Dynamic Biochemistry, Process Biotechnology and Molecular Biology ©2011 Global Science Books

Effects of Salinity on Electrolyte Levels (Na^+, K^+, and Cl^-) in the Hemolymph of the Pacific White Shrimp *Litopenaeus vannamei*

Alfredo Mena-Herrera[1] • Marco Agustín Liñán-Cabello[1*] • Fernando Díaz-Herrera[2]

[1] Universidad de Colima, Facultad de Ciencias Marinas, Km 19.5 Carretera Manzanillo-Barra de Navidad, Manzanillo Colima Mexico CP 28860 Mexico
[2] Centro de Investigación Científica y de Educación Superior de Ensenada, B. C. Mexico

Corresponding author: * **linanm@ucol.mx**

ABSTRACT

Pacific white shrimp (*Litopenaeus vannamei*) were consecutively exposed for 3 h each to salinity of 40, 30, 20, 10 and 0‰, and the concentrations of Na^+, K^+, and Cl^- in their hemolymph were measured. In females, the Na^+ concentrations in hemolymph were highest (304.1 mmol/l) after exposure to 20‰ salinity and the K^+ concentrations (5.32 mmol/l) were highest at 10‰ salinity, meanwhile in males, the Na^+ concentrations in hemolymph were highest (316.0 mmol/l) after exposure to 40‰ salinity and the K^+ concentrations (5.62 mmol/l) were highest at 30‰ salinity. The Cl^- concentrations in males decreased with salinity, with concentrations of 261.5, 254.6, 237.1, 224.5, and 191.0 mmol/l at 40, 30, 20, 10 and 0‰ salinity, respectively. Similar decreases were seen in females, except that the Cl^- concentration at 10‰ salinity was 247.6 mmol/l. The overall interaction effect of Na^+, K^+, and Cl^- differed significantly in males and females ($p < 0.05$). These results indicate that hemolymph electrolyte concentrations generally decrease with salinity, indicating that shrimp osmoregulatory and ionoregulatory activities adjust to ionic gradients in their environment.

Keywords: chloride, ionic regulation, hemolymph, potassium, sodium

INTRODUCTION

The Pacific white shrimp (*Litopenaeus vannamei*) (Boone 1931) lives naturally in salinities ranging from 1‰ to 40‰, and therefore exhibits both hyper-osmotic regulation at low salinities and hypo-osmotic regulation at high salinities. The physiological capability of penaeid shrimp to osmoregulate (i.e. regulate their internal salt and water balance) develops gradually while shrimp are still in the postlarval stages. Salinity is one of the most important abiotic factors in aquaculture, with many species of crustacean exhibiting some degree of euryhalinity (Pequeux 1995; Zhang *et al.* 2007). At low salinity, these shrimp must selectively retain salts and excrete excess water. When exposed to variations in salinity, the shrimp maintain their internal osmotic concentration, either higher or lower than that of the medium. Particularly, *L. vannamei* exhibits a pattern hyperosmotic regulation at low salinities and a pattern of hypoosmotic regulation in high, with an isosmotic point between 25 to 26 psu (Castille and Lawrence 1981; Diaz *et al.* 2001; Gong *et al.* 2004).

The osmoregulatory ability of aquatic animals can be determined by comparing the osmolality of their hemolymph at various salinities (Lignot *et al*. 2000; Thanh *et al.* 2010) with the osmolality of the medium. Furthermore, the osmoregulatory capacity of penaeid shrimps at different salinities can be used to monitor their physiological condition and assess the effects of stressors (Sang and Fotedar 2004). The osmoregulatory capacity and the osmotic concentration of hemolymph decrease with increasing shrimp size (Gong *et al*. 2004). Moreover, both the osmoregulatory capacity and hemolymph osmolality of these shrimp vary with the concentration gradients of Na^+ and Cl^- ions between the hemolymph and the growth environment (Chim *et al*. 2003). Age-associated variations in shrimp osmoregulatory capacity and the ability to manipulate the concentrations of individual ions in shrimp remain inadequately studied (McGraw and Scarpa 2004). Aquatic organisms have developed adaptations to variations in environmental factors. Such adaptability allows shrimp to live normally in environments that have suboptimal physical and chemical parameters such as salinity. It has been confirmed that shrimp gradually lose their osmoregulatory capacity in low-salinity medium as they develop from juveniles to adults (Gong *et al*. 2004; Hurtado *et al.* 2007) and, further, that the ionic constituents of shrimp hemolymph are predominantly (88%) sodium and chloride (Chen and Chen 1996).

Previous studies (Chen and Lin 1998; Lemaire *et al.* 2002) have shown a linear relationship between medium osmolality and hemolymph osmolality in the fleshy prawn (*Peneaus chinensis*) and the blue prawn (*P. stylirostris*). Physiological changes in certain crustaceans, including the white shrimp *L. vannamei*, are, however, less clear. Studies of osmolarity and baseline concentrations of oxyhemocyanin, protein, Na^+, K^+, Cl^-, Mg^{2+}, and Ca^{2+} in hemolymph in relation to sex, size, and molt stage in *L. vannamei* have indicated that electrolyte concentrations vary with the molt cycle, with no significant differences between males and females (Cheng *et al*. 2002). Low levels of osmolarity and low concentrations of hemolymph protein, oxyhemocyanin, Na^+, K^+, and Cl^- during the post-molt period were found to be associated with water uptake during molting. The molt cycle in several species of crustaceans has nutritional, environmental and physiological aspects. During intermolt stage "C", their exoskeleton has sufficient hardness to provide protection and support for handling. Only shrimp at intermolt stage "C", determined by microscopic observation of uropod scales (Robertson *et al*. 1987; Yano *et al*. 1988), were selected, because Na+, K+, and Cl^- concentrations during postmolt periods have been shown to be affected by water uptake at molting (Cheng *et al*. 2002).

Aquatic organisms may be classified according to their tolerance to changes in salinity. This is the case for most species of penaeid shrimp, such as *L. vannamei*. These shrimp are generally cultured in freshwater, to which they become gradually acclimated. The ionic composition of the

Received: 6 April, 2010. Accepted: 3 June, 2010.

Original Research Paper

Table 1 Effects of salinity on concentrations of Na^+, K^+, and Cl^- in the hemolymph of male and female *L. vannamei*.

Treatments	Na^+, mmol/l		K^+, mmol/l		Cl^-, mmol/l	
	Male	Female	Male	Female	Male	Female
40 S‰	316.0 ± 4.1 bc	298.6 ± 5.9 b	4.86 ± 0.2 bc	4.13 ± 0.1 abc	261.5 ± 4.3 b	234.8 ± 6.8 b
30 S‰	307.1 ± 7.6 b	286.1 ± 10.5 b	5.62 ± 0.2 b	4.84 ± 0.1 b	254.6 ± 6.7 b	242.5 ± 6.1 b
20 S‰	303.5 ± 4.5 b	304.1 ± 3.9 b	4.88 ± 0.2 b	4.64 ± 0.1 b	237.1 ± 3.6 b	236.0 ± 2.6 b
10 S‰	246.8 ± 3.1 b	284.9 ± 4.8 b	4.37 ± 0.1 a	5.32 ± 0.1 b	224.5 ± 2.8 b	247.6 ± 5.3 b
0 S‰	186.1 ± 1.3 a	179.5 ± 0.7 a	3.72 ± 0.1 a	3.62 ± 0.1 a	191.0 ± 2.5 a	196.4 ± 3.3 a
Control tank 40 S‰ (*n* = 16)	328.8 ± 3.9	315.0 ± 3.2	5.49 ± 0.9	4.51 ± 1.1	271.5 ± 5.6	248.0 ± 4.9

All results reported as mean ± SEM (write what SEM means)
Different letters in the same electrolyte and column show significant differences (p < 0.05), based on one-way analysis of variance followed by Tukey's HSD test.

medium seems to be more important than its salinity. For example, solutions of sodium chloride alone are not suitable for shrimp culture at any salinity, although the ions in seawater most important for osmoregulation are chloride and sodium.

Some research (Davis *et al*. 2004; Gong *et al.* 2004; Araneda *et al.* 2008), suggests that, if the salinity is adequate, calcium, potassium and magnesium are the most important ions for shrimp survival. Any of these ions can be limiting, but lack of potassium is often the most important factor affecting shrimp (Davis *et al*. 2004; Roy *et al*. 2007).

In culturing crustaceans, it is important to understand the effects of changes in salinity on hemolymph ions and on their physiological condition. This can allow prediction of the effects of stress caused by variations in the environment, such as low-salinity medium or freshwater. To determine the basal concentrations of electrolytes in shrimp, we assessed the effects of exposure to different salinities on concentrations of the electrolytes Na^+, K^+, and Cl^- in the hemolymph of the shrimp *L. vannamei*.

MATERIALS AND METHODS

Three hundred male (mean weight 30.1 ± 1.29 g) and 300 female (mean weight 40.1 ± 8.3 g) shrimp cultured in seawater for 8 months in earth ponds at the University of Colima, Manzanillo, Colima ("Campus El Naranjo") were transported to the laboratory, introduced randomly at a male: female ratio of 1: 1 into three fiberglass tanks (each 5 m in diameter) at densities of 10 shrimp/ m^2, and allowed to acclimate to indoor laboratory conditions at seawater salinity (35‰) for 1 week. The salinity was gradually increased, in increments of 1.0‰/d, to 40‰, by adding artificial sea salt Sol y Sal de Colima, Cuauhtémoc, Colima, México), and the shrimp were maintained under these conditions for 7 d to allow their bodily fluids to stabilize (Castille and Lawrence 1981). During this time shrimp were fed a commercial diet, composed of 40.0% crude protein, 8.0% crude lipid, 4.0% crude fiber, 10.0% ash and 12.0% moisture, at 3.0% of their body weight, twice daily (09:00 and 18:00).

Freshwater was added to the tank to decrease the salinity of the water salinity. The volume of water in the tank was kept constant. The shrimp were exposed for 3 h each to water with salinity of 40, 30, 20, 10 and 0‰. Upon each transfer, samples of hemolymph were taken from eight male shrimp and eight female shrimp. The shrimp were not fed during the experiment to avoid providing minerals through their feed. The temperature was 28.0–28.5°C throughout the experiment.

Using a syringe (25 G, 1 ml), 0.2–0.3 ml hemolymph samples were drawn from the region of the hemolymphatic sinus, located between the cephalothoracic ganglion of the neural cord and the fluted muscle, and adjusted to 2.0% (w/v) sodium citrate to prevent coagulation. Care was taken to prevent the hemolymph from mixing with air, especially to ensure that bubbles did not form during suction. Samples were made homogenous by rotation of the syringe. Analysis "in situ" was achieved by injection of samples into an EasyVet Medical Analyzer (Medica Corporation, Bedford, MA; USA), which measured concentrations of Na^+, K^+, and Cl^- in hemolymph. Normality, independence, and homogeneity were assessed using Bartlett and Kolmogorov-Smirnov tests (Zar 1994), with two-way analysis of variance (ANOVA) followed by a multiple comparison test (Tukey) was employed to evaluate significant differences (HSD) in the electrolyte concentrations, in relation to categories sex (males-females) and salinity changes. All tests were performed using Statistica® v 6.0 (Tulsa, OK, USA).

RESULTS

We observed a significant difference between males and females in the effects of salinity on hemolymph Na^+ concentrations ($p < 0.05$). In males, the mean concentration of Na^+ in hemolymph decreased with exposure to lower salinity, with Na^+ concentrations of 316.0, 307.1, 303.5, 246.8 and 186.1 mmol/l observed after exposure of male shrimp to 40, 30, 20, 10 and 0‰ salinity, respectively. In females, the Na^+ levels showed a different pattern, being maximal at a salinity of 20‰ and minimal at a salinity of 0‰. Following exposure of female shrimp to 40, 30, 20, 10 and 0‰ salinity, the concentrations of Na^+ in their hemolymph was 298.6, 286.1, 304.1, 284.9 and 179.5 mmol/l, respectively. Hemolymph K^+ concentrations also differed significantly between males and females ($p<0.05$). Exposure of male shrimp to 40, 30, 20, 10 and 0‰ salinity resulted in mean hemolymph K^+ concentrations of 4.86, 5.62, 4.88, 4.37 and 3.72 mmol/l, respectively, whereas exposure of female shrimp to these salinities resulted in mean K^+ concentrations of 4.13, 4.84, 4.64, 5.32, and 3.62 mmol/l, respectively. Finally, Cl^- concentrations in hemolymph differed significantly between males and females ($p < 0.05$), being 261.5, 254.6, 237.1, 224.5, and 191.0 mmol/l in males, and 234.8, 242.5, 236.0, 247.6 and 196.4 mmol/l in females exposed to 40, 30, 20, 10 and 0‰ salinity, respectively (**Table 1**).

When Na^+ concentrations were compared between males and females relative to salinity gradients, no significant difference between the two sexes was observed. Na^+ concentrations decreasing with decreasing salinity in both genders. In females the Na^+ concentration was highest at 20‰ salinity, but decreased as the shrimp were progressively exposed to medium of lower salinity (**Fig. 1**).

The hemolymph concentration of K^+ differed significantly between males and females ($p = 0.05$) (data not shown, being highest (5.62 mmol/l) in males at 30‰ salinity and decreasing to 3.72 mmol/l at 0% salinity. Analysis using Tukey's test showed a significant effect of salinity gradient in females, with K^+ concentration being highest (5.32 mmol/L) at 10‰ salinity and lowest (3.62 mmol/l) at 0% salinity (**Fig. 2**).

The hemolymph concentration of Cl^- in males decreased with decreasing salinity, with no significant difference seen between males and females ($p > 0.05$). In females, however, hemolymph Cl^- concentration peaked after exposure to 30‰ (242.5 mmol/l) and 10‰ (247.6 mmol/l) salinity (**Fig. 3**).

In comparison to the above groups of shrimp, the hemolymph of 16 male shrimp maintained throughout in medium of 40‰ salinity had a mean Na^+ concentration of 328.8 ± 3.9 mmol/l, a mean K^+ concentration of 5.49 ± 0.9 mmol/l, and a mean Cl^- concentration of 271.5 ± 5.6 mmol/l, whereas the hemolymph of 16 female shrimp had a mean Na^+ concentration of 315.0 ± 3.9 mmol/l, a mean K^+ concentration of 4.51 ± 1.1 mmol/l, and a mean Cl^- concentration of 248.0 ± 4.9 mmol/l (**Table 1**).

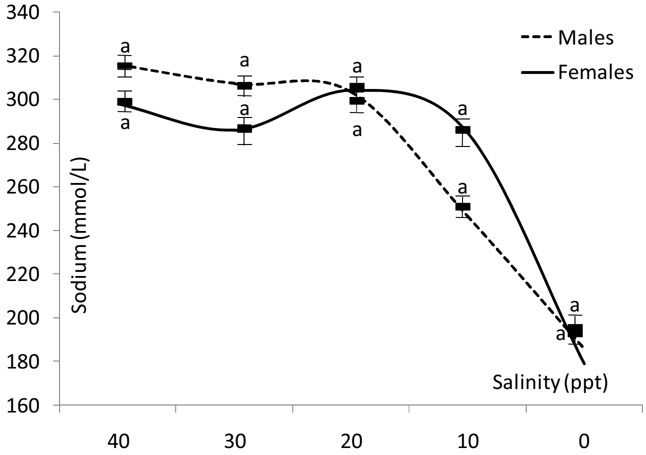

Fig. 1 Mean ± SEM concentrations of sodium in the hemolymph of male and female shrimp, *Litopenaeus vannamei*, following exposure to different gradients of salinity. (n = 8 each; p < 0.001). Different letters in the same salinity show significant differences (p < 0.05).

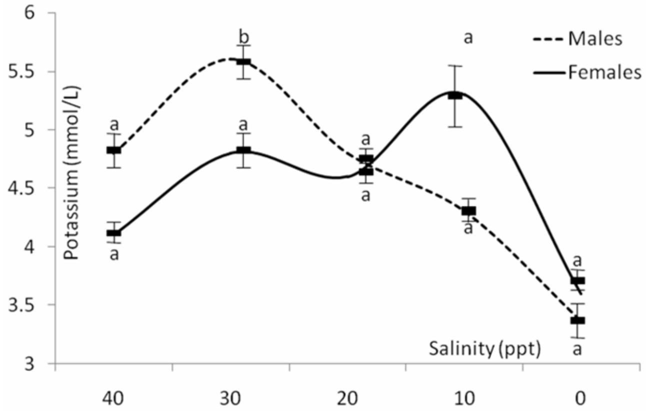

Fig. 2 Mean ± SEM concentrations of potassium in the hemolymph of male and female shrimp, *Litopenaeus vannamei*, following exposure to different gradients of salinity. (n = 8 each, p < 0.001). Different letters in the same salinity show significant differences (p < 0.05).

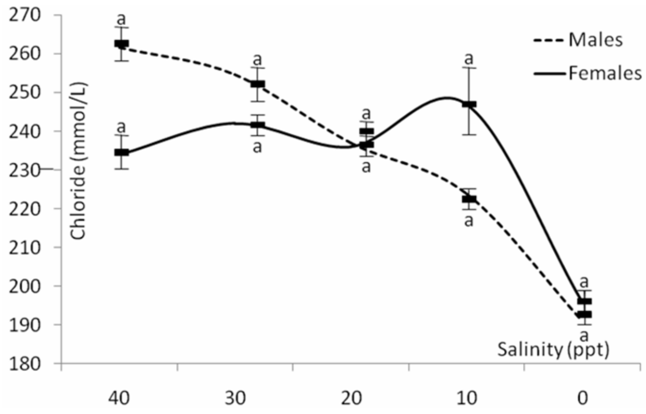

Fig. 3 Mean ± SEM concentrations of chlorine in the hemolymph of male and female shrimp, *Litopenaeus vannamei*, following exposure to different gradients of salinity. (n = 8 each, p = 0.0046). Different letters in the same salinity show significant differences (p < 0.05).

DISCUSSION

One problem associated with differences in the ionic compositions of hemolymph in the shrimp *L. vannamei* lies in the variation between the external concentrations of electrolytes in seawater and the need of the shrimp to acclimate, when necessary, to freshwater. Shrimp have to maintain their hemoelectrolyte concentrations within a certain range while salinity varies. Thus, inner and out concentrations may not be perfectly correlated. The ionic gradients of Na^+, K^+, and Cl^- in hemolymph decrease with decreasing salinity, indicating that these concentrations depend greatly on the osmoregulatory capacity of the organism. In seawater, however, the ions most important for osmoregulation are Cl^- and Na^+, either of which may be limiting, whereas, in freshwater, the concentration of K^+ may be the most important ion affecting shrimp (Davis *et al*. 2004), with K^+ concentration having significant impacts on shrimp growth, molting, feeding and nutrient retention (Chang *et al*. 2006). At high salinities, the hemolymph osmolality of "weak osmoregulators" may exhibit a close parallel association with the isoosmotic line due to either a disruption in osmoregulation or a strategy to reduce the osmotic gradient between the hemolymph and the environment (Pequeux 1995). This capacity allows interactions with external sources of Na^+ and K^+, leading to a control of internal ion levels and optimizing internal physiological functions (Zhu *et al*. 2004). Therefore, in K^+-deficient inland saline water, the physiological requirement for K^+ may be met by the diet (Araneda *et al*. 2008). We observed a maximum Na^+ concentration (316.0 mmol/l) in males at 40‰ salinity and a minimum (186.1 mmol/l) in freshwater. In females, however, the maximum Na^+ concentration was at 20‰ salinity (304.1 mmol/l) and the minimum (179.5 mmol/l) was in freshwater. Thus, males can maintain a Na^+ concentration in hemolymph at salinities of 20–40‰, decreasing markedly at lower salinities. In females, showed a tendency to maintain high levels of Na+ in the range of 10-20%, which could suggest a possible difference in electrolyte retention, but yet not significantly different compared to the males. In contrast, significant gender-associated differences in the ionic concentrations of *L. vannamei* hemolymph at the molt stage were not observed (Cheng *et al*. 2002), with mean Na+. In contrast to that reported by Cheng *et al*. (2002), significant gender-associated differences in the ionic concentrations of *L. vannamei* hemolymph at the molt stage were not observed with mean Na^+ concentrations for males and females of 350.6 mmol/l and 352.9 mmol/l, respectively.

Our shrimp were stressed by changes in salinity, whereas in a previous study (Cheng *et al*. 2002), the shrimp were not stressed. Moreover, during molting, growing crustaceans absorb water, resulting in an expanded, new, flexible exoskeleton that hardens rapidly.

In males, the maximum (5.62 mmol/l) and minimum (3.72 mmol/l) K^+ concentrations in hemolymph were observed at 30‰ and 0‰ salinity, respectively, whereas in females the maximum (5.32 mmol/l) and minimum (3.72 mmol/l) were observed at 10‰ and 0‰ salinity, respectively. These were lower than concentrations previously observed; 7.99 mmol/l for males and 8.17 mmol/l for females (Cheng *et al*. 2002) and 10.5 mmol/l overall (Sowers *et al*. 2006). These differences among studies may have been due to differences in shrimp size, particularly because electrolyte concentrations have been reported to increase with shrimp size. In addition, differences among studies may have been due to differences in the ionic compositions of the baths used and/or the balance of ions between the environment and the shrimp hemolymph (McGraw and Scarpa 2004), differences can also be due to different experimental design, methodologies and tests used to analyze ion. In some euryhaline shrimp, hemolymph potassium concentrations can be regulated to some extent by Na/K ATPase when environmental potassium concentrations varied greatly (Dall and Smith 1981). In continental waters, potassium chloride must be added to shrimp cultures to maintain viability, thereby increasing the concentration of K^+ in the medium (Boyd *et al*. 2002). Such manipulations may affect basal physiological metabolism, with *L. vannamei* reared in potassium-deficient seawater displaying anorexia, low-activity, poor growth and even death (Zhu *et al*. 2004).

In males, maximum (261.5 mmol/l) and minimum (was 191.0 mmol/l) Cl^- concentrations were observed at 40‰ and 0‰ salinity, respectively, whereas in females, the maximum (242.5 mmol/l) and minimum (196.4 mmol/l) concentrations were observed at 30% and 0‰ salinity, respectively. In contrast, Cheng *et al*. (2002) reported concentrations of 268.1 mmol/l in males and 266.6 mmol/l in females at low salinity.

The differences in concentrations of Na^+, K^+, and Cl^- between treated and control shrimp may have been due to exposure to higher salinity after a 15-h starvation diet. At extreme salinities (40‰), stress caused by starvation may decrease general metabolism (Palacios *et al*. 2004).

CONCLUSIONS

We have shown here that the ionic composition of the hemolymph of the shrimp *L. vannamei* varies significantly with sex, and that the concentrations of these electrolytes decrease as salinity decreases. The ionic composition of hemolymph is largely dependent on the ionic concentration of the external environment; this affects osmotic balance and the physiological functions of the organism. Studies are necessary to determine the rapidity of ionic absorption and homoeostasis in shrimp hemolymph.

ACKNOWLEDGEMENTS

This project was supported by the Foundation of Ramón Alvarez-Buylla of Aldana and the Sciences Marine Faculty of the Colima University, Mexico.

REFERENCES

Araneda M, Pérez EJ, Gasca-Leyva G (2008) White shrimp *Penaeus vannamei* culture in freshwater at three densities: Condition state based on length and weight. *Aquaculture* **283**, 13-18

Boyd CE, Thunjai T, Boonyaratpalin M (2002) Sales disueltas en agua de cultivo de camarón en tierras continentales y baja salinidad. *Boletín Nicovita* **7**, 1-14

Castille FL, Lawrence AL (1981) The effect of salinity on the osmotic, sodium and chloride concentrations in the haemolymph on euryhaline shrimp on the genus *Penaeus*. *Comparative Biochemistry Physiology* **68A**, 75-80

Chen JC, Chen CT (1996) Changes of osmotic and electrolyte concentration in the haemolymph of *Penaeus japonicus* exposed to ambient ammonia. *Comparative Biochemistry Physiology* **114C**, 35-38

Chen JC, Lin JN (1998) Osmotic concentration and tissue water of *Peneaus chinensis* juveniles reared at different salinity and temperature levels. *Aquaculture* **164**, 173-181

Cheng W, Liu CH, Yan DF, Chen JC (2002) Haemolymph oxyhemocyanin, protein, osmolality and electrolyte levels of whiteleg shrimp *Litopenaeus vannamei* in relation to size and molt stage. *Aquaculture* **211**, 325-339

Chim I, Bouveret R, Lemaire P, Martin JLM (2003) Tolerance of the shrimp *Litopenaeus stylirostris*, (Stimpson 1894), to environmental stress: interindividual variability and selection potential for stress-resistant individuals. *Aquaculture Research* **34**, 629-632

Dall W, Smith DM (1981) Ionic regulation of four species of penaeid prawn. *Journal Experimental Marine Biology Ecology* **55**, 219-232

Davis AD, Samocha TM, Boyd CE (2004) Acclimating Pacific White Shrimp, *Litopenaeus vannamei*, to Inland, low-salinity Waters. Southern Regional Aquaculture Center. SRAC Publication 2600

Díaz F, Farfán C, Sierra E, Re AD (2001) Effects of temperature and salinity fluctuation on the ammonium excretion and osmoregulation of juveniles of *Penaeus vannamei* Boone. *Marine Freshwater Behavior and Physiology* **34**, 93-104

Gong H, Jiang DH, Lightner DV, Collins D, Brock D (2004) A dietary modification approach to improve the osmoregulatory capacity of *Litopenaeus vannamei* cultured in the Arizona desert. *Aquaculture Nutrition* **10**, 227-236

Hurtado MA, Racotta IS, Civera R, Ibarra L, Hernández-Rodríguez M, Palacios E (2007) Effect of hypo- and hypersaline conditions on osmolality and Na+/K+-ATPase activity in juvenile shrimp (*Litopenaeus vannamei*) fed low- and high-HUFA diets. *Comparative Biochemical Physiology* **147C**, 703-710

Lemaire P, Bernard E, Martínez-Paz JA, Chin L (2002) Combined effect of temperature and salinity on osmoregulation of juvenile and sub adult *Penaeus stylirostris*. *Aquaculture* **209**, 307-317

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

McGraw WJ, Scarpa J (2004) Mortality of freshwater-acclimated *Litopenaeus vannamei* associate with acclimation rate, habituation period, and ionic challenge. *Aquaculture* **236**, 285-296

Palacios E, Bonilla A, Luna D, Racotta IS (2004) Survival, Na+/K+-ATPase and lipid responses to salinity challenge in fed and starved white pacific shrimp (*Litopenaeus vannamei*) postlarvae. *Aquaculture* **234**, 497-511

Pequeux A (1995) Osmotic regulation in crustaceans. *Journal Crustacean Biology* **15**, 1-60

Robertson L, Bray W, Leung-Trujillo J, Lawrence A (1987) Practical molt staging of *Penaeus setiferus* and *Penaeus stylirostris*. *Journal World Aquaculture Society* **18**, 180-185

Roy LA, Davis DA, Saoud IP, Henry RP (2007) Effects of varying levels of aqueous potassium and magnesium on survival, growth, and respiration of the Pacific white shrimp, *Litopenaeus vannamei*, reared in low salinity waters. *Aquaculture* **262**, 461-469

Sang HM, Fotedar R (2004) Growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) reared a different salinities. *Aquaculture* **234**, 601-614

Sowers AD, Young SP, Grosell M, Browdy CL, Tomasso JR (2006) Haemolymph osmolality and cation concentration in *Litopenaes vannamei* during exposure to artificial sea salt or a mixed-ion solution: Relationship to potassium flux. *Comparative Biochemistry Physiology* **145**, 176-180

Thanh DTTH, Jasmani S, Jayasankar V, Wilder M (2010) Na/K-ATPase activity and osmo-ionic regulation in adult whiteleg shrimp *Litopenaeus vannamei* exposed to low salinities. *Aquaculture* **304**, 88-94

Yano I, Tsukimura BT, Sweeney JN, Wyban JA (1988) Induced ovarian maturation of *Penaeus vannamei* by implantation of lobster ganglion. *Journal of the World Aquaculture Society* **19**, 204-209

Zar JH (1994) *Biostatistical Analysis*, Prentice-Hall Inc, Englewood Cliffs, New Jersey, USA, pp 275-360

Zhang P, Zhang X, Li J, Huang G (2007) The effects of temperature and salinity on the swimming ability of whiteleg shrimp, *Litopenaeus vannamei. Comparative Biochemistry and Physiology* **147A**, 64-69

Zhu C, Dong S, Wang F, Huang G (2004) Effects of Na/K ratio in seawater on growth and energy budget of juvenile *Litopenaeus vannamei*. *Aquaculture* **234**, 485-496