrupted aeration through air stones was provided.

Sample collection

Ten female shrimp per group were collected monthly at days 0, 30, 60, 90 and 120. They were transferred alive to the laboratory of the Center of Excellence for Marine and Biotechnology, Chulalongkorn University, Bangkok, Thailand. They were kept in rectangular concrete tanks (two 800 l concrete tanks with filtration) at ambient temperature (28-30ºC) for at least 12 hours. For hormone analysis, hemolymph was withdrawn from the ventral sinus of each female shrimp using a 24Gx1 syringe (NIPRO) containing 500 µl of anticoagulant (1 mg Na_2- EDTA/ml, Lab-Scan Asia, Thailand). Each sample was stored at -80ºC for further hormone extraction and protein concentration determination by the Bradford method (Bradford, 1976). The whole shrimp was immediately dissected and target tissues including hepatopancreas and gonads were separately transferred into 2.0 ml Twist Top Vials, deep frozen in liquid nitrogen and stored at -80ºC for RNA extraction.

Progesterone (P_4) and 17β-estradiol (E_2) extraction and analysis

The samples were extracted and analyzed at the Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. The method used to take the hemolymph sample of shrimps was according to the method of Kamonpatana *et al*. (1979). A 300 µl hemolymph of female shrimp was used and pipetted into10 ml assay tube containing 3 ml of cold diethylether (4ºC) for 2 minutes. Then, all sample tubes were stored at -70 ºC for 15 minutes until the solution was clearly separated. The supernatant was immediately transferred into a new assay vial for vacuum drying then stored at -30ºC until further hormone analysis.

Radioimmunoassay Procedure

The radioimmunoassay for dry hemolymph samples was performed by utilizing standard progesterone (cat. No. 24614) (Merck, Germany) and 1, 2, 6, 7^{-3}H P_4/E_2 RIA kit (Amersham, UK) according to the manufacturer's guideline. The process is as follows: a 100 µl of phosphate buffer solution (PBS), pH 7, was added to the dry extract (2 replications) in 10 ml vials and mixed by vortex mixer, then incubated at 4ºC for at least 12 hours. After incubation, 100 µl of progesterone antiserum (antibody; As-P) was added into the incubated vial and mixed. The mixture was then incubated at room temperature for 1 hour. After incubation, 100 µl of [(1, 2, 6,7^{-3}H) P_4] was added and incubated at 4ºC for at least 12 hours. Another 200 µl of ice-cold dextran-coated charcoal was added and incubated at 4ºC for 20 minutes. Then, the vial was centrifuged at 3,000 rpm for 15 minutes. The supernatant was transferred into a counting vial and 4 ml of scintillation [0.01%1,4-bis-214 Methyl-5-Phenyloxyzolyl-Benzole (POPOP), 0.3 %P-Terphynyl (pTP) dissolved in 2.5 ml of toluene solution was added, and then

incubated at room temperature for 24 hours before being counted by Liquid Scintillation Counter (LSC). Hemolymph progesterone concentration was measured and compared with the progesterone standard curve. The standard graph was plotted and the concentration of progesterone was obtained from the standard plot. The cross-reactivity of this antibody is given in Table 1.

Table 1. Percent recovery of progesterone (P_4) and 17β-estradiol (E_2) used in radioimmunoassay.

Hormones addition (pg/ml)		Tube number	Concentration ($\bar{X} \pm SD$)		Recovery (%)	
E_2	P_4	-	E_2	P_4	E_2	P_4
0	0	5	BS	BS	-	-
5	5	5	3.87 ± 0.61	4.87 ± 1.54	96.2	90.3
10	10	5	9.11 ± 1.04	9.34 ± 2.12	98.2	95.8
25	25	5	25.08 ± 2.25	24.37 ± 2.11	99.2	98.4

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) extraction and analysis

$PGF_{2\alpha}$ solution was extracted from hemolymph samples using glass homogenizer tubes containing 1 ml of extracted solution (water: ethanol, 1:4) and 50 µl acetic acid. The homogenate was mixed and immediately centrifuged at 3,000 rpm for 5 minutes at 4ºC following supernatant collection. The hemolymph $PGF_{2\alpha}$ levels in an extract solution was quantified using a Sep Pak (C18) mini column (Alltech, USA). The eluted solution was evaporated under the vacuum dried drying and re-dissolved in twenty microliter of methanol (Tahara and Yano, 2003). The supernatant was then injected onto a Prevail C_{18} column (4.6 mm x 15 cm I.D.) (Alltech, MD, USA) and run on an isocratic solvent of 30 % acetonitrile (CH_3CN) per 70% HPLC water (3:7, v/v) (pH 2 by 17mM phosphoric acid) at a flow rate of 1.0 ml/min. The $PGF_{2\alpha}$ peak of the sample was measured at 200 nm. Hemolymph $PGF_{2\alpha}$ levels was calculated and compared with the $PGF_{2\alpha}$ standard curve (Figure 1).

Total RNA preparation and first-strand cDNA synthesis

Half micrograms of ovary and the hepatopancreas tissues were homogenized in TRI-REAGENT (Molecular Research Center, Cincinnati, OH, USA) following the protocol of the manufacturer. One and a half micrograms of total RNA from ovary and hepatopancreas of female *L. vannamei* broodstock were reverse transcribed to the first strand cDNA using an ImProm-II™ Reverse Transcription System Kit (Promega, USA) according to the manufacturer's protocol using 0.5 µg of oligo dT_{15} primer and appropriate amount of DEPC-treated H_2O in a final volume of 20 µl to prime cDNA synthesis at 42°C for 90 minutes. The quality and quantity of freshly synthesized first strand cDNA of females *L. vannamei* broodstock was spectrophotometrically examined (OD_{260}

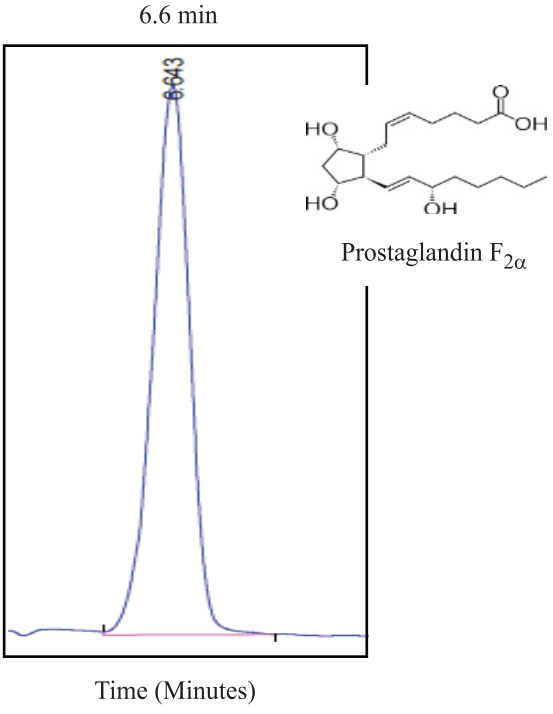

Figure 1. Chromatogram of Prostaglandin F_{2alpha} ($PGF_{2\alpha}$) standard measured by Reverse-phase High Performance Liquid Chromatography (HPLC).

$/OD_{280}$) and electrophoretically analyzed by 1.2% agarose gel. The obtained first strand cDNA template was kept at -20 °C until use.

Reverse transcription polymerase chain reaction (RT-PCR)

Total RNA extracted from ovary and hepatopancreas of female *L. vannamei* broodstock obtained from shrimp groups 1 and 2 were used as the first strand cDNA. The expected sizes of β-actin, Vg and FAMeT transcripts were 234, 357 and 314 bp, respectively (Figure 2).

To detect β-actin transcript, β-actin (F) (5′-GCGAGAAATCGTTCGTGAC-3′) and β-actin (R) (5′-TCTGGTGGATGTT GAGGTAGTA-3′) primers designed from the EST clone containing β-actin sequence from *L. vannamei* (GenBank accession no. AF300705) were used. PCR amplification was performed as follows: 1 cycle of 94ºC for 3 minutes for initial denaturation, followed by 29 cycles of 94ºC for 30 s (denaturing step), 55 ºC for 1.0 minutes (annealing step), and 72ºC for 1 minutes (extension step) and finally the extension was performed with 1 cycle of 72ºC for 7 minutes (Figure 2).

To detect Vg transcript, Vg (F) (5′-GAACTGCTCCTTTCACTGCCTTTG-3′) and Vg(R) (5′- TATGTTCCAGTTGGTGC TCCCTT-3′) primers designed from the EST clone containing Vg sequence from *L. vannamei* ovary (GenBank accession no. AY321153) were used. PCR amplification

was performed as follows: 1 cycle of 94ºC for 3 minutes for initial denaturation, followed by 29 cycles of 94ºC for 30 s (denaturing step), 58 ºC for 1.0 minutes (annealing step), and 72ºC for 1 minutes (extension step) and finally the extension was performed with 1 cycle of 72ºC for 7 minutes (Figure 2).

To detect FAMeT transcript, FAMeT (F) (5′-ACTGCCTCACATACAACTTCATTC-3′) and FAMeT(R) (5′-ACCTCACCTGCCTGGGACTC-3′) primers designed from the EST clone containing FAMeT sequence from *L. vannamei* (GenBank accession no. AY823408) were used. PCR amplification was performed as follows: 1 cycle of 94ºC for 3 minutes for initial denaturation, followed by 29 cycles of 94ºC for 30 s (denaturing step), 58 ºC for 0.45 minutes (annealing step), and 72ºC for 1 minutes (extension step) and finally the extension was performed with 1 cycle of 72ºC for 7 minutes. 5µl of the β-actin, Vg and FAMeT amplification product was electrophoretrically analyzed though a 1.5 % agarose gel (Figure 2).

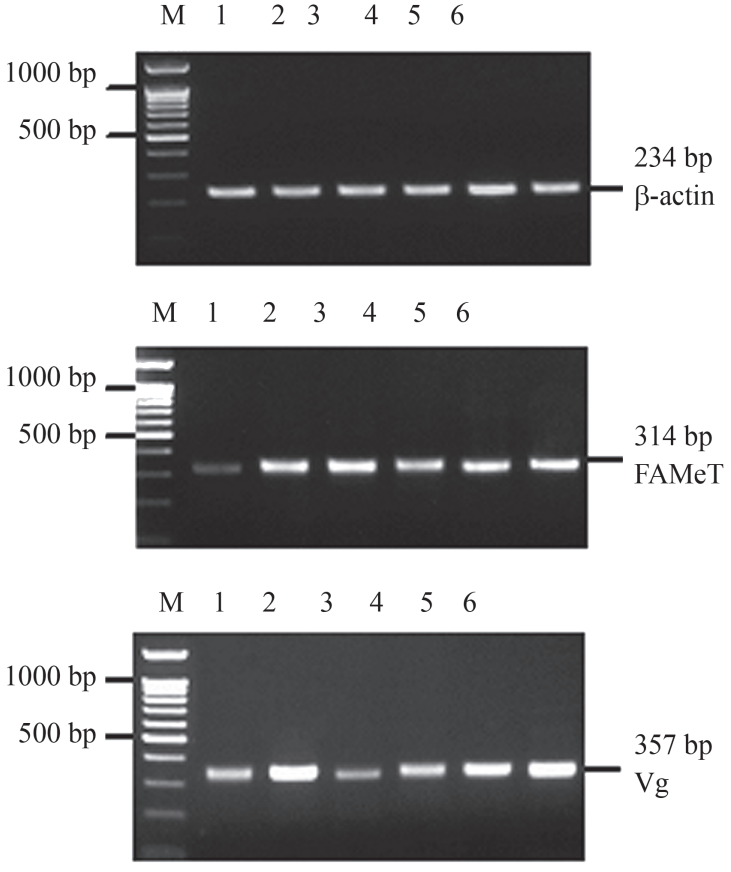

Figure 2. RT-PCR analysis of β-actin, farnesoic acid O-methyltransferase (FAMeT), and vitellogenin (Vg) gene using the first strand cDNA of ovary (lanes 1-3) and hepatopancreas (lanes 4-6) of female Pacific white shrimp (*L. vannamei*) previtellogenic stage (lane M is 100 bp DNA ladders marker).

Statistical analyses

Statistical analyses of the reproductive hormones and genes data were conducted with analysis of variance (ANOVA) followed by Duncan's multiple range test.

RESULTS

Gonadosomatic index (%)

There were no significant differences in changes in GSI of both females at 15 and 30 ppt. However, compared between initial study to day 30, day 30 to 60, day 60 to 90, and day 90 to 120, GSI observed from 15 and 30 ppt continually increased ($P>0.05$). (GSIs of shrimp at 15 ppt were 1.53, 2.70, 1.19, and 1.17 fold while at 30 ppt, GSIs were 4.45, 1.32, 1.10, and 1.14 fold, compared between initial study to day 30, day 30 to 60, day 60 to 90, and day 90 to 120, respectively) (Figures 3 and 4).

Changes in hemolymph P_4 E_2 and $PGF_{2\alpha}$ levels of female L. vannamei shrimp

Hemolymph P_4 level at 15 ppt was highest at day120 while the lowest at initial study. Obviously, there were similar P_4 levels at 30 ppt. The P_4 level observed from 15 ppt rapidly increased from initial study to day 60. Afterwhich, there was a slight decrease in day 90. Then, it gradually increased and peaked again in day120. P_4 level at 30 ppt showed similar patterns with those in 15 ppt. There was a rapid increase ($P<0.05$) from day 0 to day 60. Then, there was a decrease from day 90 till day120. The hemolymph P_4 levels at 15 ppt were 4.4, 1.51, 1.11, and 1.28 fold, while at 30 ppt, the levels were 24.6, 1.42, 0.72, and 0.92 fold, comparing between initial study to day 30, day 30 to 60, day 60 to 90, and day 90 to 120, respectively (Figure 3).

Hemolymph E_2 level of 15 ppt rapidly increased ($P<0.05$) from day 30 to day 60. After that there was a slight decrease in day 90. Then, it gradually increased and peaked again in day120. Hemolymph E_2 level of 30 ppt rapidly increased ($P>0.05$) from the beginning to the end of experiment. The hemolymph E_2 level at 15 ppt were 2.52, 5.67, 0.68, and 1.27 fold, while at 30 ppt, the levels were 11.06, 2.07, 1.48, and 1.18 fold at days 30, 60, 90, and 120, respectively, compared between initial study to days 30, days 30 to 60, days 60 to 90, and days 90 to 120, respectively (Figure 3).

The similar hemolymph $PGF_{2\alpha}$ levels were shown in both 15 and 30 ppt. $PGF_{2\alpha}$ level slightly increased ($P>0.05$) from days 30 to 120. The hemolymph $PGF_{2\alpha}$ levels at 15 ppt were 0.66, 2.70, 1.19, and 1.17 fold, while at 30 ppt the levels were 3.64, 1.82, 0.23, and 4.60 fold compared between initial study to day 30, days 30 to 60, days 60 to 90, and days 90 to 120, respectively (Figure 3).

Changes of Vitellogenin gene (Vg)

The mean expression levels of Vg gene in ovary and hepatopancreas of female *L. vannamei* at 15 and 30 ppt are presented in Figure 4. The RT-PCR results of Vg gene in ovary and hepatopancreas at 15 and 30 ppt indicated that the expression levels continually increased from the beginning to the end of the experiment. However, mean

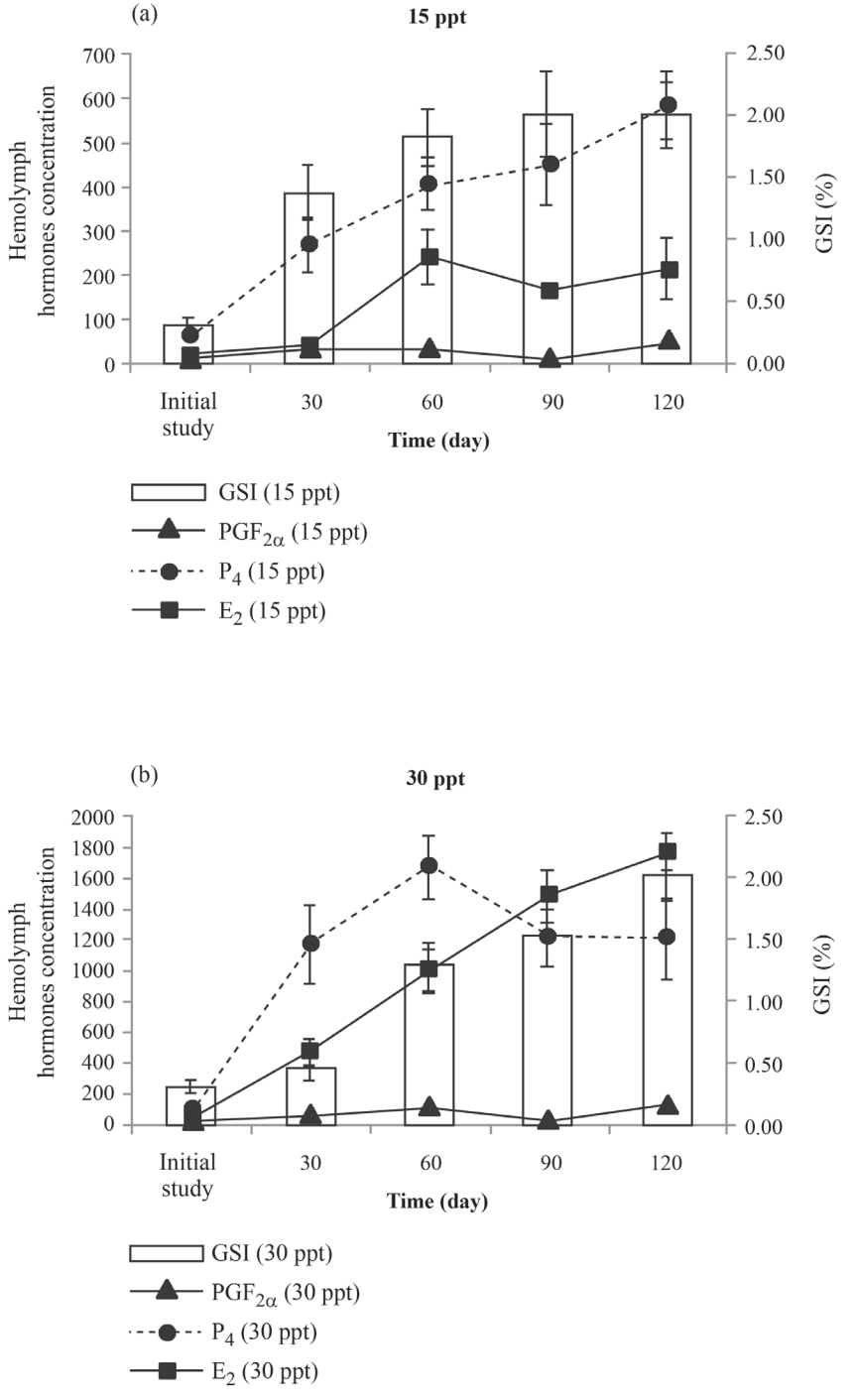

Figure 3. Comparison between changes in hemolymph progesterone (P_4), 17β-estradiol (E_2), Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) level, and gonadosomatic index (GSI) of female Pacific white shrimp (*L. vannamei*) at previtellogenic stage at 15 ppt (a) and 30 ppt (b).

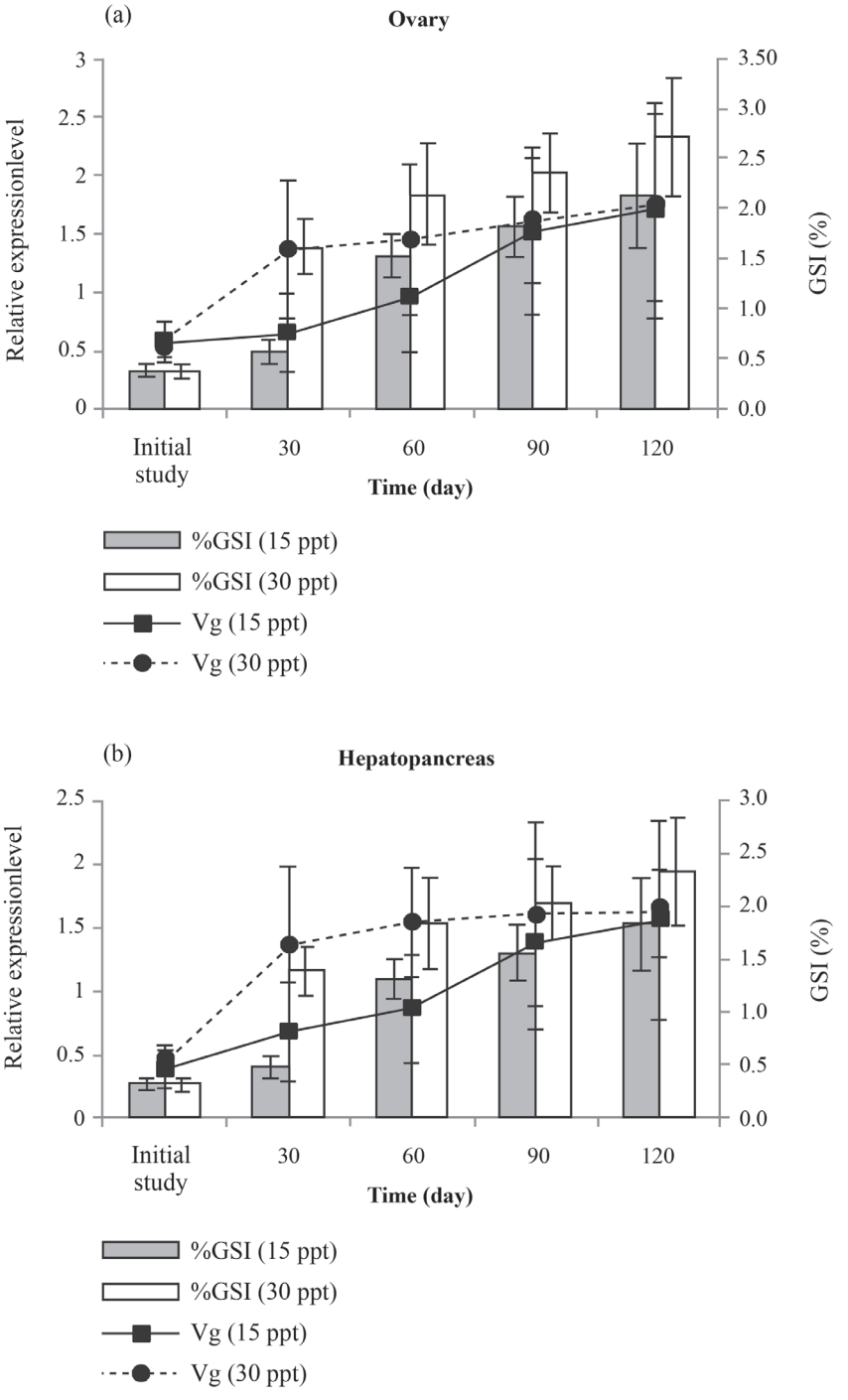

Figure 4. Comparison between relative expression pattern of vitellogenin gene (Vg) in ovary (a), hepatopancreas (b), and gonadosomatic index (GSI) of female Pacific white shrimp (*L. vannamei*) at previtellogenic stage, at 15 ppt and 30 ppt.

expression levels of Vg gene in ovary and hepatopancreas at 30 ppt was significantly ($P>0.05$). At 15 ppt, the mean expression levels of Vg gene in ovaries were 1.16, 1.16, 1.15 and 1.12 fold. For 30 ppt, levels were 2.52, 1.12, 1.11, and 1.07 fold, compared between initial study to day 30, days 30 to 60, days 60 to 90, and days 90 to120, respectively. The mean expression levels of Vg gene in hepatopancreas were 1.71, 1.41, 1.61, and 1.13 fold at days 30, 60, 90, and 120, respectively. For 30 ppt, the levels were 3.11, 1.12, 1.11, and 1.01 fold, compared between initial study to day 30, days 30 to 60, days 60 to 90, and days 90 to 120, respectively (Figure 4).

Changes of farnesoicacid O-methyltransferase gene (FAMeT)

In ovary, relative expression of FAMeT gene of 15 ppt observed from the beginning of experiment to day 90 period slightly increased ($P>0.05$) and then the level was stable until the end of the experiment. Meanwhile, the expression levels of FAMeT gene of 30 ppt rapidly increased from the beginning to day 30 ($P>0.05$), then, slightly increased from days 30 to 60. After that, the levels were stable (from day 60) until the end of the experiment.

In hepatopancreas, the expression levels of FAMeT gene of female shrimp at 15 ppt slightly increased throughout the study period, with the highest level showing in day120 ($P>0.05$). At the same time, the expression levels of FAMeT of 30 ppt increased rapidly and the highest level was reached in day 60, and then decreased until day 90, after that there was a slight increase from days 90 until 120. At 15ppt, the mean expression levels of FAMeT gene in ovaries were 1.84, 1.22, 1.33, and 1.04 fold. For 30 ppt, levels were 3.0, 1.13, 1.0, and 1.0 fold, compared between initial study to day 30, days 30 to 60, days 60 to 90, and days 90 to 120, respectively. The mean expression levels of FAMeT gene in hepatopancreas were 2.03, 1.16, 1.07, and 1.12 fold. For 30 ppt, it were 5, 1.2, 0.82, and 1.17 fold, compared between initial study to day 30, day 30 to 60, day 60 to 90, and day 90 to 120, respectively (Figure 5).

DISCUSSION

Reproductive status, in terms of changes in hemolymph P_4 E_2 and $PGF_{2\alpha}$ levels, GSI, mean expression levels FAMeT and Vg gene in ovary and hepatopancreas of female *L. vannamei* shrimp, was determined after observation at 15 ppt and 30 ppt during days 0 to120. In the study, they were compared between initial study to day 30, day 30 to 60, day 60 to 90, and day 90 to 120. No significant changes in GSI of female *L. vannamei* shrimp were observed at 15 ppt. A gradual increase in shrimp GSI at the beginning of the study was 0.32% and 1.82% at the end of the experiment. Similar patterns of GSI were reported in female *L. vannamei* shrimp at 30 ppt. Shrimp GSI started to rapidly increase at day 30 (1.31%) to day 60 (1.83%) and slowly increased at day 120 (2.32%). For gonad development, there were significant differences in GSI of female shrimp between 15 and 30 ppt. This indicated that the regulation of crustacean reproductive status, in terms of ovarian development, depends on environmental factors especially

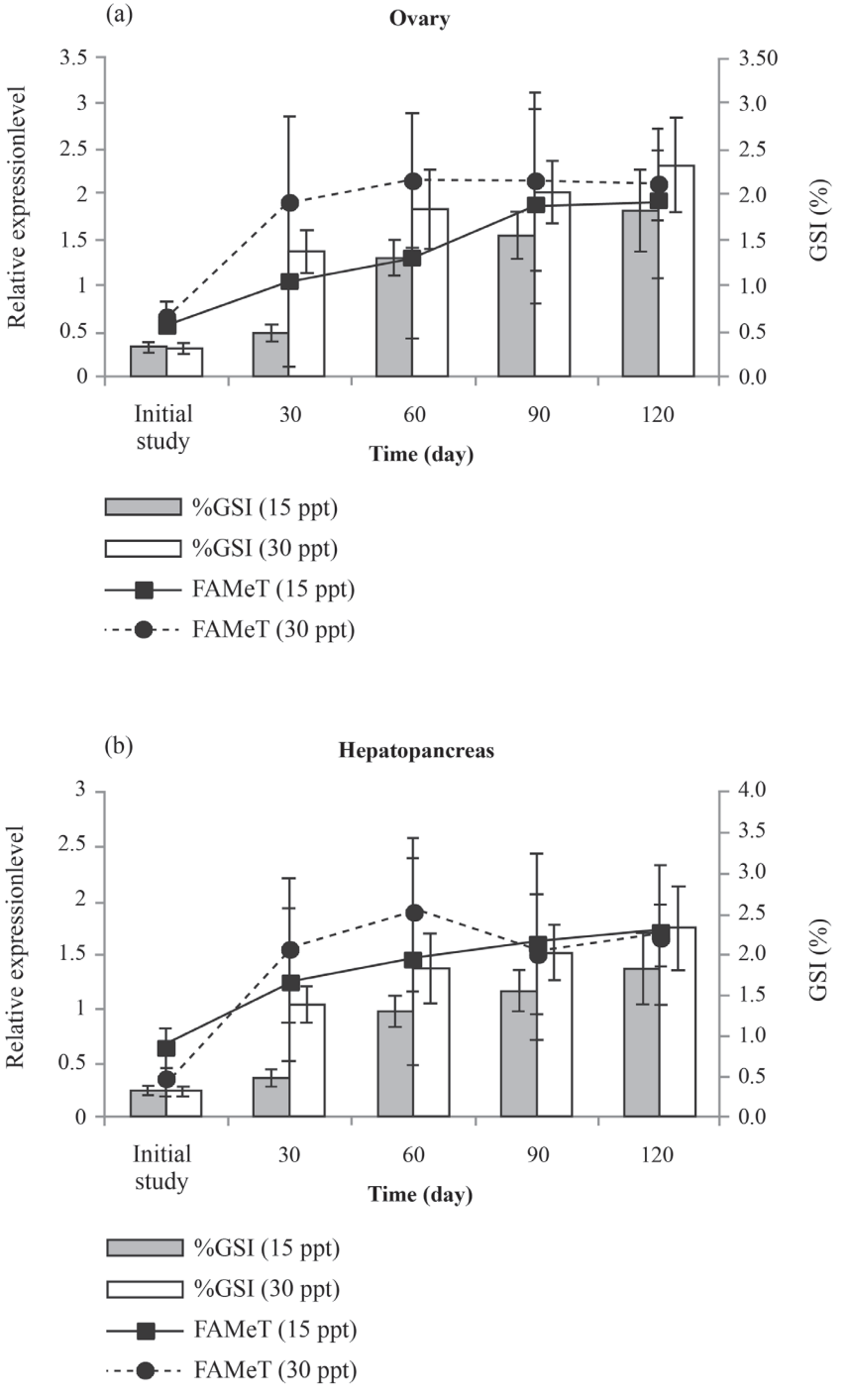

Figure 5. Comparison between relative expression pattern of farnesoicacid O-methyltransferase gene (FAMeT) in ovary (a), hepatopancreas (b), and Gonadosomatic Index (GSI) of female Pacific white shrimp (*L. vannamei*) at previtellogenic stage of 15 ppt and 30 ppt.

salinity which affects endocrine control as studied earlier in several crustacean species. Ghazy *et al*. (2009) reported that the low salinity significantly influenced negatively the growth survival and reproduction rates of the water flea, *Daphnia magna* which was studied under laboratory conditions. This suggested that salinity may play a significant role or become a limiting factor during oocytes maturation. The occurrence of shrimp GSI was always found at the beginning of oocyte development process, generally known as induced by vertebrate-type steroid hormones such as P_4, 17α-OHP_4, E_2 and PGs (Tsukimura and Kamemoto, 1991; Fingerman, 1997). Meunpol *et al*. (2007) reported that P_4 and 17α-OHP_4, extracted from natural polychaetes (*Perinereis* sp.), induced ovarian development of female black tiger prawns *Penaeus monodon* via increasing oocyte diameter and percentages of vitellogenic oocytes. In the present study, there were comparisons between hemolymph P_4 E_2 $PGF_{2\alpha}$ levels and GSI of female *L. vannamei* shrimp observed in 15 and 30 ppt. The levels were gradually increased from the beginning of experimental study to the end of experiment. The importance of vertebrate-type steroid has been demonstrated as gonad-inhibiting hormone and gonad-stimulating hormone (Reddy *et al*., 2004) which have played an importa role in reproductive physiology control including ovarian development, ovulation, and spawning of female black tiger prawns *Penaeus monodon* (Meunpol *et al*., 2010), female kuruma *prawn Marsupenaeus japonicas* (Tahara and Yano, 2003; 2004). In previous study of female kuruma prawn *Marsupenaeus japonicas* (Tahara and Yano, 2003; D'Croz *et al*.,1988) demonstrated that haemolymph $PGF_{2\alpha}$ and PGE_2 levels highest in previtellogenic stage ovaries and gradually decreased toward maturation that is supported the an important role of vertebrate-type steroid hormones in crustacean. While the $PGF_{2\alpha}$ data demonstrated that the hemolymph $PGF_{2\alpha}$ levels gradually increased from the beginning of experimental study to the end of experiment. However, Hemolymph $PGF_{2\alpha}$ levels of female *L. vannamei* shrimp observed from 30 ppt were generally higher than those of 15 ppt. Hemolymph P_4 levels significantly increased from the beginning of the study till the end of the experiment. Previous research suggested that P_4 may directly enhance maturation via vitellogenesis stimulation or act as a precursor to Vitellogenesis Stimulating Ovarian Hormone (VSOH) (Fingerman *et al*., 1993). In female kuruma prawn *Marsupenaeus japonicas*, the vertebrate-type steroid hormones which detected in the hemolymph may be controlled the activity of the ovaries and hepatopancreas (Okumura *et al*., 2004). Interestingly, the expression levels of Vg and FAMeT mRNA in hepatopancreas and ovary of females *L. vannamei* shrimp at 15 ppt and 30 ppt were increased towards shrimp GSI and also hemolymph P_4 E_2 and $PGF_{2\alpha}$ levels. Vg gene is an egg yolk precursor protein expressed in the female shrimp which measured in shrimp hemolymph. It has been found to reflect the degree of female shrimp ovarian development before eyestalk ablation (Arcos *et al*. 2011) and also expressed only in ovaries and hepatopancreas but not in other tissues of female shrimp (Hiransuchalert *et al*., 2013). Similarly, the function of FAMeT catalyzes the methylation of farnesoic acid (FA) to produce MF in the terminal step of MF synthesis and it also plays a role in the

regulation of MF synthesis which enhance vitellogenesis and metamorphosis of these animals. For example, Silva Gunawardene *et al*. (2001) reported that FAMeT in the shrimp, *Metapenaeus ensis* is expressed throughout ovarian maturation in the nerve and eyestalk suggesting a possible role of FAMeT in the regulation of reproduction.

CONCLUSION

Based on the findings of this study, the following conclusions were made: the hemolymph P_4, E_2, $PGF_{2\alpha}$ levels and shrimp GSI which observed at 30 ppt were generally higher than those at 15 ppt. This might be an indication that gonadal maturation, which was indicated by the elevation of shrimp GSI, depends on the control of salinity. The levels of Vg and FAMeT gene expression in ovaries and hepatopancreas of female shrimp obtained at 15 and 30 ppt increased towards shrimp GSI. But relative expression patterns of Vg and FAMeT gene of female shrimp at 30 ppt were higher than those at 15 ppt and the mean expression levels of Vg and FAMeT in hepatopancreas were higher than in ovaries. In conclusion, it was clearly shown that salinity played an important role over shrimp ovarian development which could be proved at different levels ie. morpholical changes (GSI), hormonal changes and gene expression levels changes.

ACKNOWLEDGEMENT

This research was financially supported by Kasetsart University Research and Development Institute (KURDI) of Kasetsart University

LITERATURE CITED

Arcos, F. G., Ibarra, A. M. and L.S. Racotta. 2011. Vitellogenin in hemolymph predicts gonad maturity in adult female *Litopenaeus (Penaeus) vannamei* shrimp. **Aquaculture.** 316: 93-98.

Boone, L. 1931. A collection of anomuran and macruran crustacea from the bay of Panama and freshwater of the Canal Zone. **Bulletin of the American Museum of Natural History.** 63: 137-189.

Bradford, M. 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein dye binding. **Anal Biochem.** 28:350-356.

Chen, S.H., Lin C.Y. and C.M. Kuo. 2004. Cloning of two crustacean hyperglycemic hormone isoforms in freshwater giant prawn *Macrobrachium rosenbergii*: evidence of alternative splicing. **Mar Biotech.** 6: 83-94.

Coman, G. J., Arnold, S. J., Jones, M. J. and N. P. Preston. 2007. Effect of rearing density on growth, survival and reproductive performance of domesticated *Penaeus monodon*. **Aquaculture.** 264: 175-183.

D'Croz, L, Wong, L.V, Justine, G. and M. Gupta. 1988. Prostaglandins and related compounds from the polychaete worm *Americonuphis reesi* Fauchald (Onuphidae) as possible inducers of gonad development in penaeid shrimps. **Rev Biol Trop.** 36:331–332.

Fingerman, M. 1995. Endocrine mechanisms in crayfish, with emphasis on reproduction and neurotransmitter regulation of hormone release. **Am. Zool.** 35: 68-78.

Fingerman, M. 1997. Roles of neurotransmitters in regulating reproductive hormones release and gonad maturation in decapod crustaceans. **Invertebr. Reprod. Dev.** 31:47-54.

Ghazy, M. M., Habashy, M. M. Kossa, F.I. and E. Y. Mohammady. 2009. Effects of Salinity on Survival, Growth and Reproduction of the Water Flea, *Daphnia magna*. **Nature & Science.** 7: 28-42.

Hiransuchalert, R., Thamniemdee, N., Khamnamtong, B., Yamano, K. and S. Klinbunga. 2013. Expression profiles and localization of vitellogenin mRNA and protein during ovarian development of the giant tiger shrimp *Penaeus monodon*. **Aquaculture.** 412–413: 193-201.

Hoa, N. D., Wouters, R., Wille, M., Thanh, V., Dong, T. K., Van Hao, N. and P. Sorgeloos. 2009. A fresh-food maturation diet with an adequate HUFA composition for broodstock nutrition studies in black tiger shrimp *Penaeus monodon*. **Aquaculture.** 297: 116-121.

Kamonpatana, M., D. F.M. Van De Wie, W. Koops, L. Dumtong, Ngramsuriyaroj, C. and S. Usanakornkul. 1979. Oestrus control and early pregnancy diagnosis in swamp buffalo: comparison of enzyme immunoassay and radio immunoassay for plasma progesterone. **Theriogenology.** 11(5): 399-409.

Limsuwan, C. and P. Chanratchakool. 2004. **Shrimp Industry of Thailand.** Magic Publication Co, Ltd. Bangkok. 206 pp.

Nguyen, M. T., Barnes, A. C., Mather, P. B., Li, Y. T. and R. E. Lyons. 2010. Single nucleotide polymorphisms in the actin and crustacean hyperglycemic hormone genes and their correlation with individual growth performance in giant freshwater prawn *Macrobrachium rosenbergii*. **Aquaculture.** 301: 7-15.

Meunpol, O., Meejing, P. and S. Piyatiratitivorakul. 2005. Maturation diet based on fatty acid content for male *Penaeus monodon* (Fabricius) broodstock. **Aquaculture Research.** 36: 1216-1225.

Meunpol, O., Iam-Pai, S., Suthikrai, W. and S. Piyatiratitivorakul. 2007. Identification of progesterone and 17a-hydroxyprogesterone in polychaetes (*Perinereis* sp.) and the effects of hormone extracts on penaeid oocyte development in vitro. **Aquaculture.** 270: 485–492.

Meunpol, O., Duangjai, E., Yoonpun, R. and S. Piyatiratitivorakul. 2010. Detection of prostaglandin E_2 in polychaete *Perinereis* sp. and its effect on *Penaeus monodon* oocyte development in vitro. **Fisheries Science.** 76: 281-286.

Morera, Y., Lugo, J. M., Ramos, L., Rodriguez-Ramos, T., Huberman, A. and M. P. Estrada. 2013. A chimeric recombinant crustacean hyperglycemic hormone from *Litopenaeus schmitti* (Burkenroad) obtained as C-terminus fusion protein boost hemolymph glucose concentration in *Litopenaeus vannamei*. **Aquaculture Research.** 44: 1066-1075.

Okumura, T., and K. Sakiyama. 2004. Hemolymph levels of vertebrate-type steroid hormones in female kuruma prawn *Marsupenaeus japonicus* (Crustacea: Decapoda: Penaeidae) during natural reproductive cycle and induced ovarian development by eyestalk ablation. **Fisheries Science.** 70: 372-380.

Palacios, E., Carreño, D., Rodrígez-Jaramillo, M.C. and I.S. Racotta. 1999. Effect of eyestalk ablation on maturation, larval performance, and biochemistry of *Penaeus vannamei* broodstock. **J. Appl. Aquac.** 9: 1–23.

Reddy, P.S., Reddy, P.R.and G.P.C. Nagaraju. 2004. The synthesis and effects of prostaglandins on the ovary of the crab *Oziotelphusa senex senex.* **Gen Comp Endocrinol.** 135: 35–4.

Sainz-Hernandez, J. C., Racotta, I. S., Dumas, S. andJ. Hernandez-Lopez. 2008. Effect of unilateral and bilateral eyestalk ablation in *Litopenaeus vannamei* male and female on several metabolic and immunologic variables. **Aquaculture.** 283: 188-193.

Shinji, J., Kang, B., Okutsu, T., Banzai, K., Ohira, T., Tsutsui, N. and M. Wilder. 2012. Changes in crustacean hyperglycemic hormones in Pacific whiteleg shrimp *Litopenaeus vannamei* subjected to air-exposure and low-salinity stresses. **Fisheries Science.** 78: 833-840.

Silva Gunawardene, Y.I.N., Chow, B.K.C., He, J.G. and S.M. Chan. 2001. The shrimp FAMeTcDNA is encoded for a putative enzyme involved in the methylfarnesoate (MF) biosynthetic pathway and is temporally expressed in the eyestalk of different sexes. **Insect Biochem. Mol. Biol.** 31: 1115–1124.

Tahara, D.and I.Yano. 2003. Development of hemolymph prostaglandins assay systems and their concentration variations during ovarian development in the kuruma shrimp, *Penaeus japonicus*. **Aquaculture.** 220: 791–800.

Tahara, D. and I. Yano. 2004. Development-related variations in prostaglandin and fatty acid content of ovary in the kuruma shrimp (*Marsupenaeus japonicus*). **Comp Biochem Phys.** 137:631–637.

Tsukimura, B. and F.I. Kamemoto. 1991. In vitro stimulation of oocytes by presumptive mandibular secretions in the shrimp *Penaeus vannamei*. **Aquaculture.** 92: 59–66.

Treerattrakool, S., Panyim, S. and A. Udomkit. 2011. Induction of ovarian maturation and spawning in *Penaeus monodon* broodstock by double-stranded RNA. **Mar Biotechn.** 13: 163-169.