Tuyển Tập Nghiên Cứu Biển, 2004, XIV: 169 - 182

ÁP SUẤT THẨM THẤU MÁU, HÀM LƯỢNG NƯỚC TRONG CƠ VÀ ẢNH HƯỞNG CỦA VIỆC ĐƯA RA NGOÀI KHÔNG KHÍ ĐẾN KHẢ NĂNG ĐIỀU HÒA ÁP SUẤT THẨM THẤU CỦA TÔM GÂN (*PENAEUS LATISULCATUS* KISHINOUYE, 1896) NUÔI Ở CÁC ĐỘ MẶN KHÁC NHAU

Huỳnh Minh Sang
Viện Hải Dương Học (Nha Trang)

TÓM TẮT *Áp suất thẩm thấu máu và hàm lượng nước trong cơ của Tôm Gân (Penaeus latisulcatus) được xác định khi nuôi ở độ mặn 10, 22, 34 và 46‰ sau 60 ngày và khả năng điều hòa áp suất thẩm thấu của tôm nuôi ở các nồng độ mặn 10, 22, 34 và 46‰ được xác định khi đưa ra ngoài không khí 7, 14 và 21 phút. Áp suất thẩm thấu của máu tăng khi tăng độ mặn môi trường nuôi và trọng lượng của tôm. Điểm trung hòa áp suất thẩm thấu được tính từ mối tương quan giữa áp suất thẩm thấu máu và áp suất thẩm thấu của môi trường của Tôm Gân là 28,87, 29,46 và 31,73‰ tại thời điểm nuôi 0, 20 và 60 ngày nuôi, ứng với trọng lượng cơ thể là 2,95 ± 0,26; 4,02 ± 0,47 và 5,79 ± 0,64 g. Lượng nước trong cơ giảm khi độ mặn môi trường nuôi tăng. Khả năng điều hòa áp suất thẩm thấu của tôm nuôi ở độ mặn 10‰ giảm sau 14 phút đưa ra ngoài không khí. Kết quả nghiên cứu trên chỉ ra rằng tôm dùng ít năng lượng để điều hòa áp suất thẩm thấu khi nuôi ở độ mặn 22 và 34‰ hơn khi nuôi ở các độ mặn khác. Kết quả cho thấy loại tôm này nên được nuôi ở độ mặn từ 22 đến 34‰.*

HAEMOLYMPH OSMOLALITY, TAIL MUSCLE MOISTURE CONTENT AND THE EFFECTS OF AIR EXPOSURE ON THE OSMOREGULATORY CAPACITY OF THE WESTERN KING PRAWN (*PENAEUS LATISULCATUS* KISHINOUYE, 1896) REARED AT DIFFERENT SALINITIES

Huynh Minh Sang
Institute of Oceanography (Nha Trang)

ABSTRACT *Haemolymph osmolality and tail muscle moisture content of the western king prawn, Penaeus latisulcatus, (2.95 ± 0.26 g mean initial weight), were measured when reared at 10, 22, 34 and 46 ppt salinities for 60 days. In addition, osmoregulatory capacity (OC) of the western king prawn (5.37 ± 0.1 g mean initial weight) from four salinities (10, 22, 34 and 46 ppt) was determined following 7, 14 and 21 minutes air-exposure. Haemolymph osmolality increased with an increase in medium salinity and weight of the prawns. Isosmotic points calculated from regression between haemolymph and medium osmolality were 28.87, 29.46 and 31.73 ppt at 0, 20 and 60 days of culture, when body weights were 2.95 ± 0.26; 4.02 ± 0.47; 5.79 ± 0.64 g respectively. Tail muscle moisture content of the western king prawn decreased with an increase in salinity. OC of the western king prawn at*

salinity 10 ppt was reduced (P < 0.05) when exposed to 14 minutes of air. The results indicate that the prawns spent less energy (P < 0.5) for osmoregulation at 22 and 34ppt of salinity than at other salinities. The results suggest that this prawn should be cultured in range of salinity from 22 to 34 ppt.

I. INTRODUCTION

Salinity is one of the most important abiotic factors affecting the growth and survival of aquatic organisms. The biological effects of this factor are complex and wide ranging (Parado-Estepa *et al.* 1993; Kumlu, Jones 1995; Kumlu *et al.* 1999; Kumlu *et al.* 2000). While temperature is the most important modifier of energy flow, and hence growth, in aquatic organisms, salinity imposes the greatest additional load on the metabolic requirement of an animal (Ponce-Palafor *et al.* 1997).

In penaeid shrimps, effects of salinity are best understood by investigating the osmoregulatory mechanism, which has been used as a tool to monitor physiological conditions and the effects of stressors (Lignot *et al.* 2000). Lignot (2000) reviewed the ability of penaeid shrimps to osmoregulate (evaluated by the osmoregulatory capacities) and found it to be sensitive to pollutants such as oil (Anderson *et al.* 1974), pesticides (Anderson *et al.* 1974; Lignot *et al.* 1997) and metals (Bambang *et al.* 1995). Change in ambient salinity may disrupt the osmotic balance in penaeid prawns. This results in prawns have to use a considerable amount of energy to readjust the osmotic balance (Chen *et al.* 1996).

Osmoregulatory capacity (OC) of penaeid prawns in different salinities can be used as a tool in monitoring physical condition and the effect of stress. The effects of salinity on haemolymph osmolality, tail muscle moisture content and osmoregulatory capacity of penaeid prawns have been widely studied. The haemolymph osmolality linearly increases with increasing salinity (Cheng, Liao 1986; Ferraris *et al.* 1986a; Ferraris *et al.* 1986b; Charmantier-Daures *et al.* 1988; Diwan *et al.* 1989; Allan, Maguire 1992; Chen *et al.* 1995; Chen, Lin 1998). The OC also changes with species, size, nutritional condition and developmental stages (Lignot *et al.* 2000).

Western king shrimp (*Penaeus latisulcatus*), also called bamboo shrimp, is one of the most economically valuable crustaceans in Australia. They are widely distributed throughout the Indo-West Pacific region (Racek, Dall 1965; Penn 1980; Dore, Frimodt 1987). Western king shrimps are found around most of coastal Australia (Racek, Dall 1965; Penn 1980; Dore, Frimodt 1987; Kailola *et al.* 1993). While research has been conducted into haemolymph osmolality and tail muscle moisture content of several penaeid species reared at different salinities, there has been limited research about those issues of the western king prawn. The purpose of this research is to determine the haemolymph osmolality and tail muscle moisture content of the western king prawn reared at different salinities. In addition, this paper presents the changes of osmoregulatory capacity of the western king prawn

reared at different salinities effected by air exposure.

II. MATERIALS AND METHODS

1. Experiment 1

1.1. Experimental animals

Western king prawns were collected using drag nets in the Peel-Harvey Estuary (32° 55'S, 115° 42'E) near Mandurah Bridge in Western Australia on 6th and 11th September 2002. Over 200 prawns were caught during the two nights between 8:00 to 11:00 pm. The average weight of collected prawns was 2.95 ± 0.26 g. The prawns were then transported to the Aquatic Science Research Unit, Curtin University of Technology, Perth, Western Australia and acclimatised to the laboratory conditions. They were kept at the seawater salinity (35 ppt) and fed with green mussels (*Mytilus edulis edulis)* daily until the experiment began.

1.2. Experimental design

Sixteen 125 L plastic cylindrical tanks, with a diameter of 59 cm were used in this experiment. Each tank was coupled to an external bio-filter and protein skimmer (Figure 1). The water temperature was maintained at 25°C by controlling the air temperature in the laboratory. Aeration was provided to every tank during the experiment.

Each experimental tank was filled with 80 L seawater (salinity of 35 ppt) and stocked with 11 prawns. The prawns were then acclimatised to salinities of 10, 22, 34 and 46 ppt in replicates of four by a procedure described by Chen *et al*. (1995). For salinities of 10, 22 and 34 ppt, salinity was adjusted with freshwater with a decrease of 3 ppt per day. For salinity of 46 ppt, concentrated seawater (60 ppt) was added and the salinity was increased 2 ppt per day. For reducing the salinity, the volume of water in each experimental tank replaced by freshwater was calculated as the following equation:

$$Vr = 80 \times (1 - Sa/Sb).$$

Where: Vr: Volume of water replaced by freshwater. Sa: Salinity after adjustment. Sb: Salinity before adjustment.

For increasing the salinity, the volume of water in each experimental tank replaced by concentrated seawater (60 ppt) was calculated as the following equation:

$$Vr = 80 \times (Sa - Sb)/(60 - Sb)$$

Where: Vr: Volume of water replaced by concentrated seawater (60 ppt). Sa: Salinity after adjustment. Sb: Salinity before adjustment.

The prawns were then reared for 60 days.

During the course of the experiment, the prawns were fed with mussel (*Mytilus edulis edulis*) till satiation. The satiation level was calculated to be 7 % of the total biomass of the prawns per day during the preliminary experiment. The feed was provided once a day at 10:00 am. Before feeding commenced, faecal matter and any uneaten food were siphoned out to minimise the organic load.

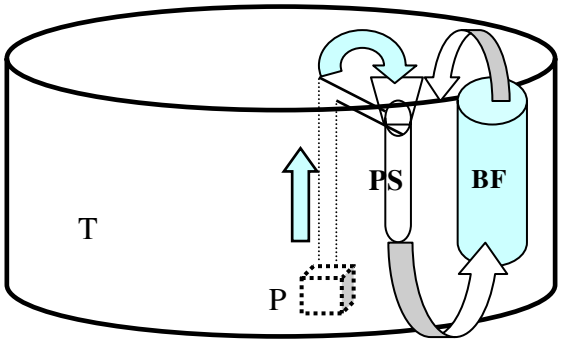

Figure 1: **Diagrammatic design outline of an experimental tank (the arrows show the direction of water flow).**
P: Internal pump
PS: Protein skimmer
BF: Bio-filter containing coral media
T: 125 L tank

1.3. Data collection

+ Haemolymph osmolality analysis

The haemolymph osmolality of one prawn from each tank was measured at 0, 20 and 60 days of the experimental period. 0.05 mL of haemolymph was extracted from the pericardial cavity through the intersegmental membrane between the cephalothorax and the first abdominal segment using 0.5 mL syringe containing 0.1 mL of precooled (10^0C) anticoagulant (0.1% glutaraldehyde in 0.2 M sodium cacodylate, pH 7.0). 23-gauge needle was used to extract the haemolymph. Osmolality of the mix solution was measured by Cryoscopic Osmometer – Osmomet 030. Osmolality of blank anticoagulants was also measured and the haemolymph osmolality was calculated using following equation: Haemolymph osmolality = 3 x Osmolality of mix – 2 x Osmolality of anticoagulant. Isosmotic range was calculated based on the regression line between haemolymph osmolality and medium osmolality at the points that haemolymph osmolality was equal to the medium osmolality.

+ Tail muscle moisture content analysis

Tail muscle moisture content of one randomly sampled prawn from each tank was measured at 0, 20 and 60 days of the experimental period. The tail muscle (i.e. the complete mass of muscle in the abdomen of the prawn) weighed was then dried to constant weight at 100°C for 24 hours. The tail muscle moisture content (moisture content) (TM%).

TM% = 100 x (Wtwet – WTdry) / Wtwet.

2. Experiment 2

Western king prawns (average weight 5.37 ± 0.1 g) were collected using the same method described in the experiment 1. The prawns were distributed into 12 experimental tanks (Figure 1) separately filled with 70 L of oceanic water (35 ppt), a density of 20 animals was contained in each tank. The prawns were maintained in these tanks for 7 days before the commencement of the acclimatisation.

The prawns were acclimatised to salinities 10, 22, 34 and 46 ppt by a procedure described above so that each

salinity concentration was represented in the replicates of three. After acclimatisation, the prawns were reared for 7 days using the culture method applied in experiment 1. The prawns were then starved for 1 day to bring them at the same nutritional status.

Only twelve prawns in inter-moult stage of development were left in each tank for the trial and the air exposure procedure was as follow:

Out of 12 western king prawn, 9 prawns from each tank were exposed to air by placing them into a foam box, leaving the remaining 3 in the water. After 7 minutes of air-exposure, 3 of the 9 prawns were tagged by cutting the edge of outer - right uropodite and released back into their respective tanks. After a further 7 minutes, 3 of 6 remaining prawns were tagged by cutting the edge of outer – left uropodite and were released back into the same tank. 7 minutes later, the remaining 3 prawns from foam box were tagged by cutting the inner – right uropodite and released back into their original salinity condition. Thus there were four groups of prawns, the first group was not exposed to air at all whereas the 2^{nd}, 3^{rd} and 4^{th} groups were exposed to air for 7, 14 and 21 minutes, respectively. Three hours after releasing back into their original salinity condition, the haemolymph of the individual prawns was collected to determine the osmolality as described above.

3. Data analysis

SPSS statistical program version 10 was used to analyse the data. Results were presented as means ± SE (Standard error). ANOVA (analysis of variance), Independent Sample T tests and LSD (Least significant difference) post hoc tests (Fowler & Cohen 1990) were used to determine the significant differences between growth, survival, haemolymph osmolality and organosomatic indices of the prawns reared at 10, 22, 34 and 46 ppt of salinity. All significant tests were at $P < 0.05$ levels.

III. RESULTS

1. Haemolymph osmolality

Haemolymph osmolality of the western king prawn increased with an increase of salinity. The lowest haemolymph osmolality was at a salinity of 10 ppt (676.25 ± 29.20 mOm/kg at the beginning and 664.00 ± 57.70 mOsm/kg after 20 days of rearing period). Those values were significantly lower ($P < 0.05$) than values obtained at salinities of 22, 34 and 46 ppt. The prawn reared at a salinity of 46 ppt showed the highest haemolymph osmolality compared to prawns at the other salinities (977.75 ± 15.31; 1001.00 ± 41.66 and 1102 ± 50.27 mOsm/kg at commencement, 20 and 60 days of the rearing period, respectively). Those values were significantly higher ($P < 0.05$) than the haemolymph osmolality of prawns reared at salinities of 10, 22 and 34 ppt. There were significant changes ($P < 0.05$) in the haemolymph osmolality between the beginning and 60 days of the rearing period of prawns at a salinity of 46 ppt. At a salinity of 22 ppt, the highest haemolymph osmolality of prawns was observed after 20 days of rearing which was significantly higher ($P < 0.05$) than those at the beginning and at 60 days of the rearing period (Table 1).

Table 1: **Haemolymph osmolality (mOsm/kg) of western king prawns reared at different salinities**

Day	Salinity			
	10 ppt	**22 ppt**	**34 ppt**	**46 ppt**
0	$_{1}676.25 \pm 29.20^{a}$	$_{1}845.00 \pm 14.54^{b}$	$_{1,2}899.00 \pm 12.18^{b}$	$_{1}977.75 \pm 15.31^{c}$
20	$_{1}664.00 \pm 57.70^{a}$	$_{2}951.00 \pm 19.05^{b,c}$	$_{1}834.00 \pm 54.71^{b}$	$_{1,2}1001.00 \pm 41.66^{c}$
60		$_{1}854.00 \pm 13.63^{a}$	$_{2}930.00 \pm 5.79^{a}$	$_{2}1102 \pm 50.27^{b}$
Media O.P	$_{2}333.00^{a}$	$_{3}668.00^{b}$	$_{2}1011.00^{c}$	$_{3}1332.00^{d}$

Data in the same column having different subscript letters (1,2,3…) are significantly different at ∝ level of 0.05. Data in the same row having different superscript letters (a, b, c…) are significantly different at ∝ level of 0.05.

The linear relationships between the haemolymph osmolality and the medium osmolality at different rearing times are shown in table 2 and figure 2. Isosmotic points that were determined based on regression lines between both the haemolymph and medium osmolality are shown in table 2. Isosmotic points increased with the increase in both the length of rearing times and weights of prawns.

Table 2: **Relationship between haemolymph osmolality (Y) and medium osmolality (X) of western king prawns reared at different salinities**

Days of culture	Day 0	Day 20	Day 60
Equation	Y = 0.2871 X + 609.47	Y = 0.2665 X + 639.69	Y = 0.3718 X + 588.88
R^2	0.9398	0.5895	0.9440
Isosmotic point (mOsm/kg)	854.80	871.78	937.09
Isosmotic point (ppt)	28.87 ppt	29.46 ppt	31.73 ppt
Weight (g)	2.95 ± 0.26	4.02 ± 0.47	5.79 ± 0.64

2. Tail muscle moisture content

Tail muscle moisture content of the western king prawn decreased with an increase in salinity. After the acclimatization period, it was highest at a salinity of 10 ppt (82.43 ± 1.681%) and lowest at a salinity of 46 ppt (71.03 ± 3.221%). Tail muscle moisture content was significantly higher ($P < 0.05$) in prawns reared at a salinity of 10 ppt compared to those at salinities of 22, 34 and 46 ppt. However, there were no significant differences in muscle moisture content in prawns raised at salinities of 22, 34, and 46 ppt. Tail muscle moisture content was also highest at a salinity of 10 ppt at 20 days of culture (80.37 ± 0.526%), which was significantly higher ($P < 0.05$) than those at salinities of 34 and 46 ppt. There was no significant difference ($P > 0.05$) in tail muscle moisture content between salinities of 22 and 34 ppt, but it was significantly higher ($P < 0.05$) at a salinity of 22 ppt compared to a salinity of 46 ppt. Tail muscle moisture of the prawn did not change significantly ($P>0.05$) during the experimental time in the four salinities (Table 3).

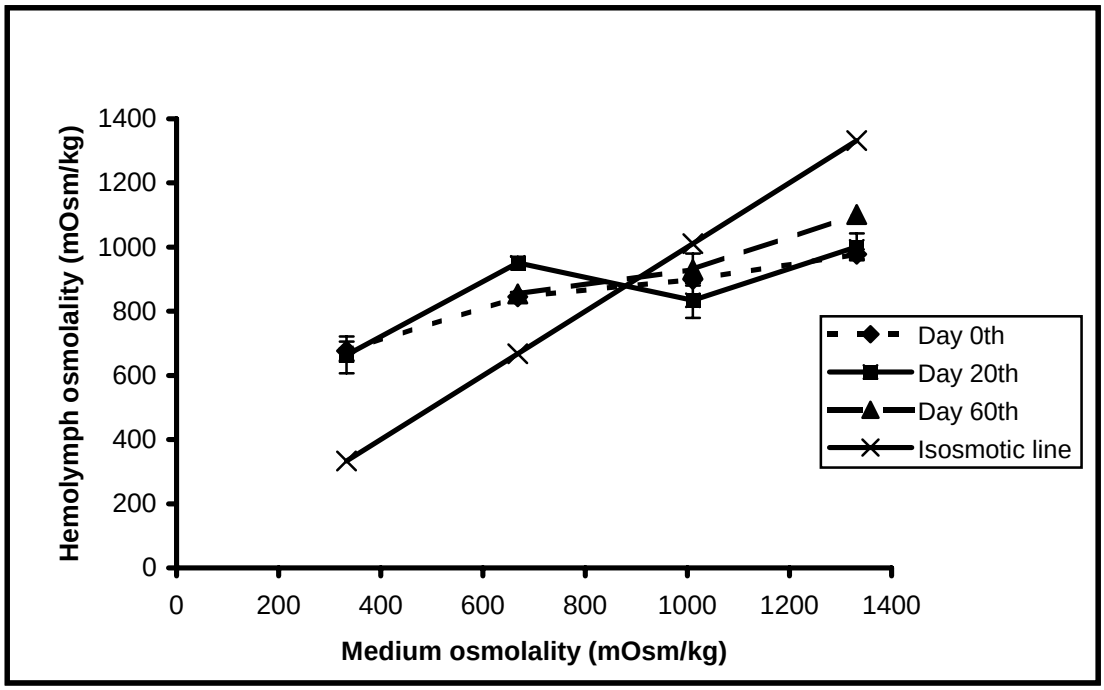

Figure 2: **Relationship between haemolymph osmolality and medium osmolality of western king prawns reared at different salinities**

Table 3: **Tail muscle moisture content of western king prawns reared at different salinities**

Day	Salinity			
	10 ppt	**22 ppt**	**34 ppt**	**46 ppt**
0	$_{1}82.43 \pm 1.681^{a}$	$_{1}76.17 \pm 8.974^{b}$	$_{1}74.62 \pm 0.787^{b}$	$_{1}71.03 \pm 3.221^{b}$
20	$_{1}80.37 \pm 0.526^{a}$	$_{1}77.16 \pm 0.591^{b}$	$_{1}76.25 \pm 0.496^{b,c}$	$_{1}74.71 \pm 0.377^{c}$
60		$_{1}76.25 \pm 0.037^{a}$	$_{1}76.11 \pm 0.527^{a}$	$_{1}75.75 \pm 0.733^{a}$

Data in the same row having different superscript letters (a, b, c…) are significantly different at∝ level of 0.05.

3. Osmoregulatory capacity (OC)

Osmoregulatory capacity of the prawns from 10 ppt salinity significantly declined ($P < 0.05$) after 14 minutes of air-exposure. In other salinities, OC of the western king prawn changed insignificantly subjected to different length of air exposure (Table 4).

The relationship between medium osmolality and haemolymph osmolality of western king prawns reared at different salinities and subjected to different lengths of air exposure was presented in table 5.

Table 4: **Osmoregulatory capacity of western king prawns reared at different salinities and subjected to different lengths of air exposure**

Time exposed (min.)	Salinity			
	10 ppt	22 ppt	34 ppt	46 ppt
0	$_1475.50 \pm 27.06^a$	$_1253.75 \pm 14.25^b$	$_184.00 \pm 42.91^c$	$_1192.75 \pm 28.01^b$
7	$_1460.50 \pm 30.92^a$	$_1188.50 \pm 59.20^b$	$_168.25 \pm 32.96^c$	$_1195.75 \pm 9.03^b$
14	$_2288.75 \pm 79.28^a$	$_1147.50 \pm 55.04^{a,b}$	$_155.50 \pm 13.99^b$	$_1173.25 \pm 56.51^a$
21	$_2263.25 \pm 34.21^a$	$_1106.25 \pm 52.25^b$	$_120.25 \pm 5.66^c$	$_1139.50 \pm 39.49^b$

Data for each species in the same column having different subscript (1, 2, 3…) letters are significantly different at ∝ level of 0.05. Data in the same row having different superscript letters (a, b, c…) are significantly different at∝ level of 0.05.

Table 5: **The relationship between medium osmolality and haemolymph osmolality of western king prawns reared at different salinities and subjected to different lengths of air exposure.**
(Y: Haemolymph osmolality, X: medium osmolality)

Length of air-exposure (mins.)	Equation	R^2	Isosmotic points	
			mOsm/kg	Ppt
0	Y = 0.2971 X + 700.72	0.8617	996.76	33.79
7	Y = 0.334 X + 654.16	0.9313	982.22	33.29
14	Y = 0.5418 X + 442.91	0.9992	966.63	32.75
21	Y = 0.6057 X + 385.18	0.9977	976.87	33.10

IV. DISCUSSION

The relationship between medium osmolality and haemolymph osmolality of western king prawns reared at different salinities and subjected to different lengths of air exposure was presented in table 5.

Various researchers (Williams 1960; Bishop *et al.* 1980; Castille, Lawrence 1981; Rodriguez 1981; Chen, Fang 1986; Cheng, Liao 1986; Ferraris *et al.* 1986a; Ferraris *et al.* 1986b; Ferraris *et al.* 1987; Charmantier-Daures *et al.* 1988; Diwan *et al.* 1989; Allan, Maguire 1992; Vargas-Albores, Ochoa 1992; Chen, Cheng 1993; Lin *et al.* 1993; Chen, Lin 1994a; Chen, Lin 1994b; Bambang *et al.* 1995; Chen *et al.* 1995; Funge-Smith *et al.* 1995; Lignot *et al.* 1997; Chen, Lin 1998; Lignot *et al.* 1998; Fang *et al.* 1999; Lignot *et al.* 1999; Lemaire *et al.* 2002) have used different protocols to determine the osmolality of crustacean haemolymph. Chen *et al.* (1994) measured haemolymph osmolality without adding any anti-coagulant solution to the haemolymph. The current research has indicated that the haemolymph of *P. latisulcatus* coagulates quickly rendering it impossible to measure osmolality of the haemolymph. The research has also indicated that the osmolality of serum is significantly higher ($P < 0.05$) than the haemolymph which is in contradiction from the research of Funge-Smith *et al.* (1995) who used serum osmolality of freshwater prawns (*Macrobrachium rosenbergii*) as haemolymph osmolality. The process of coagulation removes the haemolymph cells and some bound metals (Funge-Smith *et al.* 1995) and

thus increases the osmolality of serum in comparison to haemolymph.

The protocol adapted to measure the haemolymph osmolality in the current research overcomes some limitations of the previous methods. The protocol permits the usage of whole blood (and its dilutions) by anticoagulants for the purpose of measuring osmolality. The protocol was further tested by using several solutions (replacing the haemolymph) and the obtained results were consistent.

In crustaceans, osmoregulation is an important environmental adaptation (Pequeux, 1995). The results of the present study have clearly shown that the western king prawn is hypo-osmoregulator when the ambient salinity is above isosmotic point and hyper-osmoregulator when the ambient salinity is below the isosmotic point. This adaptation is a typical of many brackish water crustaceans (Mantel, Farmer 1983; Lemaire *et al.* 2002).

Previous studies by Chen & Lin (1998) and Lemaire *et al*. (2002) have shown a linear relationship between medium osmolality and haemolymph osmolality of the fleshy prawn (*P. chinensis*) and the blue prawn (*P. stylirostris*). The present study confirms this relationship by proving that the haemolymph osmolality of the western king prawn increases with an increase in the medium osmolality. Since the deviation from the slope in isosmotic line (slope = 1) reflects the degree of regulation, western king prawns (slope = 0.2665, 0.2871 and 0.3718) are efficient regulators when they are compared to fleshy prawns (*P. chinesis*) (Chen, Lin 1998), pink prawns (*P. duroraum*) (Castille, Lawrence 1981) and green tiger prawns (*P. semisulcatus*) (Clark 1992). The present study also indicates that the western king prawn is a less efficient osmoregulator than northern brown prawn (*P. azecus*), Indian white prawn (*P. indicus*), giant tiger prawn *(P. monodon)*, white prawn (*P. setiferus),* blue prawn and the white leg prawn (*L. vannamei*) (Chen, Lin 1998).

The isosmotic point of the western king prawn in the experiment 1 ranges from 854.80 to 937.09 mOsm/kg, which is equivalent to 28.87 to 31.73 ppt according to prawn weight. It is clear that the isosmotic point of the western king prawn has a positive relationship with size. This study confirms the conclusion of Chen & Lin (1998) and Lignot *et al*. (1999) that the osmotic point of penaeid prawn changes according to development stages. As deviation from the slope in isosmotic line (slope = 1) reflects the degree of regulation, the present study also shows that, western king prawns of a lower weight (2.95 ± 0.26 g) at the beginning of experiment (slope = 0.2871) are more efficient osmoregulators than prawns of a higher weight (5.79 ± 0.64 g) at 60 days of rearing (slope = 0.3718). This result agrees with the finding of Lignot *et al*. (1999) when studying the juvenile blue prawns (*P. stylirostris*) that this prawn decreases the absolute hypo-osmoregulatory capacity with increase of wet weight. In wild populations prawns living in estuaries, lagoons and coastal areas are strong hypo-regulators but their hypo-regulation capability decreases, as they become adults and migrate back to the open sea (Lignot *et al.* 1999).

The significant increase in the haemolymph osmolality of the western king prawn reared at a salinity of 46 ppt after 60 days indicates that the

prawn reduces osmoregulatory capacity in high salinity. Contrastly, western king prawns at salinities of 22 and 34 ppt change the haemolymph osmolality at 20 days and recover at 60 days of rearing. This result suggests that prawns at salinities of 22 and 34 ppt can maintain their osmoregulatory capacity. This can be explained by the fact that salinities of 22 and 34 ppt are close to the isosmotic point and prawns in these salinities spend less energy on osmoregulation than prawns at a salinity of 46 ppt. At salinities of 22 and 34 ppt, a dominant amount of intake energy will be used for metabolic compared to salinities outside this range.

The results obtained from experiment 2 indicate that different development stages of western king prawns have different haemolymph osmolality and isosmotic points. In the present experiment, the prawns with a weight of 5.37 ± 0.1 g seem to be less efficient in osmoregulation (slope of the regression line between haemolymph osmolality and medium osmolality is 0.2971) than the prawns with a lower weight in experiment described in previous sections (weight = 2.95 ± 0.26 g, slope = 0.2871). The isosmotic points of prawns in the present experiment range from 31 to 33 ppt, which is higher than the isosmotic points of the prawns in the previous experiment.

The present study shows that the tail muscle moisture content of the western king prawn decreases with increases in salinity. Similarly, tail muscle moisture content of the fleshy prawn has a negative correlation to medium salinity (Chen *et al*. 1995). The significantly higher ($P < 0.05$) moisture content of the tail muscle of prawns reared at a salinity of 10 ppt compared to higher salinities indicates the depletion of energy reserves in the tail muscle of prawns at a salinity of 10 ppt during the course of experiment.

Osmoregulatory capacity (OC) has been used as a tool in monitoring the physiological condition of many aquatic invertebrates (Lignot *et al.* 2000). Lignot's (2000) review has shown that the OC of penaeid prawns was influenced by various stressors including air-exposure. Air exposure has been used as stressors to evaluate the health status of several crustacean species. In giant tiger prawn, air-exposure significantly changes blood glucose level (Hall, Ham 1998). Air-exposure also reduces oxygen consumption, changes heart rates and the haemolymph acid-base status of the southern rock lobster *(Jasus edwarddsii*) (Taylor, Waldrom 1997). It also has a significant effect on the immune system and on health status of the western rock lobster (*Panulirus cygnus*). Air exposure can also result in a significant increase in the haemolymph clotting time, a reduction in total haemocyte counts, granular cell numbers and an increase in bacteraemia (Fotedar *et al.* 2001). The present study indicates that air exposure can reduce the OC of the western king prawn. Reduction in OC of the western king prawn caused by air exposure can also be used to evaluate the health status. Prawns in good health and condition will maintain their OC, whereas prawns in a stressed condition reduce their OC when exposed to air. The more stress a prawn suffers results in a greater reduction in OC.

The slopes obtained from the regression lines between rearing medium osmolality and haemolymph

osmolality in the western king prawn showed an increasing trend with the increase in air-exposure lengths. The deviation from the slope of isosmotic line (slope = 1), which reflects the degree of regulation, thus decreases with an increase in air-exposure lengths. The significant reduction in OC of western king prawns at 10 ppt indicates that this prawn does not have good tolerance for low salinity (salinity < 10 ppt). In this salinity prawns use more energy for osmoregulation and are thus more susceptible to diseases because less energy is available for the immune system.

Results obtained from the analysis of the haemolymph osmolality, tail muscle moisture content and OC of the western king prawn suggest that this prawn should be cultured in range of salinity from 22 to 34 ppt.

ACKNOWLEDGMENT

The author would like to thank David Prangnell for his help in the collection of the western king prawn and Dr. Ravi Fotetar for his critical comments on the manuscript.

REFERENCES

1. Allan G. L., G. B. Maguire, 1992. Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon* Fabricius. Aquaculture 107, 1, 33 - 47.
2. Anderson J. W., J. M. Neff, S. R. Petrocelli, 1974. Sublethal effects of oil, heavy metals and PCBs on marine organisms. Academic Press, New York.
3. Bambang Y., P. Thuet, M. Charmantier-Daures, J. P. Trilles, G. Charmantier, 1995. Effects of copper on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* Bate (Crustacea, Decapoda). Aquatic Toxicology 33, 125-139.
4. Bishop J. M., J. G. Gosselink, J. H. Stones, 1980. Oxygen consumption and haemolymph osmolality of brown shrimp, *Penaeus aztecus*. In Fish Bulletin. Vol. 78, 741-757.
5. Castille F. L. J., A. L. Lawrence, 1981. The effect of salinity on the osmotic, sodium, and chloride concentration in the hemolymph of euryhaline shrimp of the genus *Penaeus*. Comparative Biochemistry and Physiology 68, 75-80.
6. Charmantier-Daures M., P. Thuet, G. Charmentier, J. P. Trilles, 1988. Salinity tolerance and osmoregulation in post-larvae of *Penaeus japonicus* and *Penaeus chinensis*. Effect of temperature. Aquatic Living Resources 1, 4, 267-276.
7. Chen I. M., L. S. Fang, 1986. Metabolic and osmotic response of *Metapenaeus monoceros* (De Haan) subjected to sudden salinity changes. In The First Asian Fish Forum. Eds. Maclean, J. L., Dizon, L. B., Hosillos, L. V. Asian Fish Society. Manila, Philippines, 629-632.
8. Chen J. C., S. Y. Cheng, 1993. Haemolymph osmolality, acid-base balance and shift of ammonotelic to ureotelic excretory pattern of *Penaeus japonicus* exposed to ambient ammonia. Comparative Biochemical Physiological 106C, 3, 733-737.

9. Chen J. C., J. L. Lin, 1994a. Osmolality and chloride concentration in the hemolymph of subadult *Penaeus chinensis* subjected to different salinity levels. Aquaculture 125, 167-174.
10. Chen J. C., J. L. Lin, 1994b. Responses of haemolymph osmolality and tissue water of *Penaeus chinensis* Osbeck juveniles subjected to sudden change in salinity. Marine Biology 120, 1, 115-121.
11. Chen J. C., J. N. Lin, 1998. Osmotic concentration and tissue water of *Peneaus chinensis* juveniles reared at different salinity and temperature levels. Aquaculture 164, 173 - 181.
12. Chen J. C., J. N. Lin, C. T. Chen, M. N. Lin, 1996. Survival, growth and intermolt period of juvenile *Penaeus chinensis* (Osbeck) reared at different combinations of salinity and temperature. Journal of Experimental Marine Biology and Ecology 204, 169 - 178.
13. Chen J. C., M. N. Lin, Y. Y. Ting, J. N. Lin, 1995. Survival, haemolymph osmolality and tissue water of *Penaeus chinensis* juvenile acclimated to different salinity and temperature levels. Comparative Biochemistry and Physiology 110, 3, 253 - 258.
14. Cheng J. H., I. C. Liao, 1986. The effect of salinity on the osmotic and ionic concentrations in the hemolymph of *Penaeus monodon* and *P. penicillatus*. In Asian Fisheries Forum. Eds. Maclean, J. L., Dizon, L. B., Hosillos, L. V. Manila (Philippines), 633-636.
15. Clark J. V., 1992. Physiological responses of adult *Penaeus semisulcatus* (de Haan) to changes in salinity. Comparative Biochemistry and Physiology 101A, 1, 117-119.
16. Diwan A. D., A. Laxminarayana, V. S. Kakati, 1989. Osmoregulatory ability of *Penaeus monodon* (Fabricius) in relation to varying salinities. Indian Journal of Fisheries 36, 1, 53 - 57.
17. Dore I., C. Frimodt, 1987. An Illustrated Guide to Shrimp of the World. Van Nostrand Reinhold. New York.
18. Fang W., Q. Lai, H. Wang, 1999. Effect of molting and abrupt salinity change on the osmolality and ionic concentrations in the hemolymph of *Penaeus chinensis*. Journal of Fisheries China 23, 22-27.
19. Ferraris R. P., F.D.P., E. G. Estepa, D. Jesus, J. M. Ladja, 1986a. Osmo-regulation in *Penaeus monodon*: effects of molting and external salinity (eds). The First Asian Fisheries Forum, AFS. Manila.
20. Ferraris R. P., F. D. Parado-Estepa, D. Jesus, J. M. Ladja, 1987. Osmotic and chloride regulation in the hemolymph of the tiger prawn *Penaeus monodon* during molting in various salinities. Marine Biology 95, 3, 377-385.
21. Ferraris R. P., F. D. Parado-Estepa, J. M. Ladja, E. G. De Jesus, 1986b. Effect of salinity on the osmotic, chloride, total protein and calcium concentration in the hemolymph of prawn *Penaeus monodon* (Fabricius). Comparative Biochemistry and Physiology 83, 701-708.
22. Fotedar S., E. Tsvetnenko, L. Evans, 2001. Effect of air exposure on the immune system of the rock

lobster *Panulirus cygnus*. Marine and Freshwater Research 52, 1351 - 1355.

23. Fowler J. & L. Cohen, 1990. Practical statistics for field biology, Chichester, New York - Brisbane - Toronto - Singapore.
24. Funge-Smith S. J., A. C. Taylor, J. Whitley, J. H. Brown, 1995. Osmotic and ionic regulation in the giant Malaysian freshwater prawn, *Macrobrachium rosenbergii* (de Man), with special reference to strontium and bromine. Comparative Biochemical Physiological 110A, 4, 357-365.
25. Hall M. R., E. H. V. Ham, 1998. The effects of different types to stress on blood glucose in the giant tiger prawn *Penaeus monodon*. Journal of the World Aquaculture Society 29, 3, 290 - 299.
26. Kailola P., M. J. Williams, P. C. Stewart, R. E. Reichelt, A. McNee, C. Grieve, 1993. Australian Fisheries Resources. Bureau of Resource Science, Department of Primary Industries and Energy, and Fisheries Resource and Development Corporation - South Australian Research and Development Institute. Canberra Australia.
27. Kumlu M., O. T. Eroldogan, M. Aktas, 1999. The effect of salinity on larval growth, survival and development of *Penaeus semisulcatus* (Decapoda: Penaeidae). Israeli Journal of Aquaculture - Bamidgeh 51, 3, 114-121.
28. Kumlu M., O. T. Eroldogan, M. Aktas, 2000. Effect of temperature and salinity on larval growth, survival and development of *Penaeus semisulcatus*. Aquaculture 188, 1-2, 167-173.
29. Kumlu M., D. A. Jones, 1995. Salinity tolerance of hatchery-reared postlarvae of *Penaeus indicus* H. Milne Edwards originating from India. Aquaculture 130, 2-3, 287-296.
30. Lemaire P., E. Bernard, J. A. Martinez-Paz, L. Chin, 2002. Combined effect of temperature and salinity on osmoregulation of juvenile and subadult *Penaeus stylirostris*. Aquaculture 209, 307-317.
31. Lignot J. H., J. C. Cochard, G. Charmantier, 1998. Effects of fenitrothion on survival, osmoregulation and acetylcholinesterase activity of *Penaeus stylirostris* and *P. vannamei* (Crustacea, Decapoda). Aquatic Toxicology 41, 277-299.
32. Lignot J. H., J. C. Cochard, C. Soyez, P. Lemaire, G. Charmantier, 1999. Haemolymph osmolality according to nutritional status, moulting stage and body weight of *Penaeus stylirostris*. Aquaculture 170, 79-92.
33. Lignot J. H., C. Spanings-Pierrot, G. Charmantier, 2000. Osmoregulatory capacity as a tool in monitoring the physiological condition and the effect of stress in Crustaceans. Aquaculture 191, 209-245.
34. Lignot J. H., J. P. Triles, G. Charmantier, 1997. Effects of an organophosphorus insecticide, fenitrothion, on survival and osmoregulation of various developmental stages of the shrimp *Penaeus japonicus* (Crustacea: Decapoda). Marine Biology 128, 307-316.

35. Lin H. P., P. Thuet, J. P. Trilles, R. Mounet_Guillaume, G. Charmantier, 1993. Effect of ammonia on survival and osmoregulation at various developmental stages of the shrimp *Penaeus japonicus*. Marine Biology 117, 591-598.

36. Mantel L. H., L. L. Farmer (eds.), 1983. The Biology of Crustacean. Academic Press, N. Y./London/Paris.

37. Parado-Estepa F. D., J. A. Llobrera, R. Salde, 1993. Survival and metamorphosis of *Penaeus monodon* larvae at different salinity levels. Aquaculture 45, 1, 3-7.

38. Penn J. W., 1980. Spawning and Fecundity of the Western King Prawn, *Penaeus latisulcatus* Kishinouye, in Western Australia Water. Australian Journal of Marine and Freshwater Research 32, 21 - 35.

39. Pequeux A., 1995. Osmotic regulation in Crustaceans. Journal of Crustacean Biology 15, 1-60.

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

41. Racek A. A., W. Dall, 1965. Littoral Penaeinae (Crustacea: Decapoda) from Northern Australia, New Guinea and adjacent waters. Verh. K. Akad. Wel. Amsterdam, Afd. Natuukkd 56, 3, 1 - 119.

42. Rodriguez G. A., 1981. Osmoregulation and total serum protein of two species of penaeidean shrimp from Pacific coast of Mexico. Crustacean Biology 1, 3, 392 - 400.

43. Taylor H. H., F. M. Waldrom, 1997. Respiratory responses to air-exposure in the southern rock lobster, *Jasus edwardsii* (Hutton) (Decapoda: Palinuridae). Marine and Freshwater Research 48, 889-897.

44. Vargas-Albores F., J. L. Ochoa, 1992. Variation of pH, osmolality, sodium and potassium concentration in the haemolymph of sub-adult blue shrimp (*Penaeus stylirostris*) according to size. Comparative Biochemical Physiological 102A, 1, 1-5.

45. Williams A. B., 1960. The influence of temperature on osmotic regulation in two species of estuarine shrimp (Penaeus). Biology Bulletin 115, 560-571.