Muresk Institute

Division of Resources and Environment

# Growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn
# (*Penaeus latisulcatus* Kishinouye, 1896) reared at different salinities

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## Declaration

This thesis contains no material, which has been accepted for the award of any other degree or diploma in any university.

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

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## Abstract

The western king prawn (*Penaeus latisulcatus*) is one of the most economically valuable species of crustacean in Australia. The experiment was carried out for 60 days to determine the growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (2.95 ± 0.26 g mean initial weight) reared at 10, 22, 34 and 46 g/L of salinities. In addition, haemolymph osmolality and osmoregulatory capacity (OC) of the western king prawn (5.37 ± 0.1 g mean initial weight) reared at salinities (10, 22, 34 and 46 g/L) were determined following 7, 14 and 21 minutes of air exposure and compared with the brown tiger prawn (*P. esculentus*). Mean final weight, total length, carapace length and specific growth rate (SGR) of the western king prawn were highest at a salinity of 34 g/L. Moult increments (in weight and total length) of the western king prawn were not significantly different (P > 0.05) when reared at four different salinities. Food conversion ratios were lowest in prawns reared at salinities of 22 and 34 g/L. Survival of the western king prawn was highest at a salinity of 22 g/L and lowest at a salinity of 10 g/L. Haemolymph osmolality of the western king prawn increased with an increase in salinity and weight. Isosmotic points of the western king prawn calculated from regression lines between haemolymph and medium osmolality were 28.87, 29.46 and 31.73 g/L at 0, 20 and 60 days of rearing (accordingly to 2.95 ± 0.26; 4.02 ± 0.47; 5.79 ± 0.64 g body weight), respectively. Tail moisture content of the western king prawn decreased with the increase of salinity. After 60 days of rearing, the lowest hepatopancreas moisture content of the prawns was at a salinity of 22 g/L. Wet weight and dry weight hepatosomatic indices of the prawns were highest when reared at a salinity of 22 g/L. Wet weight and dry weight tail muscle indices of the prawns were highest at a salinity of 34 g/L. Isosmotic points of the western king prawn were 33.79; 33.29; 32.75 and 33.10 g/L at 0, 7, 14, and 21 minutes of air exposure, respectively. Isosmotic points of the brown tiger prawn were 30.89; 31.89; 32.09 and 31.07 g/L at 0, 7, 14, and 21 minutes of air exposure, respectively. Air exposure reduced OC of both the western king prawn and brown tiger prawn. OC of both species at a salinity of 10 g/L was reduced significantly after 14 minutes of air exposure. Twenty-one minutes of air exposure did not change OC of the western king prawn reared at salinities of 22, 34 and 46 g/L. OC of brown tiger prawn reared at 22 g/L decreased after 21 minutes of air exposure while OC of the brown tiger

prawn reared at 46 g/L decreased after 7 minutes of air exposure. The results indicate that both species spent less energy on osmoregulation at 34 g/L salinity than at other salinities.

The results suggest that the optimum salinity for rearing of western king prawns ranges from 22 g/L to 34 g/L. Salinities of 10 and 46 g/L are unsuitable for rearing brown tiger prawns and salinity 10 g/L is unsuitable for rearing western king prawns. Furthermore, a salinity range from 30 g/L to 32 g/L is suitable for the culture of brown tiger prawns.

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# 1. PREAMBLE

Salinity is one of the most important factors concerned in culture of marine organisms generally and penaeid prawns especially. The purpose of this report is to present the results of study on the effects of salinity on growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (*P. latisulcatus*) reared at different salinities.

This report comprises seven main sections. The first is the introduction, which includes the rationale, aims and objectives of the research. The second section is the literature review, which mentions about systematics, distribution and general biology of a typical penaeid prawn. While there is no published information on the effects of salinity on growth, survival, moult, food conversion ratio, tissue water, haemolymph osmolality and osmoregulatory capacity of the western king prawn, studies carried out on similar species such as the giant tiger prawn (*P. monodon*), green tiger prawn (*P. semisulcatus*), fleshy prawn (*P. chinensis*) and other penaeid species are highlighted in the literature review. Some of the studies on freshwater crayfish on organosomatic indices are also cited. The third section describes the materials and methods applied in the study. The fourth section presents results from the study. The fifth section is the discussion, in which explanations of the results and comparisons with results of previous studies are given. An additional experiment was carried out in order to compare the effects of air exposure on osmoregulatory capacity of the western king prawn another penaeid species, the brown tiger prawn (*P. esculentus*). This experiment is presented in section six. The seventh section concludes the main findings of the study and offers recommendation on future research. Two manuscripts from this report have been submitted to Applied Aquaculture and Aquaculture for publication. The titles are attached in Appendix 14.

## 2. INTRODUCTION

### 2.1 Rationale for research

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 prawns, salinity is one of the most important environmental parameters affecting the survival, growth and metabolism. Change in ambient salinity may disrupt the osmotic balance in penaeid prawns. This results in prawns having to use a considerable amount of energy to readjust their osmotic balance (Chen et al. 1996). The effects of salinity on the growth and survival of several species of penaeid prawns have been examined by a large number of researchers and reviewed by Dall et al. (1990). Different penaeid species have different ranges of salinity tolerance (Dall et al. 1990). A salinity range of 15-25 g/L is considered to be the optimal range for giant tiger prawn (P. monodon) culture (Ferraris et al. 1986a; Chen et al. 1995). The fastest growth of juvenile fleshy prawns (P. chinensis) is achieved over the salinity range 20 – 30 g/L at 30 °C (Chen et al. 1995; Zhang et al. 1999). The highest survival of juvenile whiteleg prawns (Litopenaeus vannamei) is at salinities above 20 g/L (Ponce-Palafor et al. 1997). The fastest growth of brown tiger prawns (P. esculentus) is at 30 g/L salinity (O' Brien 1994).

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, tissue water and osmoregulatory capacity of penaeid prawns have been widely studied. Haemolymph osmolality linearly increases with increasing salinity (Cheng & Liao 1986; Ferraris et al. 1986a; Ferraris et al. 1986b; Charmantier-Daures et al. 1988; Diwan at el. 1989; Allan & Maguire 1992; Chen et al. 1995; Chen & Lin 1998). Lignot et al. (2000) in a review noted that different species of penaeid prawns have different osmoregulatory capacities

responding to stress factors such as fuel oil, copper, low pH, starvation and ammonia. The OC also changes with size, nutritional condition and developmental stages.

While research has been conducted into the culture of several penaeid species, there has been limited research into the aquaculture of western king prawns (*P. latisulcatus*) although their broad rearing parameters have been established (Kathirvel *et al.* 1986). This species has a wide distribution throughout the Indo-West Pacific region (Racek & Dall 1965; Penn 1980; Dore & Frimodt 1987) and found around most of coastal Australia (Racek & Dall 1965; Penn 1980; Dore & Frimodt 1987; Kailola *et al.* 1993). The wild-caught western king prawn is mainly sold in the prawn markets of Australia and Asia (Andrew & Bowen 1992). An important step in assessing the suitability of the western king prawn for sustainable aquaculture (including artificial breeding, grow out, post harvest technique) is to investigate the optimum salinity for their growth and survival. However, there are no published data on the effects of different salinities on growth and survival of the western king prawn prawns and underlying physiological mechanism.

## 2.2 Aim and objectives

### 2.2.1 Aim

To determine the growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn reared at different salinities. The above aim is achieved by meeting following objectives.

### 2.2.2 Objectives

To determine:

- weight, total length and carapace length, specific growth rate, moult increment, moult interval; food conversion ratio (FCR) and survival of western king prawns reared at salinities of 10, 22, 34 and 46 g/L.
- haemolymph osmolality and isosmotic range of western king prawns reared at salinities of 10, 22, 34 and 46 g/L.
- organosomatic indices (hepatosomatic and tail muscle indices) of western king prawns reared at salinities of 10, 22, 34 and 46 g/L.

# 3. LITERATURE REVIEW

## 3.1 Systematics

Two Suborders, the Dendrobranchiata and the Pleocyemata comprise the Order Decapoda. Dendrobranchiata is comprised of two Superfamilies: Penaeoidea (which includes the family Penaeidae) and Sergestoidea. The most commercially important marine species, including those most commonly used for aquaculture, belong to the family Penaeidae (Allan 1993). The systematic classification, following Bowman & Abele (1982), of the western king prawn is as follows:

Suprageneric – Arthropoda

Phylum, Subphylum or Superclass Crustacea

Class Malacostraca

Subclass Eucarida

Order Decapoda

Suborder Dendrobrachiata

Superfamily Penaeoidea

Family Penaeidae

Genus Penaeus

Species *Penaeus latisulcatus* Kishinouye, 1896

## 3.2 Distribution

Western king prawns are widely distributed in the Indo-West Pacific region. They are found from Southeast Africa north to the Red Sea, in the Persian Gulf, around India, Southeast Asia and throughout the Malay Archipelago to Japan and Korea (Racek & Dall 1965; Penn 1980; Dore & Frimodt 1987). In Australia, western king prawns are found around most of coastal areas except New South Wales and Victoria (Racek & Dall 1965; Penn 1980; Dore & Frimodt 1987; Kailola *et al.* 1993). This prawn lives on hard bottoms of sand, sandy mud or gravel and prefers shallower marine water down to about 90 m (Dore & Frimodt 1987). The distribution of the western king prawn is affected by sediment type (Somer 1987).

## 3.3 General biology

The life cycle of the western king prawn comprises two stages: an oceanic adult stage and an inshore juvenile stage (Kangas 1999). It inhabits hypersaline environments at certain stages of its life history. Spawning females, however, have only been found in water of oceanic salinities (35 g/L) (Penn 1980; Rothlisberg & Jackson 1987).

The general reproductive process of the western king prawn is the same as in other common closed thelycum penaeid species (Penn 1980; Dall et al. 1990). The male transfers its sperm capsule (spermatophore) into the female thelycum using his petasma. For successful insertion, the female needs to have moulted recently so that the plates of the thelycum are open. When mature the eggs are released into the water column. At this time, sperm and eggs are fertilised (Penn 1980; Dall et al. 1990; Kangas 1999). The larvae pass through six nauplial and six protozoel substages before moulting to the megalopal stages with functional pleopods (Shokita 1984).

Microscopically and macroscopically, reproductive organ development can be determined when the carapace length (CL) of the western king prawn is longer than 12 mm. The joining of the petasma occurs at about 23 mm CL in males whereas females develop a completed thelycum at the CL size of about 25 mm. Mated females are rarely observed at a size below 28 mm CL (Penn 1980). The smallest females of the western king prawn found inseminated are 27 mm CL and the frequency of inseminated females increases up to 42 mm CL. Above this size, almost all of the females are inseminated (Penn 1980; Courtney & Dredge 1988). In the Gulf of Thailand, female western king prawns are found sexually mature primarily in February and March. The average size for sexual maturity of females is 16.21 cm CL (Roongratri 1993). In the Gulf of Suez, Egypt, the highest abundance of ripe female western king prawns ready for spawning is observed from October to March. The new recruits occur at the end of the fishing months (April-May). The smallest mature female western king prawns in the Gulf are about 10.8 cm in length and this is reached in the first year of life. The ripe female western king prawns in the Gulf of Suez show wide range of ova diameter distribution from 175 to 625 µm. During the period from October to December, a high number of ripe females with ova diameter ranged from 350 to 400 µm is observed. The number of eggs produced is strongly

correlated with the total body length and is the best predictor of fecundity of western king prawns in the Suez Gulf waters (Abdel *et al.* 1996). In Queensland's East Coast, Australia, western king prawns have a year-round spawning with the peak months from autumn to spring (Penn 1980; Courtney & Dredge 1988).

Fecundity of female western king prawns varies significantly with the size of the individual. The number of eggs released at each spawning ranges from 105,000 (123 mm total length (TL) female) to 650,000 (217 mm TL female) (Penn 1980; Kangas 1999).

Salinity and distance from the mouth of the Peel-Harvey Estuary in Western Australia exerts a marked influence over the distribution and abundance of prawns within the estuary. The extent of the preferred sandy substratum is also assumed to affect western king prawn abundance (Potter & Manning 1991). By using eye – and time dependent seasonal growth models in analysis of tag/recapture data in Gulf St. Vincent, South Australia, the results show that in nature, western king prawns exhibit a strong seasonality in growth, with the males growth cycle lagging two weeks behind that of females. The carapace length of males grows fastest two weeks after the start of autumn, shows no growth two weeks before the start of spring, shrinks until the middle spring and thenresumes its positive growth for another cycle, whereas the CL of females grows continuously throughout the year but at a slower rate in certain months (Xiao & MacShane 2000).

Larvae of western king prawns are found in water with a temperature range of $20.5^0$ - $30.6^0$ C and a salinity range of 28.6 – 34.9 g/L. This temperature and salinity range may be the suitable range for reproduction as well as growth and survival of larvae (Rothlisberg & Jackson 1987). Nursery areas for western king prawns are intertidal sand/mudflats and there is little daily variation of prawn abundance and distribution within one site. Large differences in abundance of juvenile prawns are observed throughout Gulf St. Vincent, South Australia with the highest prawn concentration in the northern part of the Gulf (Kangas & Jackson 1998).

## 3.4 Salinity

Different species of penaeid prawns, or even different populations of the same species adapted to different salinity regimes, have different salinity tolerances (Rao 1958). The optimum salinity for culture would be close to isosmotic condition so that animals would need to expend the least possible energy maintaining osmotic balance, and the oxygen requirement would also be low (Panikkar 1968). Juvenile stages of penaeid prawns are more tolerant to adverse salinities than adult stages (Charmantier *et al.* 1988). Important salinity affects on penaeid prawns as follows:

### 3.4.1 Growth and survival

Studies of the giant tiger prawn (*P. monodon*) show that a sudden change of salinity beyond their optimum range results in a poor osmotic response and mortality (Ferraris *et al.* 1986a). A salinity range of 15-25 g/L is considered to be optimal for giant tiger prawn culture (Chen 1985). This species could be acclimatised and cultured in low salinity water bodies with maintenance of strict environmental regulations for sustainable yield (Wanninayake *et al.* 2001). A salinity range from 9 to 20 g/L does not influence growth rate of the giant tiger prawn and large fluctuations at salinities from 4 to 20 g/L are not a major factor influencing their survival (Navas & Sebastian 1989). The highest mean hatching rate of eggs of the giant tiger prawn is obtained at the temperature – salinity combination of 23 $^0$C – 33 g/L, followed by 28 °C-33 g/L and 33 °C-33 g/L. Survival rate of nauplius to first protozoel stage of the giant tiger prawn is highest at 28°C-33 g/L, followed by 33 °C-33 g/L and 23 °C-33 g/L with moulting time of 50, 45 and 75 hours, respectively (Reyes 1984). The nauplius stage has a significantly higher survival rate at 32 – 36 g/L than at 28 g/L salinity (Reyes 1984; Parado-Estepa *et al.* 1993). At protozoel stages, similar survival and metamorphosis of the giant tiger prawn is obtained at salinity range from 28 to 40 g/L. Within the salinity range of 20 to 36 g/L, the mysis of the giant tiger prawn exhibits similar survival rates but metamorphosis is significantly faster at 28 g/L and 32 g/L (Parado-Estepa *et al.* 1993). Fifteen-day old postlarvae (PL15) cannot survive at 0 g/L salinity but higher salinity increases the survival rate (Tangtrongpiros *et al.* 1999). For a direct change of salinity in the range of 3 to 30 g/L, survival rate of post-larvae varies between 76% and 100%. With sudden change of salinity in the range of 1 g/L to 28 g/L, survival of juveniles varies between 2.5% to 100% with the prawns being able to survive and to grow well in

over 3 g/L salinity sea water (Zhang *et al.* 1989). Giant tiger prawns can grow satisfactorily in low salinity ponds (salinity < 6 g/L) even when the salinity is decreased after 60 days of culture to almost freshwater level (0.16 g/L) indicating the variability of semi-intensive culture of this species in a low salinity environment (Saha *et al.* 1999).

For the green tiger prawn (*P. semisulcatus*), the protozoel larvae require an acclimatisation rate of 1 g/L per 15 min when exposed to a salinity change of over 5 g/L. The larvae display better tolerance to high rather than low salinities. The lowest and highest critical salinities appear to be 23 g/L and 55 g/L, respectively. Early protozoel larvae show a high tolerance to hypersalinities but only for a few days. Larval growth and development are better in a range of salinity from 25 to 40 g/L. The optimal salinity for larval culture of the green tiger prawn has been estimated to lie between 30 and 35 g/L (Kumlu *et al.* 1999). Salinity affects several characters in protozoea I (Z1) including carapace length and a section of the first antenna (Jackson 1994). The optimum temperature and salinity combination for the culture of the green tiger prawn is 30 g/L and 30 °C (Kumlu *et al.* 2000).

The optimum growth of the fleshy prawn (*P. chinensis*) juveniles is achieved over the range 20 – 30 g/L at 30 °C (Chen *et al.* 1996; Zhang *et al.* 1999). There is no difference in growth of fleshy prawn juvenile among four different salinities (10, 20, 30 and 40 g/L) (Chen *et al.* 1995). Postlarvae of fleshy prawns can live in fresh water for several days if the acclimatisation rate is 3% of salinity decrease per day (Ma *et al.* 1999). The hatching rate of fleshy prawn eggs increases with an increase in salinity within the range of 20 – 31 g/L with the highest rate occurring at 31 g/L (Hur & Kim 1996). Survival rate of fleshy prawns are 75%, 88% and 76% for the prawns reared at 20, 25 and 30 g/L after 60 days, respectively. Moulting frequencies are the highest for prawns reared at 20 g/L salinity and the lowest for prawns reared at 5 and 40 g/L salinity (Chen *et al.* 1992).

Normal eggs and healthy nauplii of the banana prawn (*P. merguiensis*) are produced when spawning and egg incubation is carried out in water salinities of greater than 20 g/L. Larvae reared at a salinity of 15 g/L result in total mortality (Choo 1987). The optimum survival and growth of banana prawn larvae in ponds is achieved at

salinities above 15 g/L (Ruttanagosrigit & Musig 1982). Salinities of 5 g/L and 10 g/L are lethal and result in the mortality of mysis stage 3 (M3) larvae of banana prawns (Prasad *et al.* 1988). Survival of banana prawn juveniles is low at a salinity of less than 15 g/L (Prasad *et al.* 1991; Saldanha & Achuthankutty 2000). The weight of the banana prawn progressively increases with an increase of salinity up to 40 g/L (Saldanha & Achuthankutty 2000). When rearing banana prawns at different combinations of temperature and salinity, Stapes & Heales (1991) concluded that the juveniles of the banana prawn show the highest survival as well as greatest increase in wet and dry weight, protein, fat and energy value at 20 $^0$C and 20 g/L salinity. The optimum temperature and salinity for growth in length are 31 °C and 30 g/L. The optimum temperature and salinity combination for the greatest increase in biomass and production are 28 °C and 25 g/L.

The optimum survival of the whiteleg prawn (*L. vannamei*) juvenile is achieved between temperatures of 20 and 30°C and salinities above 20 g/L. The fastest growth is obtained between temperatures of 25 and 35°C with little difference among salinities (Ponce-Palafor *et al.* 1997). Juveniles of the whiteleg prawn can obtain a high survival rate (98%– 100%) in a culture salinity of 2, 4 and 8 g/L for 70 days in a semi-closed recirculating system. This demonstrates that whiteleg prawns can be cultured in low salinity water with good growth and survival (Samocha *et al.* 1998). The highest growth in weight of the whiteleg prawn is obtained at salinity of 5 and 15 g/L (Bray *et al.* 1994). PL22 of whiteleg prawns being transferred directly from seawater into lower salinities of 16, 8, 4 and 2 g/L tolerate the change better than PL8 (Ogle *et al.* 1988).

The optimum growth of the brown tiger prawn (*P. esculentus*) is achieved at 30 °C and 30 g/L salinity (O' Brien 1994). Salinity below 20 g/L is essential for faster growth and better survival of the young of the northern brown prawn (*P. aztecus*). Postlarvae of the northern brown prawn have a higher salinity tolerance than the juveniles. The best growth and survival rate for the young of the northern brown prawn are in the salinities of 8.5 and 17 g/L (Venkataramaiah *et al.* 1973). The optimum salinity for the adaptation of white prawn postlarvae, *P. setiferus*, to water with low oxygen concentration is between 5 and 15 g/L and is 25 g/L for white prawn *P. schimitti* postlarvae (Rosas *et al.* 1997). Low temperature (10-14°C)

decreases the tolerance of the kuruma prawn (*P. japonicus*) postlarvae to low salinities. This influence is less important in the fleshy prawn (*P. chinensis*) postlarvae. Mortality of the kuruma prawn postlarvae is the lowest when the medium is isosmotic to haemolymph (Charmantier-Daures *et al.* 1988). Survival of the golden prawn (*P. californiensis*) larvae is not significantly different at salinities of 30, 33, and 36 g/L and there is also no significant difference in the time taken to reach larval sub-stages at these different salinities (Porchas *et al.* 2000). Growth and survival of the golden prawn postlarvae is better when the prawns are reared at a salinity that is the same as the salinity of the larvae-culture than those nursed at a higher salinity. The best growth and survival during the nursing of the golden prawn postlarvae is obtained at a salinity of 33 g/L (Martinez *et al.* 1996). Culture salinity affects final total length and organosomatic indices (lipid composition) but does not have any effect on survival of the caramote prawn (*P. kerathurus*) postlarvae (Mourente & Rodriguez 1997). Temperature and salinity show a marked influence on the incubation period and nauplius development of the redtail prawn (*P. penicillatus*). Redtail prawn nauplii can develop into the protozoel stage when the salinity ranges from 23 g/L to 35g/L. At a salinity of more than 40 g/L, the nauplii can develop into the protozoel stage but they are unable to swim and their survival rate is low (Shi 1981).

The survival rate of the Indian white prawn (*P. indicus*) postlarvae is the highest at a salinity of around 16 g/L and the lowest at a salinity of more than 50 g/L. However, maximum weight increments and protein conversion efficiency are recorded at a salinity of 33 g/L. The most suitable salinity to grow Indian white prawn postlarvae is from 16.48 g/L to normal seawater (35 g/L) (Vijayyakumaran 1999). The Indian white prawn postlarvae display the best performance in growth, survival and total biomass increments at salinities between 20 – 30 g/L from PL20 to PL60. While the postlarvae up to 60 have gained resistance to high salinity (40 – 50 g/L), low salinity (10 g/L) is lethal in the PL45 stage of the Indian white prawn (Kumlu & Jones 1995). Rapid development of the embryo and the maximum hatching of Indian white prawn eggs are obtained when the temperature and salinity are maintained at 28 – 30 °C and 28 – 30 g/L, respectively (Hossain *et al.* 1991). During transportation, the salinity of 20 – 25 g/L is considered to be the best condition for the Indian white prawn postlarvae (Sakikumar & Vadhyar 1996).

### 3.4.2 Moult

Temperature, salinity and pH significantly influence the moulting and growth of early juveniles of the Indian white prawn (*P. indicus*). Temperature, salinity and pH of 31°C, 15 g/L and 8 ± 0.2 are considered to be the optimal levels for fast growth with highest growth increments. Outside the optimum level, increased moult frequency does not produce more gains in weight or length (Vijayan & Diwan 1995). Moulting frequency of the fleshy prawn (*P. chinensis*) juveniles is lowest for the prawns reared at 20 g/L and highest for the prawns reared at 5 and 40 g/L (Chen *et al.* 1992). Salinity had no effect on the duration or time of the moult cycle of the giant tiger prawn (*P. monodon*) (Parado-Estepa *et al.* 1989).

### 3.4.3 Food conversion ratio (FCR)

The highest net energy conversion efficiency of the Indian white prawn (*P. indicus*) postlarvae is obtained when fed with clam in 33.05 g/L salinity. Protein efficiency ratio varies significantly between salinities of 16.48, 33.05 and 44.90 g/L (Vijayyakumaran 1999). In the giant tiger prawn (*P. monodon*) both the food conversion ratio (FCR) is higher in low salinity (0.16 – 6.25 g/L) than in higher salinity (4.60-19.42 g/L) (Saha *et al.* 1999). The maximum carbon intake of the fleshy prawn is obtained at a salinity of 13 g/L, but at a salinity of 20 g/L the carbon conversion efficiency is the highest. The mechanism of salinity effect on carbon growth of the prawns fed with polychaete worm is significantly larger than prawns fed with a formulated diet. Their carbon conversion efficiency was 26.80% and 13.78% of carbon intake, respectively (Zhang *et al.* 1999). A salinity of 20 g/L is considered to be the optimum salinity at which the highest food consumption and production of the banana prawn (*P. merguiensis*) juvenile is obtained. The optimum FCR and protein efficiency ratio (PER) of banana prawns are also recorded in this salinity. At higher salinities, the banana prawn FCR is poor and there is a considerable decrease in growth and food consumption (Vinod *et al.* 1996).

### 3.4.4 Haemolymph osmolality and tissue water

The renal organs of crustaceans are considered to be responsible for maintaining the balance of haemolymph osmolality. This organ comprises the maxillary (shell) glands and the antennal (green) glands. These pairs of glands are said to be the

remnants of a segmental excretory system. In general, the glands have three principal parts, an internal end-sac, an excretory tubule, and an exit duct, which sometimes enlarges into a bladder. Marine crustaceans are more or less isosmotic with their medium, however, they show a considerable degree of ionic regulation. Sodium concentration is usually a little higher in the haemolymph than in seawater, and this may result in low concentrations of other cations. In lobsters, magnesium concentration is 14% of the seawater magnesium concentration; the sodium is considerably higher (111% of the seawater concentration). Calcium concentration in the haemolymph of most crustaceans is often considerably higher than in seawater and varies with the stages in moulting cycle.

Penaeid prawns are hyper-osmotic regulators at the medium osmolality lower and hypo-osmotic regulators at the medium osmolality higher than their haemolymph osmolality (Charmantier *et al.* 1988). For hyper-osmoregulating crustaceans, the gills are the primary sites for both passive and active uptake of salts (Mantel & Farmer 1983). For hypo-osmoregulating crustaceans (e.g. *Metapenaeus bennettae*), water uptake and salt excretion takes place in the gut and gills are not responsible for major salt excretion. Free amino acids are important osmotic effectors in crustaceans and, as they also contribute to the flavour of seafood, fluctuation at salinity concentrations can affect the taste of aquaculture products (Dall 1967).

The giant tiger prawn (*P. monodon*) is an osmoconformer in the salinity range of from 15 to 35 g/L. The salinity at which haemolymph is isomotic to the ambient seawater is 23 – 25 g/L (Cawthorne *et al.* 1983). For the giant tiger prawn, a duration of 48 hours is considered to be essential to adjust to the new medium (Diwan *et al.* 1989). Osmotic and chloride concentrations in the haemolymph of the giant tiger prawn vary with both the moult stage and salinity of the medium (Ferraris *et al.* 1987). Giant tiger prawn juveniles are stronger in osmotic and ionic regulation than adults. The salinity range over which osmoregulation is performed efficiently is 3 – 50 g/L for juveniles and 15 – 50 g/L for adults (Cheng & Liao 1986). Haemolymph osmoregulation of the giant tiger prawn is worse at salinity above 35 g/L (Allan & Maguire 1992). Active secretion of magnesium by the antennal gland enables the giant tiger prawn to maintain hypoionic levels of magnesium in the haemolymph (Lin *et al.* 2000). Haemolymph osmolality of the fleshy prawn (*P. chinensis*)

increases with an increase in salinity, and decreases with an increase in temperature. Tissue water of the fleshy prawn decreases with an increase in both the medium and haemolymph osmolality (Chen *et al.* 1995; Chen & Lin 1998). Haemolymph urea of the kuruma prawn (*P. japonicus*) increases with an increase in salinity, and haemolymph oxyhemocyanin decreases with an increase in salinity (Chen & Chen 1996). The salinity ranges over which osmoregulation is performed efficiently are 5-30 g/L for the redtail prawn (*P. penicillatus*) juvenile and 15-30 g/L for the adult (Cheng & Liao 1986).

Green tiger prawn (*P. semisulcatus*) intermoult adults have been shown to be a relatively poor osmoregulator. They respond to 18 g/L salinity with a fall in respiration rate and mortality. They have a narrow optimal survival range of salinity tolerance (Clark 1992).

Haemolymph osmolality of the fleshy prawn (*P. chinensis*) has a linear relationship (slope = 0.189) with external osmolality over the range 5 to 40 g/L of salinity. Fleshy prawn juveniles grow fastest in the isosmotic environment or slightly higher, those in the hyperosmotic environment grow at the second fastest rate and those in the hypoosmotic environment grow at the lowest rate (Chen *et al.* 1992). Haemolymph osmolality of the fleshy prawn is inversely related to temperature and tissue water is inversely related to salinity. Tissue water decreases with an increase in the medium osmolality, and decreases with an increase in haemolymph osmolality (Chen & Lin 1994b; Chen *et al.* 1995; Chen & Lin 1998). Isosmotic points at which the haemolymph osmolality equals the medium osmolality of some penaeid prawns are reviewed by Chen & Lin (1998) and are presented in Table 1.

### 3.5 Osmoregulatory capacity and stress of penaeid prawns

Osmoregulatory capacity (OC) is used as a tool in monitoring the physiological condition and the effect of stress in crustaceans. OC is defined as the difference between osmolality of haemolymph and the external medium, at a given salinity (Charmantier-Daures *et al.* 1988; Lignot *et al.* 2000). The measurement of haemolymph osmolality to evaluate OC has been successful in numerous crustaceans from small isopod and gammarid species (Charmantier 1975; Lignot *et al.* 2000) to penaeid prawns, lobsters and crabs (Castille & Lawrence 1981; Dall 1981; Ferraris *et*

*al.* 1987; Lignot *et al.* 2000; Lin *et al.* 2000). When the evaluation of OC requires a change in salinity, animals must be kept in the new medium until full osmotic equilibration (Charmantier-Daures *et al.* 1988; Lignot *et al.* 2000). OC also changes with size, nutritional condition and developmental stages. Therefore, only starved animals at the same size and developmental stage, and at intermoult stages must be used for any trial (Lignot *et al.* 2000). The effects of different stress factors on OC of penaeid prawns and reviewed by Lignot *et al.* (2000) and are presented in Table 2.

**Table 1.** Isosmotic point of some penaeid prawns from Chen & Lin 1998.

| Species | Size(g) | Slope | Isosmotic points | | Reference |
| --- | --- | --- | --- | --- | --- |
| | | | mOsm/kg | g/L | |
| *Penaeus aztecus* | | | | | |
| | - | 0.26 | 712 | 25.6 | (Bishop *et al.* 1980) |
| | - | 0.38 | 745 | 25.6 | (Castille & Lawrence 1981) |
| | - | 0.40 | 715 | 25.7 | (Howe *et al.* 1982) |
| *P. chinensis* | | | | | |
| | 12.7 | 0.39 | 707 | 25.0 | (Chen & Lin 1994a) |
| | PL20-PL60 | | 700 | | (Charmantier-Daures *et al.* 1988) |
| | Adults | | 780 | | |
| *P. duorarum* | | | | | |
| | - | 0.32 | 855 | - | (Williams 1960) |
| | - | 0.40 | 768 | 26.3 | (Castille & Lawrence 1981) |
| *P. esculentus* | | | | | |
| | 31.8 | - | - | 30.0 | (Dall 1981) |
| *P. indicus* | | | | | |
| | 5-10 | 0.24 | 780 | 26.0 | (Parado-Estepa *et al.* 1987) |
| *P. japonicus* | | | | | |
| | PL20-PL60 | | 820-830 | | |
| | Adults | | 880 | | (Charmantier-Daures *et al.* 1988) |
| *P. merguiensis* | | | | | |
| | 21.2 | - | - | 27.0 | (Dall 1981) |
| *P. monodon* | | | | | |
| | Pl | 0.28 | 780 | 23-25 | (Cawthorne *et al.* 1983) |
| | 10 | 0.26 | 698 | 23.7 | (Ferraris *et al.* 1986b) |
| | 30 | 0.31 | 752 | 25.6 | |
| | Juvenile | | 730 | | (Cheng & Liao 1986) |
| | Adults | | 750 | | |
| | Adults | | 770 | 26.5 | (Fang *et al.* 1992) |

**Table 1.** (Continued)

| Species | Size(g) | Slope | Isosmotic points | | Reference |
|---------|---------|-------|------------------|-------|-----------|
| | | | mOsm/kg | g/L | |
| *P. plebejus* | | | | | |
| | 28.8 | - | - | 30.0 | (Dall 1981) |
| *P. penicillatus* | | | | | |
| | Juvenile | | 747 | | (Cheng & Liao 1986) |
| | Adults | | 800 | | |
| *P. semisulcatus* | | | | | |
| | 20-70 | 0.73 | 930 | 33.0 | (Clark 1992) |
| *P. setiferus* | | | | | |
| | - | 0.27 | 824 | 26.8 | (Williams 1960) |
| | - | 0.23 | 680 | 23.3 | (Castille & Lawrence 1981) |
| *P. stylirostris* | | | | | |
| | - | 0.16 | 699 | 24.0 | (Castille & Lawrence 1981) |
| *L. vannamei* | | | | | |
| | - | 0.21 | 718 | 24.7 | (Castille & Lawrence 1981) |
| *Metapenaeus bennettae* | | | | | |
| | 20.4 | - | - | 23.0 | (Dall 1981) |
| *M. ensis* | | | | | |
| | - | - | 700 | 22.0 | (Chen & Fang 1986) |

**Table 2.** The effect of different stress factors on osmoregulatory capacity (OC), osmoregulation (OR) and ionic regulation of different species of penaeid prawns

| Stress | Species | Dose | Effect | Reference |
|---|---|---|---|---|
| Fuel oil | *Penaeus aztecus* | 20% solution of water soluble fraction | No effect on OR at 10 and 30 g/L. | (Anderson *et al.* 1974) |
| Fenitrothion | *P. japonicus* (Adult) | 0.25, 0.5 µg/l | Decreases OR by 58% in seawater | (Lignot *et al.* 1997) |
| | *P. japonicus* (juveniles) | 0.5, 0.75 µg/l | Time- and dose-dependent decreases OC up to 62% at 37 g/L and 18% at 19 g/L. | (Lignot *et al.* 1997) |
| | *P. stylirostris* (juvenile) | 6,8,10,12 µg/l | Time- and dose-dependent decreases OC up to 22% at 36 g/L | (Lignot *et al.* 1998) |
| Copper | *P. japonicus* | 0.5-1.5 mg/l | Time- and dose-dependent decreases OC up to 100% at 37 g/L and up to 33% at 17 g/L | (Bambang *et al.* 1995) |
| Ammonia | *P. japonicus* (juveniles) | 16, 48 mg/l  32, 38, 64 mg/l | Dose- dependent decreases OC up to 100% at 36 g/L.  Dose- dependent decreases OC up to 40% at 15 g/L | (Lin *et al.* 1993) |
| Low Ph | *P. monodon* (juveniles) | pH 5.6 | Decreases OC by 15% at 30 g/L, and 7% at 15 g/L | (Allan & Maguire 1992) |
| Starvation | *P. stylirostris* (juveniles) | 24 h | Decreases OR by 6% in seawater | (Lignot *et al.* 1999) |

## 3.6 Organosomatic indices as indicators of crustacean condition

### 3.6.1 Role of hepatopancreas in crustacean metabolism

The hepatopancreas is located in the cephalothoraxes of crustaceans, occupying most of its space, above the midgut. It is a trilobed structure with two lobes projecting forward and the third to the rear of the carapace. The organ is in close contact with the haemolymph and is involved in digestive enzyme (proteinases, peptidases, lipases and amylases) synthesis and secretion, lipid and carbohydrate metabolism, and storage of calcium and some heavy metals. The hepatopancreas is connected to the digestive tract with tubes guiding the nutrients in and enzymes out of the organ. The tubes open to the midgut, and the nutrients, after they have been carefully processed and converted into fine particulate matter in the complex gastric system, are actively transported into the hepatopancreas by co-ordinated contraction of the muscle network investing the hepatopancreas (Holdich & Reeve 1988). The nutrients, entering the tubules of the hepatopancreas, are processed by the R, F or B cells. R cells absorb luminal nutrients, store and metabolize lipids and glycogen and absorb heavy metals from the haemolymph, F cells synthesize enzymes and pack them into vacuoles and absorb heavy metals before they enter the haemolymph, absorb luminal nutrients and may even develop into B cells, while B cells are the storage and secretion bodies of the digestive enzymes. E cells are earlier developmental stages of R or F cells (Dall & Moriarty 1983).

The hepatopancreas acts as the main storage site of lipids in crustaceans and lipid concentrations of up to 92% of dry mass have been measured in the hepatopancreas of crayfish (Suprunovich *et al.* 1983). The marron (*Cherax tenuimanus*) hepatopancreas can act as a detoxifying organ for DDT, in feed (Francesconi *et al.* 1995). The hepatopancreas serves as a calcium storage site in some crustaceans to allow rapid initial postmoult calcification (Sardà *et al.* 1989).

### 3.6.2 Crustacean condition

Hepatosomatic indices could be used to evaluate the condition of the crayfish. Other parameters from the environment, i.e. population density, food resources, water quality, etc., have to be taken into account when the results are interpreted (Mannonen & Henttonen 1995). Jussila & Mannonen (1996), Jussila (1997) studied the effects of nutrients on organosomatic indices of crayfish and suggested that the combination of growth and organosomatic indices could give a more reliable basis for the overall condition or performance estimates than either of the indices alone. Therefore, a high hepatosomatic index and low hepatopancreas moisture content in crayfish was necessary but not solely, a condition for fast growth. Furthermore, good condition in crayfish can be taken as a key marginal prerequisite for fast growth. Crayfish condition, when estimated from hepatopancreatic indices, has to be evaluated with caution. Previous studies on the wild populations of the co-species have to be examined, and any differences from what is observed in wild populations should be considered carefully. Also, parameters in the experimental environment, and in the crayfish itself (growth components, mineralization, stress indicators, etc.), have to be considered together with the hepatopancreatic indices to achieve the widest possible support for the interpretations.

Noble crayfish (*Astacus astacus*) had hepatopancreas moisture contents ranging from 70.4 to 75.7% in females and from 74.0 to 81.8% in males. The relationship between marron (*C. tenuimanus*) and noble crayfish hepatopancreas moisture content and total energy content is linear. This gives an opportunity to estimate the total energy reserves of a crayfish by measuring its hepatopancreas moisture content (Jussila 1997). The availability of supplemental feed and population density both affected the hepatopancreas moisture content and size in the red swamp crayfish (*Procambarus clarkii*) (McClain 1995). Prolonged starvation decreased hepatosomatic ratios in marron (Evans *et al.* 1992). When culturing the marron and feeding with diet containing 20% protein (D1), D1 mix with cod liver oil (D2), D1 mix with sunflower oil (D3) and D1 mix with these two oil (D4), Fotedar *et al.* (1998) stated that marron fed with D1 resulted in significantly higher moisture content in the hepatopancreas and tail muscle tissues than all other diets. Marron receiving no formulated feed and free-ranging outside the experimental cages showed the lower hepatosomatic and tail muscle indices than the caged marron receiving formulated feed (D1 – D4). An

experiment of rearing marron in an intensive culture system has indicated that wet and dry hepatosomatic indices are higher and moisture content of hepatopancreas is lower in marron fed with diets containing reference diets compared to starved marron and marron fed with their own exuviae alone. Dry tail muscle index of starved marron was significantly lower ($P < 0.05$) than on marron fed with their reference diet alone, marron fed with their exuviae alone and on marron fed with reference diet plus their own exuviae. There were no significantly difference in wet tail muscle indices of marron among any dietary treatments (Fotedar *et al.* 2000).

Starvation increases whole body water content in juvenile red claw crayfish (*Cherax quadricarinatus*), which is similar to the changes in water content in the hepatopancreas of red swamp crayfish (*P. clarkii*) fed with different diets (Gu *et al.* 1996). Female noble crayfish deplete energy reserves from their hepatopancreas during the ovarian maturation process. This is observed as high hepatopancreas moisture content, up to 80%, compared to reproductively inactive females that have 60-70% moisture levels (Huner *et al.* 1990).

Studies on the effects of different diets on the hepatosomatic indices of the western rock lobster (*Panulirus cygnus*) Tsvetnenko *et al.* (1999) suggested that the hepatosomatic indices of western rock lobsters decreased when fed artificial moist pelleted diets. The wet muscle-somatic index decreased in western rock lobsters fed blue mussel (*Mytilus edulis edulis*) and artificial diets after 3 months of culture. The dry muscle-index decreased in western rock lobsters fed artificial diets, but did not decrease in the lobsters fed a natural diet. Low dry indices of lobsters fed on an artificial diet compared to natural diets indicate the depletion of energy reserves in both the digestive gland and tail muscle. While there were no significant differences between growth rates in animals fed artificial diets, the significant differences observed in several of organosomatic indices proves the usefulness of tissues parameters as condition indicators.

## 4. MATERIALS AND METHODS

### 4.1 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 (Figures 1 & 2) on $6^{th}$ and $11^{th}$ 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 g/L) and fed with blue mussels (*Mytilus edulis edulis*) daily until the experiment began.
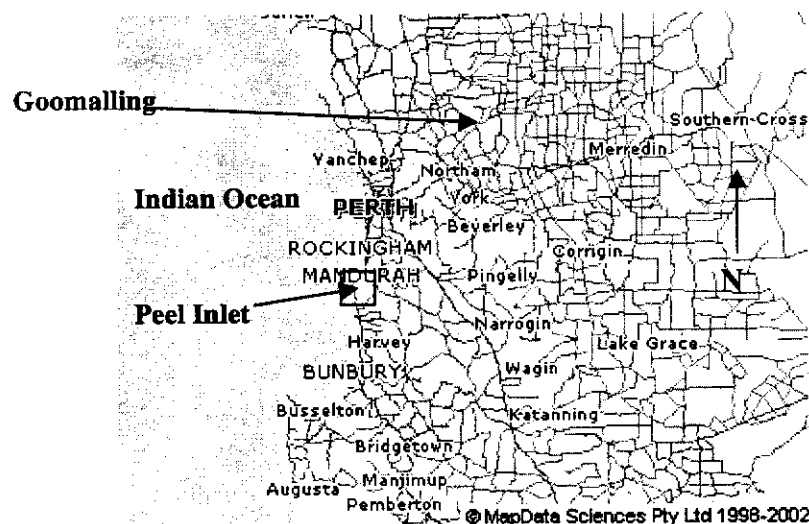


**Figure 1.** Location of Peel Inlet in South West Western Australia.

### 4.2 Experiment design and setup

Sixteen 125 L plastic cylindrical tanks, with a diameter of 59 cm were used in this experiment. The tanks were painted black and the tops covered with black plastic to reduce the light penetration and prevent prawns from jumping out. Small holes were cut in the black plastic to allow sufficient gaseous exchange. Each tank was coupled to an external bio-filter and protein skimmer (Figure 3). Water was dropped back into each tank from its top (49 cm height) to ensure sufficient aeration and the water temperature was maintained at 25 °C by controlling the air temperature in the laboratory.

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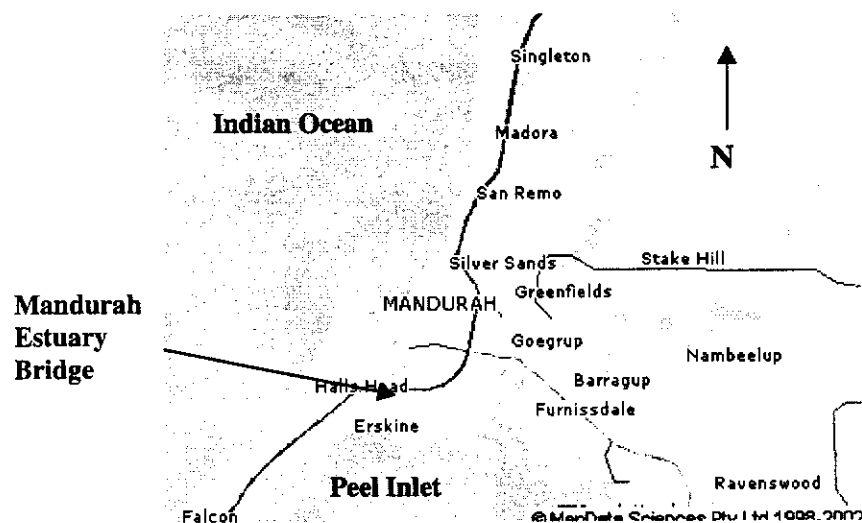
**Figure 2.** Mandurah Estuary Bridge, where the prawns were collected for the experiment.

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

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

Where         Vr: Volume of water replaced by fresh water.

                 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 g/L) was calculated as the following equation:

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

Where         Vr: Volume of water replaced by concentrated seawater (60 g/L).

                 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. Temperature was maintained at 25°C in every cultured tank using an electric heater in the wet laboratory. Aeration was provided to every tank during the experiment. Nitrate, nitrite, ammonium and pH of water were measured weekly using nitrate, nitrite, ammonium and pH test kits (Hagen Brand).
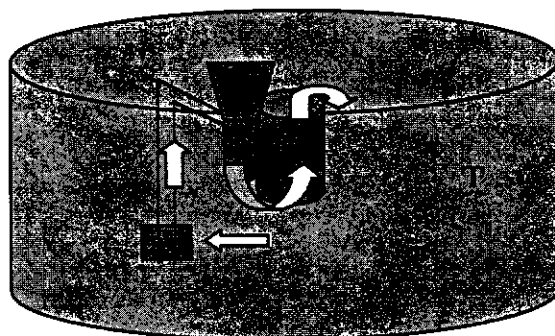


**Figure 3.** Diagrammatic design outline of an experimental tank (the arrows show the direction of water flow).
P: Internal pump;
PS: Protein skimmer
BF: Bio-filter containing coral media
T: 125 L tank

## 4.3 Data collection

### 4.3.1 Growth and survival

All the prawns were measured for total weight (W), total length (TL) and carapace length (CL) at 0 (immediately after acclimatisation), 15 30, 45 and 60 days of experimental period. Specific growth rates (both in term of weight and length) and survival rates were calculated using the following equations:

$$SGR_l = 100 \times (\ln TLf - Ln Tlo) / t$$

Specific growth rate in weight (%):

$$SGR_w = 100 \times (\ln Wf - Ln Wo) / t$$

Where $SGR_l$ and $SGR_w$ are specific growth rate in length and weight respectively; TLf and Wf are total length and weight at time t and Tlo, Wo are total length and weight at the beginning.

Survival rate (%):

$$S = 100 \times (n_t/n_o)$$

Where $n_t$ is the number of prawn at time t and $n_o$ is the number of prawns at the commencement (11 prawns).

Moult increments (%) represented the increment increase of weight and total length, carapace length between two successive moults using the following equations:

$$Mi = 100 \times (Wa - Wb) / Wb$$

$$Mi = 100 \times (Tla - TLb) / TLb \text{ and}$$

$$Mi = 100x (Cla - CLb) / CLb$$

Where Mi is the moult increment Wa, Tla and Cla are total weight, total length and carapace length after the second moult and Wb, TLb and CLb are total weight, total length and carapace length after the first moult.

Moult intervals were measured by the time between two successful moults. Food conversion ratio was calculated based on the quantity of food consumed and the increase in biomass as follow:

$$FCR = Fc / \Delta W$$

Where FCR is food conversion ratio, Fc is total food consumed and $\Delta W$ is total increase in weight.

### 4.3.2 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 a 0.5 mL syringe containing 0.1 mL of precooled (10 $^{0}$C) 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 using a Cryoscopic Osmometer – Osmomet 030. Osmolality of blank anticoagulants was also measured and the haemolymph osmolality was calculated using the 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.

### 4.3.3 Organosomatic indices analysis

Organosomatic indices of one, randomly sampled prawn from each tank were 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) and hepatopancreas (all lobes of the hepatopancreas) of the prawn were weighed to determine the wet hepantosomatic index (Hiw) and wet tail muscle index (Tiw) using the following equations:

$$Hiw = Whwet \times 100 / W$$

Where Whwet is weight of wet hepatopancreas (g) and W is total weight of prawn.

$$Tiw = Wtwet \times 100 / W$$

Where Wtwet is weight of wet tail muscle.

The whole hepatopancreas and tail muscles were then dried to constant weight at 100 °C for 24 hours. The hepatopancreas moisture content (HM%) and tail muscle moisture content (TM%), dry hepatosomatic index (Hid) and dry tail muscle index (Tid) were calculated using the following equations:

$$MH\% = 100 \times (Whwet - WHdry) / Whwet$$

Where WHdry is weight of dry hepatopancres (g)

$$TM\% = 100 \times (Wtwet - WTdry) / Wtwet$$

Where WTdry is weight of dry tail muscles (g)

$$Hid = WHdry \times 100 / W$$
$$Tid = WTdry \times 100 / W$$

### 4.4 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 g/L of salinity. All significant tests were at $P < 0.05$ levels.

# 5. RESULTS

## 5.1 Growth

### 5.1.1 Total weight, total length and carapace length

Weight of the western king prawn reared at different salinities is shown in Table 3. Prawns, after 15 days of rearing at four different salinities, showed no significant difference (P > 0.05) in total weigh. The highest total weigh (6.06 ± 0.511 g after 45 days and 6.21 ± 0.504 g after 60 days of rearing) was achieved at a salinity of 34 g/L, which were significantly higher (P < 0.05) than achieved at a salinity of 46 g/L (4.23 ± 0.752 g after 45 days and 4.27 ± 0.761g after 60 days of rearing). The significant increases (P < 0.05) in total weight were at salinities of 22 and 34 g/L after 30 days of rearing. At a salinity of 46 g/L, there was no significant increase (P > 0.05) in total weight after 60 days of rearing.

**Table 3.** Weight (mean ± S.E. (g)) of western king prawns reared at different salinities.

| Day | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1$2.94 ± 0.164$^a$ | $_1$2.96 ± 0.289$^a$ | $_1$2.99 ± 0.171$^a$ | $_1$2.98 ± 0.348$^a$ |
| 15 | $_1$2.99 ± 0.394$^a$ | $_{1,2}$3.99 ± 0.563$^a$ | $_{1,2}$3.98 ± 0.488$^a$ | $_1$3.04 ± 0.204$^a$ |
| 30 | | $_{2,3}$4.37 ± 0.399$^a$ | $_{2,3}$4.42 ± 0.377$^a$ | $_1$3.33 ± 0.305$^a$ |
| 45 | | $_3$5.05 ± 0.193$^{a,b}$ | $_4$6.06 ± 0.511$^a$ | $_1$4.23 ± 0.752$^b$ |
| 60 | | $_3$5.13 ± 0.137$^{a,b}$ | $_4$6.21 ± 0.504$^a$ | $_1$4.27 ± 0.761$^b$ |

*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.*

There were no significant difference (P > 0.05) in total length and carapace length of prawns reared at a salinity of 10 g/L after 15 days and 46 g/L after 60 days. The significant increases (P < 0.05) in total length were achieved at salinities of 22 g/L after 30 days and 34 g/L after 45 days. The significant increases in carapace length were obtained at a salinity of 22 g/L after 15 days and at a salinity of 34 g/L after 30

days of rearing. The highest total length (87.34 ± 4.076 mm) of prawns was obtained at a salinity of 34 g/L after 45 days, which was significantly higher (P < 0.05) than when prawns were reared at a salinity of 46 g/L (73.65 ± 4.630 mm). The highest carapace length of prawns was at a salinity of 34 g/L (19.47 ± 0.819 mm) after 30 days, which was significantly higher (P < 0.05) than at a salinity of 46 g/L (17.19 ± 0.759). However, there were no differences in total length and carapace length of prawn reared at salinities of 34 and 22 g/L (Figure 4; Figure 5; Appendix 1 and Appendix 2).
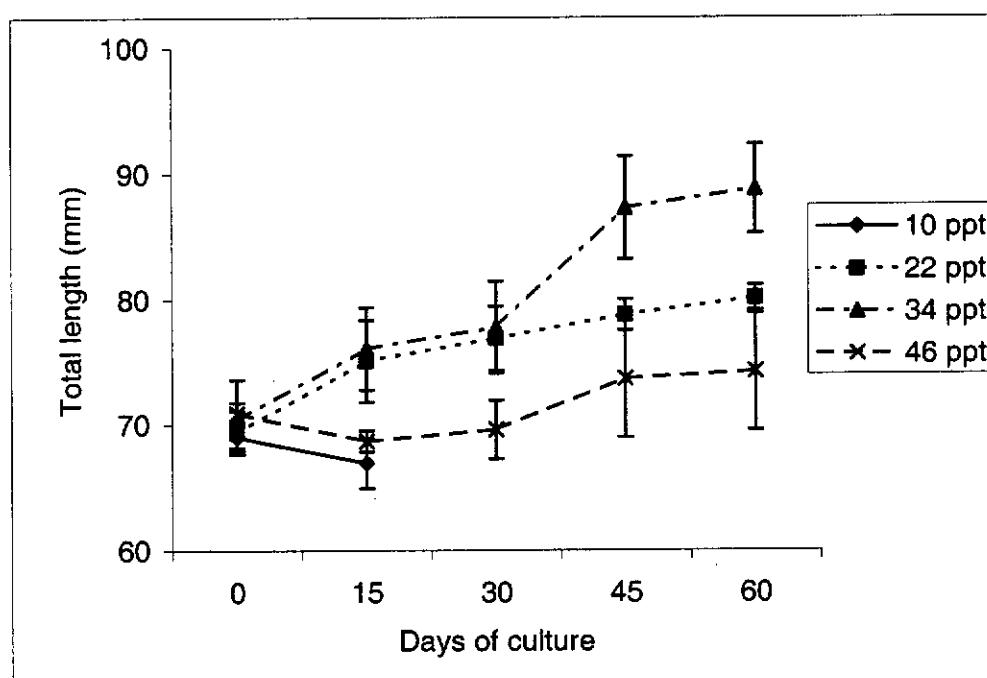


**Figure 4.** Total length (mean ± S.E. (mm)) of western king prawns reared at different salinities.

The relationship between total weight and total length and between total weight and carapace length of prawn reared at four different salinities are shown in Table 4. The results show that western king prawns reared at salinities of 22, 34 and 46 g/L increased in total weight faster and increased in carapace length slower than prawns reared at a salinity of 10 g/L. However, there are no significant differences in the rate of either total weight or total length increments of prawns reared in four different salinities.
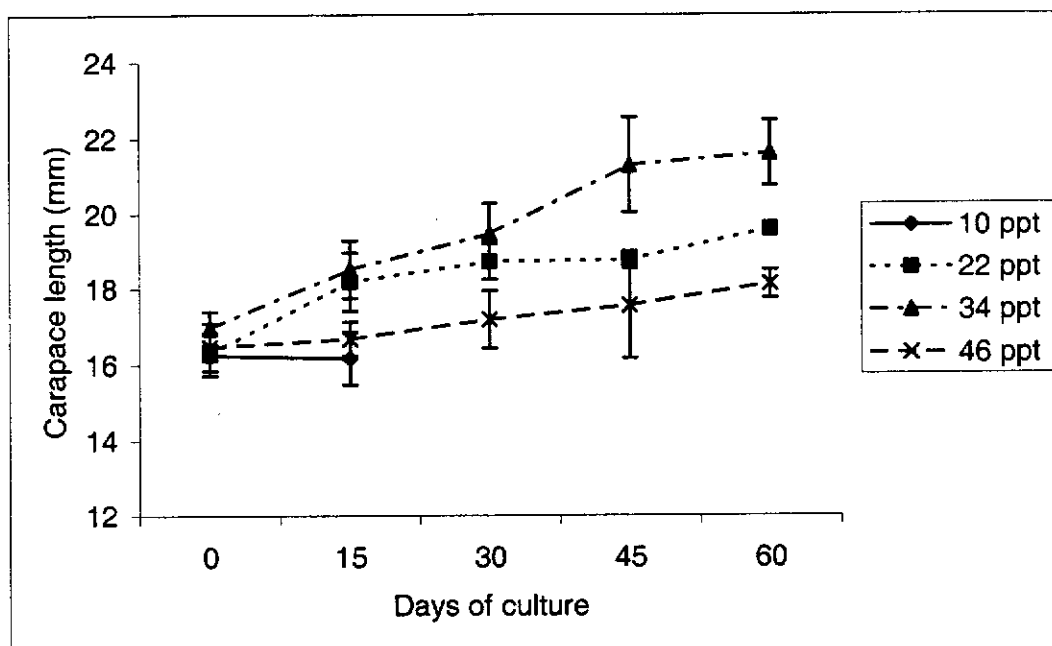
**Figure 5.** Carapace length (Mean ± S.E. (mm)) of western king prawns reared at different salinities.

**Table 4.** Weight – total length, weight – carapace length relationship of western king prawns reared at different salinities.

| Salinity | Weight (g) – Total length (mm) Relationship | | Weight (g) – Carapace length (mm) relationship | |
|---|---|---|---|---|
| | Equation | $R^2$ | Equation | $R^2$ |
| 10 g/L | $W = 0.0015\ TL^{1.8955}$ | 0.9563 | $W = 0.0794\ CL^{1.1477}$ | 0.9223 |
| 22 g/L | $W = 0.0011\ TL^{1.9526}$ | 0.9106 | $W = 0.0384CL^{1.7221}$ | 0.9275 |
| 34 g/L | $W = 0.0025\ TL^{1.764}$ | 0.9194 | $W = 0.0381CL^{1.7142}$ | 0.9240 |
| 46 g/L | $W = 0.003\ TL^{1.7238}$ | 0.9389 | $W = 0.0518\ CL^{1.6132}$ | 0.9239 |

**5.1.2 Moult increment**

There were no significant differences ($P > 0.05$) in increase of total weight, total length and carapace length of prawns at salinities of 22, 34 and 46 g/L after two successive moults. The time intervals between two successive moults were also not significantly different ($P > 0.05$) in different salinities. However, the trend showed that the highest increase in total weight was in prawns reared at a salinity of 34 g/L (11.71 ± 0.225 %); in total length was in prawns reared at a salinity of 46 g/L (2.96 ±

0.909 %) and in carapace length in prawn reared at a salinity of 22 g/L (3.93 ± 0.577 %) (Table 5).

**Table 5.** Moult increment of western king prawns reared at different salinities.

| Moult increment | Salinity | | |
| --- | --- | --- | --- |
| | 22 g/L | 34 g/L | 46 g/L |
| Weight (%) | 11.61± 0.988 [a] | 11.71 ± 0.225 [a] | 9.60 ± 0.665 [a] |
| Total length (%) | 2.74 ± 0.983 [a] | 2.89 ± 0.440 [a] | 2.96 ± 0.909 [a] |
| Carapace length (%) | 3.93 ± 0.577 [a] | 3.56 ± 0.786 [a] | 2.54 ± 0.641 [a] |
| Time period (day) | 16.33 ± 0.717 [a] | 16.50 ± 0.540 [a] | 16.17 ± 0.425 [a] |

### 5.1.3 Specific growth rate (SGR)

Specific growth rate in term of total weight and total length of prawns were highest at a salinity of 34 g/L (1.154 ± 0.151% in total weight and 0.345 ± 0.045% in total length), which was significantly higher (P < 0.05) than when prawns were reared at a salinity of 46 g/L (0.667 ± 0.137% in total length and 0.101 ± 0.050% in total length) and 10 g/L (-0.056 ± 0.216% in total weight and –0.212 ± 0.283% in total length). There was no significant difference (P > 0.05) in SGR in total weight of prawns reared at a salinity of 22 compared to a salinity of 46 g/L. SGR in total length of prawn reared at a salinity of 22 g/L was significantly higher (P < 0.05) compared to those reared at a salinity of 46 g/L. Negative SGR in weight and total length were observed when prawns were reared at a salinity of 10 g/L. Prawns reared at salinities of 22 and 34 g/L had the similar SGR in total weight and length (Figure 6, Figure 7; Appendix 3 and Appendix 4).
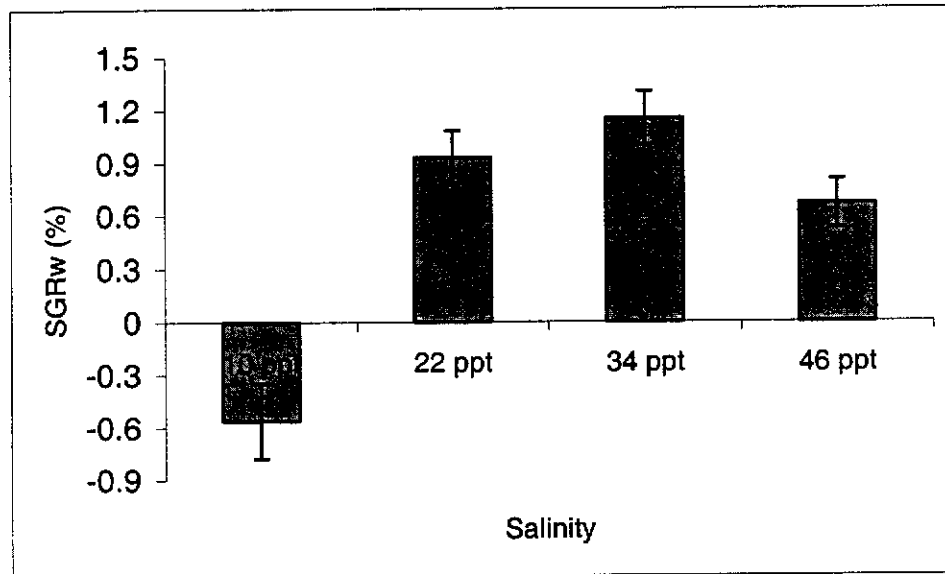
**Figure 6.** SGR in total weight of western king prawns reared at different salinities.
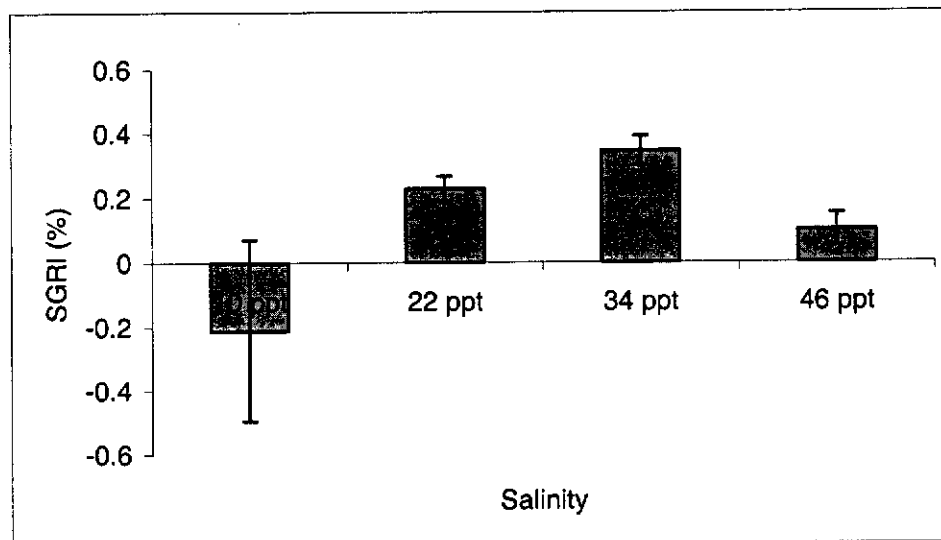


**Figure 7.** SGR in total length of western king prawns reared at different salinities.

### 5.1.4 Food conversion ratio (FCR)

The FCR data for prawns reared at a salinity of 10 g/L could not obtained because of negative growth. The highest FCR was obtained in prawns reared at a salinity of 46 g/L (7.83 ± 1.24), which was significantly higher (P < 0.05) than in prawns reared at salinities of 22 and 34 g/L (3.26 ± 0.36 and 3.34 ± 0.51). The prawns reared at salinities of 22 and 34 g/L showed no significant difference (P > 0.05) in FCR (Figure 8 and Appendix 5).
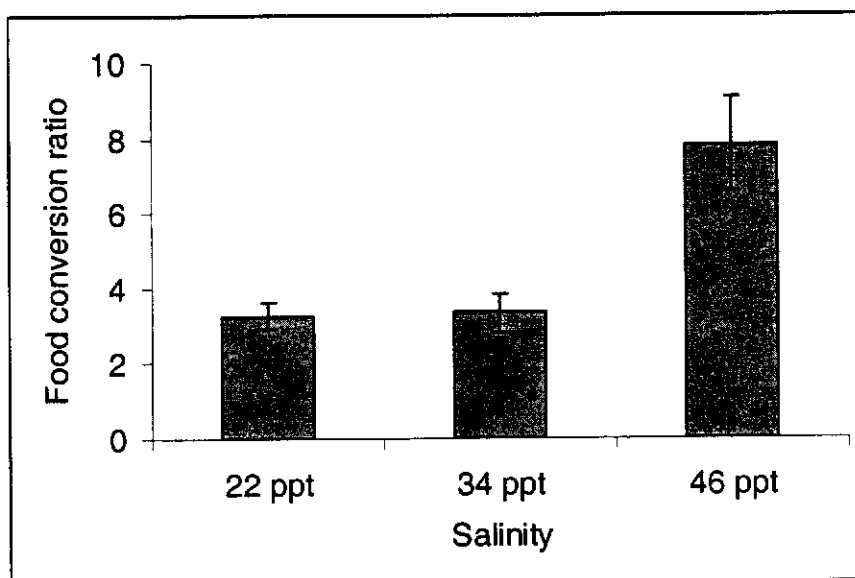
**Figure 8.** FCR of western king prawns reared at different salinities.

## 5.2 Survival

There were no mortalities in the four different salinities during the acclimatization period. After 15 days of rearing, the prawns showed high mortality (80%) at a salinity of 10 g/L. The highest survival rate at 15 days was obtained at a salinity of 22 g/L (68.18 ± 5.87 %), which was significantly higher (P < 0.05) than survival at salinities of 10 g/L (20.45 ± 5.72 %) and 46 g/L (38.64 ± 4.35 %) (Figure 9 and Appendix 6).

After 30 days, the highest survival was also in 22 g/L (40.91 ± 5.87 %). This was significantly higher (P < 0.05) than in 34 g/L (25.00 ± 4.35 %) and 46 g/L (13.64 ± 2.62 %). After 60 days, the highest survival rate was obtained at a salinity of 22 g/L (22.73 ± 5.87 %). This was significantly higher (P < 0.05) than the survival rate at a salinity of 46 g/L (6.82 ± 2.27) but not significantly higher than the survival of prawns reared at a salinity of 34 g/L (Figure 9 and Appendix 6).

Prawn survival reduced significantly (P < 0.05) after 15 and 30 days of culture at four salinities and remained stable after 30 days at salinities of 34 and 46 g/L while it was stable at a salinity of 22 g/L after 45 days of culture (Figure 9 and Appendix 6).
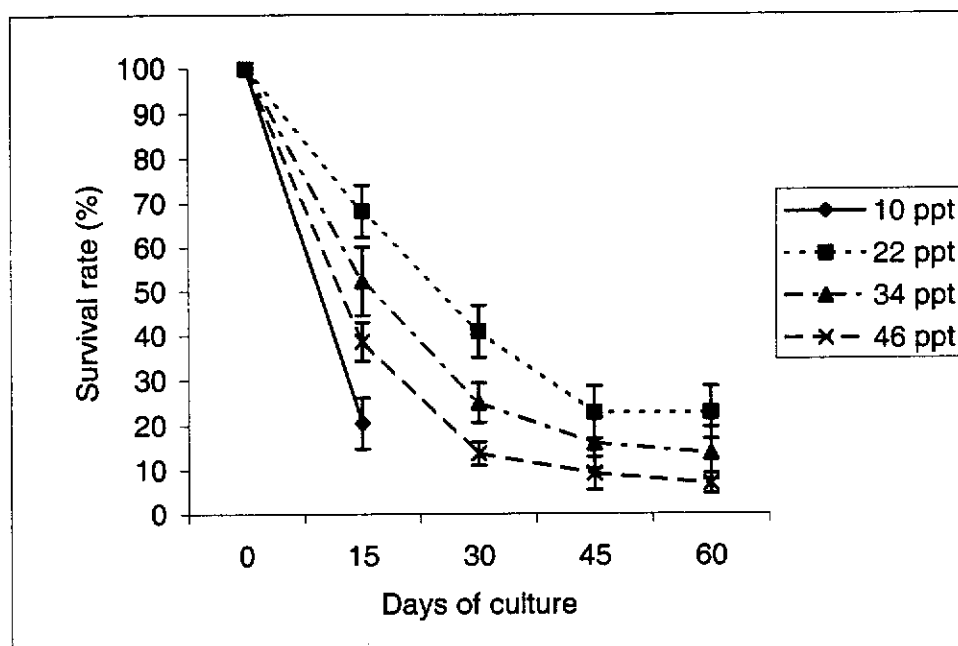
**Figure 9.** Survival rate of western king prawns reared at different salinities.

## 5.3 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 g/L (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 g/L. The prawn reared at a salinity of 46 g/L 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 g/L. 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 g/L. At a salinity of 22 g/L, the highest haemolymph osmolality of prawns was observed after 20 days of rearing which was significantly higher ($P < 0.05$) than at the beginning and at 60 days of the rearing period (Figure 10 and Appendix 7).
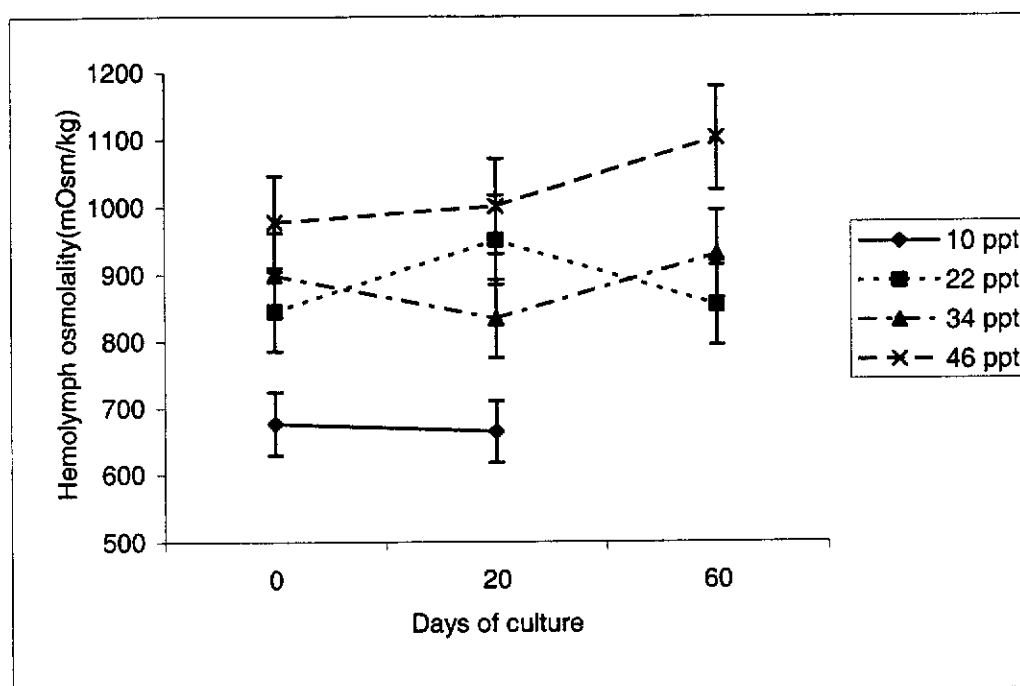
**Figure 10.** Haemolymph osmolality (mOsm/kg) of western king prawns reared at different salinities.

The linear relationships between the haemolymph osmolality and the medium osmolality at different rearing times are shown in Figure 11 and Table 6. Isosmotic points that were determined based on regression lines between both the haemolymph and medium osmolality are shown in Table 6. Isosmotic points increased with the increase in both the length of rearing times and weights of prawns.

**Table 6.** 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 (g/L) | 28.87 g/L | 29.46 g/L | 31.73 g/L |
| Weight (g) | 2.95 ± 0.26 | 4.02 ± 0.47 | 5.79 ± 0.64 |
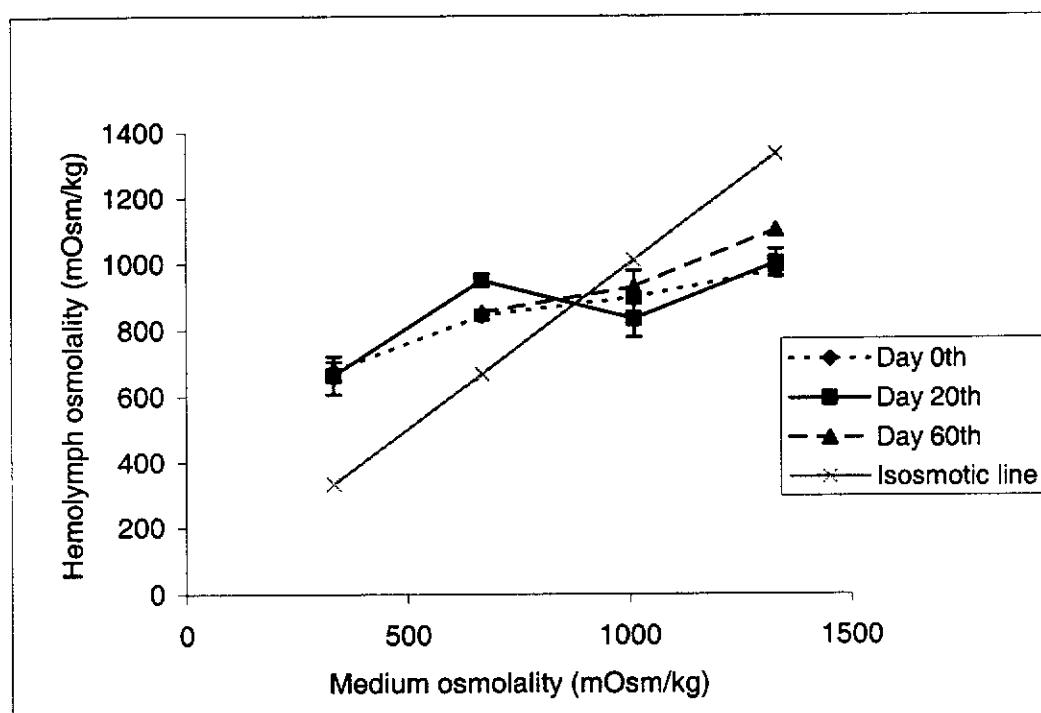
**Figure 11.** Relationship between haemolymph osmolality and medium osmolality of western king prawns reared at different salinities.

## 5.4 Moisture content

### 5.4.1 Hepatopancreas moisture content

Following acclimatization, hepatopancreas moisture was highest in prawns reared at a salinity of 10 g/L (73.40 ± 1.736 %). It was significantly higher (P < 0.05) than in the prawns reared at salinities of 22 and 34 g/L (63.79 ± 1.986 % and 62.01 ± 2.094%) but not significantly higher (P > 0.05) in prawns reared at a salinity of 46 g/L (69.86 ± 0.705%). Hepatopancreas moisture was also highest at a salinity of 10 g/L at 20 days of culture (75.81 ± 1.302%) but not significantly higher (P > 0.05) in those reared at salinities of 22 and 34 g/L (69.38 ± 2.706 and 72.75 ± 3.105 %). The lowest hepatopancreas moisture was at a salinity of 46 g/L (68.00 ± 1.769%), which was significantly lower (P < 0.05) than in those measured at a salinity of 10 g/L. At 60 days, the lowest hepatopancreas moisture content was at a salinity of 22 g/L, which was significantly lower (P < 0.05) than at salinities of 34 and 46 g/L (Figure 12 and Appendix 8).
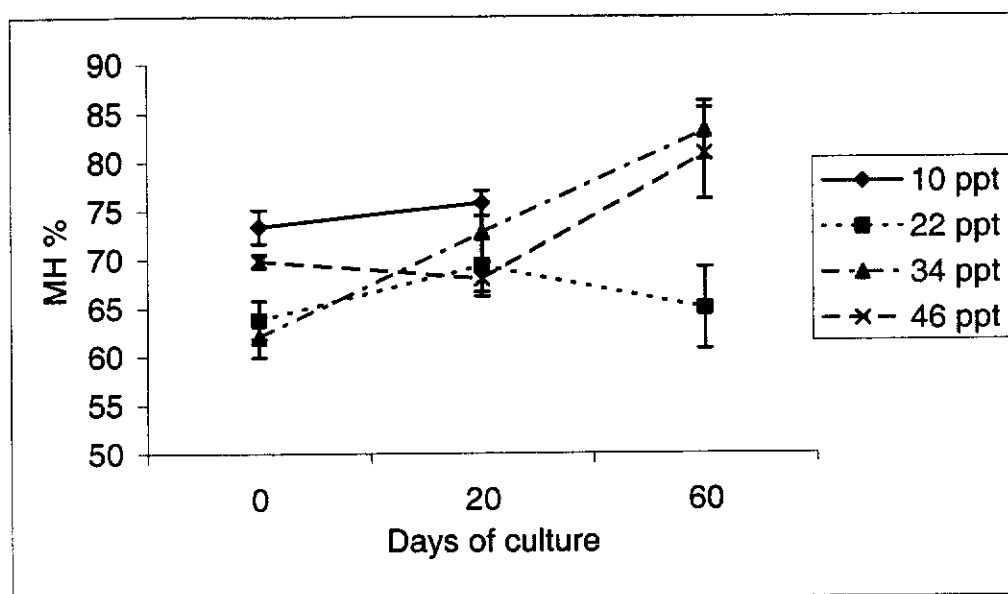
**Figure 12.** Moisture content (%) of hepatopancreas of western king prawns reared at different salinities.

At a salinity of 34 g/L, hepatopancreas moisture increased significantly (P < 0.05) after 20 days and 60 days of culture. At a salinity of 46 g/L, it was significantly higher (P < 0.05) at 60 days compared to the beginning and at 20 days of culture. There was no significant change (P > 0.05) in hepatopancreas moisture content of prawns reared at a salinity of 22 g/L (Figure 12 and Appendix 8).

### 5.4.2 Tail muscle moisture content

Tail muscle moisture content decreased with an increase in salinity. After the acclimatization period, it was highest at a salinity of 10 g/L (82.43 ± 1.681%) and lowest at a salinity of 46 g/L (71.03 ± 3.221%). Tail muscle moisture content was significantly higher (P < 0.05) in prawns reared at a salinity of 10 g/L compared to those at salinities of 22, 34 and 46 g/L. However, there were no significant differences in muscle moisture content in prawns raised at salinities of 22, 34, and 46 g/L. Tail muscle moisture content was also highest at a salinity of 10 g/L at 20 days of culture (80.37 ± 0.526%), which was significantly higher (P < 0.05) than at salinities of 34 and 46 g/L. There was no significant difference (P> 0.05) in tail muscle moisture content between salinities of 22 and 34 g/L, but it was significantly higher (P < 0.05) at a salinity of 22 g/L compared to a salinity of 46 g/L. Tail muscle moisture did not change significantly (P>0.05) during the experimental time in the four salinities (Figure 13 and Appendix 9).
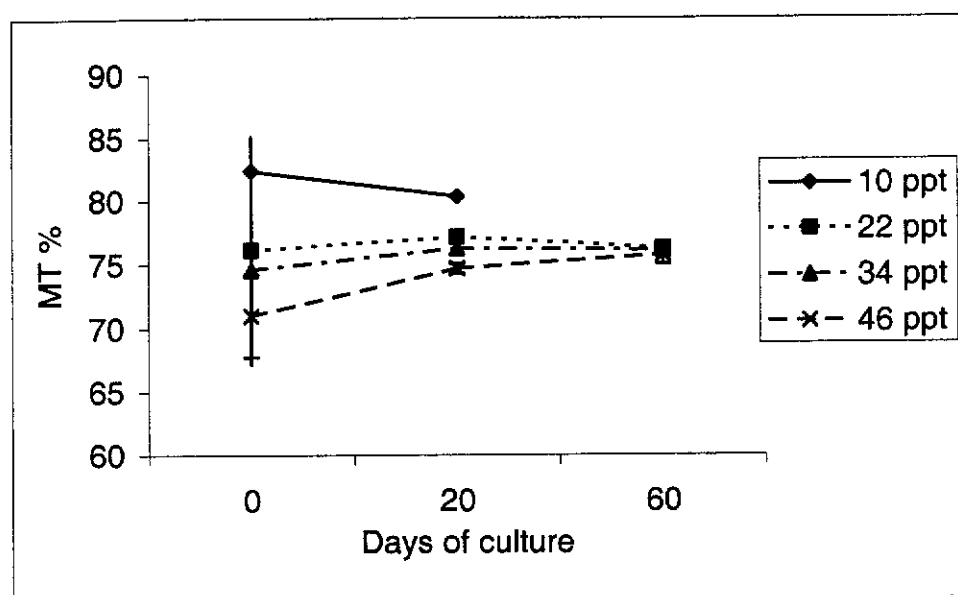
**Figure 13.** Moisture content of tail muscle of western king prawns reared at different salinities.

## 5.5 Organosomatic indices

### 5.5.1 Hepatosomatic indices

The wet weight hepatosomatic index of the prawn was highest at a salinity of 22 g/L (1.36 ± 0.073 %), which was significantly higher (P < 0.05) than at salinities of 10, 34 and 46 g/L of salinity and the lowest was at a salinity of 46 g/L (0.86 ± 0.028%). There was no significant difference in the wet weight hepatosomatic index of prawn reared at four different salinities after 20 days of culture. At 60 days of culture, the lowest index was at a salinity of 46 g/L (1.19 ± 0.088), which was significantly lower (P < 0.05) than at salinities of 22 and 34 g/L. Wet weight hepatosomatic index of the prawns reared at salinities of 22, 34 and 46 g/L changed significantly (P < 0.05) during the culture period. There was a significant increasing trend (P < 0.05) of the wet weight hepatosomatic index at a salinity of 34 g/L during the culture time (Figure 14 and Appendix 10).
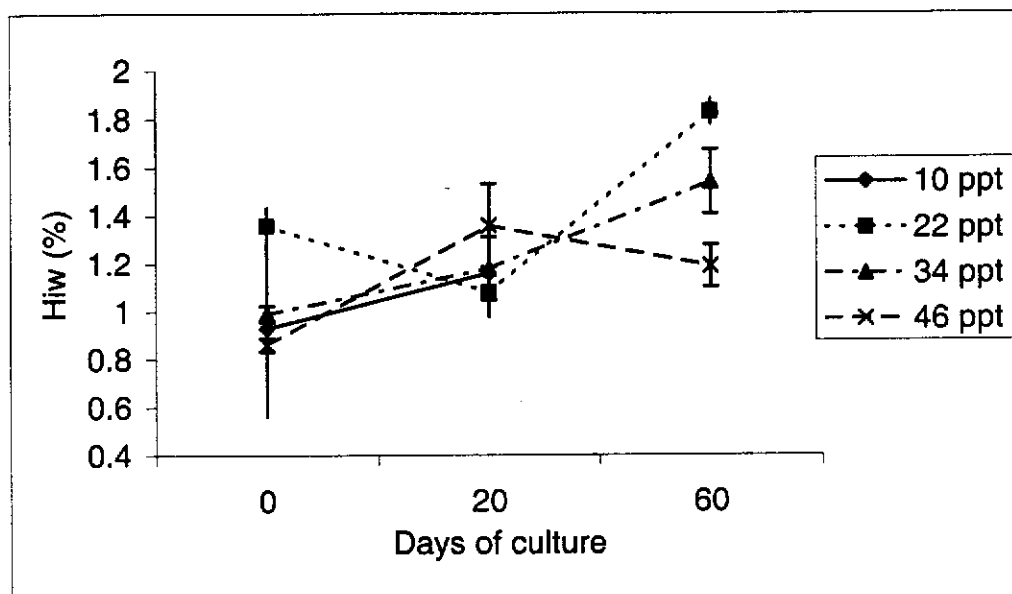
**Figure 14.** Wet weight hepatosomatic index of western king prawns reared at different salinities.

The dry weight hepatosomatic index of prawns was highest at a salinity of 22 g/L (0.49 ± 0.041%) and lowest at a salinity of 10 g/L (0.24 ± 0.092 %). The dry weight hepatosomatic index of prawns reared at a salinity of 22 g/L was significantly higher (P < 0.05) compared to prawns reared at salinities of 10 and 46 g/L. However, at a salinity of 22 g/L the dry weight hepatosomatic index was not significantly higher (P > 0.05) compared to 34 g/L. After 60 days, the highest dry weight hepatosomatic index was at a salinity of 22 g/L, which was significantly higher than that at salinities of 22 and 34 g/L (Figure 15 and Appendix 11).

There was no significant change in the dry weight hepatosomatic index of prawns reared at salinities of 10 g/L and 34 g/L as culture time increased. However, at salinities of 22 and 46 g/L, it changed significantly (P < 0.05) during the rearing time. In 22 g/L salinity, the highest index was 0.64 ± 0.092 % at 60 days and at a salinity of 46 g/L the highest index was 0.43 ± 0.052% at 20 days (Figure 15 and Appendix 11).
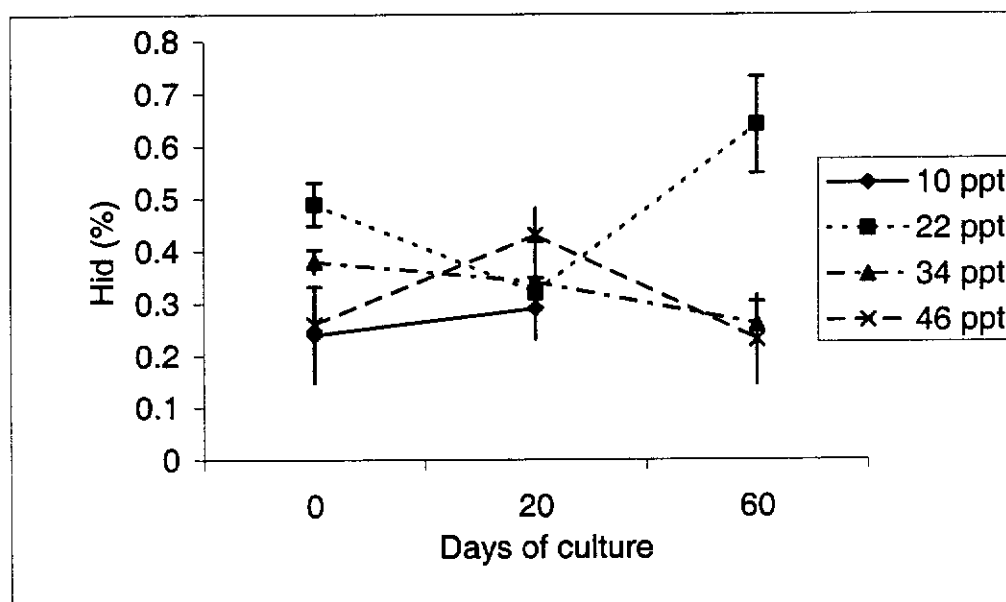
**Figure 15.** Dry weight hepatosomatic index of western king prawns reared at different salinities.

### 5.5.2 Tail muscle indices

After 60 days rearing the wet weight tail muscle index of the prawns was significantly higher ($P < 0.05$) at a salinity of 34 g/L compared to 46 g/L. At salinities of 10, 22 and 46 g/L, there were no significant changes ($P > 0.05$) in the wet weight tail muscle index during the rearing periods. However, at a salinity of 34 g/L, there was an increasing trend and the wet weight tail muscle index was significantly higher ($P < 0.05$) after 60 days (Figure 16 and Appendix 12).

The dry weight tail muscle index of the prawns was highest in 46 g/L salinity (6.30 ± 1.21%), which was significantly higher ($P < 0.05$) than at a salinity of 10 g/L (3.05 ± 1.045%) but not significantly higher ($P > 0.05$) than at 22 and 34 g/L. After 60 days, the dry weight tail muscle index of prawns was the highest at a salinity of 34 g/L (6.84 ± 0.31%), which was significantly higher ($P < 0.05$) than at a salinity of 46 g/L (5.33 ± 0.34) but not significantly higher ($P > 0.05$) than at 22 g/L of salinity. At salinities of 10, 22 and 46 g/L, there were no significant changes ($P > 0.05$) in the dry weight tail muscle index during the culture periods. At a salinity of 34 g/L, there was an increasing trend and the dry weight tail muscle index was significantly higher ($P < 0.05$) at 60 days (Figure 17 and Appendix 13).
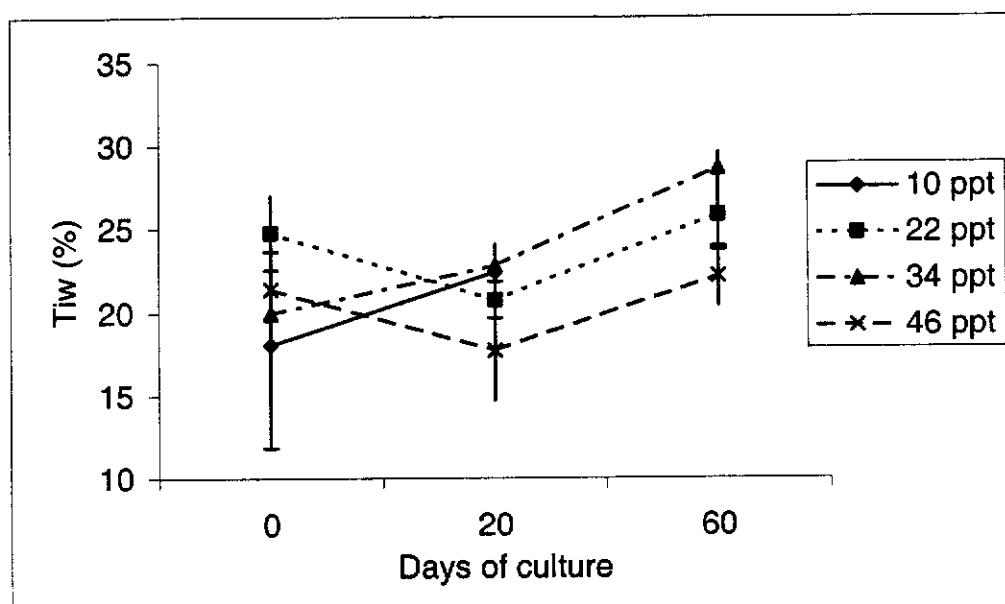
**Figure 16.** Wet weight tail muscle index of western king prawns reared at different salinities.
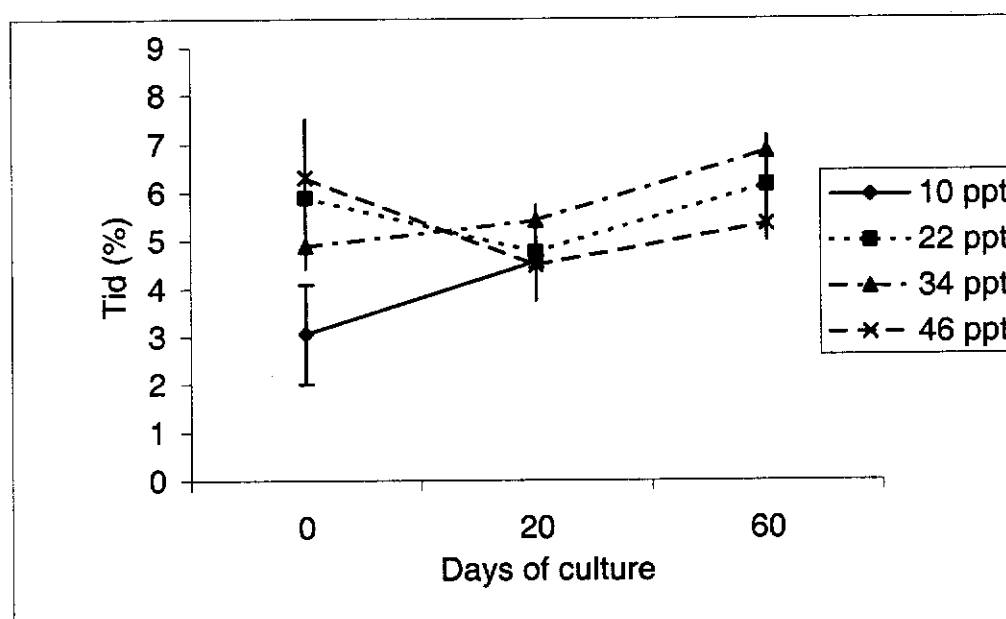


**Figure 17.** Dry weight tail muscle index of western king prawns reared at different salinities.

## 5.6 Environmental parameter of cultured tanks

During the experiment, the temperature was kept constant at 25 °C. pH was highest at a salinity of 10 g/L (8.09 ± 0.077) but it was not significantly higher (P < 0.05) than at other salinities. $NO_3$ was highest at a salinity of 22 g/L (13.70 ± 2.050 mg/l), but not significantly higher than at other salinities. $NO_2$ was highest at a salinity of 34 g/L (0.34 ± 0.075 mg/l) but not significantly higher (P > 0.05) than at other

salinities. Total ammonical nitrogen were not significantly different among salinities (Table 7).

Table 7. Mean environmental parameters of rearing tank.

| Parameters | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| T ($^{o}$C) | 25 | 25 | 25 | 25 |
| PH | $8.09 \pm 0.077^{a}$ | $7.97 \pm 0.038^{a}$ | $7.93 \pm 0.084^{a}$ | $7.93 \pm 0.016^{a}$ |
| NO$_3$ (mg/l) | $11.25 \pm 0.433^{a}$ | $13.70 \pm 2.050^{a}$ | $11.68 \pm 2.330^{a}$ | $8.53 \pm 0.602^{a}$ |
| NO$_2$ (mg/l) | $0.26 \pm 0.013^{a}$ | $0.27 \pm 0.020^{a}$ | $0.34 \pm 0.075^{a}$ | $0.25 \pm 0.030^{a}$ |
| Total ammonia-nitrogen (mg/l) | $0.064 \pm 0.0043^{a}$ | $0.065 \pm 0.0042^{a}$ | $0.139 \pm 0.0246^{a}$ | $0.139 \pm 0.0114^{a}$ |

# 6. DISCUSSION

## 6.1 Growth and survival

The present study shows that salinity is an extremely important factor affecting the growth and survival of the western king prawn. Growth performance of the western king prawn is achieved differently in different salinities. The significant reduction in survival rate of prawns at salinity of 10 g/L after 15 days rearing indicates that western king prawns cannot tolerate low salinity (salinity < 10 g/L). After 20 days of rearing, only 3 prawns were alive in the four replicates of 10 g/L salinity and they were used for osmotic pressure analysis while there were still a considerable number of prawns at other salinities. The higher survival rate of western king prawns at salinities of 22 and 34 g/L in this study suggests that the prawns have a good tolerance for a salinity range from 22 to 34 g/L. Based on those growth factors (weight gain, total length gain, specific growth rate and FCR, weight - length relationship), it can be assumed that the optimum range of salinity for growth and survival of western king prawns would includes a range from 22 to 34 g/L.

The optimum salinity conditions for growth and survival of western king prawns are similar to those estimated for the fleshy prawn (*P. chinensis*), green tiger prawn (*P. semisulcatus*), banana prawn (*P. merguiensis*), brown tiger prawn (*P. esculentus*); golden prawn (*P. californiensis*) and the Indian white prawn (*P. indicus*). However, the optimum salinity condition for growth and survival of western king prawns is different to those of the giant tiger prawn (*P. monodon*), banana prawn larvae, whiteleg prawn (*L. vannamei*) and the brown prawn (*P. aztecus*). This demonstrates that each species of penaeid prawn has a different salinity tolerance. However, it is likely that most juveniles of penaeid prawns would show maximum growth at salinities around 25 to 35 g/L (Table 8).

Based on the salinities preference for growth and survival it is suggested that culture of the western king prawn should be performed in the range of salinity from 22 to 34 g/L. This range of salinity is common in most coastal areas in Australia (Abdel *et al.* 1996). This implies that culture of this prawn is definably possible in coastal areas of Australia.

**Table 8.** Optimum salinity range for growth and survival of some penaeid prawns.

| Species | Development stages | Salinity (g/L) | Reference |
|---|---|---|---|
| *Penaeus monodon* | Juveniles | 15 –25 | (Chen 1985) |
| *P. semisulcatus* | Larvae | 30 – 35 | (Kumlu *et al.* 2000) |
| *P. chinensis* | Juveniles | 20 – 30 | (Chen *et al.* 1996) |
| *P. merguiensis* | Larvae | 15 | (Ruttanagosrigit & Musig 1982) |
| | Juveniles | 25 | (Stapes & Heales 1991) |
| *L. vannamei* | Juveniles | 5 –15 | (Bray *et al.* 1994) |
| *P. esculentus* | Juveniles | 30 | (O' Brien 1994) |
| *P. aztecus* | Juveniles | 8.5- 17 | (Venkataramaiah *et al.* 1973) |
| *P. californiensis* | Postlarvae | 33 | (Martinez *et al.* 1996) |
| *P. indicus* | Postlarvae | 17 –35 | (Vijayyakumaran 1999) |

## 6.2 Haemolymph osmolality

### 6.2.1 Methods applied to measure the haemolymph osmolality

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 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*). The process of coagulation removes the haemolymph cells and some bound metals (Funge-Smith *et al.* 1995) and thus increases the osmolality in comparison to haemolymph. However, the research has not shown any significant differences between the osmolality of plasma and haemolymph in the target penaeid species.

The protocol adapted to measure the haemolymph osmolality in the current research does overcome 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 results obtained were consistent.

### 6.2.2 Haemolymph osmolality and isosmotic point

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 the isosmotic point and a 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 the isosmotic line (slope = 1) reflects the degree of regulation, western king prawns (slope = 0,2665, 0.2871 and 0.3718) are efficient regulators when 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 the 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 present study ranged from 854.80 to 937.09 mOsm/kg, which is equivalent to 28.87 to 31.73 g/L 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 the 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) in studies on the juvenile blue prawns (P. stylirostris) that this prawn decreases the absolute hypo-osmoregulatory capacity with increasing 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 g/L after 60 days indicates that the prawn reduce osmoregulatory capacity in high salinity. By contrast, western king prawns at salinities of 22 and 34 g/L 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 g/L can maintain their osmoregulatory capacity. This can be explained by the fact that salinities of 22 and 34 g/L are close to the isosmotic point and prawns in these salinities spend less energy on osmoregulation than prawns at a salinity of 46 g/L. At salinities of 22 and 34 g/L, a dominant amount of intake energy will be used for metabolic compared to salinities outside this range. This explains why the western king prawn growth better and survival rates at salinities of 22 and 34 g/L, compared to growth and survival rates at salinities of 46 g/L and 10 g/L.

Results obtained from the analysis of the haemolymph osmolality of the western king prawn strongly supports the conclusion above that this prawn should be cultured in the salinity range from 22 to 34 g/L.

## 6.3 Moisture content of the hepatopancreas and tail muscle

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

The hepatopancreas serves as the main digestive gland in crustaceans responsible for both absorbing of nutrients from the digestive tract and excreting fluids enabling the breakdown of nutrients. The hepatopancreas is also the main energy reserve in crustaceans and changes in its size and moisture content show it is affected by nutrient status, developmental stage, growth and moulting (Holdich & Reeve 1988; Huner *et al.* 1990; Jussila 1997) and could be used to determine the condition of a crustacean (Huner *et al.* 1990; Evans *et al.* 1992). The present study shows that moisture content of the hepatopancreas, thus energy content, can also be changed by extreme salinities. This indicates that, in extreme salinity conditions, western king prawns use energy from the digestive gland for osmoregulation to maintain the osmotic balance in the body.

## 6.4 Hepatosomatic and tail muscle indices

Hepatopancreatic and tail muscle indices have been successfully used as indicators of crustacean condition. The use of these parameters in the evaluation of crustaceans has been reviewed by Jussila (1997). A large hepatopancreas size, especially when it related to low hepatopancreas moisture content, can be taken as an indicator of good condition in crustaceans (Mannonen & Henttonen 1995; McClain 1995; Jussila 1997). The present study indicates that hepatopancreatic and tail muscle indices can also be used to evaluate the condition of the western king prawn. There were no significant differences (P > 0.05) in indices of prawns reared in 46 g/L of salinity after 20 days, compared to prawns reared at salinities of 22 and 34 g/L. However, the lowest wet weight hepatosomatic index and tail muscle index of prawns in 46 g/L after 60 days indicates that prawns have to use more energy from the hepatopancreas for osmoregulation at a salinity of 46 g/L compared to salinities of 22 and 34 g/L.

Those results indicate that the western king prawn may stress in the high salinity condition. Those results also explain why the FCR of the western king prawn was the highest in a salinity of 46 g/L and growth was higher in a salinity of 34 g/L.

Combined evaluation of several indicators such as weight and moisture content of the hepatopancreas, the wet and dry hepatosomatic indices and the wet and dry weight of tail muscle to body weight ratios are an effective method to evaluate the condition of prawn. The use of only one index may not sufficiently reflect the condition of the prawns (Fotedar 1998). The study showed that wet hepatopancreatic index can be used as acute indicator of stress caused by salinity whereas dry hepatopancreatic index can be used for chronic indicator.

The highest dry weight hepatosomatic index of prawns grown in a salinity of 22 g/L suggests that the prawns have to use a lower level of reserved energy for osmoregulation at a salinity of 22 g/L compared to 34 g/L. Rearing in salinities of 22 and 34 g/L resulted in similar growth and survival of prawns however, wet and dry hepatosomatic indices were altered by these salinities. Wet hepatosomatic index was getting influenced during acclimatisation whereas dry hepatosomatic index after 60 days of experiment remained unchanged. The usefulness of organosomatic indices in the present study confirms the finding of (Tsvetnenko et al. 1999) where organosomatic indices were different between western rock lobsters (Panulirus cygnus) fed with different artificial diets while the growth rate showed no significant difference. This suggests that the combination of growth, survival and organosomatic indices could give a more reliable basis for the overall condition or performance estimates.

# 7. THE EFFECTS OF AIR EXPOSURE ON THE HAEMOLYMPH OSMOREGULATORY CAPACITY OF BROWN TIGER PRAWNS AND WESTERN KING PRAWNS REARED AT DIFFERENT SALINITIES

## 7.1 Introduction

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).

Brown tiger shrimps (P. esculentus) have a wide natural distribution from mid New South Wales to the sub-tropical Australian waters around Shark Bay in Western Australia (Grey et al. 1983; Keys 2003). They are commercially fished on the east coast of Queensland, the Gulf of Carpentaria, Northern Territory and the north-western coast of Western Australia (Grey et al. 1983). Environmental parameters, such as water temperature and salinity vary greatly over this range, suggesting that P. esculentus may be suitable for farming over a wide range of geographical areas and conditions (Keys 2003). P. esculentus is also considered to be an alternative candidate to black tiger shrimps (P. monodon) for Australian shrimp farmers (Crocos et al. 2000; Keys 2003). They survive between the salinity range of 5 to 55 g/L (Rothlisberg & Jackson 1987). Under laboratory conditions, optimum growth is achieved at 30 °C and 30 g/L salinity and optimum survival is achieved at 30 °C and 30 g/L salinity (O' Brien 1994).

Air exposure has been used as a stressor to evaluate the health status of several crustacean species (Hall & Ham 1998; Taylor & Waldrom 1997; Fotedar et al. 2001). Moreover, transport of live prawns will result in the animals experiencing air exposure. Being exposed to air during live transport may affect the health status of the animals. The experiment described in the previous sections indicated that western king prawns have good tolerance in salinities of 22 and 34 g/L whereas they are stressed in salinities of 10 and 46 g/L. The purpose of this experiment was to

determine how the osmoregulatory capacity of western king prawns reared at 10, 22, 34 and 46 g/L change under air exposure in comparison with brown tiger prawns (*P. esculentus*). The specific objective was to determine the haemolymph osmolality and osmoregulatory capacity of western king prawns and brown tiger prawns reared at salinities of 10, 22, 34 and 46 g/L following 7, 14 and 21 minutes of air exposure.

## 7.2 Materials and methods

### 7.2.1 Experimental animals

Brown tiger prawns with an average weight of 0.94 ± 0.037 g were transferred from a hatchery in Broome, Western Australia (17° 58'S, 122° 14'E) to the Aquatic Science Research Unit, Curtin University of Technology, Perth, Western Australia. Western king prawns (average weight 5.37 ± 0.1 g) were collected using the same method described in the fourth section. Each species were distributed into 12 experimental tanks (Figure 3) separately filled with 70 L of oceanic water (35 g/L). Brown tiger prawns were stocked at a density of 30 animals per tank and western king prawns were stocked at a density of 20 animals per tank. The prawns were maintained in these tanks for 7 days before the commencement of the acclimatisation.

### 7.2.2 Experimental design and data collection

Each species of prawn were acclimatised to salinities of 10, 22, 34 and 46 g/L by a procedure described by Chen *et al.* (1995) so that each salinity concentration was represented in the replicates of three tanks for each species. After acclimatisation, the prawns were reared for 7 days. During the pre-acclimatisation, acclimatisation and subsequent rearing period, prawns were fed with chopped green mussels till satiation. The satiation level was calculated to be 7 % of the total biomass of the prawns per day. 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. The prawns were then starved for 1 day to bring them to the same nutritional status.

Only twelve prawns in inter-moult stage of development were left in each tank for the trial and both species were exposed to air using the following procedure:

Out of 12 brown tiger prawns, 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 the outer right uropodite and released back into their respective tanks. After a further 7 minutes, 3 of the 6 remaining prawns were tagged by cutting the edge of the 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 of which was not exposed to air at all whereas the 2nd, 3rd and 4th groups were exposed to air for 7, 14 and 21 minutes, respectively. The same procedure of air-exposure was carried out for the western king prawn. Three hours after release back into their original salinity condition, the haemolymph of the individual prawns was collected to determine the osmolality as described in the fourth section.

## 7.3 Results

### 7.3.1 Haemolymph osmolality

The haemolymph osmolality of brown tiger prawns and western king prawns when exposed to air and raised in different salinities, are presented in Table 9. In general, the haemolymph osmolality of both species increased with increasing medium salinity. In brown tiger prawns the highest haemolymph osmolality was from a salinity of 46 g/L, which was significantly higher (P < 0.05) than prawns from salinities of 10 and 22 g/L. The highest haemolymph osmolality of the western king prawn was also from a salinity of 46 g/L and was significantly higher (P < 0.05) than prawns from 10, 22 and 34 g/L. Medium salinity of 10 g/L resulted in the significantly lowest (P < 0.05) haemolymph osmolality in both species. Irrespective of the duration of air exposure both species of penaeid prawn showed significantly different (P < 0.05) haemolymph osmolality than their external medium. There was a positive relationship between the duration of air exposure and haemolymph osmolality of both species from a salinity 46 g/L whereas this trend was reversed when prawns were kept in 10 and 22 g/L of salinity.

Different air exposure lengths to both species of prawn when raised in 34 g/L salinity did not significantly alter (P > 0.05) their haemolymph osmotic pressures. However,

air-exposure for more than 14 minutes significantly (P < 0.05) reduced the haemolymph osmolality of both species in 10 g/L salinity. The haemolymph osmolality of the western king prawn at 46 g/L of salinity was higher than the haemolymph osmolality of the brown tiger prawn. Air exposure significantly (P < 0.05) changed the haemolymph osmolality of the brown tiger prawn at 22 g/L salinity after 21 minutes of air exposure and at 46 g/L salinity, after 7 minutes of air exposure.

The haemolymph osmolality of the two species as a function of the medium osmolality are shown in Figures 18 and 19. Table 10 shows isosmotic points and regression equations between the haemolymph osmolality and medium osmolality of the two species of penaeid prawns. For both species, the slopes obtained from the regression lines between both the haemolymph and medium osmolality increased with an increase in the length of air exposure. The slopes for brown tiger prawns were lower than the slopes for western king prawns except when prawns were exposed to air for 7 minutes. Isosmotic points of western king prawns (32.75 to 33.79 g/L) were higher than isosmotic points of brown tiger prawns (30.89 to 32.09 g/L).

**Table 9.** Mean haemolymph osmolality (mOsm/kg) ± SE of brown tiger prawns and western king prawns (data in brackets) reared at different salinities and subjected to different lengths of air exposure.

| Length of air-exposure (min.) | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1$781.66 ± 18.09$^a$ ($_1$808.50 ± 27.06$^a$) | $_1$891.00 ± 10.96$^b$ ($_1$921.75 ± 14.25$^b$) | $_1$950.00 ± 22.60$^{b,c}$ ($_1$927.00 ± 21.45$^b$) | $_1$972.00 ± 23.81$^c$ ($_1$1139.25 ± 28.01$^c$) |
| 7 | $_{1,2}$687.66 ± 39.75$^a$ ($_1$793.50 ± 30.92$^a$) | $_1$886.00 ± 40.11$^b$ ($_1$856.50 ± 59.20$^{ab}$) | $_1$925.00 ± 27.62$^b$ ($_1$947.25 ± 35.84$^b$) | $_2$1105.33 ± 49.36$^c$ ($_1$1136.25 ± 9.03$^c$) |
| 14 | $_2$669.00 ± 36.86$^a$ ($_2$621.75 ± 79.28$^a$) | $_1$882.00 ± 14.17$^b$ ($_1$802.50 ± 65.20$^a$) | $_1$952.00 ± 8.88$^b$ ($_1$1000.50 ± 34.43$^b$) | $_2$1104.33 ± 27.44$^c$ ($_1$1158.75 ± 56.51$^c$) |
| 21 | $_2$637.33 ± 18.77$^a$ ($_2$596.25 ± 34.21$^a$) | $_2$748.00 ± 28.47$^b$ ($_1$771.75 ± 53.90$^b$) | $_1$935.00 ± 23.51$^c$ ($_1$1005.75 ± 12.63$^c$) | $_2$1175.66 ± 25.56$^d$ ($_1$1192.50 ± 39.49$^d$) |
| **Medium Osmolality** | $_3$333 ± 0.00$^a$ | $_3$668 ± 0.00$^b$ | $_2$1011 ± 0.00$^c$ | $_3$1332 ± 0.00$^d$ |

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

**Table 10.** The relationship between medium osmolality and haemolymph osmolality of brown tiger prawns and western king prawns (data in brackets) 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 | G/L |
| 0 | Y = 0.1891 X + 740.57 (Y = 0.2971 X + 700.72) | 0.9168 (0.8617) | 913.27 (996.76) | 30.89 (33.79) |
| 7 | Y = 0.3816 X + 578.24 (Y = 0.334 X + 654.16) | 0.9442 (0.9313) | 941.91 (982.22) | 31.89 (33.29) |
| 14 | Y = 0.4117 X + 557.67 (Y = 0.5418 X + 442.91) | 0.9635 (0.9992) | 947.93 (966.63) | 32.09 (32.75) |
| 21 | Y = 0.539 X + 423.34 (Y = 0.6057 X + 385.18) | 0.9718 (0.9977) | 918.31 (976.87) | 31.07 (33.10) |
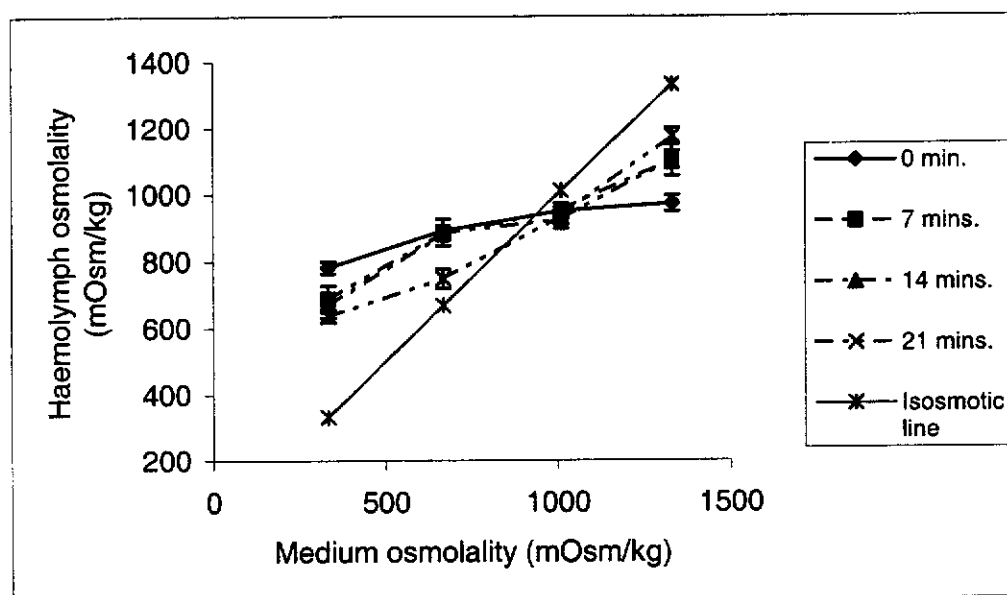
**Figure 18.** The relationship between medium osmolality and haemolymph osmolality of brown tiger prawns reared at different salinities and subjected to different lengths of air exposure.
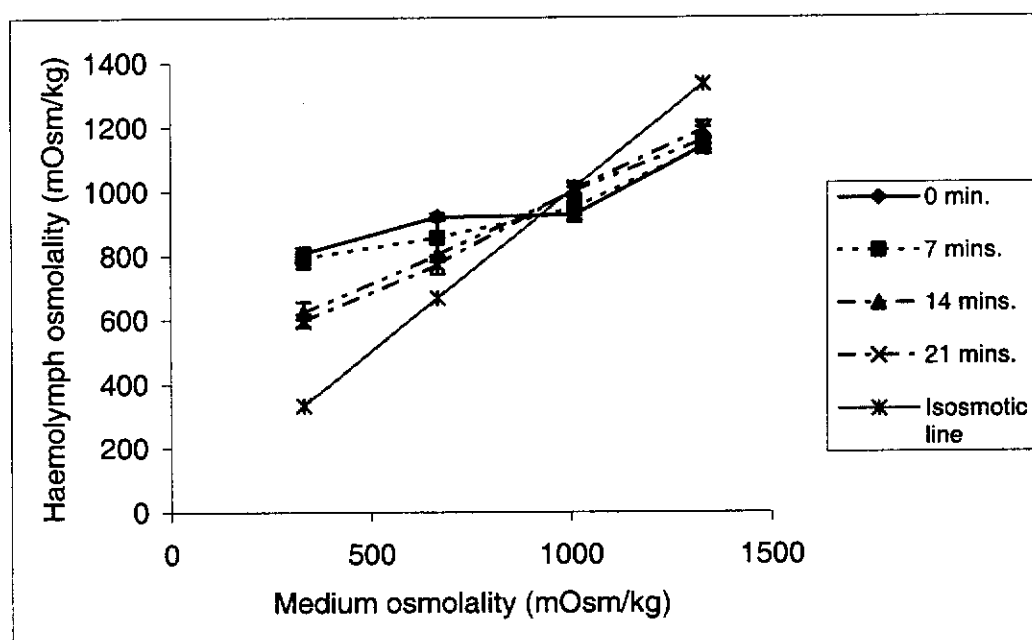


**Figure 19.** The relationship between medium osmolality and haemolymph osmolality of western king prawns reared at different salinities and subjected to different lengths of air exposure.

### 7.3.2 Osmoregulatory capacity (OC)

Osmoregulatory capacity of both species of prawn from 10 g/L salinity significantly declined ($P < 0.05$) after 14 minutes of air-exposure. The OC of the brown tiger prawn from 22 g/L salinity exposed to air for 21 minutes (80.00 ± 28.47 mOsm/kg)

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was significantly lower (P < 0.05) than in prawns exposed for 7 and 14 minutes. There were no significant differences (P > 0.05) in the OC of the two species of prawn from 34 g/L of salinity subjected to different lengths of air-exposure. Osmoregulatory capacity of brown tiger prawns from a salinity of 46 g/L significantly (P< 0.05) declined after being exposed to air for 7 minutes (Table 11).

**Table 11.** Osmoregulatory capacity of western king prawns (data in brackets) and brown tiger prawns reared at different salinities and subjected to different lengths of air exposure.

| Time exposed (min.) | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1$448.66 ± 18.09$^a$ ($_1$475.50 ± 27.06$^a$) | $_1$223.00 ± 10.96$^b$ ($_1$253.75 ± 14.25$^b$) | $_1$61.00 ± 22.60$^c$ ($_1$84.00 ± 42.91$^c$) | $_1$360.00 ± 23.81$^d$ ($_1$192.75 ± 28.01$^b$) |
| 7 | $_{1,2}$354.66 ± 39.75$^a$ ($_1$460.50 ± 30.92$^a$) | $_1$218.00 ± 40.11$^b$ ($_1$188.50 ± 59.20$^b$) | $_1$86.00 ± 27.62$^c$ ($_1$68.25 ± 32.96$^c$) | $_2$226.33 ± 49.36$^{a,b}$ ($_1$195.75 ± 9.03$^b$) |
| 14 | $_2$336.00 ± 36.86$^a$ ($_2$288.75 ± 79.28$^a$) | $_1$214.00 ± 14.17$^b$ ($_1$147.50 ± 55.04$^{a,b}$) | $_1$59.00 ± 8.88$^c$ ($_1$55.50 ± 13.99$^b$) | $_2$227.67 ± 27.44$^b$ ($_1$173.25 ± 56.51$^a$) |
| 21 | $_2$304.33 ± 18.77$^a$ ($_2$263.25 ± 34.21$^a$) | $_2$80.00 ± 28.47$^{bc}$ ($_1$106.25 ± 52.25$^b$) | $_1$76.00 ± 23.51$^b$ ($_1$20.25 ± 5.66$^c$) | $_2$156.33 ± 25.56$^c$ ($_1$139.50 ± 39.49$^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.*

## 7.4 Discussion

The results 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 g/L, which are higher than the isosmotic points of the prawns in the previous experiment. In addition, the present study indicates that brown tiger prawns (slope = 0.1891), when not exposed to air, are better osmoregulators than western king prawns (slopes = 0.2665; 0.2871; 0.2971; 0.3718). In the present study, the isosmotic points of the brown tiger prawn ranges from 913.27 to 947.93 mOsm/kg which is equivalent to 31 to 32 g/L. O'

Brien (1994) showed that optimum growth of the brown tiger prawn occurs at 30 g/L, which is close to the isosmotic point (between 31 and 32 g/L) where prawns spend less energy on osmoregulation. At the isosmotic point the energy uptake is predominantly utilised for growth, development and tissue repair.

The isosmotic points of both the western king and brown tiger prawns are higher than the northern brown, fleshy, pink, Indian white, kuruma, giant tiger, red tail (*P. penicillatus*), blue, white leg and greasyback prawns (Chen & Lin 1998). The results show that the western king and brown tiger prawns have higher salinity preferences than the northern brown, fleshy, pink, Indian white, kuruma, giant tiger, red tail, blue, white leg and greasyback prawns.

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 side 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 both the western king and brown tiger prawns. Reduction in OC in both the western king and brown tiger prawns 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 both species showed an increasing trend with the increase in air-exposure lengths. Thus, 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. This indicates that the reduction in OC of both the western king and brown tiger prawns is positively related to air exposure lengths. The significantly lower OC of brown tiger prawns at 10 g/L and 46 g/L after 7 and 14 minutes of air exposure suggests that salinities of 10 and 46 g/L are not suitable for culturing this species. Under these salinities, the prawns may use more energy to regulate the haemolymph osmolality required to maintain their osmolality balance. The results also show that, at 34 g/L, prawns spent less energy than in other salinities (10, 22 and 46 g/L) for osmoregulation. When being subjected to stressors, prawns still kept the OC to maintain the osmolality of the haemolymph. The significant reduction in OC of western king prawns at 10 g/L indicates that this prawn does not have good tolerance for low salinity (salinity $< 10$ g/L). 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. The results explains why western king prawns have low performance in growth and low survival rate (described in previous sections) at salinities of 10 and 46 g/L.

# 8. CONCLUSIONS AND RECOMMENDATIONS

## 8.1 Conclusions

Optimum salinity range for rearing the western king prawn is from 22 g/L to 34 g/L. A salinity of 34 g/L provided maximum weight, SGR, total length and carapace length of the western king prawn whereas food conversion ratio (FCR) was lowest in prawns reared at salinities of 22 and 34 g/L. Survival rate of the western king prawn was highest at a salinity of 22 g/L and lowest at a salinity of 10 g/L after 15 days of rearing. After 60 days of rearing the survival rate was highest at a salinity of 22 g/L and lowest at a salinity of 46 g/L.

Haemolymph osmolality of the western king prawn and the brown tiger prawn increased with an increase in salinity. Isosmotic points of the western king prawn were 28.87, 29.46 and 31.73 g/L at 0, 20 and 60 days of rearing, respectively. Isosmotic points of the brown tiger prawn were 30.89 to 32.09 g/L. Air exposure reduced the osmoregulatory capacity of both the western king and brown tiger prawns. The osmoregulatory capacity of both species at a salinity of 10 g/L decreased significantly after 14 minutes of air exposure. Twenty-one minutes of air exposure did not change the OC of western king prawns reared at salinities of 22, 34 and 46 g/L. The OC of brown tiger prawns reared in 22 g/L salinity decreased after 21 minutes of air exposure. The OC of brown tiger prawns reared in 46 g/L decreased after 7 minutes of air exposure.

Tail moisture content of western king prawn decreased with an increased medium salinity. After 60 days of rearing, the lowest hepatopancreas moisture content of western king prawn was at a salinity of 22 g/L. Both wet and dry weight hepatosomatic indices of the western king prawn were highest in prawns reared at a salinity of 22 g/L. These indices were lowest in the prawns reared at a salinity of 46 g/L. Both wet weight and dry weight tail muscle indices of the western king prawn were highest in prawns reared at a salinity of 34 g/L.

## 8.2 Recommendations

Future research should focus on the following aspects:

Determining the optimum salinity for growth and survival of various development stages of the western king prawn.

Determining the osmoregulatory capacity of different developmental and moult stages of the western king prawns at different salinities.

Investigating the ionic (calcium, potassium and sodium) regulatory ability of various developmental stages of the western king prawn reared at different salinities.

Understanding the physiological mechanism to regulate various ions when the western king prawn are reared at different salinities.

Finding the relationship between ionic regulatory budgets with various growth related parameters like moult increment, food conversion ratio and specific growth rate.

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# APPENDIX

**Appendix 1.** Total length (mean ± S.E. (mm)) of western king prawns reared at different salinities

| Day | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1$69.03 ± 1.104$^a$ | $_1$69.39 ± 1.703$^a$ | $_1$70.39 ± 1.389$^a$ | $_1$70.89 ± 2.706$^a$ |
| 15 | $_1$66.94 ± 1.998$^a$ | $_{1,2}$75.08 ± 3.277$^b$ | $_1$76.07 ± 3.318$^b$ | $_1$68.70 ± 0.85$^{a,b}$ |
| 30 | | $_2$76.89 ± 2.598$^a$ | $_{1,2}$77.80 ± 3.705$^a$ | $_1$69.62 ± 2.322$^a$ |
| 45 | | $_2$78.79 ± 1.240$^{a,b}$ | $_{2,3}$87.34 ± 4.076$^a$ | $_1$73.65 ± 4.630$^b$ |
| 60 | | $_2$80.15 ± 1.012$^{a,b}$ | $_3$88.84 ± 3.536$^a$ | $_1$74.23 ± 4.667$^b$ |

*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.*

**Appendix 2.** Carapace length (Mean ± S.E. (mm)) of western king prawns reared at different salinities.

| Day | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1$16.25 ± 0.129$^a$ | $_1$16.28 ± 0.562$^a$ | $_1$16.69 ± 0.435$^a$ | $_1$16.47 ± 0.624$^a$ |
| 15 | $_1$16.17 ± 0.689$^a$ | $_2$18.19 ± 0.779$^{a,b}$ | $_{1,2}$18.52 ± 0.765$^b$ | $_1$16.68 ± 0.445$^{a,b}$ |
| 30 | | $_2$18.73 ± 0.475$^{a,b}$ | $_{2,3}$19.47 ± 0.819$^a$ | $_1$17.19 ± 0.759$^b$ |
| 45 | | $_2$18.77 ± 0.230$^{a,b}$ | $_3$21.30 ± 1.258$^a$ | $_1$17.55 ± 1.377$^b$ |
| 60 | | $_2$19.59 ± 0.172$^{a,b}$ | $_3$21.61 ± 0.864$^a$ | $_1$18.13 ± 0.382$^b$ |

*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.*

**Appendix 3.** SGR in weight of western king prawns reared at different salinities.

| Day period | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 15-0 | $-0.056 \pm 0.216^a$ | $1.895 \pm 0.318^b$ | $1.785 \pm 0.497^b$ | $0.251 \pm 0.926^b$ |
| 30-0 | | $1.306 \pm 0.109^a$ | $1.279 \pm 0.166^a$ | $0.405 \pm 0.687^a$ |
| 45-0 | | $1.218 \pm 0.274^a$ | $1.477 \pm 0.089^a$ | $0.728 \pm 0.740^a$ |
| 60-0 | | $0.941 \pm 0.198^a$ | $1.149 \pm 0.051^a$ | $0.561 \pm 0.556^a$ |
| 30-15 | | $0.718 \pm 0.412^a$ | $0.775 \pm 0.393^a$ | $0.559 \pm 0.514^a$ |
| 45-15 | | $0.880 \pm 0.563^a$ | $1.324 \pm 0.232^a$ | $0.963 \pm 0.459^a$ |
| 60-15 | | $0.624 \pm 0.364^a$ | $0.938 \pm 0.168^a$ | $0.662 \pm 0.306^a$ |
| 45-30 | | $1.041 \pm 0.753^a$ | $1.714 \pm 0.198^a$ | $1.634 \pm 0.450^a$ |
| 60-30 | | $0.576 \pm 0.354^a$ | $0.940 \pm 0.132^a$ | $0.846 \pm 0.228^a$ |
| 60-45 | | $0.116 \pm 0.125^a$ | $0.166 \pm 0.074^a$ | $0.058 \pm 0.005^a$ |
| Mean | $-0.056 \pm 0.216^a$ | $0.931 \pm 0.152^{b,c}$ | $1.154 \pm 0.151^b$ | $0.667 \pm 0.137^c$ |

*Data in the same row having different superscript letters (a, b, c...) are significantly different at $\propto$ level of 0.05.*

**Appendix 4.** SGR in total length of western king prawns reared at different salinities.

| Day period | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 15-0 | $-0.212 \pm 0.283^a$ | $0.512 \pm 0.129^b$ | $0.503 \pm 0.218^b$ | $-0.196 \pm 0.276^b$ |
| 30-0 | | $0.399 \pm 0.044^a$ | $0.324 \pm 0.112^a$ | $-0.058 \pm 0.201^a$ |
| 45-0 | | $0.283 \pm 0.046^a$ | $0.441 \pm 0.068^a$ | $0.076 \pm 0.226^a$ |
| 60-0 | | $0.241 \pm 0.038^a$ | $0.360 \pm 0.037^a$ | $0.070 \pm 0.171^a$ |
| 30-15 | | $0.166 \pm 0.106^a$ | $0.145 \pm 0.229^a$ | $0.079 \pm 0.154^a$ |
| 45-15 | | $0.169 \pm 0.122^a$ | $0.421 \pm 0.066^a$ | $0.230 \pm 0.166^a$ |
| 60-15 | | $0.151 \pm 0.088^a$ | $0.319 \pm 0.076^a$ | $0.171 \pm 0.112^a$ |
| 45-30 | | $0.171 \pm 0.206^a$ | $0.505 \pm 0.185^a$ | $0.374 \pm 0.116^a$ |
| 60-30 | | $0.143 \pm 0.109^a$ | $0.311 \pm 0.108^a$ | $0.213 \pm 0.060^a$ |
| 60-45 | | $0.114 \pm 0.027^a$ | $0.117 \pm 0.082^a$ | $0.052 \pm 0.018^a$ |
| **Mean** | $-0.212 \pm 0.283^a$ | $0.229 \pm 0.038^b$ | $0.345 \pm 0.045^b$ | $0.101 \pm 0.050^c$ |

*Data in the same row having different superscript letters (a, b, c...) are significantly different at $\propto$ level of 0.05.*

**Appendix 5.** FCR of western king prawns reared at different salinities.

| Salinity (g/L) | Mean ± S.E. | Max | Min |
|---|---|---|---|
| 22.00 | $_1$3.26 ± 0.36 | 4.27 | 2.71 |
| 34.00 | $_1$3.34 ± 0.51 | 4.38 | 2.26 |
| 46.00 | $_2$7.83 ± 1.24 | 11.29 | 5.54 |

*Data in the same column having different subscript letters (1,2,3...) are significantly different at ∝ level of 0.05.*

**Appendix 6.** Survival rate of western king prawns reared at different salinities.

| Day | Salitnity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1$100 | $_1$100 | $_1$100 | $_1$100 |
| 15 | $_2$20.45 ± 5.72 [a] | $_2$68.18 ± 5.87 [b] | $_2$52.27 ± 7.76 [b,c] | $_2$38.64 ± 4.35 [a,c] |
| 30 | 0 | $_3$40.91 ± 5.87 [a] | $_3$25.00 ± 4.35 [b] | $_3$13.64 ± 2.62 [b] |
| 45 | 0 | $_4$22.73 ± 5.87 [a] | $_3$15.91 ± 5.72 [a] | $_3$9.09 ± 3.71 [a] |
| 60 | 0 | $_4$22.73 ± 5.87 [a] | $_3$13.64 ± 5.87 [a,b] | $_3$6.82 ± 2.27 [b,c] |

*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.*

**Appendix 7.** Haemolymph osmolality (mOsm/kg) of western king prawns reared at different salinities.

| Day | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1$676.25 ± 29.20[a] | $_1$845.00 ± 14.54[b] | $_{1,2}$899.00 ± 12.18[b] | $_1$977.75 ± 15.31[c] |
| 20 | $_1$664.00 ± 57.70[a] | $_2$951.00 ± 19.05[b,c] | $_1$834.00 ± 54.71[b] | $_{1,2}$1001.00 ± 41.66[c] |
| 60 | | $_1$854.00 ± 13.63[a] | $_2$930.00 ± 5.79[a] | $_2$1102 ± 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.*

**Appendix 8.** Hepatopancreas moisture content (%) of western king prawns reared at different salinities.

| Day | Salinity | | | |
| --- | --- | --- | --- | --- |
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1$73.40 ± 1.736$^a$ | $_1$63.79 ± 1.986$^b$ | $_1$62.01 ± 2.094$^b$ | $_1$69.86 ± 0.705$^a$ |
| 20 | $_1$75.81 ± 1.302$^a$ | $_1$69.38 ± 2.706$^{ab}$ | $_2$72.75 ± 3.105$^{a,b}$ | $_1$68.00 ± 1.769$^b$ |
| 60 | | $_1$64.97 ± 4.225$^a$ | $_3$83.23 ± 2.981$^b$ | $_2$80.85 ± 4.664$^b$ |

*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.*

**Appendix 9.** Tail muscle moisture content of western king prawns reared at different salinities.

| Day | Salinity | | | |
| --- | --- | --- | --- | --- |
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1$82.43 ± 1.681$^a$ | $_1$76.17 ± 8.974$^b$ | $_1$74.62 ± 0.787$^b$ | $_1$71.03 ± 3.221$^b$ |
| 20 | $_1$80.37 ± 0.526$^a$ | $_1$77.16 ± 0.591$^b$ | $_1$76.25 ± 0.496$^{b,c}$ | $_1$74.71 ± 0.377$^c$ |
| 60 | | $_1$76.25 ± 0.037$^a$ | $_1$76.11 ± 0.527$^a$ | $_1$75.75 ± 0.733$^a$ |

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

**Appendix 10.** Wet weight hepatosomatic index of western king prawns reared at different salinities.

| Day | Salinity | | | |
| --- | --- | --- | --- | --- |
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1$0.93 ± 0.367$^a$ | $_1$1.36 ± 0.073$^b$ | $_1$0.99 ± 0.034$^a$ | $_1$0.86 ± 0.028$^a$ |
| 20 | $_1$1.16 ± 0.182$^a$ | $_2$1.08 ± 0.058$^a$ | $_1$1.18 ± 0.133$^a$ | $_2$1.36 ± 0.173$^a$ |
| 60 | | $_3$1.83 ± 0.053$^a$ | $_2$1.54 ± 0.134$^a$ | $_{1,2}$1.19 ± 0.088$^b$ |

*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.*

**Appendix 11.** Dry weight hepatosomatic index of western king prawns reared at different salinities.

| Day | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1 0.24 \pm 0.092^{a}$ | $_{1,2} 0.49 \pm 0.041^{b}$ | $_1 0.38 \pm 0.023^{a,b}$ | $_1 0.26 \pm 0.011^{a}$ |
| 20 | $_1 0.29 \pm 0.059^{a}$ | $_1 0.32 \pm 0.028^{a}$ | $_1 0.34 \pm 0.078^{a}$ | $_2 0.43 \pm 0.052^{a}$ |
| 60 | | $_2 0.64 \pm 0.092^{a}$ | $_1 0.26 \pm 0.043^{b}$ | $_1 0.23 \pm 0.086^{b}$ |

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

**Appendix 12.** Wet weight tail muscle index of western king prawns reared at different salinities.

| Day | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1 18.06 \pm 6.230^{a}$ | $_1 24.79 \pm 2.227^{a}$ | $_1 19.93 \pm 1.377^{a}$ | $_1 21.42 \pm 2.233^{a}$ |
| 20 | $_1 22.42 \pm 0.538^{a}$ | $_1 20.77 \pm 1.101^{a}$ | $_1 22.78 \pm 1.264^{a}$ | $_1 17.72 \pm 2.989^{a}$ |
| 60 | | $_1 25.84 \pm 2.090^{a,b}$ | $_2 28.62 \pm 0.968^{a}$ | $_1 22.17 \pm 1.766^{b}$ |

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

**Appendix 13.** Dry weight tail muscle index of western king prawns reared at different salinities.

| Day | Salinity | | | |
|---|---|---|---|---|
| | 10 g/L | 22 g/L | 34 g/L | 46 g/L |
| 0 | $_1 3.05 \pm 1.045^{a}$ | $_1 5.90 \pm 0.53^{b}$ | $_1 4.89 \pm 0.47^{a,b}$ | $_1 6.30 \pm 1.21^{b}$ |
| 20 | $_1 4.55 \pm 0.126^{a}$ | $_1 4.75 \pm 0.34^{a}$ | $_1 5.40 \pm 0.32^{a}$ | $_1 4.47 \pm 0.73^{a}$ |
| 60 | | $_1 6.14 \pm 0.56^{a,b}$ | $_2 6.84 \pm 0.31^{a}$ | $_1 5.33 \pm 0.34^{b}$ |

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

**Appendix 14.** List of manuscripts submitted

1. The effects of air exposure on haemolymph osmoregularoty capacity of brown tiger shrimp (*Penaeus esculentus*) and western king shrimp (*P. latisulcatus*) reared at different salinities.

2. Growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (*Penaeus latisulcatus* Kishinouye, 1896) reared at different salinities.