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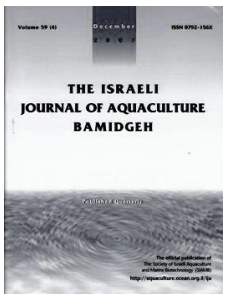

Effects of Salinity Fluctuation on Gene Expression Profiles of Female *Litopenaeus vannamei* Broodstocks

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Keywords: *Litopenaeus vannamei*; salinity; ovary; hepatopancreas; germ cell; *vitellogenin*

Abstract

In this study, the effects of salinity fluctuation on gene expression patterns in ovary and hepatopancreas of *Litopenaeus vannamei* were analyzed. The expression level of *vitellogenin* in the ovaries were significantly, markedly, and slightly, inhibited respectively in the hepatopancreas after brooders were transferred from 30‰ to 20‰ or 40‰ salinity for 4 days. The expression levels of the development of germ cell related genes *Dmc1*, proliferating cell nuclear antigen (*PCNA*) and *cyclophilin A* were significantly reduced at 20‰ or 40‰ salinity. The transcript level of shrimp ovarian peritrophin (*SOP*) was significantly reduced at 20‰ salinity, and the expression level of ovary specific gene *mucin-5 AC* was significantly higher at 40‰ salinity. Four stress related genes, cytoplasmic carbonic anhydrase (*LvCAc*), Na^+/K^+-ATPase alpha subunit (*LvNK*), V-H^+-ATPase subunit A (*LvVH*), and heat shock protein 90 (*LvHSP90*), were significantly up-regulated at 40‰ salinity in the ovaries. In the hepatopancreas, the transcript level of fatty acid synthase (*LvFAS*) was significantly inhibited under 40‰ salinity. The mRNA levels of stress related genes *LvNK*, *LvVH,* and *LvHSP90,* were significantly lower at 40‰ salinity. Results indicated that changes in salinity caused transcription inhibition of germ cell and ovarian development related genes, and the expression patterns of stress related genes in the ovaries and hepatopancreas were quite different.

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Introduction

Pacific white shrimp (*Litopenaeus vannamei*) is native to the seacoast of the Pacific Ocean. It is one of the most important species targeted in commercial aquaculture, because of its rapid growth, adaptability to a wide range of salinity and temperature variations, disease resistance, and low demand for dietary protein. *L. vannamei* is an omnivorous species and can grow normally given feed with a 20% protein ratio. Survival water temperature for *L. vannamei* ranges between 13-40℃ but the optimum water temperature for growth and ingestion is 23-30℃. In addition, *L. vannamei* has good osmoregulatory ability with a wide range of salinity adaptation from 1-50 g/L (Li et al., 2010).

Environmental factors especially physical and chemical ones can affect the developmental process of *L. vannamei*. Much research has focused on effects of temperature, salinity, illumination, ammonia nitrogen, nitrate nitrogen, dissolved oxygen, pH or COD on the growth of juvenile shrimp, however, only little has been undertaken to analyze the effect of temperature, salinity, or illumination on gonadal development. Salinity variation is directly related to osmoregulation (Robles *et al.,* 2014) and it is one of the main environmental factors which affect metabolism, growth, and nutrition demands of marine animals. *L. vannamei* is an euryhaline species which can adjust osmotic pressure according to salinity variation of growth environment. Conversion rates, growth, and other physiological processes are highest when shrimp are cultured in isotonic point salinity. Analysis of the effects of salinity and temperature on the growth of *L. vannamei* found that survival ratio and growth are best at 33 to 40‰ (Ponce-Palafox *et al.* 1997). GDH activity in gills of *L. vannamei* was found to increase in enhanced environmental salinity, while GDH activity is directly related to protein synthesis and decomposition (Chen *et al.,* 2009). Salinity also influences fatty acid assimilation, composition, and distribution in *L. vannamei*. The composition and content of free amino acids are also influenced by different salinities (Liang *et al.,* 2000; Li *et al.,* 2009), while protein, fatty acid, and free amino acid affect gonadal development.

Gonad quality can be influenced by many factors. Salinity affects energy consumption, ingestion, growth, and conversion efficiency which cause diversity in protein accumulation, amino acid composition and digestive enzyme activity of shrimp. Thus, salinity causes an indirect impact on gonadal development by influencing metabolism and physiological activities, however, little research has focused on the molecular mechanism. In this study, we analyzed the effects of salinity fluctuation on expression levels of ovary development and stress related genes in the hepatopancreas and ovary of female *L. vannamei* broodstocks.

Materials and Methods

Animals and experimental treatments

The brooders used in this study were bought from Guangxi Academy of Fishery Sciences, Nanning, China. They were 15 months old weighing 50-60 g, cultured in 30‰ salinity at 28℃. These females, after spawning, were transferred directly to three salinity treatments (5 shrimps per treatment): from 30‰ to 20‰ and 40‰ with no acclimation. After 4 days, the ovaries and hepatopancreas were dissected and immediately immersed in liquid nitrogen and stored at -80℃ until RNA isolation.

RNA isolation and cDNA synthesis

Total RNA of ovary or hepatopancreas were isolated using Easyspin Plus kit (Aidlab, China). RNA quality and quantity were determined by NanoDrop (Aoseng, Hangzhou, China) and agrose gel analysis. 1 μg total RNA was used for cDNA synthesis according to protocol of PrimeScript™ RT reagent kit with gDNA Eraser (Takara, Dalian, China). The cDNAs were stored at -20℃ after dilution.

Transcription analysis of genes in ovary or hepatopancreas

Primers for Real-Time RT-PCR analysis were designed according to gene sequences using Primer Premier 5.0 after the primer specificity was analyzed. The gene accession numbers and primer sequences are listed in Table 1. The C_T values were determined in a

10 μl reaction volume containing 5 μl SYBR® *Premix Ex Taq*™ II (Tli RNaseH Plus) (Takara, Dalian, China), 0.2 μM each of gene-specific primers, and 1 μl cDNA (10 ng RNA) by using StepOnePlus system (ABI, America). Relative transcript levels were calculated relative to the transcript amount of the constitutively expressed *18s rRNA* gene as follows: RTL = $1000*2^{-\Delta CT}$.

Table 1. Primers used for Real Time RT-PCR.

Gene	*Accession No.*	*Forward (5′→3′)*	*Reverse (5′→3′)*
18s rRNA	AF186250.1	GGAATGATGGAACAGGACC	ATCCTTGGCAGATGCTTTC
vitellogenin	KM077131.1	GAACCCTAAGGCTATCATCACTG	AGGTCGCTCTTCCATCTTTACT
Dmc1	HQ116385.1	CGAGGAATACAACGTGTCTGTC	ATTCGGGGCTGTCGTAGAT
PCNA	JN034913.1	ATTGCCTTCTGGGGAGTTC	CAAGCAAAGGTGAGCGTGA
cyclophilin A	JN546074.1	ATCCCCAACTTCATGTGCC	TGTCCAGCCAGGAGGTTTT
SOP	KX880374	CCAGATGGCATGGTATTCG	GGTACAGGCAAGGGTTTTCA
mucin-5 AC	GDUV01014128	GCCAGATGTTCAGCAGATG	TCTACGAAGCAAGCCATTTC
CAc	HM991703.2	ACCTATTCTGGCTCCCTCACT	GTTCATCCTCTGGGCAACAC
NK	JQ996559.2	CTGACTGGAGAATCCGAACC	CAAACCAGCAATACGACCC
VH	HM163157.1	TCGCTACATCATCCTGCTCA	GGGCTTGCCACTGTATTCTT
HSP90	HQ008268.1	AGCTTGGCCTGGGTATTGA	TGGGTGAAGATTACGGTATGG
FAS	HM595630.1	GAAGGGGAAAGAATCAGGTG	AGAGCGTGTTCAGAGGGTTG

Statistical analysis

Data of Real-Time RT-PCR analysis were processed using SPSS package (version #16.0), analysis of variance (ANOVA) was performed on the data sets, with the mean value and S.E. of each treatment. SigmaPlot (version #10) was used for the graphics.

Results

Expression patterns of vitellogenin mRNA in ovary and hepatopancreas

Vitellogenin (VTG) is synthesized both in the ovaries and hepatopancreas of *L. vannamei*. Transcription of VTG mRNA in ovaries was reduced by more than 60% after being transferred to 20‰ or 40‰ salinity from 30‰ salinity for 4 days (Fig. 1a). The VTG transcript level was slightly inhibited in the hepatopancreas after shrimps were subjected to salinity changes (Fig. 1b).

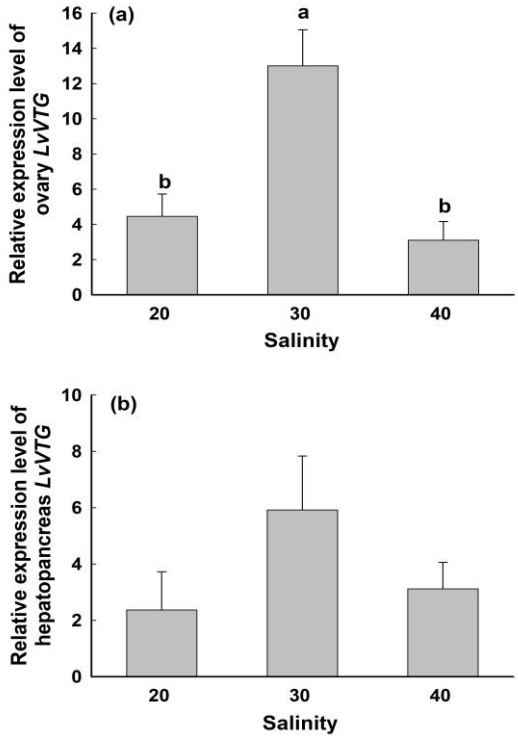

Fig. 1. Expression profiles of VTG in ovary and hepatopancreas of *L. vannamei* under different salinities. The ovary (a) and hepatopancreas (b) of shrimp cultured at 20‰, 30‰ or 40‰ salinity for 4 days were sampled and the expression level of VTG was analyzed. Results are means ± S.E. (n=5). Different letters indicate the mean values are significantly different between samples isolated from three salinities by LSD test (p <0.05).

Expression profiles of ovary development related genes

Expression levels of three genes related to germ cell development were analyzed in the ovaries after the females were subjected to salinity fluctuations (Fig. 2). Transcription levels of *Dmc1*, *PCNA,* and *cyclophilin A,* were significantly inhibited; mRNA levels *LvDmc1*, *LvPCNA,* and *cyclophilin A,* decreased more than 60%, 70%, and 40%, at 20‰ or 40‰ salinity, than at 30‰ salinity, respectively.

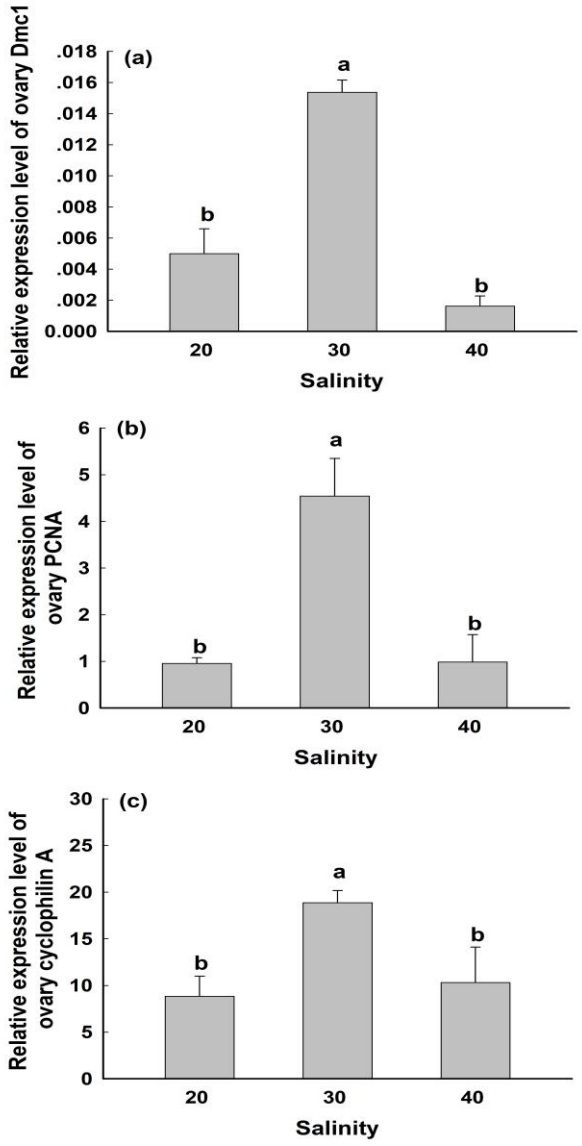

Fig. 2. Effects of different salinities on expression levels of germ cell development related genes of *L. vannamei.* The expression levels of Dmc1 (a), PCNA (b) and cyclophilin A (c) were measured after shrimp were cultured in 20‰, 30‰ or 40‰ salinity for 4 days. Different letters indicate the mean values are significantly different between samples isolated from three salinities by LSD test (p <0.05).

Two other genes related to ovarian development were also determined (Fig. 3). The transcript level of *SOP* at 20‰ salinity was 44% lower compared to 30‰ salinity (Fig. 3a). As opposed to *SOP*, the mRNA level of ovary specific expressed gene *mucin-5 AC,* was significantly up-regulated at 40‰ salinity (Fig. 3b).

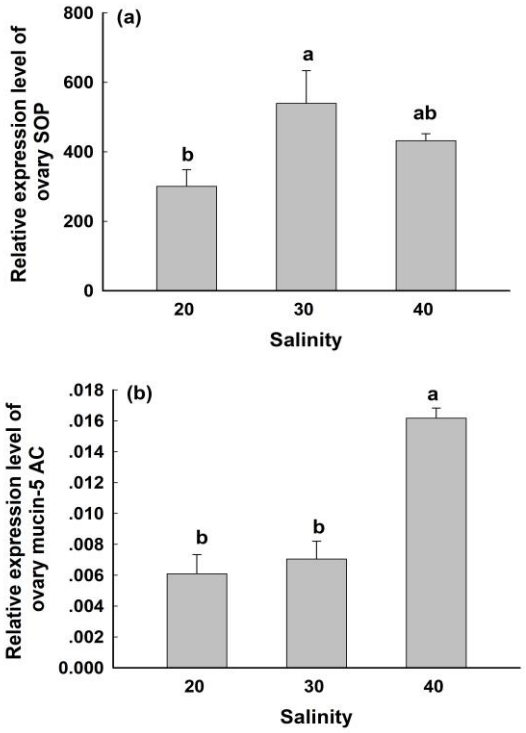

Fig. 3 Effects of different salinities on expression levels of ovary development related genes of *L. vannamei.* The expression levels of SOP (a) and mucin-5 AC (b) were measured after shrimp were treated with 20‰, 30‰ or 40‰ salinity for 4 days. Different letters indicate the mean values are significantly different between samples isolated from three salinities by LSD test (p <0.05).

In the control treatment, the mRNA levels of these five genes, *LvDmc1* and *mucin-5 AC* had the lowest expression level, and the relative transcript level of *SOP* was 20 and 100 times higher than *cyclophilin A* and *LvPCNA*, respectively.

Expression profiles of stress regulation related genes in ovary and hepatopancreas

As shown in Fig. 4, the transcript levels of four genes related to stress regulation (*LvCAc*, *LvNK*, *LvVH* and *LvHSP90*) were significantly up-regulated in the ovaries after broodstocks were subjected to 40‰ salinity. The expression levels of these four genes were more than 2.5, 1.2, 1.2 and 1.5 times up-regulated, respectively. However, the expression levels of these four genes were slightly enhanced in 20‰ or 30‰ salinity.

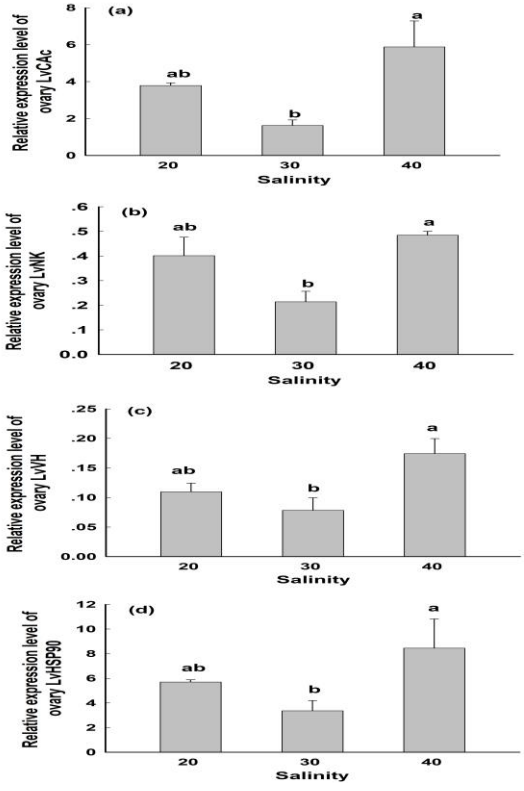

Fig. 4 Expression patterns of four genes involved in stress regulation in ovary of *L. vannamei*. After treatment with different salinities (20‰, 30‰ or 40‰) for 4 days, the ovaries of shrimp were collected and expression levels of LvCAc (a), LvNK (b), LvVH (c) and LvHSP90 (d) were determined. Different letters indicate the mean values are significantly different between samples isolated from three salinities by LSD test (p <0.05).

In the hepatopancreas, the mRNA level of fatty acid synthase encoded gene (*LvFAS*) decreased by more than 80% at 40‰ salinity, and slightly decreased at 20‰ salinity (Fig. 5a). mRNA levels of these three stress related genes (*LvNK*, *LvVH,* and *LvHSP90*) were significantly lower at 40‰ salinity than 20‰ or 30‰ salinity (Fig. 5b-d). Expression levels of LvNK and LvHSP90 decreased more than 60% and 90% in 40‰ salinity than in 30‰ salinity.

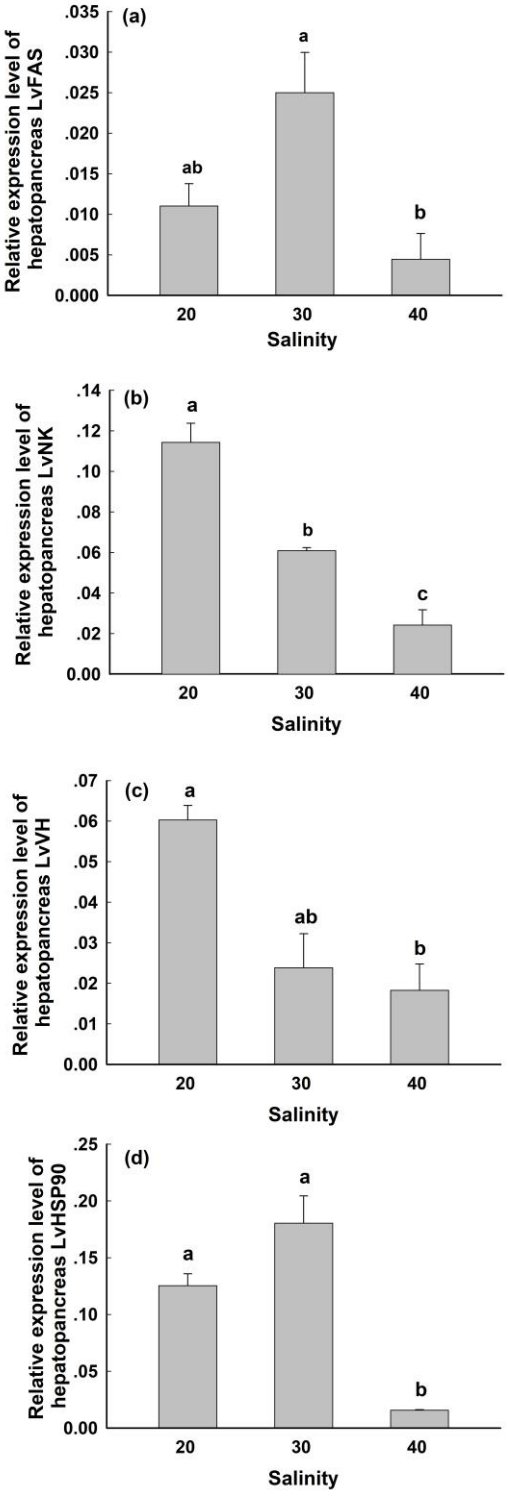

Fig. 5 Expression patterns of fatty acid synthase and three stress regulation related genes in hepatopancreas of *L. vannamei*. After treatment with 20‰, 30‰ or 40‰ salinity for 4 days, the expression levels of LvFAS (a), LvNK (b), LvVH (c) and LvHSP90 (d) in hepatopancreas of shrimps were determined. Different letters indicate the mean values are significantly different between samples isolated from three salinities by LSD test (p <0.05).

In the control group, the mRNA levels of *LvNK*, *LvVH* were more than double in the ovaries than in hepatopancreas, while the expression level of *LvHSP90* was over 16 times higher in the ovaries.

Discussion

Vitellogenesis and oogenesis are two delicately regulated processes required for ovarian maturation in crustaceans (Tsukimura, 2001). The ovarian development of oviparous animals is characterized by the accumulation of a major yolk protein (vitellin) in the oocytes, and the precursor of vitellin is called vitellogenin (VTG). In *L. vannamei*, VTG is expressed both in the ovary and in the hepatopancreas (Raviv *et al.,* 2006). In this study, the expression level of VTG in the ovaries was significantly reduced after the broodstocks were transferred from 30‰ to 20‰ or 40‰ salinity for 4 days. This step inhibited the maturation of oocytes but not the expression level of VTG in the hepatopancreas. This suggests that the synthesis of vitellogenin is not affected by salinity fluctuations.

LvDmc1 is expressed specifically in premeiotic oogonia/spermatogonia and can be used as an indicator for germ cell development (Okutsu *et al.,* 2010). In *Marsupenaeus japonicus*, *mjPCNA* plays an important role in the testis and ovary development, especially in the process of mitosis and meiosis (Shen *et al.,* 2010). In this study, the reduction of tree germ cell development related genes *LvDmc1*, PCNA and cyclophilin A in *L. vannamei* suggests possible inhibition of germ cell development caused by salinity fluctuations. Shrimp ovarian peritrophin (SOP) is a major protein in jelly layer (JL) and cortical rods (CRs) and plays a role in the protection of spawned eggs. SOP was first isolated from the ovaries of *Penaeus semisulcatus*. It is abundant in the cytoplasm of vitellogenic oocytes and was detected in the extracellular CRs at the end of vitellogenesis (Khayat *et al.,* 2001). A current study showed that transcript level of SOP was reduced after transfer of crayfish from 30‰ to 20‰ salinity, indicating that oocyte maturation was inhibited in the process. Mucin-5 AC is an ovary specific expressed gene in *L. vannamei* (Peng *et al.,* 2015); its function is unknown in shrimp. Further studies are needed to analyze SOP function and mucin-5 AC in ovarian development of *L. vannamei*.

Osmoregulatory capacity is seen as a tool to monitor the physiological state of penaeid shrimp in different adverse conditions under artificial rearing (Lignot *et al.,* 2000). Cytoplasmic carbonic anhydrase and Na^+/K^+-ATPase are the two most studied in crustacean osmoregulatory system. They belong to transport-related enzymes and specific ion transport proteins respectively (Liu et al., 2015; Jasmani et al., 2010). V-H^+-ATPase is also a transport-related enzyme, which participates in osmoregulation (Faleiros *et al.,* 2010; Li *et al.,* 2015). In the current study, the transcription levels of LvCAc, LvNK, and LvVH in the ovary were significantly up-regulated at 40‰ salinity which indicates that the three genes regulate intracellular and extracellular fluid volumes and regulate the hemolymph ionic/osmotic balance under high salinity. HSPs can be used as cell stress markers that are well recognized in many species (Wahid *et al.,* 2007). The expression level of LvHSP90 was significantly up-regulated in the ovaries under salinity fluctuation. However, expression levels of LvNK, LvVH and LvHSP90 in the hepatopancreas were significantly reduced under 40‰ salinity. We speculated that high salinity environments may be harmful to the hepatopancreas and cause low expression of LvNK, LvVH, and LvHSP90.

Fatty acid synthase (FAS) is an important enzyme that catalyzes the synthesis of long-chain fatty acids from acetyl-CoA and malonyl-CoA (Smith *et al.,* 2003). It is known to be a complex multi-functional enzyme that plays an important role in energy homeostasis. In sea bass, dietary and endogenous fatty acids are crucial for male and female gonadogenesis, fecundity, and embryo and larval development (Asturiano *et al.,* 2001). Hepatic and ovarian FAS mRNA expression levels significantly increase at vitellogenesis and postvitellogenesis relative to previtellogenesis in silver pomfret (Peng *et al.,* 2017). In *L. vannamei*, the LvFAS play a role in white spot syndrome virus and *Vibrio parahaemolyticus* infections (Yang *et al.,* 2011; Zuo *et al.,* 2017). No study is focused on its role during ovary development. In this study, mRNA expression level of LvFAS in hepatopancreas was significantly reduced at 40‰ salinity, which indicates that fatty acid synthesis and energy balance was disrupted by salinity fluctuation.

In conclusion, the results of this study showed that salinity fluctuation caused inhibition of ovarian maturation. The response of stress related genes in ovary and hepatopancreas resulted in different response mechanisms in these two organs.

Acknowledgments

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References

Asturiano J.F., Sorbera L.A., Carrillo M., Zanuy S., Ramos J., Navarro J.C. and N. Bromage, 2001. Reproductive performance in male European sea bass (*Dicentrarchus labrax* L.) fed two PUFA-enriched experimental diets: a comparison with males fed a wet diet. *Aquaculture*, 194:173-190.

Chen Z.Z., Shen Y.C., Zhu C.H., Wu Z.H. and X.H. Huang, 2009. Effects of salinity and nutrition on glutamate dehydrogenase and pyruvate carboxylase activity in *Litopenaeus vannamei*. *Modern Agricultural Sci.*, 16(7):12-14.

Faleiros R.O., Goldman M.H.S., Furriel R.P.M. and J.C. McNamara, 2010. Differential adjustment in gill Na^+/K^+- and V-ATPase activities and transporter mRNA expression during osmoregulatory acclimation in cinnamon shrimp *Macrobrachium amazonicum* (Decapoda, Palaemonidae). *J. Exp. Biol.*, 213:3894-3905.

Jasmani S., Jatasankar V., Shinji J. and M.N. Wilder, 2010. Carbonic anhydrase and Na/K-ATPase activities during molt cycle of low salinity-reared white shrimp *Litopenaeus vannamei*. *Fisheries Sci.*, 76:219-225.

Khayat M., Babin P.J., Funkenstein B., Sammar M., Nagasawa H., Tietz A. and E. Lubzens, 2001. Molecular characterization and high expression during oocyte development of a shrimp ovarian cortical rod protein homologous to insect intestinal peritrophins. *Biol. Reprod.*, 64:1090-1099.

Li L., Jia Y., Li P., Yin S., Zhang G., Wang X., Wang Y., Wang X., Zang X. and Y. Ding, 2015. Expression and activity of V-H^+-ATPase in gill and kidney of marbled eel *Anguilla marmorata* in response to salinity challenge. *J. Fish Biol.*, 87:28-42.

Li C.C., Yeh S.T. and J.C. Chen, 2010. Innate immunity of the white shrimp *Litopenaeus vannamei* weakened by the combination of a *Vibrio alginolyticus* injection and low salinity stress. *Fish Shellfish Immun.*, 28:121-127.

Li E.C., Chen L.Q., Xiong Z.Q., Lin C., Peng S.M. and L.H. Liu, 2009. Effects of salinity on protein accumulation, amino acid composition and transaminase activity in tissues of *Litopenaeus vannamei*. *Acta Hydrobiologica Sinia*, 33(3):532-538.

Liang M.Q., Wang S.W., Han Q.W., Wang J.L. and Q. Chang, 2000. Analysis and comparison of fatty acids in mariculture and low salt cultured *Litopenaeus vannamei*. *Progress in Fishery Sci.*, 30(1):87-91.

Lignot J.H., Spanings-Pierrot C. and G. Charmantier, 2000. Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in crustaceans. *Aquaculture*, 191:209-245.

Liu M.Q., Liu S.G., Hu Y.Y. and L.Q. Pan, 2015. Cloning and expression analysis of two carbonic anhydrase genes in white shrimp *Litopenaeus vannamei*, induced by pH and salinity stresses. *Aquaculture*, 448:391-400.

Okutsu T., Kang B.J., Miwa M., Yoshizaki G., Maeno Y. and M.N. Wilder, 2010. Molecular cloning and characterization of *Dmc1*, a gene involved in gametogenesis, rom the whiteleg shrimp *Litopenaeus vannamei*. *Fisheries Sci.*, 76:961-969

Peng J.X., Wei P.Y., Zhang B., Zhao Y.Z., Zeng D.G., Chen X.L., Li M. and X.H. Chen, 2015. Gonadal transcriptomic analysis and differentially expressed genes in the testis and ovary of the pacific white shrimp (*Litopenaeus vannamei*). *BMC Genomics*, 16:1006.

Peng S., Shi Z., Gao Q., Zhang C. and J. Wang, 2017. Dietary n-3 LC-PUFAs affect lipoprotein lipase (LPL) and fatty acid synthase (FAS) activities and mRNA expression during vitellogenesis and ovarian fatty acid composition of female silver pomfret (*Pampus argenteus*) broodstock. *Aquaculture Nutr.*, 23:692-701.

Ponce-Palafox J., Martinez-Palacios C.A. and L.G. Ross, 1997. The effects of salinity and temperature on the growth and survival rates of juvenile white shrimp, *Penaeus vannamei*, Bonne, 1931. *Aquaculture*, 157:107-115.

Raviv S., Parnes S., Segall C., Davis C. and A. Sagi, 2006. Complete sequence of *Litopenaeus vannamei* (Crustacea: Decapoda) vitellogenin cDNA and its expression in endocrinologically induced sub-adult females. *Gen. Comp. Endocr.*, 145:39-50.

Robles J., Charmantier G., Boulo V., Lizarraga-Valdez J., Enriquez-Paredes L.M. and I. Giffard-Mena, 2014. Osmoregulation pattern and salinity tolerance of the white shrimp *Litopenaeus vannamei* (Boone, 1931) during post-embryonic development. *Aquaculture*, 422:261-267.

Shen B.L., Wang Y.L., Chen Y., Wang G.D., Lin P. and Z.H. Zou, 2010. Molecular cloning of proliferating cell antigen and its differential expression analysis in the developing ovary and testis of Penaeid shrimp *Marsupenaeus japonicus*. *DNA Cell Biol.*, 29(4):163-170.

Smith S., Witkowski A. and A.K. Joshi, 2003. Structural and functional organization of the animal fatty acid synthase. *Progress Lipid Res.*, 42: 289-317.

Tsukimura B., 2001. Crustacean vitellogenesis: its role in oocyte development. *Am Zool*, 41:465-476.

Wahid A., Gelani S., Ashraf M. and M.R. Foolad, 2007. Heat tolerance in plants: an overview. *Environ. Exp. Bot.*, 61:199-223.

Yang L., Liu J., Liu M., Qian M.R., Zhang M.Z. and H.J. Hu, 2011. Identification of fatty acid synthase from the Pacific white shrimp, *Litopenaeus vannamei* and its specific expression profiles during white spot syndrome virus infection. *Fish Shellfish Immun.*, 30(2):744-749.

Zuo H.L., Gao J.F., Yuan J., Deng H.W., Yang L.W., Weng S.Q., He J.G. and X.P. Xu, 2017. Fatty acid synthase plays a positive role in shrimp immune responses against *Vibrio parahaemolyticus* infection. *Fish Shellfish Immun.*, 60:282-288.